Example: Nonlinear Standard Addition Curves

This is the standard addition data, collected using atomic absorption (a technique notorious for nonlinear calibration curves).

vol (mL) of aliquots conc (ppm) of standard spikes number of data pts

We must correct for the effects of dilution (due to the std spike) on the signal:

i := 0.. n - 1
(MathCAD needs subscript)
$$y_{corr_{i}} := y_{i} \cdot \frac{\left(V_{a} + x_{i}\right)}{V_{a}}$$

$$y_{corr}^{T} = \begin{bmatrix} 0.2380 & 0.4468 & 0.6270 & 0.7853 & 0.9093 \end{bmatrix}$$
corrected for dilution due to spikes

Now let's look at the standard addition plot, along with the best-fit line of the data.

$$\mathbf{b}_0 := \text{intercept}(\mathbf{x}, \mathbf{y}_{\text{corr}})$$
 $\mathbf{b}_0 = 0.2650$ $\mathbf{b}_1 := \text{slope}(\mathbf{x}, \mathbf{y}_{\text{corr}})$ $\mathbf{b}_1 = 1.6812$

Let's calculate the std deviation of the residuals for model comparison purposes

$$y_{hat} := b_1 \cdot x + b_0 \qquad \text{res} := y_{corr} - y_{hat} \qquad v := n - 2 \qquad s_{res} := \sqrt{\frac{1}{v} \cdot \left[\sum_i \left(res_i\right)^2\right]} \qquad s_{res} = 0.0296$$
residuals



The standard additions plot is shown here with a best-fit line. The inset plot shows the residuals. There definitely seems to be some structure to the plot (ie, the residuals are nonrandom), suggesting that a linear regression model is not appropriate.

So let's try a second-order polynomial fit of the std add'n data.

Let's do a second-order fit of the data, then calculate the std dev of the residuals

$$f(x) := \begin{bmatrix} 1 & x & x^2 \end{bmatrix}^T \qquad b := linfit(x, y_{corr}, f) \qquad b^T = \begin{bmatrix} 0.2377 & 2.2280 & -1.3672 \end{bmatrix}$$
(defines regression
model in MathCAD)

$$g_{hat_i} := b_2 \cdot (x_i)^2 + b_1 \cdot x_i + b_0 \qquad res := y_{corr} - y_{hat} \qquad v := n - 3 \qquad s_{res} := \frac{1}{v_i} \cdot \sum (res_i)^2 \qquad s_{res} = 2.0690 \cdot 10^{-3}$$

degrees of freedom
$$\sqrt{\frac{v}{i}}$$

This is better than the best-fit line.

Here is the data again, along with the LS quadratic fit.



This time, the fit looks better. The residuals are more randomly distributed about the fit, and the s_{res} statistic also favors the quadratic model. The problem we run into now is this: the standard additions method for analyte quantitation assumes a linear relationship between signal and analyte concentration. To be more precise, the method assumes that the calibration function is linear when extrapolated to the baseline. The following figures illustrate this point.



As mentioned, in order for the standard addition equations to work, extrapolation to the baseline must be linear. This assumption is more justifiable if the calibration function is linear for the standard addition data (eg, the plot on the left). If the standard addition data are nonlinear (above right) linear extrapolation might still be appropriate; however, the evidence to support this assumption is much weaker.

The calibration function for atomic absorption is usually linear for small absorbances, showing curvature toward the baseline as the concentration increases - exactly the behavior demonstrated by the data in this problem. Thus, we may claim with some legitimacy that, even though our standard addition data is slightly nonlinear, the extrapolation to the baseline is linear, since this encompasses smaller absorbance values. In other words, the response was still (at least approximately) linear for the analyte present in the sample.

With this assumption, we can now extrapolate to the baseline.

$$V_{prime} := \frac{b_0}{b_1} \qquad V_{prime} = 0.1067 \qquad \text{volume (in mL) of added standard that contains the same amount of analyte as was originally present in the aliquot (before spiking)}$$
$$C_a := C_{std} \cdot \frac{V_{prime}}{V_a} \qquad C_a = 0.0427 \qquad \text{analyte concentration (in ppm) in original aliquot}$$

Since 1ppm is assumed equivalent to 1 μ g/mL, we can now determine the "extractable" concentration of Cd in the original soil sample

mass
$$Cd := C_a \cdot 100$$
 mass $Cd = 4.2674$ mass (in µg) of Cd extracted from the soil sample

$$C_{soil} := \frac{mass Cd}{1.2901}$$
 $C_{soil} = 3.3078$ concentration (in ppm) of extractable cadmium in the original soil sample

This is a point estimate. Let's calculate the standard error in this estimate.

$$x_{bar} := mean(x)$$
 $x_{bar} = 0.2000$ $S_{xx} := 4 \cdot Var(x)$ $S_{xx} = 0.1000$

$$s_{V} := \frac{s_{res}}{b_{1}} \cdot \sqrt{1 + \frac{1}{5} + \frac{\left(V_{prime} + x_{bar}\right)^{2}}{S_{xx}}} \qquad s_{V} = 1.3586 \cdot 10^{-3} \qquad \text{std error in V_prime}$$

$$s_{Ca} := s_{V} \cdot \frac{C_{std}}{V_{a}} \qquad s_{Ca} = 5.4344 \cdot 10^{-4} \qquad s_{Csoil} := s_{Ca} \cdot \frac{100}{1.2901} \qquad s_{Csoil} = 0.0421$$

std error in Ca $s_{csoil} := s_{Ca} \cdot \frac{100}{1.2901} \qquad s_{csoil} = 0.0421$

t := qt(.975, 2) t = 4.3027

 $t \cdot s_{Csoil} = 0.1812$

t-crit with 2 df width of 95% confidence interval

The concentration of extractable cadmium in the soil sample is 3.31 ± 0.18 ppm (95% CL)