United States Environmental Protection Agency Perchlorate Method 332.0 via Microbore and Capillary Chromatographic Formats: Statistical Evaluation of the Use of ^{18}O-Perchlorate Internal Standard with Deionized-Water Matrices

L.E. Vanatta[1,*] and **R.W. Slingsby**[2]

[1]Air Liquide-Balazs, Dallas, TX and [2]Dionex Corporation, Sunnyvale, CA

Abstract

A statistically sound evaluation is made of the United States Environmental Protection Agency's perchlorate Method 332.0 by tandem ion chromatography–mass spectrometry–mass spectrometry. Two microbore formats and one capillary format are used with a deionized-water matrix. The evaluation is made for raw peak-area data and for analyte responses scaled by the internal standard, over an analyte concentration range of 0.25 to 200 µg/L. Results indicate that: (*i*) the internal-standard signal is suppressed by the analyte in both microbore formats; (*ii*) the analyte signal is not affected by the internal standard; (*iii*) models for the calibration curves usually contain bias; (*iv*) the measurement uncertainty is similar in magnitude for both the peak-area- and ratio-based curves.

Introduction

When a mass spectrometer with an electrospray interface (ESI) is used as a quantitative detector such as in an ion chromatograph–mass spectrometer–mass spectrometer (IC–MS–MS) system, it is normally recommended to use internal standards; most preferably, a stable-labeled analog. Internal standards (ISTDs) are used to scale the analyte data, in an attempt to correct for method and system variations (run-to-run), including suppression (or enhancement) of analyte signal due to the presence of matrix components. Because stable-labeled forms are used, the ISTD co-elutes with the analyte and is (in theory) thus subjected to the same variations as the analyte. There can still be signal suppression (or enhancement), depending on the concentration ratio of the analyte and ISTD (and possibly total ionic load), and these effects should be investigated over the expected range of the calibration plot.

* Author to whom correspondence should be addressed: email Lynn.Vanatta@airliquide.com

Several researchers in the environmental field have addressed the challenges associated with tandem liquid chromatography (LC) and atmospheric-pressure-ionization mass spectrometry (API-MS). Reemtsma (1) reviewed the literature for techniques (both separation and detection) to quantify polar organic pollutants in water at trace levels. Stüber and Reemtsma (2) addressed matrix effects in environmental samples by investigating three calibration methods: (*i*) external-standard in pure solvent; (*ii*) ISTD; and (*iii*) external-standard in matrix. Bester (3) addressed the topic of quality assurance in LC–MS work. Hedrick and Munch (4) described the use of ^{18}O-perchlorate in United States Environmental Protection Agency (USEPA) Method 332.0 using single-quadrupole MS detection. Using USEPA Method 331, Wendelken et al. (5) performed calibration and recovery studies for perchlorate in water matrices with the goal of determining detection and reporting limits, and overall method uncertainty. In no case has anyone investigated (in a statistically detailed manner) the ISTD-based calibration process for tandem-MS data. However, there is a benefit to understanding how various factors affect the resulting calibration curves, and to conducting such an investigation across the concentration ranges that are useful in daily practice.

USEPA Method 332.0 (6) covers the determination of perchlorate in water using IC–MS and IC–MS–MS with ISTD calibration. The ISTD is an ^{18}O-labeled perchlorate. This procedure covers a very low concentration range (~0.05 µg/L to 20 or 25 µg/L) in water; a 100-µL injection volume and microbore (i.e., 2-mm column i.d.) format are used. ISTD-based calibration curves for this range often exhibit correlation coefficients (R^2) of ca. 0.999; external-standard calibration can provide R^2 values of approximately 0.998 under the same conditions. No literature to date has: (*i*) discussed wider (higher) calibration ranges for Method 332.0; (*ii*) investigated regression diagnostics for this method's data (including the ability of

R^2 to indicate linearity); (*iii*) taken a close look at the performance of ISTDs in wide calibration ranges; or (*iv*) investigated the possible advantages of using smaller injection volumes.

In this paper, Method 332.0 with tandem MS–MS detection was used for the determination of perchlorate in wide calibration ranges (i.e., 0.25–200 µg/L). Two instrumental formats were used: (*i*) microbore (i.e., 2-mm i.d.) with both 100-µL and 15-µL injection volumes and (*ii*) capillary (i.e., 0.4–mm i.d.) with a 4-µL injection volume. When an ISTD was used, ^{18}O-perchlorate was added. In all work, the solvent was deionized water, because perchlorate's behavior in this medium must be understood before more complicated matrices can be investigated successfully.

The statistical goals of the study were to use sound techniques to: (*i*) evaluate sources of variation in the response data (both with and without scaling via the ISTD), and (*ii*) construct calibration curves and their associated prediction intervals; in all statistical work, the confidence level was 95%. The practical goals of the research were to use the statistical results to gain insight into: (*i*) the trade-offs related to using ISTDs and (*ii*) the advantages and disadvantages of using various instrumental formats.

Experimental

Instrumentation

The ion chromatograph used in the microbore work was the Dionex ICS 3000 (Dionex Corp., Sunnyvale, CA) that included an analytical pump, a postcolumn solvent pump, an eluent generator, a conductivity detector, an autosampler, and a column compartment. The IC used in the capillary work was a prototype with equivalent modules. The API 2000 triple-quadrupole MS (ABI-Sciex, Toronto, Quebec, Canada) had a pneumatically and thermally assisted electrospray ion source with a moveable electrospray probe. A grounding adaptor was in-line to eliminate the buildup of voltage between the conductivity and the MS–MS detectors. Analyst software version 1.4.2 (ABI-Sciex) with prototype Chromeleon (Dionex) software was used to control the capillary instrumentation and data collection. Chromeleon DCMSLink for Analyst software version 2.0 (Dionex) was used for microbore control and data collection. JMP 7.0 (SAS Institute, Cary, NC) was used for statistical analysis of the results.

Chromatography supplies and chemicals

The IonPac AS20 analytical column (250 × 2-mm i.d., Dionex) was used for the microbore work and a prototype AS20 (250 × 0.4-mm i.d.) was used for the capillary work. In both cases, the eluent was 45 mM KOH, which was supplied by an eluent generator. Electrolytic suppressors were used in both systems (the Anion Self-Regenerating Suppressor 300 in 2-mm format and a prototype in capillary format). Native perchlorate ($^{35}Cl^{16}O_4^-$, 1000 µg/mL, AccuStandard, New Haven, CT) and stable-labeled ^{18}O-perchlorate ($^{35}Cl^{18}O_4^-$, 1 mg/L, Dionex) were used to prepare standards. Acetonitrile (Burdick and Jackson, Muskegon, MI) was used for post-suppressor solvent addition to the mass spectrometer.

Standards

A stock solution of sodium perchlorate was prepared at 10 mg/L concentration. Standards of perchlorate were prepared in deionized water at 0.25, 0.5, 1, 5, 10, 25, 50, 75, 100, 150, 200 µg/L; a blank was also prepared. The ISTD was added to the blank and all standards at 5 µg/L. The 1-ppm stock ISTD was added to the native standard using a calibrated Eppendorf (Westbury, New York) pipette. Native standards were produced by weighing. These standards were analyzed in quintuplicate; on each day, a data set was collected using a given instrumental format. (Because some of the capillary sample sequences for the IC and for the MS had to be coordinated using the prototype software, in some runs, a slightly different number of replicates were actually analyzed for some concentrations. Also, three replicates of a 125-µg/L standard were included for the capillary format. On Day 1 of the microbore-15 µL configuration, 5 replicates of the 125-µL standard were analyzed, but the 0.25-µL solution was not tested.) Within each replicate, the concentrations were analyzed in random order.

For each format, an "intertwined" sequence was analyzed once. This schedule was based on the random quintuplicate pattern described previously. However, after each standard (which contained ISTD at 5 µg/L), a matching standard without ISTD was analyzed. The analyte concentrations included were: blank; 1, 5, 10, 25, 50, 150, and 200 µg/L.

IC–MS–MS conditions

The IC–MS–MS system was configured in the typical manner [i.e., separator–suppressor-conductivity cell–static mixer (to allow infusion of acetonitrile)–mass spectrometer]. The position of the probe in the ESI was optimized for the 2-mm and 0.4-mm formats separately.

The IC-system conditions were as follows. For the microbore work, both the eluent and the acetonitrile flow rates were 0.3 mL/min, and the suppressor current was 50 mA. For the capillary instrument, these same parameters were 0.015 mL/min and 15 mA, respectively.

The flow-rate and injection volume were scaled according to the theoretical relationship of [column i.d.$_{(1)}$ / column i.d.$_{(2)}$]^2. Thus, the theoretical scaling factor between a 2-mm i.d. format and a 0.4-mm i.d. format is found by the relationship of $2^2/0.4^2$, which is 25. This factor assumes all elements of the systems are scaled properly and the detector behaves the same in both scales (7,8). Injection volumes of 100 µL for the 2-mm system (the volume suggested in Method 332.0) and 4 µL for the capillary systems use the 25× factor. A 15-µL injection volume was used in the 2-mm format to test whether a lower sample load would affect the regression results (as compared

Table I. Conditions for the SRM Channels*

SRM channels	Time (mS)	DP (V)	FP (V)	EP (V)	CE (V)	CXP (V)
107/89	600	–25	–300	–5	–38	–13
99/83	600	–50	–320	–10	–35	–12

* CE = collision energy; CXP = cell exit potential; DP = declustering potential; EP- entrance potential; and FP = focusing potential.

to the 100-µL format).

The conditions for the mass spectrometer in the microbore and capillary formats were as follows: (*i*) probe temperature = 475ºC; (*ii*) probe voltage = –4.2 kV; (*iii*) curtain gas = 20; (*iv*) collision gas = 4; and (*v*) gas 1/gas 2 = 50/50 psi. The probe position was optimized for each format separately. Details for the selected-reaction-monitoring (SRM) channels are given in Table I.

Results and Discussion

Signal suppression

When stable-labeled ISTDs are used, there is a necessary coelution of analyte with its stable-labeled analog. Suppression (or enhancement) of a response from ESI-MS–MS detection can be caused by such a coelution with another substance. For example, if there is a large concentration difference between two species or very different electron affinities, or both, then the ion present in higher concentration/higher electron affinity can carry more of the electrospray current than does the other ion. Also, when the overall ionic load is too high, then repulsion at the ESI interface can lower the efficiency of ions entering the detector. Thus, signal suppression may have significant effects on the raw response data, as well as on the subsequent regression curves that are generated [using either: (*i*) response ratios (i.e., analyte peak area/ISTD peak area) vs. analyte concentration or (*ii*) raw peak areas (PAs) vs. analyte concentration].

Analysis of raw response data

Before calibration curves were constructed, the responses (i.e., raw PAs and, where applicable, ratios) were studied statistically to investigate their behavior. These analyses were conducted to help illuminate the trade-offs involved in using ISTDs for quantifying IC–MS analyses. For the ISTD, the raw peak areas were studied as a function of: (*i*) analyte concentration and (*ii*) day (within each format). For the analyte, these responses were studied as a function of: (*i*) presence or absence of ISTD (PAs only) and (*ii*) day (within each format).

ISTD responses

Comparison as a function of analyte concentration. For both microbore injection volumes, the ISTD PAs showed a statistically significant decline as the concentration of the analyte increased. With the 15-µL injection volume, these responses dropped (over the course of the concentration range; i.e., from a blank to a 200-µg/L standard) from 64,000 to 45,000 on the first day, and from 85,000 to 54,000 on the second day. With the 100-µL injection volume, the peak areas dropped (over the same concentration range) from 512,000 to 230,000 on Day 1, and from 558,000 to 230,000 on Day 2. Thus, for these two formats, the suppression of the ISTD signal by the presence of the analyte increased with concentration. As was mentioned in the "Signal suppression" section, signal suppression is common in electrospray work. The exact mechanism behind this particular occurrence is unknown, and its determination was beyond the scope of this research.

For the capillary 4-µL arrangement, there was no significant change in the ISTD peak area as concentration of the analyte increased. Such consistency suggests that the lack of suppression is due to the small injection volume and thus a lower ionic load.

Besides analyzing the PAs of the ISTDs in each data set, the standard deviations (SD) of these responses were calculated at each analyte concentration. The goal was to determine if the noisiness of the PAs changed with analyte concentration. For the capillary 4 µL, the SD behaved randomly. However, for Day 2 of the microbore 15 µL, there was a clear trend downward as analyte concentration increased. Especially for Day 1 of the microbore 100 µL, the SD for the low concentrations (i.e., the six concentrations between 10 ppb and the blank) were much greater than were these statistics for the higher concentrations. (Specifically, on Day 1, the SD for the low end ranged from 11,000 to 74,000; for the high end, the values were only form 3,000 to 7,000. For Day 2 for the microbore 100 µL, four of the six values were 9,000 or greater.)

The use of ISTDs will always introduce at least some noise into the data, but the hope is that the effects will be more than offset by the minimizing of ESI-instability problems. However, if the ISTD noisiness is high, and especially if it is inconsistent over the working range of the method, then the final results may be affected significantly.

Comparison as a function of day. At each concentration, the PAs of the ISTD were compared between days. The Student's t-test was used to evaluate each pair of means. The microbore 15-µL and capillary 4-µL formats had significant differences (for the ISTD PAs) day-to-day at all concentrations except 0.25 µg/L for the capillary mode. With the microbore 100-µL configuration, approximately half of the concentrations (i.e., 0.5, 1, 10, 50, and 75 µg/L) displayed differences. Such variability between days is not uncommon with electrospray interfaces.

Analyte responses

Comparison as a function of presence or absence of ISTD. At each concentration within the "intertwine" studies, the peak areas from the with-ISTD analyses were compared with the without-ISTD injections. As mentioned previously, the Student's t-test was used to evaluate each pair of means. With only one exception (i.e., 150 µg/L for the capillary 4-µL format), the presence of the ISTD did not have a statistically significant effect on the peak area of the analyte itself. (In the case of the exception, the peak areas were larger when the ISTD was not present.) Thus overall, the presence of the ISTD at the 5-µg/L level did not cause signal suppression of the analyte itself, even when the analyte concentration was as much as 20 times lower (i.e., at 0.25 µg/L).

Comparison as a function of day. At each concentration, raw peak areas and ratios were compared between days, again using the Student's t-test to evaluate each pair of means.

For raw peak areas, each format exhibited a statistically significant difference at all but a few concentrations. The exceptions were: (*i*) 25 µg/L for microbore 15 µL; (*ii*) 5 and 10 µg/L for microbore 100 µL; and (*iii*) 0.25, 0.5, 75, and 125 µg/L for capillary 4 µL. As with the ISTD PAs, these results were prob-

ably attributable to day-to-day variations in electrospray detection and suggest the need for close monitoring of the calibration plots' performances (and possibly for frequent recalibration) when PA alone is used as the response.

When the ratios were compared between days, there were very few statistically significant differences for the microbore 15-µL and capillary 4-µL formats. Exceptions were: (*i*) 25, 100, and 200 µg/L for the former and (*ii*) 5, 25, 150, and 200 µg/L for the latter. However, differences were present for the microbore 100-µL configuration, except for 0.25, 1, 5, and 200 µg/L. These results indicate that, in general, scaling the analyte response provides greater day-to-day stability when the injection volume (and therefore ionic load) is kept relatively low (i.e., 15 µL and 4 µL).

Calibration curves

Introduction. Calibration curves are used to transform response data (typically, in arbitrary units such as peak area) into concentration, which can be used in calculations, etc. Construction of such a plot utilizes regression, a statistical technique that involves the choice of a fitting technique [typically, ordinary least squares (OLS) or weighted least squares (WLS)] and a model [typically, straight line (SL) or quadratic; higher-order choices are usually impractical, because it is difficult at best to invert the associated equation to predict sample concentrations]. The two choices are made totally independent of each other.

In order to make a statistically sound choice of both a model and a fitting technique, several replicates of several concentrations should be analyzed; a minimum of 5 replicates of each of 5 concentrations is recommended (9). While large designs may sound daunting at first, they can be carried out easily by varying the concentration of daily check standards (which are routinely analyzed in most laboratories) and analyzing them in random order. Because day-to-day variability should be incorporated into any study design, collecting such data over a period of several days is appropriate.

Although the assumption is sometimes made that WLS (with the weight = $1/x$) should be used in all cases, the fitting technique of choice depends on the behavior of the SD of the responses (and solely on this behavior). If the SD trends with concentration, then WLS is needed; otherwise, OLS is used. The OLS-WLS decision is made by modeling the SD of the responses. Once these data are calculated at each concentration, they are plotted (as the y-values) versus concentration (as the x-values). A straight line is fitted, using OLS; the equation is $y = a + bx$. If the slope of the line is significant, then WLS is needed. Weights must be generated and then applied to the raw responses when the model is fitted to the data. Noisier data have lower weights (and thus influence the fitting of the line less) than do "nicely behaved" values. The formula for the weights is basically the reciprocal square of the formula for the fitted straight line [i.e., $1 / (a + bx)^2$; see Reference 10 for further details] .

The choice of a model depends solely on where there may be curvature in the raw-response data. Ideally, at each concen-

Table II. Summary of Regression Results (For Both PA- and Ratio-Related Curves) for the Full-Concentration-Range Calibration Plots*

	Summary data: full concentration range PAs					
	Micro 15 µL Day 1	**Micro 15 µL Day 2**	**Micro 100 µL Day 1**	**Micro 100 µL Day 2**	**Cap 4 µL Day 1**	**Cap 4 µL Day 2**
Fitting technique	WLS	OLS	OLS	WLS	WLS	WLS
Model	Quadratic†	Quadratic†	Quadratic†	Quadratic†	Quadratic†	Straight line†
R^2	0.956	0.998	0.998	0.992	0.998	0.995
Prediction-interval half-width @ 0.25 µg/L‡	10	5	5	2	0.2	0.9
Prediction-interval half-width @ 200 µg/L‡	76	14	14	56	24	20
	Ratios					
	Micro 15 µL Day 1	**Micro 15 µL Day 2**	**Micro 100 µL Day 1**	**Micro 100 µL Day 2**	**Cap 4 µL Day 1**	**Cap 4 µL Day 2**
Fitting technique	OLS	WLS	WLS	WLS	WLS	WLS
Model	Straight line†	Straight line†	Straight line†	Straight line†	Quadratic†	Straight line
R^2	0.993	0.994	0.989	0.997	0.999	0.998
Prediction-interval half-width @ 0.25 µg/L‡	11	0.7	3	1	1	1
Prediction-interval half-width @ 200 µg/L‡	15	20	32	15	14	11

* See the "Calibration curves" section for details. Micro = microbore format; Cap = capillary format.
† Model exhibited bias.
‡ Confidence level = 95%; units = µg/L

tration, the calibration curve will pass exactly through the mean of the responses (i.e., the model is adequate and thus unbiased). The decision on adequacy should be based on the residuals pattern (residual equals observed response minus predicted response) and on a statistical test called a lack-of-fit test; the value of R^2 is not reliable for making this judgment (11, 12).

Associated with any regression (e.g., calibration) curve is a prediction interval, which estimates (at a given confidence level; recall that in this paper, 95% is used) the uncertainty in any transformation made via the curve (12). Knowing the magnitude of this uncertainty is critical to assessing the usefulness of the calculated concentration. Thus, a prediction interval should always be constructed along with a regression curve and the interval's half-width (which can be read directly from the regression graph itself, using either crosshairs in the software program or a ruler with a hard-copy of the plot) should be reported along with any concentration result. The format for reporting is: "concentration ± half-width (in concentration units) of the prediction interval."

The width of the prediction interval depends on the noise inherent in the instrumental data and the number of data points in the calibration design (in general, the width decreases as the number of points increases). If WLS is needed as the fitting technique, then the interval will flare as concentration changes.

If reality departs drastically from ideal (i.e., if the selected model contains bias), then the prediction interval must be corrected for the bias. The result is a jagged pair of limits (13). In such cases, the uncertainty (which now includes the bias that results from the inadequate model as well as the imprecision in the measurement itself) in the plus direction will not be the same as the uncertainty in the minus direction. However, the uncertainties still can be read directly from the graph, as described previously.

Once a robust calibration curve (with its associated prediction interval) has been developed, its performance should be monitored via check standards. At least a high and a low concentration should be analyzed and tracked over time. As long as these quality-control data fall within the tolerance limits set by the laboratory, then the calibration curve can continue to be used, without the need for recalibration.

Calibration-curve results: full-concentration range. Subsequent to the analysis of the raw data (see the "Analysis of raw response data" section), the calibration curves and their associated prediction intervals were generated for evaluation. Table II summarizes the results from the regression work for each format on each day. Figures 1 through 3 compare (for each format/response combination) the calibration curves and prediction intervals for the two days.

In the cases where WLS was needed, the SD of the responses

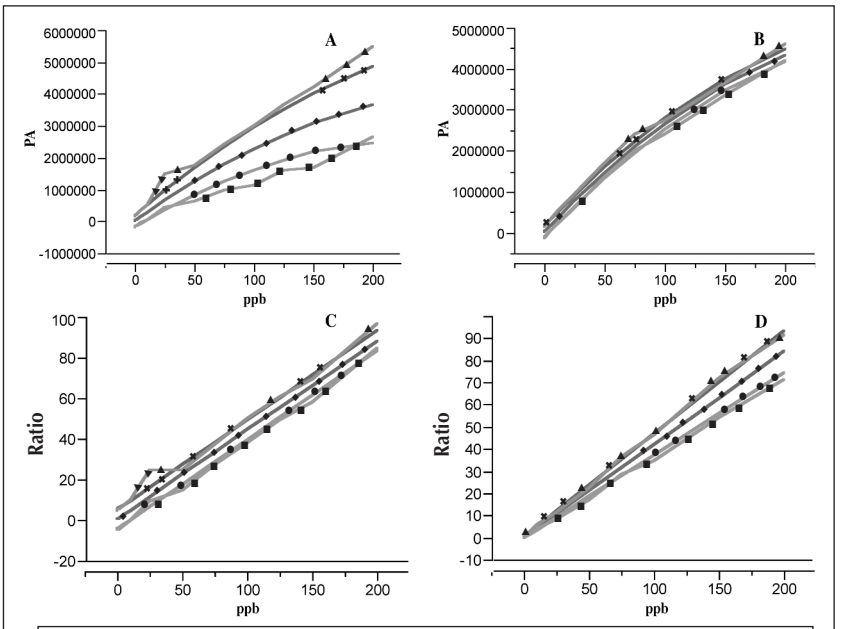

Figure 1. PA versus concentration for the microbore-15 µL format on Day 1 (A) and Day 2 (B). Ratio versus concentration for the microbore-15 µL format on Day 1 (C) and Day 2 (D). See the "Calibration curves" section for details.

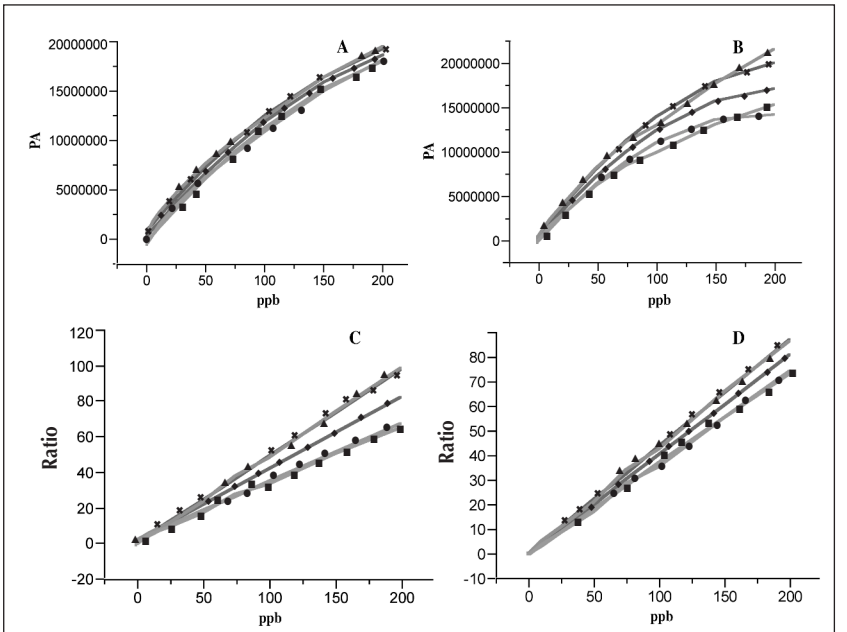

Figure 2. PA versus concentration for the microbore-100 µL format on Day 1 (A) and Day 2 (B). Ratio versus concentration for the microbore-100 µL format on Day 1 (C) and Day 2 (D). See the "Calibration curves" section for details.

always trended upwards as concentration increased. The range of the values of the weights varied, depending on the data set in question. High values (for the blanks) ranged from 0.6 to 4.2; low values (for 200 µg/L) extended from 0.0004 to 0.05. [By comparison, using a weight of 1/*x* for these studies always results in a range from a high of 4.0 (for 0.25 µg/L) to a low of 0.005 (for 200 µg/L). Additionally, the 1/*x* protocol does not permit the calculation of a weight for blanks, because 1 divided by 0 is infinity.

It should be noted that the results in Table II reflect the inadequacy of R^2 in predicting the adequacy of the model. All but one R^2 value is ca. 0.99 or better, yet all but one of these values is associated with a model that has bias. In addition, the adequate SL for the ratio curve on Day 2 of the capillary format has an R^2 of 0.998, while the inadequate quadratic for the ratio curve on Day 1 of that format has an R^2 of 0.999.

Especially in the low-concentration region of the plots, the uncertainty results indicate that use of ratios to construct calibration curves does not necessarily result in a lower uncertainty than is obtainable via the PA data, at least in a deionized-water matrix. Indeed, use of PAs for the capillary format resulted in a Day 1 uncertainty that is approximately five times lower than is the corresponding ratio-generated value.

For both microbore formats in general, the uncertainties varied from day to day; however, for the capillary format, both results appear to be relatively more stable, day-to-day. Nevertheless, it should be kept in mind that most of the models exhibited bias and the procedure to adjust the prediction intervals is not perfect; thus, the reported uncertainty estimates are inherently more variable than are such estimates made from unbiased models.

Calibration-curve results: low-concentration range. Because Method 332.0 typically is used over only a restricted low-level range (i.e., 0.5 µg/L to 20 or 25 µg/L), the low-end data (i.e., blank; 0.25, 0.5, 1, 5, 10, 25 µg/L) were analyzed separately for each format on each day. The goal was to make direct format/day comparisons between the full- and restricted-concentration ranges for: (*i*) model choice, (*ii*) fitting-technique choice, and (*iii*) presence or absence of bias in the model.

Table III shows the results. Comparison with Table II shows that for both concentration

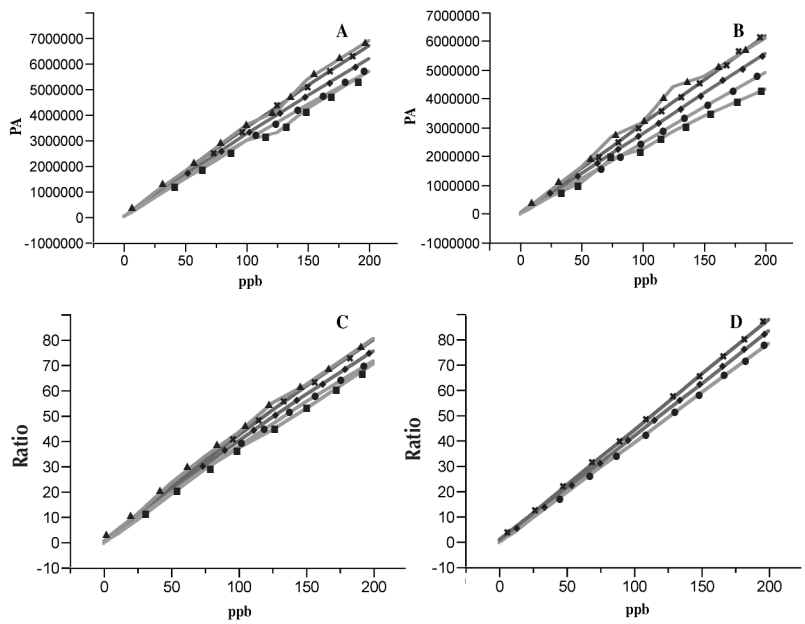

Figure 3. PA versus concentration for the capillary-4 µL format on Day 1 (A) and Day 2 (B). Ratio versus concentration for the capillary-4 µL format on Day 1 (C) and Day 2 (D). See the "Calibration curves" section for details.

Table III. Summary of Regression Results (For Both PA and Ratio-Related Curves) for the Low-Concentration-Range Calibration Plots*

	Summary data: low concentrations PAs					
	Micro 15 µL Day 1	**Micro 15 µL Day 2**	**Micro 100 µL Day 1**	**Micro 100 µL Day 2**	**Cap 4 µL Day 1**	**Cap 4 µL Day 2**
Fitting technique	WLS	WLS	OLS	OLS	WLS	WLS
Model	Quadratic	Quadratic†	Quadratic	Quadratic†	Straight line†	Straight line†
	Ratios					
	Micro 15 µL Day 1	**Micro 15 µL Day 2**	**Micro 100 µL Day 1**	**Micro 100 µL Day 2**	**Cap 4 µL Day 1**	**Cap 4 µL Day 2**
Fitting technique	WLS	WLS	WLS	OLS	WLS	WLS
Model	Quadratic	Quadratic†	Quadratic†	Quadratic†	Straight line†	Straight line

* See the "Calibration curves" section for details. Micro = microbore format; Cap = capillary format.
† Model exhibited bias.

ranges, the choices of model and fitting technique vary considerably. Additionally, for both concentration ranges, there typically is a lack of fit (i.e., presence of bias) in the better model, be it a straight line or a quadratic.

Conclusions

This statistical study evaluated EPA Method 332.0 (using tandem MS–MS detection) to determine perchlorate in deionized-water. Both external-standard and ISTD calibration techniques were examined.

An important conclusion of the research is that the data should be allowed to "speak" for themselves. If there is curvature, then a model other than a straight line should be used; if neither a SL or a quadratic model is adequate, but the better is chosen for use, then the prediction interval should be corrected for bias. The measuring stick for curve evaluation should be the residuals pattern and the lack-of-fit test, not R^2. If there are trends in data noisiness, then WLS should be used instead of OLS, and weights should be based on modeling of the responses' SDs. While it is true that most chromatography programs do not include all of the statistical techniques used in this research, statistical-software packages do. Analysts are advised to consult a statistician for help with utilizing sound regression diagnostics. Also, the analyst must decide if any statistically significant differences that are seen in study data (including the results presented here) are practically important; statistics cannot make this decision for the user.

Throughout these calibration studies, the better model was biased in most instances. However, when the prediction intervals for the full-concentration range were adjusted, the bias effects were typically found to be minimal. The study results also indicate that restricting the calibration range to the low-concentration range does not improve the ability to generate the typically desired linear plot (fitted with OLS), or to have calibration curves that are bias-free.

In the microbore format, there was increased signal suppression (as a function of analyte concentration) of the ISTD's PA. Thus, the calculation of ratios in such circumstances introduces bias into the results. If the PAs had not trended downwards, then the ratios for the higher concentrations would have been lower than they were. As a result, a SL would not have been an appropriate regression model for ratio-based calibration curves. (This point must be kept in mind when working with difficult matrices, where interfering material can affect the signal strength of both the analyte and the ISTD even more; recovery studies are crucial to obtaining reliable analyses of such samples.) On the other hand, the lack of signal suppression in the capillary format suggests that having a lower injection volume may be advantageous when ratio-based curves will be used.

In all formats, calibration curves (from 0.25 to 200 µg/L) with fairly similar prediction-interval widths could be generated using either PAs or ratios (i.e., for a given format, the lower width was sometimes found with the PA curve and sometimes found with the ratio plot). If the possibility of frequent recalibration (including at least a few replicates of several concentrations) is acceptable, then an ISTD probably is not required; this observation is especially true in the capillary format, where lower uncertainties were achievable in the low-concentration range using PAs. Again, though, only the user can decide the practical importance of any statistical results. It also should be emphasized that matrix effects were minimal in this deionized-water-based study; more complicated sample types may make the use of PAs less tractable.

References

1. T. Reemtsma. Liquid chromatography - mass spectrometry and strategies for trace-level analysis of polar organic pollutants. *J. Chromatogr. A* **1000:** 477–501 (2003).
2. M. Stüber and T. Reemtsma. Evaluation of three calibration methods to compensate matrix effects in environmental analysis with LC-ESI-MS. *Anal. Bioanal. Chem.* **378:** 910–916 (2004).
3. K. Bester. Quantification with HPLC-MS/MS for environmental issues: quality assurance and quality assessment. *Anal. Bioanal. Chem.* **391:** 15–20 (2008).
4. E. Hedrick and D. Munch. Measurement of perchlorate in water by use of an ^{18}-O isotopic standard and ion chromatography with mass spectrometric detection. *J. Chromatogr. A* **1039:** 83–88 (2004).
5. S. Wendelken, L.E. Vanatta, D.E. Coleman, and D.J. Munch. Perchlorate in water via US Environmental Protection Agency Method 331. Determination of method uncertainties, lowest concentration minimum reporting levels, and Hubaux-Vos detection limits in reagent water and simulated drinking water. *J. Chromatogr. A* **1118:** 94–99 (2006).
6. E. Hedrick, T. Behymer, R. Slingsby, and D. Munch. Determination of perchlorate in drinking water by ion chromatography with suppressed conductivity and electrospray ionization mass spectrometry. United States Environmental Protection Agency Method 332.0. EPA Document #: EPA/600/R-05/049, Revision 1.0, March 2005. http://www.epa.gov/microbes/m_332_0.pdf
7. J.P. Chervet, M. Ursem, and J.P. Salzmann. Instrumental requirements for nanoscale liquid chromatography. *Anal. Chem.* **68:** 1507–1512 (1996).
8. K. Vanhoutte, W. Van Dongen, I. Hoes, F. Lemière, E. L. Esmans, H. Van Onckelen, E. Van den Eeckhout, R.E. J. van Soest, and A.J. Hudson. Development of a nanoscale liquid chromatography/electrospray mass spectrometry methodology for the detection and identification of DNA adducts. *Anal. Chem.* **69:** 3161–3168 (1997).
9. D. Coleman and L. Vanatta. Statistics in analytical chemistry. Part 5. Calibration: calibration design. *Amer. Lab.* **35(Jun.):** 30–31 (2003). www.iscpubs.com.
10. D. Coleman and L. Vanatta. Statistics in analytical chemistry. Part 8. Calibration diagnostics. *Amer. Lab.* **35(Nov.):** 40–41 (2003). www.iscpubs.com.
11. D. Coleman and L. Vanatta. Statistics in analytical chemistry. Part 9. Calibration diagnostics (continued). *Amer. Lab.* **36(Feb.):** 64–66 (2004). www.iscpubs.com.
12. D. Coleman and L. Vanatta. Statistics in analytical chemistry. Part 10. Calibration diagnostics (concluded). *Amer. Lab.* **36(Mar.):** 46–48(2004). www.iscpubs.com.
13. D. Coleman and L. Vanatta. Statistics in analytical chemistry. Part 16. Calibration example 5 (concluded). *Amer. Lab.* **35(May):** 37–39 (2005). www.iscpubs.com.

Manuscript received November 19, 2008;
Revision received January 22, 2009.