ACCOUNTING FOR NONUNIFORM VARIANCE IN ASSAY CALIBRATION

Leon Aarons, Department of Pharmacy, University of Manchester, M13 9PL

The conventional method of determining the line of best fit for a linear calibration is the method of ordinary least squares (OLS). For this method to work efficiently the error in the data must be randomly and normally distributed about the fitted line and the error variance must be constant i.e. independent of either the x or y variable. If the variance is not uniform then OLS will be inefficient in that although the asymptotic estimates of the intercept and slope are unbiased the precision of these estimates will be poor. In the situation where the variance in the data is not uniform then weighted least squares (WLS) should be used in which the squared deviation of each data point from the fitted line is weighted by the reciprocal of the variance of that data point. The practical problem of using WLS is that in many instances the variance in the data is not known.

A more systematic approach to the problem is to use the method of extended least squares (ELS) suggested by Sheiner and Beal (1980). ELS is a maximum likelihood method which attempts to determine both a structural model and a variance model. The objective function that is minimized in order to obtain the parameters, p, of the calibration is

$$\Phi(p,\xi,y) = \sum_{i=i}^{N} \frac{(y_i - f(p,x_i))^2}{v(p,\xi,y_i)} + \ln(v(p,\xi,y_i))$$

where $f(p,x_i)$ is the structural model - in this case a straight line - and $v(p,\xi,y_i)$ is the variance model which contains additional parameters, ξ.

The three methods (OLS, WLS and ELS) were applied to ibuprofen calibration data obtained from a reverse phase HPLC assay. Calibrations consisted of a plot of peak height ratio of ibuprofen to the internal standard against ibuprofen concentration. At an ibuprofen concentration of 50 mg. litre^{-1} the variance in peak height ratio was 25 times greater than at a concentration of 5 mg. litre^{-1}. The calibration lines obtained by each method were quite similar but the precision of the OLS calibration was significantly worse than either the WLS or ELS lines.

In order to determine the precision of estimation of the calibration lines the assay sensitivity was calculated. For this purpose sensitivity is defined as the ibuprofen concentration that gives rise to a 20 per cent coefficient of variation in the assay (Aarons 1981). Only OLS and ELS can be used to make this estimate as in WLS the variance is only known at the concentrations of the standards used in this assay. The variance model used in the ELS method was of the form

$$v = \xi_1 + \xi_2 \cdot (y)^{\xi_3}$$

The sensitivities calculated by the OLS and ELS methods were 11.1 and 3.4 mg. litre^{-1} respectively. As the coefficient of variation of the peak height ratio of the lowest standard used (5 mg. litre^{-1}) was 13.4 per cent the OLS result is clearly far too high whereas the ELS result is much more reasonable.

Although ELS requires a microcomputer it is more efficient than traditional OLS methods and more powerful than WLS methods which require detailed prior knowledge of the variance model.

Aarons, L. (1981). The Analyst. 106, 1249 - 1254.
Sheiner, L.B., Beal, S.L. (1980). J. Pharmacokin. Biopharm, 8, 553 - 571.

0022-3573/82/120086P-01$02.50/0