Calibration Curve – Any good in replication and successive dilution for plotted points?

An analytical instrument generally needs to be calibrated before measurements made on prepared samples, through construction of a linear regression between the analytical response and the concentration of the analyte.

Replication

Replication in standard calibration is found to be useful if replicates are genuinely independent. It improves precision by increasing the number of replicates, n, and provides additional checks on the calibration solution preparation and on the precision of different concentrations.

It may be noted that increasing the independent concentration points has actually little benefit after a certain extent. After having six calibration points, it can be shown that any further increase in the number of observation in calibration has relatively modest effect on the standard error of prediction for a predicted x value unless such number of points increases very substantially, say to 30 which of course is not practical. Instead, independent replication can be recommended as a method of improving uncertainties. However, independent replication is accordingly a viable method of increasing n when the best performance is desired.

However replication suffers from an important drawback. Most analysts can testify seeing the effect of simply injecting a calibration standard solution twice, i.e. the plotted residuals appear in close pairs but are clearly not independent. This is essentially useless for improving precision. Worse, it artificially increases the number of freedom for simple linear regression, giving a misleading small prediction interval. Ideally, therefore, replicated observations should be entirely independent, using different calibration solutions if at all possible. Otherwise it is best to first examine replicated injections to check for outlying differences and then to calculate the calibration based on the mean value of y for each distinct concentration.

There is one side effect of replication that may be useful. If means of replicates are taken, the distribution of errors in the mean tend to be the normal distribution as the number of replicates increases, regardless of parent distribution. The distribution of the mean of as few as three replicates is very close to the normal distribution even with fairly extreme departure from normality. Averaging three or more replicates can therefore provide more accurate statistical inference in critical cases where non-normality is suspected.

Successive dilutions

A common pattern of calibration that we usually practice is doing a successive dilution, resulting in logarithmically decreasing concentrations (for example, 16, 8, 4. 2 and 1 mg/L). This is simple and has the advantage of providing a high upper calibrated level, which may be useful in analyzing routine samples that occasionally show high values.

However, this layout has several disadvantages. First, errors in dilution are multiplied at each step, increasing the volumetric uncertainties, and perhaps worse, increasing the risk of any undetected gross dilution error (especially if the analyst commits the cardinal sin of using one of the calibration solutions as a QC sample as well!). Second, the highest concentration point has high leverage, affecting both the gradient and y-intercept of the line plotted; errors at the high concentration will cause potentially large variation in results. Third, departure in linearity are easier to detect with fairly even spaced points. In general, therefore, equally spaced calibration points across the range of interest are preferred.