**Alternative and Modified Analytical Laboratory Methods**

#### DEP-QA-001/01



**FLORIDA DEPARTMENT OF ENVIRONMENTAL PROTECTION**

**2600 BLAIR STONE ROAD**

**TALLAHASSEE, FLORIDA 32399-2400**

**Aquatic Ecology and Quality Assurance Section**

Table of Contents

[1.0. Introduction 2](#_Toc156463151)

[1.1. Limited-Use Method 2](#_Toc156463152)

[1.2. Statewide-Use Method 2](#_Toc156463153)

[2.0. Demonstration of Capability (DOC) 2](#_Toc156463154)

[3.0. Determination of Method Detection Limit and Practical Quantitation Limit 3](#_Toc156463155)

[3.1. Method Detection Limit (MDL) determination in clean matrix 3](#_Toc156463156)

[3.2. MDL, Precision and Accuracy determinations in the applicable environmental matrix …………………………………………………………………………………………………..3](#_Toc156463157)

[3.3. Determination of Practical Quantitation Limit (PQL) 4](#_Toc156463158)

[4.0. Equivalency Study (if required) 4](#_Toc156463159)

[4.1. Overlap of Confidence Intervals 4](#_Toc156463160)

[4.2. Pair-wise Comparisons of Co-Collected Samples 5](#_Toc156463161)

[5.0. Inter-laboratory Collaborative Study for Statewide-Use Methods 6](#_Toc156463162)

[5.1. Collaborative studies using consensus-based standards 6](#_Toc156463163)

[5.2. Conducting the inter-laboratory study 7](#_Toc156463164)

[6.0. Approval of Alternative and Modified Methods 7](#_Toc156463165)

[7.0. Required Documentation for Method Validations 7](#_Toc156463166)

[7.1. Contents of the validation documentation 7](#_Toc156463167)

[7.2. Documentation for Inter-Laboratory Collaborative Studies Conducted for Statewide-Use Methods 10](#_Toc156463168)

[Appendix A: Glossary 11](#_Toc156463169)

[Appendix B: Calculations and Applicable Formulae 13](#_Toc156463170)

[B-1. Formulas for Calculating Precision and Accuracy 13](#_Toc156463171)

[B-2. Method Detection Limits and Practical Quantitation Limits 13](#_Toc156463172)

[Appendix C: References 16](#_Toc156463173)

# Introduction

The requirements for approval of alternative and modified laboratory methods for use in Department-related work activities are described in Rule 62-160.330, F.A.C. Alternative and modified methods must be demonstrated as appropriate for use to meet the specific data quality objectives of the Department program activity or project for which the methods are being used. The applicable requirements in this document shall be met for all laboratory method validations required by the Department.

This document discusses requirements for method validation documentation, which may include a demonstration of capability, determination of method detection limits and practical quantitation limits, demonstration of method equivalency and inter-laboratory, collaborative method validation studies. Submitted validation information must be sufficient to demonstrate the efficacy of the proposed method for the scope of approval requested. The requirements in this document do not apply to laboratory methods used for research that may be contracted or required by the Department. The submitted method validation documentation will be evaluated based on its intended use, and if approved, designated for one of the two following categories of use:

* 1. Limited-Use Method - a laboratory procedure that is validated for testing environmental samples by a single laboratory for purposes specified in the scope of the approval (e.g., for specified analytes, facility or site locations or types, sample matrices such as effluent, groundwater, drinking water, fresh or marine surface waters, soils, sediments or chemical wastes). A limited-use method is validated by and approved by the Department for the analytical laboratory requesting approval, and may not be used by another analytical laboratory.
	2. Statewide-Use Method – an alternative or modified laboratory procedure that is validated for testing environmental samples and approved for unlimited use by all persons and organizations, based on the information and data provided in the method validation documentation.

# Demonstration of Capability (DOC)

Validation information for alternative and modified methods must demonstrate laboratory capability to perform the proposed method in a representative, clean matrix. When laboratory certification is required according to Rule 62-160.300, F.A.C., applicable requirements for method validation shall be met, and, applicable initial and ongoing demonstrations of capability shall be performed as required in the appropriate testing module of the TNI Standard (EL-V1-ISO-2016-Rev 2.1), which is incorporated by reference in paragraph 62-160.800(3)(b), F.A.C., and, if applicable, as required in the published method. In addition, the requirements for the use of non-standard methods, including validation of methods, as discussed in Module 2 of the 2016 TNI Standard shall be met for the use of any alternative or modified methods requiring laboratory certification. The following describes DOC requirements for a typical chemistry method.

* 1. Examples of clean matrix include analyte-free water, sand, soil or other materials that do not contain detectable method analytes or method interferences.
	2. The DOC requires a determination of precision, accuracy and method detection limit (MDL).
		1. See section 3.1 below for the determination of MDL.
		2. See section 3.3 below for the determination of the practical quantitation limit (PQL).
		3. The DOC and MDL study may be combined for efficiency by analyzing a sufficient number of replicate aliquots prepared at 1-to-4 times the determined PQL for the proposed method.

# Determination of Method Detection Limit and Practical Quantitation Limit

Determine the MDL and PQL for the proposed alternative or modified method according to the following applicable requirements.

* 1. Method Detection Limit (MDL) determination in clean matrix

If a published method or Department rule does not specify a protocol for determining the MDL, one of the procedures specified in Appendix B-2 belowmay be usedto calculate the MDL.

* + 1. Use a clean matrix as described in section 2.1 above as the sample matrix (e.g., analyte-free water with no detectable analytes of interest and no analytical interference).
		2. Use all target analytes and any proposed surrogates (if applicable) for the fortification (spike) compounds.
		3. Calculate the MDL using the statistics and formulae of the required or selected MDL method.
	1. MDL, precision and accuracy determinations in the applicable environmental matrix

If the Department has determined that the DOC and MDL should be calculated using a representative sample of environmental matrix (field sample) in order to evaluate the effect of potential interferences, validation information must be provided according to the following requirements.

* + 1. Use the appropriate matrix applicable to the proposed method (e.g., drinking water, surface water, wastewater, groundwater, saline water, soil, sediment). The matrix selected must be free of target compounds (i.e., analytes below the MDL of method) but representative of the sample types likely to be analyzed.
		2. Fortify spiked samples with all target analytes and proposed surrogates and/or internal standards (if applicable).
		3. Conduct the study using one of the procedures specified in Appendix B-2 or the specific protocol for determining the MDL as required by a published method or Department rule..
		4. Refer to Appendix B-1 for appropriate formulae for calculating Accuracy (as % Recovery) and Precision (e.g. RPD or % RSD).
		5. Use the statistics and formulae of the required or selected MDL procedure to determine the MDL.
	1. Determination of Practical Quantitation Limit (PQL)

The PQL for the proposed method must be determined by a defined procedure and/or criteria. For example, the PQL may be chosen to be the lowest calibration standard used for a calibration curve. Alternatively, the PQL may be indicated as the concentration at which the method has been demonstrated to achieve a specified range of precision and accuracy. The procedure and/or criteria used to define the PQL must be included in the laboratory standard operating procedure developed for the method, or in the laboratory quality manual.

* + 1. When laboratory certification is required according to Rule 62-160.300, F.A.C., the laboratory practical quantitation limit (PQL) shall also be determined, verified and documented according to all certification requirements.
		2. When laboratory certification is not required, the PQL may be determined and verified by any technically justifiable and scientifically sound method appropriate for the test.
		3. Regardless of how determined, the Department requires documentation of the PQL determination for the proposed method.

# Equivalency Study (if required)

If required by the Department for a specific program activity or project, a proposed alternative or modified method must be shown to be equivalent to the replaced method or original, un-modified method at the 95% confidence level. The following types of equivalency studies are examples of protocols that will be considered by the Department. Depending on the scope of approval requested and the best available information about matrix interferences or other method performance concerns that may affect the analyses of specific sample types, the Department will specify which type of study to use for an equivalency demonstration, as further discussed in the sections below. NOTE: The same analyst or workgroup may conduct the analyses for each method, or additional analysts or workgroups may participate in the study, but the analyst and workgroup combinations should be chosen to reflect the routine operations of the laboratory when using the proposed method.

* 1. Overlap of Confidence Intervals

Using the relevant field sample matrix shown to be free of the analytes of concern (groundwater, surface water, soil, etc.), prepare a minimum of seven (7) replicates for both the proposed method and the replaced or unmodified method. The spiking level must be at the known or estimated PQL of the replaced or unmodified method. NOTE: The replicates may be prepared in a clean matrix if the Department has determined that the intended use of the method will be limited to the analyses of samples with little or no potential for matrix interferences.

* + 1. The number of replicates is dictated by the determination of the MDL (for example, n=7 replicates, if Appendix B of 40 CFR 136 is followed). A combined, DOC/MDL study may be used for the determination of confidence interval overlap (see section 2.2.3, above).
		2. Statistical Evaluation for Overlap of Confidence Intervals:

The following formulas use information derived from the EPA procedure for determining the MDL using seven replicate samples. Other procedures for the MDL determination may be used, and the number of replicates can be increased. The appropriate statistics for the degrees of freedom must be used. A combined DOC/MDL study (section 2.2.3) may also be used to develop confidence intervals.

* + - 1. The 95% confidence interval estimate of the MDL is derived from percentiles of the chi square over degrees of freedom distribution (see Appendix B of 40 CFR 136). In the case of 7 replicates, the lower and upper control limits (LCL and UCL) of this skewed interval are calculated as (in this example, the PQL is defined as a multiple of the MDL):

**Practical Quantitation Limit LCL = 0.64 x PQL UCL = 2.20 x PQL**

**Method Detection Limit LCL = 0.64 x MDL UCL = 2.20 x MDL**

* + - 1. The 95% confidence interval of the accuracy (as %R) is directly proportional to the 95% confidence interval of the mean value X, which is calculated using the Students'-t distribution factors. In this case, the interval for <%R> is symmetric and can be calculated as:

**Accuracy LCL = [%R] \* [1 - 0.0093 x (%RSD)]**

**UCL = [%R] \* [1 + 0.0093 x (%RSD)]**

* + 1. Equivalency is shown if the MDL, PQL, precision and accuracy of the proposed alternative or modified method are comparable (i.e., statistically equal at the 95% confidence level, CL) to, or better than, the same quality control indicators in the replaced or unmodified method.
			1. The corresponding confidence intervals for MDL and PQL for the alternative or modified method must be either lower than or overlap with the corresponding confidence intervals of the replaced or unmodified method.
			2. The accuracy confidence interval [%R] of the alternative or modified method must overlap the corresponding confidence interval of the replaced or unmodified method.
	1. Pair-wise Comparisons of Co-Collected Samples

The Department may determine that increased confidence in the equivalency study is necessitated after a review of the proposed method and all available information about method performance, including sample matrix effects that may impact the analysis. In this case, the Department may specify that replicate samples must be co-collected from appropriate field sample sources and analyzed by both the proposed method and the replaced or unmodified method in order to conduct a pair-wise evaluation of the compared methods for statistical differences in results. The sampling procedure and the location of field sample sources must be determined by the Department in collaboration with the requester before conducting the study.

* + 1. The statistical certainty of the comparison increases with the number of paired samples. A greater number of samples is needed if the results are potentially highly variable due, for example, to the matrix or other environmental conditions. Typically, for optimal statistical robustness, a minimum of 30 samples or measurements should be taken over a variety of conditions for which the alternative or modified procedure will be used, according to the scope of approval requested.
		2. If 30 samples are logistically difficult or considered to be too numerous, the number may be modified with the prior consent of the Department after a review of any relevant information provided.
		3. The analytical results derived from the co-collected replicates (paired samples) must be analyzed for statistical difference at the 95% confidence level.
		4. The following tests are examples of conventional pair-wise comparisons that may be used to evaluate results. Other statistical tests may be proposed by the requester, but must be approved by the Department before submitting the validation information.
			- Student’s t-test
			- Bland and Altman's Limits of Agreement and Tolerance Interval
			- Wilcoxon Signed Rank Test
			- Fisher Sign Test
			- Empirical Tolerance Intervals
		5. Equivalency between compared methods is demonstrated when there is no statistical difference between the methods after evaluation of paired samples results according to section 4..2.3 above. However, the Department may conclude that, in the case where a statistical difference is shown, the practical significance of the difference is negligible for the intended data use (e.g., differences in concentration results between the two methods are inconsequential with respect to a critical value, such as a water quality criterion).

# Inter-laboratory Collaborative Study for Statewide-Use Methods

If an alternative or modified method is proposed for statewide use according to Rule 62-160.330, F.A.C., the Department requires validation with a collaborative study conducted by multiple independent laboratories in order to investigate the efficacy and robustness of the proposed method for specified site or environmental conditions, sample types, sample matrices, waste streams, analytes, or other specifications applicable to the scope of approval requested (see, for example, the Association of Official Analytical Collaboration (AOAC) International standard in section 5.1.1 below, where at least five and up to eight laboratories must be enlisted for the study). Prior to conducting the inter-laboratory collaborative study, contact the Department to ensure that the specific details of the study design will be acceptable for all relevant data quality objectives.

* 1. Collaborative studies using consensus-based standards

The Department accepts the design of collaborative studies using consensus-based standards according to the following:

* + 1. Conduct an inter-laboratory collaborative study following the specifications established by AOAC International (Reference 1). This standard is incorporated by reference in Rule 62-160.800, F.A.C.
		2. Alternatively, conduct an inter-laboratory collaborative study that is developed and validated based on procedures published by any nationally recognized consensus-based standards organization such as ASTM International.
	1. Conducting the inter-laboratory study

Perform the inter-laboratory study according to all instructions and recommendations in the applicable consensus-based standard, and as follows:

* + 1. Use a clean sample matrix, as described in section 2.1, above, which is representative of the type(s) of matrix(ices) for which the method is being proposed (e.g., analyte-free water). The sample matrix must be free of target compounds.
		2. Follow the AOAC or other consensus standard recommendations for the minimum number of participating laboratories, minimum number of samples (concentrations) and minimum number of replicates of each sample. The selected range of concentration for study samples should bracket relevant critical values (section 5.2.3, below) and should otherwise be chosen to meet Department data quality objectives.
		3. Appropriate statistical tests must be used in the analysis of the reported analytical data. Normal or parametric statistics are generally sufficient to test for the significance of the data and for carrying out the analysis of variances. Follow the AOAC or other consensus standard recommendations for the use of statistical evaluations of the data. The reproducibility of the data at each concentration level, determined with replicates (generally two replicates, as recommended by AOAC) must be less than the pre-established critical value for the method to be validated, such as a regulatory limit or minimum concentration target for quantitation.

# Approval of Alternative and Modified Methods

An alternative or modified method shall be considered appropriate for use if the Department determines that the technical justification and submitted validation information establish that the alternative or modified method provides accuracy, precision, reliability and method detection limit(s) equivalent to, or better than, those of the replaced or unmodified method, when required by the Department in order to meet specified data quality objectives. In addition, when required by the Department according to the criteria and requirements described in section 4, above, an alternative or modified method must be shown to be equivalent to the replaced or unmodified method at the 95% confidence level. The Department will evaluate submitted validation information for potential approval based on all applicable requirements described in this document according to the scope of approval requested.

# Required Documentation for Method Validations

Submit the following validation documentation to the Department for review. Submissions for statewide-use validations must include all relevant documentation discussed in the AOAC or other consensus-based standard (see section 5, above).

* 1. Contents of the validation documentation

Provide the name(s), mailing address, email address and telephone number of individual(s) preparing the documentation, and the organization name for which the validation information is being submitted for approval.

* + 1. Include a statement specifying the reason for requesting approval of the alternative or modified method for the specified analytes. Clearly specify the justification(s) for proposing the alternative method instead of the replaced method, or the reason(s) for using the proposed modified method. Clearly specify all parts of the unmodified method that have been changed, and the explanations for using the proposed modifications. Describe the specific application for which the procedure is proposed, according to criteria such as type of field site, environmental conditions, sample types, facility location, specified permit(s), sample matrix (e.g., effluent, surface water, groundwater, drinking water, or soil), or type of waste stream, and the specific uses of data that will be applicable to use of the procedure. This statement must also identify the specific Department project(s) or program activity(ies) for which the proposed method will be used.
		2. A cover letter (or email) containing the above information may be submitted, and must accompany the laboratory SOP for the proposed method, along with the complete set of validation data and any other information necessary for the Department’s complete review.
		3. A complete description of the proposed alternative or modified method must be submitted to the Department for review, written in the format of a laboratory SOP that includes stepwise instructions, discussions about interferences, and all calibration and quality control performance criteria necessary for acceptable qualitative and quantitative analyses. If a published method is proposed as an alternative method without modification, and will be performed by the laboratory exactly as published, the laboratory SOP is not required. A copy or webpage link to the published method must be provided to the Department. Please note that the Department will not be responsible for purchasing copies of methods that are copyrighted materials.
		4. The laboratory SOP must include the following:
			- **Title Page** - Identify the method (e.g., *Analysis of XYZ Pesticide and its Metabolites in Groundwater by HPLC*).
			- **Scope and Application** - Describe the scope and applicability of the method, including the matrix or matrices for which the method is applicable.
			- **Target Analyte List** - Include a list of the applicable analyte(s) for which the method has been developed, the limits of detection and concentration ranges for each, and any precautionary notes about detection limits or ranges.
			- **Summary of Method -** Give a brief description of the method, such as sample preparation, type of instrumentation used, detectors, confirmation requirements; and types of standards used (internal/external).
			- **Definitions -** Define any terms that may not be commonly understood, or that have multiple meanings.
			- **Interferences** - Discuss interferences that may result from processing and analysis of samples (e.g., from solvents, reagents, glassware and other sample processing hardware). Discuss procedures necessary to reduce or eliminate such interferences (e.g., glassware cleaning). Discuss matrix interferences and how to reduce or compensate for their effects.
			- **Safety -** Address all safety aspects of sample handling, processing and disposal (e.g., OSHA regulations, health effects of chemicals used and precautionary measures).
			- **Apparatus and Materials -** Describe or identify all sample processing and analytical equipment, instruments and materials. This requirement includes sample containers, glassware and ancillary equipment (i.e., water baths, balances, etc.).
			- **Reagents and Standards -** Describe the preparation of all reagents and standards. Include precautions and/or specifications for reagent and standard grades.
			- **Calibration -** Describe the procedures for initial calibration of the method, the method for generating the calibration curve (for example, linear regression, quadratic fit or other calibration models or procedures), procedures for continuing calibration verifications and the acceptance criteria for initial and continuing calibration checks.
			- **Quality Control -** Address all QC measures needed for initial demonstration of capability and for routine analysis. Include frequency of QC measurements and all acceptance criteria.
			- **Sample Collection, Preservation and Handling** - Address sample type (grab, composite), required container and preservation, maximum holding times and any special precautions that might be needed when collecting the samples. Sample collection information differing from or not addressed by DEP SOP requirements must be included.
			- **Sample Extraction/Preparation -** Describe the protocols used to extract, digest or prepare the sample prior to analysis.
			- **Sample Cleanup and Separation -** Describe any protocols needed to separate the analyte(s) of interest from the matrix.
			- **Sample Analysis -** Describe all protocols relating to sample analysis. Include instrument conditions, column type (if applicable), solvent or temperature programs, etc.
			- **Calculations -** Include all formulas used in calculating final concentrations.
			- **Confirmation -** Include protocols used to confirm the presence of the analyte (for example, GC/MS, second column, alternative wave length).
			- **Data Assessment -** Include all procedures to be used in assessing the data, including quality control acceptance criteria.
			- **Corrective Actions -** Include all measures that will be taken if a quality control measure or other measures of performance are not acceptable. Discuss contingencies for handling unacceptable data.
			- **Method Performance –** Employ a table format for clarity where applicable. Summarize the method detection limit, practical quantitation limit, quality control acceptance ranges and other pertinent information (for example, retention times, extraction/cleanup efficiency).
			- **Pollution Prevention and Waste Management -** Include all measures to prevent pollution, and how waste products such as solvents, extracts and digestates are handled.
			- **Tables, Diagrams, Flowcharts -** Include any applicable tables or figures.
			- **References -** include any applicable reference citations.
		5. Provide specific validation and initial demonstration of capability data, to include:
			- Copies of QC data: Results for QC check standards, initial and continuing calibration verification (ICV and CCV) standards, spikes (all types), blanks (all types) surrogates, etc. Include the assigned values (expected analyzed values) for all QC samples and calibration standards, and the acceptance criteria established for all QC samples and calibration standards.
			- All calibration data: concentration of standards, calculation of response factors and calibration curves. If linear regression is applicable, include the acceptance criteria for the resulting correlation coefficient; or, provide corresponding data for other calibration models or procedures.
			- All calculations pertaining to MDL, precision and accuracy determinations.
			- All applicable instrumentation analytical output data (run data) such as chromatograms, spectra, reports of sample results and calibration reports.
		6. If an equivalency study is required, additionally include the raw data, calculations and the complete description of statistical analysis for the study as specified in section 4.0.
	1. Documentation for Inter-Laboratory Collaborative Studies Conducted for Statewide-Use Methods
		1. In addition to the documentation required for all validations described above (section 7.1), ensure the following information is included for collaborative studies:
			+ A detailed description of the collaborative inter-laboratory method validation study identifying all participating laboratories;
			+ Reduced and raw analytical data for each laboratory; and,
			+ A complete description of statistical analysis of the study data and a discussion of the results and associated conclusions.

# Appendix A: Glossary

**Alternative Method** – an analytical laboratory method that is intended to be used in place of an existing Department-approved laboratory method specified in a Department rule, permit, order or contract.

**Data Quality Objectives** (DQOs) - a set of qualitative and quantitative requirements that environmental data must achieve to be acceptable for use by a specific Department program for an indicated use of the resulting data. The requirements pertain to the quality of the data in terms of precision, accuracy, sensitivity, selectivity, representativeness and comparability.

**Department** - the Florida Department of Environmental Protection, also referred to as “FDEP” or "DEP”.

**Florida Department of Health’s Environmental Laboratory Certification Program (DOH ELCP)** - a laboratory certification program recognized by the National Environmental Laboratory Accreditation Program (NELAP) as an authority with responsibility and accountability for granting accreditation for specified fields of laboratory testing. The standards used by the DOH ELCP are those as specified in Chapter 64E-1, F.A.C. and established by The NELAC Institute (TNI), which operates NELAP.

**Limited-Use Method** – an alternative or modified laboratory analytical method that is validated for testing environmental samples by a single laboratory (location, branch, etc.) for purposes specified in the scope of the approval (e.g., for specified analytes, facility or site locations or types, sample matrices such as effluent, groundwater, drinking water, fresh or marine surface waters, soils, sediments or chemical wastes). A limited-use method is approved by the Department for the analytical laboratory requesting approval, and may not be used by another analytical laboratory.

**Method Detection Limit (MDL)** - The method detection limit (MDL) is defined as the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results. For laboratories conforming with the 2016 TNI Standard, the MDL is defined as the minimum result which can be reliably discriminated from a blank with a predetermined confidence level. An MDL is analyte-and matrix-specific and is laboratory-dependent. For an MDL study, all sample processing steps of the analytical method shall be included for each analyte and matrix proposed for the method scope and applicability. The MDL may be determined following procedures specified in Appendix B-2 unless otherwise specified by a published method for which the laboratory is seeking approval as a modified or alternative method, or, the MDL may otherwise be determined by any technically justifiable and scientifically sound method. A specific determinative MDL procedure must be used if required by the Department to meet DQOs for a specific program activity or data use for which the method is proposed (such as may be required in a Department rule).

**Method Modification and Modified Method** - a method or procedure that includes any change that alters the scope, applicability, specifications, steps, performance criteria or any other requirements described in a published laboratory analytical method.

**Method Validation** - the process by which a laboratory substantiates the performance of an alternative or modified method. Alternative and modified methods must be validated for the scope and applicability proposed by the requester or laboratory using the method.

**The NELAC Institute** - The NELAC Institute (TNI) is a non-profit organization whose mission is to foster the generation of environmental data of known and documented quality through an open, inclusive, and transparent process that is responsive to the needs of the environmental sampling and testing community. TNI operates a national accreditation program, whereby all entities involved in the generation of environmental measurement data within the United States are accredited to one uniform, rigorous, and robust program.

**National Environmental Laboratory Accreditation Program (NELAP)** - is operated by the TNI and establishes and implements a program for the accreditation of environmental laboratories.

**Practical Quantitation Limit (PQL)** - the lowest level that can be reliably achieved during routine laboratory operating conditions within specified limits of precision and accuracy.

**Quality Control** - is defined as the overall system of measurement activities whose purpose is to control the quality of environmental data to meet established data quality objectives for a specified data use.

**Statewide-Use Method** – an alternative or modified laboratory procedure that is validated for testing environmental samples and approved for unlimited use by all persons and organizations, based on the information and data provided in the method validation documentation.

# Appendix B: Calculations and Applicable Formulae

1. **Formulas for Calculating Precision and Accuracy**

Use the following formulas for calculating the precision and accuracy of test measurements and the associated acceptance ranges:

* 1. **Precision**

Calculate the precision of replicate samples using one of the following three formulas:

* + 1. Percent Relative Standard Deviation:

Where: X = Mean (average) of the data points

s = Standard deviation calculated as:

s = {[S(X-Xi)2]¸(n-1)}0.5

X = mean value of measured concentrations (µg/L)

Xi = value of each measured concentration (µg/L)

n = number of determinations

* + 1. Relative Percent Difference:

Where: A = concentration in sample A

B = concentration in sample B

* + 1. Industrial Statistic\*:

Where: A = concentration in sample A

B = concentration in sample B

\*The industrial statistic may be used in place of %RSD or RPD, if routinely calculated by the laboratory to monitor precision.

* 1. **Accuracy (as % Recovery)**

Determine the accuracy (as % recovery) by calculating the % recovery of a known amount of analyte from the fortified (spiked) sample as follows:

1. Method Detection Limits and Practical Quantitation Limits
	1. **Method Detection Limit (MDL)**

An MDL is analyte-and matrix-specific and is laboratory-dependent. For an MDL study, all sample processing steps of the analytical method must be included for each analyte and matrix proposed for the method scope and applicability. The MDL may be determined following the procedures specified below unless otherwise required by a published method for which the laboratory is seeking approval as a modified or alternative method, or, the MDL may otherwise be determined by any technically justifiable and scientifically sound procedure. A specific MDL procedure must be used if required by the Department to meet DQOs for a specific program activity or data use for which the method is proposed (e.g., if required in a Department rule).

NOTE: The MDL is not equivalent to the Instrument Detection Limit (IDL) which cannot be used in place of the MDL. The IDL is determined using multiple analyses of standards and is useful in determining an experimental concentration level to use when fortifying samples for the MDL determination. The MDL is determined by processing samples through the entire sample preparation and analytical procedure (not just analysis).

The laboratory must determine the MDL using the protocol specified in the published laboratory method, if applicable. If the protocol for determining detection limits is not specified, one of the following three protocols may be used:

1. EPA - "Definition and Procedure for the Determination of the Method Detection Limit Revision 2" (2016), 40 CFR Part 136, Appendix B (Reference 2);
2. IUPAC- “Nomenclature in Evaluation of Analytical Methods including Detection and Quantification Capabilities”, Pure & Appl. Chem., Vol. 67, No. 10, pp. 1699-1723, 1995 (Reference 3);
3. Hubaux and Vos- “Decision and Detection Limits for Linear Calibration Curves”, Analytical Chemistry, Vol. 42, No. 8, July 1970, pp. 849-855 (Reference 4).

The method endorsed by the International Union of Pure and Applied Chemistry (IUPAC) is derived from the method published by Lloyd Currie and assumes a constant error model within a small concentration region. This method sets the MDL at a critical value intended to exclude 99% of the analytical noise population from reportable levels. The Appendix B to Part 136, Title 40 method for determining the MDL is designed for use with a variety of physical and chemical methods. It incorporates all sample processing steps used by the laboratory for an analytical method, including all steps of the method for samples analyzed over a prescribed period of time and distributed across all instruments to which the MDL will be applied. The laboratory calculates the MDL by determining the standard deviation of a set of spiked samples (MDLs) and method blanks (MDLb) and assigns either an initial MDL or an ongoing verification of the MDL, as applicable. The method published by Hubaux and Vos is based on a variable error model and the effect of concentration on the resulting noise distribution is considered in determining the detection limit. While this technique is more robust than that of other models, considerably more effort is required to develop method detection limits.

NOTE: Any methods that support compliance monitoring and reporting for EPA's National Pollutant Discharge Elimination System (NPDES) program, or, for compliance with monitoring requirements in rules authorized under the Safe Drinking Water Act must use the 40 CFR method for determining the MDL. See applicable Department rules for this requirement. If a Department rule refers to a later revision of the 40 CFR Part 136 MDL method, follow the requirements in the later version.

* 1. **Practical Quantitation Limit (PQL)**

The Practical Quantitation Limit (PQL) is the lowest level that can be reliably achieved during routine laboratory operating conditions within specified limits of precision and accuracy. Typically, the PQL is 3-5 times the MDL, and it represents a practical and routinely achievable detection level with a relatively good certainty than any reported value is reliable. The PQL for the proposed method must be determined by a defined procedure and/or criteria. For example, the PQL may be chosen to be the lowest calibration standard used for a calibration curve. Alternatively, the PQL may be indicated as the concentration at which the method has been demonstrated to achieve a specified range of precision and accuracy (e.g., %R= 70% - 130%; RPD ≤20%). See section 3.3 above for applicable requirements for determining the PQL. The procedure and/or criteria used to define the PQL must be included in the laboratory standard operating procedure developed for the method, or, in the laboratory quality manual.

# Appendix C: References

* + 1. “Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis”, Appendix D, Official Methods of Analysis of AOAC INTERNATIONAL, 19th edition (2012).
		2. EPA - "Definition and Procedure for the Determination of the Method Detection Limit - Revision 2", 40 CFR Part 136, Appendix B (12-13-16 edition);
		3. IUPAC- “Nomenclature in Evaluation of Analytical Methods including Detection and Quantification Capabilities”, Pure & Appl. Chem., Vol. 67, No. 10, pp. 1699-1723, 1995 6.
		4. Hubaux, A., G. Vos, “Decision and Detection Limits for Linear Calibration Curves”, Analytical Chemistry, Vol. 42. No. 8, pp. 849-855, July 1970