Calibration Options For Triple Quadrupole LC-MS Data

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AIMS: Calibration with LC-MS data may be accomplished with different combinations of curve fit, weighting, and origin treatment. Agilent’s Mass Hunter Quantitative Analysis software contains a calibration curve fit assistant, which allows comparison of over 100 different calibration options. Curve fit choices include linear, quadratic, power, logarithmic, second order logarithmic, and average of response factors. Weighting choices include equal, inverse x or y, inverse² x or y, and logarithmic. Origin choices include ignore, include, and force. The calibration curve assistant ranks calibration options by r², standard error, or maximum % residual error. We used the curve fit assistant to compare various calibration modes while validating an analysis of zopiclone and benzodiazepines in blood.

METHODS: Data are from the validation of the analysis of 16 benzodiazepines and zopiclone from blood extracts, acquired on an Agilent 6410 QQQ LC-MS. Blood calibrators of 25, 50, 125, 250, and 500 ng/mL, linearity check samples at 10 and 1000 ng/mL, and a control blood specimen were extracted and analyzed on multiple occasions. Endpoints included accuracy and precision of extrapolated values for the linearity check samples, and precision for the control. Although the software allowed disabling selected points, only calibrations utilizing all data points were evaluated.

RESULTS: Using data from this study, r² was not a sensitive measure of linearity or goodness of fit for calibration data. Maximum % residual error was a useful measure for ranking calibration curve options. As expected, inverse weighting was preferable to equal weighting for linear curve fits. When calibration data deviated from linearity, extrapolation from a linear calibration curve, below the lowest calibrator, gave inaccurate results. Several nonlinear curve fit options demonstrated low maximum residual error, and good accuracy for extrapolated values.

CONCLUSIONS: Mass Hunter’s calibration curve assistant provides extensive data for evaluation of calibration options, which are useful for method validation. Inverse weighting is generally preferable to equal weighting of linear calibration curves, because of data heteroscedasticity. When linear curve fitting is applied to nonlinear chromatographic data, extrapolation below the lowest calibrator gives inaccurate results. Properly validated nonlinear curve fits may be useful in some QQQ LC-MS applications.

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