United States
Environmental Protection
Agency

Protocol for EPA Approval of New Methods for Organic and Inorganic Analytes in Wastewater and Drinking Water

March 1999

U.S. Environmental Protection Agency
Office of Water
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Foreword

Within the U.S. Environmental Protection Agency (EPA), the Office of Water (OW) publishes test procedures (analytical methods) for analysis of wastewater and drinking water. Listed at parts 136 and 141 of Title 40 of the *Code of Federal Regulations* (CFR), these methods are authorized for use in data gathering and environmental monitoring under the Clean Water Act (CWA) and the Safe Drinking Water Act (SDWA). These methods have been developed by EPA, by consensus standards organizations, and by others. Many of these methods, especially methods published before 1990, are prescriptive with limited ability to modify procedures or change technologies to accommodate specific situations. There has been a growing awareness within EPA and the analytical community that the requirement to use prescriptive measurement methods and technologies to comply with Agency regulations has unintentionally imposed a significant regulatory burden and created a barrier to the use of innovate environmental monitoring technology.

This document gives specific instructions to external organizations regarding the validation, submission, and EPA approval of applications for the approval of new methods to determine inorganic and organic analytes. EPA anticipates that the standardized procedures described herein should expedite the approval of new methods, encourage the development of innovative technologies, and enhance the overall utility of the EPA-approved methods for compliance monitoring under National Pollution Discharge Elimination System (NPDES) permits and national primary drinking water regulations (NPDWRs).

This document is not a legal instrument and does not establish or affect legal obligations under Federal regulations. EPA reserves the right to change this protocol without prior notice.

All questions regarding the guidelines presented in this document should be directed to:

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1.0 INTRODUCTION

1.1 Background and Objectives

1.1.1 Clean Water Act and Safe Drinking Water Act

CWA section 304(h) requires the EPA Administrator to promulgate guidelines establishing test procedures for data gathering and monitoring compliance with published guidelines. EPA's approval of analytical methods is authorized under this section of CWA, as well as the general rulemaking authority in CWA section 501(a). The section 304(h) test procedures (analytical methods) are specified at 40 CFR part 136. They include *Methods for Chemical Analysis of Water and Waste* (MCAWW); the 600- and 1600-series methods; methods published by consensus standards organizations; methods used by the U.S. Geological Survey; methods developed by the environmental community; and other methods referenced in CWA regulations. EPA uses these test procedures to support development of effluent limitations guidelines approved at 40 CFR parts 400 - 499, to establish compliance with (NPDES) permits issued under CWA section 402, for implementation of the pretreatment standards issued under CWA section 307, and for CWA section 401 certifications.

The SDWA requires the EPA Administrator to promulgate National Primary Drinking Water Regulations (NPDWRs) that specify maximum contaminant levels (MCLs) or treatment techniques for listed drinking water contaminants (section 1412). In addition, section 1445(a) of SDWA authorizes the Administrator to establish regulations for monitoring to assist in determining whether persons are acting in compliance with the requirements of SDWA. EPA's approval of analytical test procedures is authorized under these sections of SDWA, as well as the general rulemaking authority in SDWA section 1450(a).

SDWA section 1401(1)(D) specifies that NPDWRs contain criteria and procedures to ensure a supply of drinking water that dependably complies with MCLs, including quality control (QC) and testing procedures to ensure compliance with such levels and to ensure proper operation and maintenance of drinking water supply and distribution systems. These test procedures (analytical methods) are approved at 40 CFR part 141. They include MCAWW methods; the 200-, 300-, and 500- series methods; and other methods referenced in SDWA regulations. EPA uses these test procedures to establish MCLs under SDWA section 1412 and to establish monitoring requirements under SDWA Section 1445(a).

1.1.2 40 CFR 136.4, 136.5 and 141.27

Requirements for approval of alternate analytical techniques (methods) are specified at 40 CFR 136.4 and 136.5 for wastewater methods and at 40 CFR 141.27 for drinking water methods. These requirements are the basis for the Agency's alternate test procedure (ATP) program for water methods. Under the ATP program, an organization may submit an application for approval of a modified version of an approved method or for approval of a new method to be used as an alternate to an approved method. The submitting organization is responsible for validating the new or modified method. The Agency reviews the ATP validation package and, if required, promulgates successful applications in the CFR. Rulemaking is required when a new or revised method is added to the list of approved methods in the CFR. The ATP and rulemaking processes make heavy demands on stakeholder, contractor, EPA, and *Federal Register* resources. These processes can require several months to approve a minor method modification and a year or more to promulgate a major modification or a new technology. Because advances in analytical technology continue to outpace the capacity of OW's method approval program, the program has been

under-utilized and slow to respond to emerging technologies. This protocol is intended to specify a more rapid and less resource intensive process for approval of new technologies.

1.1.3 Description of Document

This protocol details the requirements for approval of new methods to be included at 40 CFR part 136 or 141. A new method is a set of procedures that has been written in the seventeen section standard EPA format as detailed in the *Guidelines and Format for Methods to be Proposed at 40 CFR Part 136 or Part 141*; contains standardized QC elements with associated QC acceptance criteria; employs a determinative technique for an analyte of concern that differs from determinative techniques employed for that analyte in methods previously approved at 40 CFR part 136 or 141 and employs a determinative technique that is as sensitive and/or selective as the determinative techniques in all methods previously approved for the analyte.

The new methods approval program provides chemists with the opportunity to utilize best professional judgement to enhance compliance monitoring. Approval for a new method may be sought when the new method reduces analytical costs, overcomes matrix interferences problems, improves laboratory productivity, or reduces the amount of hazardous materials used and/or produced in the laboratory. The new methods approval program thus can serve as a mechanism for gaining approval of innovative technologies for use in compliance monitoring programs. The protocol described in this document is designed to reduce the barriers to gaining acceptance of new methods, to spur the development and use of new technologies, and to expedite the review and approval process for gaining acceptance of a new method. A method developer may apply to gain approval for the use of a new method for determination of an analyte of interest to the NPDES or NPDWR monitoring programs by developing and validating the new method using either the procedures described in this document or the classical interlaboratory validation procedures provided by organizations such as ASTM[1] and AOAC-International.[2,3] While EPA can be contacted at any point for assistance, EPA's main role will be to review the application for completeness and to determine acceptability. Consequently, EPA will be able to approve new methods for use more quickly and efficiently.

1.2 Tiered System for Validation of New Methods

EPA recognizes that a formal interlaboratory method validation may not be suitable for all situations and may be prohibitively costly to implement, especially for small laboratories and regulated entities. Therefore, EPA has developed a three-tiered, cost-effective approach to method validation that classifies the intended use of a new method and requires a method validation study that reflects the level of use associated with each tier. An applicant would have to determine the most appropriate tier for the new method and develop QC acceptance criteria using the procedures specified in Appendix D of this protocol. The three method validation tiers are listed below.

Tier 1 methods may only be used by a single laboratory (limited-use) for one or more matrix type(s). A matrix type 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, POTW sludge, and in-process streams in the Atlantic and Gulf Coast Hand-shucked Oyster Processing subcategory are each a matrix type. Tier 1 validation requires a single laboratory validation study in the matrix type(s) of interest.

Tier 2 methods may be used by all laboratories (nationwide use) for only one matrix type. Validation requires a three-laboratory validation study.

Tier 3 methods may be used by all laboratories (nationwide use) for all matrix types. Validation requires a nine-laboratory validation study.

2.0 APPLICATION REQUIREMENTS

Every new method application shall be made in triplicate and include a completed new method approval application form (provided in Appendix A) with required attachments.

2.1 Submission Addresses

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

Table 1: Submission of New Method Applications

TIER	LEVEL OF USE	APPLICANT	SUBMIT APPLICATION TO[1]	APPROVAL AUTHORITY
Tier 1	Limited Use for Wastewater	EPA Regional laboratories	EPA Regional Administrator[2] (Regional ATP coordinator)	EPA Regional Administrator
		States, commercial laboratories, individual dischargers, or permitees in States that do not have authority	EPA Regional Administrator[2] (Regional ATP coordinator)	
		States, commercial laboratories, individual dischargers, or permitees in States that have authority	Director of State Agency issuing the NPDES permit[2]	
Tier 2	Nationwide Use	All applicants	Director, Analytical Methods Staff, EPA Headquarters	EPA Administrator
Tier 3	Nationwide Use	All applicants	Director, Analytical Methods Staff, EPA Headquarters	EPA Administrator

[1] See Appendix B for EPA addresses.

[2] The Regional ATP coordinator may choose to forward Tier 1 (LU) applications to the Director of the Analytical Methods Staff (AMS) for an approval recommendation.

Upon receipt of the application, AMS staff will assign an identification number to the application. The applicant should use the identification number in all future communications concerning the application.

2.2 Application Information

Information required on the new method application form includes: the name and address of the applicant; the date of submission of the application; the method number and title of the proposed new method; the analytes(s) for which the new method is proposed; the type of application (i.e., wastewater, drinking water, or a combined wastewater/drinking water application); the level of use desired (i.e., limited use or nationwide use); the tier level at which the proposed new method will be validated; and, for limited-useapplications, the applicant's NPDES permit number, the issuing agency, the type of permit and the discharge serial number if applicable.

The following items must be submitted with the application: the justification for proposing the new method; the proposed new method prepared in standard EPA format; the method validation study report, including supporting data; and, 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.

Before proceeding with the new method validation, the Agency strongly encourages an applicant to submit its validation study plan for EPA review and comment.

The elements required for a complete application at each tier are presented in Table 2. EPA must receive all required application information and attachments before the application is considered complete.

Table 2. Application Requirements

Tier	Level of Use	Application Requirements
Tier 1	Limited Use	• Completed application form • Justification for new method • Method in EPA format • Validation study report
Tier 2	Nationwide Use	• Completed application form • Justification for new method • Method in EPA format • Validation study report • Method development information and documentation
Tier 3		

2.2.1 Justification for New Method

The entity that proposes a new method should provide a brief justification for why the new method is being proposed. Examples include but are not limited to: the new method successfully overcomes some or all of the interferences associated with the approved method; the new method significantly reduces the amount of hazardous wastes generated by the laboratory; or the cost of analyses are significantly reduced when using the new method.

2.2.2 Standard EPA Method Format

In accordance with the standard EPA format advocated by EPA's Environmental Monitoring Management Council (EMMC), methods must contain 17 specific topical sections in a designated order. The 17 sections listed in Appendix C to this document are mandatory for all methods. Additional numbered sections, may be inserted after Section 11.0, *Procedure*, as appropriate for a particular method. For more detailed information on the EPA format for proposed methods, see the Guidelines and Format document.[4]

2.2.3 Validation Study Report

The applicant must conduct a validation study and provide a comprehensive validation study report with the new method application. The validation study report must include the following elements:

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

These elements are described in Section 3.6.

2.2.4 Method Information and Documentation to Facilitate EPA Preparation of Preamble and Docket

For Tier 2 and 3 applications, the new method will be approved by the EPA Administrator through rulemaking. In these cases, the applicant shall provide to EPA information and documentation that will aid EPA in preparing the preamble and docket for the proposed rule that will be published in the *Federal Register*. Information to be provided includes: a detailed background and summary of the method, a discussion of QC acceptance criteria development, and a description and discussion of the interlaboratory method validation study and any other method studies conducted during method development and validation. Specifically, the applicant shall 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 indicates whether the method is associated with a sampling method
- Identifies the matrix(ces) 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 performing the test and data analysis
- Describes the QC acceptance criteria development process and gives example calculations

- Lists options to the method, if applicable
- Discusses in a summary fashion the acceptability criteria for the method
- Describes and discusses the validation study report, including study design and objectives, study limitations, study management, technical approach, data reporting and validation, results, data analysis discussion, and conclusions

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. In addition to method information, the applicant must provide copies of all relevant supporting documents used in developing the method, for EPA's inclusion in the rule docket.

2.3 Proprietary Information in Applications

All information provided to the Federal government is subject to the requirements of the Freedom of Information Act. Therefore, any proprietary information submitted with the proposed new method application should be marked as 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*. Allegedly confidential 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 so state.

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 assure that such efforts will be effective in light of the possibility of prior disclosure or widespread prior dissemination of the information. Methods to be proposed in the *Federal Register* cannot be claimed as confidential.

3.0 METHOD VALIDATION

3.1 Introduction

Method validation is the process by which a method developer substantiates the performance of a new method. New methods must be validated to prove that they accurately measure the concentration of an analyte in an environmental sample. If, during a compliance inspection or audit, it is determined that a regulated party is using an unvalidated new method, the data generated by the unvalidated method will be considered unacceptable for compliance monitoring or reporting. The validation requirements listed below were developed to reflect the level of intended use of the method. This is accomplished through a three-tiered approach, as shown in Table 3.

Table 3: Tiered Validation Strategy

Tier Level	Laboratory Use	Applicable to . . .
Tier 1	Single Laboratory (Limited-use or LU)	One or more matrix types from any industry; (Excluding PWSs)
Tier 2	All Laboratories (Nationwide use or NW)	One matrix type within one industrial subcategory; or all PWSs
Tier 3	All Laboratories (Nationwide use or NW)	All matrix types from all industrial subcategories

Under Tier 1, single laboratories will be allowed to validate and use new test methods without the burden of conducting an interlaboratory validation study, whereas new methods intended for multi-laboratory use in a given industrial subcategory (Tier 2) or for multi-laboratory use for all industrial subcategories (Tier 3) require interlaboratory testing.

3.2 Summary of Validation Requirements

EPA has developed a tiered validation approach that coordinates validation requirements with the level of intended use of the new method. Tier 1 (LU) represents validation in a single laboratory, Tier 2 (NW) represents interlaboratory validation in one industrial subcategory, and Tier 3 (NW) represents interlaboratory validation in multiple matrix types. New methods may be used after validation at the appropriate level is performed and formal approval is granted by the appropriate authority. Tier 1 (LU) contains two levels of validation, depending on whether the individual laboratory will be applying the new method to a single matrix type or to multiple matrix types. The Tier 1- Single Matrix Type category allows the laboratory to apply the new method to a single matrix type. The Tier 1- Multiple-Matrix Type category allows a single laboratory to apply the new method to an unlimited number of matrix types after the method has been validated on a minimum of nine matrix types.

Table 4 summarizes the validation requirements for wastewater new methods. Table 5 summarizes the validation requirements for drinking water new methods. Only Tier 2 (NW) validations are applicable

to drinking water because the Office of Ground Water and Drinking Water (OGWDW) no longer approves Tier 1 (LU) new methods and the drinking water program regulates a limited number of matrix types.

Table 4. Summary of Validation Requirements for New Methods for the Analysis of Wastewater [1]

	Number of		Number of Analyses Required			
Method Application	**Labs**	**Matrix types**	**IPR- reagent water[2]**	**IPR- sample matrix[3]**	**MS/MSD**	**MDL[4]**
Tier 1-Single-lab						
First matrix type	1	1	4	4	0	7
Each additional matrix type (8 max.)	1	1	0[5]	0[5]	2	0[5]
Tier 2-Multi-lab, single matrix type	3	1	12	0	6[6]	21
Tier 3-Multi-lab, multiple matrix types All matrix types	9[7]	9	36	0	18[6]	63

Notes:

(1) Numbers of analyses in this table do not include background analyses or additional QC tests such as calibration, blanks, etc. Validation requirements are based on the intended application of the method. Nine would be the maximum number of matrix types (or facilities) that would be required to validate a new wastewater method at Tier 1 or 3.

(2) IPR reagent water analyses would be used to validate method performance and to establish QC acceptance criteria for initial precision and recovery (IPR) and ongoing precision and recovery (OPR) for a new method. The required number of IPR analyses, except as noted under footnote 6, would be four times the number of laboratories required to validate a new method because each laboratory would perform a 4-replicate IPR test.

(3) IPR sample matrix analyses would be 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 would not be required for validation of Tier 2 or 3 new methods because this variability data would be obtained from MS/MSD tests.

(4) A method detection limit (MDL) test would be performed in each laboratory using the new method. 40 CFR part 136, Appendix B, requires a minimum of seven analyses per laboratory to determine an MDL. Each lab involved in validation of a new wastewater method would demonstrate that the new method would achieve the detection limits specified in the regulations at 40 CFR parts 136 and/or in another EPA specified documents.

(5) The MDL, reagent water IPR, and sample matrix IPR tests would not have to be repeated after the first matrix type or facility was validated.

(6) The MS/MSD analyses would establish MS/MSD recovery and precision for the new method. The required number of MS/MSD analyses would be two times the number of facilities or matrix types tested.

(7) The number of laboratories and samples would vary if a conventional interlaboratory study is used.

Table 5. Summary of Validation Requirements for New Methods for the Analysis of Drinking Water [(1)]

Method Application	Number of		Number of Analyses Required			
	Labs	PWSs	IPR- reagent water[(2)]	IPR- sample matrix[(3)]	MS/MSD	MDL[(4)]
Tier 2-Multilab	3	3	12	0	6[(4)]	21

Notes:

(1) Numbers of analyses in this table do not include background analyses or additional QC tests such as calibration, blanks, etc.

(2) IPR reagent water analyses would be used to validate method performance and to establish QC acceptance criteria for initial precision and recovery (IPR) and ongoing precision and recovery (OPR) for a new method.

(3) IPR sample matrix analyses would not be required for validation of Tier 2 new methods because this variability data would be obtained from MS/MSD tests.

(4) A method detection limit (MDL) test would be performed in each laboratory using the new method. 40 CFR part 136, Appendix B, requires a minimum of seven analyses per laboratory to determine an MDL.

All new methods must be validated to demonstrate that the method is capable of yielding reliable data for compliance monitoring purposes. All validation study results must be documented in accordance with the requirements outlined below.

3.3 Tier 1, 2, and 3 Validation Studies

The tiered approach to validation encourages laboratories to take advantage of new technologies, overcome matrix interference problems, lower detection limits, improve the reliability of results, lower the costs of measurements, and improve overall laboratory productivity without undertaking costly and time-consuming interlaboratory studies. 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 2 studies are expected to be used by water supply laboratories, dischargers, and state and municipal laboratories repetitively testing samples from multiple sites within the same industrial subcategory on a routine basis. Tier 3 studies are expected to be used by vendors, commercial laboratories, dischargers, and state and municipal laboratories testing a wide variety of sample matrices from diverse sites. Tier 3 also is expected to be used by vendors seeking nationwide approval of a new technology.

3.3.1 Tier 1 Validation Studies (for Wastewater Only)

The primary intent of Tier 1 is to allow use of a new method by a single laboratory. Tier 1 can be applied to one or more matrix types.

Tier 1 - Single Matrix Type

Tier 1- single matrix type validation studies are performed in a single laboratory on a single matrix type. Results of the validation study and the method are applicable in this laboratory to this matrix type only and cannot be used by another laboratory or for another matrix type.

Tier 1 - Multiple Matrix Types

If a laboratory intends to apply the method to more than one matrix type, the laboratory must validate the method on each matrix type. Nine matrix types for new methods for the analysis of wastewater is the maximum number of matrix types to which the new method must be applied to demonstrate that it will likely be successful for all other matrix types. EPA chose this upper limit of matrix tests for Tier 1- multiple matrix types validation, because the maximum number of matrices tested should not be greater than the number required for Tier 3 validation of a wastewater method (nine). Therefore, nine different wastewater matrix types is the number after which a test on each subsequent matrix type is not required. The specific tests to be conducted on the first wastewater matrix type and those for each additional matrix type are enumerated in Table 4. In all cases, the laboratory must try to determine if the measurement result for the target analyte using a new matrix type differs from the result obtained in a reagent water matrix or in a previously validated matrix type.

Matrices that must be tested for Tier 1- multiple matrix type validation of a new method are given in Table 6. As with a Tier 1- single matrix type validation study, Tier 1- multiple matrix type validation studies are performed in a single laboratory and, therefore, cannot be transferred to another laboratory. If a method is validated by a single laboratory in two to eight discrete matrix types, the validation is applicable to those matrix types only. However, once a laboratory has validated the method on nine matrix types, and those matrix types possess the characteristics required in Table 6, the validation is applicable to all other matrix types.

If results of Tier 1- multiple matrix type validation studies are to be applied to a different medium (e.g., air, water, soil, sludge), each medium must be represented in the samples tested in the validation study.

Table 6. Matrix Types Required for Multiple Matrix Type Validation Studies

1. Effluent from a POTW
2. ASTM D 5905 - 96, *Standard Specification for Substitute Wastewater*
3. Sewage sludge, if sludge will be in the permit
4. ASTM D 1141 - 90 (Reapproved 1992), *Standard Specification for Substitute Ocean Water*, if ocean water will be in the permit
5. Drinking water, if the method will be applied to drinking water samples
6. Untreated and treated wastewaters to a total of nine matrix types

At least one of the above wastewater matrix types must 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

3.3.2 Tier 2 Validation Studies for Wastewater and Drinking Water

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 in a single industry. Since drinking water is considered a single matrix type and PWSs represent a single industry, Tier 2 facilitates nationwide use of a new method for the analysis of drinking water.

EPA believes that implementation of Tier 2 will encourage the development and application of techniques that overcome matrix interference problems, lower detection limits, improve the reliability of results, lower the costs of measurements, and improve overall laboratory productivity when analyzing samples from a given industry.

Significant industries within Tier 2 are: PWSs, publicly-owned treatment works (POTWs), and individual industrial subcategories that are defined in the regulations at 40 CFR parts 405 - 503. At present, there are approximately 650 industrial subcategories defined in the Part 405 - 503 regulations, each of which constitutes an individual industry under this protocol.

Tier 2 validation studies are performed in a minimum of three laboratories. Samples of the same matrix type (e.g., drinking water, final effluent, extraction-stage effluent,) are collected from one or more facilities in the same industrial subcategory. In all cases, the laboratory must try to determine if the measurement result for the target analyte using a new method differs from the result obtained in a reagent water matrix or in a previously validated matrix type or PWS sample.

Drinking water sources tested for Tier 2 validation of a new method for the analysis of drinking water must include samples collected from PWSs with water quality characteristics that are sufficiently different so that sample matrix effects, if any, can be observed. Selection of suitable PWSs requires a knowledge of the chemistry in the method. Analysts may review an applicable approved or published method for indications of matrix effects that are unique to the analyte separation and measurement technologies used in the new method. Water quality characteristics that can affect analysis of drinking water samples include, but are not limited to: pH, total organic carbon content, turbidity, total organic halogen content, ionic strength, sulfate contamination, metal contamination, and trihalomethane contamination of the drinking water sample.

For POTWs, if a new method is validated on final effluent only, that method would be applicable to final effluent only, and the title of the method must reflect that the method is applicable to final effluent only. If influent to treatment, primary effluent, and sludges are to be monitored, the method must be validated separately on these sample matrix types.

In contrast to Tier 1, once a new method has been validated, the validation study results can be transferred to other laboratories, and the other laboratories may freely use the method, as long as the method is applied to analysis of samples of the validated matrix type from 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 method must be validated on that matrix type separately.

3.3.3 Tier 3 Validation Studies

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. The increased flexibility at Tier 3 should allow vendors to establish that new devices and reagents produce results that are acceptable for compliance monitoring purposes, and should allow commercial laboratory chains to apply new technologies and techniques throughout their chain of laboratories to 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. The minimum requirements for sample matrix types that must be used in the validation study are given in Table 6. If the method is to be applied to more than one sample medium (e.g., air, water, soil, sludge), a separate validation must be performed on each medium.

When validating a method directed at overcoming a matrix interference problem in a specific matrix type, a minimum of three samples representative of those matrix types must be included in the matrix types required by Item 6 in Table 6. For example, if a new method is intended to overcome matrix interferences associated with effluents containing high concentrations of polymeric materials from indirect industrial discharges in the Thermoplastic Resins subcategory of the Organic Chemicals, Plastics, and Synthetic Fibers industrial category, the method must be tested on a minimum of three such discharges. Where possible, EPA will assist the method developer in identifying sources for samples of such discharges.

3.4 Development of a Validation Study Plan

Prior to conducting Tier 1, 2, or 3 validation studies, the organization responsible for conducting the study should prepare a detailed study plan. The validation study plan should contain the elements described in Sections 3.4.1 through 3.4.6 of this document.

3.4.1 Background

The Background section of the validation study plan must do the following:

- Identify the new method
- Identify the program use of the new method (drinking water or waste water or both)
- Include a summary of the new method
- Describe the reasons for development of the new method, the logic behind the technical approach of the new method, and the result of 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 or EMMI numbers
- 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 must measure total metals.

3.4.2 Objectives

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

3.4.3 Study Management

The Study Management section of the validation study plan should do the following:

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

3.4.4 Technical Approach

The Technical Approach section of the validation study plan should do the following:

- 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

3.4.5 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.

3.4.6 Limitations

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

3.5 Detailed Procedures for Conducting Validation Studies

When validating new methods, laboratories must adhere to the standardized QC elements detailed in the proposed new method. Laboratories must use a reference matrix (usually, reagent water) and field samples for the validation study.

3.5.1 Method Compilation

Prior to conducting a validation study, the organization responsible for modifying the method should detail the full method in accordance with EPA's Guidelines and Format document.[4] If the organization that develops a new method is a consensus standards organization or government organization with a standardized format, that format may be used. The documented method should be distributed to each laboratory participating in the validation study to ensure that each laboratory is validating the same set of procedures.

3.5.2 Method Detection Limit Study

Each laboratory participating in the Tier 1, 2, or 3 validation study shall use the procedures specified in the new method and perform an MDL study in accordance with the procedure given at 40 CFR part 136, Appendix B.

For validation studies of a new method, each laboratory participating in the study must use the results of the MDL study to determine a minimum level (ML) of quantitation. Determination of an ML for new drinking water methods is encouraged but not required, because the regulations at 40 CFR part 141 specify detection and sometimes quantitation limits for all regulated analytes.

3.5.3 Calibration

Following completion of the MDL study, each laboratory participating in the study must perform a multi-point calibration in accordance with the procedures specified in the new method. However, a single-point calibration is allowed if the < 2% relative standard deviation (RSD) criteria given in Table D-1 (see Appendix D) are met. The method developer shall use the data from the laboratories participating in the study to develop linearity criterion.

3.5.4 Initial Precision and Recovery

After successfully calibrating the instrument, each laboratory participating in the study shall perform initial precision and recovery (IPR) analyses using the procedures specified in the method. The IPR consists of analyses of four replicates of reagent water spiked with the analytes of interest. The method developer shall 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 ML.

3.5.5 Field Sample Analyses

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

3.5.5.1 Tier 1 - Single (First) Matrix Type

In a Tier 1- single matrix type study performed to validate a new method, the laboratory must analyze four spiked replicates of the matrix type to which the new method will be applied. The replicate samples must 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 must determine the background concentration of an unspiked aliquot. In all, Tier 1- single matrix type validation studies of new methods will require analysis of five field samples (one background and four matrix). The organization responsible for developing the method must use the results of these sample analyses to develop MS/MSD precision and recovery QC acceptance criteria.

3.5.5.2 Tier 1 - Multiple (Additional) Matrix Types

In Tier 1- multiple matrix type studies performed to validate new methods, the laboratory must determine QC acceptance criteria using a single matrix of interest as outlined in Section 3.5.5.1, and determine the background concentration and analyze an MS/MSD pair for each additional matrix type being tested, up to a total of eight additional matrix types. For a method to be validated for each additional matrix type, the results of the background/MS/MSD samples must fall within the QC acceptance criteria determined in the single matrix. Because three field sample analyses are required for each matrix type (one background, one MS, and one MSD), and between two and nine matrix types may be tested, a Tier 1- multiple matrix type validation study will require analysis of 8 - 29 samples.

3.5.5.3 Tier 2 Validation Studies

In a Tier 2 validation study, each of the three laboratories will determine the background concentration and analyze an MS/MSD pair on the sample it receives. Because there are three laboratories, each of which performs three analyses (one background, one MS, and one MSD), Tier 2 validation studies will require analysis of 9 samples. The laboratory responsible for developing the new method must use the results of these samples analyses to develop MS/MSD precision and recovery QC acceptance criteria.

3.5.5.4 Tier 3 Validation Studies

In a Tier 3 validation study, each of the nine laboratories participating in the study will determine the background concentration and analyze an MS/MSD pair on the sample it receives. Because there are a total of nine laboratories, each performing three field sample analyses (one background, one MS, and one MSD), a Tier 3 validation study will require analysis of 27 samples. The laboratory responsible for developing the new method must use the results of these samples analyses to develop MS/MSD precision and recovery QC acceptance criteria.

3.5.6 Ongoing Precision and Recovery

If the field samples discussed in Section 3.5.5 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 must analyze an OPR sample with each batch. The concentration of the OPR sample must be as stated in the method being validated.

The organization responsible for developing the method must use the results of the IPR tests described above in Section 3.5.4 to develop OPR recovery criteria as described in Appendix D (Section 9.0).

3.5.7 Calibration Verification

If the field samples discussed in Section 3.5.5 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 must verify calibration as described in the method.

The organization responsible for developing the method must use the results of the calibration sequence described above in Section 3.5.3 to develop QC acceptance criteria for the calibration verification analyses as described in Appendix D (Section 9.0).

3.5.8 Contamination Level in Blanks

Each laboratory that participates in a Tier 1, 2, or 3 validation study must 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 must 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.

3.5.9 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 must 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.

3.5.10 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 relative retention times of the analytes of interest.

For validation of a new method, each laboratory participating in the study must use the results of these sample analyses to develop absolute and relative retention time QC acceptance criteria.

3.5.11 Further Validation Studies

After completing the Tier 1, 2, or 3 validation studies of new methods, the organization responsible for developing the method must 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 must 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.

3.6 Validation Study Report

Laboratories or other organizations responsible for developing new methods at Tier 1, 2, or 3 must document the results of the validation study in a formal validation study report that is organized and contains the elements 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 must be sufficient to enable EPA to evaluate the performance of a new method. If data are collected by a contract laboratory, the organization responsible for using the method (e.g., permittee, POTW, PWS, 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. The validation study plan 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 (if developed) that were made in implementing the study.

The validation study report must contain the elements described in Sections 3.6.1 through 3.6.10.

3.6.1 Background

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

- Include a method summary
- Describe the reasons for and developing the new method, the logic behind the technical approach to the new method, and the result of the new method
- Identify the matrices, matrix types, and/or media to which the method is believed to be applicable
- List the analytes measured by the method including corresponding CAS Registry or EMMI 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

3.6.2 Study Design and Objectives

The Study Design and Objectives section of the validation study report must describe the study design and identify overall objectives and data quality objectives of the study. Any study limitations must be identified. The validation study plan may be appended to the validation study report to provide the description of the study design. If no validation study plan was prepared, the study design must be described in this section (see Section 3.4 for required elements of the study design).

3.6.3 Study Implementation

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

- Identify the organization that was responsible for managing the study
- Identify the laboratories, facilities, and other organizations that participated in the study; describe how participating laboratories 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 requirements 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

3.6.4 Data Reporting and Validation

This section of the validation study report must describe the procedures that were used to report and validate study data. EPA will not establish a standard format for analytical data submission because of the large variety of formats currently in use.

3.6.5 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 3.6.11).

3.6.6 Development of QC Acceptance Criteria

The validation study report must contain a section that describes the basis for development of QC acceptance criteria for all of the required QC tests. The requirements for developing QC acceptance criteria are detailed in Appendix D of this protocol (Section 9).

3.6.7 Data Analysis/Discussion

This section of the validation study report must provide a statistical analysis and discussion of the study results.

3.6.8 Conclusions

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

3.6.9 Appendix A - The Method

A detailed step-by-step analytical method prepared in accordance with EPA's Guidelines and Format document[4], must be appended to the validation study report.

3.6.10 Appendix B - Validation Study Plan

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

3.6.11 Appendix C - Supporting Data

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

3.6.11.1 Raw Data

The Results section of the validation study report must include 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. The raw data are method specific and may include any of the following:

- Sample numbers or other identifiers used by the both the regulated entity and the laboratory
- 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 must 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 required by the new method. Data must 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 must be provided. For instruments involving data systems (e.g., GC/MS), raw data on magnetic tape or disk must be made available on request.

3.6.11.2 Example Calculations

The validation study report must 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.

4.0 EPA REVIEW AND APPROVAL

4.1 EPA Review of Applications

All requests for approval of proposed new methods will undergo review by EPA. Limited-use new methods (Tier 1) will be approved through an EPA letter of approval. New methods proposed for nationwide-use (Tiers 2 and 3) will be approved through rulemaking. Proposed test procedures prepared under this protocol should demonstrate an improvement over current EPA- approved methods 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 Analytical Methods Staff (AMS) at EPA Headquarters will review all nationwide-use new methods and will review limited-use applications if requested by the EPA Regional Office or State Agency. AMS may be assisted in its technical review by contractor personnel. When a formal new method application is received, AMS will first check the documentation for completeness. If the documentation is incomplete, AMS will contact the applicant and request missing documentation before proceeding with its review.

At a minimum, an application must include a completed new method application form, the method in EPA standard format (or other standard format - see Section 3.5.1), and a Validation Study Report with supporting data, before AMS will review the package. If these elements are present, AMS will begin an internal review of the new method for scientific merit, consistency, and appropriateness. The internal review at EPA may involve multiple programs and workgroups. Should any problems or questions arise during the review, EPA or its technical support contractor will communicate with the applicant to resolve outstanding issues. Depending on the circumstances, EPA may return the application to the applicant for revision. Internal review of proposed new methods will involve the three steps briefly described below.

The first step of EPA's technical review will evaluate the description of the proposed method and assess the new methods applicability for approval at 40 CFR parts 136 or 141. If the proposed method is not applicable to 40 CFR parts 136 or 141 and/or the method description is not acceptable, EPA will recommend rejection of the application. If this information is acceptable, the evaluation will proceed.

In the second step of EPA's review, the performance of the new method will be evaluated. The performance (sensitivity, precision and recovery) of the method is based on data provided by the applicant and the development of QC acceptance criteria. If method performance is acceptable, the review will continue.

As the third and final step, EPA will perform a detailed audit of the proposed 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 on floppy disks with the application. Data files should be in IBM-PC compatible format, suitable for input directly into statistical analysis software, such as the Trimmed Spearman-Karber, Probit, Dunnett, and ICP programs.

4.2 Approval Recommendation

EPA will complete its review and notify the applicant of EPA's recommendation. For limited-use applications, the Regional Administrator will issue the formal approval for limited-use of the new method. For all nationwide use applications (Tiers 2 or 3), AMS 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.

4.3 Rulemaking Process

Using the information provided with the new method application to develop the preamble, EPA will prepare the proposed rule for approval, complete the rule docket, pass the draft rule through internal 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 four months, and may take longer depending on the nature of the method.* When published, the proposed rule requests public comment and allows a specified comment period, generally 30 to 60 days. At the end of the comment period, EPA will forward any significant comments to the method applicant for 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 the comment 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 and/or 141 in the next CFR update. *It generally requires a minimum of eight months 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.*

5.0 REFERENCES

1. ASTM, 1994. *Standard Practice for Determination of Precision and Bias of Applicable Methods of Committee D-19 on Water.* Designation D-2777-86 (Reapproved 1994). *Annual Book of ASTM Standards,.* Vol. 11.04.

2. Youden, W.J. and E.H. Stiener, 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, D.C. EPA- 821-B-96-003.

6.0 APPENDIX A - APPLICATION FORM

EPA Office of Water New Method Application Form for Chemical Analytes			
Applicant Name and Address:			*EPA use only* **Case No.**
Date Application Submitted:			
Method Number & Title:			
Analyte(s):			
Type (WW, DW, or WW/DW):			
Level of Use: **(LU or NW)**		**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:			
☐ **Justification for New Method**			
☐ **Method in standard EPA format**			
☐ **Validation Study Plan (optional)**			
☐ **Validation Study Report**			
☐ **Method Information for Preamble**			
☐ **Method Documentation for Docket**			
☐ **Other _____**			
Submit Application and Attachments in Triplicate			

7.0 APPENDIX B - EPA HEADQUARTERS AND REGIONAL CONTACTS

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8.0 APPENDIX C - STANDARD EPA METHOD FORMAT

The following is a listing of the 17 EMMC-required method sections. Applicants should consult the Method Guidelines and Format document [4] for a detailed description of the required 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 must 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 must 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.

Note: To ensure data quality, water methods must specify a comprehensive laboratory QA program. The method must contain the standard QC elements and specify QC acceptance criteria for each of those elements in accordance with Appendix D of this protocol

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.

9.0 APPENDIX D - QUALITY CONTROL REQUIREMENTS

9.1 Introduction

All new methods must 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 requires 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 requirements 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.

9.2 Standardized Quality Control Tests

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

- calibration linearity
- calibration verification
- absolute and relative retention time precision (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 reference sample

These tests are described in Sections 9.2.1 - 9.2.10 below.

9.2.1 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.

9.2.1.1 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." For nearly all analytical instruments and systems, the standard deviation 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."

Current analytical instrument data reduction packages often include several different statistical options for analysts, including both weighted and unweighted regression[1]. The unweighted or simple regression is often the default choice. However, it leads to tremendously inefficient use of the calibration data and totally ignores the common observation that instrument responses have non-constant variances. In a weighted regression, it is possible to allow the more precise calibration points (typically the lower level standards) to more heavily influence the resulting calibration curve. Therefore, weighted regression is the most appropriate choice for nearly all analytical systems and instruments. Application of weighted regression to the 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. EPA plans to provide a supplement to this protocol that discusses the details of these regressions.

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

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

where y_i and x_i are corresponding pairs of instrument response and analyte amount, n is the number of calibration points, and w_i is the variance or squared error of response y_i. With the assumption that the

[1] An unweighted regression weights all points equally (hence the term unweighted) but the weighted regression weights the more precise points more heavily.

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

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

The proportionality coefficient is estimated as the average 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 average ratio is termed the "response factor" (RF). For isotope dilution, the internal standard is a labeled compound and EPA has designed the average 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 136, Appendix A.

9.2.1.2 Calibration linearity

The calibration linearity specification establishes a break point between a straight line through the origin and other forms of calibration (described below). The break point is specified as a maximum relative standard deviation (RSD) of the:

- relative response (RR) for isotope dilution calibration,
- response factor (RF) for internal standard calibration, or
- calibration factor (CF) for external standard calibration,

below which an averaged RR, RF, or CF may be used. If the RSD is greater than the limit specified, another form of calibration must be used.

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 at the minimum level of quantitation (ML) and a minimum of two additional points

- determining the RSD of the RR, RF, or CF.

Depending on the resulting RSD, calibration during the subsequent validation study must be performed at the minimum number of points shown in Table D-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 9.3).

Table D-1: Minimum Number of Points Required for Calibration[1]

Percent RSD[2]	Minimum Number of Calibration Points
0 - <2	1[3]
2 - <10	3
10 - <25	5
>25	7

[1] Based on Rushneck et al., 1987. Effect of number of calibration points on precision and accuracy of GC/MS, in Proceedings of Tenth Annual Analytical Symposium, USEPA: Washington, DC.

[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 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-logistic 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 ensures that a unique analyte amount or concentration corresponds to 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 an averaged RR, RF, or CF, on a straight line not through the origin, or on a calibration curve.

9.2.2 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 (½) the highest calibration point. Using a concentration this 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 (as defined by linearity criteria in Table D-1), specifications for calibration verification are developed to define the allowable deviation of the RR, RF, or CF of the calibration verification standard from the averaged RR, RF, or CF of the initial calibration. If a line with a non-zero intercept or a higher-order curve is used for calibration, specifications for calibration verification must be defined as a maximum allowable deviation of the verification standard from the calibration line or curve.

For calculation of analyte concentrations in field samples, the averaged RR, RF, or CF, 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 an averaged RR, RF, or CF, 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.

9.2.3 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 is developed for identification of an analyte by external standard measurements, and an RRT precision specification is developed for (1) each analyte relative to its labeled analog by isotope dilution measurements, (2) each labeled compound relative to its internal standard for isotope dilution measurements, and (3) each analyte relative to an internal standard for internal standard measurements.

9.2.4 Initial Precision and Recovery

The initial precision and recovery (IPR) test, also termed a "startup test," is used for initial demonstration of 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.

9.2.5 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 separates 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) in the same as the concentration used in the IPR test. Specifications are developed for the permissible range of recovery for each analyte.

9.2.6 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.

9.2.7 Surrogate or Labeled Compound Recovery

The surrogate or labeled compound recovery is used to assess the performance of the method on each sample. For this test, 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.

9.2.8 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.[2]

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[2] For analytes, such as oil and grease, that adhere to container walls and cannot be adequately homogenized, it is not possible to divide a spiked aliquot into two replicate aliquots. In these cases, two field samples are collected and each field sample is spiked with identical concentrations of the analytes of interest to produce an MS and MSD sample.

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.

9.2.9 Demonstration of Method Detection Limit

Nearly all of the 40 CFR part 136, Appendix A, methods 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 achievement of an MDL that meets acceptable criteria. The MDL must be determined according to the procedures specified at 40 CFR part 136, Appendix B. The Appendix B, MDL calculation and analytical procedure is described in Section 9.3.1.1.

9.2.10 Reference Sample Analysis

The most common reference sample is a Standard Reference Material (SRM) from the National Institute of Standards and Technology (NIST). The reference sample and the period for its use are specified in each method. EPA is considering setting acceptance criteria for standard reference materials to be within some percentage of the stated value based on the variability of measurement for that analyte. One possible indicator of that variability is the relative standard deviation calculation for the initial precision and recovery samples. Corrective action to be taken when the acceptance criteria are not met should involve identifying the samples affected, determining the amount of the effect, and if the effect is significant, determining the impact of the effect on the environmental samples analyzed.

9.3 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 9.3.1, 9.3.2, and 9.3.3, respectively. Interlaboratory study data are required 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 9.3.2 or 9.3.3, 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 this protocol, *Guidelines for Collaborative Study*

Procedures to Validate Characteristics of a Method of Analysis, JAOAC *72* No. 4, 1989, *Use of Statistics to Develop and Evaluate Analytical Methods* (published by AOAC-International), ASTM Standard D-2777 (published by ASTM), or other well-established and documented principles.

The statistical procedures described in Sections 9.3.2 and 9.3.3 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.[3] 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 and in the Method 1625 validation study report.[4]

9.3.1 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.

9.3.1.1 Method detection limit and minimum level

An MDL must be determined for each target analyte using the procedure detailed at 40 CFR part 136, Appendix B. This procedure involves spiking seven replicate aliquots of reference matrix or the sample matrix with the analytes of interest at a concentration within one to five times the estimated MDL. The seven aliquots are then carried through the entire analytical process, and the standard deviation of the seven replicate determinations is calculated. The standard deviation is multiplied by 3.14 (the Student's *t* value at 6 degrees of freedom) to form the MDL. If the spike level is greater than five times the determined MDL, the spike level must be reduced and the test repeated until the MDL is within a factor of five of the spike level. 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. In addition, if the analytical system or instrument fails to produce a positive response for any of the seven replicates (i.e., produces a zero or negative result), the MDL procedure must be repeated at a higher spike level. To assure that the MDL is reliable, the optional interative procedure in Step 7 of the MDL procedure must be performed, the

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[3] Appendix I, "Estimation of Variance Components", of the *Interlaboratory Validation of U.S. Environmental Protection Agency Method 1625A*, available from the EPA Sample Control Center operated by DynCorp, Alexandria, VA 22314, 703/519-1140.

[4] *Interlaboratory Validation of U.S. Environmental Protection Agency Method 1625A.* See above.

F-ratio test criteria met, and the pooled MDL from the two levels must be the MDL specified in the method.

The ML is established by multiplying the MDL by 3.18 and rounding 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's (ACS) limit of quantitation (LOQ) (P.S. Porter et al., *Environ. Sci. Technol.*, 22, 1988).

9.3.1.2 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_{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 9.2.1.2) and it is unnecessary to establish a limit for calibration linearity.

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

(1) Determine the average response factor ($\overline{RF}$), calibration factor ($\overline{CF}$), or relative response ($\overline{RR}$) for each analyte from the initial calibration:

$$\overline{RF} = (RF_1 + RF_2 + ... + RF_n)/n$$

where *n* is the number of calibration points.

(2) Determine the RSD_{CAL} using $\overline{RF}$, $\overline{CF}$,or $\overline{RR}$ and the standard deviation (s) of the RF, CF, or RR for each analyte from the initial calibration. The RSD_{CAL} is determined by:

$$RSD_{CAL} = 100s/(\overline{RF})$$

(3) Develop $RSD_{CAL,max}$ as follows:

$$RSD_{CAL,max} = \text{Minimum } (35\%, kRSD_{CAL})$$

where *k* is 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 numerator and denominator. 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 $RSD_{CAL,max}$ is 35%.

Note: In the above equations, the $\overline{RF}$ and RF terms should be replaced by $\overline{CF}$ and CF or $\overline{RR}$ and RR terms where appropriate.

9.3.1.3 Calibration verification

Calibration verification criteria are specified as allowable percentage deviations from the response factor ($\overline{RF}$), calibration factor ($\overline{CF}$), or relative response ($\overline{RR}$) 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 and 3.0 for a five point calibration.

(2) Calculate the upper and lower QC acceptance criteria for the response or calibration factors for each analyte by developing a window around the average response factor found in the initial calibration by:

$$\text{Lower limit (\%)} = \frac{\overline{RF} - ks}{\overline{RF}}$$

$$\text{Upper limit (\%)} = \frac{\overline{RF} + ks}{\overline{RF}}$$

where *k* is the multiplier determined in Step 1 and s is the standard deviation determined in 9.3.1.2, Step 2.

Note: In the above equations, the $\overline{RF}$ terms should be replaced by $\overline{CF}$ or $\overline{RR}$ terms where appropriate.

9.3.1.4 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 9.4.1.5 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 9.2.4.

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) Calculate the average percent recovery ($\overline{X}$), the standard deviation of recovery (s), and the relative standard deviation ($RSD_{IPR}=100s/\overline{X}$) of the four IPR results.

(2) IPR QC acceptance criterion for precision - To approximate a 95% confidence interval for precision, the RSD_{IPR} is multiplied by the square root of the 95th percentile of an F distribution with *n* - 1 degrees of freedom in the numerator and the denominator, where *n* is the number of IPR data points. The resulting multiplier on RSD_{IPR} for four data points will then be 3.0, and the QC acceptance criterion for precision in the IPR test ($RSD_{IPR,max}$) is calculated as follows:

$$RSD_{IPR,max} = 3.0RSD_{IPR}.$$

(3) IPR QC acceptance criteria for recovery - Calculate the QC acceptance criteria for recovery in the IPR test by constructing a window around the average percent recovery ($\overline{X}$). This factor comes from the 97.5th percentile of the t-distribution for n - 1 degrees of freedom, multiplied by $\sqrt{1.15(1+1)+(1/4+1/n)}$, where n is the number of IPR data points, to account for interlaboratory variability and the estimation of the mean. For four data points, this simplifies to 5.3s, and the limits are as follows:

Lower limit (%) = $\overline{X}$ - 5.3s
Upper limit (%) = $\overline{X}$ + 5.3s

(Based on EPA's interlaboratory validation study of Method 1625, the additional variance due to interlaboratory variability is estimated as $1.15s^2$.)

(4) OPR QC acceptance criteria for recovery - A similar multiplier is used as for the IPR test but the second factor is $\sqrt{1.15(1+1)+(1+1/n)}$, so the multiplier is 6.0 for 4 IPR data points. Calculate the QC acceptance criteria for recovery in the OPR test by constructing a window around the average percent recovery $\overline{X}$. For 4 IPR data points, the limits would be:

Lower limit (%) = $\overline{X}$ - 6.0s
Upper limit (%) = $\overline{X}$ + 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 40 CFR part 136, Appendix A, methods (49 FR 43234).

9.3.1.5 Matrix spike and matrix spike duplicate

As noted above, 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 MS/MSD analyses. Sample matrix IPR tests are performed by: (1) determining the background concentration of the sample matrix, (2) 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 9.3.1.1, or one to five times the background concentration of the sample, whichever is greater, and (3) 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 average percent recovery ($\overline{X}$) 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 IPR aliquots) around the average percent recovery ($\overline{X}$) (derived the same as for the OPR test above):

Lower limit (%) = $\overline{X}$ - 6.0s
Upper limit (%) = $\overline{X}$ + 6.0s

Note: For highly variable methods, it is possible that the lower limit for recovery for both IPR and OPR analysis 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 40 CFR part 136, Appendix A, methods (49 *FR* 43234).

Calculate the QC acceptance criteria for the relative percent difference between the MS and MSD as follows:

(1) Calculate the relative standard deviation (RSD) of the recoveries of each target analyte in the sample matrix IPR aliquots as follows:

$$RSD_{IPR} = 100s/\overline{X}$$

(2) Calculate the maximum allowable relative percent difference (RPD_{max}) by multiplying the RSD_{IPR} by $\sqrt{2}$ 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 IPR data points, the calculation simplifies to:

$$RPD_{max} = 4.5RSD$$

9.3.1.6 Absolute and relative retention time

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

$$\text{Lower limit} = \overline{RT} - ts\sqrt{1 + \frac{1}{n}}$$

$$\text{Upper limit} = \overline{RT} + ts\sqrt{1 + \frac{1}{n}}$$

The relative retention time upper and lower limits are determined by replacing $\overline{RT}$ with $\overline{RRT}$ in the equations above. The t value is the 97.5th percentile of a t distribution with n - 1 degrees of freedom, where n is the number of retention time or relative retention time values used.

9.3.1.7 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 average of the blank measurements plus two standard deviations of the blank measurements.

9.3.1.8 Reference sample

Establish the QC acceptance criteria for the reference sample based on the error provided with the reference sample.

9.3.2 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). If the matrix type being validated is drinking water, then tests shall be run on a drinking water matrix collected from three different sources or on three drinking water samples that each have different characteristics.

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.

9.3.2.1 Method detection limit and minimum level

Each laboratory participating in the MDL study must perform an MDL test as described in Section 9.2.9 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 MDLs by comparing 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.

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

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:

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

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 9.3.1.1.

9.3.2.2 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 9.2.1.2). If the initial RSD is < 2%, a one- or two-point calibration can be used (see Section 9.2.1.2) and it is unnecessary to establish a limit for calibration linearity.

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

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

(2) Calculate the relative standard deviation of the RF, CF, or RR of each laboratory and analyte as:

$$RSD_i = \frac{100 s_i}{\overline{RF}_i}$$

where s_i and $\overline{RF}_i$ are the standard deviation and mean of the RFs for laboratory *i*.

(3) Calculate the pooled RSD of the RF, CF or RR 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:

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

(4) Calculate the maximum RSD_{CAL} of the RF, CF, or RR for each analyte as follows:

$$RSD_{CAL,max} = \text{Minimum}(35\%, k RSD_{pool})$$

where *k* is 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 $RSD_{CAL,max}$ is 35%.

Note: In the above equations, the $\overline{RF}$ and RF terms should be replaced by $\overline{CF}$ and CF or $\overline{RR}$ and RR terms where appropriate.

9.3.2.3 Calibration verification

The calibration verification criterion is stated as a maximum relative distance between the average RF obtained by a future laboratory's initial calibration ($\overline{RF}_{INIT}$) and the RF obtained from its calibration verification standard (RF_{VER}).The maximum allowable deviation is based on the pooled relative RSD_{pool} obtained from Section 9.3.2.2.

(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 relative distance as follows:

$$100 * \frac{\left|\overline{RF}_{INIT} - RF_{VER}\right|}{\overline{RF}_{INIT}} \leq k_{VER} RSD_{pool}$$

For example, if the calibration verification criterion, calculated as *k*Ver * RSD_{pool}, equals 17%, then the difference between the mean RF from the initial calibration and the RF from the cal ver sample must be less than or equal to 17% of the initial mean RF.

Note: In the above equations, the $\overline{RF}_{INIT}$ and RF_{VER} terms should be replaced by $\overline{CF}_{INIT}$ and CF_{VER} or $\overline{RR}_{VER}$ and RR_{VER} terms where appropriate.

9.3.2.4 Initial and ongoing precision and recovery

For the IPR and OPR tests, QC acceptance criteria are calculated using the average 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 average 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 lab means $\overline{X}_{(lab\ 1)}$, $\overline{X}_{(lab\ 2)}$, $\overline{X}_{(lab\ 3)}$), and the pooled within-laboratory standard deviation (s_w). s_w is calculated as the square root of the mean of all within-laboratory variances. For example for 3 laboratories:

$$s_w = \sqrt{\frac{s^2_{(lab\ 1)} + s^2_{(lab\ 2)} + s^2_{(lab\ 3)}}{3}}$$

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

(2) IPR QC acceptance criterion for precision - To calculate a 95% confidence interval for precision, the RSD (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 (RSD_{max}) is calculated as follows:

$$RSD_{IPR,max} = 1.9RSD_{IPR}$$

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

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

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

$$s_c = \sqrt{\frac{4}{3}s_b^2 + \frac{1}{20}s_w^2} \quad .$$

(4) Calculate the QC acceptance criteria for recovery in the IPR test by constructing a ± 3.2 s_c window around the average 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):

$$\text{Lower limit}(\%) = \overline{X} - 3.2s_c$$
$$\text{Upper limit}(\%) = \overline{X} + 3.2s_c$$

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.

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

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

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

$$s_c = \sqrt{\frac{4}{3} s_b^2 + \frac{4}{5} s_w^2} \quad .$$

(6) Calculate the QC acceptance criteria for recovery in the OPR test by constructing a ± 2.6 s_c window around the average 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):

$$\text{Lower limit}(\%) = \overline{X} - 2.6 s_c$$
$$\text{Upper limit}(\%) = \overline{X} + 2.6 s_c$$

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 twice the number of laboratories will serve for most situations.

9.3.2.5 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 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 of the 3 pairwise means (s_b), and the pooled within-laboratory standard deviation (s_w) for each target analyte (see Section 9.3.2.4).

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

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

where m = the number of laboratories. For three labs,

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

(3) MS/MSD QC acceptance criteria for recovery - Calculate the QC acceptance criteria for recovery in the MS/MSD test by constructing a $\pm 2.6 s_c$ window around the average 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):

$$\text{Lower limit}(\%) = \overline{X} - 2.6 s_c$$
$$\text{Upper limit}(\%) = \overline{X} + 2.6 s_c$$

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 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 40 CFR part 136, Appendix A methods.

(4) MS/MSD QC acceptance criteria for relative percent difference (RPD) - To evaluate a 95% confidence interval 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 $\sqrt{2}$, 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:

$$RPD_{max} = 4.5 RSD.$$

9.3.2.6 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 average RTs and RRTs based on different operating conditions will result

in the establishment of erroneously wide windows. It is advised, therefore, 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 average retention time, $\overline{RT}$, (or average relative retention time, $\overline{RRT}$), and the corresponding standard deviation (s) for each analyte and standard. Determine the upper and lower retention time (or relative retention time) limits using the following:

$$\text{Lower limit} = \overline{RT} - ts_{avg}\sqrt{1 + \frac{1}{n}}$$

$$\text{Upper limit} = \overline{RT} + ts_{avg}\sqrt{1 + \frac{1}{n}}$$

where the t value is the 97.5th percentile of a t distribution with n - 1 degrees of freedom and where n is the number of retention time or relative retention time data values to be used.

9.3.2.7 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 average of the blank measurements plus two standard deviations of the blank measurements.

9.3.2.8 Reference sample

Establish the QC acceptance criteria for the reference sample based on the error provided with the reference sample.

9.3.3 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). Because data gathered from nine laboratories lends itself to the statistical procedures used for interlaboratory method validation studies, the procedures suggested by ASTM and AOAC-International are particularly applicable and those procedures are preferred for development of QC acceptance criteria. However, QC acceptance criteria may also be 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.

9.3.3.1 Method detection limits and minimum levels

Each laboratory participating in the validation must perform an MDL study as described in Section 9.3.1.1. 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.

$$\mathrm{MDL}_{\mathrm{pooled}} = \sqrt{\frac{d_1\left(\frac{\mathrm{MDL}_{(\mathrm{Lab1})}}{t_{(0.99,d_1)}}\right)^2 + d_2\left(\frac{\mathrm{MDL}_{(\mathrm{Lab2})}}{t_{(0.99,d_2)}}\right)^2 + \ldots d_m\left(\frac{\mathrm{MDL}_{(\mathrm{Labm})}}{t_{(0.99,d_m)}}\right)^2}{d_1 + d_2 + \ldots d_m}}\; t_{(0.99,\,d_1 + d_2 + \ldots d_m)},$$

where m = the number of laboratories, and d_i = one less than 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:

$$\mathrm{MDL}_{\mathrm{pooled}} = \sqrt{\frac{\mathrm{MDL}^2_{(\mathrm{Lab\,1})} + \mathrm{MDL}^2_{(\mathrm{Lab\,2})} + \ldots \mathrm{MDL}^2_{(\mathrm{Lab\,9})}}{9}}\;\frac{2.41}{3.14}$$

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 9.3.1.1.

9.3.3.2 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 9.2.1.2). If the initial RSD is < 2%, a one- or two-point calibration can be used (see Section 9.2.1.2) and it is unnecessary to establish a limit for calibration linearity.

The RSD and the RSD limit for the response factor, calibration factor, or relative response is determined as follows:

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

(2) Calculate the relative standard deviation of the RF, CF, or RR of each laboratory and analyte as:

$$\mathrm{RSD}_i = \frac{100 s_i}{\overline{\mathrm{RF}_i}}$$

where s_i and $\overline{RF}_i$ are the standard deviation and mean of the RFs for laboratory *i*.

(3) Calculate the pooled RSD of the RF, CF or RR 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:

$$RSD_{pool} = \sqrt{\frac{\overline{RSD}_1^2 + \overline{RSD}_2^2 + \ldots + \overline{RSD}_9^2}{9}}$$

(4) Calculate the maximum RSD for each analyte by the following:

$$RSD_{CAL,max} = \text{Minimum } (35\%, kRSD_{pool}),$$

where *k* is 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 nine laboratories using a three-point calibration (*n* = 3), the value of *k* is 1.9, and for nine laboratories using a five-point calibration (*n* = 5), the value of *k* is 1.6. The maximum allowable specification for $RSD_{CAL,max}$ is 35%.

Note: In the above equations, the $\overline{RF}$ and RF terms should be replaced by $\overline{CF}$ and CF or $\overline{RR}$ and RR terms where appropriate.

9.3.3.3 Calibration Verification

The calibration verification criterion is stated as a maximum relative distance between the average RF obtained by a future laboratory's initial calibration ($\overline{RF}_{INIT}$) and the RF obtained from its calibration verification standard (RF_{VER}).The maximum allowable deviation is based on the pooled relative RSD_{pool} obtained from Section 9.3.3.2.

(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 relative distance as follows:

$$100 * \frac{\left| \overline{RF}_{INIT} - RF_{VER} \right|}{\overline{RF}_{INIT}} \leq k_{VER} RSD_{pool}$$

For example, if the calibration verification criterion, calculated as *k*Ver * RSD_{pool}, equals 17%, then the difference between the mean RF from the initial calibration and the RF from the cal ver sample must be less than or equal to 17% of the initial mean RF.

Note: In the above equations, the $\overline{RF}_{INIT}$ and RF_{VER} terms should be replaced by $\overline{CF}_{INIT}$ and CF_{VER} or $\overline{RR}_{VER}$ and RR_{VER} terms where appropriate.

9.3.3.4 Initial and ongoing precision and recovery

For the IPR and OPR tests, QC acceptance criteria are calculated using the average 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 average 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 *m* laboratories (the standard deviation of the m laboratory averages $\overline{X}_{lab1}$, $\overline{X}_{lab2}$, ..., $\overline{X}_{labm}$), and the pooled within-laboratory standard deviation (s_w) (For calculation of s_w, see Section 9.3.2.4). Note: the organization responsible for developing the method must ensure that all laboratories are spiking IPR and OPR samples at the same concentration.

(2) IPR QC acceptance criteria for precision - To calculate a 95% confidence interval for precision, the RSD (computed as s_w divided by $\overline{X}$) is multiplied by the square root of the 95th percentile F value with 3 degrees of freedom in the numerator and $m(n - 1)$ degrees of freedom in the denominator, and *n* is the number of data points per laboratory. The resulting multiplier for nine laboratories will be 1.7. The QC acceptance criterion for precision in the IPR test ($RSD_{IPR,max}$) for 9 laboratories and 5 data points per laboratory is calculated as follows:

$$RSD_{IPR,max} = 1.7RSD$$

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

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

where *m* = the number of laboratories, and *n* = the number of data points per laboratory. For 9 laboratories and 5 data points per laboratory,

$$s_c = \sqrt{\frac{10}{9}s_b^2 + \frac{1}{20}s_w^2} \quad .$$

(4) Calculate the QC acceptance criteria for recovery in the IPR test by constructing a ± 2.3 s_c window around the average percent recovery ($\overline{X}$, 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):

$$\text{Lower limit}(\%) = \overline{X} - 2.3s_c$$
$$\text{Upper limit}(\%) = \overline{X} + 2.3s_c$$

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.

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

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

where *m* = the number of laboratories, and *n* = the number of data points per laboratory. For 9 laboratories and 5 data points per laboratory,

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

(6) Calculate the QC acceptance criteria for recovery in the OPR test by constructing a ± 2.1 s_c window around the average percent recovery ($\overline{X}$, 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):

$$\text{Lower limit}(\%) = \overline{X} - 2.1s_c$$
$$\text{Upper limit}(\%) = \overline{X} + 2.1s_c$$

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 twice the number of laboratories will serve for most situations.

9.3.3.5 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 and MSD performance criteria as follows.

(1) Calculate the mean percent recovery over all labs ($\overline{X}$) and the between-laboratory standard deviation (s_b) of the mean results for each of the nine laboratories and also the pooled within-laboratory standard deviation (s_w) for each target analyte using the MS and MSD analyses (see Section 9.3.3.4)

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

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

where m = the number of laboratories. For nine labs,

$$s_c = \sqrt{\frac{10}{9} s_b^2 + \frac{1}{2} s_w^2}$$

(3) MS/MSD QC acceptance criteria for recovery - Calculate the QC acceptance criteria for recovery in the MS/MSD test by constructing a ± 2.2 s_c window around the average 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):

$$\text{Lower limit}(\%) = \overline{X} - 2.2 s_c$$
$$\text{Upper limit}(\%) = \overline{X} + 2.2 s_c$$

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 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 40 CFR part 136, Appendix A methods.

(4) MS/MSD QC acceptance criterion for relative percent difference (RPD) - To calculate a 95% confidence interval 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 95% percentile F value with 1

degree of freedom in the numerator and *m* degrees of freedom in the denominator multiplied by $\sqrt{2}$. The resulting multiplier on the RSD for nine laboratories will be 3.2. The QC acceptance criterion for precision in the MS/MSD test (RPD_{max}) is calculated as follows:

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

9.3.3.6 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 average RTs and RRTs based on different operating conditions will result in the establishment of erroneously wide windows. It is advised, therefore, that the organization developing the method specify to the participating laboratories the chromatagraphic 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.

(1) Using replicate RT and/or RRT data, calculate the upper and lower QC acceptance criteria for each analyte using the procedures in the calibration verification test in section 9.4.1.3.

(2) Determine the average retention time, $\overline{RT}$ (or average relative retention time, $\overline{RRT}$), and the corresponding standard deviation (s) for each analyte and standard. Determine the upper and lower retention time (or relative retention time) limits using the following:

$$\text{Lower limit} = \overline{RT} - ts\sqrt{1 + \frac{1}{n}}$$

$$\text{Upper limit} = \overline{RT} + ts\sqrt{1 + \frac{1}{n}}$$

where the t value is the 97.5th percentile of a t distribution with n - 1 degrees of freedom, where n is the number of retention time or relative retention time data values to be used.

9.3.3.7 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 average of the blank measurements plus two standard deviations of the blank measurements.

9.3.3.8 Reference sample

Establish the QC acceptance criteria for the reference sample based on the error provided with the reference sample.

10.0 APPENDIX E - Data Reporting Form

This appendix provides an example data reporting form. The form illustrates those aspects of data reporting which are expected, regardless of the specific format used; 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 electronic versions of data can be very helpful in expediting the review of an ATP. Data files should be in IBM-PC compatible format, suitable for input directly into statistical analysis software, such as the Trimmed Spearman-Karber, Probit, Dunnett, and ICP programs.

New Method Data Form†

Method Title*		Revision Date	__/__/__

*Include Method Number and Revision Number

Please record all data and quality control (QC) acceptance criteria from your validation study using this 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 1 Studies (Single Laboratory Use): Complete **1** form for each matrix type.
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 IPR Matrix Study (include units): _____

	Tier 1	Tier 2 or 3			
IPR Matrix Data					

MS Concentration	
MSD Concentration	
Background Concentration	

New Method QC Acceptance Criteria

Calibration		Spike	IPR Recovery0 and Precision			OPR Recovery		MS/MSD Recovery and RPD			MDL/ML	
Points	Lin	Conc	Low	High	Precision	Low	High	Low	High	RPD	MDL	ML

† **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.**