amc technical brief

Editor: M Thompson Analytical Methods Committee AMCTB No 22 March 2006

deviations and confidence limits of the estimated concentrations, and the conditions under which their uncertainties can be minimised. Often these uncertainties are disturbingly large. When the calibration graph is linear, straightforward equations are available to achieve these outcomes.

The line of regression of *y* on *x*

In most calibration experiments we make the assumption that the uncertainties in the concentrations (x) of the standards are negligible compared with those of the output signals (y) of the analytical instrument. The graph thus plotted is *the line of* *We can illustrate this using a non-chem* *row) for concentration* x_0.

ical example. If we determine the weights of a number of infants of known ages and plot them on a graph (for simplicity, we assume it to be linear) then, to use the line of regression of y on x, it will clearly be right to plot the weights as y and the ages as x. Different children of the same age do not all weigh the same, and there will be measurement errors too, whereas the infants' ages will be known exactly. The normal use of such a graph would be to estimate by interpolation the *average weight of a child of a given age*, i.e. we would find a y-value from an x-value. Such an estimate would naturally have an associated uncertainty, as the slope and intercept of the graph would be uncertain because of the scatter of the points. (The standard deviations of the slope and intercept are readily given by programs such as Excel).

In analytical work, however, we use the same type of graph *to estimate* x_0

'Inverse confidence limits'

Because this interaction of error contributions is rather complex, we tend to use a simplified version of the necessary equations. If we use an *un-weighted* calibration approach (in which the y-direction random error is assumed to be the same for all x values, so that all the points on the graph have the same weight, or importance) then the equation for s_0 is:

$$s_0 = \frac{s_{y/x}}{b}\left[\frac{1}{m} + \frac{1}{n} + \frac{(y_0 - \bar{y})^2}{b^2 \sum_i (x_i - \bar{x})^2}\right]^{\frac{1}{2}} \qquad (1)$$

In (1) the n calibrating points on the graph have means $\bar{x}$ and $\bar{y}$, the test material is measured m times giving a mean response value y_0, b is the slope of the graph, and $s_{y/x}$ is given by:

$$\left[\frac{\quad^{2}}{2}\right]^{\frac{1}{2}}$$

(reflecting the use of inverse regression) or *fiducial limits* (Draper and Smith, 1998).

The approximation inherent in equation (1) is valid if:

$$\frac{t^2 s_{y/x}^2}{b^2 \sum_i (x_i - \bar{x})^2} < 0.05 \qquad (3)$$

The t-statistic is used as above. In analytical calibrations, equation (3) is almost always valid: unless the data are very poor the function often has a value of <0.01.

Example

We can apply these equations to a simple and typical example of a good-quality calibration graph:

y	0.099	0.187	0.274	0.347	0.426	0.489
x	0	5	10	15	20	25

The data and regression line are shown in Figure 2. It is easy to show that in this case b = 0.0157, $s_{y/x}$