STANDARD OPERATING PROCEDURE
GUIDE FOR ANALYTICAL METHOD DEVELOPMENT

KEY WORDS
Quality control, method detection limit, reporting limit, method development, method validation, storage stability, spike, blank, laboratory specifications.

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1.0 INTRODUCTION

1.1 Purpose

The SOP is designed to be a guide for registrants and associated laboratories submitting analytical methods to DPR for the quantification of pesticides in aqueous and/or sediment samples. This document supersedes portions of SOP Chemistry Quality Control QAQC001.00.

1.2 Definitions

1.2.1 **Aliquot** refers to a portion of the sample extract used for analysis.

1.2.2 **Analyte(s)** refers to the compound(s) tested for by this method.

1.2.3 **Blank** refers to a sample with no detectable amount of pesticide. Blanks are used to check for contamination or to prepare QC samples (e.g., **method blank, blank-matrix, reagent blank,** and **field blank** samples).

1.2.4 **Calibration Standard** refers to a solution prepared for a secondary standard or stock solution. This standard is used to calibrate the instrument's response.

1.2.5 **Extract** refers to the final sample which contains the pesticide residue in a solvent.

1.2.6 **Extraction Set** refers to a group of samples extracted and processed at the same time.

1.2.7 **Limit of Detection (LOD)** is the concentration equivalent to a signal, due to the analyte of interest, which is the smallest signal that can be distinguished from background noise. Use of LOD may replace MDL.

1.2.8 **Limit of Quantitation (LOQ)** is the level above which quantitative results may be obtained. It is typically reported as equal to 10 times the standard deviation of the results for a series of replicates used to determine LOD.

1.2.9 **Method Detection Limit (MDL)** as defined by the US EPA (40 CFR, Part 136, Appendix B) is "the minimum concentration of a substance that can be measured and reported with 99% confidence that the

analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix...."

1.2.10 **Quality Control (QC)** refers to the performance check of the sample analysis against a standard. This assesses the precision of sample results.

1.2.11 **Relative Standard Deviation (RSD)** is the standard deviation multiplied by 100 and divided by the mean.

1.2.12 **Reporting Limit (RL)** is 3–5 times the MDL depending on the analytical method and matrix.

1.2.13 **Spike** refers to a known amount of pesticide added. These QC samples are used to check the precision and accuracy of a method.

1.2.14 **Standard** refers to the laboratory analytical standard.

1.2.15 **Storage Stability** is the stability of residues during sample storage prior to their analysis. A storage stability study may validate the residue's rate of decomposition in a representative matrix.

2.0 GENERAL PROCEDURES

These guidelines are meant to be a starting point for registrant submitted analytical methods. The procedures outlined below are the minimum QC measures to be reported as a part of a fully validated method.

2.1 General Method Development - Analytical methods should utilize mass spectrometry or tandem mass spectrometry paired with either gas chromatography or liquid chromatography. Justification must be provided to utilize an alternative analysis.

2.1.1 **Standard** - Standard solution concentrations shall be validated, prior to use, by checking the standard's chromatographic purity using a secondary standard prepared at a different time or obtained from a different source.

2.1.2 **Method Detection Limit Determination** - The MDL is typically determined by US EPA method 40 CFR, Part 136, Appendix B, which is provided in Appendix 1. LOD may be substituted for MDL. Replicates used for MDL determination must have analyte recoveries

between 70–120% of spiked concentration with a relative standard deviation (RSD) <20%.

2.1.3 **Reporting Limit Determination** - The RL is set at 3–5 times the MDL depending on the matrix and instrument. RLs for natural surface water should be no greater than 10% of the lowest acute toxicity (LC_{50}) for an aquatic organism. RLs for sediment should be no greater than 1 µg/kg for sediment or 10% of lowest acute sediment toxicity value (LC_{50}) if available. Submitted methods may substitute LOQ for RL.

2.1.4 **Method Validation** – Method validation requires a minimum of five replicate analyses at two matrix spike concentrations (typically LOQ and 10XLOQ). Method validation recoveries are acceptable between 70–120% with a relative standard deviation (RSD) <20%.

2.1.5 **Storage Stability** - A 28-day (minimum duration) storage stability study in both surface water and sediment matrices. The objective of the storage stability test is to determine how long environmental samples could be stored and still remain viable. Surface water matrices should be stored in a refrigerator between 2-7 ^{0}C and sediment samples stored in a freezer below 0 ^{0}C for the duration of the test. The test should include at least four sampling intervals and two replicate samples at each interval to validate residue's rate of decomposition in representative matrices. Clean water and sediment matrices should be spiked with analyte of interest at an environmentally relevant concentration (i.e., 10X LOQ). Analysis should be conducted with fully validated analytical methods and acceptable recovery range (70–120%) and RSD (<20%)

2.2 Quality Assurance and Quality Control (QA/QC) - The following analyses are to be conducted by the performing laboratory on a continual basis. Each aqueous or sediment extraction set should include 10% QC samples.

2.2.1 **Reagent Blanks** - 1–2 per extraction set

2.2.2 **Blank-Matrix Spikes** - 1–3 per extraction set

2.2.3 **Matrix Spikes** - 1–3 per extraction set

2.2.4 **Replicate Sample Analyses** - analyzing multiple aliquots of a single sample will give a better estimate of the method precision (minimum of seven for MDL determination).

2.2.5 **Replicate Extract Analyses** - multiple analyses of a single extract will give a separate estimate of the precision of the extraction and analysis processes.

3.0 QC PROCEDURES FOR ADDITIONAL MATRICES

3.1 Sediment Method Development - The MDL is typically determined by US EPA method 40 CFR, Part 136, Appendix B. The above QC procedures must be followed for this matrix.

3.2 Wastewater Method Development – The MDL is typically determined by US EPA method 40 CFR, Part 136, Appendix B. The MDL for wastewater may vary depending on interferences within the sample matrix. Interferences will vary between sample sources depending on the municipality. The above QC procedures must be followed for this matrix.

4.0 DATA REPORTING REQUIREMENTS

4.1 QC Results - The following QC results should be submitted for review: the concentration of all blanks, the concentration detected for all spikes and the amount added for all spikes.

4.2 Reporting Method Results - For all method validation parameters both the individual results and the calculated values (i.e., MDL or LOD, RL or LOQ) should be submitted for review. The individual results used for any calculation should be clearly identified in a final report.

5.0 ANALYTICAL METHODS REVIEW

Prior to submittal to DPR, the analytical method(s) should be reviewed by the laboratory's quality assurance officer, laboratory liaison or lead chemist. DPR's Environmental Scientists will review submitted methods for completeness and accuracy.

6.0 REFERENCES

Segawa et al. (1995) Chemistry Laboratory Quality Control Standard Operating Procedure. QAQC001.00. California Department of Pesticide Regulation.

US EPA (2015) Protection of the Environment: Environmental Protection Agency, Subchapter D- Water Programs. 40 CFR, Part 136, Appendix B.

APPENDIX 1 - U.S. EPA (2015) Method Detection Limit Determination**APPENDIX 1**

APPENDIX B TO PART 136—DEFINITION AND PROCEDURE FOR THE DETERMINATION OF THE METHOD DETECTION LIMIT—REVISION 1.11

Definition

The method detection limit (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 and is determined from analysis of a sample in a given matrix containing the analyte.

Scope and Application

This procedure is designed for applicability to a wide variety of sample types ranging from reagent (blank) water containing analyte to wastewater containing analyte. The MDL for an analytical procedure may vary as a function of sample type. The procedure requires a complete, specific, and well defined analytical method. It is essential that all sample processing steps of the analytical method 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.

The MDL procedure was designed for applicability to a broad variety of physical and chemical methods. To accomplish this, the procedure was made device- or instrument-independent.

Procedure

1. Make an estimate of the detection limit using one of the following:

(a) The concentration value that corresponds to an instrument signal/noise in the range of 2.5 to 5.

(b) The concentration equivalent of three times the standard deviation of replicate instrumental measurements of the analyte in reagent water.

(c) 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.

(d) Instrumental limitations.

It is recognized that the experience of the analyst is important to this process. However, the analyst must include the above considerations in the initial estimate of the detection limit.

2. Prepare reagent (blank) water that is as free of analyte as possible. Reagent or interference free water is defined as a water sample in which analyte and interferent concentrations are not detected at the method detection limit of each analyte of interest. Interferences are defined as systematic errors in the measured analytical signal of an established procedure caused by the presence of interfering species (interferent). The interferent concentration is presupposed to be normally distributed in representative samples of a given matrix.

3. (a) If the MDL is to be determined in reagent (blank) water, prepare a laboratory standard (analyte in reagent water) at a concentration which is at least equal to or in the same concentration range as the estimated method detection limit. (Recommend between 1 and 5 times the estimated method detection limit.) Proceed to Step 4.

(b) If the MDL is to be determined in another sample matrix, analyze the sample. If the measured level of the analyte is in the recommended range of one to five times the estimated detection limit, proceed to Step 4.

If the measured level of analyte is less than the estimated detection limit, add a known amount of analyte to bring the level of analyte between one and five times the estimated detection limit.

If the measured level of analyte is greater than five times the estimated detection limit, there are two options.

(1) Obtain another sample with a lower level of analyte in the same matrix if possible.

(2) The sample may be used as is for determining the method detection limit if the analyte level does not exceed 10 times the MDL of the analyte in reagent water. The variance of the analytical method changes as the analyte concentration increases from the MDL, hence the MDL determined under these circumstances may not truly reflect method variance at lower analyte concentrations.

4. (a) Take a minimum of seven aliquots of the sample to be used to calculate the method detection limit and process each through the entire analytical method. Make all computations according to the defined method with final results in the method reporting units. If a blank measurement is required to calculate the

measured level of analyte, obtain a separate blank measurement for each sample aliquot analyzed. The average blank measurement is subtracted from the respective sample measurements.

(b) It may be economically and technically desirable to evaluate the estimated method detection limit before proceeding with 4a. This will: (1) Prevent repeating this entire procedure when the costs of analyses are high and (2) insure that the procedure is being conducted at the correct concentration. It is quite possible that an inflated MDL will be calculated from data obtained at many times the real MDL even though the level of analyte is less than five times the calculated method detection limit. To insure that the estimate of the method detection limit is a good estimate, it is necessary to determine that a lower concentration of analyte will not result in a significantly lower method detection limit. Take two aliquots of the sample to be used to calculate the method detection limit and process each through the entire method, including blank measurements as described above in 4a. Evaluate these data:

(1) If these measurements indicate the sample is in desirable range for determination of the MDL, take five additional aliquots and proceed. Use all seven measurements for calculation of the MDL.

(2) If these measurements indicate the sample is not in correct range, reestimate the MDL, obtain new sample as in 3 and repeat either 4a or 4b.

5. Calculate the variance (S^2) and standard deviation (S) of the replicate measurements, as follows:

$$S^2 = \frac{1}{n-1}\left[\frac{\sum_{i=1}^{n} x_i^2 - \left(\sum_{i=1}^{n} X_i\right)^2}{n}\right]$$

$$S = \left(S^2\right)^{\frac{1}{2}}$$

where:

X_i; i=1 to n, are the analytical results in the final method reporting units obtained from the n sample aliquots and Σ refers to the sum of the X values from i=1 to n.

6. (a) Compute the MDL as follows:

$$MDL = t_{(n-1,1-\alpha=.99)} \quad (S)$$

where:

MDL = the method detection limit

$t_{(n-1,1-\alpha=.99)}$ = the students' t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom. See Table.

S = standard deviation of the replicate analyses.

(b) The 95% confidence interval estimates for the MDL derived in 6a are computed according to the following equations derived from percentiles of the chi square over degrees of freedom distribution (χ^2/df).

LCL = 0.64 MDL

UCL = 2.20 MDL

where: LCL and UCL are the lower and upper 95% confidence limits respectively based on seven aliquots.

7. Optional iterative procedure to verify the reasonableness of the estimate of the MDL and subsequent MDL determinations.

(a) If this is the initial attempt to compute MDL based on the estimate of MDL formulated in Step 1, take the MDL as calculated in Step 6, spike the matrix at this calculated MDL and proceed through the procedure starting with Step 4.

(b) If this is the second or later iteration of the MDL calculation, use S^2 from the current MDL calculation and S^2 from the previous MDL calculation to compute the F-ratio. The F-ratio is calculated by substituting the larger S^2 into the numerator S^2_A and the other into the denominator S^2_B. The computed F-ratio is then compared with the F-ratio found in the table which is 3.05 as follows: if $S^2_A/S^2_B<3.05$, then compute the pooled standard deviation by the following equation:

$$S_{pooled} = \left[\frac{6S_A^2 + 6S_B^2}{12}\right]^{\frac{1}{2}}$$

if $S^2_A/S^2_B>3.05$, respike at the most recent calculated MDL and process the samples through the procedure starting with Step 4. If the most recent calculated MDL does not permit qualitative identification when samples are spiked at that level, report the MDL as a concentration between the

current and previous MDL which permits qualitative identification.

(c) Use the S_{pooled} as calculated in 7b to compute The final MDL according to the following equation:

$$MDL=2.681\ (S_{pooled})$$

where 2.681 is equal to $t_{(12,1-\alpha=.99)}$.

(d) The 95% confidence limits for MDL derived in 7c are computed according to the following equations derived from precentiles of the chi squared over degrees of freedom distribution.

LCL=0.72 MDL

UCL=1.65 MDL

where LCL and UCL are the lower and upper 95% confidence limits respectively based on 14 aliquots.

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

Number of replicates	Degrees of freedom (n-1)	$t_{(n-1,.99)}$
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
00	00	2.326

Reporting

The analytical method used must be specifically identified by number or title ald the MDL for each analyte expressed in the appropriate method reporting units. If the analytical method permits options which affect the method detection limit, these conditions must be specified with the MDL value. The sample matrix used to determine the MDL must also be identified with MDL value. Report the mean analyte level with the MDL and indicate if the MDL procedure was iterated. If a laboratory standard or a sample that contained a known amount analyte was used for this determination, also report the mean recovery.

If the level of analyte in the sample was below the determined MDL or exceeds 10 times the MDL of the analyte in reagent water, do not report a value for the MDL.

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