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Improving quantitative precision and throughput by reducing calibrator use in liquid chromatography-tandem mass spectrometry

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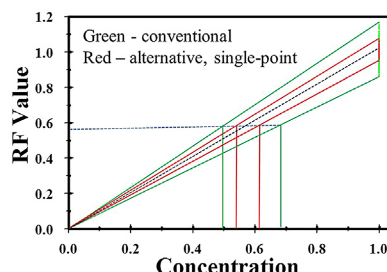
HIGHLIGHTS

- Use of a weighted single point calibration approach improves quantitative precision.
- A weighted response factor approach incorporates historical calibration information.
- Several scenarios are discussed with regard to their influence on quantitation.

GRAPHICAL ABSTRACT



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ABSTRACT

To improve efficiency in our mass spectrometry laboratories we have made efforts to reduce the number of calibration standards utilized for quantitation over time. We often analyze three or more batches of 96 samples per day, on a single instrument, for a number of assays. With a conventional calibration scheme at six concentration levels this amounts to more than 5000 calibration points per year. Modern LC-tandem mass spectrometric instrumentation is extremely rugged however, and isotopically labelled internal standards are widely available. This made us consider whether alternative calibration strategies could be utilized to reduce the number of calibration standards analyzed while still retaining high precision and accurate quantitation.

Here we demonstrate how, by utilizing a single calibration point in each sample batch, and using the resulting response factor (RF) to update an existing, historical response factor (HRF), we are able to obtain improved precision over a conventional multipoint calibration approach, as judged by quality control samples. The laboratory component of this study was conducted with an existing LC tandem mass spectrometric method for three androgen analytes in our production laboratory. Using examples from both simulated and laboratory data we illustrate several aspects of our single point alternative calibration strategy and compare it with a conventional, multipoint calibration approach. We conclude that both the cost and burden of preparing multiple calibration standards with every batch of samples can be reduced while at the same time maintaining, or even improving, analytical quality.

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1. Introduction

We recently described efforts to improve efficiency, and reduce the cost of clinical LC/MS/MS determinations by reducing the

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number of calibration standards used over time [1]. There we summarized work demonstrating an improvement in the precision obtained, as measured by QC results for three androgen analytes, through use of a single point calibration update strategy. In the clinical laboratory setting many tests are performed at high volume with dedicated instruments running a single assay over several years. This makes efficiency improvements particularly attractive.

Reducing calibration standard use has long been an interest of ours [2] as we have often had the opportunity to evaluate agreement of calibration curves over multiple runs and days. Others have made similar evaluations, describing various strategies and advantages of these approaches [3–5]. Taylor et al. [6] described use of single point calibration for therapeutic drug monitoring of tacrolimus by LC-MS/MS in 1997. That same year Renman and Jagner [7] illustrated how alternative calibration strategies can perform better than conventional approaches using simulations in several different scenarios. An example is given demonstrating improvements in precision obtained using five replicates of a single point calibrator in comparison with single replicates at five different concentrations.

More recently, Tan et al. [8] applied similar concepts to both simulations and actual data sets from three methods in a bioanalytical setting. They compared different numbers of calibration standard levels, and numbers of replicates at each level, and evaluated accuracy under differing degrees of imprecision. They concluded that not only do reduced calibration standard strategies save time and cost, they are more robust than conventional multipoint calibration.

In 2007 Nilsson and Eklund [9] published on use of a single, isotopically labelled, IS concentration to quantify a model compound after demonstrating linearity over the range of interest. This is essentially an isotope dilution technique. As such, one might trace use of single point calibration in mass spectrometry to the late 1930's when Rittenburg and colleagues [10,11] began using isotope dilution to quantify amino acids in protein hydrolysates. They used ^{15}N-labeled glutamic acid, aspartic acid, or glycine added to a fibrin sample after boiling the protein in 20% HCL. Knowing the concentration of labeled amino acid (y) and the ^{15}N content (C_0), they calculated the percentage of each amino acid in the sample (x) by determining the isotope ratio (C) in the sample mixture (Equation (1)).

$$x = \left(\frac{C_0}{C} - 1 \right) y \qquad (1)$$

We can think of the parenthetical term as equivalent to analyte to internal standard (IS) peak area ratio (PAR) and y as the inverse of the slope, or 1/m. The equation therefore has a linear form with zero intercept:

$$x = \frac{PAR}{m} \qquad (2)$$

Since slope is determined by the labeled amino acid concentration (y), the unlabeled concentration can be calculated by experimental determination of the parenthetical term. This is equivalent to a single point calibration strategy. We highlight this work to illustrate that single point calibrations have been in use in mass spectrometry for quite some time.

We have often seen at meetings, and in MS vendor literature, how stable PAR measurements can be over many, or even thousands of injections on LC tandem mass spectrometers. If instrument response (as defined by PAR) is linear over a given range and reasonably stable over time, a single calibrator should serve as an accurate indicator of the slope for quantitation. Using a historically-based weighting technique (Equation (3), definitions) we obtain a measure of slope that fluctuates less around the presumed "true" slope, providing improved analytical precision and, with one assumption, improved accuracy as well, the assumption being that slopes obtained by each method are equivalent.

Because this approach will be considered new or foreign to many laboratorians, we have undertaken to describe our strategy in more detail than previously. We provide examples of how it performs under several scenarios using both simulated and real data. Our purpose in this work is to illustrate that the approach suggested behaves in a largely predictable fashion and to highlight several differences between it and a conventional multipoint calibration approach.

2. Definitions

PAR = peak area ratio of analyte to Internal Standard (IS).
RF = PAR/unit calibrator concentration.
(RF is equivalent to the slope of the regression line used for quantitation).
HRF= HIstorical response factor.
PRf = provisional response factor.
CRf= Current response factor.
W = weighting factor

$$\mathrm{CRF} = \mathrm{W} \times \mathrm{HRF} + (\mathrm{PRF} - \mathrm{W} \times \mathrm{PRF}) \qquad (3)$$

3. Methods

Single calibrator quantitation was performed in the fashion described previously [1], with a weighting factor (W) of 0.75 used for production sample batches. We determined a starting point "historical response factor" (HRF) along with standard deviations for each compound from multiple analyses of the high level calibrator at the outset of the study. Once established, additional provisional response factor (PRF) values, from each calibrator analysis, were accepted for incorporation if within ± 3 SD of the starting point mean. Values outside these bounds were excluded and the HRF brought forward for quantitation of samples in the run. QC acceptance criteria are the same as those ordinarily used (Westgard rules).

Simulations and plots were created using PSI-Plot (version 10, Poly Software International, Pearl River, NY). Data points were generated using the random Gaussian number generator with user defined mean and variance. Simply, values were first generated to represent calibrator, QC, and internal standard peak areas. As an example, a mean of 1.0 with CV of 6% would be specified by use of a variance of 0.0036. From the values generated an analyte to IS peak area ratio was then calculated from calibrator peak areas to generate PRFs for subsequent determination of CRF by Equation (3). Simulated QC concentrations were then calculated by division of QC peak area ratios by the CRF corresponding to each QC data point.

Reference materials androstenedione, testosterone, dehydroepiandrosterone (DHEA), were purchased from Sigma–Aldrich (St. Louis, MO, testosterone) or Steraloids (Newport, RI) and internal standards from CDN Isotopes (Pointe-Claire, Quebec, Canada) or Sigma–Aldrich (d3-testosterone). Solvents were J.T. Baker brand (Avantor, Center Valley, PA) and water prepared in-house using an 18 MOhm resin purification system. Formic acid and hydroxylamine hydrochloride were from Fluka (Sigma–Aldrich, St. Louis, MO).

Instrumentation consisted of an AB Sciex (Concord, ON) API 5500 triple quadrupole mass spectrometer with TurboIonSpray source ionization operated in positive ion mode. A two-

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