

concentration was selected to perform the MDL test.

(b) Conducting an iterative procedure involving analyses of additional replicates to verify the reasonableness of the MDL (required for method development).

(c) Determining the MDL in additional relevant matrices or in multiple laboratories.

4.0 Procedure

4.1 Estimate the detection limit of the method

If the purpose of determining the MDL is to verify laboratory performance using a specific method or to determine the MDL in a specific matrix, the laboratory should use the MDL published in the method as the initial estimate. If the MDL is being determined for other reasons (e.g., method development), the experience of the laboratory is important to properly estimate the detection limit. The laboratory must include at least one of the following considerations in producing this initial estimate:

4.1.1 The concentration of analyte that produces an instrument signal/noise in the range of 2.5 to 5 for those instances in which an instrument is used for the determination.

4.1.2 The concentration approximately equal to three times the standard deviation of replicate measurements of the analyte in a blank. If analysis of the blank produces no response (zero), use the concentration approximately equal to three times the standard deviation of replicate measurements at the lowest concentration that always produces a response.

4.1.3 A concentration in the region of constant or effectively constant standard deviation at low concentrations. This assumes that the model of Glaser et al. (1981), which includes a low concentration region where the standard deviation of the measurement error is constant or effectively constant, is suitable to describe the measurement process for the analytical method under consideration.

4.1.4 The lowest concentration that can be detected by analyzing samples containing successively lower concentrations of the analyte.

4.2 Select the matrix to be used to develop the MDL. The MDL is typically determined in a reference matrix. However, it may be determined in a real-world matrix to verify that the MDL required for a specific application can be achieved in that matrix.

4.2.1 Reference Matrix

The most common reference matrix is reagent water. Reagent water is defined as water in which the analyte and interferences are not detected at the MDL or, if this is the initial estimate, detected at the detection limit estimated in section 4.1. An interference is defined as a systematic error in the measured analytical signal caused by the presence of a substance other than the analyte. Other common reference matrices are sand as a reference matrix for soils, sediments, and other solid samples; and corn oil as a reference matrix for tissue samples. After selecting the reference matrix to be tested, proceed to section 4.3.

4.2.2 Matrices other than a reference matrix

4.2.2.1 If the MDL determined in a matrix other than a reference matrix is sufficient to meet requirements of the specific application (e.g., the laboratory is able to meet the MDL required for compliance monitoring or published in the method), it is not necessary to determine the MDL in a reference matrix.

4.2.2.2 If the purpose of a matrix-specific MDL is to determine the effects of matrix interferences in a real-world sample, the laboratory also must determine the MDL in a reference matrix to demonstrate the laboratory's ability to apply the method in the absence of interferences.

Note to Section 4.2.2.2: A laboratory seeking to develop a matrix-specific MDL for a specific method must use the same cleanup steps that will be used for analysis of samples.

4.3 Establish the test concentration range and prepare test samples. Establish the test concentration range per section 4.3.1. Prepare the test samples from a reference matrix per section 4.3.2, or from an alternative matrix per section 4.3.3. Prepare a sufficient quantity of the matrix to provide samples for a minimum of seven analyses.

Note to Section 4.3: For analytes for which a single-volume (bulk) sample or spiked single-volume sample would result in non-homogenous replicates (e.g., for determination of "oil and grease"), or for which preparation of a spiked single-volume sample is impractical, a minimum of seven individual aliquots should be prepared at the test concentration.

4.3.1 Establish the test concentration range as follows.

4.3.1.1 If verifying an MDL that is published in an analytical method, the test concentration should be no more than five times the published MDL.

4.3.1.2 If verifying an MDL required to support a regulatory objective or the objective of a specific study or program, the test concentration should be no more than one third the compliance or target limit.

4.3.1.3 If performing an MDL study for a new or revised method, the test concentration should be no more than five times the detection limit estimated in section 4.1.

4.3.1.4 If performing an iteration (see section 4.8), the test concentration should be no more than five times the MDL determined in the most recent iteration.

4.3.2 Preparing test samples from a reference matrix

If a blank sample produces an acceptable signal (see section 4.3.2), spiking is not required; otherwise, spike the reference matrix at the concentration established in section 4.3.1. Proceed to section 4.4.

Note to Section 4.3.2: The laboratory must ensure that the levels in blanks are not too high. Otherwise, the resulting MDL produced may be artificially biased. For a spiked sample, the concentration of the contaminant in the blank should not be a significant portion of the total concentration since this also could result in an artificial bias for the MDL. It is important to spike the analyte at the proper concentration (section 4.3) to ensure the MDL is determined accurately.

4.3.3 Preparing test samples from a matrix other than a reference matrix

Analyze three aliquots of the sample matrix to characterize the concentration of the target analyte(s) present in the matrix.

4.3.3.1 If the average measured concentration of the analyte in the matrix is less than five times the concentration established in section 4.3.1, proceed to section 4.4.

4.3.3.2 If the average measured concentration of the analyte in the matrix is less than the concentration range established in section 4.3.1, spike the matrix to bring the concentration of the analyte to the established concentration range and proceed to section 4.4.

4.3.3.3 If the average measured concentration of the analyte in the matrix is greater than the concentration range established in section 4.3.1, reduce the concentration of the analyte to the established concentration range, using one of the following techniques before proceeding to section 4.4:

4.3.3.3.1 Selectively remove the analyte from the matrix.

4.3.3.3.2 Obtain another matrix with a lower concentration of the analyte.

4.3.3.3.3 Dilute a sample of the matrix with the appropriate reference matrix. For example, if the matrix is aqueous, dilute the sample with reagent water.

Note to Section 4.3.3.3.3: Dilution should be used only if the analyte cannot be selectively removed (3.3.3.1) or if another matrix with a lower analyte concentration cannot be obtained (3.3.3.2) because dilution of the sample has the potential to dilute any interferences present.

4.4 Perform the analyses

4.4.1 The analyses in section 4.4.3 (optional pre-test of estimated detection limit) and 4.2 (MDL analyses) must be performed using all of the routinely employed calibration, sample handling, processing, and result calculations specified in the analytical method. For example, many methods contain multiple sample cleanup options; any and all cleanup options routinely used to analyze a sample must be used when analyzing the replicate samples prepared in section 4.3.

4.4.2 Similarly, if the analytical method employs recovery-correction or blank-correction procedures for calculating results, those procedures must be used when calculating results of an analysis of each aliquot. If a recovery- or blank-correction procedure is not specified in the test method, such correction must not be used.

4.4.3 Optional pre-test

It may be economically and technically desirable to evaluate the estimate of the detection limit (section 4.1) before proceeding with determination of the MDL in section 4.5. This pre-test attempts to ensure that the MDL study is being conducted at the correct concentration to prevent repeating the entire study; it may be particularly useful when the analytical costs are high. To evaluate the estimated detection limit, proceed as follows:

4.4.3.1 Process three aliquots of the test sample prepared in section 4.3 through the entire method, per section 4.5.

4.4.3.2 Calculate the standard deviation of results for the three aliquots as follows: