Technical Tip

SPE Method Validation – Common Terms

Establishing Linearity

The purpose of establishing **linearity** in method development is to show and ensure that the data collected falls in the workable portion of the curve of the relationship between analyte concentration and signal response or biological effect or response factor, etc. Establishing the line, curve or graph that represents this relationship is as easy as graphing low concentrations of analyte and the corresponding response factor to very high levels or concentrations of analyte and the corresponding response factors on the "X"-axis and "Y"-axis, respectively.

With this graph or curve there is a linear portion of the line or curve where the concentration-response relationship is most desirable to work within to ensure that analyte response is linearly proportional to analyte concentration. On the low end of the curve we have the lower limit of detection and then eventually LLOQ [see past tech tips for explanation], and as the analyte increases in concentration the response factor increases exponentially (increasing slope), then linearly (near constant slope), and then again towards the upper range of analyte concentration the curve begins to level-off with decreasing slope.

On this graph if we analyze concentrations that are well above the linear portion, the response factor will undoubtedly be almost the same value for each concentration analyzed and may not correlate sufficiently, which is analytically undesirable. Likewise, if extremely low concentrations are analyzed the response factor may not be detectable or may not correlate well with the increase in analyte concentration and is thereby undesirable as well.

Demonstrating acceptability of linearity data is as easy as examining the correlation coefficient, "r", and the "y"-intercept of the regression line that is generated from the curve or analyte concentration versus response plot. An "r" value of >.99, which is a well-correlated regression line to the plotted data points, is generally considered evidence of a significant fit of the data to the regression line. Also the "y"-intercept should be much lower than the response obtained for the analyte concentration required to generate the linear portion of the curve. In other words, the regression line should have a significant positive slope.

Typically, linearity is established using a minimum of 5 or 6 standards whose concentration span is 80-120% of the target concentration range. If the "r" value is <.99, an explanation must be submitted with the validation, but this depends on the method validation governing body to approve. The purpose is to show that the range of concentrations analyzed is reasonably within a range that shows intuitive values for response factors, relatively. The goal of linearity is simply to show reasonable data in a reasonable range using scientific rigor.

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