Designation: D8272 – 19

Standard Guide for Development and Optimization of D19 Chemical Analysis Methods Intended for EPA Compliance Reporting[1]

This standard is issued under the fixed designation D8272; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This guide identifies procedures for use in developing and optimizing new or modified Subcommitees D19.05 and D19.06 test methods intended for regulatory compliance reporting in EPA drinking water and wastewater programs. This guide may also be useful for developing test methods for emerging contaminants that may not yet have regulatory requirements.

1.2 This guide also cites statistical procedures that are useful in the single laboratory characterization and optimization and in the inter-laboratory studies (ILSs).

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *This guide offers an organized collection of information or a series of options and does not recommend a specific course of action. This document cannot replace education or experience and should be used in conjunction with professional judgment. Not all aspects of this guide may be applicable in all circumstances. This ASTM standard is not intended to represent or replace the standard of care by which the adequacy of a given professional service must be judged, nor should this document be applied without consideration of a project's many unique aspects. The word "Standard" in the title of this document means only that the document has been approved through the ASTM consensus process.*

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1129 Terminology Relating to Water

D1141 Practice for the Preparation of Substitute Ocean Water

D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water

D4841 Practice for Estimation of Holding Time for Water Samples Containing Organic and Inorganic Constituents

D5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis

E178 Practice for Dealing With Outlying Observations

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

E1169 Practice for Conducting Ruggedness Tests

E1488 Guide for Statistical Procedures to Use in Developing and Applying Test Methods

E1601 Practice for Conducting an Interlaboratory Study to Evaluate the Performance of an Analytical Method

E2857 Guide for Validating Analytical Methods

E2935 Practice for Conducting Equivalence Testing in Laboratory Applications

E3080 Practice for Regression Analysis

2.2 *Code of Federal Regulations (CFR) Standard:*[3]

40 CFR Part 136, Appendix B Definition and Procedure for the Determination of the Method Detection Limit

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this standard, refer to Terminology D1129.

[1] This guide is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.02 on Quality Systems, Specification, and Statistics.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from U.S. Government Printing Office, Superintendent of Documents, 732 N. Capitol St., NW, Washington, DC 20401-0001, http://www.access.gpo.gov.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *technical change, n*—modification to a test method, that could affect the outcome of a result.

3.2.2 *certified reference material, n*—reference material, accompanied by a certificate, having a value, measurement uncertainty, and stated metrological traceability chain to a national metrology institute.

3.2.3 *characterization, n*—a combination of the distinctive things that make up a test method.

3.2.4 *development (of a method), n*—an empirical series of steps that determine how to set up and run a chemical operation.

3.2.5 *formulation, n*—defined mixture or blend of reagents used to perform a chemical test.

3.2.5.1 *Discussion*—It often happens that two analysts, after preparing the same mixture, will get different results. This is due to variability of raw materials, or intricacies of technique. Small modifications of a formulary that seem insignificant but could have a significant impact on results.

3.2.6 *optimization (of a method), n*—a series of experiments consisting of systematic variations in an attempt to define critical steps of a new or modified test method in which important errors can be made.

3.2.6.1 *Discussion*—Optimization helps define the exact steps an analyst should take to ensure data obtained meets the accuracy and precision requirements of the test method.

3.2.7 *validation (of an analytical method), n*—confirmation, by the provision of objective evidence and examination, that a test method meets performance requirements and is suitable for its intended use.

3.2.7.1 *Discussion*—Test method validation is a practice performed by laboratories to demonstrate their capability of obtaining results that meet the specifications of the test method. In environmental testing, test method validation usually consists of establishing the calibration range, determination of minimum detectable concentration, and determination of precision and bias.

4. Significance and Use

4.1 The ASTM guidance manual, *Form and Style for ASTM Standards*,[4] Section A21, requires a precision and bias statement in all ASTM test methods. Section A21.2.2 states:

> Precision shall be estimated in accordance with the interlaboratory test program prescribed in Practice E691, Conducting an Interlaboratory Study to Determine the Precision of a Test Method, or by an interlaboratory test program that yields equivalent information, for example, a standard practice developed by an ASTM technical committee.

4.2 Practice D2777, Section 1.1, states:

> This practice establishes uniform standards for estimating and expressing the precision and bias of applicable test methods for Committee D19 on Water. Statements of precision and bias in test methods are required by the *Form and Style for ASTM Standards*, "Section A21. Precision and Bias (Mandatory)." In principle, all (ASTM Committee D19) test methods are covered by this practice.

4.3 Practice D2777, Section 1.2, requires a task group proposing a new test method to carry out a collaborative study from which concentration limits, repeatability and reproducibility precision and bias statements are developed.

4.3.1 This guide describes options for developing and optimizing chemical test methods for Committee D19, not implementation of a test method by a laboratory. Refer to Guide E2857 for procedures used in validating existing test methods for your laboratory.

4.3.2 The collaborative study described in Practice D2777 is not the test method validation. The collaborative study verifies the new test method is reproducible among different laboratories, different instruments/apparatus, and different analysts.

4.3.3 Practice D2777, Section 6.1, assumes the test method has already been optimized prior to conducting the collaborative study.

4.4 Practice D2777, Section 4 (Summary of Practice), requires, a collaborative study only after the task group has assured itself that preliminary evaluation work is complete and the test method has been written in its final form.

4.5 Practice D2777, Section 5.2 (Significance and Use), requires the collaborative test corroborates the test method write up (preliminary evaluation) within the limits of the test design.

4.5.1 The assumption is that the collaborative study is a fair evaluation of the inter-laboratory variability when using the test method to analyze the matrices, and concentration ranges specified in the test method.

4.6 Practice D2777, Section 6 (Preliminary Studies), requires considerable pilot work on a test method should precede the determination of precision and bias (collaborative study). This pilot work evaluates such variables as:

4.6.1 Representative Sampling,
4.6.2 Suitability of containers,
4.6.3 Preservation requirements,
4.6.4 Identification of interferences,
4.6.5 Holding times (Practice D4841),
4.6.6 Concentration range,
4.6.7 Quantitation ranges,
4.6.8 Concentration and preparation of reagents,
4.6.9 Reagent standardization,
4.6.10 Shelf life of reagents,
4.6.11 Calibration,
4.6.12 QC, and
4.6.13 Sample size.

4.7 Potentially significant factors are investigated in advance and are controlled in the written test method that is distributed for the collaborative test.

4.8 Only after the proposed test method has been thoroughly tried and proved and reduced to unequivocal written form should a collaborative test be conducted.

4.9 The Committee D19 test method is written in two steps:

4.9.1 *Step I*—Single laboratory characterization or optimization (Practice D2777, Section 6.3.1.1).

[4] ASTM guidance manual, *Form and Style for ASTM Standards* (the "Blue Book"), available from ASTM International Headquarters, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959.

4.9.2 *Step II*—Collaborative study (Practice D2777, Section 6.3.1.2).

4.10 This document is a guide to Committee D19 task groups developing chemical test methods.

5. Summary of Guide

5.1 There are two options for test method development. See Fig. 1 for a diagram of the sequence of steps.

5.1.1 *Modification* of an existing test method requires proof of equivalence, or comparability study (Practice D2777, Section 1.3).

5.1.1.1 For the potential acceptance by EPA, the modified ASTM test method must be the same analytical technique as the existing approved test method.

5.1.1.2 Changes in extraction procedures, digestion procedures, colorimetric reagent ratios, or reagent formulation require demonstration of equivalency on non-interfering samples, a demonstration that the change removed an interference, and a new collaborative study.

5.1.1.3 All sample preservation, holding times, and sample extraction for the existing test method apply to the new ASTM test method, or the task group must collect new data to demonstrate that the test method is equally effective on non-interfering samples and a conduct a new collaborative study.

5.1.1.4 If the test method is modified to overcome an interference, updates apparatus, or comprises a technical change as determined by the task group, a limited single laboratory study comparing the pre- and post- modified test method and a new collaborative study is required.

5.1.1.5 Demonstrate equivalence according to Practice D2777, Section 7.7, or establish equivalence using Practice E2935. Summarize results in a research report.

5.2 *New Test Method Development* is an empirical process of determining how to set up and run a series of chemical operations resulting in a numerical measurement within defined precision and bias. These steps will become the standard test method.

5.2.1 This process is used if there is currently no approved or standardized test method, or the task group is significantly (significance is determined by the subcommittee) modifying an existing test method.

5.2.2 Development of new test methods requires more extensive single laboratory studies than modification to make equivalent test methods.

5.2.3 Most Committee D19 test methods will be new test methods.

5.3 A summary of a sequence of four phases in test method development is outlined below. (The sequence may be modified to adapt to specific situations. A test method may have already passed through some of the phases prior to involvement of ASTM.

5.3.1 *Design Phase:*

5.3.1.1 A task group consists of task group chair, who will also serve as the technical contact, and is comprised of a small

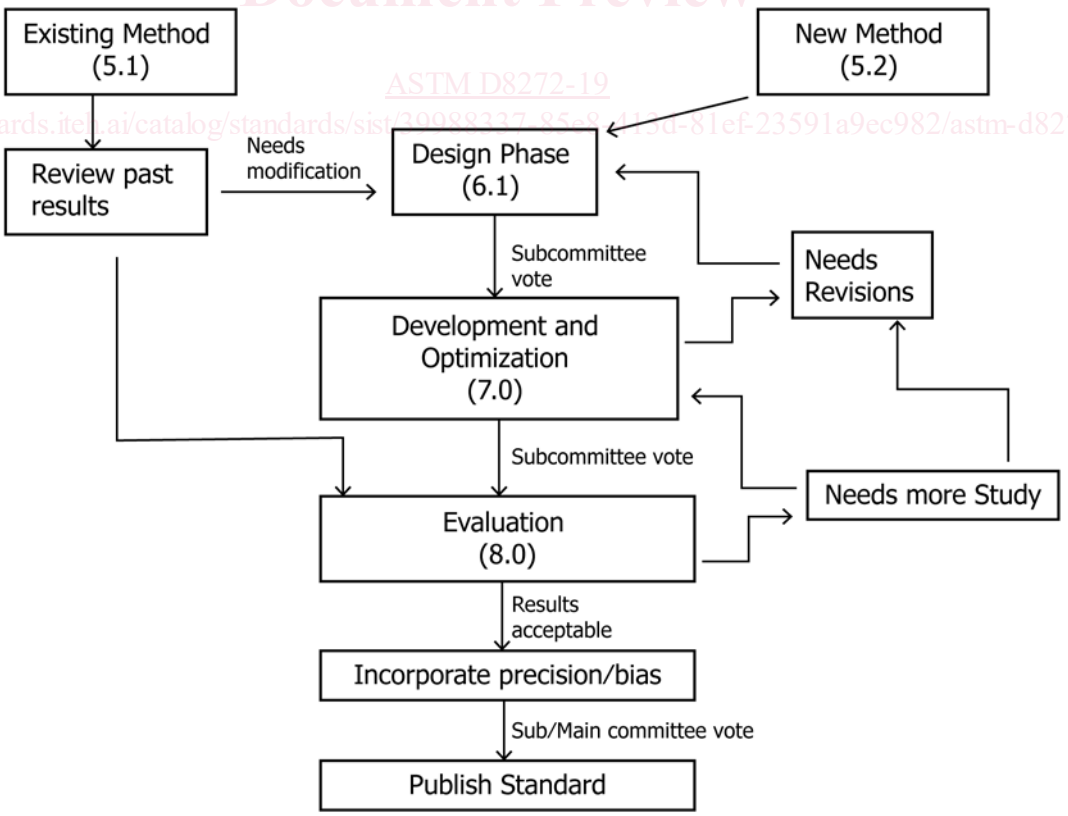

FIG. 1 Sequence of Steps

number of individual experts who volunteer or are appointed by the subcommittee to work on the specific project is established.

5.3.1.2 The task group decides the Scope and the Significance and Use sections of the test method.

5.3.1.3 The design phase includes the formalization of the Scope and the Significance and Use sections.

5.3.1.4 The design phase may describe a general approach to a test method, but does not involve any statistical studies.

5.3.1.5 The design phase is discussed further in Section 6.

5.3.2 *Development Phase:*

5.3.2.1 Conduct studies to determine the appropriate operations, instruments, reagents, and variables to study or not to study further on various matrices and concentration ranges as defined in the Scope section.

5.3.2.2 These studies may be single laboratory studies consisting of a series of smaller experiments in sequential order. Or preferably, several members of the task group laboratories study and test available options.

5.3.2.3 The draft test method is prepared in ASTM format with sampling requirements and preliminary test results on samples and known reference materials presented at a meeting or during a subcommittee ballot.

5.3.2.4 Some task groups may choose to complete this phase using the ASTM collaboration site and not take a standard to ballot until after the validation phase.

5.3.3 *Optimization Phase:*

5.3.3.1 This phase includes statistically controlled procedures described in references[5, 6] and Guide E1488 that systematically and progressively record and compare the outcome of experiments to create a series of steps/operations. These operational steps compile into a written test method. These steps may include, but are not limited to the collection and documentation of sample collection, preservation and preservation checks, holding time, reagent preparation and shelf life, contributions to variability, interferences, and interference checks with and without analyte(s) present, calibration range, and method detection limit (MDL) studies.

5.3.3.2 Perform repeatability tests consisting of seven replicate injections of standards and spikes (Practice D2777, Section 7.6.1) at a minimum of three concentrations in all applicable matrices (Practice D2777, Section 7.6.4).

5.3.3.3 If two or more task group laboratories are available, conduct preliminary multiple laboratory variability studies. Fewer than seven replicates may be made by each laboratory as long as there are at least six "degrees of freedom" for repeatability (Practice D2777, Section 7.6.5).

5.3.3.4 Prepare provisional precision and bias statements based on the smaller study. Perform ruggedness testing (Practice E1169), eliminating flexibility for a user to modify significant variables in the written test method.

5.3.3.5 Use Practice D5847 and include a quality control (QC) section with acceptance limits based on single laboratory study data in the draft test method. Alternatively, use the ILS data as shown in Table X1.4 to calculate matrix spike recovery per matrix and across matrices. Table X1.4 also shows how to calculate precision and bias using the ILS data. Compile results in a proposal for a collaborative study.

5.3.3.6 The draft standard should now be of sufficient form for use in an ILS. Instruct laboratories that they must meet the QC and bias acceptance criteria.

5.3.3.7 Ballot at sub-committee level to approve the draft standard prior to conducting an ILS. The subcommittee chair may use an administrative negative to restrict automatic advancement to main committee balloting.

5.3.3.8 The test method may be approved with a single laboratory or preliminary multiple laboratory variability studies in accordance with Practice D2777, Section 1.7, and the *Form and Style for ASTM Standards*,[4] Section A21.2.3.

5.3.4 *Evaluation Phase, or Inter-Laboratory Study (ILS):*

5.3.4.1 The purpose of the evaluation phase is to measure how well the new test method operates at different laboratories/locations and to quantify acceptable differences in different laboratories.

5.3.4.2 The data collected provides guidance to users of the test method on how well different instrument setups and users function on various materials.

5.3.4.3 For some standard test methods, it may also be useful to collect data on variation associated with day-to-day effects or for different calibration times.

5.3.4.4 The ILS should include a wide range of matrices and analyte concentrations. Compile the results in a research report.

5.3.4.5 Ballot the draft at subcommittee and main committee concurrently.

5.3.4.6 All ASTM test methods require an ILS by Practice E691 or an equivalent, committee specific procedure. Committee D19 typically performs ILSs according to Practice D2777, Section 1.2, within the first re-approval cycle.

6. Design Phase — Proposing Modified and New Test Methods and Assignment of Task Group

6.1 *Proposal of a New Test Method at Committee D19:*

6.1.1 An ASTM member, a visitor, or a task group proposes new test methods to the appropriate subcommittee chair or at an ASTM subcommittee meeting. The assigned technical contact opens a work item with ASTM. If approved, ASTM will assign a work item number.

6.1.2 The subcommittee determines whether the request is for an equivalent test method or a new test method and creates a task group if the test method is needed.

6.1.2.1 Prior to subcommittee approval to work on a new standard, a rationale for the standard is prepared. The rationale should include:

(1) The need for the standard,

(2) The intended use of the standard,

(3) A list of potential stakeholders, and

(4) An invitation to all members to join and take part in development of the standard.

6.1.3 The ASTM task group will determine the specifications of new test methods during the design, development, and validation phases.

[5] Youden, W. J., and Steiner, E. H., *Statistical Manual of the Association of Official Analytical Chemists*, Association of Official Analytical Chemists, Washington, DC, 1975.

[6] Wernimont, G. T, *Use of Statistics to Develop and Evaluate Analytical Methods*, AOAC International, Gaithersburg MD, 1985.

TABLE 1 Examples of Representative Wastewater Matrices

POTW effluent
Synthetic Ocean Water (Practice D1141)
Soil Extract (if soil extracts may be analyzed by the test method or else add another POTW)
Groundwater (>500 ppm TDS), may be 500 mg/L TDS of Synthetic Ocean Water in Reagent Water
Surface water (>2 ppm TOC), may be Reagent Water with 2 ppm TOC from humic acid
Drinking Water
Municipal POTW Pretreatment
Industrial Effluent — Treated
Industrial Effluent — Treated

TABLE 2 Representative Matrices for Drinking Water

Finished Tap Water
Surface Water (containing about 2 mg/L organic matter as humic acid). Assumes target analyte not a constituent of humic acid.
Ground Water (containing about 500 mg/L TDS)

6.1.4 The test method scope, purpose, applicability, and performance characteristics are decided by the task group and documented prior to commencing development or validation work.

6.1.5 *Literature Search:*

6.1.5.1 Locate all current and previous versions and references for the proposed test method. Search for reference methods from databases for NEMI, ASTM, Standard Methods, EPA, or other similar sources.

6.1.5.2 Consult with other ASTM task group members and establish a collaboration site to gather and compile historical information about the test method.

6.1.5.3 Review all versions of the test method (if applicable), noting previous changes and previously noted problem areas.

7. Development and Optimization of Modified and New Test Methods

7.1 *Test Method Performance Characteristics:*

7.1.1 Conduct statistically designed[5, 6] single laboratory studies or studies among a few laboratories to establish method performance. These studies determine specific operational and analytical steps that, once in written form, become the test method. Two or more task group members can share work to speed the process. They can also get a sense of reproducibility between their laboratories by analyzing the same samples. Following are items that should be evaluated for each test method.

7.1.1.1 *Selectivity*—Determine what other constituents in a sample may cause a response in addition to the analyte. Test matrix blanks and fortified matrix blanks preferably at three or more different concentrations spanning the expected range of the test method. Repeat with synthetic or actual matrices containing expected interferences. If possible, devise spot tests enabling rapid checks for interferences. Use Table 1 and Table 2 for examples of matrices. Document any interference and their upper concentration limits. In addition to the matrices specified in Table 1 and Table 2, analyze any available Certified Reference Materials (CRM).

7.1.1.2 *Instrument Calibration*—Define the calibration technique and calibration model. Allow the calibration model to fit the data. If feasible, measure each calibration level in triplicate (replicated) to evaluate random error associated with instrument response.

NOTE 1—If a titration or gravimetric test method, describe necessary steps to standardize reagents, or obtain accurate weights on the balance. For example, the average of four replicate determinations of Normality results in a value with ½ the random error of a single titration. In addition, test different volumes of sample to document effect on sample size.

7.1.1.3 *Bias/Trueness*—Evaluate reference materials (if available). Evaluate systematic errors by comparing the average of multiple replicates with the certified value. If reference materials are not available, then plot method results of up to nine different matrices analyzed at up to five different concentrations against known spiked values. Prepare a scatter plot for each matrix, and insert a regression line with equations (Practice E3080). Slopes should be normally distributed with no outliers (Practice E178). Another option is to overlay each regression line in one plot. The lines from each matrix should overlay within the precision of replicates from a clean matrix. An outlying slope is easily detected visually. See Fig. 2 for an overlay plot of nitrate spikes of three concentrations in eleven matrices. Calculate spiked concentration after subtracting the un-spiked concentration. Exclude matrices with high un-spiked concentration because dilution removes matrix effects. The matrices in Fig. 2 included known CRMs, drinking water, and wastewater.

7.1.1.4 *Repeatability*—Analyze three to eight consecutive measurements (preferably at least seven) (Practice D2777, Section 7.6.1) from each calibration standard encompassing the entire range of the test method and expressed as standard deviation or percentage relative standard deviation to evaluate the repeatability of the test method in an interference free matrix. Then analyze three to eight consecutive measurements (same as used for bias determination) of up to nine different representative matrices to evaluate repeatability in real samples. Use an *F* test to compare standard deviations between matrices. For a better comparison, use as many replicates as possible.

7.1.1.5 *Reproducibility*—Carry out the repeatability measurements in triplicate on a minimum of six consecutive (or nonconsecutive) days. Use the differences in the means to estimate the reproducibility of the test method. Use this test if a collaborative study is not practical (such as short holding time analytes, or on-line analyzers). Calculate according to Table X1.3 substituting laboratories with days. Otherwise, the collaborative study evaluates reproducibility.

7.1.1.6 *Quantitation Limit and Range*—The lower limit of the quantitation range (called limit of quantitation (LOQ), or minimum level (ML)) should be equal or close to the lowest calibration standard in the test method. A quantitation range may be wider than the calibration range due to dilution or concentration steps.

7.1.1.7 *Method Detection Limit (MDL)*—ASTM validation for test methods intended for EPA compliance monitoring should define the MDL (if applicable) according to the EPA definition. Follow the 40 CFR Part 136, Appendix B, procedure

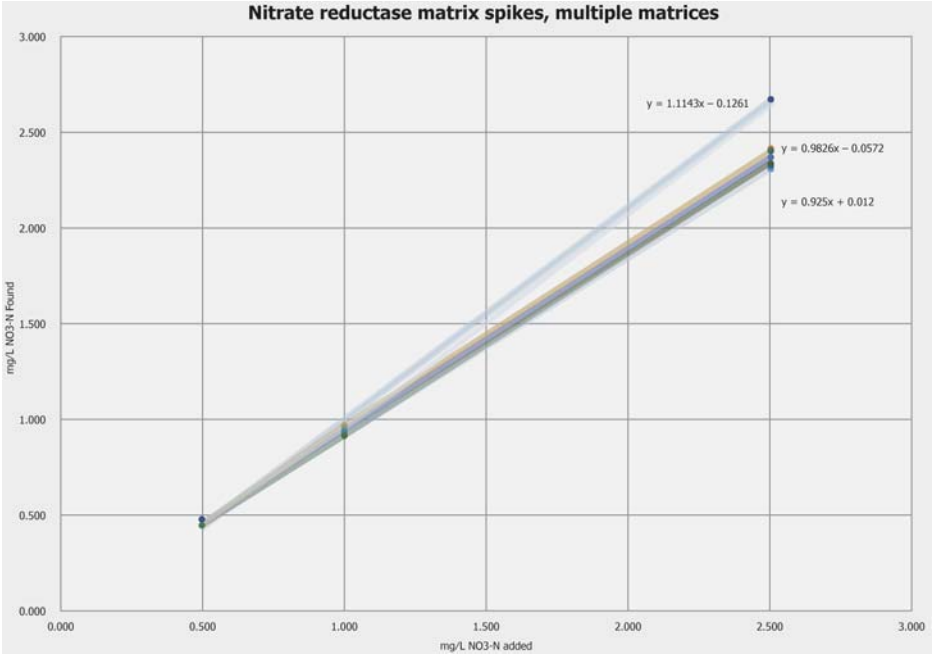

FIG. 2 Evaluation of Matrix Interferences for Nitrate

for determining the MDL, or using the detection limit procedure for the applicable regulatory program.

7.2 *Optimization of Operating Parameters:*

7.2.1 *Sample Collection and Preservation*—Conduct studies to determine minimum sample size required, sample container and container materials, preservatives, and holding time. Describe checks in the procedure for verification that preservation criteria were met.

7.2.1.1 Follow Practice D4841 to determine holding time using different sample containers, preservatives, and storage temperatures.

7.2.2 *Reagent Preparation and Storage*—Conduct literature research or, preferably, shelf life studies to determine reagent containers, container materials, and storage temperature. Note in the procedure, for example, when reagents change color, a precipitate forms, or when analyte response falls above or below acceptable limits.

7.2.3 Conduct preliminary reproducibility studies by following 7.1.1.5, or comparing results between a few task group laboratories.

7.2.4 Prepare a preliminary precision and bias statement based on single laboratory repeatability and accuracy, or on the smaller study from 7.2.3.

7.2.5 Perform ruggedness testing according to Practice E1169 or similar. Use results of ruggedness test to eliminate flexibility for a user to modify significant variables in the written test method. Include in the written test method what may, or may not be modified.

7.2.6 The draft standard should now be of sufficient form for use in an ILS. Ballot the draft at sub-committee level to approve the draft standard prior to conducting an ILS.

8. Evaluation Phase – Conduction an Inter-Laboratory Study (ILS)

8.1 Prepare an ILS plan and present along with the subcommittee balloted validated test method to the D19 subcommittee for review. The purpose of the ILS is to measure how well the new test method operates at different laboratories/locations and how large a difference in results using the test method in two laboratories is acceptable. The data collected provides guidance to users of the test method on how well different instrument setups or users function on various materials, or both. For some test methods, it may also be useful to collect data on variation associated with day-to-day effects or for different calibration times. The ILS should include a wide range of reproducible matrices, and analyte concentrations.

8.1.1 Write the ILS study plan to comply with Practice D2777.

8.1.2 Document deviations from Practice D2777.

8.2 The subcommittee chair presents their recommendation at the D19 Technical Operations subcommittee meeting.

8.3 The D19 Technical Operations subcommittee votes to approve the ILS and records the results of the vote in the meeting minutes. The Technical Operations subcommittee chair or a designee shall record any deviations from Practice D2777 into the meeting minutes. Once approved, the task group may begin the ILS.

8.4 Alternatively, you may register the work item for assistance from the ASTM ILS Program. The ASTM ILS Program can assist in finding laboratories and contracting suppliers and, unless specifically asked to design the ILS according to Practice D2777, they will use Practice E691 (*Form and Style for ASTM Standards*,[4] A21.2.2). Approval at