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ENVIRONMENTAL MONITORING AND ASSESSMENT PROGRAM (EMAP) CHEMICAL METHOD EVALUATION GUIDANCE

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DISCLAIMER

The U.S. Environmental Protection Agency through its Aquatic Research Division funded and managed the preparation of the guidance described here under Contract No. 68-C1-0022. It has been reviewed by management of the Environmental Monitoring and Assessment Program (EMAP) and the National Exposure Research Laboratory (NERL), U.S. Environmental Protection Agency (USEPA). It has further been subjected to the Agency's peer and administrative review process and has been approved for publication. The mention of trade names or commercial products does not constitute endorsement or recommendation for use.

FOREWORD

The U.S. Environmental Protection Agency (USEPA) is developing the Environmental Monitoring and Assessment Program (EMAP) to determine the current status and trends in the condition of our nation's ecological resources on regional and national scales. EMAP's goal is to monitor the condition of the nation's ecological resources, thereby contributing to decisions on environmental protection and management. EMAP's monitoring efforts will operate on regional scales over periods of years to decades and will involve collecting data from all ecosystems. EMAP data will enable policy makers, scientists, and the public to evaluate the success of current policies and programs and to identify emerging problems before they become widespread or irreversible. EMAP's ecological status and trends data will allow decision makers to assess objectively whether or not the nation's ecological resources are responding positively, negatively, or not at all to regulatory programs.

This document has been prepared to assist scientists and managers in selecting and evaluating chemical laboratory measurement methods for producing EMAP data. It is the first in a series of documents intended to ensure that the methods used to generate EMAP data will provide the quality required of the task. Subsequent documents in this series will provide guidance for use when evaluating biological laboratory methods, field measurement methods, and field collection methods. Each evaluation guidance document will provide guidance for use in determining if: 1) method documentation is adequate to meet the needs of EMAP, and 2) a method produces data of sufficient quality to meet the needs of EMAP.

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SECTION 1
INTRODUCTION

1.1 Background

1.1.1 As originally conceived, EMAP consisted of seven Coordination Groups: Quality Assurance, Indicators, Information Management, Design and Statistics, Assessment and Reporting, Landscape Characterization, and Methods. These groups coordinated the collection of data from seven Resource Groups: Rangelands, Agroecosystems, Forests, Inland Surface Waters, the Great Lakes, Estuaries, and Landscapes. This organizational structure is currently undergoing change, but the guidance provided here will serve any long term environmental monitoring effort. As one of several coordination activities being undertaken by EMAP-Methods, this document provides guidance on chemical method evaluation in support of the larger EMAP goals of developing indicators that produce comparable data at regional and national scales. The coordination activities being undertaken by EMAP-Methods include providing method format guidance, identifying and organizing EMAP methods in use, and providing guidance for evaluating EMAP methods. EMAP-Methods has the following objectives:

- To provide guidance for preparing methods in a standard method format that includes written specifications for the style and content of EMAP methods and method manuals;
- To establish a database on the EPA Wide Area Information Server (WAIS) and Web Server containing the full text of all EMAP methods;
- To establish a relational database in the EMAP Information Management System containing full text and abstracted versions of all EMAP methods to be directly tied to analytical data sets; and
- To develop a method evaluation protocol suited to the requirements of EMAP that ensures that methods used by the Resource Groups are consistent and produce comparable data.

1.1.2 Method manuals produced and used by the Resource Groups are in different stages of development. Field and laboratory method manuals have been preprared for pilot, demonstration, and full implementation stages of EMAP. Resource Groups have prepared these methods and manuals in the style most familiar to them. EMAP-Methods has prepared the document entitled "EMAP Methods Format Guidance" (USEPA, 1995) to meet the objective of providing standardization for method and method manual preparation within EMAP. The method format presented in that document, and required for use with EMAP methods, is the Environmental Monitoring Methods Council (EMMC) format. Specifically, this format is required for use with field collection, field measurement, and biological and chemical laboratory methods. In addition to describing the required method format, the "EMAP Methods Format Guidance" (USEPA, 1995) provides specifications and guidance for preparing hardcopy and electronic versions of methods and method manuals. This document is available over the Internet at the following address: **http://www.epa.gov/docs/emap/coordgrp/methods**.

1.1.3 One of the databases under development will become a part of the EPA WAIS and Web Servers and will provide public access to the full text of methods and related guidance documents.

The other database will form the EMAP-Methods relational database, which will provide authorized users with on-line access to the full text and abstracted versions of methods. The relational database will link analytical data to methods information to support compliance with EMAP's "20 year rule," which states that all EMAP data must be sufficiently documented to be fully understandable in 20 years. The databases will provide method users with a rapid way of determining if an existing method for a given parameter has been used within EMAP. The databases will facilitate the coordination of methods issues across the program by providing a means of tracking the introduction of new methods into the program, as well as method changes through time. Standard documentation is essential for the entry and retrieval of methods into and from the databases and; therefore, certain format specifications provided in the "EMAP Methods Format Guidance" (USEPA, 1995) were defined specifically to support their use.

1.1.4 EMAP-Methods is responsible for developing a method evaluation protocol that will support EMAP's Performance-Based Method System (PBMS). Methods used within EMAP are typically selected from scientific literature or are newly developed. This method evaluation protocol provides guidance on the validation and acceptance of methods obtained from various sources. The protocol is intended to assist method evaluators in determining if the methods used within EMAP are adequately documented and whether the methods meet the needs of the program. EMAP-Methods will provide a set of four evaluation guidance documents, one for each of the following types of methods: field collection, field measurement, biological laboratory, and chemical laboratory methods. Each of these documents will provide the standard format for EMAP methods with specific information pertinent to the targeted discipline. The documents will also provide instructions for performing and evaluating method validation activities and method comparability studies. These instructions are presented to assist scientists and managers in determining if candidate method performance is adequate to meet the needs of EMAP.

1.2 Chemical Laboratory Method Evaluation Guidance

1.2.1 This document has been written to provide practical guidance for chemical laboratory method evaluation and method approval. It will enable scientists and managers to evaluate whether a candidate method is fully documented and if it will provide data that meet EMAP MQOs. This document provides a standardized procedure for establishing any candidate method as an approved EMAP method.

1.2.2 To apply the guidance on method evaluation provided in this document, the following must be true.

- A parameter to be measured has been identified;
- A candidate method has been used or proposed to measure the parameter; and
- Measurement Quality Objectives (MQOs) for the measurement have been defined by the program and are available to method assessors.

1.2.3 Any candidate method will be accepted for EMAP use once the method has been shown to be completely documented and capable of meeting EMAP quality objectives. A previously used

or new candidate method may become an approved EMAP method if the method has been completely documented in the EMMC method format and:

- Has been validated and shown through validation to meet MQOs; **or**
- Has been compared to a validated method and shown to meet or exceed the performance specifications of that method.

1.2.4 Outline of Guidance: This document provides guidance to method users for characterizing and documenting method performance, and to method assessors for evaluating method performance and accepting or rejecting methods based on performance data. The following paragraphs describe the framework of the guidance provided.

Section 2 summarizes the procedures required to characterize, document, and evaluate method performance for approval. This section provides an overview of the guidance contained in subsequent sections of this document.

Section 3 provides a discussion of the criteria that are used to evaluate the performance of candidate chemical methods during the approval process.

Section 4 provides a description of the EMMC method format to assist in preparing written chemical methods and evaluating the completeness of method documentation. The "EMAP Methods Format Guidance" (USEPA, 1995) contains detailed instructions for preparing methods in the EMMC format and should be used to obtain document formatting specifications. To become accepted for future use in performing EMAP measurements, methods must be documented in the EMMC format.

Section 5 describes the procedures required to document and evaluate a method using the EMAP Method Validation, and assists method evaluators in determining if a candidate method produces data that meet quality objectives. The EMAP Method Validation is required to approve a new candidate method as an EMAP method, when the candidate method has not previously been validated, and when no approved EMAP method is available for comparison. The required elements of the EMAP Method Validation are performed to characterize the method's performance in terms of precision, percent recovery, method range, and method detection limit (MDL). This section of the guidance also introduces ruggedness testing, which may be used to characterize the method's performance in producing acceptable data over a reasonable range of analytical conditions.

Section 6 describes the procedures required to document and evaluate a method using the EMAP Demonstration of Method Comparability. This section is intended to assist method evaluators in determining if a candidate method produces data that meet or exceed the performance specifications of an approved EMAP method. The procedures described will only be used when previously approved EMAP methods are available for comparison.

SECTION 2
METHOD PERFORMANCE EVALUATION

2.1 Summary

2.1.1 Selecting appropriate measurement methods is a critical task for EMAP. A method evaluation protocol for EMAP should ensure that the methods used are reliable in producing data of sufficient quality to meet the needs of EMAP. The EPA publishes validated test methods that are designated as appropriate for complying with various regulations. These test methods are fully documented and have been extensively tested and characterized to assure that they meet specific measurement requirements under a variety of conditions likely to be encountered in their intended application. The process for approving EPA validated methods for EMAP use consists of determining: 1) if the method is written in the EMMC format; and 2) if the method's performance specifications meet MQOs.

2.1.2 To allow for technological improvements and to enable laboratories utilizing varying methodologies to participate in EMAP, the EMAP method evaluation protocol addresses the approval of alternate methods for use within the program. For EPA, approval of alternate methods has required extensive alternative test procedures to show equivalence to validated methods (i.e., the "Requirements for Approval of Alternate Test Procedures for Inorganic and Organic Analytes in National Pollutant Discharge Elimination System Monitoring" (USEPA, 1993)). Recently, the PBMS used by other Federal agencies has been approved for use by EPA. This approach provides method performance specifications and permits the use of validated methods, as well as alternate or adapted methods that meet the specifications provided.

2.1.3 EMAP has chosen to apply a PBMS to its method evaluation and approval process. This approach will be used to allow laboratories participating in EMAP the flexibility to utilize measurement methods other than those supplied by EPA. Furthermore, the approach will enable laboratories to provide methods to EMAP when EPA validated methods are not available for a parameter of interest. This document provides a formal approach to method approval for EMAP. This method evaluation guidance has been developed to support the desired PBMS **and** to control the propagation of multiple methods. To achieve both objectives, the requirements for method approval consist of a combination of elements of the "Single-Laboratory Method Validation Protocol" (USEPA, 1985) and elements of the PBMS to ensure that:

- the methods used for EMAP are adequately documented;
- the methods used for EMAP perform reliably in meeting MQOs;
- method selection is not restricted to EPA validated methods only; and
- the use of multiple methods for measuring the same analytes or properties will not result in a loss of quality or comparability among the data.

2.2 Method Approval For Previously Used Methods

2.2.1 Candidate methods may be methods that have already been used in pilot and/or demonstration stages of EMAP, or they may be new to the program. Because no formal review and approval procedures were set forth prior to this guidance, the methods already in use have been used without having undergone the procedures detailed here. These methods will be "grandfathered" only to the extent that they will be entered into the methods database regardless of their approval status to permit traceability to the data that has already been produced. For data collection or generation subsequent to this guidance, however, formally approved methods must be used for collecting and/or generating the data.

2.2.2 Previously used, but non-approved methods must be taken through the evaluation procedures issued in this guidance for approval for continued or future use. To reduce the burden of validating these methods at this time, the guidance permits the use of an abbreviated evaluation and approval process. The flowchart presented in *Figure 1: Evaluation and Approval Procedures for Previously Used Methods* (p.7) summarizes the requirements for approving methods that have been previously used to generate EMAP data. These methods become approved EMAP methods when they have been completely documented. As used here, "completely documented" means that the method is written in the EMMC format, including all method performance specifications such as precision, percent recovery, method range, and MDL. All candidate methods must meet the complete documentation specification.

2.2.3 *Section 4 - Method Content*, provides guidance for preparing the method in the EMMC format. To meet the requirement for documenting method performance, data must be available in a level of detail at least as rigorous as that described in *Section 5 - EMAP Method Validation*. If this information is not included in the written method in sufficient detail, the method must be treated as a new method. All new methods must undergo one of the procedures described *subsection 2.3 - Method Approval for New Candidate Methods.*

2.3 Method Approval For New Candidate Methods

2.3.1 For new candidate methods, the evaluation and approval process will depend upon whether the method has undergone a method validation and whether an approved EMAP method is available for the parameter to be measured. Throughout this guidance, the "Single-Laboratory Method Validation Protocol" (USEPA, 1985) is cited as an acceptable alternate to the EMAP Method Validation. Furthermore, wherever the "Single-Laboratory Method Validation Protocol" (USEPA, 1985) is cited, any validation protocol may be substituted, provided that the substituted protocol is at least as rigorous as the "Single-Laboratory Method Validation Protocol" (USEPA, 1985) The evaluation and approval procedures for new candidate methods are summarized in *Figure 2: Evaluation and Approval Procedures for New Candidate Methods* (p.8). If the method is written in the EMMC format and is an EPA validated method, it will be approved for EMAP use if the method's performance specifications are evaluated and determined to meet defined MQOs. If the method is not an EPA validated method, it must undergo one of the procedures described in subsections 2.3.4 and 2.3.5, as appropriate.

2.3.2 The guidance for evaluating and approving new methods presented in this document has been compiled from existing information for the convenience of EMAP method users and assessors. The EMAP Method Validation procedures have been adapted from the "Single-Laboratory Method Validation Protocol" (USEPA, 1985). The EMAP Demonstration of Method Comparability procedures have been adapted from the "Guidance on the Evaluation of Safe Drinking Water Act Compliance Monitoring Results from Performance-Based Methods" (USEPA, 1994). Sections of these two documents have been selected and modified to define the minimum requirements for characterizing and documenting the performance of candidate EMAP methods.

2.3.3 While this guidance provides two procedures for obtaining method approval, the requirements for method approval have been designed to ensure that all approved EMAP methods are traceable to a formally validated method. All previously used methods must be evaluated for adherence to the requirements presented here before they are used to generate additional data and before they may be used as the basis of method comparisons.

2.3.4 For new candidate methods that have not been validated according to the "Single-Laboratory Method Validation Protocol" (USEPA, 1985) or according to another validation procedure which is at least as rigorous, the evaluation process will depend upon whether an approved EMAP method is available for the parameter to be measured. When no approved EMAP method is available for comparison to a new candidate method, the EMAP Method Validation must be conducted and evaluated to assess the method's performance. The procedures outlined in *Section 5 - EMAP Method Validation* constitute the minimum requirements for characterizing, documenting, and evaluating the performance and reliability of candidate methods when method comparability cannot be assessed. A method will be approved for EMAP use when an evaluation of validation results determines that the method meets MQOs.

2.3.5 Once a method has been written in the EMMC method format and method validation has shown that the method meets MQOs, the method may be used as the approved EMAP method to which a new candidate method's performance is compared. Using the approved EMAP method, the EMAP Demonstration of Method Comparability is conducted to assess the candidate method's performance. If the performance characteristics of the candidate method are determined to meet or exceed the performance characteristics of the approved method, the candidate method will be approved for use.

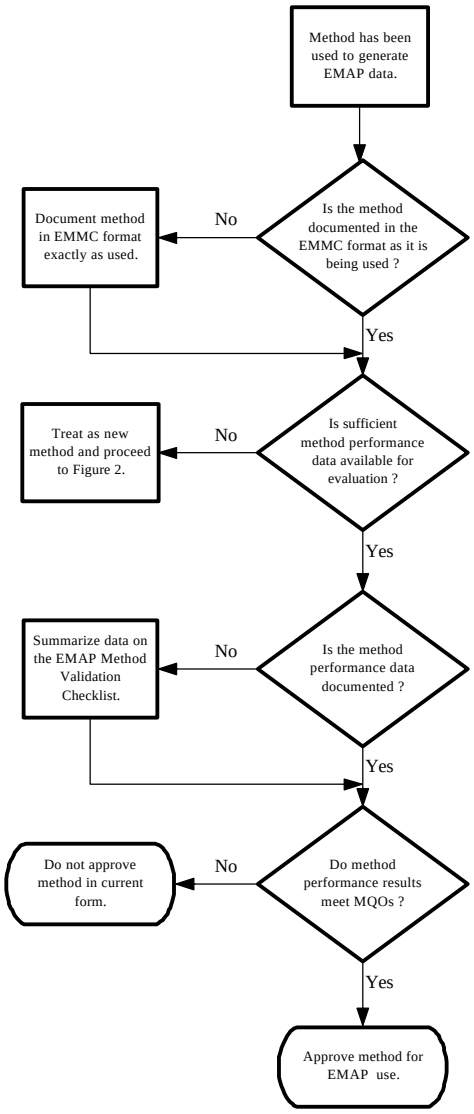
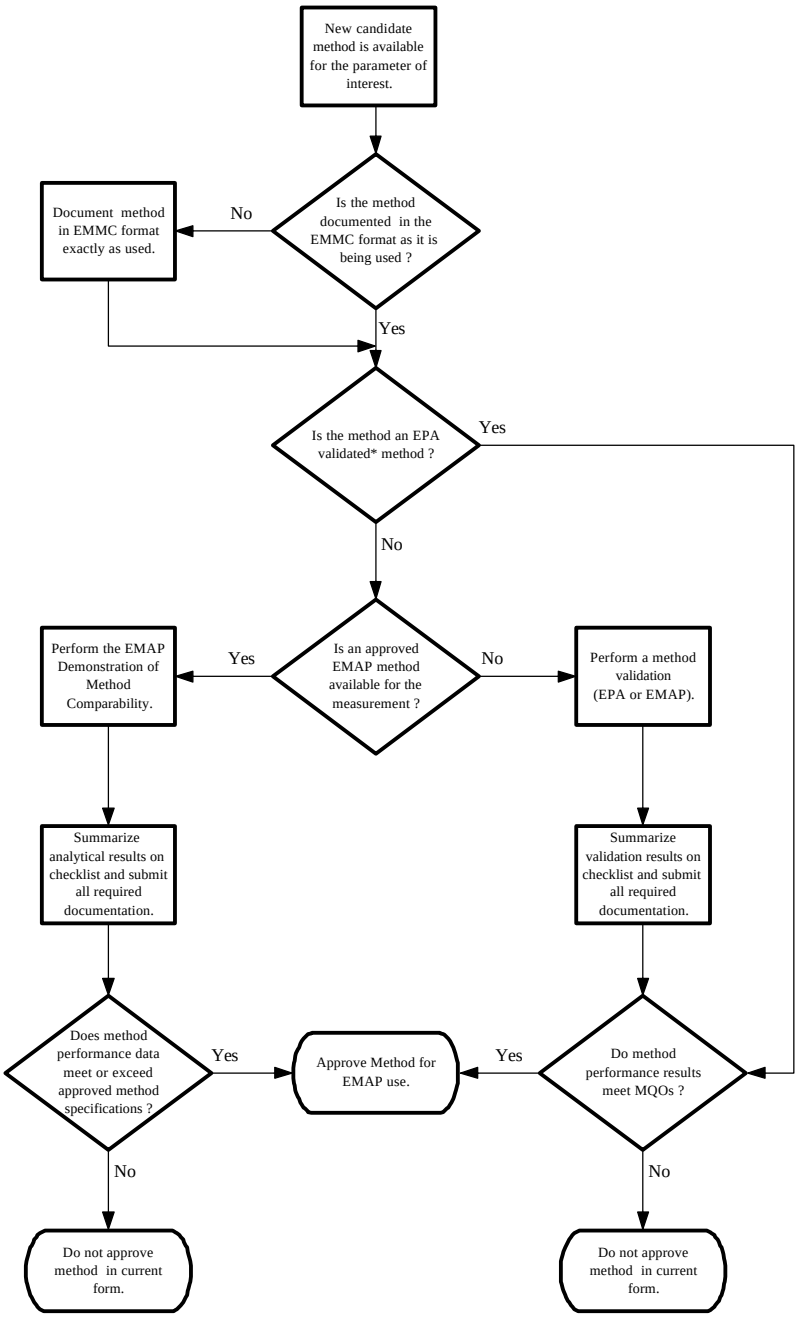


Figure 1: Evaluation and Approval Procedures for Previously Used Methods



* Throughout this guidance, "EPA validated" means validated according to the "Single-Laboratory Method Validation Protocol" (USEPA, 1985).

Figure 2: Evaluation and Approval Procedures for New Candidate Methods

SECTION 3
METHOD PERFORMANCE CRITERIA

3.1 Summary

3.1.1 Performance criteria or data quality indicators are quantitative and qualitative descriptors that are used to interpret the degree of acceptability of data to the user. Data quality indicator information is calculated and kept to support the scientific integrity of EMAP data. The principal data quality indicators addressed in this guidance are bias, percent recovery, precision, Method Detection Limit (MDL), and selectivity. This section of the guidance provides working definitions of these indicators.

3.1.2 The most important factor in evaluating a candidate method for approval for EMAP use is determining whether the candidate method produces data that meet the quality objectives of the EMAP Resource Group. MQOs are defined by the Resource Group based upon the intended use(s) of the data. MQOs place numerical requirements on the value of data quality indicators to ensure that the quality of EMAP data is sufficient to support EMAP's goals. Project planners will prepare the project's Quality Assurance Project Plan (QAPP) containing the MQOs. The QAPP should contain the MQOs with a discussion of how they are determined and how data will be evaluated relative to them. For environmental data to be useful to EMAP, the methods used to generate the data must meet these MQOs. This document's focus, therefore, is placed on evaluating the performance of chemical laboratory methods by analyzing data quality indicators and comparing analytical results to MQOs. Method users and assessors must retrieve the MQOs from the appropriate QAPPs to evaluate whether a candidate method produces data of adequate quality.

3.2 Bias and Percent Recovery

3.2.1 Bias: In chemical measurements, bias is the difference between a measured value and the true value of the quantity measured. Bias represents the systematic error of the measurement due to sampling and analytical systems. Bias is determined from repeat analysis of reference (certified) materials or samples fortified with known amounts of analytes of interest. For repeated measurements of samples with known composition, bias may be calculated in absolute or relative terms with the following equations:

$$Bias,\ B = \bar{x} - T$$

-or-

$$B(\%) = \frac{\bar{x} - T}{T} \times 100$$

where $\bar{x}$ equals the mean value for a set of measurements and T equals the theoretical true value of the evaluation sample.

3.2.2 Percent Recovery: In chemical measurements, percent recovery is the ratio of the measured value and the true value of a quantity measured. Percent recovery is estimated through the analysis

of quality control matrix samples or reagent blanks that are spiked with known amounts of target analytes. When reagent blank samples are used, the percent recovery of the spiked analyte is determined with the following equation:

$$Percent\ Recovery = \frac{\overline{x}}{T} \times 100$$

where $\overline{x}$ equals the mean value for a set of measurements and T equals the theoretical true value of the evaluation sample.

Alternatively, environmental samples or standard matrix samples may be used for analysis. When these samples are used, unspiked samples must be analyzed to determine if background concentrations of target analytes are present in the sample. Percent recovery in a spiked sample matrix that contains a background concentration is calculated using the following equation:

$$Percent\ Recovery = \frac{\overline{x} - \overline{B}}{T} \times 100$$

where $\overline{x}$ equals the mean value for a set of measurements, T equals the theoretical true value of the evaluation sample, and $\overline{B}$ equals the mean background concentration in the unspiked sample.

3.2.3 Because bias is not commonly used to quantify method performance, the EMAP Demonstration of Method Comparability and the EMAP Method Validation require determinations of percent recovery for the assessment of candidate method performance. For the EMAP Demonstration of Method Comparability, the percent recovery of the candidate method will be evaluated by comparison to the percent recovery of an approved EMAP method for the same measurement. For the EMAP Method Validation, the percent recovery of the candidate method will be evaluated by comparison to MQOs for percent recovery obtained from the appropriate QAPP.

3.3 Precision

3.3.1 Precision is the degree to which a set of replicate measurements agree. It may be expressed as standard deviation, variance, or range. Precision represents the random error of the measurement due to sampling and analytical systems. The error due to analytical systems is estimated from repeated measurements of split samples or spikes in the laboratory. Precision may be expressed in absolute terms as the standard deviation, *s*, or in relative terms as the relative standard deviation, RSD. Precision is calculated with the following equations:

$$s = \sqrt{\frac{\sum (x_i - \overline{x})^2}{(n - 1)}}$$

-or-

$$RSD = \frac{s}{\bar{x}} \times 100$$

where x_i is an individual measurement, $\bar{x}$ is the mean value of the set of measurements, and n is the number of measurements in the set.

3.3.2 The EMAP Demonstration of Method Comparability and the EMAP Method Validation require replicate sample analyses to determine the precision of a candidate chemical laboratory method. Respectively, the determined precision will be evaluated by comparison to the precision of an approved EMAP method for the same measurement or by comparison to MQOs obtained from the appropriate QAPP.

3.4 Method Detection Limit

3.4.1 The MDL is the lowest level at which the method can be expected to reliably measure the analyte or property of interest. The MDL is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. MDLs are determined by analyzing at least seven replicate samples, spiked at a concentration at or near an estimated limit of detection. MDLs are analyzed over time and/or across sample batches to include long-term variation in the estimate. They are calculated from the standard deviation between these replicate measurements as follows:

$$MDL = t_{n-1,0.01} \times s$$

where $t_{n-1,0.01}$ equals the upper first percentile point of the *t*-distribution with *n-1* degrees of freedom and *s* equals the standard deviation of the replicate measurements (see equation in subsection 3.3.1).

3.4.2 Both the EMAP Demonstration of Method Comparability and the EMAP Method Validation require that the MDL be determined, documented, and evaluated for method approval.

3.5 Selectivity

3.5.1 Selectivity refers to the method's ability to selectively identify and measure the constituents of interest in the presence of interferences and, in the case of multiple analyte methods, in the presence of all other constituents of interest. Interferences are determined through the analysis of reagent or standard matrix blank samples. Comparisons of blank analyses to those of spiked standard matrices or sample matrices will allow method users to assess the method's selectivity.

3.5.2 The EMAP Demonstration of Method Comparability and the EMAP Method Validation require that method interferences be identified. Whenever known interferences reduce the method's selectivity, the method may be deemed unacceptable for the specific application. However, if the method employs procedures that effectively minimize or eliminate the effects of the interferences, the method may be approved for use.

SECTION 4
METHOD CONTENT

4.1 Summary

4.1.1 As noted in the introduction to this document, methods used to produce EMAP data are required to be completely documented and properly formatted. EMAP-Methods stresses the importance of consistent method preparation and documentation across the program for two reasons. First, standardized documentation is necessary to ensure that all written EMAP methods contain information that is appropriately detailed to meet EMAP needs. Second, standardized documentation will be essential to storing and retrieving methods from the methods database, which will enable the tying of data sets to methods and the tracking of methods through time.

4.1.2 To address the issue of standardization, the "EMAP Methods Format Guidance" (USEPA, 1995) was prepared. The guidance requires the use of the EMMC method format for all EMAP measurement methods. The following subsections of this guidance document provide the sections of the EMMC method format with content requirements and suggestions for preparing the method sections for application to chemical laboratory methods. These requirements and suggestions are presented to assist authors in preparing methods and to aid evaluators in assessing methods' content.

4.2 EMMC Method Format

4.2.1 All chemical laboratory methods used to produce EMAP data are required to be written in the EMMC method format, containing the following sections, numbered as shown:

1. Scope and Application
2. Summary of Method
3. Definitions
4. Interferences
5. Personnel Health and Safety
6. Equipment and Supplies
7. Reagents and Standards
8. Sample Collection, Preservation, and Storage
9. Quality Control
10. Calibration and Standardization
11. Procedure
12. Data Analysis and Calculations
13. Method Performance
14. Pollution Prevention
15. Waste Management
16. References
17. Tables, Diagrams, Flowcharts, and Validation Data (optional final section)

4.2.2 Each of method sections one through 16 must be addressed in the written method. If any of these sections do not apply to the method, the section heading must be listed as shown, with "Not Applicable" or "N/A" appearing below. If *Section 17. Tables, Diagrams, Flowcharts, and Validation Data* is not needed, the heading for this section is not required to be included in the written method.

1. Scope and Application

Describe the purpose of the method (i.e., what chemical analyte or attribute is being measured). Include target analyte lists for the method and Chemical Abstract Service Registry Numbers for chemical analytes. Identify all matrices for which the method applies.

2. Summary of Method

Provide a brief summary of the method's major steps. The purpose of the summary is to provide a succinct overview of the technique to aid the reviewer or data user in evaluating the method and the data. List sample volumes, extraction, digestion, concentration, and other preparation steps employed, analytical instrumentation and detection systems, and qualitative and quantitative data analysis techniques used.

3. Definitions

List definitions of terms relevant to the method, or terms with which the reader may be unfamiliar. This section is a useful location for defining quality control samples and acronyms. For extensive lists of definitions, a glossary may be attached to the end of the method.

4. Interferences

Describe any known or potential problems, such as sample or equipment contamination, instrument noise, etc. that may be encountered during the performance of the method. Describe the effects of any known or potential interferences on method performance. Describe any procedures employed by the laboratory to prevent or minimize interferences or contamination problems.

5. Personnel Health and Safety

Describe special precautions needed to ensure personnel safety during the performance of the method. Safety issues discussed here should be limited to those that are method-specific and that are beyond the scope of routine laboratory practices. Include information regarding specific toxicity of target analytes or reagents. List personal protective equipment required for personnel performing the method.

6. Equipment and Supplies

List all nonconsumable supplies or instruments needed to perform the method. Include material specifications (glass, plastic, stainless steel, etc.) when needed. Use generic language whenever

possible; however, when specific equipment is necessary, this should be stated clearly. When computational or data analysis software is used, include user-generated instructions for using the software here.

7. Reagents and Standards

List all reagents and standards required to perform the method, along with necessary grades or quality. Provide detailed instructions for preparation of reagents and standards. Reference specific suppliers when necessary.

8. Sample Collection, Preservation, and Storage

Provide requirements and instructions for sample collection, preservation, shipment, and storage conditions. For laboratory methods, extensive detail about sample collection, preservation, and shipment are typically not required. These aspects should be listed here, but need only be addressed as necessary for performance of the laboratory method. This section should describe any actions required in response to problems with collection, preservation, etc.. For example, provide details regarding actions that are required to handle samples when they have been received with a loss of integrity (i.e., without appropriate preservation or in broken sample containers). When holding times are known and their effects have been studied, specify requirements in this section, along with instructions for actions to be taken if holding times are exceeded.

9. Quality Control

Provide all Quality Control (QC) requirements of the method. Describe the types of QC samples analyzed, and the frequency of their use. Describe the analysis of QC samples, including calculations such as percent recovery. List data acceptance criteria, along with procedures for dealing with data that do not conform to requirements. This section may be used to describe data review procedures, documentation requirements, etc..

10. Calibration and Standardization

Describe the initial method or instrument calibration. Indicate calibration frequency requirements, the range of calibration standards required, the number of calibration standards required, performance specifications, and corrective actions that are taken when performance criteria are not met. Verification or continuing calibration procedures may be described here or in *11. Procedure*.

11. Procedure

Present the complete instructions for using the method, describing all sample processing, instrumental analysis, or physical analysis steps in detail.

12. Data Analysis and Calculations

Provide instructions for qualitative data analysis procedures, such as those employed for chemical analyte identification. Provide instructions for quantitative data analysis, including calculations and definitions of constants. When analytical and/or computational software is used to automate analysis, refer to any proprietary instructional documentation here and provide user-generated instructions for use of the software.

13. Method Performance

Present method performance data, including precision, percent recovery, MDLs, and limitations of the method. All information necessary to evaluate whether the method will meet MQOs should be summarized in this section.

14. Pollution Prevention

Cite practices employed to minimize or prevent pollution that may be attributable to the method. The following guidelines for the content of this section have been taken from Method 350.1 (EPA/600/R-93/100).

Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address waste generation. When wastes cannot be feasibly reduced at the source, EPA recommends recycling as the next best option.

The quantity of a chemical or reagent purchased should be based on expected usage during its shelf life and on the disposal costs of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

For information about pollution prevention that may be applicable to laboratories and research institutions, consult the document "Less is Better: Laboratory Chemical Management for Waste Reduction", available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036, (202) 872-4477.

15. Waste Management

Describe proper disposal of waste and samples specific to the method. The following guidelines for the content of this section have been taken from Method 350.1 (EPA/600/R-93/100).

EPA requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations.

Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. EPA urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any waste discharge permit and regulations, and complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions.

For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in *14. Pollution Prevention*.

16. References

List source documents or publications. Each method must be a free-standing document, in which all information necessary for the method user to perform the method may be found. Procedural steps or instructions may not be referenced as being found elsewhere and must be included in full within the written method.

17. Tables, Diagrams, Flowcharts, and Validation Data

Tables, diagrams, flowcharts, and validation data may be included in the written method as a final section, as an attachment to the method, or dispersed throughout the method, as appropriate.

4.3 Evaluation of Method Documentation

4.3.1 Method documentation should be evaluated with regard to conformance to the EMMC format, and with regard to completeness. The following questions should be asked when evaluating method documentation.

- Is the method written in the EMMC format?
- Are all EMMC method sections represented in the method?
- Is "Not Applicable or N/A" used in place of needed information?

4.3.2 If the method is written in the EMMC format, with all sections represented, and containing all needed information, the method meets the minimum documentation requirements. A method that meets minimum documentation requirements should undergo further evaluation to determine the method's suitability for its intended use. Otherwise, the method should be revised to meet these requirements before additional evaluation takes place.

4.3.3 Methods that have been used for pilot and demonstration stages of EMAP and that are completely documented as described in this section, including all required method performance data, will be considered approved. In the event that a method is determined to conform to the EMMC format, but some or all of the method performance data is missing or incomplete, the organization using the method must produce the missing or incomplete data. This data must be produced by completing the procedures described in *Section 5 - EMAP Method Validation* or those described in *Section 6 - EMAP Demonstration of Method Comparability*. Once method performance data is available, it shall be compared to MQOs to determine the method's capability of meeting EMAP needs.

SECTION 5
EMAP METHOD VALIDATION

5.1 Summary

5.1.1 Any method intended to be used for EMAP measurements must be determined to be valid or to perform comparably to a valid method. A method that has been taken through the validation procedures described in this section or through the more exhaustive "Single-Laboratory Method Validation Protocol" (USEPA, 1985) and has been shown to meet MQOs will be considered a valid and approved EMAP method. Once a method has become an approved EMAP method, new candidate methods may be compared to that method for approval, as described in *Section 6 - EMAP Demonstration of Method Comparability*. All method comparisons must stem from an initial validated method. Because the EMAP Method Validation is intended to provide a solid foundation for later method comparisons, its requirements are rigorous.

5.1.2 The EMAP Method Validation is used to determine a method's precision, percent recovery, method range, and MDL. Ruggedness testing is recommended, but not required, to characterize the method's performance in producing acceptable data over a reasonable range of analytical conditions. The data generated during validation are compared to MQOs to determine the method's suitability for measuring the parameter of interest. A completely documented method that is determined to meet MQOs, through evaluation of the data obtained from the EMAP Method Validation, will be approved as an EMAP method. Alternatively, the "Single-Laboratory Method Validation Protocol" (USEPA, 1985) may be substituted for the EMAP Method Validation to characterize the method's performance.

5.1.3 The EMAP Method Validation must take place when a new method is proposed for use and no approved method is available for comparison. Furthermore, any method used to perform EMAP measurements, regardless of its prior use within the program, must contain the method performance data described in this section. If such data is unavailable, the method must undergo the EMAP Method Validation. After the validation steps outlined in this section have been completed, the information and statistics generated should be included in the EMMC method format to complete the document.

5.2 EMAP Method Validation

5.2.1 Two procedures are required to be performed for the EMAP Method Validation. These procedures have been derived from the "Single-Laboratory Method Validation Protocol" (USEPA, 1985) and are described in detail in *Appendix B: Method Range and Method Detection Limit* and *Appendix C: Matrix Validation*.

This section provides a brief description of the method range and method detection limit determinations, of the matrix validation, and of ruggedness testing, which is highly recommended although not required as part of the EMAP Method Validation. *Figure 3: The EMAP Method Validation* (p.21) contains a flowchart that summarizes the sample analyses required of the EMAP Method Validation. The three aspects of performance testing outlined in this figure may be performed in any sequence desired. *Appendix D: Statistics for Determining Outliers, Significance,*

and Confidence Intervals provides instructions for calculating the statistics required by these analyses. *Appendix E: Statistical Example* provides examples of the calculations to further aid method users and evaluators.

5.2.2 Method Range and Method Detection Limit (MDL): Method validations must include a determination of the method's range, which is the sample concentration range over which the method is valid. The method range is determined by analyzing replicate standard matrix samples spiked at multiple concentration levels spread over the instrument range. The method range is bounded by the highest and lowest concentrations of analytes that produced acceptable results for precision and percent recovery when processed through the entire analytical procedure. The MDL is determined by analyzing at least seven replicate samples, spiked at a concentration at or near an estimated limit of detection. MDLs are analyzed over time and/or across sample batches to include long-term variation in the estimate. *Appendix B: Method Range and Method Detection Limit* contains detailed instructions for performing determinations of method range and MDL.

5.2.3 Matrix Validation: Method precision, percent recovery, and range must be determined for all matrices for which the use of the method is intended. Validation for a single matrix consists of a determination of analyte background levels from the analysis of unspiked matrix samples, the analysis of replicate spiked matrix samples, and a comparison of the results obtained for precision and percent recovery to the performance requirements of the method. Replicate samples must be analyzed at a minimum of two concentration levels, the high and low end of the determined method performance range. If acceptable results are not obtained, intermediate concentrations are tested. The range for the matrix is then defined from the lowest to the highest concentration that produced acceptable results for precision and percent recovery when processed through the entire analytical procedure. Matrix validation procedures are described in detail in *Appendix C: Matrix Validation*.

5.2.4 Ruggedness Testing (optional): Ruggedness testing is performed to determine method sensitivity or insensitivity to deviations from method-specified conditions such as extraction temperatures, solvent ratios, sorbent hydration, flow rates, etc. A ruggedness test is conducted to show that reasonable and allowable differences in these conditions will not cause any important change in analytical results. Methods which show extreme sensitivity to slight condition variations may be unsuitable if such conditions are not easily controlled.

Whenever a method has the potential to be sensitive to changes in specified conditions, method performance testing should be conducted under varied conditions. Specifically, the method range and MDL, and matrix validations described previously should be reevaluated under the varied conditions. Samples should be analyzed under varied conditions within a range of conditions that might be encountered in normal application of the method. For example, if a sample extraction procedure requires heating a sample at a specified temperature with a device of known variability, the method's sensitivity to changes in temperature may be assessed by performing the extraction at two temperatures - the high and low extremes of the heating device's known variation around the desired extraction temperature. If method performance is shown to meet MQOs at both extremes, the method is considered insensitive to expected variation in extraction temperature.

Some methods may require a more detailed approach to ruggedness testing. If the method has the potential to show sensitivity to several method conditions, a carefully designed ruggedness test is advised to examine the effects of variations of multiple conditions and combinations of conditions simultaneously. A designed experiment will enable this simultaneous examination of several combinations of conditions, while minimizing the number of required analyses. When a designed experiment is needed, consult "Chemometrics: A Textbook" (Vandeginste, 1988) or the "Statistical Manual of the A.O.A.C." (Youden, 1975) for proper procedures.

When method validation includes ruggedness testing, a method that is approved as an EMAP method will be approved for use under all conditions where ruggedness testing results met MQOs. When ruggedness testing is not performed, a method that is approved as an EMAP method will be approved for use only under the exact conditions of the method validation.

5.3 Required Documentation

5.3.1 Method validations must be supported by data sufficient to allow an independent reviewer to verify every measurement and calculation generated by the providing laboratory. The data must be clearly labeled, compiled, and maintained in an organized manner.

5.3.2 The documentation required to support the EMAP Method Validation consists of the following items:

- The EMAP Method Validation Checklist for Chemical Methods and any required attachments (see *Appendix A: EMAP Method Validation Checklist for Chemical Methods* and *subsection 5.4 - Checklist*).

- Raw data that will allow an independent reviewer to verify each determination and calculation performed by the laboratory. The documentation must support the tracing of data from sample preparation and spiking through the final results reported. Because different methods will employ different instrumentation or equipment to measure the parameter of interest, the specific format of raw and calculated data may differ by method.

- The Certification Statement (see *subsection 5.5 - Certification Statement*).

5.3.3 In developing the PBMS, EPA has designed a checklist to aid the facility attempting to employ a performance-based method in assessing and documenting specific aspects of the performance-based method. EMAP-Methods has adapted this checklist for use in documenting and evaluating candidate method performance as part of its method approval system. The checklist is presented in *Appendix A: EMAP Method Validation Checklist for Chemical Methods*. The first page of the checklist summarizes information about the conditions under which the validation was conducted. The second page summarizes the analytical results obtained during validation. In addition to the checklist, method performance data generated during a method validation must be included in the written method in *Section 13. Method Performance* and in *Section 17. Tables, Diagrams, Flowcharts, and Validation Data* when needed (see *subsection 4.2 - EMMC Method Format* for descriptions of these method sections).

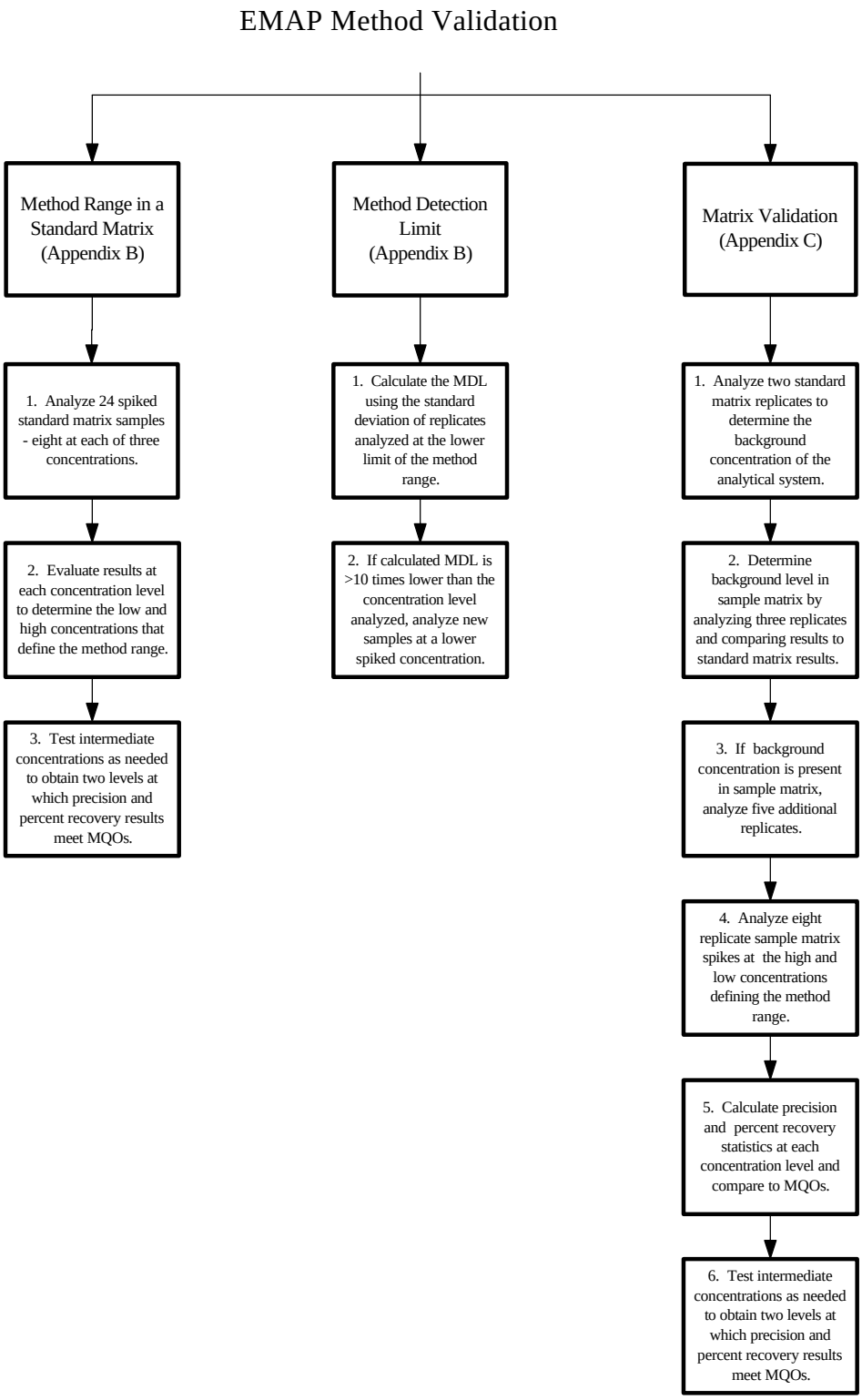


Figure 3: The EMAP Method Validation

5.4 Checklist

5.4.1 Header information: The header information required on each page of the EMAP Method Validation Checklist for Chemical Methods includes the following items:

- **Date:** The "date" entered on each page should be the date of submission of the information to the Resource Group evaluating the method's performance.

- **Facility Name:** The "facility name" should be the name of the facility proposing the use of the candidate method.

- **Resource Group/Program:** For EMAP's purposes, the "Resource Group/Program" is the name of the Resource Group to whom the results will be reported.

- **Matrix Type:** The "matrix type" refers to the specific environmental sample matrices to which the candidate method has been applied and for which approval is being sought. Because most evaluations of a method will involve matrix-specific performance measures, a separate checklist should be prepared for each matrix tested.

- **Analyte or Class of Analytes:** The last header field is used to indicate the analyte or class of analytes to which the candidate method applies. When a method applies to a large number of analytes, a class of analytes may be entered in this field. If such classification is used, a separate list of analytes and their respective Chemical Abstract Service Registry Numbers must be attached to the checklist.

5.4.2 With the exception of the certification statement, each page of the checklist also contains the field "Page # of #" for consecutive pagination of the checklist.

5.4.3 Checklist Items: The two pages of the EMAP Method Validation Checklist for Chemical Methods are used to summarize the conditions of validation testing and the results obtained. The items on the checklist are consecutively numbered, as shown in the following descriptions, for easy reference.

1. Written candidate method (EMMC format) attached?: The first item on the checklist is a simple "yes/no" question regarding the required method write-up. The details of the method must be written in the EMMC format as described in *Section 4 - Method Content*. Conformance to the required method format is essential to EMAP's goal of achieving program consistency.

2. Title and date of QAPP containing MQOs: This field identifies the QAPP that contains the MQOs to which the performance specifications of the candidate method are being compared.

3. Method range tested (w/units): This range should reflect the actual range of sample concentrations that were tested and must include the concentration units. These concentration units are typically expressed in terms of mass/mass or mass/volume. The range should not be expressed in terms of the concentrations of the standards tested.

4. Linear working range (w/units): The linear working range is the range of concentrations over which the analytical system exhibits a linear relationship between the amount of material introduced into the instrument and the instrument's response. Linearity is typically measured as either a linear regression for inorganic analytes or as the RSD of the response factors or calibration factors for organic analytes. Traditional performance specifications consider any regression line with a correlation coefficient (r) of 0.995 or greater as linear. For organic analytes, an RSD of 25% or less is typically considered linear. Using these limits for linearity as guidance, enter the method's linear working range in this field.

5. Concentrations of calibration standards w/units corresponding to final sample concentration: The concentrations of the calibration standards are entered in the next field. The number of calibration standards may vary for different methods.

6. Calibration curve attached?: The calibration curve is the graphical representation of the instrument response versus the concentration of the calibration standards. For methods that use linear regression or another statistical curve, the calibration curve must be included with any data submission. For methods that use the RSD of the calibration results as a measure of linearity, the curve is not needed if the RSD specifications are met. However, calibration curves must be attached for methods where the instrument response is not sufficiently linear to pass the RSD specifications.

7. Slope of calibration regression line: If a regression line is calculated, its equation should be entered in this field.

8. Relative Standard Deviation (RSD) of calibration factors: If the RSD calculation is used to determine a calibration's linearity, the result should be entered in this field.

9. Sample preservatives and holding times: If holding times have been evaluated, include the conclusions of the evaluation in this field.

10. Interferences: In this field, enter information on any known or suspected interferences with the candidate method. While interferences may be difficult to predict, they may be indicated by unacceptable spike recoveries, especially when such interferences are not noted when testing a clean matrix such as reagent water.

11. Qualitative identification criteria used: Qualitative identification criteria are crucial to the accurate assessment of contaminants and will vary depending on the analytical technique employed. Enter all relevant criteria used for target analyte identification. These criteria should include items such as retention time, spectral wavelengths, co-elution of peaks for specific m/z's, ion abundance ratio requirements, etc. If the list of criteria is lengthy, attach it to the checklist on a separate sheet and enter "Attached" in this field.

12. Surrogates used: If surrogate compounds are added to samples prior to preparation, which is typically done for organic analyses, indicate the specific surrogates used in the candidate method.

13. Surrogate recovery limits established: Summarize the surrogate recovery limits that have been established for the candidate method. For methods with multiple surrogates, include a range of recovery limits, and attach a detailed list to the checklist. These recovery limits may be derived from specific performance tests conducted during the EMAP Method Validation or based on a scientifically defensible consensus of expected performance.

14. Method Range Determination

Method range in a standard matrix: Enter the range of concentrations in a standard matrix for which the method was determined to perform reliably during method validation.

Standard matrix used: Enter the standard matrix used to determine the method range.

Spike levels: Enter the theoretical concentrations of analytes in the standard matrix after spiking. The concentrations should be expressed in the same units in which the sample results will be expressed, not in the concentration units of the spiking solution.

Source of spiking material: If reference materials obtained from commercial sources are used, indicate the sources. If externally prepared materials are not available, spiking materials must be prepared in the laboratory and this should be indicated on the checklist, along with the source of all neat materials used.

Number of replicates: In this field, indicate the number of replicate sample analyses that were used. The minimum number of replicates required for the method range determination is sixteen - eight replicates at the high concentration and eight replicates at the low concentration of the method range determined.

15. Matrix Validation

Sample matrix used: Enter the matrix used to determine the valid method range.

Spike levels: Enter the theoretical concentrations of analytes in the sample matrix after spiking. The concentrations should be expressed in the same units in which the sample results will be expressed, not in the concentration units of the spiking solution.

Source of spiking material: If reference materials obtained from commercial sources are used, indicate the sources. If externally prepared materials are not available, spiking materials must be prepared in the laboratory and this should be indicated on the checklist, along with the source of all neat materials used.

Number of replicates: In this field, indicate the number of replicate sample analyses that were used. The minimum number of replicates required for the matrix validation is five.

16. Performance Characteristics: These items summarize the method range, MDL, precision, and percent recovery results for the candidate method. For multiple analyte methods, it would be impractical to enter the results for each analyte in these fields. Therefore, for multiple analyte methods, enter the range of results in the precision and percent recovery fields and attach an analyte-specific summary to the checklist. In the left-hand column for each of these criteria, enter the MQO obtained from the QAPP indicated on page 1 of the checklist. In the right-hand column for each of these criteria, enter the results of validation testing for the candidate method.

Method range determined: Enter the method range determined during matrix validation. For multiple analyte methods, enter "Attached" and attach a full listing of results to the checklist. If approval is being sought for more than one sample matrix, attach a full listing of results for each selected matrix.

Method Detection Limit (MDL): Enter the MDL determined during validation, including the number of replicates analyzed.

Precision: The precision statistics determined during matrix validation, at both the high and low concentrations that define the method range, are entered in this field.

Percent Recovery: The percent recovery estimates determined during matrix validation, at both the high and low concentrations which define the method range, are entered in this field.

5.5 Certification Statement

Appendix A: EMAP Method Validation Checklist for Chemical Methods contains the certification statement required to be documented with every EMAP Method Validation. This statement is intended to ensure that the laboratory or organization that provided and tested the candidate method assumes full responsibility for method performance and for the data provided on the checklist.

5.6 Evaluation of Method Performance

5.6.1 Evaluation of the EMAP Method Validation will consist of ensuring that the required documentation is in place and complete and that the candidate method meets or exceeds the MQOs specified in the appropriate EMAP QAPP. Laboratories proposing candidate methods for approval must perform all required performance testing, document the results, and submit the documentation to the appropriate Resource Group. The Resource Group is then responsible for evaluating the documentation and determining the approval status of the proposed candidate method.

5.6.2 Incomplete documentation will not be evaluated for approval. Whenever required information is missing or incomplete, the providing laboratory or organization will be required to complete the information and resubmit the completed documentation for approval. Once the documentation has

been determined to be complete, method evaluators will assess the method's suitability for EMAP's needs.

5.6.3 With documentation requirements met, method assessors may evaluate the method by using the documentation to answer the following questions.

Is the method specific (or selective) for the analyte? Evaluation of the analysis of spikes and standards, along with noted interferences, will enable the method assessor to determine the method's selectivity. If the method is not specific for the analyte, it will not be approved in its current form. The method may be revised and retested if desired. Otherwise, the method is not approved for EMAP use.

Do the method's precision and percent recovery results meet or exceed MQOs? The results of the EMAP Method Validation are available for this evaluation. If the precision and percent recovery estimates determined during performance testing do not meet or exceed MQOs, the method will not be approved in its current form. The method may be revised and retested if desired. Otherwise, the method is not approved for EMAP use.

Does the MDL meet or exceed MQOs? If the MDL of the candidate method does not meet or exceed the MDL requirement specified in the QAPP, the method will not be approved in its current form. The method may be revised and retested if desired. Otherwise, the method is not approved for EMAP use.

Is the method valid over the range of conditions expected in normal use? As noted in *subsection 5.2 - EMAP Method Validation*, conducting ruggedness testing is an optional aspect of the EMAP Method Validation. If ruggedness testing is performed and all other requirements for method approval have been met, the method will be approved for use under all conditions for which the method was tested and determined to perform acceptably. If ruggedness testing is not performed, the method will be approved for use only for the conditions under which the validation was conducted.

5.6.4 Multi-Analyte Methods: For methods that measure more than one parameter, method approval will be more complicated than with single parameter methods. For example, an organic method that measures pesticides will measure a number of compounds in a single analysis. The EMAP Method Validation and the EMAP Demonstration of Method Comparability will involve characterizing the method's performance for each compound measured. Likewise, method evaluation will involve evaluating the method's performance for each compound measured. In multiple parameter methods, the possibility exists that the performance statistics of some of the method's analytes will meet requirements, while others do not.

Whenever the performance statistics of some fraction of a method's analytes fail to meet MQOs, any of three potential decisions may be made regarding approval of the method for EMAP use. The method may be approved for use in measuring only those parameters whose performance statistics met requirements; the method may be approved "as is"; or the method may be rejected. Criteria for making this determination are not addressed in this guidance. Method acceptance criteria fall within

the responsibilities of the Resource Groups. Along with MQOs, specific method acceptance criteria should be defined by the Resource Group and included in the appropriate QAPP.

SECTION 6
EMAP DEMONSTRATION OF METHOD COMPARABILITY

6.1 Summary

6.1.1 For most measurements needed for EMAP, methods are available that have either been validated by EPA or have already been widely used within EMAP. As described in *Section 4 - Method Content*, when these methods are completely documented and evaluated with respect to MQOs, they will become approved EMAP methods. With approved methods available for performing EMAP measurements, the Resource Groups will be able to recommend their use when seeking laboratories to perform chemical analyses. Utilizing a PBMS, the laboratories may then adopt the recommended method or may alternatively propose to use a modified version of the method or an entirely new method to perform the needed measurement.

6.1.2 When an alternate or modified method is proposed for use in performing EMAP measurements, the laboratory proposing the new candidate method must demonstrate and document the candidate method's performance using one of two procedures. The laboratory may choose to determine the method's precision, percent recovery, method range, and MDL by performing the EMAP Method Validation as described in *Section 5 - EMAP Method Validation*. Alternatively, the laboratory may perform the EMAP Demonstration of Method Comparability to characterize method performance.

6.1.3 The EMAP Demonstration of Method Comparability is performed to compare the performance statistics of a new candidate method to the performance statistics of an approved EMAP method. The EMAP Demonstration of Method Comparability is adequate for method assessment only when an approved EMAP method is available for measuring the parameter of interest. For an alternate method to gain approval, the candidate method must be written in the EMMC method format and the EMAP Demonstration of Method Comparability must be successfully completed.

6.2 EMAP Demonstration of Method Comparability

6.2.1 The procedures specified for the EMAP Demonstration of Method Comparability have been adapted from the "Guidance on the Evaluation of Safe Drinking Water Act Compliance Monitoring Results from Performance-Based Methods" (USEPA, 1994). Much of the first chapter of that document is repeated here, slightly modified for EMAP's use. The analyses required for the EMAP Demonstration of Method Comparability include replicate spike analyses and MDL determination.

Figure 4: The EMAP Demonstration of Method Comparability (p.30) contains a flowchart which summarizes the sample analyses required of these procedures. *Appendix C: Matrix Validation* provides detailed instructions for spiking and analyzing replicate matrix spike samples. *Appendix B: Method Range and Method Detection Limit* describes the MDL determination. Method users should refer to these appendices when performing the EMAP Demonstration of Method Comparability.

6.2.2 Replicate Spike Analyses: To demonstrate comparability, at least five aliquots of a spiked sample matrix are processed through the entire analytical procedure. The concentration of each

analyte is measured and the results are used to evaluate the method's precision and percent recovery.

- **Matrix:** Replicate analyses are required to be performed in at least two matrices: a reference matrix and in each sample matrix for which the method is intended. The reference matrix will vary depending upon the analysis performed and the sample matrix. A variety of "clean" matrices such as reagent water, clean sand, sediment, or soil may be used during performance testing. The results of the analyses of clean matrices, when compared to those of sample matrices, may yield important information about method interferences. The sample matrix evaluated must be the same matrix as the samples to which the method will routinely be applied.

- **Spike levels:** For most analytical techniques, the precision and percent recovery of the measurement technique are directly related to concentration of the analyte. As the concentration of the analyte increases, the absolute error of the measured concentration will increase, while the relative error will decrease. Therefore, the choice of the concentration at which to demonstrate performance is critical. Use the approved EMAP method as a guide in choosing the concentration at which to spike the replicate aliquots for the comparability study. The spiking level must be no higher than the spiking level in the approved method. If the approved method does not specify a concentration level for spiking, spike the sample matrix at one to five times the MDL for each analyte of interest. If the sample matrix is known to contain the analytes of interest at appropriate levels (one to five times the MDL), spiking may not be required.

- **Source of spiking material:** Whenever possible, it is preferable to use a spiking solution or material prepared by or obtained from an external source. Reference materials for various analytes are available from a number of commercial sources. If externally prepared materials are not available, spiking materials must be prepared in the laboratory.

- **Number of replicates:** The minimum number of replicate aliquots required for the EMAP Demonstration of Method Comparability is five.

- **Precision:** The precision is calculated as the RSD of the replicate measurements, using (*n-1*) degrees of freedom.

- **Percent recovery:** The percent recovery is calculated as the ratio of the measured value to the true value of the spiked analyte, multiplied by 100.

6.2.3 Method Detection Limit: The MDL is determined by analyzing at least seven replicate samples, spiked at a concentration at or near an estimated limit of detection. MDLs are analyzed over time and/or across sample batches to include long-term variation in the estimate. *Appendix B: Method Range and Method Detection Limit* contains detailed instructions for determining the MDL.

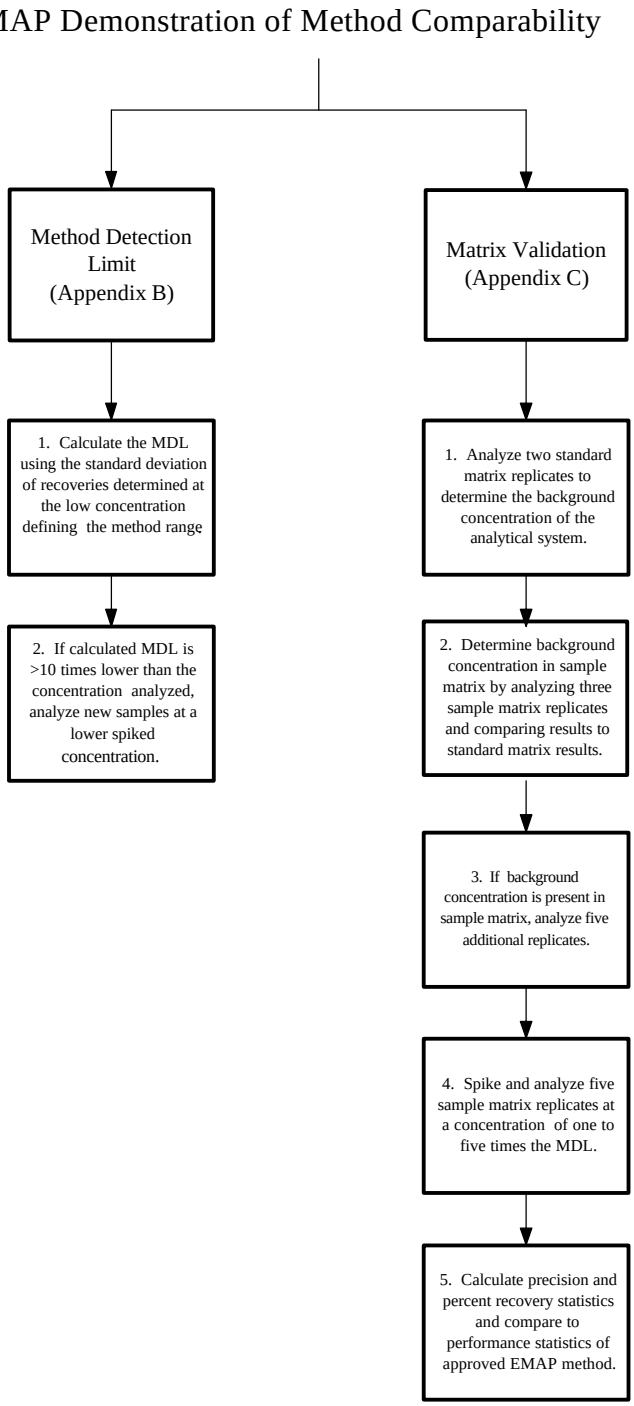


Figure 4: The EMAP Demonstration of Method Comparability

6.3 Required Documentation

6.3.1 Demonstrations of method comparability must be supported by data sufficient to allow an independent reviewer to verify every measurement and calculation generated by the laboratory. In

a PBMS, the data must be clearly labeled, compiled, and maintained in an organized manner and available for inspection at the facility.

6.3.2 The documentation required to support the EMAP Demonstration of Method Comparability consists of the following items:

- The EMAP Demonstration of Method Comparability Checklist for Chemical Methods and any required attachments (see *Appendix F: EMAP Demonstration of Method Comparability Checklist for Chemical Methods* and *subsection 6.4 - Checklist*).

- A summary of all quality control results required by the approved EMAP method and the candidate method.

- Raw data that will allow an independent reviewer to verify each determination and calculation performed by the laboratory. The documentation must support the tracing of data from sample preparation and spiking through the final results reported. Because different methods will employ different instrumentation or equipment to measure the parameter of interest, the specific format of raw and calculated data may differ by method.

- The Certification Statement (see *subsection 6.5 - Certification Statement*).

6.3.3 The evaluation checklist developed for documenting the EMAP Demonstration of Method Comparability is very similar to the list adapted for use with the EMAP Method Validation. As with the latter, the former checklist is intended to aid method evaluators and other users in assessing method performance and its documentation. The checklist for the EMAP Demonstration of Method Comparability is presented in *Appendix F: EMAP Demonstration of Method Comparability Checklist for Chemical Methods*. The first page of the checklist summarizes information about the conditions under which the comparability testing was performed. The second page of the checklist summarizes the analytical results obtained during performance testing.

6.4 Checklist

6.4.1 Header information: The header information required on each page of the EMAP Demonstration of Method Comparability Checklist for Chemical Methods includes the following:

- **Date:** The "date" entered on each page should be the date of submission of the information to the Resource Group evaluating the method's performance.

- **Facility Name:** The "facility name" should be the name of the facility proposing the use of the candidate method.

- **Resource Group/Program:** For EMAP's purposes, the "Resource Group/Program" is the name of the Resource Group to whom the results will be reported.

- **Matrix Type:** The "matrix type" refers to the specific environmental sample matrices to which the candidate method has been applied and for which approval is being sought. Because most

evaluations of a method will involve matrix-specific performance measures, a separate checklist should be prepared for each matrix tested.

- **Analyte or Class of Analytes:** The last header field is used to indicate the analyte or class of analytes to which the candidate method applies. When a method applies to a large number of analytes, a class of analytes may be entered in this field. If such classification is used, a separate list of analytes and their respective Chemical Abstract Service Registry Numbers must be attached to the checklist.

6.4.2 With the exception of the certification statement, each page of the checklist also contains the field "Page # of #" for consecutive pagination of the checklist for submission.

6.4.3 Checklist Items: The two pages of the EMAP Demonstration of Method Comparability Checklist for Chemical Methods are used to summarize the condition of comparability testing and the analytical results obtained. The items on the checklist are consecutively numbered, as shown in the following descriptions, for easy reference.

1. Written candidate method (EMMC format) attached?: The first item on the checklist is a simple "yes/no" question regarding the required method write-up. The details of the method must be written in the EMMC format as described in *Section 4 - Method Content*. Conformance to the required method format is essential to EMAP's goal of achieving program consistency.

2. Title and date of approved EMAP method: This field identifies the accepted EMAP method to which the performance-based method is being compared.

3. Copy of approved EMAP method maintained on-site?: This item is another "yes/no" question regarding required paperwork. Although a copy of the approved EMAP method is not required to be attached to the checklist submitted for approval, a copy should be maintained at the laboratory.

4. Listing of differences between candidate method and approved EMAP method attached?: The submission for evaluation must include a side-by-side listing of the differences between the candidate method and the approved EMAP method. The listing should contain each section of the EMMC method format of the approved EMAP method and the candidate method. The left-hand column will list pertinent aspects of the approved EMAP method, while the right-hand column will list pertinent aspects of the candidate method. The comparison should focus on differences between the two procedures in technique, not minor variations in the glassware used or the concentrations of specific reagents. The listing should highlight any differences between method performance data. All method performance information must be included in the comparison in the method sections for Scope and Application, Interferences, Quality Control, and Method Performance.

5. Performance range tested (w/units): This range should reflect the actual range of sample concentrations that were tested and must include the concentration units. These concentration units are typically expressed in terms of mass/mass or mass/volume. The range should not be expressed in terms of the concentrations of the standards tested.

6. Linear working range (w/units): The linear working range is the range of concentrations over which the analytical system exhibits a linear relationship between the amount of material introduced into the instrument and the instrument's response. Linearity is typically measured as either a linear regression for inorganic analytes or as the RSD of the response factors or calibration factors for organic analytes. Traditional performance specifications consider any regression line with a correlation coefficient (r) of 0.995 or greater as linear. For organic analytes, an RSD of 25% or less is typically considered linear. Using these limits for linearity as guidance, enter the method's linear working range in this field.

7. Concentrations of calibration standards w/units corresponding to final sample concentration: The concentrations of the calibration standards are entered in the next field. As noted on the checklist, the concentration of the lowest standard must be one to four times the MDL. The number of calibration standards may vary for different methods. Under the PBMS, the concentrations of the calibration standards must bracket the sample concentrations. No sample result may be reported that is above the calibration range of the method unless that sample or extract has been diluted for analysis. Similarly, no result may be reported that is below the calibration range specified in the method.

8. Calibration curve attached?: The calibration curve is the graphical representation of the instrument response versus the concentration of the calibration standards. For methods that use linear regression or another statistical curve, the calibration curve must be included with any data submission. For methods that use the RSD of the calibration results as a measure of linearity, the curve is not needed if the RSD specifications are met. However, calibration curves must be attached for methods where the instrument response is not sufficiently linear to pass the RSD specifications.

9. Slope of calibration regression line: If a regression line is calculated, its equation should be entered in this field.

10. Relative Standard Deviation (RSD) of calibration factors: If the RSD calculation is used to determine a calibration's linearity, the result should be entered in this field.

11. Sample preservatives and holding times: If holding times have been evaluated, include the conclusions of the evaluation in this field.

12. Interferences: In this field, enter information on any known or suspected interferences with the candidate method. While interferences may be difficult to predict, they may be indicated by unacceptable spike recoveries, especially when such interferences are not noted when testing a clean matrix such as reagent water. It may also be useful to indicate whether known interferences with the approved EMAP method are not interferences with the candidate method, and vice versa.

13. Qualitative identification criteria used: Qualitative identification criteria are crucial to the accurate assessment of contaminants and will vary depending on the analytical technique employed. Enter all relevant criteria used for target analyte identification. These criteria should include items such as retention time, spectral wavelengths, co-elution of peaks for specific m/z's, ion abundance ratio requirements, etc. The accepted EMAP method should be used as

guidance when specifying identification criteria if the instrumental techniques are similar. If the list of criteria is lengthy, attach it to the checklist on a separate sheet and enter "Attached" in this field.

14. Surrogates used: If surrogate compounds are added to samples prior to preparation, which is typically done for organic analyses, indicate the specific surrogates used in the candidate method.

15. Surrogate recovery limits established: Summarize the surrogate recovery limits that have been established for the candidate method. For methods with multiple surrogates, include a range of recovery limits, and attach a detailed list to the checklist. These recovery limits may be derived from specific performance tests performed during the EMAP Demonstration of Method Comparability, adopted from the approved EMAP method, or based on a scientifically defensible consensus of expected performance.

16. Testing conditions:

Matrix used: Enter the matrix used to perform comparability testing.

Spike levels: When the standard matrix aliquots are spiked, enter the theoretical concentrations of analytes after spiking. The concentrations should be expressed in the same units in which the sample results will be expressed, not in the concentration units of the spiking solution. If spiking is not required, enter that information in this field. Include the background concentrations of the analytes found in the standard matrix.

Source of spiking material: If reference materials obtained from commercial sources are used, indicate the sources. If externally prepared materials are not available, spiking materials must be prepared in the laboratory and this should be indicated on the checklist, along with the source of all neat materials used.

Number of replicates: In this field, indicate the number of replicates that were used. The minimum number of replicates required is five.

17. Performance Characteristics: The last three items on the checklist summarize the precision, percent recovery, and MDL results for the candidate method. For multiple analyte methods, it would be impractical to enter the results for each analyte in these fields. Therefore, for multiple analyte methods, enter the range of results in the precision and percent recovery fields, and attach an analyte-specific summary to the checklist. In the left-hand column for each of these criteria, enter the performance specification from the accepted EMAP method. In the right-hand column, enter the results of performance testing for the candidate method.

Precision: The precision statistics determined from the analysis of replicate aliquots are entered in this field.

Percent Recovery: The percent recovery estimates determined from the analysis of replicate aliquots are entered in this field.

Method Detection Limit (MDL): The MDL determined for each method analyte is entered in this field.

6.5 Certification Statement

Appendix F: EMAP Demonstration of Method Comparability Checklist for Chemical Methods contains the certification statement required to be documented with every EMAP Demonstration of Method Comparability. This statement is intended to ensure that the laboratory or organization that provided and tested the candidate method assumes full responsibility for method performance and for the data provided on the checklist.

6.6 Evaluation of Method Performance

6.6.1 Evaluation of the EMAP Demonstration of Method Comparability will consist of ensuring that the required documentation is in place and complete and that the candidate method meets or exceeds the performance specifications of the approved EMAP method. Laboratories proposing candidate methods for approval must perform all required performance testing, document the results, and submit the documentation to the appropriate Resource Group. The Resource Group is then responsible for evaluating the documentation and determining the approval status of the proposed candidate method.

6.6.2 Incomplete documentation will not be evaluated for approval. Whenever required information is missing or incomplete, the providing laboratory or organization will be required to complete the information and resubmit the completed documentation for approval. Once the documentation has been determined to be complete, method evaluators will assess the method's suitability for EMAP's needs.

6.6.3 With documentation requirements met, method assessors may evaluate the method by using the documentation to answer the following three questions.

Is the method specific (or selective) for the analyte? Evaluation of the analysis of spikes and standards, along with noted interferences, will enable the method assessor to determine the method's selectivity. If the method is not specific for the analyte, it will not be approved in its current form. The method may be revised and retested if desired. Otherwise, the method is not approved for EMAP use.

Do the method's precision and percent recovery results meet or exceed those of the approved EMAP method? The results of the EMAP Demonstration of Comparability are available for this evaluation. If the precision and percent recovery estimates determined during performance testing do not meet or exceed those of the approved EMAP method, the method will not be approved in its current form. The method may be revised and retested if desired. Otherwise, the method is not approved for EMAP use.

Does the MDL meet or exceed the MDL specifications of the approved EMAP method? If the MDL of the candidate method does not meet or exceed the MDL specifications of the

approved EMAP method, the method will not be approved in its current form. The method may be revised and retested if desired. Otherwise, the method is not approved for EMAP use.

6.6.4 At the discretion of the Resource Group, method assessors may find it beneficial to consider a number of additional Quality Control (QC) checks when evaluating candidate methods. The following list provides examples of the types of checks that may be useful:

- Verification of linear working range
- Calibration verification
- External QC samples
- Performance evaluation studies
- Surrogate recoveries

6.6.5 Multi-Analyte Methods: As noted in subsection 5.6.4, for methods that measure more than one parameter, method approval will be more complicated than with single parameter methods. The EMAP Method Validation and the EMAP Demonstration of Method Comparability will involve characterizing the method's performance for each compound measured. Likewise, method evaluation will involve evaluating the method's performance for each compound measured. In multiple parameter methodds, the possibility exists that the performance statistics of some of the method's analytes will meet requirements, while others do not.

Whenever the performance statistics of some fraction of a method's analytes fail to meet performance objectives, any of three potential decisions may be made regarding approval of the method for EMAP use. The method may be approved for use in measuring only those parameters whose performance statistics met requirements; the method may be approved "as is"; or the method may be rejected. Criteria for making this determination are not addressed in this guidance. Method acceptance criteria fall within the responsibilities of the Resource Groups. Along with MQOs, specific method acceptance criteria should be defined by the Resource Group and included in the appropriate QAPP.

SECTION 7
REFERENCES

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APPENDICES

Appendix A: EMAP Method Validation Checklist for Chemical Methods

Date: **Page __ of __**
Facility Name:
Resource Group/Program:
Matrix Type:
Analyte or Class of Analytes:

Conditions	Measurement Quality Objectives (MQOs)	Summary of Results
1. Written candidate method (EMMC format) attached?	---	Y/N
2. Title and date of QAPP containing MQOs	---	Y/N
3. Method range tested (w/units)		
4. Linear working range (w/units)		
5. Concentrations of calibration standards w/units corresponding to final sample concentration (lowest standard must be one to four times MDL)		
6. Calibration curve attached?	---	Y/N
7. Slope of calibration regression line (if applicable)		
8. Relative Standard Deviation (RSD) of calibration factors (if applicable)		
9. Sample preservatives and holding times		
10. Interferences		
11. Qualitative identification criteria used		
12. Surrogates used (if applicable)		
13. Surrogate recovery limits established		

EMAP Method Validation Checklist for Chemical Methods

Date: **Page __ of __**
Facility Name:
Resource Group/Program:
Matrix Type:
Analyte or Class of Analytes:

Analytical Results*

14. Method Range Determination		
Method range in a standard matrix		
Standard matrix used		
Spike levels (w/units corresponding to final sample concentration)		
Source of spiking material		
Number of replicates (minimum n=8)		
15. Matrix Validation		
Sample matrix used		
Spike levels (w/units corresponding to final sample concentration)		
Source of spiking material		
Number of replicates (minimum n=5)		
16. Performance Characteristics**	**Measurement Quality Objectives (MQOs)**	**Validation Results**
Method range determined (in the sample matrix)		
Method detection limit (MDL) (w/units and number of replicate samples analyzed)		
Precision (RSD [n-1] of replicate measurements)		
Percent Recovery		

* Complete one page for each matrix tested.

** For multiple analyte methods, enter the range of results obtained, and attach a full listing of results to the checklist.

EMAP Method Validation
Certification Statement*

Date: **Page __ of __**
Facility Name:
Resource Group/Program:
Matrix Type:
Analyte or Class of Analytes:

We, the undersigned, CERTIFY that:

1. The candidate method provided for EMAP use has undergone the EMAP Method Validation (or the "Single-Laboratory Method Validation Protocol" (USEPA, 1985)) and has met the MQOs of the approved QAPP.

2. A copy of the candidate method, written in the EMMC format, is attached to this checklist and is available on-site.

3. The data associated with the EMAP Method Validation are complete (including the mandatory copy of this checklist), all raw data necessary to reconstruct and verify the analyses have been retained, and the associated information is available for review.

Name and Title	Signature	Date
Name and Title	Signature	Date
Name and Title	Signature	Date
Name and Title	Signature	Date

*This certification form must be completed when the EMAP Method Validation is performed to approve a candidate method for EMAP use.

Appendix B: Method Range and Method Detection Limit

The method range is the concentration range over which the method is valid. In actual application of the method, the method range is used to determine when sample dilution is required or when a smaller sample size should be used for analysis. The Method Detection Limit (MDL) is defined as the minimum concentration of a substance that can be identified, measured, and reported with 99 percent confidence that the analyte concentration is greater than zero. The MDL is used to judge the significance of a single measurement in a future sample.

To determine the method range, eight standard matrix samples, spiked at each of three concentrations spread over the instrumentation range, are processed through the entire analytical procedure. The results are compared to the precision and percent recovery performance requirements to determine the concentration range over which the method is valid. For each method analyte, both an upper limit and a lower limit for the method range are determined. The lower limits are then used to calculate the MDL for each analyte.

1. Procedure

1.1 Determine the three spike concentrations for each method analyte.

1.1.1 The low spike concentration (L) should be one of the following, whichever is higher:

(a) The concentration value that corresponds to the lower limit of the instrumentation range.

(b) The concentration value that corresponds to a signal-to-noise ratio in the range of 2.5 to 5. If the criteria for qualitative identification of the analyte are based upon pattern recognition techniques, the least abundant signal necessary to achieve identification must be considered in making the estimate.

(c) The concentration value that corresponds to three times the standard deviation of replicate instrumental measurements for the analyte in reagent water.

(d) The concentration value that corresponds to the region of the standard curve where there is a significant change in sensitivity at low analyte concentration (i.e., a break in the slope of the standard curve).

1.1.2 The high spike concentration (H) should be the concentration that defines the upper limit of the instrument range.

1.1.3 The middle spike concentration (M) should be the geometric mean of the low and high spike concentrations as calculated from the following equation:

$$M = (H \times L)^{1/2}$$

1.2 Spike eight standard matrix samples with each method analyte at each of the three spike concentrations.

1.3 Process the spiked samples through the entire analytical procedure in random order. Make all calibrations and computations according to the procedure and express the concentrations found in the procedure reporting units.

2. Evaluation of Results

2.1 Test the concentrations found for outliers.

2.1.1 For each analyte, test the concentrations found at each of the three spike concentrations for outliers at the 1 percent significance level (see *Appendix D: Statistics for Determining Outliers, Significance, and Confidence Intervals*). Check each data point declared an outlier for calculation and/or recording errors. Eliminate any outlying data point.

2.1.2 If the results from *step 2.1.1* produce less than six data points for any analyte-concentration combination, analyze new standards as needed to assure a minimum of six data points for each analyte at each concentration.

2.1.3 If new standards were analyzed in *step 2.1.2*, return to *step 2.1.1*. Otherwise, proceed to *step 2.2*.

NOTE: The completion of *step 2.1* will result in a minimum of six data points for each analyte-concentration combination. Because the outlier detection procedure eliminates data on an analyte-by-analyte basis and the analysis of new standards produces data for all analytes, different numbers of data points may be obtained for the three concentrations for some analytes.

2.2 Calculate standard deviation statistics and compare them to the precision performance requirement for the method.

2.2.1 Calculate the standard deviation (s) and the RSD of the method for each analyte-concentration combination.

2.2.2 Calculate a 100 (1-.01/N_a) percent confidence interval for the RSD of each analyte-concentration combination, where N_a is the number of analytes for which the method is being validated (*Appendix D: Statistics for Determining Outliers, Significance, and Confidence Intervals*). If any of the lower limits of the confidence intervals calculated are greater than the precision performance requirement, the method has failed to meet the precision performance requirement for that analyte-concentration combination.

2.3 Calculate percent recovery statistics and compare them to the performance requirements.

2.3.1 Calculate the percent recovery of the method for each analyte-concentration combination.

2.3.2 For each analyte-concentration combination, calculate a 100 (1-.01/N_a) percent confidence interval for the true percent recovery of the method where N_a is the number of analytes for which

the method is being validated (*Appendix D: Statistics for Determining Outliers, Significance, and Confidence Intervals*). If any of the upper limits of the confidence intervals are less than the lower performance requirement or if any of the lower limits of the confidence intervals are greater than the upper performance requirement, the method has failed to meet the percent recovery performance requirement of the corresponding analyte-concentration combination.

2.4 Determine the range of the method for each method analyte. The upper limit of the method range for an analyte is the highest analyte concentration that met both the precision and percent recovery performance requirements. The lower limit of the method range for an analyte is the lowest analyte concentration tested such that all concentrations included in the method range passed both the precision and percent recovery performance requirements. If the upper and lower limits are equal for a single analyte, then the method has failed for that analyte and the analyte should be eliminated from the method.

2.5 Determine the MDL for each analyte.

2.5.1 Compute the MDL as follows:

$$MDL = t_{n-1,0.01}(s)$$

where $t_{n-1,0.01}$ = the upper first percentile point of the Student's *t* distribution with *n-1* degrees of freedom (see *Table B-1: Upper First Percentile Points of the Student's t Distribution*). *s* = the standard deviation of the concentrations found for the analyte at the spike concentration that defines the lower limit of the method range as determined in *step 2.4*. *n* = the number of replicate analyses from which the standard deviation is calculated.

2.5.2 If 10 times the calculated MDL is less than the spike concentration from which the standard deviation for the MDL calculation was obtained, the calculated MDL is not reasonable. In this case, a lower spike concentration should be selected for analysis. The procedures outlined in *steps 1.1* through *1.3* should be repeated using the new spike concentration.

2.5.3 Compute the 95% confidence limits for the MDL obtained as shown in *Appendix D: Statistics for Determining Outliers, Significance, and Confidence Intervals*.

2.5.4 Modify the written method to include the calculated MDL values. The standard matrix used to determine the MDL must be identified with the MDL value. In addition, report the analyte concentration used to calculate the MDL and the percent recovery.

Table B-1: Upper First Percentile Points of the Student's *t* Distribution

Degrees of Freedom ***(n-1)***	$t_{n-1,0.01}$
5	3.365
6	3.143
7	2.998
8	2.896
9	2.821
10	2.764
11	2.718
12	2.681
13	2.650
14	2.624

Appendix C: Matrix Validation

Matrix validation consists of a determination of the method precision, percent recovery, and range for a minimum of two matrices - the standard matrix and a representative sample matrix. The number of matrices to be tested will depend upon the end use of the method. The method must be validated for a standard matrix and for each sample matrix for which it will be used. Although it is not possible to list appropriate matrix types for all methods for which validation may be required, some representative sample matrices are provided in *Table C-1: Representative Sample Matrices for Matrix Validation* along with the relevant standard matrix. In all cases, the matrices selected for validation are assumed to be homogenous.

A validation for a single sample matrix consists of a determination of analyte background levels and the analysis of five matrix samples at each of two spike concentrations. The results obtained are statistically compared to the results obtained for the standard matrix during method range determination. Finally, the results obtained are compared to the precision and percent recovery performance requirements for the method.

Spike concentrations to be used for matrix validation are the high and low concentrations which define the method range for the standard matrix. If acceptable results are not obtained at these concentrations, an intermediate spike concentration is tested. The method range for a matrix is then defined to be the range from the lowest spike concentration that produced acceptable results to the highest spike concentration that produced acceptable results for precision and percent recovery.

Table C-1: Representative Sample Matrices for Matrix Validation

Method Application	Standard Matrix	Appropriate Representative Sample Matrix
Drinking water	Reagent water	Drinking water
Wastewater	Reagent water	POTW aqueous outfall Industrial wastewater Surface water (lake, river)
Sewage sludge	Wet (H_2O) diatomaceous earth	POTW sludge of $\geq$2 suppliers Sediments
Organic Liquids	Toluene, hexane, $MeCl_2$	Organic waste stream Waste solvents
Solid Waste	Wet (H_2O or organic solvent) diatomaceous earth	Soil Stillbottoms from manufacturer

1. Procedure

1.1 Select the sample matrices to be used in the method validation. Obtain samples of each selected matrix.

1.2 For each sample matrix, determine if a background level of any method analyte exists.

1.2.1 Analyze two method blank samples consisting of the standard matrix that have been processed through the entire analytical procedure to establish the background level of the analytical system.

1.2.2 Process three replicates of the sample matrix through the entire analytical procedure. Any response above the response obtained for the method blank samples for any method analyte indicates that a background level of that analyte is present in the sample matrix.

NOTE: The analyses conducted to determine the background concentration of method analytes will indicate other instances when a matrix is inappropriate for the validation. For example, a matrix that interferes with the detection device employed in the method or that will not allow the chromatographic system to return to baseline conditions within a reasonable length of time would be considered inappropriate. The experience of the analyst is important in determining the appropriateness of a matrix.

1.2.3 If a sample matrix contains a background level of any method analyte, process five additional samples of that matrix through the entire analytical procedure and proceed to *step 1.3*. Otherwise, proceed to *step 1.5*.

1.3 Test the concentrations found for outliers.

1.3.1 For each matrix-analyte combination in which a background level was detected, test the eight concentrations found for outliers at the 1 percent significance level (*Appendix D: Statistics for Determining Outliers, Significance, and Confidence Intervals*). Check each data point declared an outlier for calculation and/or recording errors. Correct any errors and retest for outliers. Eliminate any outlying data points.

1.3.2 If *step 1.3.1* results in less than five data points for any matrix-analyte combination in which a background level was detected, analyze new matrix samples as needed to assure a minimum of five data points for each matrix-analyte combination.

1.3.3 If new samples were analyzed in *step 1.3.2*, return to *step 1.3.1*. Otherwise, proceed to *step 1.4*.

1.4 For each sample matrix, determine the mean background concentration of each analyte.

1.4.1 If no response was found for an analyte in *step 1.2.2*, the background concentration for that matrix-analyte combination is assumed to be zero.

1.4.2 For matrix-analyte combinations in which a background level was detected in *step 1.2.1*, the mean background concentration ($\bar{B}$) is the average of the data points for that matrix-analyte combination after *step 1.3*.

1.4.3 If the background concentration of any method analyte is higher than the middle spike concentration (M) used to determine the method range, the matrix is inappropriate for the validation and should be eliminated.

1.5 Choose spike concentrations for the matrix validation. A spike concentration is the final concentration of a matrix sample after spiking, and is equal to the background concentration of an analyte plus the amount of analyte spiked into the matrix (T).

1.5.1 The low spike concentration for an analyte should be the lowest concentration determined by the procedures outlined in *Appendix B: Method Range and Method Detection Limit* that is at least five times higher than the background level of that analyte.

1.5.2 The high spike concentration for an analyte should be the concentration that defines the upper limit of the method range for that analyte.

1.6 Prepare a spiking solution containing each method analyte in a solvent compatible with the matrix being evaluated. The concentration of analytes in the spiking solution should be such that the volume of spiking solution added to a matrix sample to produce the desired spike concentration does not exceed 1 percent of the volume of the matrix sample. This limitation does not apply to spiking solutions where the solvent used to prepare the spiking solution is identical to the bulk of the matrix.

1.7 For each sample matrix, prepare five samples at each spike concentration using the spiking solution. Record the amount of each analyte added to the samples.

1.8 For each sample matrix, process all samples through the entire analytical procedure in random order. Make all calibrations and computations according to the method and express the concentrations found in the method reporting units.

2. Evaluation of Results

2.1 Test the concentrations found for outliers.

2.1.1 For each matrix-analyte-concentration combination, test the concentrations found for outliers at the 1 percent significance level (*Appendix D: Statistics for Determining Outliers, Significance, and Confidence Intervals*). Check each data point declared an outlier for calculation and/or recording errors. Correct any errors and retest for outliers. Eliminate any outlying data point for which no explanation is found.

2.1.2 If *step 2.1.1* results in less than five data points for any matrix-analyte-concentration combination, analyze new samples as needed to assure a minimum of five data points for each matrix-analyte-concentration combination.

2.1.3 If new samples were analyzed in *step 2.1.2*, return to *step 2.1.1*. Otherwise, proceed to *step 2.2*.

2.2 For each matrix, calculate mean percent recovery statistics and compare them to the corresponding statistics for the standard matrix and to performance requirements.

2.2.1 For each matrix, calculate the mean percent recovery for each analyte at both the high and low spike concentration, correcting for any background level of the analyte determined by the procedure outlined in *Appendix B: Method Range and Method Detection Limit*.

2.2.2 For each matrix-analyte-concentration combination, calculate a 100 (1-.025/N_a) percent confidence interval for the difference between the mean percent recovery in the sample matrix and the percent recovery at the corresponding concentration in the standard matrix (*Appendix B: Method Range and Method Detection Limit*), where N_a is the number of analytes for which the method is being validated (*Appendix D: Statistics for Determining Outliers, Significance, and Confidence Intervals*). If any of the confidence intervals do not contain the value zero, the bias in the sample matrix is significantly different from the percent recovery in the standard matrix for the corresponding matrix-analyte-concentration combination.

2.2.3 For each matrix-analyte-concentration combination, calculate a 100 (1-.025/N_a) percent confidence interval for the mean percent recovery, where N_a is the number of analytes for which the method is being validated (*Appendix D: Statistics for Determining Outliers, Significance, and Confidence Intervals*). If the upper limit of any of the confidence intervals is less than the lower performance requirement, or if the lower limit of any of the confidence intervals is greater than the upper performance requirement, the method has failed to meet the percent recovery performance requirements for the corresponding matrix-analyte-concentration combination.

2.2.4 If the method fails to meet the bias requirement and the bias in the sample matrix is significantly different from the bias in the standard matrix, the method is declared to have failed the matrix validation for that matrix-analyte-concentration combination.

2.3 For each matrix, calculate standard deviation statistics and compare them to the corresponding statistics for the standard matrix and to the precision performance requirement.

2.3.1 For each matrix, calculate the standard deviation for each analyte and the RSD for each analyte at both the high and low concentration.

2.3.2 For each matrix-analyte-concentration combination, calculate a 100 (1-.025/N_a) percent confidence interval for the ratio of the standard deviation in the sample matrix to the standard deviation in the standard matrix at the corresponding concentration, where N_a is the number of analytes for which the method is being validated (*Appendix D: Statistics for Determining Outliers, Significance, and Confidence Intervals*). If any of the confidence intervals do not contain the value 1, the standard deviation in the sample matrix is significantly different from the standard deviation in the standard matrix for the corresponding matrix-analyte-concentration combination.

2.3.3 For each matrix-analyte-concentration combination, calculate a 100 (1-.025/N_a) percent confidence interval for the true RSD of the method, where N_a is the number of analyte for which the

method is being validated (*Appendix D: Statistics for Determining Outliers, Significance, and Confidence Intervals*). If the lower limit of any of the confidence intervals is greater than the precision performance requirement, the method has failed to meet the precision performance requirement for the corresponding matrix-analyte-concentration combination.

2.3.4 If the method fails to meet the precision performance requirement and the standard deviation in the sample matrix is significantly different from the standard deviation in the standard matrix, the method is declared to have failed the matrix validation for the corresponding matrix-analyte-concentration combination.

2.4 If the method has not failed for any matrix-analyte-concentration combination in *steps 2.2.4* and *2.3.4*, proceed to *step 2.6*. If a failure has occurred in either step, determine whether an intermediate spike concentration should be tested.

2.4.1 If the failure occurred at both the high and low spike concentrations, no intermediate spike concentration should be tested.

2.4.2 If the failure occurred at only the high or low spike concentration, but no spike concentration for which standard matrix samples were analyzed (*Appendix B: Method Range and Method Detection Limit*) falls between the high and low spike concentrations, no intermediate spike concentration should be tested.

2.4.3 If the failure occurred at only the high or low spike concentration, and at least one intermediate spike concentration is available, an intermediate spike concentration should be tested according to *step 2.5*.

2.5 For each failed matrix-analyte-concentration combination identified in *step 2.4.3*, choose and test an intermediate spike concentration.

2.5.1 The intermediate spike concentration to test for an analyte is the spike concentration used to determine the method range that falls between the high and low spike concentrations. If more than one intermediate spike concentration is available, the one nearest the spike concentration that caused failure should be used.

2.5.2 Prepare five samples of a matrix to contain each failed analyte at the intermediate spike concentration using the spiking solution. Record the amount of each analyte added to the samples.

2.5.3 Process the five samples through the entire analytical procedure.

2.5.4 For each additional set of analyses performed in *step 2.5.3*, perform the tests, calculations, and comparisons in *steps 2.1* through *2.4*. For these steps, the intermediate spike concentration should be considered the high or low concentration, whichever caused the failure for the matrix-analyte-concentration combination.

2.6 For each matrix, determine the method validity and range.

2.6.1 A method is valid for an analyte in a particular matrix if two spike concentrations investigated for that matrix-analyte combination do not result in failure. These two spike concentrations may be the original high and low concentrations or the high and low concentrations combined with a subsequently investigated intermediate concentration.

2.6.2 A method is not valid for a matrix-analyte combination that caused failure at both the initial high and low concentrations.

2.6.3 A method is not valid for a matrix-analyte combination that caused a failure at either the initial high or low concentration and all intermediate spike concentrations.

2.6.4 If a failure occurs for a single analyte in all matrices tested, the method is not valid for that analyte and the analyte should be deleted from the method. If a method fails for the majority of analytes in all matrices tested, the method is not valid.

2.6.5 The method range for each valid matrix-analyte combination extends from the lowest to the highest spike concentration that did not result in failure.

Appendix D: Statistics for Determining Outliers, Significance, and Confidence Intervals

This appendix explains how to perform the statistical calculations and analyses required of the performance tests described in previous sections of this document. The primary reference for these techniques is "Statistical Methods" (Snedecor and Cochran, 1980). This text and additional references are cited within this appendix where appropriate. Full citations are provided in *Section 8 - Statistical References* at the end of the appendix. See *Appendix E: Statistical Example* for examples of the calculations contained here.

1. Using Statistical Tables

1.1 This section describes methods for using reference tables to determine the distributional deviates needed in the statistical calculations contained in this document. The particular tables referenced in this section can be found in "Statistical Methods" (Snedecor and Cochran, 1980). Similar tables may be found in nearly any general statistics text. Note that reference tables for a given distribution may take many forms. Therefore, it is important to read the description of the table in order to understand what the table provides and how to find the particular value needed in a calculation. The required values may also be obtained using certain statistical packages. Examples are provided for obtaining the distributional deviates using the SAS System.

1.2 The Standard Normal Distribution

1.2.1 This section describes methods for obtaining required distributional deviates from the standard normal distribution. The distributional deviates take the following form: z_δ is the deviate from the standard normal distribution such that:

$$P(z \geq z_\delta) = \delta$$

1.2.2 To find this upper-tail deviate in a table that provides $P(0 \leq z \leq z_\delta)$, the desired δ must be subtracted from 0.5 to obtain the appropriate value for use with the standard normal distribution table.

1.2.3 Scan the standard normal distribution table to find the value of 0.5 - δ. The first portion of standard normal deviate associated with this value can be found in the leftmost column of the row in which the value of 0.5 - δ was found. The hundredths place for the deviate value is found in the top row of the column in which the value was found. Since standard normal tables generally provide areas expressed to four decimal places, the value of δ should be rounded to four decimal places.

1.2.4 To illustrate, consider calculating 100 (1-.01/N_a)% confidence intervals for some parameter where N_a = 2. In this case, δ = .01/(2N_a) = 0.0025 and 0.5 - δ = 0.4975. Scanning the standard normal table, it can be seen that this area corresponds to z = 2.81.

1.2.5 If the area in question does not appear in the table, there are two methods to determine the appropriate value of z. The simplest method is to take the larger of the two z-scores whose associated probabilities bracket the value in question, giving a conservative estimate. The other

method is to interpolate between the two *z*-scores whose associated probabilities bracket the value in question. See *subsection 1.3.6* of this appendix for instructions on performing linear interpolation.

1.2.6 If the probability being sought is associated with more than one value of *z*, the conservative estimate is to choose the highest *z*-score associated with the value. For example, if N_a = 5, then δ = .01/(2*5) = 0.001 and 0.5 - δ = 0.4990. Scanning the table reveals that 0.4990 is associated with three values of *z*: 3.08, 3.09, and 3.10. The largest of these values, 3.10, would be selected for use in calculations.

1.2.7 The required standard normal deviate may be obtained using statistical software. In the SAS System, the PROBIT function returns deviates from the standard normal distribution for a given probability. The form of the function is as follows:

$$value = PROBIT \quad (1 - \delta);$$

Because the PROBIT function returns values associated with quantiles of the standard normal distribution, (i.e., *z* such that $P(Z \leq z_\epsilon) = \epsilon$), 1 - δ is the appropriate parameter for the function. Note that other statistical software may not perform in the same manner. Consult the software user's guide to determine the proper function and syntax.

1.3 The Student's *t* Distribution

1.3.1 This section describes methods for obtaining required distributional deviates from the Student's *t* distribution. The distributional deviates take the following form: $t_{df,\delta}$ is the deviate from the Student's *t* distribution with df degrees of freedom such that:

$$P(t_{df} \geq t_{df,\delta}) = \delta$$

1.3.2 This value can be found in a table such as the one contained in "Statistical Methods" (Snedecor and Cochran, 1980), which provides two-tailed critical values (i.e., $P(|t_{df}| \geq t_{df,\epsilon})$). The first row of the table indicates the level of the two-tailed probability. Locate the appropriate column for ϵ and find the value of *t* in the row corresponding to the appropriate number of degrees of freedom. In this case, $\epsilon = 2 \cdot \delta$.

1.3.3 Because in these sections the calculation of δ usually involves division by N_a, a column for the exact level of δ required may not appear in the table. Because the distribution of *t* is different for each value of *df*, it would be too cumbersome to provide a complete table (similar to the standard normal distribution table) for each number of degrees of freedom. Rather, values corresponding to certain commonly chosen levels of δ are provided for the different degrees of freedom. Linear interpolation between tabular values is preferable to the conservative estimate of using the tabular value for the probability value closest to, but less than δ. Because the difference between this conservative estimate and the actual value required can be relatively large, linear interpolation between adjacent tabular values is recommended to enhance calculation results.

1.3.4 It is more common for tables of the Student's *t* distribution to provide one-tailed probabilities rather than two-tailed probabilities. In this case, the value of δ is as defined in *subsection 1.3.1*, one

half of the value that would be used in a two-tailed table. The remainder of the procedure is the same as noted in the previous subsection.

1.3.5 To illustrate, consider calculating 100 (1-.01/N_a)% confidence intervals for some parameter where N_a = 4. To use a two-tailed *t* table, set δ = .01/(N_a) = 0.0025. If *n* = 10, then *df* = *d* - 1 = 9. Using the row of the two-tailed table corresponding to 9 degrees of freedom, the deviate corresponding to δ = 0.0025 is bracketed by $t_{9,.005}$ = 3.690 and $t_{9,.001}$ = 4.781.

1.3.6 Linear interpolation between the two adjacent tabular values is performed as follows:

δ:	0.005	0.0025	0.001
t_δ:	3.690	?	4.781

First calculate:

$$\frac{0.0025 - 0.001}{0.005 - 0.001} = 0.375$$

Rearranging the above equation gives:

$$0.0025 = 0.001 + 0.375\ (0.005 - 0.001)$$

The desired value of the *t* distribution can be found by replacing the δ values with the corresponding values of t_δ in the above equation as shown:

$$t_\delta = 4.781 + 0.375\ (3.690 - 4.781) = 4.372$$

1.3.7 To use a one-tailed *t* table for the example above, set δ = .01/(2N_a) = 0.00125 and proceed as above. Some tables will provide values associated with quantiles of the Student's *t* distribution, (i.e., *t* such that P($t_{df} \le t_{df,\epsilon}$) = ϵ). When using a table of this form, use 1 - δ in place of δ.

1.3.8 The required Student's *t* distribution deviate may be obtained using statistical software. In the SAS System, the TINV function returns deviates from the Student's *t* distribution for a given probability. The form of the function is as follows:

$$value = TINV\ (1 - \delta,\ df\ );$$

Because the TINV function returns values associated with quantiles of the Student's *t* distribution, (i.e. *t* such that P($t_{df} \le t_{df,\epsilon}$) = ϵ), 1 - δ is the appropriate parameter for the function. Note that other statistical software may not perform in the same manner. Consult the software user's guide to determine the proper function and syntax. The value obtained using the computer software will probably not be the same as the result of the linear interpolation performed above. The computer-produced values are usually more accurate than the results found by simple linear interpolation.

1.4 The *F* Distribution

1.4.1 This section describes methods for obtaining required distributional deviates from the *F* distribution. The distributional deviates take the following form: $F_{df_n,df_d,\delta}$ is the deviate from the *F* distribution with df_n degrees of freedom in the numerator and df_d degrees of freedom in the denominator such that:

$$P(F_{df_n,df_d} \geq F_{df_n,df_d,\delta}) = \delta$$

1.4.2 This value can be found in tables such as those contained in "Statistical Methods" (Snedecor and Cochran, 1980), which provides upper-tail critical values (i.e., $P(F_{df_n,df_d} \geq F_{df_n,df_d,\delta})$. In most tables, numerator degrees of freedom are listed across the top of the table (columns) and the denominator degrees of freedom are listed down the left side of the table (rows). Either individual tables are provided for various values of δ or rows are provided for the values of δ within each level of df_d. Locate the appropriate position in the table for df_n and df_d.

1.4.3 As with the Student's *t* distribution only certain commonly chosen levels of δ are provided in the table and the exact value of δ required may not appear in the table. Linear interpolation between tabular values is preferable to the conservative estimate of using the tabular value for the probability value closest to, but less than δ. Because the difference between this conservative estimate and the actual value required can be relatively large, linear interpolation between adjacent tabular values is recommended to enhance calculation results. Tables of the *F* distribution may not contain the particular combination of numerator and denominator degrees of freedom required by the calculation. In this case, linear interpolation between available degrees of freedom should be performed as well.

1.4.4 In general, only upper-tail values are provided in tables of the *F* distribution. Although the *F* distribution is not symmetric, the following relationship locates lower-tail critical values using upper-tail critical values:

$$F_{df_n,df_d,1-\delta} = \frac{1}{F_{df_d,df_n,\delta}}$$

The lower-tail critical value is simply the reciprocal of the corresponding upper-tail critical value with the numerator and denominator degrees of freedom reversed. Some tables will provide values associated with quantiles of the *F* distribution, (i.e., *F* such that $P(F_{df_n,df_d} \leq F_{df_n,df_d,\epsilon}) = \epsilon$). When using a table of this form, use 1 - δ in place of δ. The smallest value of δ represented in tables of the *F* distribution from "Statistical Methods" (Snedecor and Cochran, 1980) is $\delta = 0.005$. Deviates corresponding to values of δ less than 0.005 will need to be obtained from other tables or by using statistical software.

1.4.5 To illustrate, consider calculating 100 (1-.01/N_a)% confidence intervals for a ratio of standard deviations where $N_a = 2$. For this problem, $\delta = .025/(2N_a) = 0.00625$. If $n_1 = 8$ and $n_2 = 10$, then $df_n = n_1 - 1 = 7$ and $df_d = n_2 - 1 = 9$. In this case, $\delta = 0.00625$ is bracketed by values of $\delta = 0.01$ and $\delta = 0.005$. The use of two tables in "Statistical Methods" (Snedecor and Cochran, 1980) is required

for this problem. In the appropriate tables, using the columns for 7 numerator degrees of freedom and the rows for 9 denominator degrees of freedom, $F_{7,9,.01} = 5.62$ and $F_{7,9,.005} = 6.88$.

1.4.6 Linear interpolation between the available tabular values is performed as follows:

δ:	0.01	0.00625	0.005
F_δ:	5.62	?	6.88

First calculate:

$$\frac{0.00625 - 0.005}{0.01 - 0.005} = 0.25$$

Rearranging the above equation gives:

$$0.00625 = 0.005 + 0.25(0.01 - 0.005)$$

The desired value of the F distribution can be found by replacing the δ values with the corresponding values of F_δ in the above equation as shown:

$$F_\delta = 6.88 + 0.25(5.62 - 6.88) = 6.565$$

1.4.7 The lower-tail deviate is obtained in a similar fashion. As above, tabular values associated with the values of δ that bracket the desired level are obtained using the following relationship:

$$F_{7,9,1-.00625} = \frac{1}{F_{9,7,.00625}}$$

From the table, $F_{9,7,.01} = 6.71$ and $F_{9,7,.005} = 8.51$. Linear interpolation is then performed, as above.

δ:	0.01	0.00625	0.005
F_δ:	6.71	?	8.51

The first two calculations will be the same as the linear interpolation step performed above. Only the step replacing the values of δ with the corresponding F_δ values in the equation above needs to be redone.

$$8.51 + 0.25(6.71 - 8.51) = 8.06$$

Therefore,

$$F_{7,9,1-.00625} = \frac{1}{F_{9,7,.00625}} = \frac{1}{8.06} = 0.124$$

1.4.8 The required *F* distribution deviates may be obtained using statistical software. In the SAS System, the FINV function returns deviates from the *F* distribution for a given probability. The form of the function is as follows:

$$value = FINV(1 - \delta, df_n, df_d);$$

Because the FINV function returns values associated with quantiles of the *F* distribution, (i.e., *F* such that $P(F_{df_n,df_d} \leq F_{df_n,df_d,\epsilon}) = \epsilon$), 1 - δ is the appropriate parameter for the function. Note that other statistical software may not perform in the same manner. Consult the software user's guide to determine the proper function and syntax. The values obtained using the computer software will probably not be the same as the results of the linear interpolation performed above. The computer-produced values are usually more accurate than the results found by simple linear interpolation.

1.5 The χ^2 Distribution

1.5.1 This section describes methods for obtaining required distributional deviates from the χ^2 distribution. The distributional deviates take the following form: $\chi^2_{df,\delta}$ is the deviate from the χ^2 distribution with *df* degrees of freedom such that:

$$P(\chi^2_{df} \geq \chi^2_{df,\delta}) = \delta$$

1.5.2 This critical value can be found in a table such as the one contained in "Statistical Methods" (Snedecor and Cochran, 1980). Because the χ^2 distribution is not symmetric and there is no relationship to obtain lower-tail values using upper-tail values, tables of the χ^2 distribution must provide critical values for both tails of the distribution. The χ^2 tables are usually organized with the probability values (δ) across the top of the table (columns) and the degrees of freedom down the left side of the table (rows), similar to most tables of the Student's *t* distribution. Locate the appropriate column for δ and find the value of χ^2 in the row corresponding to the appropriate number of degrees of freedom.

1.5.3 As with the Student's *t* and *F* distributions, because the calculation of δ usually involves division by N_a, a column for the exact level of δ required may not appear in the table, making linear interpolation necessary. Linear interpolation between available values is preferable to the conservative estimate of using the tabular value for the column in the table with the probability value closest to, but less than δ. Because the difference between the nearest available deviate and the actual value required can be relatively large, linear interpolation between adjacent tabular values is recommended to enhance calculation results. The format of some tables may provide values associated with quantiles of the χ^2 distribution, (i.e., χ^2 such that $P(\chi^2_{df} \leq \chi^2_{df,\epsilon}) = \epsilon$). When using a table of this form, use 1 - δ in place of δ. The smallest value of δ represented in tables of the χ^2 distribution from "Statistical Methods" (Snedecor and Cochran, 1980) is δ = 0.005. Deviates corresponding to values of δ less than 0.005 will need to be obtained from other tables or by using statistical software.

1.5.4 To illustrate, consider calculating a 95% confidence interval for the MDL of a particular analytical method. In this case, δ = .05/2 = 0.025. If *n* = 10, then *df* = *n* - 1 = 9. Using the row of

the χ^2 table corresponding to 9 degrees of freedom and the column for $\delta = 0.025$, $\chi^2_{9,.025} = 19.02$. Similarly, $\chi^2_{9,.975} = 2.70$.

1.5.5 If the particular value of δ is not represented in the χ^2 table, linear interpolation to determine the desired value may be performed (see *subsection 1.3.5*).

1.5.6 The required χ^2 distribution deviates may be obtained using statistical software. In the SAS System, the CINV function returns deviates from the χ^2 distribution for a given probability. The form of the function is as follows:

$$value = CINV\ (1 - \delta, df);$$

Because the CINV function returns values associated with quantiles of the χ^2 distribution, (i.e., χ^2 such that $P(\chi^2_{df} \leq \chi^2_{df,\epsilon}) = \epsilon$), 1 - δ is the appropriate parameter for the function. Note that other statistical software may not perform in the same manner. Consult the software user's guide to determine the proper function and syntax. For values of δ not represented in a table of the χ^2 distribution, values obtained using the computer software will probably not be the same as the results of a linear interpolation calculation. The computer-produced values are usually more accurate than the results found by simple linear interpolation.

2. Testing for Outliers in a Single Set of Data

2.1 This section describes the maximum normed residual outlier test to detect outliers in a set of data. x_i is used to denote the i^{th} data point and n is the number of data points in the data set. This procedure assumes that the data are a random sample from a normal distribution.

2.2 If n is four or less, it is not possible to perform an outlier test.

2.3 If n is greater than four, compute the average of the sample observations:

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

2.4 For each data point x_i, compute the absolute deviation from the mean:

$$d_i = |x_i - \bar{x}|$$

2.5 The largest of the d_i values and the sum of the squared d_i are used to compute the Maximum Normed Residual (MNR) statistic as follows:

$$MNR = \frac{\max\{d_i\}}{\sqrt{\sum_{i=1}^{n} d_i^2}}$$

2.6 Compare the MNR statistic to the critical value corresponding to the sample size *n*. Critical values of the MNR statistic are provided in *Table D-1: Critical Values for the MNR Outlier Test at the 1% Level of Significance*.

2.6.1 If the MNR statistic is greater than the critical value, the data point associated with the maximum d_i value is declared an outlier and this data point is eliminated from the data set.

2.6.1.1 If more than four data points remain in the data set, return to *subsection 1.3* and perform the test calculations again using the reduced data set. The sample size, *n*, will be reduced by one.

2.6.1.2 If only four data points remain, terminate the outlier test.

2.6.2 If the MNR statistic is less than or equal to the critical value, conclude that there are no outliers in the data set and terminate the outlier test.

TABLE D-1: CRITICAL VALUES FOR THE MNR OUTLIER TEST AT THE 1% LEVEL OF SIGNIFICANCE

n	Critical Value
5	0.882
6	0.882
7	0.873
8	0.860
9	0.844
10	0.827
11	0.811
12	0.795
13	0.779
14	0.764
15	0.750

3. The Percent Relative Standard Deviation (RSD) and Approximate Confidence Intervals

3.1 This section describes the procedures for calculating approximate confidence intervals for the RSD also known as the percent Coefficient of Variation (CV) of an analytical method. The notation and formulas that follow assume that the distribution of the sampled population is normal. The sampled population should not be assumed to be normal if the RSD is too large. Generally, if the sample RSD is greater than 33%, the sampled population should not be assumed to be normal (Johnson and Welch, 1940). Additional notation will be introduced for the RSD when it is not expressed as a percentage, which is to be used in calculating limits.

3.2 Compute the average of the sample observations for each analyte-concentration combination:

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

3.3 Compute the standard deviation of the sample observations for each analyte-concentration combination:

$$s = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n - 1}}$$

3.4 Compute the percent RSD for each analyte-concentration combination:

$$RSD = \frac{s}{\bar{x}} \times 100$$

The notation RSD′ will be used to denote the relative standard deviation not expressed as a percentage. RSD′ is calculated as follows:

$$RSD' = \frac{s}{\bar{x}}$$

3.5 Compute an approximate 100 (1-α)% confidence interval for the RSD′ of each analyte-concentration combination (Johnson and Welch, 1940), where α = the significance level (.01/N_a, .025/N_a, etc.) as follows:

LOWER LIMIT:

$$\frac{100 \cdot \sqrt{n}}{\frac{\sqrt{n}}{RSD'} - Z_{1-\frac{\alpha}{2}} \sqrt{1 + \frac{n}{2(n-1)(RSD')^2}}}$$

UPPER LIMIT:

$$\frac{100 \cdot \sqrt{n}}{\frac{\sqrt{n}}{RSD'} - Z_{\frac{\alpha}{2}} \sqrt{1 + \frac{n}{2(n-1)(RSD')^2}}}$$

where n is the number of sample observations in the analyte-concentration combination and z_δ is the standard normal deviate such that $P[Z \geq z_\delta] = \delta$.

4. Calculating Confidence Intervals for the Mean Percent Recovery of an Analytical Method

4.1 This section describes the procedures for calculating confidence intervals for the mean percent recovery of an analytical method with and without a background level of analyte present. The calculations in this section assume the independence of x and B. Note that if x and B are not independent, the estimates of the mean percent recovery and associated confidence intervals will be over or under estimated.

4.2 If a background level of analyte is present in the matrix, compute the percent recovery as follows:

$$PR = \frac{\bar{x} - \bar{B}}{T} \cdot 100$$

where x the average of the concentrations found, B = the average of the background concentrations found, and T = the amount of analyte spiked into the replicate samples.

4.3 Compute upper and lower 100 (1-α)% confidence limits for the percent recovery (Moser and Stevens, 1992), where α = the significance level (.01/N_a, .025/N_a, etc.), as follows:

LOWER LIMIT:

$$PR - \frac{100}{T} t_{v,\frac{\alpha}{2}} \sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$$

UPPER LIMIT:

$$PR + \frac{100}{T} t_{v,\frac{\alpha}{2}} \sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$$

where n_1 is the number of replicate samples at the concentration being considered, s_1 is the standard deviation of the n_1 concentrations found, n_2 is the number of replicate samples analyzed to determine the background level, s_2 is the standard deviation of the n_2 background concentrations found, and $t_{v,\delta}$ is the deviate from the Student's t distribution with v degrees of freedom such that $P[t_v \geq t_{v,\delta}] = \delta$. This value can be found in a table of the Student's t distribution (see *subsection 1.3*). The following procedure is used to determine the degrees of freedom, v:

$$v = \frac{(Q_1 + Q_2)^2}{\frac{Q_1^2}{n_1 - 1} + \frac{Q_2^2}{n_2 - 1}}$$

$$\text{where} \quad Q_1 = \frac{s_1^2}{n_1} \quad \text{and} \quad Q_2 = \frac{s_2^2}{n_2}$$

The result of the preceding calculation is usually not an integer. Common practice is to round down to the nearest integer.

4.4 If no background level of analyte is present in the matrix, compute the percent recovery as follows:

$$PR = \frac{\bar{x}}{T} \cdot 100$$

where x the average of the concentrations found and T = the amount of analyte spiked into the replicate samples.

4.5 Compute upper and lower 100 (1-α)% confidence limits for the percent recovery, where α = the significance level (.01/N_a, .025/N_a, etc.), as follows:

$$\text{UPPER LIMIT}:$$
$$PR + \frac{100}{T} t_{n_1 - 1, \frac{\alpha}{2}} \frac{s_1}{\sqrt{n_1}}$$

$$\text{LOWER LIMIT}:$$
$$PR - \frac{100}{T} t_{n_1 - 1, \frac{\alpha}{2}} \frac{s_1}{\sqrt{n_1}}$$

where n_1 is the number of replicate samples at the concentration being considered, s_1 is the standard deviation of the n_1 concentrations found, and $t_{n_1-1,\delta}$ is the deviate from the Student's t distribution with n_1-1 degrees of freedom such that

$$P(t_{n_1 - 1} \geq t_{n_1 - 1, \delta}) = \delta$$

This value can be found in a table of the Student's t distribution (see *subsection 1.3*).

5. Calculating Confidence Intervals for the Method Detection Limit for an Analyte

5.1 This section describes the procedures for calculating confidence intervals for the method detection limit (MDL) for an analyte for a given analytical method.

5.2 Calculate the MDL as follows:

$$MDL = t_{n-1,0.01}(s)$$

where n is the number of replicate samples at the spike concentration that defines the lower limit of the method range, s is the standard deviation of the n concentrations found, and $t_{n-1,\delta}$ is the deviate from the Student's t distribution with $n-1$ degrees of freedom such that $P[t_{n-1} \geq t_{n-1,\delta}] = \delta$. This value can be found in a table of the Student's t distribution (see *subsection 1.3*).

5.3 Compute upper and lower 100 $(1-\alpha)$% confidence limits for the MDL, where α = the significance level (generally 0.05) as follows:

$$LOWER\ \ LIMIT: \quad MDL \sqrt{\frac{n-1}{\chi^2_{n-1,\frac{\alpha}{2}}}}$$

$$UPPER\ \ LIMIT: \quad MDL \sqrt{\frac{n-1}{\chi^2_{n-1,1-\frac{\alpha}{2}}}}$$

where n is the number of replicate samples at the spike concentration that defines the lower limit of the method range and $\chi^2_{n-1,\delta}$ is the deviate from the χ^2 distribution such that $P[\chi^2_{n-1} \geq \chi^2_{n-1,\delta}] = \delta$. The tabular value can be found in a table of the χ^2 distribution (see *subsection 1.5*).

6. Calculating Confidence Intervals for the Difference in Two Percent Recovery Means

6.1 This section describes the procedures for calculating confidence intervals for the difference in two percent recovery means. The first percent recovery value is assumed to be from a sample with no background level of analyte present (see *subsection 4.4*). The second percent recovery value may or may not include an adjustment for a background level of analyte present (see *subsection 4.2*). The calculations in this section assume the independence of $\bar{x}_1$, $\bar{x}_2$, and B. Note that if $\bar{x}_1$, $\bar{x}_2$, and B are not independent, the estimates of the mean percent recoveries and associated confidence limits will be over or under estimated.

6.2 If there is a background level of analyte present, compute upper and lower 100 (1-α)% confidence limits for the difference in percent recovery, PR_1 - PR_2, where α = the significance level (.01/N_a, .025/N_a, etc.), as follows:

$$LOWER\ \ LIMIT:$$

$$(PR_1 - PR_2) - 100\, z_{\frac{\alpha}{2}} \sqrt{\frac{s_1^2}{T_1^2 n_1} + \frac{s_2^2}{T_2^2 n_2} + \frac{s_3^2}{T_2^2 n_3}}$$

$$UPPER\ \ LIMIT:$$

$$(PR_1 - PR_2) + 100\, z_{\frac{\alpha}{2}} \sqrt{\frac{s_1^2}{T_1^2 n_1} + \frac{s_2^2}{T_2^2 n_2} + \frac{s_3^2}{T_2^2 n_3}}$$

where n_1 is the number of replicate samples in the first set of concentrations found, T_1 is the amount of analyte spiked into the first set of samples, s_1 is the standard deviation of the n_1 concentrations found in the first set of samples, n_2 is the number of replicate samples in the second set of concentrations found, T_2 is the amount of analyte spiked into the second set of samples, s_2 is the standard deviation of the n_2 concentrations found in the second set of samples, n_3 is the number of samples analyzed to determine the background level, s_3 is the standard deviation of the n_3 background concentrations found, and z_δ is the deviate from the standard normal distribution such that $P[z \geq z_\delta] = \delta$. This value can be found in a table of the standard normal distribution (see *subsection 1.2*).

6.3 If no background level of analyte is present in the second sample, compute the percent recovery for the second set of samples as follows:

$$PR_2 = \frac{\bar{x}_2}{T_2} \cdot 100$$

where $\bar{x}_2$ the average of the concentrations found in the second set of samples.

6.4 Compute the upper and lower 100 (1-α)% confidence limits for the difference in percent recovery, PR_1 - PR_2, where α = the significance level (.01/N_a, .025/N_a, etc.), as follows:

$$LOWER\ \ LIMIT:$$

$$(PR_1 - PR_2) - 100\, z_{\frac{\alpha}{2}} \sqrt{\frac{s_1^2}{T_1^2 n_1} + \frac{s_2^2}{T_2^2 n_2}}$$

$$UPPER\ \ LIMIT:$$

$$(PR_1 - PR_2) + 100\, z_{\frac{\alpha}{2}} \sqrt{\frac{s_1^2}{T_1^2 n_1} + \frac{s_2^2}{T_2^2 n_2}}$$

where n_1 is the number of replicate samples in the first set of concentrations found, T_1 is the amount of analyte spiked into the first set of samples, s_1 is the standard deviation of the n_1 concentrations found in the first set of samples, n_2 is the number of replicate samples in the second set of concentrations found, T_2 is the amount of analyte spiked into the second set of samples, s_2 is the standard deviation of the n_2 concentrations found in the second set of samples, and z_δ is the deviate from the standard normal distribution such that $P[z \geq z_\delta] = \delta$. This value can be found in a table of the standard normal curve or by using computer software capable of providing exact values (see *subsection 1.2*).

7. Calculating Confidence Intervals for the Ratio of Two Standard Deviations

7.1 This section describes the procedures for calculating 100 (1-α)% confidence intervals for the ratio of the population standard deviations (σ_1/σ_2) associated with two sets of concentrations, where α = the significance level ($.01/N_a$, $.025/N_a$, etc.).

7.2 The upper and lower 100 (1-α)% confidence limits for the ratio of two standard deviations, σ_1/σ_2, are computed as follows:

$$LOWER\ \ LIMIT:$$

$$\frac{s_1}{s_2}\sqrt{\frac{1}{F_{n_1-1,n_2-1,\frac{\alpha}{2}}}}$$

$$UPPER\ \ LIMIT:$$

$$\frac{s_1}{s_2}\sqrt{\frac{1}{F_{n_1-1,n_2-1,1-\frac{\alpha}{2}}}} = \frac{s_1}{s_2}\sqrt{F_{n_2-1,n_1-1,\frac{\alpha}{2}}}$$

where n_1 is the number of replicate samples in the first set of concentrations found, s_1 is the standard deviation of the n_1 concentrations found in the first set of samples, n_2 is the number of replicate samples in the second set of concentrations found, s_2 is the standard deviation of the n_2 concentrations found in the second set of samples, and $F_{n^1-1,n^2-1,\delta}$ is the deviate from the F distribution with n_1-1 numerator degrees of freedom and n_2-1 denominator degrees of freedom, such that:

$$P(F_{n_1-1,n_2-1} \geq F_{n_1-1,n_2-1,\delta}) = \delta$$

This value can be found in a table of the F distribution (see *subsection 1.4*).

8. Statistical References

Snedecor, George W. and Cochran, William G. 1980. Statistical Methods, Seventh Edition. The Iowa State University Press, pp 279-280.

Johnson, N. L. and Welch, B. L. 1940. Applications of the Non-Central *t*-Distribution. Biometrika, **31**, 362.

Moser. Barry C. and Stevens, Gary R. 1992. Homogeneity of Variance in the Two-Sample Means Test. The American Statistician, **46**, 19.

Appendix E: Statistical Example

This appendix contains examples of statistical calculations and analyses described in *Appendix D: Statistics for Determining Outliers, Significance, and Confidence Intervals*.

1. Example of Testing for Outliers in a Single Set of Data

1.1 This section contains an example of using the maximum normed residual outlier test (Snedecor and Cochran, 1980) to detect outliers in a set of data. If the sample size, *n*, is 4 or less, it is not possible to perform the outlier test. Statistical data adapted from a data set produced by Technology Applications Inc. for Environmental Monitoring Systems Laboratory, USEPA, Cincinnati, Ohio using cadmium as the analyte is found in *Table E-1: Data for Outliers Test.* The values of the cadmium are in µg/l.

Table E-1: Data for Outliers Test

n	x_i
1	1.91
2	2.02
3	2.30
4	2.20
5	2.11
6	3.15
7	1.81
8	2.14

1.2 Compute the average of the sample observation.

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

where x_i is the i^{th} observation and n is the number of data points in the data set.

For the data in this example, $n = 8$ and $\Sigma x_i = 17.64$. The calculated mean is:

$$\bar{x} = \frac{17.64}{8} = 2.205$$

1.3 Compute the absolute deviation from the mean for each data point x_i.

$$d_i = | x_i - \bar{x} |$$

The calculated deviations for this example are listed in *Table E-2: Absolute Deviations from the Mean*.

1.4 Compute the Maximum Normed Residual statistic (MNR).

$$MNR = \frac{\max \{d_i\}}{\sqrt{\sum_{i=1}^{n} d_i^2}}$$

For the data in this example,

$$MNR = \frac{0.945}{\sqrt{1.1926}} = 0.865$$

Table E-2: Absolute Deviations from the Mean

n	x_i	d_i	d_i^2
1	1.91	0.295	0.087025
2	2.02	0.185	0.034225
3	2.30	0.095	0.009025
4	2.20	0.005	0.000025
5	2.11	0.095	0.009025
6	3.15	0.945	0.893025
7	1.81	0.395	0.156025
8	2.14	0.065	0.004225

1.5 The decision rule for this test is to compare the MNR as computed in *subsection 1.4* to the critical value corresponding to the sample size, *n*, found in *Table D-1: Critical Values for the MNR Outlier Test at the 1% Level of Significance* in *Appendix D: Statistics for Determining Outliers, Significance, and Confidence Intervals*. If the computed MNR is greater than the critical value, conclude that the data point associated with the maximum d_i value is an outlier and should be eliminated from the data set. Because MNR = 0.865 is greater than the critical value, 0.860, eliminate the data point 3.15.

1.6 If more than four data points remain in the dataset, return to *subsection 1.2* and perform the calculations again using the reduced data set. The sample size, *n*, is reduced by one.

1.7 The data for this calculation of MNR is outlined in *Table E-3: Reduced Data Set*. For the data in this example, $n = 7$, $\Sigma x_i = 14.49$, and $\bar{x} = 2.07$. The calculated MNR is:

$$MNR = \frac{\max d_i}{\sum d_i^2}$$

$$MNR = \frac{0.26}{\sqrt{0.172}} = 0.627$$

Table E-3: Reduced Data Set

n	x_i	d_i	d_i^2
1	1.91	0.16	0.0256
2	2.02	0.05	0.0025
3	2.30	0.23	0.0529
4	2.20	0.13	0.0169
5	2.11	0.04	0.0016
6	1.81	0.26	0.0676
7	2.14	0.07	0.0049

1.8 Compare the MNR statistic of the reduced data set to the critical value corresponding to the reduced sample size, *n*, found in *Table D-1: Critical Values for the MNR Outlier Test at the 1% Level of Significance* in *Appendix D: Statistics for Determining Outliers, Significance, and Confidence Intervals*. The new MNR statistic, 0.627, is less than the critical value, 0.873. There are no remaining outliers in the data set. End the outlier test and use the reduced data set for all future calculations.

2. Example of Calculating the Percent Relative Standard Deviation (RSD) and Approximate Confidence Intervals

2.1 This section contains an example of calculating approximate confidence intervals for the RSD. The distribution of the sampled population is assumed to be normal. The reduced data set found in *Table E-3: Reduced Data Set* is used to illustrate the calculations.

2.2 Compute the average of the sample observations for each analyte-concentration combination:

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

For the data in this example, $n = 7$, $\Sigma x_i = 14.49$. The computed mean, $\bar{x}$, is 2.07.

2.3 Compute the standard deviation of the sample observation for each analyte-concentration combination:

$$s = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n - 1}}$$

For the data in this example:

$$s = \sqrt{\frac{0.172}{7-1}} = 0.169$$

2.4 Compute the RSD for each analyte-concentration combination:

$$RSD = \frac{s}{\bar{x}} \times 100$$

For the data in this example:

$$RSD = \frac{0.169}{2.070} \times 100 = 8.2$$

2.5 Compute the RSD′ statistic that denotes the RSD not as a percentage.

$$RSD' = \frac{s}{\bar{x}} = \frac{0.169}{2.070} = 0.082$$

2.6 Compute the 100 (1-α)% confidence interval for the RSD of each analyte-concentration combination, using the RSD′ statistic, as follows:

LOWER LIMIT:

$$\frac{\sqrt{n}}{\frac{\sqrt{n}}{RSD'} - Z_{1-\frac{\alpha}{2}}\sqrt{1 + \frac{n}{2(n-1)(RSD')^2}}}$$

UPPER LIMIT:

$$\frac{\sqrt{n}}{\frac{\sqrt{n}}{RSD'} - Z_{\frac{\alpha}{2}}\sqrt{1 + \frac{n}{2(n-1)(RSD')^2}}}$$

Where α is the the significance level (.01/N_a, .025/N_a, etc), n is the number of sample observations in the analyte-concentration combination, and z_δ is the standard normal deviate such that P[$Z \geq z_\delta$] = δ. This value can be found in a table of the Standard Normal distribution (see *subsection 1.2* in *Appendix D: Statistics for Determining Outliers, Significance, and Confidence Intervals*).

2.7 For the data in this example, at $\alpha = .05$, the confidence limits are:

$$\textit{LOWER LIMIT}: \quad \frac{\sqrt{7}}{\frac{\sqrt{7}}{0.082} - z_{1-.\frac{05}{2}}\sqrt{1 + \frac{7}{(2)(6)(0.082)^2}}} = 0.052$$

$$\textit{UPPER LIMIT}: \quad \frac{\sqrt{7}}{\frac{\sqrt{7}}{0.082} - z_{\frac{.05}{2}}\sqrt{1 + \frac{7}{(2)(6)(0.082)^2}}} = 0.190$$

The 95% confidence interval for the percent RSD is (0.052, 0.190).

3. Example of Calculating Confidence Intervals for the Mean Percent Recovery of an Analytical Method

3.1 This section contains an example of the calculation of confidence intervals for a mean percent recovery of an analytical method with a background level present. $\bar{B}$ and $\bar{x}$ are assumed to be independent. The data for this example is found in *Table E-4: Data with a Background Level Present.*

Table E-4: Data with a Background Level Present

n	x_i	Background Level
1	1.91	0.00
2	2.02	0.03
3	2.30	0.19
4	2.20	0.15
5	2.11	0.07
6	1.81	0.01
7	2.14	0.09

3.2 Compute the percent recovery as follows:

$$PR = \frac{\overline{x} - \overline{B}}{T} \cdot 100$$

where $\overline{x}$ is the average of the concentration measurements, $\overline{B}$ is the average of the background concentration measurements, and T is the amount of analyte spiked into the replicate samples.

3.3 For the data in this example:

$$PR = \frac{2.070 - 0.077}{2} \cdot 100 = 99.650$$

3.4 Compute the upper and lower 100 (1-α)% confidence limits for the percent recovery, as follows:

LOWER LIMIT:

$$PR - \frac{100}{T} t_{v,\frac{\alpha}{2}} \sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$$

UPPER LIMIT:

$$PR + \frac{100}{T} t_{v,\frac{\alpha}{2}} \sqrt{\frac{s_1^2}{n_1} + \frac{s_1^2}{n_2}}$$

where α is the significance level (.01N_a, .025N_a, etc), n_1 is the number of replicate samples at the concentration being considered, s_1 is the standard deviation of the n_1 measurements found, n_2 is the number of replicate samples analyzed to determine the background level, s_2 is the standard deviation of the n_2 background measurements found, and $t_{v,\delta}$ is the deviate from the Student's t distribution with v degrees of freedom such that $P[t_v \geq t_{v,\delta}] = \delta$. This value can be found in a table of the

Student's *t* distribution (see *subsection 1.3* in *Appendix D: Statistics for Determining Outliers, Significance, and Confidence Intervals*).

The following equations are used to compute the degrees of freedom, *v*:

$$v = \frac{(Q_1 + Q_2)^2}{\frac{Q_1^2}{n_1 - 1} + \frac{Q_2^2}{n_2 - 1}}$$

$$\text{where} \quad Q_1 = \frac{s_1^2}{n_1} \quad \text{and} \quad Q_2 = \frac{s_2^2}{n_2}$$

3.5 For the data in this example, the degrees of freedom are:

$$Q_1 = \frac{(0.169)^2}{7} = 4.08 \cdot 10^{-3} \qquad Q_2 = \frac{(0.073)^2}{7} = 7.613 \cdot 10^{-4}$$

$$v = \frac{[(4.080 \cdot 10^{-3}) + (7.613 \cdot 10^{-4})]^2}{\frac{1.664 \cdot 10^{-5}}{7-1} + \frac{5.796 \cdot 10^{-7}}{7-1}} = 8.167$$

Rounding *v* down to the nearest integer, gives $v = 8$. The confidence limits are:

LOWER LIMIT:

$$99.650 - \frac{100}{2} t_{8,\frac{.05}{2}} \sqrt{\frac{(0.169)^2}{7} + \frac{(0.073)^2}{7}}$$

$$= 91.627$$

UPPER LIMIT:

$$99.650 + \frac{100}{2} t_{8,\frac{.05}{2}} \sqrt{\frac{(0.169)^2}{7} + \frac{(0.073)^2}{7}}$$

$$= 107.672$$

The 95% confidence interval for the mean percent recovery is (91.27, 107.672).

4. Example of Calculating Confidence Intervals for the Method Detection Limit of Analytes

4.1 This section contains an example of the calculation of confidence intervals for an MDL for an analyte of a given analytical method. The data for this example is found in *Table E-3: Reduced Data Set*.

4.2 Compute the MDL as follows:

$$MDL = t_{n-1,0.01}(s)$$

where n is the number of replicate samples at the spike level that defines the lower limit of the method range, s is the standard deviation of the n measurements found, and $t_{n-1,\delta}$ is the deviate from the Student's t distribution with $n-1$ degrees of freedom such that $P[t_{n-1} \geq t_{n-1,\delta}] = \delta$. This value can be found in a table of the Student's t distribution (see *subsection 1.3* in *Appendix D: Statistics for Determining Outliers, Significance, and Confidence Intervals*).

4.3 For the data in this example:

$$MDL = t_{7-1,0.01}(0.169)$$

$$= (3.143)(0.169)$$

$$= 0.531$$

4.4 Compute upper and lower 100 $(1-\alpha)$% confidence limits for the MDL, where α = the significance level as follows:

$$LOWER\ LIMIT: \quad MDL \cdot \sqrt{\frac{n-1}{X^2_{n-1,\frac{\alpha}{2}}}}$$

$$UPPER\ LIMIT: \quad MDL \cdot \sqrt{\frac{n-1}{X^2_{n-1,1-\frac{\alpha}{2}}}}$$

where n is the number of replicate samples at the spike level that defines the lower limit of the method range, s is the standard deviation of the n measurements found, $t_{n-1,\delta}$ is the deviate from the Student's t distribution with $n-1$ degrees of freedom such that $P[t_{n-1} \geq t_{n-1,\delta}] = \delta$, and $\chi^2_{n-1,\delta}$ is the deviate from the Student's t distribution such that $P[\chi^2_{n-1} \geq \chi^2_{n-1,\delta}] = \delta$.

The tabular values can be found in tables of the Student's *t* distribution and the χ^2 distribution (see *subsections 1.3 and 1.5* in *Appendix D: Statistics for Determining Outliers, Significance, and Confidence Intervals*).

4.5 For the data in this example:

$$LOWER\ LIMIT: 0.531 \cdot \sqrt{\frac{7-1}{X^2_{7-1,.\frac{05}{2}}}} = 0.342$$

$$UPPER\ LIMIT: 0.531 \cdot \sqrt{\frac{7-1}{X^2_{7-1,1-\frac{.05}{2}}}} = 1.168$$

The 95% confidence interval for this example is (0.342, 1.168).

5. Example of Calculating Confidence Intervals for the Difference in Two Percent Recovery Means

5.1 This section contains an example of calculating confidence intervals for the difference in two percent recovery means including an adjustment for a background level of the analyte present. $\bar{B}$, $\bar{x}_1$, and $\bar{x}_2$ are assumed to be independent. The statistical data are found in *Table E-5: Data with a Background Level Present*. The spike level for the first concentration is 2 and the spike level for the second concentration is 3.

Table E-5: Data with a Background Level Present

Concentration	n	x_i	Background Level
1	1	1.91	0.00
	2	2.02	0.03
	3	2.30	0.19
	4	2.20	0.15
	5	2.11	0.07
	6	1.81	0.01
	7	2.14	0.09
2	1	2.98	0.02
	2	3.04	0.01
	3	3.12	0.13
	4	3.07	0.10
	5	2.94	0.01
	6	2.97	0.03

5.2 Compute the upper and lower 100 (1-α)% confidence limits for the difference in percent recovery PR_1 - PR_2 as follows:

LOWER LIMIT:

$$(PR_1 - PR_2) - 100\, z_{\frac{\alpha}{2}} \sqrt{\frac{s_1^2}{T_1^2 n_1} + \frac{s_2^2}{T_2^2 n_2} + \frac{s_3^2}{T_2^2 n_3}}$$

UPPER LIMIT:

$$(PR_1 - PR_2) + 100\, z_{\frac{\alpha}{2}} \sqrt{\frac{s_1^2}{T_1^2 n_1} + \frac{s_2^2}{T_2^2 n_2} + \frac{s_3^2}{T_2^2 n_3}}$$

Where α is the significance level (.01/N_a, .025/N_a, etc.), n_1 is the number of replicate samples in the first set of measurements found, T_1 is the amount of analyte spiked into the first set of samples, s_1 is the standard deviation of the n_1 measurements found in the first set of samples, n_2 is the number of replicate samples in the second set of measurements found, T_2 is the amount of analyte spiked into

the second set of samples, s_2 is the standard deviation of the n_2 measurements found in the second set of samples, n_3 is the number of replicate samples use to determine the background level, s_3 is the standard deviation of the n_3 background measurements found, and z_δ is the deviate from the standard normal deviation such that $P[z \geq z_\delta] = \delta$. This value can be found in a table of the standard normal distribution (see *subsection 1.2* in *Appendix D: Statistics for Determining Outliers, Significance, and Confidence Intervals*.

5.3 For the data in this example:

LOWER LIMIT:

$$(99.650 - 99.000) - 100\, z_{\frac{.05}{2}} \sqrt{\frac{0.169^2}{2^2 7} + \frac{0.068^2}{3^2 6} + \frac{0.066^2}{3^2 13}}$$

$$= -5.976$$

UPPER LIMIT:

$$(99.650 - 99.000) + 100\, z_{\frac{.05}{2}} \sqrt{\frac{0.169^2}{2^2 7} + \frac{0.068^2}{3^2 6} + \frac{.066^2}{3^2 13}}$$

$$= 6.626$$

The 95% confidence interval for the difference in the two percent recoveries is (5.976, 6.626).

6. Example of Calculating Confidence Intervals for the Ratio of Two Standard Deviations

6.1 This section includes an example of calculating 100 (1-α)% confidence intervals for the ratio of the population standard deviations (σ_1/σ_2) associated with two sets of measurements. The data for this example is found in *Table E-5: Data with a Background Level Present.*

6.2 Compute the upper and lower 100 (1-α)% confidence limits for the ratio of the standard deviations (σ_1/σ_2):

LOWER LIMIT:

$$\frac{s_1}{s_2} \sqrt{\frac{1}{F_{n_1-1, n_2-1, \frac{\alpha}{2}}}}$$

UPPER LIMIT:

$$\frac{s_1}{s_2} \sqrt{\frac{1}{F_{n_1-1, n_2-1, 1-\frac{\alpha}{2}}}} = \frac{s_1}{s_2} \sqrt{F_{n_2-1, n_1-1, \frac{\alpha}{2}}}$$

where α is the significance level ($.01/N_a$, $.025/N_a$, etc.), n_1 is the number of replicate samples in the first set of measurements found, s_1 is the standard deviation of the n_1 measurements found in the first set of samples, n_2 is the number of replicate samples in the second set of measurements found, s_2 is the standard deviation of the n_2 measurements found in the second set of samples, and $F_{n^1-1,n^2-1,\delta}$ is the δ deviate from the F distribution with n_1*-1* numerator degrees of freedom and n_2*-1* denominator degrees of freedom, such that:

$$P\ (F_{n_1-1,n_2-1} \geq F_{n_1-1,n_2-1,\delta}) = \delta$$

This value can be found in a table of the *F* distribution (see *subsection 1.4* in *Appendix D: Statistics for Determining Outliers, Significance, and Confidence Intervals*).

6.3 For the data in this example:

$$\textit{LOWER LIMIT}: \quad \frac{0.169}{0.068}\sqrt{\frac{1}{F_{7-1,6-1,\frac{.05}{2}}}}$$

$$= 0.941$$

$$\textit{UPPER LIMIT}: \quad \frac{0.169}{0.068}\sqrt{F_{6-1,7-1,\frac{.05}{2}}}$$

$$= 5.716$$

The 95% confidence interval for the ratio of two standard deviations is (0.941, 5.716).

Appendix F: EMAP Demonstration of Method Comparability Checklist for Chemical Methods

Date: **Page __ of __**
Facility Name:
Resource Group/Program:
Matrix Type:
Analyte or Class of Analytes:

Conditions	Approved EMAP Method Performance Specification	Summary of Results
1. Written candidate method (EMMC format) attached?	---	Y/N
2. Title and date of approved EMAP method	---	Y/N
3. Copy of approved EMAP method maintained on-site?	---	Y/N
4. Listing of differences between candidate method and approved EMAP method attached?	---	Y/N
5. Performance range tested (w/units)		
6. Linear working range (w/units)		
7. Concentrations of calibration standards w/units corresponding to final sample concentration (lowest standard must be one to four times MDL)		
8. Calibration curve attached?	---	Y/N
9. Slope of calibration regression line (if applicable)		
10. Relative Standard Deviation (RSD) of calibration factors (if applicable)		
11. Sample preservatives and holding times		
12. Interferences		
13. Qualitative identification criteria used		
14. Surrogates used (if applicable)		
15. Surrogate recovery limits established		

EMAP Demonstration of Method Comparability Checklist for Chemical Methods

Date: **Page __ of __**
Facility Name:
Resource Group/Program:
Matrix Type:
Analyte or Class of Analytes:

Analytical Results*

16. Testing Conditions		
Matrix used (reagent water, drinking water, etc.)		
Spike levels (w/units corresponding to final sample concentration)		
Source of spiking material (using external source whenever possible)		
Number of replicates (minimum n=5)		
17. Performance Characteristics	**Approved EMAP Method Performance Specification**	**Candidate Method Results**
Precision (RSD [n-1] of replicate measurements - for multiple analyte methods, give range of results and attach full listing)		
Percent recovery - for multiple analyte methods, give range of results and attach full listing)		
Method Detection Limit (MDL) (w/units and number of replicates - for multiple analyte methods, give range of results and attach full listing)		

* Complete one page for each matrix tested.

EMAP Demonstration of Method Comparability for Chemical Methods Certification Statement*

Date: **Page __ of __**
Facility Name:
Resource Group/Program:
Matrix Type:
Analyte or Class of Analytes:

We, the undersigned, CERTIFY that:

1. The candidate method proposed for EMAP use has undergone the EMAP Demonstration of Method Comparability, as specified under the EMAP PBMS, and has met the performance specifications of the approved EMAP method.

2. A copy of the candidate method, written in the EMMC format, and copies of the approved EMAP method and laboratory-specific Standard Operating Procedures (SOPs) are attached to this checklist and are available on-site.

3. The data associated with the EMAP Demonstration of Method Comparability are complete (including the mandatory copy of this checklist), all raw data necessary to reconstruct and verify the analyses have been retained, and the associated information is available for review.

Name and Title	Signature	Date
Name and Title	Signature	Date
Name and Title	Signature	Date
Name and Title	Signature	Date

*This certification form must be completed when the EMAP Demonstration of Method Comparability is performed to approve a candidate method for EMAP use.