amc technical brief

Analytical Methods Committee Editor: Michael Thompson AMCTB No 27 June 2007

Why are we weighting?

When we calibrate an analytical system, we use a calibration set with concentrations c_i, $i = 1,\ldots,n$ and measure r_i, the corresponding responses. If the values of c_i are essentially error-free, regression is usually appropriate to obtain the calibration function $r = f(c)$ that we use to estimate unknown concentrations. But should we use simple or weighted regression?

The question arises because simple regression is based on a statistical model in which the variance of an observed response is the same across the whole calibration range (Figure 1). However, this circumstance is unlikely to be strictly true in chemical analysis. In typical (but not all) analytical calibrations, the variance of the response is heteroscedastic, increasing steadily with the concentration. We can see this effect in the example data (Figure 2). (This example was chosen because, unusually, the variance in the response is large enough at higher concentrations to be visible on the scale of the figure.) In such instances, weighted regression, based on a heteroscedastic model (Figure 3), should give a more accurate answer. Differences between calibration functions estimated by the two methods are small over most of the range but could lead to serious error at low concentrations (Figure 4).

Somewhat more effort is required to execute weighted regression, so the obvious question arises: when, if at all, is that extra effort justified? The answer depends on a number of circumstances, including:

- the characteristics of the analytical system;
- the design of the calibration set (evenly spaced or otherwise);
- the calibration range in relation to the detection limit;
- the unknown concentration of the analyte in a test solution;
- the required accuracy of the analysis.

Why calibrations are heteroscedastic

Most analytical calibration functions are derived from a calibration set with concentrations from zero upwards. Such a system naturally has two separate contributions to the precision of the response at any one concentration. Firstly there is the variation in response when the concentration is zero, that is, in the calibration blank. Suppose that variation has a standard deviation σ_0. That value is equivalent to a 'naïve detection limit'* of $c_L = 3\sigma_0/S$ where, for a linear calibration, S is the sensitivity of the system (the slope of the calibration line).

The zero-point variation will also be present in the response from calibrators containing analyte. In that case, however, there will be an additional independent variation that we can usually expect to be proportional to the concentration. The standard deviation of this concentration-dependent component will be of the form $\sigma_c = SAc$, where A is a constant, the relative standard deviation of the response at high concentrations. So at any concentration c we will see a response with a standard deviation of

Eq 1. $$\sigma = \sqrt{\sigma_0^2 + \sigma_c^2} = \sqrt{\sigma_0^2 + S^2A^2c^2}\ .$$

Equation 1 accounts for changing standard deviation rather well in most cases. (Our example data are compared with this model in Figure 5.) At low concentrations (where $S^2A^2c^2 << \sigma_0^2$), we find an almost constant $\sigma \approx \sigma_0$. Simple regression would serve well for calibrations in this concentration range. At high concentrations (where $S^2A^2c^2 >> \sigma_0^2$) the function tends

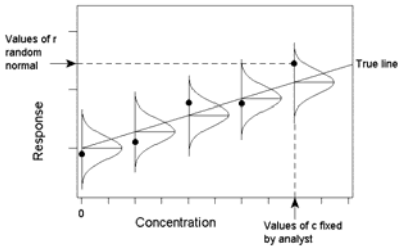

Figure 1. Model used for linear regression. The *x*-values are fixed and the *y*-values (points) are subject to measurement variation with constant variance.

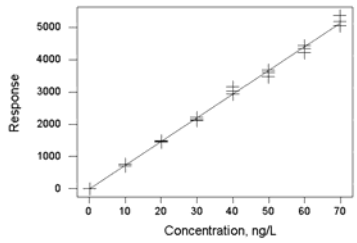

Figure 2. Calibration data (points) for ^{239}Pu determined by ETV-ICP-MS. Simple and weighted regression lines are indistinguishable at this scale. (The data can be found in *AMC Data* on www.rsc.org/amc.)

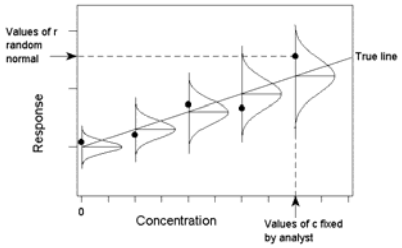

Figure 3. A heteroscedastic model of regression. The variance increases with the signal.

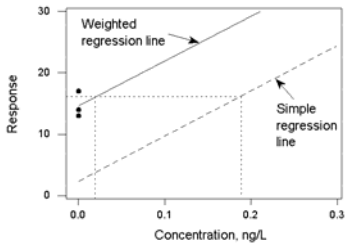

Figure 4. Same data as Figure 2, showing the zone near zero concentration. A response of (say) 16 would give rise to a serious inaccuracy if the simple regression line were used.

* 'Naïve' because, as we shall see, in most instances the system is incapable of delivering such a low value.

towards $\sigma = SAc$, that is, an almost constant relative standard deviation, and for this region weighted regression will be more accurate. But is the improvement in accuracy worthwhile? If so, where should we draw the line between our choice of methods? We can tackle this question by generalising Eq 1 across analytical methods. This is done by expressing concentration $\underline{c}$ and standard deviation $\underline{\sigma}$ in units of detection limit. This gives us

Eq 2: $\underline{\sigma} = S\sqrt{1/9 + A^2\underline{c}^2}$.

We can now investigate two commonly encountered designs for analytical calibration.

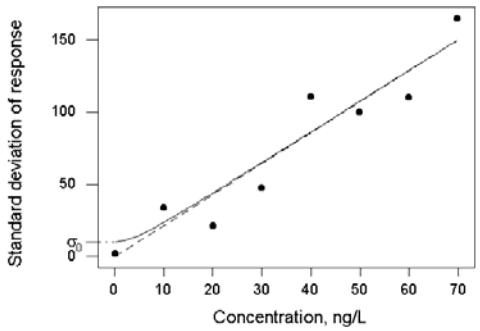

Figure 5. Example data (points) and Equation 1 (solid line) with $\sigma_0 = 10$, $S = 73.3$, $A = 0.0292$, showing the line of constant relative standard deviation $\sigma = SAc$ (dashed line). (σ_0 was estimated separately.)

Evenly spaced calibrators

Let us consider a typical calibration design—six evenly spaced concentrations from zero to a maximum of $c_{\max}$. We assume that responses are random, independent, and normally distributed with standard deviation according to Equation 2. Finally we assume a value of $A = 0.01$, as typical for instrumental methods. We can then find the confidence interval for any unknown predicted concentration after using both simple and weighted regression. (Technical Brief No 22 provides some background to this topic.) The two confidence intervals can be compared at particular predicted concentrations by considering the ratio of standard deviations (simple/weighted regression) as a function of generalised concentration. A summary of the outcome is shown in Figure 6.

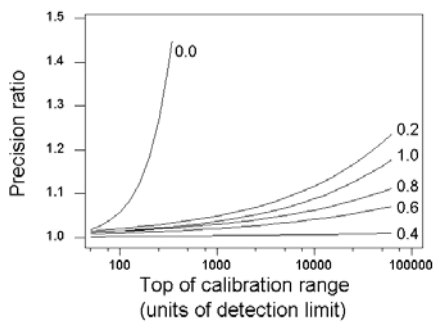

Figure 6. Precision ratio (simple regression: weighted regression) as a function of the maximum calibration concentration c_{max}, for an assumed value of $A = 0.01$. The labels on the lines show a range of example 'unknown' concentrations c relative to c_{max}. The ratio is minimal around $c/c_{\max} = 0.4$, near the centre of the calibration range.)

The model predicts that weighted regression should give a more precise result (ratio greater than unity) under all circumstances, but over much of the range the deviation from unity is small and could often be safely ignored. So, for short-range calibrations (up to say $200c_L$), any difference between the outcomes of simple and weighted regression is probably negligible. For longer range calibrations, we could often accept an increase in uncertainty of 30% (a factor of 1.3). In most measurement results this would be an imperceptible change and, in any event, a small proportion of the combined uncertainty. However, results between zero and $0.2c_{\max}$ would be detrimentally affected by inflated variance, and results near to zero severely affected. For example, with $c_{\max} = 10^5 c_L$, the precision at zero concentration is degraded by a factor of 500 by using simple regression.

(These conclusions would be modified in an obvious way if values of *A* other than 0.01 were appropriate.)

'Logarithmically-spaced' calibrators

Another commonly used design of the calibration set is where successive concentrations increase by a constant factor, plus a zero-level calibrator. For comparison with even spacing we consider a calibration set with generalised concentrations 0, 10, 100, 1000, 10000, 100000). The precisions found with weighted regression were uniformly close to those found previously for the evenly-spaced design. For simple regression, however, the results were about 25% higher than weighted regression, except below the $0.2c_{\max}$ level, where precisions were more severely affected. For example, at zero concentration, the precision was worse by a factor of about 70, considerably less than with evenly-spaced calibrators, but still substantial.

Recommendations

- For short-range calibrations (up to about 200 times the detection limit) there is no worthwhile advantage in using weighted regression.
- For longer range calibrations, weighted regression offers no worthwhile advantage unless the concentrations of interest include low levels (below 0.2 times the calibration maximum). At low levels, precision may be substantially degraded by using simple regression. Realistic detection limits will be seriously degraded unless weighted regression is used.
- For longer range calibrations, logarithmically spaced calibrators give rise to a degradation in precision in comparison with uniformly spaced calibrators if simple regression is used. If weighted regression is used, precisions are very similar to those found with uniform spacing. There is no benefit in using logarithmically spaced calibrators.

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