

MEASUREMENT UNCERTAINTY

It is one of the “dirty little secrets” that all analytical laboratories have, but often never reveal to their clients – every result that a laboratory provides has some uncertainty to it. In fact, during a very well publicized murder investigation, when a defense attorney asked the assistant district attorney for the error rate in a blood test, the assistant DA replied that their testing laboratories had no percentage of error because “they have not committed any errors” (*San Francisco Chronicle*, June 29, 1994, p.4). This assistant DA was not aware, or would not acknowledge, that every measurement has an uncertainty and an error rate. These uncertainties are caused by uncontrolled and often unknown variables, and when combined in a complex analysis, often produce a scatter in results. Unfortunately, measurement uncertainties can never be completely eliminated, and are just as likely to cause randomly high results as well as low results. The upshot is that we can never really know the true value for any quantity.

While measurement uncertainties can not be eliminated, their magnitude can be estimated. By performing repeated analyses of the same sample, we can observe the scatter in results, and quantify the scatter as a standard deviation. This is a *precision* study. The more analyses we perform, the more meaningful the standard deviation becomes, and the better the estimate of the uncertainty. When a single laboratory performs this type of a study, the *repeatability* of the method can be determined. When multiple laboratories perform this type of study through a collaborative study, the *reproducibility* of the method can be determined.

Measurement uncertainty can often be reduced by observing some good laboratory practices. Using properly calibrated instruments and well-characterized reference standards, properly training analysts, providing detailed instructions in written methods, and following these instructions exactly the same way each time can all help to minimize the uncertainty in a measurement. Usually the uncertainties become larger as the concentration of analyte in the matrix decreases, and as the complexity of the matrix increases. For example the uncertainty in an aflatoxin analysis, where the amount of analyte is in the parts per billion level, may be 20 – 30% when expressed as a relative standard deviation (RSD). The amount of uncertainty in the assay of pure acetaminophen raw material, on the other hand, may only be 1%.

When a laboratory sends out a result, you may notice as “+/-“ after the result. This is the “confidence interval,” and is an estimate of the uncertainty. Confidence intervals can be expressed at different levels of probability, such as 90%, 95%, or 99%, with 95% being the most commonly used probability. If you receive a result of “23.4 ± 0.6%” at the 95% confidence interval, it means that the laboratory is 95% confident that the true value is between 22.8% and 24.0%. This confidence interval is based not only on the level of probability, but on the number of replicate analyses used to determine the precision of the method. The better the estimate of the precision of the method, the smaller this confidence interval becomes.

For a new or developmental test method, it may not be possible to estimate the confidence interval because there isn’t enough data. Some laboratories will use a technique called “propagation of error” to estimate the uncertainty. The propagation of error technique uses mathematical transformations to factor in the uncertainties associated with all the measurement steps of the method to come up with an overall uncertainty of the method. Unfortunately, for complex methods and/or complex materials, this can provide an unreasonably low estimate of

Tampa Bay Analytical Research

the uncertainty, as the major sources of random error become variability in extractions, minor chromatographic interferences, possible sample inhomogeneities, and other sample-related factors that are not accounted for in the propagation of error technique. It is always best to make sure you know how the measurement uncertainty was determined.

The amount of uncertainty in a result dictates how many significant digits we can report in that result. The better our method precision and the more certain we are of the result, the more digits we can report. If our method can only give us an RSD on a result of 10% or more, then there is not much point in reporting out to 3 significant digits or more, as that last digit is pretty much meaningless. For most analyses, we can report out to 3 significant digits, but beyond that there is too much uncertainty (and usually little need) to report more digits. One last comment about the number of significant digits: a value of 0.00123 does not contain as many significant digits as a value of 0.12300. In the first case, the 2 zeroes to the right of the decimal are just place holders (in scientific notation, it would be 1.23×10^{-3}). In the second case, the 2 zeroes to the right of the decimal at the end indicate that we know with sufficient certainty the result out to 5 decimal places (in scientific notation it would be 1.2300×10^{-1}). The second result contains 5 significant digits, and is much more precise than the first result that contains 3 significant digits.

