3M EHS Laboratory

Standard Operating Procedure

Validation of Chromatographic Analytical Methods

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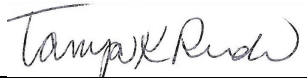

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Quality Assurance

1 Scope and Application

This procedure defines the steps for validating chromatographic analytical methods using non-extracted standards prepared in solvent or using standards prepared from matrix, prior to their use in the 3M Environment, Health, and Safety (EHS) Laboratory. This procedure is based on guidance documents published by the US Environmental Protection Agency (EPA) and by the US Food and Drug Administration (FDA).

This procedure describes the validation of analytical methods used at the 3M EHS Laboratory. Validation demonstrates that the method is suitable for its intended use. The following parameters will be evaluated during validation: Specificity, Selectivity, Minimum Reporting Level (MRL), Calibration, Precision, Accuracy, Carry-Over, Stability, and Range (sections 7.1 to 7.8). Validations for methods utilizing matrix calibration should also include absolute recovery, matrix effects, and dilution schemes. As needed for the method's intended purpose, the following optional parameters may also be evaluated: Detection Limit (DL), Ruggedness, Robustness, System Suitability, Sample Holding Time, and Performance-Based Method Quality Control. Specific study protocols or GPOs will define additional validation parameters to be evaluated, or modify the requirements of this procedure in accordance with the needs of the customer.

This procedure is applicable to non-standard methods, 3M EHS Laboratory-designed or developed methods, standard methods used outside their intended scope, methods published in scientific journals and amplifications and modifications of standard methods, to confirm that the methods are fit for the intended use. The validation shall be as extensive as is necessary to meet the needs of the given application or field of application.

Examples of compatible matrices for analysis include aqueous solutions (Millipore ASTM Type I water, buffered water, synthetic humic water (SHW), etc.), non-aqueous solutions (methanol, acetone, IPA, etc.) or aqueous/solvent mixtures, biological matrices (liver, serum, urine, etc.), environmental matrices (soil, sediment, waste water treatment plant sludge, landfill leachate, water, air). Other miscellaneous matrices (foods, packaging, chemicals, materials, products, etc.) may also be applied. This procedure is appropriate for any matrix for which the validation can be executed in accordance with the requirements of this SOP and/or customer needs.

2 Definitions

2.1 Method validation

Validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled. Alternately, method validation could be considered the determination of the suitability of a test method for a given application. This determination is generally based upon data quality parameters, such as precision, reproducibility, bias, sensitivity, limits of reliable measurement, and ruggedness of the method.

2.2 Accuracy

The closeness of agreement between an experimentally determined value and an accepted reference value. This quantity is often referred to as the recovery when using a QC sample. It is generally expressed as a percentage. Accuracy is generally expressed as the portion of the true value found in the measured value (ideally, 100% accuracy, when the measure is equal to the true value):

$$Accuracy = \frac{(Measured \times 100)}{True}$$

It is also sometimes expressed as a deviation from the true value (ideally, 0% deviation from true value, with an acceptability range expressed as, for example, ± 20% of true value.)

$$Accuracy = \frac{(Measured - True) \times 100}{True}$$

2.3 Precision / Relative Percent Difference (RPD)

ISO/IEC Guide 99:2007 defines the precision of a measurement as the closeness of the agreement between measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions. Precision is a means of evaluating random errors and is determined from the standard deviation of replicate measurements made under repeatability conditions. Under these conditions, ISO/IEC Guide 99:2007 defines the precision as synonymous with repeatability.

Precision can be expressed as the ratio of the standard deviation (σ) and the mean of multiple results using the Relative Standard Deviation (RSD), also called the coefficient of variation (CV). It is generally expressed as a percentage. In the EHS Laboratory, Precision is typically calculated as either an RSD or RPD.

$$\text{Precision} = \text{RSD} = CV = \frac{\text{standard deviation} \times 100}{\text{mean}}$$

The relative percent difference between two numbers (a and b) is the absolute difference between the two numbers divided by the mean of the two numbers. It is always expressed as a percentage.

$$RPD = \frac{|a - b|}{(a + b)/2} \times 100$$

2.4 Bias

A constant or systematic error as opposed to a random error. It manifests itself as a persistent plus or minus deviation of the method average from the accepted reference value. Bias can be assessed by comparing a measured value to an accepted reference value in a sample of known concentration or by determining the recovery of a known amount of contaminant spiked into a sample (matrix spike).

2.5 Minimum Reporting Limit (MRL)

The minimum concentration that can be reported as a quantitated value for a method analyte in a sample following analysis. This defined concentration can be no lower than the concentration of the lowest calibration standard for that analyte and can only be used if acceptable QC criteria for this standard are met.

2.6 Detection Limit (DL)

The DL is defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the reported value is greater than zero. The DL is compound dependent and is dependent on extraction efficiency, sample matrix, fortification concentration, and instrument performance.

2.7 Calibration Curve

The graphical relationship between known values and their instrumental response. Also known as a Standard Curve.

2.8 Calibration Standard

A solution prepared from a stock, intermediate or purchased standard. This solution may also be purchased.

2.9 Correlation Coefficient (r)

A measure of the degree of correlation between two variables. This term is generally used to evaluate the linearity of a Least Squares Linear regression. An r value of 0.99 is at the lower bounds of what is considered linear in the 3M EHS Laboratory. Values of r may range from –1 to +1. A value of +1 denotes perfect direct functional relationship between the two variables. A value of –1 also denotes a perfect inverse relationship. When r = 0, there is no effect of one variable upon the other variable.

2.10 Coefficient of Determination (r^2)

The square of the correlation coefficient. It is the proportion of the variation in the dependent variable that is accounted for by the independent variable.

2.11 Continuing Calibration Verification (CCV)

A standard analyzed periodically during an analytical run to verify the continued accuracy of the calibration curve. This solution may be prepared from a different source or lot # than the calibration curve standards.

2.12 Laboratory Control Sample (LCS)

An aliquot of control matrix to which known quantities of the target analytes, Internal Standards and Surrogate Recovery Standards (when applicable), are added in the laboratory at the time when samples are prepared. The LCSs are analyzed exactly like a laboratory sample and evaluate the performance of the method for that analytical batch. LCSs should be included with each batch of prepared samples.

2.13 Method Blank

An aliquot of control matrix that is treated exactly like a laboratory sample including exposure to all glassware, equipment, solvents, and reagents that are used to prepare the laboratory samples. The method blank is used to determine if test substances or other interferences are present in the laboratory environment, the reagents, or the apparatus.

2.14 Analysis Batch

A series of measurements made under repeatability conditions. A set of study samples that are prepared with calibration standards and all study specific laboratory control samples (LCSs), field matrix spike samples (FMSs), lab matrix spike samples (LMSs), trip blank matrix spike samples (TMBSs), trip blanks, and procedural blanks, and analyzed on the same instrument during a time period that begins and ends with the analysis of the appropriate continuing calibration check standards.

2.15 Sample Holding Time

The period of time between sampling and sample preparation for analysis. The sample holding time stipulated by the final method should be no longer than the time validated for the method by section 7.7 of this SOP. During routine sample analysis, samples should not be analyzed outside of the validated sample holding time without appropriate documentation (e.g. acceptable FMS or TBMS).

2.16 Field Matrix Spike (FMS)

A sample to which known quantities of the target analytes, and/or SRSs and ISs are added to the sample either with bottles spiked in the laboratory before the bottles are sent to the field for collection or spiked in the field. A known, specific volume or weight of sample must be added to the sample container. This may be accomplished by making a “fill to this level” line on the outside of the sample container or using volumetric devices in the field. The FMS is analyzed to ascertain if any matrix effects, interferences, or stability issues may complicate the interpretation of the sample analysis.

2.17 Laboratory Matrix Spike (LMS)

A laboratory matrix spike is an aliquot of a sample to which known quantities of target analytes, ISs and/or SRSs (when applicable) are added in the laboratory. The LMS is analyzed exactly like a laboratory sample to determine whether the sample matrix contributes bias to the analytical results. The endogenous concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LMS corrected for these concentrations.

2.18 Field Blank (FB)/Trip Blank (TB)

Suitable blank matrix, placed in a sample container in the laboratory and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FB is to determine if test substances or other interferences are present during transportation of the samples to the laboratory from the field. This sample is also referred to as a Trip Blank.

2.19 Trip Blank Matrix Spike (TBMS)

An aliquot of suitable matrix, to which known quantities of the target analytes, ISs and/or SRSs, are added in the laboratory prior to the shipment of the collection media. The TBMS is analyzed exactly like a study sample to help determine if the method is in control and whether a loss of analyte or analytical bias could be attributed to sample holding time, sample storage and/or shipment issues. A low and high TBMS are appropriate when expected sample concentrations are not known or may vary.

2.20 Performance-Based Method Quality Control

A combination of sample-based and analytical system-based QC elements such as calibration curves, LCSs, CCVs, LMSs, FMSs, TBs, TBMSs, internal standards, and surrogate recovery standards used during routine analysis that show that results obtained from an analysis are valid and of a known analytical uncertainty.

2.21 Selectivity

Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample.

3 Precautions

As described by the analytical method.

4 Responsibility

Management is responsible for assuring that validated methods are available for the studies conducted at the 3M EHS Laboratory.

Team Leaders are responsible for designing validation protocols and assigning/authorizing personnel to conduct validation studies.

The study director will be responsible for conducting validation studies under Good Laboratory Practices (GLP).

5 Supplies and Materials

As described by the analytical method.

6 Equipment

As described by the analytical method.

7 Procedures

The validation will be as extensive as is necessary to meet the needs of the given application; acceptability criteria may be adjusted to assure that the validated method is relevant to the client's needs and the objectives of the method application. Sections for Specificity, Selectivity, MRL, Calibration, Precision and Accuracy, Carry-Over, Stability, and Range (sections 7.1 to 7.8) should be performed for all validations. If one of these sections are omitted from any validation, the GPO or protocol shall explicitly discuss why the given section is not required for the validation. Additional sections for DL, Ruggedness, Robustness and System Suitability (section 7.9 to 7.12), Sample Holding Time, and Performance Method Quality Control may be added to the validation depending on the intended use of the method. Validations for methods utilizing matrix calibration should also include absolute recovery, matrix effects, and dilution schemes (sections 7.13 and 7.14).

When changes are made to a validated method, the influence of such changes shall be determined and where they are found to affect the original validation, a new method validation shall be performed.

7.1 Selectivity

7.1.1 Procedure:

Create six separate method blanks by extracting a minimum of six different samples of matrix blank. Preferably select samples from different sources, but samples from different individuals (or animals) for biological matrices are acceptable. In cases where there is a limited amount of blank matrix or a specific type of matrix must be used, the number of matrices analyzed may be reduced. If the method uses internal standardization, each matrix blank must be extracted both with and without the internal standard. Evaluate the presence of interferences (or endogenous target analyte) in the retention time window of the analyte(s), and internal standard if applicable. For methods that utilize a solvent calibration, the solvent used for the calibration and sample preparation should be used.

Test the analytical method to differentiate and quantify each analyte in the presence of other components in the sample. Analytes must be either chromatographically resolved, or differentiated by their mass spectra (when mass-selective detectors are used). In cases where analyte peaks co-elute, it must be shown that the co-elution does not interfere with the quantitation. To demonstrate this, compare response factors for the analytes analyzed separately and combined, and show equivalent response. The responses' RPD for both analytes must be less than 15%.

7.1.2 Documentation:

Retain the chromatograms, showing what interferences may be present as well as any other supporting data.

7.1.3 Acceptance Criteria:

Interferences (or endogenous target analyte) below 20% of the MRL (see below, 7.3) are acceptable. For analyses utilizing matrix calibration, combine all matrix samples with acceptable interferences into one bulk blank matrix sample. This bulk sample is used for the preparation of all extracted matrix standard and spiked matrix samples in the rest of this procedure. Again, in cases where there is a limited amount of blank matrix or a specific type of matrix must be used, it is not recommended to combine with other sources. If additional blank matrix is needed, another selectivity determination must be conducted. For methods that utilize a solvent calibration, if interferences are seen then additional method development steps should be done in order to find a solvent that does not interfere with the analysis.

7.2 Minimum Reporting Level (MRL) or Lower Limit of Quantitation (LLOQ)

Based on the needs of the method, either the MRL or LOQ procedure below will be used. The procedure that will be used for the validation should be documented in the project's GPO or protocol.

The MRL procedure is based on the EPA Method 537. Establish a target concentration for the MRL based on the intended use of the method. Establishing the MRL concentration too low may cause repeated failure of ongoing QC requirements. It is recommended that an MRL be established at the mean method blank concentrations + 3σ or 3 times the mean method blank concentration, whichever is greater. This value should be calculated over a period of time, to reflect variability in the method blank measurements. It is recommended that this value be used as an MRL in order to avoid reporting false positive results.

7.2.1 Procedure:

Fortify, and extract seven replicate LCSs at the proposed MRL concentration. These LCSs must follow all steps of the method. Calibrate the instrument using the range indicated by the Calibration section of this procedure (section 7.4) to analyze the MRL LCSs. The MRL LCSs shall not be included in the calibration curve. Analyze the MRL samples against the Calibration curve.

Note: If the intended use of this method also requires the determination of a DL as indicated by section 7.9 of this procedure then this MRL preparation and analysis should be done three times over three days.

7.2.2 Documentation:

Retain all data and calculations that support the determination of the MRL.

7.2.3 Acceptance Criteria:

Calculate the mean measured concentration (Mean) and standard deviation for the MRL replicates. Determine the Half Range for the prediction interval of results (HR_{PIR}) using the equation below.

$$HR_{PIR} = 3.963s$$

where

s = the standard deviation

3.963 = a constant value for seven replicates at the 99% confidence level[1]

Confirm that the upper and lower limits for the Prediction Interval of Result (PIR = Mean ± HR_{PIR}) meet the upper and lower recovery limits as shown below

The Upper PIR Limit must be ≤150% recovery.

$$\frac{Mean + HR_{PIR}}{Fortified\ Concentration} \times 100\% \leq 150\%$$

The Lower PIR Limit must be ≥ 50% recovery.

$$\frac{Mean - HR_{PIR}}{Fortified\ Concentration} \times 100\% \geq 50\%$$

The MRL is validated if both the Upper and Lower PIR Limits meet the criteria described above. If these criteria are not met, the MRL has been set too low and must be determined again at a higher concentration.

[1] The value listed here, 3.963, is the double-sided student's t-value at the 99% confidence level multiplied by $(1+(1/n))^{1/2}$ for seven replicates. For derivation of this value see Winslow, S.D., Pepich, B.V., Martin, J.J., Hallberg, G.R., Munch, D.J., Frebis, C.P., Hedrick, E.J., Krop, R.A. "Statistical Procedures for Determination and Verification of Minimum Reporting Levels for Drinking Water Methods." Environ. Sci. & Technol. 2006, 40, 281-288.

7.2.4 Alternate Procedure for LLOQ Determination

This procedure is based on the Guidance for Industry, Q2B Validation of Analytical Procedures, Methodology, published by FDA. Prepare at least four, preferably five, calibration standard solutions at low levels starting with the estimated Detection Limit (e.g., 1X, 2X, 4X, 6X and 8X DL). Inject these as at least five separate calibration curves. Perform a linear regression on each curve, and record the standard deviation σ_0 of the five y-intercepts. Perform a linear regression on the entire set of results, and record the overall slope S. Calculate the LLOQ as:

$$LOQ = 10 \times \sigma_0 / S$$

Note: The LOD can also be calculated using this method by calculating by 3.3 as opposed to 10.

7.2.5 Documentation

Print the chromatograms, the linear regressions, and the calculations of LLOQ.

7.2.6 Acceptance Criteria

There are no set criteria for the LLOQ.

The method LLOQ is the highest of the calculated LLOQ, and of the lowest level achieving acceptable precision and accuracy. Verify that the LLOQ is showing a peak S/N ratio greater than 10. Report the LLOQ both as a concentration in the calibration solution, and as a concentration in the original matrix sample.

7.3 Calibration

7.3.1 Procedure:

Prepare a range of standard solutions or matrix standards containing at least 7 different concentrations of analyte; preferably, select concentrations in an arithmetic distribution for most of the range, with a few additional concentrations near the lower end of the range (e.g., 50, 75, 100, 200, 300, 400, 500 ng/mL). The lowest calibration standard used to establish the curve must be at or below the concentration of the MRL proposed in section 7.3 of this document. The standard concentrations should span the entire expected range of concentrations of analyte, from below the expected MRL to a minimum of 150% of the highest expected test concentration. Standard solutions should be prepared/extracted exactly as described in the method. Aliquot at least three replicates of each calibration standard solution into appropriate containers for use during the validation analyses. The full curve should be analyzed at least three times during the validation.

7.3.2 Documentation:

For each calibration curve, plot concentration (x-axis) versus response (y-axis). If an internal standardization is used, the ratio of analyte response to internal standard response is used on the y-axis. Calculate the regression equation and the coefficient of determination (r^2). Typically, the origin is excluded and the regression is not forced through the origin. If the curve is forced through the origin, or the origin is included, the validity of this decision must be demonstrated. Retain a copy of the calibration curve with the equation and r^2 for the validation data packet. If response is nonlinear, use a quadratic fit (or other mathematical fit), recording in the raw data the justification for using a quadratic equation (or other mathematical fit). If weighting of the regression is selected, record in the raw data the justification for this decision and for the power selected. Retain all raw data and any supporting documentation.

7.3.3 Acceptance Criteria:

The coefficient of determination (r^2) must be at least 0.990.

Calculate the accuracy of each injection of each calibration standard. The accuracy of each injection of each calibration standard must be between 70% and 130% (except at or below the expected MRL, where 50% to 150% is acceptable). Record and report all data points.

Should the low standard concentrations show insufficient response (demonstrated by a lower accuracy than for the higher levels), that portion of the calibration may be deactivated. Should the high standard concentrations show saturation (demonstrated by a lower accuracy than for the higher levels), that portion of the calibration may be deactivated. This will affect the possible calibration range.

7.4 Method Precision and Accuracy

7.4.1 Procedure:

Fortify method blank samples at a minimum of three levels, including one at the calculated MRL (see above §7.3), a mid-level, and a level 70% of the upper limit of the calibration range. Extract a minimum of three aliquots of each level and analyze according to the method. On a separate day, extract a second set of three aliquots of each level and analyze according to the method with a fresh calibration curve.

7.4.2 Documentation:

Calculate each recovery; calculate the mean and RSD for each level for each day (intra-day statistics), and for both days combined (inter-day statistics). Retain all raw data and supporting documentation.

7.4.3 Acceptance Criteria:

The inter-day and the intra-day mean recovery (accuracy) must be between 70% and 130% at each level. The inter-day and intra-day RSD (precision) must be less than 20%.

7.5 Carry-over

Carry-over should be assessed and monitored during validation and analysis. If carryover occurs, it should be mitigated or reduced to the extent possible.

7.5.1 Procedure:

Analyze the highest acceptable curve standards as determined in the previous section, each followed by a solvent blank.

7.5.2 Documentation:

Retain all data and calculations that support the determination of carry-over.

7.5.3 Acceptance Criteria:

Carry-over in the blank sample following the high concentration standard should not be greater than 20% of the MRL and 5% for the internal standard. If it appears that carry-over is unavoidable, specific measures should be considered and tested during the validation and applied during the analysis of the study samples, so that it does not affect accuracy and precision. This could include the injection of blank samples after samples with an expected high concentration, before the analysis of the next study sample. If carry over is unavoidable then the method validation report and summary page should discuss what is needed to mitigate any effect on the integrity of the data.

7.6 Stability testing

The stability of the analytes throughout the procedure must be validated, from sample collection to final extract analysis. The chemical stability of an analyte in each matrix or solvent under specific conditions for given time intervals is assessed in several ways. Pre-study stability evaluations should cover the expected sample handling and storage conditions during the conduct of the study, including conditions in the lab, during shipment, and at all other secondary sites as applicable.

Stability determinations of the analytes may be performed concurrently with the validations described in this SOP.

7.6.1 Procedure

Prepare and analyze at least three replicates at each of the low, mid and high concentrations for all conditions that need to be challenged. All stability determinations should use samples prepared from a freshly made stock solution. Aged stability samples should be compared to freshly made calibrators. Assessments of analyte stability should be conducted in the same matrix/solvent as that of the study samples and solutions. Conditions used in stability experiments should reflect situations likely to be encountered during actual sample handling and analysis (e.g., long-term, bench top, and room temperature storage; and freeze-thaw cycles). If, during sample analysis for a study, storage conditions changed and/or exceed the sample storage conditions evaluated during method validation, stability should be established under the new conditions. Stock solution stability also should be assessed.

7.6.2 Documentation:

Retain all data and calculations that support the determination of stability intervals.

7.6.3 Acceptance Criteria:

Stability sample results should be within 25% of nominal concentrations. The stability interval is the longest storage stability sample that meets this requirement.

7.7 Range of Quantitation:

7.7.1 Procedure:

Review the test results for all the previous components (Section 7.1-7.7). Determine the range of analyte concentrations for which all components of the validation meet their respective acceptance criteria.

7.7.2 Documentation:

Record the validated range for the method.

7.7.3 Acceptance Criteria:

The range of quantitation is established for all uses of the method, unless specific sources of matrix cause this range to be reduced (e.g., interferences).

7.8 Detection Limit (DL)/Limit of Detection (LOD)/Method Detection Limit (MDL)

While DL determination is an optional component of this procedure, it may be required under some circumstances. It is the responsibility of the project lead to determine if DL determination is required based upon the intended use of the data. The appropriate fortification concentrations below will be dependent upon the sensitivity of the detection system used.

Based on the needs of the method, either the DL, LOD, or MDL procedure below will be used. The procedure that will be used for the validation should be documented in the project's GPO or protocol.

7.8.1 Procedure:

Replicate analyses for this procedure, both extraction and analyses, should be done over at least three days. Prepare at least seven replicate LCSs at a concentration estimated to be near the DL. This concentration may be estimated by selecting a concentration at 2-5 times the noise level.

NOTE: If an MRL confirmation data set meets these requirements, a DL may be calculated from the MRL confirmation data, and no additional analyses are necessary.

Calculate the DL using the following equation:

$$DL = s \times t_{(n-1,1-\alpha=0.99)}$$

where

s = standard deviation of the replicate analyses

$t_{(n-1,1-\alpha=0.99)}$ = Student's t value for the 99% confidence level with n-1 degrees of freedom

n = number of replicates

7.8.2 Documentation:

Retain all data and calculations that support the determination of the DL.

7.8.3 Acceptance Criteria:

There are no minimum or maximum criteria for detection limit. Specific requirements may be needed for regulatory purposes.

7.8.4 Alternate Procedure for LOD:

Estimate the lowest concentration where the analyte in the sample matrix is detected by analyzing at least three matrix blanks. Set the integration parameters so that very small peaks are detected. Estimate the LOD based on 3-4 times the signal to noise (S/N) ratio. Prepare a standard solution dilution at the expected LOD concentration and analyze to verify that the calculated LOD is appropriate. Then use this estimate as the lowest level used in the statistical determination of the analytical limits.

7.8.5 Documentation:

Print the chromatograms of the matrix blanks and low standard, and record the estimated LOD.

7.8.6 Acceptance Criteria:

There are no minimum or maximum criteria for limit of detection. Specific requirements may be needed for regulatory purposes.

7.8.7 Alternate Procedure for MDL

The following alternate procedure is based on the “Definition and Procedure for the Determination of the Method Detection Limit” by EPA (821-R-16-006).

Estimate the MDL from blank levels, S:N ratio between 3 and 5, 3 times the standard deviation of replicate spiked blanks, or previously determined MDLs.

Select a spiking level 2-10 times higher than the estimated MDL from the preceding paragraph.

Process a minimum of seven spiked samples and seven method blanks through all steps of the method. The samples used for the MDL must be prepared in at least three batches on three separate calendar dates and analyzed on three separate calendar dates.

Evaluate the spiking level: If any result for any individual analyte from the spiked samples does not meet the method qualitative identification criteria or does not provide a numerical result greater than zero, then repeat the spiked samples at a higher concentration.

Make all computations as specified in the analytical method and express the final results in the method-specified reporting units.

Calculate the sample standard deviation (S) of the replicate spiked sample measurements and the sample standard deviation of the replicate method blank measurements from all instruments to which the MDL will be applied.

Compute the MDL_s (the MDL based on spiked samples) as follows:

$$MDL_S = t_{(n-1,1-\propto=0.99)} S_S$$

Where:

MDL_S	=	the method detection limit based on spiked samples
$t_{(n-1,\ 1-\propto=0.99)}$	=	the Student's t-value appropriate for a single-tailed 99th percentile t statistic and a standard deviation estimate with n-1 degrees of freedom
S_S	=	sample standard deviation of the replicate spiked sample analyses

Compute the MDL_b (the MDL based on method blanks) as follows:

If none of the method blanks give numerical results for an individual analyte, the MDL_b does not apply. 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.

If some (but not all) of the method blanks for an individual analyte give numerical results, set the MDL_b equal to the highest method blank result. If more than 100 method blanks are available, set MDL_b to the level that is no less than the 99th percentile of the method blank results. For "n" method blanks where n $\geqslant$ 100, sort the method blanks in rank order. The (n * 0.99) ranked method blank result (round to the nearest whole number) is the MDL_b. For example, to find MDL_b from a set of 164 method blanks where the highest ranked method blank results are … 1.5, 1.7, 1.9, 5.0, and 10, then 164 x 0.99 = 162.36 which rounds to the 162nd method blank result. Therefore, MDL_b is 1.9 for n =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.

If all of the method blanks for an individual analyte give numerical results, then calculate the MDL_b as:

$$MDL_b = \bar{X} + t_{(n-1,1-\propto=0.99)} S_b$$

Where:

MDL_b	=	the method detection limit based on method blanks
$t_{(n-1,\ 1-\propto=0.99)}$	=	the Student's t-value appropriate for a single-tailed 99th percentile t statistic and a standard deviation estimate with n-1 degrees of freedom
S_b	=	sample standard deviation of the replicate method blank analyses
$\bar{X}$	=	mean of the method blank results (use zero in place of the mean if the mean is negative)

The MDL is the greater value of the MDL_S and MDL_b.

7.9 Ruggedness

7.9.1 Procedure:

Repeat one day of precision and accuracy, this time with all different personnel, and preferably different equipment.

7.9.2 Documentation:

Calculate each recovery; calculate the mean and RSD for each level (intra-day statistics), and compare with the intra-day statistics obtained for precision and accuracy (see above §7.5). Calculate the mean and RSD for each level using values from all three sets (inter-day statistics) to document ruggedness.

7.9.3 Acceptance Criteria:

The intra-day statistics must meet the acceptance of precision and accuracy (see above §7.5). The inter-day RSD (precision of ruggedness) must be less than 20%.

7.10 Robustness

7.10.1 Procedure:

Evaluate the method for steps where slight changes are possible during routine use of the method. Examples include slight error of preparation of solvent mixture, slight error in pH adjustment, slight error in flow rate, slight change in operating temperature. Design precision / accuracy experiments to evaluate the effect of these changes on the results.

7.10.2 Documentation:

Document the experimental design, including the magnitude of each change; each experiment should include a deviation in each direction (e.g., +pH 0.2, and –pH0.2). Calculate each recovery; calculate the mean and RSD for each change (as intra-day statistics), and combined with the results at the nominal setting (inter-day statistics).

7.10.3 Acceptance Criteria:

The inter-day and the intra-day mean recovery (accuracy) must be between 70% and 130% at each level. The inter-day and intra-day RSD (precision) must be less than 20%. Changes that result in inter-day or intra-day statistics outside these values must be identified in the method as critical for the acceptable performance of the method.

7.11 System Suitability:

7.11.1 Procedure:

Review the test results for all the previous components. Evaluate the parameters that may be used for documenting the proper operation of the analytical equipment (e.g., injection of DL-level, MRL-level, or mid-level standards, injection of solvent blanks, etc.) Establish acceptable limits (e.g., precision of the response, precision of the retention time, minimum resolution between close peaks, etc.) Demonstrate that these limits are met in at least one analytical set.

7.11.2 Documentation:

Record the parameters selected and the limits selected. Include results of the demonstration.

7.11.3 Acceptance Criteria:

The system suitability selected is established for all uses of the method before the start of any analytical set to demonstrate that the equipment is operating properly.

7.12 Absolute Recovery and Matrix Effect

This section is only appropriate for methods utilizing matrix calibration.

7.12.1 Procedure:

Absolute Recovery: Fortify method blank samples at a minimum of three levels, including one at 3-5 times the determined MRL (see above §7.3), a mid-level, and one at about 80% of the upper limit of the calibration range before extraction. Extract a minimum of three aliquots of each level and analyze according to the method.

Matrix Effects: Extract a minimum of nine aliquots of blank matrix, and fortify them after extraction at levels that would give the same theoretical concentration as for the set of samples fortified before extraction (i.e., three replicates at each of three levels, 3-5 times MRL, mid-range, and 80% of the upper limit).

Analyze absolute recovery and matrix effects extracts using a non-extracted calibration curve (standards in solvent)

7.12.2 Documentation:

Retain all data and calculations that support the determination of the absolute recovery and matrix effect.

7.12.3 Acceptance Criteria:

Calculate each recovery; calculate, separately for absolute and for matrix effects, the mean and RSD for all levels combined. There is no acceptance limit for absolute recoveries; however very low recoveries in the samples fortified before extraction (e.g., 30%) may indicate the need for revising or optimizing the method. The RSD (precision) must be less than 20% for samples fortified before extraction.

There is no acceptance limit for matrix effects recoveries; however very low or very high recoveries in the samples fortified after extraction (e.g., 30% or 150%) may indicate a matrix effect, either matrix suppression or matrix enhancement.

7.13 Dilution Scheme:

This section is only appropriate for methods utilizing a matrix calibration curve.

It is expected that samples may occasionally exceed the validated upper limit of the range of quantitation. These samples will need to be diluted to be quantitated within the validated range. The reference dilution scheme, which does not need to be validated, is to take a new aliquot of the original matrix, dilute it with blank matrix, and extract the diluted sample. Should another procedure be desirable, that procedure should be validated. Care should be taken to ensure that the dilution matrix is free of the target analyte of interest.

7.13.1 Procedure:

In particular, should it be desirable to dilute the final extract into the range with solvent (or with solvent spiked with internal standard), one over-range spiked blank matrix should be extracted in five replicates, and diluted before analysis.

7.13.2 Documentation:

Retain all data and calculations that support the determination of the dilution scheme.

7.13.3 Acceptance Criteria:

The mean recovery (accuracy) must be between 75% and 125%. The RSD (precision) must be less than 20%.

7.14 Uncertainty

After all other phases of the validation are complete, follow ETS-12-012 to estimate the method's measurement uncertainty. Include results of the evaluation in the validation report and the analytical method.

8 Records

Retain all raw data and supporting raw data for archiving.

Fill out appropriate preparation worksheets completely, making sure to include all initials and dates, along with the study number and sample identification.

Conclude the report with a statement as to whether the method is fit for the intended use.

Include in the final report a table similar to the table in Attachment A to summarize the results of the validation. It is recommended that a copy of this table be included in the SOP database with the associated method. This attachment can be used retrospectively for methods validated prior to the approval of this SOP.

Archive electronic data according to Chapter 13 SOPs.

9 Attachments

Attachment A - Validation Summary Table

10 References

FDA - Guidance for Industry - Q2B Validation of Analytical Procedures: Methodology - ICH - November 1996 http://www.fda.gov/cder/guidance/1320fnl.pdf

FDA - Guidance for Industry - Bioanalytical Method Validation - May 2001 http://www.fda.gov/cder/guidance/4252fnl.pdf

EPA Method 537: DETERMINATION OF SELECTED PERFLUORINATED ALKYL ACIDS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS). Version 1.1 September 2009, EPA Document #: EPA/600/R-08/092. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=198984&simpleSearch=1&searchAll=EPA%252F600%252FR-08%252F092+

EPA - Definition and Procedure for the Determination of the Method Detection Limit, Revision 2, December 2016, EPA 821-R-16-006.

Winslow, S.D., Pepich, B.V., Martin, J.J., Hallberg, G.R., Munch, D.J., Frebis, C.P., Hedrick, E.J., Krop, R.A. “Statistical Procedures for Determination and Verification of Minimum Reporting Levels for Drinking Water Methods.” Environ. Sci. & Technol. 2006, 40, 281-288.

11 Revisions

Revision Number	Reason for Revision
1	Modified the scope (section 1) to include other methods than 3M Environmental Laboratory-developed methods. Added a definition of method validation (section 2). Added an introductory paragraph to section 7 providing some flexibility in determining the scope of the validation, and the basis for this flexibility. Removed paragraph 7.13 describing optional tests Replaced with a new paragraph 7.13 for the estimation of measurement uncertainty Added a requirement for a conclusion of the method's fitness for use. Added ETS-12-012 to section 11
2	Removed the reference to ETS-8-29 from section 7.12. Updated format (removed revision date from first page).
3	Extensive Revision of the SOP combining ETS-4-001 and ETS-4-010. Clarified which portions of the SOP are required for all validations. Added procedures for MRL and MDL determination. Added numerous definitions from other SOPs for clarification. Reworded many portions of the SOP for clarification. Added Attachment A for the summarization of validation activities.
4	Updates made to sections 1 and 7. Removed the affected documents section, 11.

Method Number:
Method Name:
Validation LIMS Number:

Table of results (complete one table for each matrix or solvent used):

Analyte	Analyte 1	Analyte 2	Analyte 3	Analyte 4	Analyte 5	Analyte 6
MRL (Conc)						
MRL (Avg. % Recovery)						
Curve regression						
Curve r^2						
Low LCS Avg % Rec and StDev						
Mid LCS Avg % Rec and StDev						
High LCS Avg % Rec and StDev						
Carry Over % of MRL						
Quant. Range						
DL (Conc)						
Uncertainty						

Specificity and Selectivity Discussion:

Carry-over discussion: (discuss any issues with carry over with each analyte):
Analyte 1:
Analyte 2:
Analyte 3:
Analyte 4:
Analyte 5:
Analyte 6:

Stability Discussion:
Standard Stability:
Sample Stability:

Ruggedness Discussion:

Robustness Discussion:

Suggested System Suitability Tests: