APPENDIX B TO PART 136 - DEFINITION AND PROCEDURE FOR THE DETERMINATION OF THE METHOD DETECTION LIMIT - REVISION x.xx

*Definition*

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.

*Scope and Application*

The MDL procedure is designed to be a straightforward technique for estimation of the detection limit for a broad variety of physical and chemical methods. The procedure requires a complete, specific, and well defined analytical method. It is essential that all sample processing steps used by the laboratory be included in the determination of the method detection limit.

The MDL obtained by this procedure is used to judge the significance of a single measurement of a future sample. A result below the MDL is judged to be qualitatively unreliable while a result at or above the MDL is judged to be qualitatively reliable.

*Procedure*

1. Estimate the Initial MDL using one of the following:
	* The mean plus three times the standard deviation of a set of method blanks.
	* The concentration value that corresponds to an instrument signal/noise in the range of 3 to 5.
	* The concentration equivalent of three times the standard deviation of replicate instrumental measurements of spiked blanks.
	* That region of the standard curve where there is a significant change in sensitivity, i.e., a break in the slope of the standard curve.
	* Instrumental limitations.
	* Previously determined MDL

It is recognized that the experience of the analyst is important to this process. However, the analyst should include some or all of the above considerations in the initial estimate of the MDL.

1. Determine the Initial MDL
	1. Select a spiking level, typically 2 – 10 times the estimated MDL in section 1.[[1]](#footnote-1)

* 1. Process a minimum of 7 spiked blank samples and 7 method blank samples through all steps of the method, including any sample preservation. Both preparation and analysis of these samples must include at least three batches on three separate days.
		1. If there are multiple instruments that will be assigned the same MDL, then the samples must be distributed across all of the instruments.
		2. A minimum of two spiked samples and two method blank samples prepared and analyzed on different days is required for each instrument.
		3. Evaluate the spiking level: If any result for any individual analyte from the spiked blank samples does not meet the method qualitative identification criteria[[2]](#footnote-2) or does not provide a numerical result greater than zero then repeat the spikes at a higher concentration.
	2. Make all computations according to the defined method with final results in the method reporting units.
		1. Calculate the sample standard deviation (S) of the replicate spiked blank measurements and the sample standard deviation of the replicate method blank measurements from all instruments.
		2. Compute the MDLs (MDL based on spiked blanks) as follows:

$$MDLs=t\_{(n-1, 1-∝=0.99)}S$$

where:

MDLs = the method detection limit

t(n-1, 1-α = 0.99) = the students t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom. See Table 1.

S = sample standard deviation of the replicate spiked blank sample analyses.

* + 1. Compute the MDLb (MDL based on method blanks) as follows:
			1. If none of the method blanks give numerical results[[3]](#footnote-3) for an individual analyte, the MDLb does not apply.
			2. If some (but not all) of the method blanks for an individual analyte give numerical results, set the MDLb equal to the highest method blank result.
			3. If all of the method blanks for an individual analyte give numerical results, calculate the MDLb as:

 $MDL\_{b}=\overline{X}+t\_{\left(n-1, 1-∝=0.99\right)}S\_{b}$

Where:

MDLb = the MDL based on method blanks

$\overbar{X}$ = mean method blank

t(n-1, 1-α = 0.99) = the students t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom. See Table 1.

Sb = sample standard deviation of the replicate blank sample analyses.

* 1. Set the greater of MDLs or MDLb as the initial MDL.
1. Ongoing Data Collection
	1. During any quarter in which samples are being analyzed, prepare and analyze a minimum of two spiked blanks on each instrument, in separate batches if available, using the same spiking concentration[[4]](#footnote-4) used in Section 2.
	2. Ensure that at least 7 spiked blanks and 7 method blanks are completed for the annual verification.
	3. At least once per year, re-evaluate the spiking level.
		1. If more than 5% of the spiked blanks do not return positive numerical results that meet all method qualitative identification criteria, then the spiking level must be increased and the initial MDL re-determined following the procedure in Section 2.
2. Ongoing Annual Verification
	1. At least once per year, re-calculate MDLs and MDLb from the collected spiked blank and method blank results using the equations in section 2.
	2. For analytes where some but not all of the method blanks give numerical results and there are over 100 method blank results, set the MDLb equivalent to the method blank concentration that is no less than the 99th percentile of the blanks. [[5]](#footnote-5)
	3. Include data generated within the last 2 years, but only data with the same spiking level.
	4. Include the initial MDL spiked blanks if within two years.
	5. Only use data associated with acceptable calibrations and batch QC[[6]](#footnote-6).
	6. The verified MDL is the greater of the MDLs or MDLb. If the verified MDL is within a factor of 3 of the existing MDL, and fewer than 3% of the method blank results (for the individual analyte) have numerical results above the existing MDL, then the existing MDL may optionally be left unchanged. Otherwise, adjust the MDL to the new verification MDL.

ADDENDUM: DETERMINATION OF THE MDL FOR A SPECIFIC MATRIX

MDLs may be determined in specific sample matrices as well as in reagent water.

1. Analyze the sample matrix to determine the native concentration of the analyte(s) of interest.
2. If the native concentration is at a signal to noise ratio of approximately 5-20, calculate the matrix specific MDL according to Sections 3-6.
3. Calculate MDLb using method blanks, not the sample matrix, unless required by the method.
4. If the signal to noise is less than 5, the analyte(s) should be spiked to obtain a concentration that will give results with a signal to noise of approximately 10-20.
5. If the analytes(s) of interest have signal to noise greater than approximately 20, then the resulting MDL is likely to be biased high.

TABLES OF STUDENTS' t VALUES AT THE 99 PERCENT CONFIDENCE LEVEL

|  |  |  |
| --- | --- | --- |
| Number of replicates | Degrees of freedom (n-1)  | t (n-1, 0.99) |
| 7  | 6  | 3.143  |
| 8  | 7  | 2.998  |
| 9  | 8  | 2.896  |
| 10  | 9  | 2.821  |
| 11  | 10  | 2.764  |
| 16  | 15  | 2.602  |
| 21  | 20  | 2.528  |
| 26  | 25  | 2.485  |
| 31  | 30  | 2.457  |
| 61  | 60  | 2.390  |
| 100  | 100  | 2.326  |

*Documentation*

The analytical method used must be specifically identified by number or title and the MDL for each analyte expressed in the appropriate method reporting units. Data and calculations used to establish the MDL must be able to be reconstructed upon request.

The sample matrix used to determine the MDL must also be identified with MDL value. Document the mean spiked and recovered analyte levels with the MDL.

 [49 F.R. 43430, Oct. 26, 1984; 50 F.R. 694, 696, Jan. 4, 1985, as amended at 51 F.R. 23703, June 30, 1986]

1. Spiking levels in excess of 10 times the estimated detection limit may be required for analytes with poor recovery. [↑](#footnote-ref-1)
2. Qualitative identification criteria – a set of rules or guidelines for establishing the identification or presence of an analyte using a measurement system.  Qualitative identification does not ensure that quantitative results for the analyte can be obtained. [↑](#footnote-ref-2)
3. A numerical result includes both positive and negative results, including results below the current MDL, but not results of ND (not detected) commonly observed when a peak is not present in chromatographic analysis. [↑](#footnote-ref-3)
4. If any analytes are repeatedly not detected in the quarterly spike sample analysis, this is an indication that the spiking level is not high enough and should be adjusted upward. [↑](#footnote-ref-4)
5. For example, if the highest ranked method blank results for a set of 200 method blanks were 1.5, 1.7, 1.9, 5.0 and 10, the MDLb would be set to 5 (the second highest value out of 200 ranked values is the rank of 99%). For "n" method blanks where n >= 100, sort the method blanks in rank order. The  (n \* 0.99) ranked method blank result (round down to the nearest whole number) is the MDLb. Using the above example blank concentrations but with an n of 164 this time, then 164\*0.99 = 162.36 which rounds down to the 162nd method blank result. Therefore, MDLb is 1.9 for an 'n' of 164 (10 is the 164th result, 5.0 is the 163rd result, and 1.9 is the 162nd result). Alternatively, you may use spreadsheet algorithms to calculate the 99th percentile to interpolate between the ranks more precisely.

 [↑](#footnote-ref-5)
6. All routine data should be included, with the exception of batches that are rejected and the associated samples reanalyzed. [↑](#footnote-ref-6)