CAPE Technologies

High Performance Dioxin/Furan Immunoassay Kit

Technical Note TN-004

Quantitation, Calibration, and Quality Assurance for Method 4025m

Quantitation: Dioxin/furan analysis by US EPA Method 4025m using the CAPE Technologies DF1 Immunoassay Kit gives quantitative results which correlate with TEQ (per Application Note AN-008). However, just as with conventional chemical analysis, proper calibration and quality assurance are required for maximum reliability.

The DF1 immunoassay is inherently quantitative. Each immunoassay run should include 2378-TCDD standards to define a standard curve as described in Section D (Table 1) of the kit insert IN-DF1. This curve is applied to unknowns using Calculation Module C, a special purpose Microsoft Excel file available from the CAPE Technologies web site (www.cape-tech.com). Module C uses an iterative non-linear curve fitting procedure based on the same four parameter equation which is the basis for a variety of commercial immunoassay data analysis software. Module C calculates the best fit standard curve and the concentrations of unknowns based on that curve. Background information and instructions are included with Calculation Module C.

The process described above produces raw quantitative results based on the standard curve, which may or may not be an acceptable endpoint. If the goal of the analyst is relative quantitation (i.e. looking for hot spots-finding deviations from a certain baseline and estimating their concentration relative to that baseline), then no calibration adjustment is required. However, if the goal is absolute quantitation (as for virtually all dioxin analysis by GC-MS), then a calibration adjustment must be applied to the raw quantitative results. Calculation Module C has this calibration adjustment calculation built in, but the analyst must determine the actual calibration adjustment factor (CAF) and provide the QA data supporting its use.

Calibration of other 4000 series methods: In order to articulate the rationale supporting this calibration adjustment, it is helpful to first describe the approach to calibration for the other 4000 series immunoassay methods approved by the US EPA (www.epa.gov/epaoswer/hazwaste/test/4_series.htm). These methods, such as Method 4020 for PCBs, have a calibration adjustment built into the method. This adjustment is determined by the kit manufacturer and is applied on the front end, through the use of immunoassay calibrators instead of standards. Standards are solutions with the actual concentration stated, such as 1, 5, 10, and 50 µg/mL solutions of Aroclor 1248 which would be used to construct a standard curve for absolute quantitation by interpolation. Calibrators however, are generally not labeled with the actual concentration, but rather the level at which that solution allows a semiquantitative decision to be made. These may be prepared at specific pre-selected levels, such as 1, 5, 10, or 50 µg/g PCB, and the decision is made by comparing a sample raw result to the calibrator raw result from the same run. Once the kit user makes this comparison and the corresponding decision, no further data interpretation is required for that sample. This front end calibration method limits the user to the same semiquantitative decision levels as used for the kit validation study. Additionally, the calibration rationale assumes that the validation sample set supporting the calibration is equivalent to the sample set analyzed by the kit user.

The actual concentrations of these calibrators may differ from the decision level by a factor of two or more. For example, users of one of the Method 4020 PCB kits would make a decision on whether the sample PCB level is above 10 µg/g by comparing it to a calibrator in the same run that actually contains 5 µg/g PCB (an actual sample concentration of 7 µg/g would be reported as positive at 10 µg/g). The exact difference between decision level and actual concentration used for the calibrator is determined in advance as part of the validation of the kit. This is done by splitting samples and analyzing them using both the conventional method and the immunoassay, in quantitative mode and with no adjustment of the data. The resulting quantitative relationship between the two data sets is used to set the actual concentration of each calibrator, such that a slight positive bias is introduced. The exact levels are selected so that the semi-quantitative decision making process achieves a false negative rate below some chosen maximum, but at the cost of a slightly elevated false positive rate because of the deliberately introduced positive bias.

There are several good reasons why quantitative results from the PCB immunoassay and conventional methods might not follow a 1:1 relationship (regression line slope of 1), even if the correlation is excellent. These include, but are not limited to, reduced efficiency of the rapid extraction method, effects of differences in congener profile between the PCB in the sample and standard, and random variation. The front end calibration procedure described above allows compensation for all such factors together, without explicitly determining their individual contributions. This approach works because of the simplicity of the situation (i.e. relatively few factors likely to affect the calibration, plus only a small number of predefined decision levels).

The front end calibration adjustment procedure described above is effectively the same as obtaining unadjusted quantitative results, then multiplying them by a uniform adjustment factor. The back end approach to calibration for Method 4025m is similar and accomplishes the same goal, but with some very important differences. The rationale for this approach is described below.

Calibration rationale and procedure for Method 4025m: The same factors noted above which can cause the regression line slope to be less than 1 must also be dealt with when calibrating Method 4025m. However, there are more potential factors because of the increased complexity of the procedure (e.g. recovery through cleanup and solvent exchange as well as extraction) and because of the greater variability of the analyte composition (congener profile) among the population of possible samples. Potential for effect of the congener profile on Method 4025m calibration can be assessed in advance using CAPE's Calculation Module A with prior Method 8290 data from the site in question. Additionally, the combination of carbon column cleanup and aqueous analysis system creates a unique problem. Even the highest grades of commercial toluene may contain sufficient high boiling hydrocarbon residue to give the immunoassay a low bias for samples evaporated from 12 mL of toluene (as per AN-008). This bias will be the same within all samples prepared using one lot of toluene (and can therefore be removed by a simple calibration adjustment), but may vary significantly according to manufacturer, product type, and even product lot. For these and other reasons, the front end calibration approach described above for other 4000 series immunoassays is not viable for Method 4025m. Therefore Method 4025m analysis uses standards rather than calibrators, and the analyst applies a back end calibration adjustment to the raw quantitative results.

The calibration procedure supported by the above rationale is straightforward. A set of split samples is analyzed by the reference method (GC-MS) and also by Method 4025m. The comparison data set will likely have some deviation from the ideal 1:1 relationship noted above (regression line slope other than 1). A new data set of adjusted 4025m results is created by multiplying each raw 4025m result by the CAF (starting at 1). The CAF is then changed and the regression line slope is calculated for the adjusted 4025m data. The final CAF value is that which gives a regression line slope of 1 for the adjusted 4025m data. This CAF is then uniformly applied to all raw 4025m results. Once a CAF is determined, it should be checked and refined continuously using the stream of GC-MS data from ongoing quality assurance samples. On a larger project, from 5 to 20 percent of samples screened by Method 4025m should be split for conventional analysis. These are the most important quality assurance samples, but are by no means the only ones that should be run.

Notes on calibration quality: For best results, calibration adjustment should be done on a site specific basis if possible. Differences in dioxin source, sample matrix, and congener profile will all increase the variability of quantitative results and decrease the probability of successful calibration. The effect of congener profile on both correlation and calibration can be estimated in advance using Calculation Module A and Method 8290 data from the same site. More samples supporting any calibration adjustment will obviously give better results. It is theoretically possible to base a CAF on a single sample, but statistically unwise. Likewise, it is statistically best for the samples on which the CAF is based to cover as wide a concentration range as possible.

The closer the calibration samples are to the target sample population, the better the calibration adjustment will be. It is possible to use other reference samples for calibration, but the results will not be as good as when using samples from the same set as the unknowns. For example, calibration based solely on spiked samples can be used, but is less than ideal, since it will not account for extraction differences between spikes and incurred residues. Likewise, calibration based solely on unrelated samples, such as standard reference materials (SRMs), will not account for matrix differences between the SRM and the unknown samples. Additionally, it is possible that sample preparation methods for SRMs, such as lyophilization, may render the analyte less extractable by rapid methods. Hence, these samples are less than ideal as the basis for calibration adjustment.