Explanatory Document for Analytical Methods Validation Elements

1. Purpose

To describe the validation elements for an analytical method.

2. Detection Limit (MDL)

The Method Detection Limit (MDL) is the lowest concentration of an analyte in a real matrix which, when subjected to all the steps of the analytical method (including chemical extractions and pre-treatment) produces a detectable signal, statistically different with defined reliability, from the one produced by a “blank” in the same conditions. By convention, the MDL is set equal to a signal-to-noise ratio of 3. In the laboratory, background noise is estimated by calculating the standard deviation of the concentration of 10 (or more) replicates of a single low-concentration sample subjected to the complete analytical procedure. This standard deviation is then multiplied by 3 to obtain the MDL.

Applicability: In a sample analyzed by a particular analytical method, if the signal generated by the analyte results in a concentration lower than the MDL, the analyte will be considered undetected in the sample.

3. Quantification Limit (MQL)

A Method Quantification Limit (MQL) is the minimum concentration of analyte in a real matrix which, when subjected to all the steps in the analytical method (including chemical extractions and pre-treatment), may be quantified with defined reliability. By convention, the MQL is set equal to a signal-to-noise ratio of 10. In the laboratory, background noise is estimated by calculating the standard deviation of the concentration of 10 (or more) replicates of a single low-concentration sample subjected to the complete analytical procedure. This standard deviation is then multiplied by 10 to obtain the MQL.

Applicability:

1) *In a sample analyzed by a particular analytical method, if the signal produced by the analyte results in a concentration greater than the MDL but lower than the MQL, the analyte is detected but the determined concentration will be considered to be of non-negligible uncertainty.*
2) *In a sample analyzed by a particular analytical method, if the signal generated by the analyte results in a concentration greater than the MQL, the analyte is considered quantifiable and any associated result will be considered analytically reliable.*

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4. Reporting limit (RL) or minimum reported value (VMR)

Reporting limit (RL) and minimum reported value (VMR) are equivalent terms which are directly related to the context in which the analytical method is used. The VMR is the lower limit, expressed as concentration or quantity, beyond which no results obtained by the analytical method will be reported. For the sake of consistency, the VMR will also be equal to or greater than the MDL. Depending on the meaning of the results provided by a method in a particular context, the method technical manager may assign a VMR higher than the MDL or even the MQL, provided that results below the VMR are meaningful or useful in the circumstances in which the analysis was requested.

Example: In a given report, the VMR of an analytical method is set at 10 µg, while the MDL and MQL of the same method are 0.1 µg and 0.3 µg, respectively. Thus, a VMR of 10 µg reflects the fact that results below 10 µg are not relevantly applicable for their users.

5. Precision

Reliability of a method relates to the dispersion of results obtained from an experimental procedure applied repeatedly to a single sample in specific conditions. Depending on the testing conditions, reliability is expressed by the replicability or repeatability of an analytical method. The **reliability of a method is expressed by its precision.**

Replicability: is determined based on the results obtained over a number of sample replicates, each of different concentration levels. They are subjected to the same analytical procedure conducted in the same laboratory in the following conditions: same analyst, same equipment and same analytical sequence.

Repeatability: is determined based on the results obtained over a number of sample replicates, each of different concentration levels. They are subjected to the same analytical procedure conducted in the same laboratory, but at least one of the following elements needs to be different: analyst, equipment or analytical sequence.

6. Accuracy

Accuracy is concerned with correctness. Accuracy is the degree of agreement of a measured value obtained by a method with the true or expected value of analyte concentration or quantity in a sample. It is determined by comparing the average result obtained by submitting a number of samples of certified concentration to an entire analytical procedure to the concentration certified by a recognized organization (or one related thereto).

Accuracy is always determined in relation to a unit e.g. 100%. For example, a difference of ± 6% from the certified value always signifies a correctness of 94% (not 106%). The concept of bias is used to express this difference in relation to the certified value (see next section).

More specifically for a microbiology method, the true value corresponds to a culture of the target organism counted on a non-selective medium.

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7. Bias

Bias complements information on the accuracy of the method. It represents a systematic measurement error inherent in a method or caused by some artifact or idiosyncrasy of the measurement system. It indicates whether the method tends to over- or underestimates the true value. It represents the method's relative error.

For example, for a accuracy of 94%, the method might have a bias of 6% or -6%. Bias is positive if the method underestimates and negative if it overestimates.

8. Recovery

Recovery rate is the relative quantity of analyte recovered in a specific matrix. It is the difference between the measured concentration of a spiked sample and the measured concentration of the same sample unspiked, divided by the concentration of the added analyte.

9. Measurement Uncertainty

Measurement Uncertainty is a non-negative parameter, associated with a measuring result, which characterizes the dispersion of quantified values that could reasonably be assigned to the measured value. The concept of measurement uncertainty is further divided into the concepts of analytical measurement uncertainty and expanded measurement uncertainty.

- **Analytical** measurement uncertainty (**CV_a**) is a *combined* measurement uncertainty. It is obtained using the individual measurement uncertainties associated with the different input quantities in a measurement system. It is equal to the square root of the total variance obtained by adding the provided variances obtained by individual measurement uncertainties. It is calculated from the individual results obtained over several samples of different concentration levels subjected to the entire analytical procedure (e.g. 35 samples (5 levels of concentration, 7 samples per level)).
- **Expanded analytical** measurement uncertainty (**expanded CV_a**) corresponds to analytical measurement uncertainty (CV_a) expanded to provide a measurement interval within which the values reasonably attributable to the measurement provide a high level of confidence. The expanded CV_a is obtained by multiplying analytical uncertainty by a coverage factor K, which generally equals to about 2, for a 95% coverage probability.
- **Total** measurement uncertainty (**CV_T**) is also a combined measurement uncertainty that takes account of the contribution of the CV_a (analytical CV) and the uncertainty related to the sampling process (CV_E) which is estimated at 5% for pump sampling (see reference (1)).
- **Total expanded** measurement uncertainty (**expanded CV_T**) corresponds to total uncertainty (CV_T) expanded to provide a measurement interval within which the values reasonably attributable to the measurement provide a high level of confidence. It includes the contribution of analytical uncertainty (CV_a) and sampling uncertainty (CV_E - estimated at 5% for pump sampling), as well as a probability threshold of 95% by the coverage factor K, which generally equals to about 2 (see reference (1)).

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10. References

(1) IRSST, *Sampling guide for air contaminants in the workplace,* 8th edition, version 8.1 updated, 2012.