STANDARD OPERATING PROCEDURE

**ESTIMATION OF ANALYTICAL MEASUREMENT UNCERTAINTY**

# Signature and Title

**--------------------------------------------------------------------------------- -----------------**

# Director Date

**--------------------------------------------------------------------------------- -----------------**

# Laboratory Quality Assurance Date

**TABLE OF CONTENTS PAGE**

# SCOPE AND APPLICATION 3

# PURPOSE 4

# SUMMARY OF METHOD 5

# DEFINITIONS 6

# QUALITY CONTROL 10

# PROCEDURE 11

# DOCUMENTATION 12

# REFERENCES 13

# APPENDIX A: CALCULATIONS 14

# APPENDIX B: GENERAL UNCERTAINTY BUDGET 22

**APPENDIX C: EXAMPLE UNCERTAINTY BUDGET 23**

APPENDIX D: EXAMPLE SPREADSHEET 25

APPENDIX E: SOFTWARE VALIDATION 28

1. **SCOPE AND APPLICATION**
   1. This Standard Operating Procedure (SOP) applies to test methods that are within the scope of ISO/IEC 17025-1999 Standard: *General Requirements for the Competence of Testing and Calibration Laboratories* and it is based on the general rules outlined in *Guide to the Expression of Uncertainty in Measurement (GUM).* The GUM approach is recommended in ISO/IEC 17025. (17025, 5.4.6.3 Note 3)
   2. According to ISO/IEC 17025, a laboratory “shall have and shall apply procedures for estimating uncertainty of measurement.” (17025, 5.4.6.2)
   3. Where appropriate, an estimation of uncertainty must be reported with the test result. (17025, 5.10.3.1c)
   4. When estimating analytical measurement uncertainty, all significant components of uncertainty must be identified and quantified. (17025, 5.4.6.3)
   5. Components that affect analytical measurement uncertainty include sampling, handling, transport, storage, preparation, and testing. (17025, 5.4.1)
   6. Components of uncertainty that do not contribute significantly to the total uncertainty of the test result can be neglected. (17025, 5.6.2.2.1)
   7. Estimation of analytical measurement uncertainty is not required for qualitative tests with pass/fail or detect/non-detect results. However, decision uncertainty may be required by estimating Type I and Type II errors. Certain biological tests, spot tests, and immunoassay tests are included in this category.
   8. Estimation of analytical measurement uncertainty is not required for well-recognized quantitative test methods where the reference method specifies:

* bias and precision acceptance limits,
* form of presentation of the test result, and
* procedure for estimating analytical measurement uncertainty

Certain methods with well-characterized uncertainties are included in this category (e.g., NIOSH 7400). (17025, 5.4.6.2 Note 2)

* 1. Estimation of analytical measurement uncertainty is required for quantitative test methods where the estimation of uncertainty is not specified in the method. The QC-based Nested Approach for Estimating Analytical Measurement Uncertainty Spreadsheet can be used to estimate analytical measurement uncertainty when Quality Control data is available. Certain performance-based methods (published regulatory or consensus methods) are included in this category.
  2. This SOP is for use by environmental testing laboratories in the development and implementation of their quality systems.
  3. To be recognized as competent for carrying out specific environmental tests, this SOP describes the requirements that a laboratory must successfully demonstrate for the estimation of analytical measurement uncertainty.
  4. This SOP includes requirements and information for assessing competence, and for determining compliance by the organization or accrediting authority granting the accreditation or approval.
  5. Accrediting authorities may use this SOP in assessing the competence of environmental laboratories.
  6. If more stringent standards or requirements are included in a mandated test method or by regulation, the laboratory shall demonstrate that such requirements are met.
  7. If it is not clear which requirements are more stringent, the standard from the method or regulation must be followed.

# PURPOSE

* 1. The International Organization of Standardization (ISO) and the International Electrotechnical Commission (IEC) developed the ISO/IEC 17025 standard, *General Requirements for the Competence of Testing and Calibration Laboratories,* December 1999. A primary requirement in the standard is the estimation of analytical measurement uncertainty.
  2. This Standard Operating Procedure (SOP) describes the rationale and methodology for estimating analytical measurement uncertainty using the Quality Control-based Nested Approach for Estimating Analytical Measurement Uncertainty Spreadsheet. Other approaches that meet the requirements of ISO/IEC 170215 may also be used to estimate analytical measurement uncertainty.
  3. The estimation of analytical measurement uncertainty is formalized in the *U.S. Guide to the Expression of Uncertainty in Measurement*, (US GUM), published by American National Standards Institute (ANSI) in 1997. The US GUM is the ANSI adoption of the ISO *Guide to the Expression of Uncertainty in Measurement* (GUM), published in 1993, and it establishes general rules to evaluate and express uncertainty for quantitative analytical measurements.
  4. The general rules outline the process for identifying components of uncertainty, quantifying component standard uncertainty, combining standard uncertainties, expanding combined uncertainty, and reporting uncertainty.
  5. The QC-based Nested Approach for Estimating Analytical Measurement Uncertainty Spreadsheet was developed to automate estimation of analytical measurement uncertainty.
  6. Laboratory-generated quality control data is used to populate a Microsoft Excel spreadsheet that automatically partitions sources of uncertainty, quantifies uncertainty for each component, and calculates the expanded uncertainty with optional bias correction. A histogram is generated to identify significant and negligible sources of uncertainty.

1. **SUMMARY OF METHOD**
   1. The concept of analytical measurement uncertainty is widely recognized among analytical chemists. Replicate preparation and testing of a sample generates a range of results. This variability of results represents the analytical measurement uncertainty.
   2. Samples are routinely prepared and tested only once and replicate preparation and testing of environmental samples is not practical. However, any rigorous statistical determination of uncertainty based on a single test measurement is not possible. “There is no statistical basis for a confidence level statement of one measurement unless supported by a control chart or other evidence of statistical control.” (Taylor, J.K., 28)
   3. Readily available laboratory Quality Control Chart data can be used to estimate the analytical measurement uncertainty for single test results. Using the laboratory generated Quality Control Limits, a mathematical model can be constructed to systematically “back-out” component uncertainties.
   4. The steps for estimating uncertainty are incorporated into the following conceptual algorithm:

* Specify the analyte of interest that is to be quantified
* Identify the sources of analytical measurement uncertainty
* Quantify the components of analytical measurement uncertainty
* Calculate the combined and expanded analytical measurement uncertainty
  1. The first step is to state what is to be quantified (the analyte of interest). A summary of the chemical preparation and testing methods is included.
  2. The second step is to identify the sources of analytical measurement variability or uncertainty. The sources of uncertainty can be partitioned into the following general components:
  + Large-scale site population variability
  + Small-scale sample location variability
  + Field sampling and laboratory subsampling variability
  + Sample chemical preparation variability
* Sample test measurement variability

An Uncertainty Budget can be developed to tabulate analytical measurement uncertainty.

* 1. The third step is to quantify the components of analytical measurement uncertainty. A frequent approach to evaluating and expressing uncertainty of a measurement is the use of the statistical concept of the confidence interval. The confidence interval is the range of results that reasonably captures the analyte concentration with a specified probability. When the confidence interval is constructed by the statistical analysis of replicate results, the approach is a Type A evaluation of standard uncertainty (US GUM, Section 4.2). When the confidence interval is not constructed by statistical analysis of replicate results, the approach is a Type B evaluation of standard uncertainty (US GUM, Section 4.3).
  2. For statistical analysis (Type A evaluation), the standard deviation is calculated for the percent deviation (relative bias) for each quality control standard or sample. The standard deviation of analytical measurement results represents the standard uncertainty.
  3. The fourth step is to combine the individual uncertainties and then apply a “coverage factor” which is chosen on the basis of the desired level of confidence to be associated with the interval around the measurement. Coverage factors are usually 2 or 3, corresponding to intervals with levels of confidence of approximately 95% and 99%, respectively.

1. **DEFINITIONS**
   1. Acceptance limits are data quality limits specified by the test method or generated by the laboratory.
   2. Accuracy is the agreement of a single analytical measurement result to a reference value. Accuracy is a combination of random and systematic components. Random components affect the precision of the test result and systematic components affect the bias of the test result. See bias and precision.
   3. “Backing-out” is the rearrangement of the “square root-of-the-sum-of-the-squares” equation to solve for an unknown component standard uncertainty.
   4. Bias is the deviation of the mean of replicate analytical measurements from a reference analyte concentration. Relative bias is represented by analytical measurement mean minus the reference analyte concentration and the difference divided by the reference analyte concentration. See accuracy and precision.
   5. Combined standard uncertainty is the standard uncertainty of the analytical measurement result that is the sum in quadrature (square-root-of-the-sum-of-the-squares) of the component standard uncertainties.
   6. Coverage factor is the numerical factor used as a multiplier of the combined standard uncertainty to expand the uncertainty corresponding to a specific level of confidence. The Student’s *t*-distribution is used for determining the coverage factor.
   7. Duplicate samples are two samples taken from the same population and carried through certain stages of sampling and testing. Duplicate sample include field co-located duplicate samples, field-split duplicate samples, and laboratory duplicate subsamples.
   8. Expanded uncertainty is the quantity defining an interval enveloping the analytical measurement that captures a large fraction of the distribution of analyte concentrations that could be attributable to the quantity measured. The combined standard uncertainty is multiplied by the coverage factor to calculate the expanded uncertainty.
   9. Field samples are sampled and tested to represent the large-scale population distribution. Sampling usually includes primary sampling stage where the sample is extracted from the sample location and secondary sampling stage where the collected sample is reduced to a subsample after physical preparation such as milling and blending. Testing usually includes chemical preparation such as extraction and separation, and instrumental analysis.
   10. Field co-located duplicate samples are samples collected near (0.5 to 3 feet) the field sample. Co-located duplicate samples are used to quantify the variance of the sampling strategy, sample collection, preparation, and testing stages.
   11. Field-split duplicate sample is a field sample homogenized in the field and split into two or more portions that are sent to the laboratory as separate samples. Field-split duplicate samples are used to quantify the variance of the sample collection, preparation, and testing stages.
   12. Hypothesis testing is the formulation of a decision such as not rejecting the null hypothesis, or rejecting the null hypothesis and accepting the alternative hypothesis. An example of hypothesis testing is that the null hypothesis (H0)is H0 > the Action Level (AL) and the alternative hypothesis (HA) is HA < AL.
   13. Independent Calibration Verification (ICV) is a standard solution used to verify the calibration curve derived from a source independent of the instrument calibration standard. The ICV is use to quantify second source standard variance and bias.
   14. Instrument Calibration Standard (ICS) is a reference material used to standardize an analytical instrument.
   15. Instrument Performance Check (IPC) is the analyses of one of the ICSs to verified initial and continuing calibration. The IPC is used to quantify the instrumental testing repeatability variance and bias.
   16. Laboratory control sample (LCS) is a clean-matrix reference material with an established analyte concentration derived from a source independent of the instrument calibration standard. The LCS is carried through the entire chemical preparation and testing procedures. The LCS is used to quantify the variance and bias of the chemical preparation and instrumental testing stages without matrix interference. Same a laboratory fortified blank.
   17. Laboratory duplicate subsample is a portion of the collected sample that is carried through the chemical preparation and testing. The Laboratory duplicate subsample is used to quantify the variance of the chemical preparation and instrumental testing stages with matrix interferences.
   18. Laboratory fortified blank (LFB) is the same as the Laboratory Control Sample.
   19. Laboratory fortified matrix (LFM) is the same as the Matrix Spike Sample.
   20. Matrix spiked sample is a subsample spiked with reference material with an established concentration derived from a source independent of the instrument calibration standard. Matrix spiked sample are carried through the chemical preparation and testing stages. Matrix spiked samples are used to quantify the variance and bias of the chemical preparation and testing stages with matrix interference.
   21. Precision is the dispersion of replicate analytical measurements. Precision is represented by the variance, relative variance, standard deviation, relative standard deviation, or range. See accuracy and bias.
   22. QC-based Nested Approach Spreadsheet is the Microsoft Excel spreadsheet used to automatically calculate analytical measurement uncertainty.
   23. Quality Assurance (QA) is the program used to establish confidence in the quality of data generated by the laboratory. Quality Control is a component of Quality Assurance.
   24. Quality Control (QC) is the program that includes planning, implementing, monitoring, assessing, and adjusting processes that the laboratory uses to measure its capability and performance in generating quality data.
   25. Quality Control Chart is a graph of analytical measurement results for a specific QC standard plotted sequentially with upper and lower control limits (±3σ). A central line that is the best estimate of the average variable plotted, and upper and lower warning limits (±2σ) are usually included in the Quality Control Chart.
   26. Quality Control Sample is the same as the Independent Calibration Verification (ICV).
   27. Reference material is a traceable standard with an established analyte concentration.
   28. Replicate analytical measurements are two or more results representing the same sample parameter. Replicate analytical measurements are used to quantify the analytical measurement repeatability precision.
   29. Replicate samples are two or more samples representing the same population parameter.
   30. Standard uncertainty is the analytical measurement uncertainty expressed as a standard deviation. The relative standard deviation represents the relative standard uncertainty.
   31. Type I error results in hypothesis testing for rejecting the null hypothesis when it should not be rejected.
   32. Type II error results in hypothesis testing for not rejecting the null hypothesis when it should be rejected.
   33. Type A evaluation of uncertainty is the method of evaluation of uncertainty by the statistical analysis of a series of test results.
   34. Type B evaluation of uncertainty is the method of evaluation of uncertainty by means other than statistical analysis.
   35. Uncertainty is the parameter associated with the analytical measurement results that characterizes the dispersion of the values that could be reasonable attributed to the quantity measured.
   36. Uncertainty interval is the range of analyte concentrations that an analytical measurement could represent at a specified level of confidence. The relative standard deviation is used to represent the relative standard uncertainty in the QC-based Nested Approach.
2. **QUALITY CONTROL**
   1. The estimation of analytical measurement is an integral component of the Quality Assurance-Quality Control system. “One of the prime objectives of quality assurance is to evaluate measurement uncertainty.” (Taylor, J.K., 10)
   2. A component of Quality Control is laboratory generated Quality Control Charts. The use of the QC-based Nested Approach Spreadsheet is based on the bias and precision limits of Quality Control Charts.
   3. Quality Control Charts must represent the laboratory’s capability and performance, and the analytical measurement system must have a stable pattern of variation. “Until a measurement operation has attained a state of statistical control, it cannot be regarded in any logical sense as measuring anything at all.” (Taylor, J.K., 13)
   4. The QC-based estimation of analytical measurement uncertainty per analyte, matrix, and technology must be calculated when Quality Control Charts are updated. Usually Quality Control Charts are updated annually or when there is a major change in primary analytical personnel, analytical instrumentation, or analytical procedures.
   5. Though it is recognized that other sources of uncertainty contribute to total analytical measurement uncertainty, the laboratory is usually only responsible for reporting estimations of uncertainty for the analysis components of the laboratory. If the laboratory has access to field-split duplicates data or field co-located duplicate data, then sample collection and subsampling, and sampling strategy components can be quantified.
   6. Each analyst is responsible for calculating estimations of uncertainty and Quality Control assessment of the reasonableness of the calculations. The automated Microsoft Excel spreadsheet (QC-based Nested Approach for Estimating Analytical Measurement Uncertainty) calculates the analytical measurement uncertainty based on Quality Control Chart data.
   7. The person responsible for Quality Assurance must review analytical measurement uncertainty calculations at least annually. The estimation of analytical measurement uncertainty must be uniform and consistent to ensure data quality and data comparability.

#### PROCEDURE

#### If the reference method results in qualitative or semi-quantitative measurements, then the report result is an estimate and analytical measurement uncertainty is not quantified.

#### If the reference method specifies the procedure for estimation of analytical measurement uncertainty, then follow the reference method procedure.

#### If the reference method does not specify the procedure for estimating analytical measurement uncertainty, then use this procedure.

#### The analytical measurement uncertainty for each quantitative field of testing must be estimated per analyte of interest, sample matrix, and analytical technology.

#### The automated calculation of laboratory analytical measurement uncertainty requires the following Quality Control standards:

* Instrument Calibration Standard or Instrument Performance Check
* Independent Calibration Verification or Quality Control Sample
* Laboratory Control Sample or Laboratory Fortified Blank
* Matrix Spiked Sample or Laboratory Fortified Matrix

#### Acquire twenty analyses for each of the QC standards described in Section 6.5. Twenty analyses are required for the automated calculation of analytical measurement uncertainty. These data may be acquired from Quality Control Charts.

#### Subtract the reference analyte concentration from the analytical measurement result and divide the difference by the reference analyte concentration.

#### Multiply relative error by 100 to calculate the percent deviation. The percent deviation is relative deviation from the reference analyte concentration multiplied by 100. Input the percent deviation data into the QC-based Nested Approach Spreadsheet in the appropriate column. See Appendix A for the mathematical algorithm used to calculate analytical measurement uncertainty.

#### Input the following information into the spreadsheet:

* Analyte, matrix, and technology
* Confidence level
* Analytical measurement
* Units

#### The confidence level is usually 95%, but other confidence levels can be selected according to client requirements. The QC-based Nested Approach Spreadsheet presents the confidence interval associated with the analytical measurement. Bias-correction is also presented for comparison. The bias-correction is based on the recovery efficiency of the laboratory chemical preparation and instrumental analysis components.

#### Representative sampling and subsampling eliminates sampling bias and imprecision associated materialization error.

#### The Uncertainty Budget of the general analytical measurement components of uncertainty can be tabulated from the QC-based Nested Approach Spreadsheet histogram. An example Uncertainty Budget is presented in Appendix B.

#### The Uncertainty Budget of specific analytical measurement components of uncertainty can be tabulated by itemizing sources of uncertainty that may or may not affect total analytical measurement uncertainty. An example Uncertainty Budget with specific sources of uncertainty is presented in Appendix C.

#### An example spreadsheet is presented in Appendix D with copper in wastewater by ICP quality control results.

#### The data from Appendix D is used in Appendix E to validate the QC-based calculator spreadsheet software.

### DOCUMENTATION

### Documentation of a analytical measurement may require the following:

* Description of the methods used to calculate the measurement result and estimation of analytical measurement uncertainty
* Uncertainty Budget of uncertainty components
* Correction factors used to normalize (correct for bias) the data
* Report the analytical measurement result with estimated expanded uncertainty and the level of confidence

### The estimated analytical measurement uncertainty must be reported to the clients when uncertainty affects the interpretation of the analytical measurement result. (17025, 5.10.3.1 c) An example is comparing the uncertainty interval to a specification limit to determine whether the uncertainty affects compliance.

### The QC-based Nested Approach can be used to estimate the uncertainty interval and correct for bias when reporting analytical measurement results.

### REFERENCES

### ISO 17025-1999, *General Requirements for the Competence of Testing and Calibration Laboratories,* The International Organization of Standardization (ISO) and the International Electrotechnical Commission (IEC), December 1999.

### American National Standard for Expressing Uncertainty - U.S. Guide to the Expression of Uncertainty in Measurement, (US GUM), American National Standards Institute (ANSI) in 1997.

### ISO *Guide to the Expression of Uncertainty in Measurement* (GUM), 1993.

*Quality Assurance of Chemical Measurements*, Taylor, John Keenan, Lewis Publishers, 1987.

* *Environmental Analytical Measurement Uncertainty Estimation: Nested Hierarchical Approach*, Ingersoll, William Stephen, 2001.
* *QC-based Nested Approach for Estimating Measurement Uncertainty Spreadsheet*, Microsoft Excel Spreadsheet, Ingersoll, William Stephen, 2002.

**APPENDIX A: CALCULATIONS**

A.1 The mathematical model for uncertainty propagation is the Taylor series expansion.

A.1.1 The Equation A.1 is the Taylor series expansion for determining the estimated combined variance (*uc2)*:

*uc2(y) =* *(∂ f/∂ xi)2 u2(xi) +2* *(∂ f/∂ xi)(∂ f/∂ xj )u(xi,, xj)*

**Equation A.1**

A.1.2 The Taylor series expansion equation can be simplified to calculate the combined standard uncertainty in Equation A.2:

*u2c(y) =* *[c1u(x1)]2 + [c2u(x2)]2 +…+[cn u(xn)]2 + 2* *cicju(xi)u(xj)rij*

**Equation A.2**

The symbol *ci* represents *∂ f /∂ xi ,* symbol *rij* represents the correlation of *xi* and *xj*. The second term is the co-variance associated with *xi* and *xj .*The estimated co-variances or the estimated correlation coefficients are required if the variable *xi* and *xj* components are dependent. If the variable *xi* and *xj* are independent, then the co-variant term is equal to zero and the co-variant term drops out of the equation. The combined standard uncertainty estimate *uc* uses the quadrature equation or “square-root-sum-of-squares” method for combining the standard uncertainties. This equation is the law of propagation of uncertainty.

A.1.3 There two primary approaches for applying the law of propagation of uncertainty: additive and multiplicative.

A.14 If *y* is an additive function of *x1 , x2 ,…xn* , then Equation A.3 is used:



**Equation A.3**

A.1.5 If *y* is a multiplicative function of *x1 , x2 ,…xn*, then Equation A.4 is used to determine the relative combined standard uncertainty *uc,r* where *y ≠ 0* and *|y|* is the absolute value of *y*:



**Equation A.4**

A.1.6 The QC-based Nested Approach Spreadsheet is based on multiplicative combination of component efficiencies; therefore Equation A.5 is used to estimate analytical measurement uncertainty.

A.2 The QC-based Nested Approach for Estimating Analytical Measurement Uncertainty is an automated system for calculating analytical measurement uncertainty.

A.2.1 The data inputted into the Microsoft Excel QC-based Nested Approach Spreadsheet are the percent deviation of the Quality Control Chart data.

A.2.2 The ICS, ICV, LCS, and MIS standards are used to calculate analytical measurement uncertainty for the laboratory.

A.2.3 Calculate the percent deviation *(%D)* by subtracting the reference analyte concentration *(T)* from the each individual analytical measurement *(Xi),* divide the difference by *T,* and multiplying the quotient by 100 in Equation A.5:



**Equation A.5**

A.2.4 On page 1 input the *%D* of 20 Quality Control analytical measurements for the ICS, ICV, LCS and MIS in the appropriate column of the spreadsheet.

A.2.5 On page 1, when the data is inputted, the spreadsheet automatically calculates:

* Relative standard uncertainty (*ur*) of the 20 (*n*) individual *%Di* results
* Average bias (%) based on the average (%*)* of the *n* individual *%Di* results
* Average recovery (%)of the *n* individual *%Di* results

The following equations are used to calculate:

* % in Equation A.6
* %in Equation A.7
* *ur* in Equation A.8

%

**Equation A.6**



**Equation A.7**



**Equation A.8**

A.2.6 On page 2, when the data is inputted, the spreadsheet automatically calculates standard uncertainty (*s* or *ur*), recovery, and systematic error for components:

* IME – Intrinsic Measurement Effect
* SPE – Spike Preparation Effect
* PME – Preparation Method Effect
* MIE – Matrix Interference Effect
* SCE – Sample Collection Effect
* SLE – Sample Location Effect

A.2.7 The relative standard deviation of the Instrumental Calibration Standard (ICS) represents the uncertainty associated with instrumental repeatability Intrinsic Measurement Effects (IME) in Equation A.9.

*ICSur = IMEur*

## Equation A.9

A.2.8 The relative standard deviation of the Independent Calibration Verification (ICV) is a combination of IME and the Spike Preparation Effects (SPE) in Equation A.10.



**Equation A.10**

Equation A.10 is rearranged to “back-out” the SPE standard uncertainty from the known ICV and IME standard uncertainties:



A.2.9 The relative standard deviation of the Laboratory Control Sample (LCS) is a combination of IME, SPE, and the Preparation Method Effects (PME) in Equation A.11.



## Equation A.11

Equation A.11 is rearranged to “back-out” the PME standard uncertainty from the known ICV, IME, and SPE standard uncertainties:



A.2.10 The relative standard deviation of the Matrix Spiked Sample (MIS) is a combination of IME, SPE, PME, and the Matrix Interference Effects (MIE) in Equation A.12.



# Equation A.12

Equation A.12 is rearranged to “back-out” the MIE standard uncertainty from the known MIS, IME, SPE, and PME standard uncertainties:



A.2.11 The relative standard deviation of the Field-split Duplicate Sample (FSR) is a combination of IME, PME, MIE, and the Sample Collection and Subsampling Effects (SCE) in Equation A.13.



## Equation A.13

Equation A.13 is rearranged to “back-out” the MIE standard uncertainty from the known FSR, IME, PME, and MIE standard uncertainties:



A.2.12 The relative standard deviation of the Field Co-located Duplicate Sample (CLR) is a combination of IME, PME, MIE, SCE, and the small-scale Sample Location Effects (SLE) in Equation A.14.



**Equation A.14**

Equation A.14 is rearranged to “back-out” the SLE standard uncertainty from the known CLR, IME, PME, MIE and SCE standard uncertainties:



A.2.13 The large-scale natural variability of the analyte distribution inherent in the sampling site is not measured directly, but is derived by a process of sampling and testing. This process confounds the natural site population parameter mean and standard deviation. The relative standard deviation of the collection of Site Field Samples (SFS) is a combination of IME, PME, MIE, SCE, SLE, and the large-scale Sampling Site Effects (SSE) in Equation A.15.



**Equation A.15**

Equation A.15 is rearranged to “back-out” the SSE standard uncertainty from the known SFS, IME, PME, MIE, SCE, and SLE standard uncertainties:



A.2.14 The spreadsheet has a logic test to make the calculations more robust. If a component in the logic hierarchy has a standard uncertainty less than the standard uncertainty of a component lower in the logic hierarchy, then the spreadsheet reports zero for the higher component.

A.2.15 The component recovery () for IME is calculated by using Equation A.16.



**Equation A.16**

A.2.16 The component recovery () for SPE is calculated by using Equation A.17.



**Equation A.17**

A.2.17 The component recovery () for PME is calculated by using Equation A.18.



**Equation A.18**

A.2.18 The component recovery () for MIE is calculated by using Equation A.19.



**Equation A.19**

A.2.19 The component systematic error () for IME is calculated by using Equation A.20.



**Equation A.20**

A.2.20 The component systematic error () for SPE is calculated by using Equation A.21.



**Equation A.21**

A.2.21 The component systematic error () for PME is calculated by using Equation A.22.



**Equation A.22**

A.2.22 The component systematic error () for MIE is calculated by using Equation A.23.



**Equation A.23**

#### A.2.23 The analyst must select from the menu and input the percent confidence. The following table (Table A.1) is a list of available confidence levels with corresponding coverage factors based on 20 analytical measurements.

**TABLE A.1: CONFIDENCE LEVELS AND COVERAGE FACTORS**

|  |  |
| --- | --- |
| **Confidence Level**  **(Percent Confidence)** | **Two-Tailed Distribution Coverage Factor**  **(Student’s t-Value for 19 Degrees of Freedom)** |
| **80** | **1.328** |
| **90** | **1.729** |
| **95** | **2.093** |
| **99** | **2.861** |

A.2.24 After selection of the confidence level, the spreadsheet automatically presents the coverage factor and calculates the Relative Analytical Measurement Uncertainty and the Relative Systematic Error.

A.2.25 The combining relative standard uncertainty in percent for routine single test measurements (*RSTur*) is a combination of IME, PME, and MIE in Equation A.24.



**Equation A.24**

A.2.26 The combined standard uncertainty is expanded to the specified confidence level by multiplying the *RSTur* by the appropriate coverage factor.

A.2.27 The combined relative bias in percent for the routine single test measurements  is a combination of IME, PME, and MIE in Equation A.25.



**Equation A.25**

##### A.2.28 On page 3 the analyst must enter the analytical measurement and the units of the analytical measurement.

##### A.2.29 The spreadsheet automatically calculates and presents the uncertainty interval expanded to the specified level of confidence for the test result. The calculation of the confidence interval (*CI)* for the analytical measurement result (*Cm*) is presented in Equation A.26.

*CI = Cm ± (Cm)\*(k\*RSTur)*

**Equation A.26**

##### A.2.30 The spreadsheet automatically calculates and presents the bias corrected results (*CmBC*) and the bias-corrected confidence interval (*CIBC*) expanded to the specified confidence level for the test result in Equations A.27a and A.27b.

*CmBC =* 

**Equation A.27a**

*CIBC = CmBC ± (CmBC)\*(k\*RSTur)*

**Equation A.27b**

##### APPENDIX B: GENERAL UNCERTAINTY BUDGET

The general components of sampling and testing that are sources of analytical measurement uncertainty are tabulated in the following table (Table B-1).

**TABLE B-1: SOURCES OF UNCERTAINTY**

|  |  |  |  |
| --- | --- | --- | --- |
| **Uncertainty Sources** | **Source Symbol** | **Analytical Sample** | **Analytical Sample Symbol** |
| **Intrinsic (Instrumental) Measurement Effects** | **IME** | **Instrument Calibration Standard** | **ICS** |
| **Spike Preparation Effects** | **SPE** | **Initial Calibration Verification Standard** | **ICV** |
| **Preparation Method**  **Effects** | **PME** | **Laboratory Control Sample** | **LCS** |
| **Matrix Interference Effects** | **MIE** | **Matrix Interference Sample**  **Matrix Spike/ Duplicate Sample** | **MIS**  **MS/MSD** |
| **Sample Collection Effects** | **SCE** | **Field Replicate (Duplicate) Sample**  **(Collected from same location and during same sampling event time)** | **FSR** |
| **Sample Location Effects** | **SLE** | **Co-Located (Same Location) Sample**  **(Collected 0.5 – 3 feet away from field sample)** | **CLR** |
| **Sampling Site Population Effects** | **SSE** | **Site field sample collected from the environmental site for the study** | **SFS** |

An example of general uncertainty budget components of sampling and testing that contribute the analytical measurement are presented in the follow table (Table B-2).

**TABLE B-2: GENERAL UNCERTAINTY BUDGET**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Component** | **Symbol** | **Relative Standard Uncertainty** | **Probability Distribution** | **Sensitivity Coefficient** | **Relative Uncertainty Contribution** |
| **Intrinsic**  **Instrumental Measurement**  **Effects** | **IME** | **2%** | **Normal** | **1** | **1** |
| **Laboratory Preparation Method**  **Effects** | **PME** | **4%** | **Normal** | **1** | **2** |
| **Sample Matrix Interference Effects** | **MIE** | **6%** | **Normal or Lognormal** | **1** | **3** |
| **Sample Collection and Subsampling Effects** | **SCE** | **8%** | **Normal or Lognormal** | **1** | **4** |
| **Sampling Strategy**  **Effects** | **SSE** | **9%** | **Normal or Lognormal** | **1** | **4.5** |
| **Sampling Site Media Contamination Effects** | **SME** | **14%** | **Normal or Lognormal** | **1** | **7** |

**APPENDIX C: EXAMPLE UNCERTAINTY BUDGET FOR METHOD 3050B**

|  |  |  |  |
| --- | --- | --- | --- |
| **Uncertainty Source** | **Uncertainty Interval (99.7% CL)** | **Evaluation Type** | **Distribution** |
| **Weighing-Boat Weight**  **(Top Loader Balance)** | **5 +/-0.05 g** | **Type A** | **Normal** |
| **Sample Weight Wet (Tared)**  **(Top Loader Balance)** | **996 +/-10 g** | **Type A** | **Normal** |
| **Drying Temperature**  **(Thermometer)** | **30 +/-4 o C** | **Type B** | **U-shaped** |
| **Drying Time**  **(Analog Clock)** | **24 +/- 2 hours** | **Type B** | **Rectangular** |
| **Particle Size Reduction - #10 sieve 2 mm**  **(Milling Machine)** | **1+/- 1 mm** | **Type B** | **Triangular** |
| **Homogenization**  **(Tumbler Blending Machine)** | **30+/-2 rpm** | **Type B** | **Triangular** |
| **Tumbler Time**  **(Analog Clock)** | **18+/-2 hours** | **Type B** | **Rectangular** |
| **Weight of the Dried Sample + Boat Dry**  **(Top Loader Balance)** | **664 +/- 7 g** | **Type A** | **Normal** |
| **Weighing-Boat Weight**  **(Analytical Balance)** | **1+/-0.001 g** | **Type A** | **Normal** |
| **Weight of the Subsample (Tared)**  **(Analytical Balance)** | **2+/-0.002 g** | **Type A** | **Normal** |
| **Quantitative Transfer Efficiency**  **(From Weighing-Boat to Beaker)** | **99+/-1%** | **Type B** | **Triangular** |
| **Spike Volume**  **(Eppendorf Pipette)** | **0.5 +/- 0.005 mL** | **Type A** | **Normal** |
| **Spike Concentration**  **(Manufacture’s Reagent Purity)** | **995 +/- 10 mg/L** | **Type B** | **Rectangular** |
| **Hot-Plate/Hot-Block/Microwave Digestion Temperature (Thermometer)** | **95 +/-5 o C** | **Type B** | **U-shaped** |
| **Extraction Time**  **(Analog Clock)** | **4.75+/-0.25 hours** | **Type B** | **Rectangular** |
| **Nitric Acid Volume**  **(Transfer Pipette)** | **10 +/- 1 mL** | **Type B** | **Triangular** |
| **Nitric Acid Concentration**  **(Manufacture’s Reagent Purity)** | **69.5 +/- 0.5 %** | **Type B** | **Rectangular** |
| **Hydrogen Peroxide Volume**  **(Transfer Pipette)** | **10+/-3 mL** | **Type B** | **Triangular** |
| **Hydrogen Peroxide Concentration**  **(Manufacture’s Reagent Purity)** | **30.5+/-1.5%** | **Type B** | **Rectangular** |
| **Hydrochloric Acid Volume**  **(Transfer Pipette)** | **10+/-1 mL** | **Type B** | **Triangular** |
| **Hydrochloric Acid Concentration**  **(Manufacture’s Reagent Purity)** | **36.5+/-0.5%** | **Type B** | **Rectangular** |
| **Extraction Efficiency**  **(Matrix Interference)** | **96+/-2%** | **Type B** | **Triangular** |
| **Quantitative Transfer Efficiency**  **(From Beaker to Graduated Cylinder)** | **99.5+/-0.5%** | **Type B** | **Triangular** |
| **Dilution Volume**  **(Graduated Cylinder)** | **100+/-3 mL** | **Type A** | **Normal** |

EXPLANTIONS OF DISTRIBUTIONS FOR METHOD 3050B

Normal Distribution

The normal distribution is usually determined statistically. The normal distribution is based on the central limit theorem where random sampling results in a normal distribution of data regardless of the underlying distribution of the quantity measured.

*S = a/3 or approximately 0.33 a*

# Where *S* is the standard deviation and *a* is ½ the range of values.

# Triangular Distribution

The triangular is more conservative than normal. The triangular is used when the variation limits are known (lowest and highest), and it is known that it is a better probability (most likely) of finding values close to the mean value that further away from it.

***S = a/(6)0.5 or approximately 0.41 a***

# Rectangular Distribution

Rectangular is more conservative than the triangular. The upper and lower bounds of range of data is estimated, but the distribution is not known so all results are assumed to be equally likely. For example, the throw of a dice has a rectangular distribution. 1, 2, 3, 4, 5,and 6 are equally likely and the probability is 1/6 or 0.167 that 1 to 6 will occur. There is a zero probability that <1 or >6 will occur. It is often used when information is derived from calibration certificates and manufacturer’s specifications.

***S = a/(3)0.5 or approximately 0.58 a***

**U-shaped Distribution (Sine Wave)**

U-shaped is more conservative than rectangular and the U-shaped returns a higher equivalent standard deviation value for the same variation width, +/- a. U-shaped distribution is not as rare as it seems. Cyclic events, such as temperature of a hot plate, oven, or furnace, often yield uncertainty contributors that fall into this sine-wave pattern. Another example is the power cycle of a microwave digestion system. If we assume that the amplitude of the signal is sinusoidal, the distribution for incident voltage is the U-shaped distribution. There is a better probability of finding values close to the variation limits than around the mean value.

***S = a/(2)0.5 or approximately 0.71 a***

**APPENDIX D: QC-BASED NESTED APPROACH FOR ESTIMATING ANALYTICAL MEASUREMENT UNCERTAINTY EXAMPLE SPREADSHEET**

## D-1: Page 1

D1.1 The analyte of interest, sample matrix, and analytical technology is entered as “Copper in Wastewater by ICP”.

D1.2 For the ICS, ICV, LCS, and MIS, 20 replicate analytical measurement results are entered.



**D-2: Page 2**

D-2.1 The confidence level is selected from: 80%, 90%, 95%, and 99%.

D-2.2 The confidence level is entered as “95”.



**D-3: Page 3**

D-3.1 The analytical measurement result is entered as “10”.

D-3.2 The units are entered as “mg/L”.



**APPENDIX E: SOFTWARE VALIDATION BASED ON DATA IN APPENDIX D**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Replicate Number** | **ICS** | | | ICV | | |
| ***%D*** | ***%D -%*** | ***(%D-%)2*** | ***%D*** | ***%D -%*** | ***(%D-%)2*** |
| **1** | **1.1** | **-0.4** | **0.14** | **0.5** | **-0.6** | **0.40** |
| **2** | **0.8** | **-0.7** | **0.52** | **0.1** | **-1.0** | **1.07** |
| **3** | **0.4** | **-1.1** | **1.25** | **1.0** | **-0.1** | **0.02** |
| **4** | **2.0** | **0.6** | **0.31** | **1.2** | **0.1** | **0.00** |
| **5** | **1.0** | **-0.5** | **0.24** | **0.2** | **-0.9** | **0.87** |
| **6** | **1.2** | **-0.3** | **0.07** | **0.4** | **-0.7** | **0.54** |
| **7** | **1.7** | **0.3** | **0.07** | **1.2** | **0.1** | **0.00** |
| **8** | **3.7** | **2.3** | **5.06** | **0.9** | **-0.2** | **0.06** |
| **9** | **1.1** | **-0.4** | **0.18** | **0.1** | **-1.0** | **1.07** |
| **10** | **3.1** | **1.6** | **2.63** | **1.3** | **0.2** | **0.03** |
| **11** | **2.0** | **0.5** | **0.27** | **0.9** | **-0.2** | **0.06** |
| **12** | **0.7** | **-0.8** | **0.61** | **1.0** | **-0.1** | **0.02** |
| **13** | **0.4** | **-1.1** | **1.17** | **2.0** | **0.9** | **0.75** |
| **14** | **0.9** | **-0.6** | **0.34** | **0.2** | **-0.9** | **0.87** |
| **15** | **1.4** | **-0.1** | **0.01** | **1.0** | **-0.1** | **0.02** |
| **16** | **1.9** | **0.4** | **0.18** | **1.4** | **0.3** | **0.07** |
| **17** | **2.0** | **0.5** | **0.27** | **1.5** | **0.4** | **0.13** |
| **18** | **1.5** | **0.0** | **0.00** | **1.7** | **0.6** | **0.32** |
| **19** | **1.6** | **0.1** | **0.01** | **3.0** | **1.9** | **3.48** |
| **20** | **1.1** | **-0.4** | **0.14** | **3.1** | **2.0** | **3.86** |

###### ICV



%= 32.6/20 =1.1%

=100+1.1=101.1%





###### ICS



%=12.7/20=1.5%

=100+1.5=101.5%





## Equation A.6

## Equation A.7

## Equation A.8

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Replicate Number** | **LCS** | | | MIS | | |
| ***%D*** | ***%D -%*** | ***(%D-%)2*** | ***%D*** | ***%D -%*** | ***(%D-%)2*** |
| **1** | **4.0** | **-1.4** | **2.06** | **12.0** | **7.3** | **53.29** |
| **2** | **0.5** | **-4.9** | **24.35** | **1.4** | **-3.3** | **10.89** |
| **3** | **1.5** | **-3.9** | **15.48** | **8.0** | **3.3** | **10.89** |
| **4** | **1.7** | **-3.7** | **13.95** | **3.7** | **-1.0** | **1.00** |
| **5** | **0.1** | **-5.3** | **28.46** | **12.0** | **7.3** | **53.29** |
| **6** | **2.2** | **-3.2** | **10.47** | **0.4** | **-4.3** | **18.49** |
| **7** | **0.4** | **-5.0** | **25.35** | **3.6** | **-1.1** | **1.21** |
| **8** | **0.3** | **-5.1** | **26.37** | **0.1** | **-4.6** | **21.16** |
| **9** | **0.5** | **-4.9** | **24.35** | **2.7** | **-2.0** | **4.00** |
| **10** | **15.0** | **9.6** | **91.49** | **17.0** | **12.3** | **151.29** |
| **11** | **20.0** | **14.6** | **212.14** | **30.0** | **25.3** | **640.09** |
| **12** | **0.4** | **-5.0** | **25.35** | **3.7** | **-1.0** | **1.00** |
| **13** | **4.0** | **-1.4** | **2.06** | **1.5** | **-3.2** | **10.24** |
| **14** | **0.6** | **-4.8** | **23.38** | **5.0** | **0.3** | **0.09** |
| **15** | **1.5** | **-3.9** | **15.48** | **1.4** | **-3.3** | **10.89** |
| **16** | **5.0** | **-0.4** | **0.19** | **20.0** | **15.3** | **234.09** |
| **17** | **24.0** | **18.6** | **344.66** | **3.5** | **-1.2** | **1.44** |
| **18** | **3.0** | **-2.4** | **5.93** | **5.0** | **0.3** | **0.09** |
| **19** | **13.0** | **7.6** | **57.23** | **-24.0** | **-28.7** | **823.69** |
| **20** | **11.0** | **5.6** | **30.97** | **-13.0** | **-17.7** | **313.29** |

###### MIS



%= 32.6/20 =4.7% =100+4.7=104.7%



###### LCS



%=12.7/20=5.4%

=100+5.4=105.4%





## Equation A.6

## Equation A.7

## Equation A.8

ICSur = IMEur=0.84%

## Equation A.9

**If** *ICSur > ICVur*, **then** *SPEur* is estimated as zero, **else**:



**Equation A.10**

**If** *ICVur > LCSur*, **then** *PMEur* is estimated as zero, **else**:



## Equation A.11

**If** *LCSur > MISur***, then** *MIEur* is estimated as zero, **else**:



# Equation A.12



**Equation A.16**



**Equation A.17**



**Equation A.18**



**Equation A.19**

=(101.5-100)=1.5%

**Equation A.20**



**Equation A.21**



**Equation A.22**



**Equation A.23**



**Equation A.24**





**Equation A.25**

*CI = Cm ± (Cm)\*(k\*RSTur)=* 10 ± (10\*2.093\*0.1113) = 10 ± 2.3 *mg/L*

**Equation A.26**

*CmBC =* 

**Equation A.27a**

*CIBC = CmBC ± (CmBC)\*(k\*RSTur)*= 9.5 ±(9.5\*2.093\*0.1113) = 9.5 ± 2.2 *mg/L*

**Equation A.27b**