

A Statistical Comparison of the Behavior of the Perchlorate Internal Standard in Microbore and Capillary Format IC-ESI-MS/MS

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ABSTRACT

When a mass spectrometer with an electrospray interface is used as a quantitative detector, such as in an IC-MS/MS system, use of internal standards is normally recommended, preferably stable-labeled isotopic internal standards. Internal standards are used to correct for method and system variations, run-to-run, including suppression of analyte signal due to the presence of matrix components. Since the stable-labeled forms are used, the internal standard co-elutes with the analyte and is thus subjected to the same variations as the analyte. There can be signal suppression depending on the concentration ratio of the analyte and internal standard, and this effect should be investigated over the expected range of the calibration plot. The use of internal standards is a well-accepted technique for LC-MS and IC-MS methods where quantification is needed.

US EPA method 332.0 covers the determination of perchlorate in water using IC-MS and IC-MS/MS with internal-standard calibration. The internal standard is an ¹⁸O-labeled perchlorate. This method covers a very low concentration range, low ppt to low ppb, in water. Using standard statistical algorithms such as those available in chromatography software packages, it is common to achieve correlation coefficients of 0.999–0.9999 with internal standard calibration over the calibration range of 0.05–20 ppb using a 100 µL injection volume in deionized water. External standard calibration can provide R² values of 0.998–0.999 under the same conditions. No literature to date has discussed wider (higher) calibration ranges or has taken a close look at the performance of internal standards in wide calibration ranges.

In this poster, we investigate the use of ¹⁸O-perchlorate internal standard for the determination of perchlorate in very wide calibration ranges, 0.25–200 ppb, using a 100 µL injection volume in a 2 mm ID format and a 4 µL injection volume in a 0.4 mm ID format. We attempt to draw some conclusions regarding the benefits of internal standards, the tradeoffs in very clean systems, and the behavior at high analyte concentrations. In all work, the solvent was deionized (DI) water, since perchlorate's behavior in this medium must be understood before more complicated matrices can be investigated successfully.

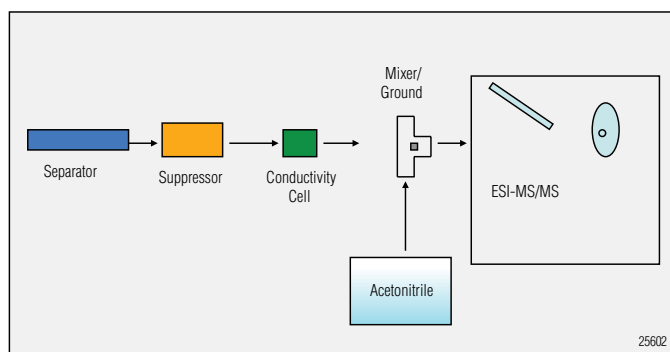


Figure 1. Schematic of an IC-MS/MS system.

Table 1 shows the ion chromatography conditions for the microbore and capillary formats used in this study. The API 2000™ mass spectrometer was used in all of the studies.

Table 1. Microbore and Capillary IC System Conditions		
Format	2 mm microbore	0.4 mm capillary
Analytical Column	IonPac® AS20, 250 × 2 mm ID	Prototype AS20, 250 × 0.4 mm ID
Eluent	45 mM KOH	45 mM KOH
Suppression	ASRS® 300 suppressor	Prototype capillary suppressor
Analytical Flow Rate	300 µL/min	15 µL/min
Injection Volume	15–100 µL	1–10 µL
Postcolumn Solvent Flow Rate	Matches analytical flow rate	Matches analytical flow rate
ESI Heat/Gas	ESI heat/gas required	ESI gas, but not heat, required

Mass Spectrometer

Instrument: API 2000 (Applied Biosystems)

Interface: Electrospray (ESI)

2 mm Conditions:

Temperature:	475 °C
Curtain gas:	20
Collision gas:	4
Spray voltage:	-4500 V
Gas 1/Gas 2:	50/50

MRM	Time (msec)	DP	FP	EP	CE	CXP
107/89	600	-25	-300	-5	-38	-13
99/83	600	-50	-320	-10	-35	-12

Capillary Conditions:

Temperature: 150 °C

All other parameters same as 2 mm, except the ESI probe position was re-optimized.

Figure 2 is a schematic showing the proximity of the ESI spray to the inlet orifice on the mass spectrometer. The probe position must be repositioned at each flow rate in order to achieve maximum s/n. The position of the ESI capillary is optimized to achieve a target “spot” similar to that shown.

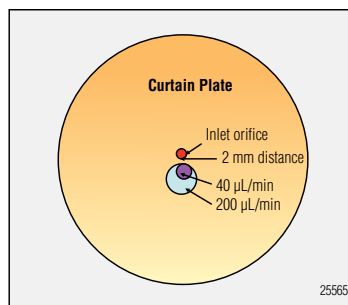


Figure 2. Transferring the ESI spray droplets to the mass analyzer.

Experimental Design

- Microbore 2 mm format, 5 replicates, randomized, 2 separate days
 - 100 µL injection volume
- Capillary 0.4 mm format, 5 replicates, randomized, 2 separate days
 - 4 µL injection volume
- 11 non-zero ppb concentrations (plus blanks):
 - 0.25, 0.5, 1, 5, 10, 25, 50, 75, 100, 150, 200
- $^{35}\text{Cl}^{16}\text{O}_4^-$ = the analyte
 - $^{35}\text{Cl}^{18}\text{O}_4^-$ = the internal standard (ISTD), present at 5 ppb
- The confidence level for all work = 95%
- The matrix = deionized (DI) water

Statistical Analyses

Studying

- Analyte responses [i.e., raw peak area (PA) and ratio*] as a function of:
 - Format
 - Presence or absence of ISTD (PAs only)
 - Day (within each format)
- ISTD peak area as a function of:
 - Day (within each format)
 - Analyte concentration
- Calibration curves for each day of each format

*Ratio = [(PA-analyte) ÷ (PA-ISTD)]

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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. Our choice of 100 μL for the 2 mm system and 4 μL for the capillary systems uses the 25x factor; we test whether or not the peak-area response is the same (i.e., perfectly scaled, meaning no statistically significant difference), given the details of our systems.

For peak areas, the responses did not scale at any concentration. Interestingly enough, however, at some concentrations, the responses scaled when the RATIO of peak area/internal standard was used. Table 2 shows the concentrations where scaling worked; concentrations with no statistically significant difference are marked with a “yes.”

At each concentration, in general, there was no statistically significant difference between analyte PAs when ISTD was present versus when ISTD was not present. The only exception was 150 ppb for the capillary format.

Table 2. Scaling of Peak Area/Internal Standard Ratio											
Analyte Conc./ 5 ppb ISTD Conc.	0.25 ppb	0.5 ppb	1 ppb	5 ppb	10 ppb	25 ppb	50 ppb	75 ppb	100 ppb	150 ppb	200 ppb
Scaled Response?	no	no	no	no	no	yes	no	yes	yes	yes	yes

ESI-MS/MS Signal Suppression

- For the microbore format, the internal standard peak areas showed a statistically significant decline as the concentration of the analyte increased. See Figure 3.
- For the capillary format, there was no statistically significant change in internal standard peak area as the concentration of the analyte increased. See Figure 4.

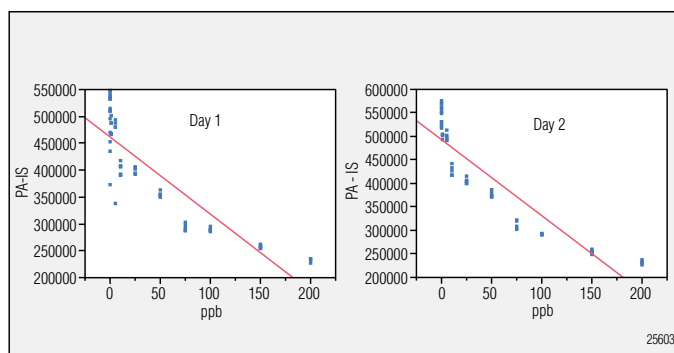


Figure 3. Microbore 2 mm system with a 100 μL injection volume. The slopes of the lines show a downward trend, indicating that the analyte suppresses the ISTD signal (PA-IS) as concentration increases. Using a 15 μL injection volume a similar downward trend was observed.

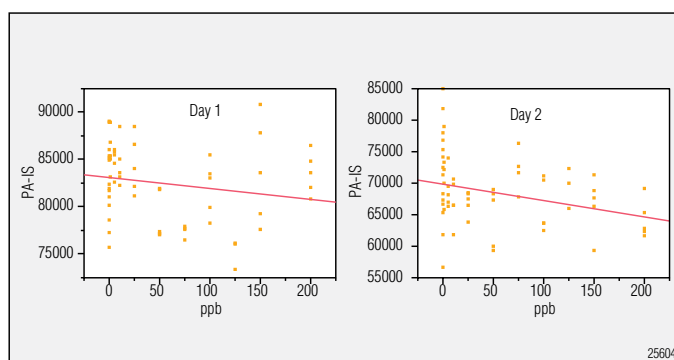


Figure 4. Capillary 0.4 mm system with a 4 μL injection volume. The slopes of the lines show no trend in ISTD area (PA-IS) as a function of concentration, indicating that no suppression by the analyte is occurring.

Calibration Curves

Calibration curves are used to transform response data (typically, in arbitrary units such as peak area) into concentration, which can be used in calculations. Construction of such a plot utilizes regression, a statistical technique that involves the choice of a fitting technique [e.g., ordinary least squares (OLS), weighted least squares (WLS)] and a model (e.g., straight line, quadratic). The two choices are made totally independent of each other.

The fitting technique depends solely on the behavior of the standard deviation (SD) of the responses. If the SD trends with concentration, the WLS is needed; otherwise, OLS is used. The OLS-WLS decision is made by modeling the standard deviation of the responses. Once these data are calculated at each concentration, they are plotted versus concentration. 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 data 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., $1/(a+bx)^2$].

The choice of a model depends solely on where there may be curvature in the raw-response data. Ideally, at each concentration, 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 a statistical test called a lack-of-fit test; the value of R^2 is not reliable for making this judgment.

Associated with any regression (e.g., calibration) curve is a prediction interval, which estimates (at a given confidence level) the uncertainty in any transformation made via the curve. 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 (up to a point, 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. The smoothness of each limit’s curve depends on how well the calibration function (i.e., model) explains the data. If reality departs drastically from this ideal, then the model contains bias and the prediction interval must be corrected for the bias. The result is a jagged pair of limits.

For any calibration study (at the chosen confidence level), the detection limit (DL) in concentration units is the total width of the prediction interval, measured at the PA level corresponding to $x = 0$ on the plot.

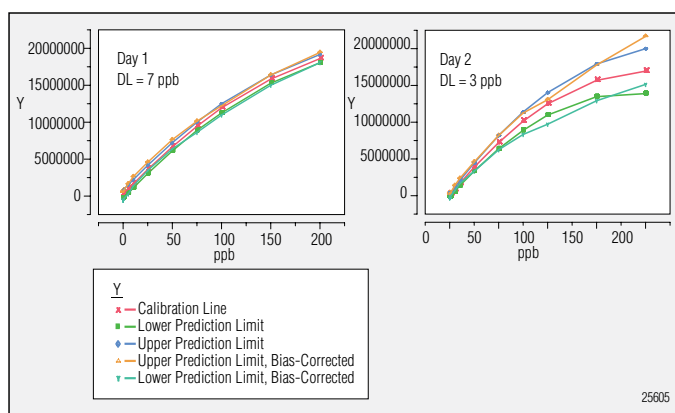


Figure 5. PA prediction intervals and DLs for 2 mm format, 100 μ L injection volumes.

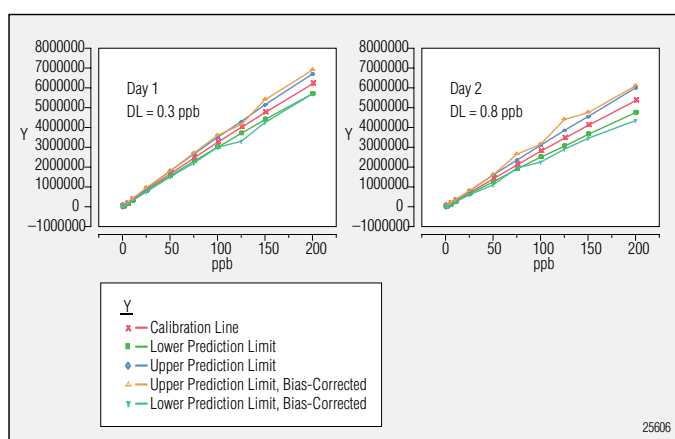


Figure 6. PA prediction intervals and DLs for 0.4 mm format, 4 μ L injection volumes.

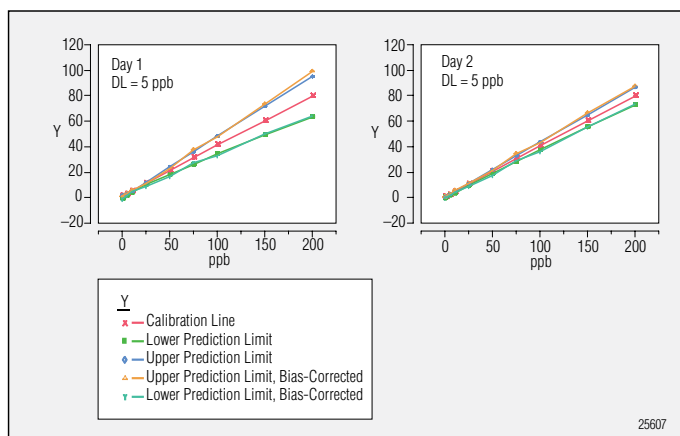


Figure 7. Ratio prediction intervals and DLs for 2 mm format, 100 µL injection volumes.

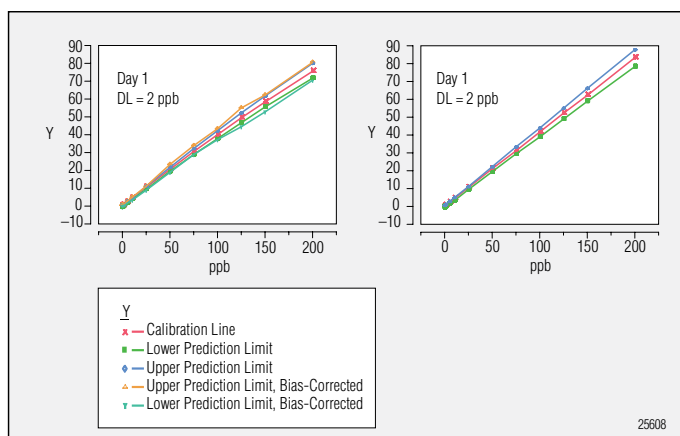


Figure 8. Ratio prediction intervals and DLs for 0.4 mm format, 4 µL injection volumes.

Table 3 summarizes the regression results for each format on each day of the study. DLs and uncertainties are at 95% confidence and have the units of ppb.

Table 3. Peak Area				
	Microbore 100 µL Day 1	Microbore 100 µL Day 2	Capillary 4 µL Day 1	Capillary 4 µL Day 1
Fitting technique	OLS	WLS	WLS	WLS
Model	Quadratic	Quadratic	Quadratic	Quadratic
R ²	0.998	0.991	0.998	0.995
DL (ppb)	7	3	0.3	0.8
Uncertainty @ 200 ppb	14	64	23	28
Ratio				
Fitting technique	WLS	WLS	WLS	WLS
Model	Straight line	Straight line	Straight line	Straight line
R ²	0.989	0.997	0.999	0.998
DL (ppb)	5	1	2	2
Uncertainty @ 200 ppb	39	18	13	11

For calibrations based on PAs or ratios, Tables 4A and 4B, respectively, summarize (for each day, at each concentration) the weights applied to these responses; these weights are based on the formula for the standard-deviation modeling. Also shown are the values for 1/x, a weighting protocol that is often used but that is statistically unwise.

Table 4A. Peak Area					
ppb	Microbore 100 µL (Day 1)	Microbore 100 µL (Day 2)	Capillary 4 µL Day 1	Capillary 4 µL Day 2	1/x
0	Weights not required	2.38	4.19	3.68	Infinity
0.25		2.31	3.34	3.18	4.00
0.5		2.24	2.73	2.79	2.00
1		2.11	1.92	2.18	1.00
5		1.39	0.36	0.60	0.20
10		0.91	0.13	0.23	0.10
25		0.37	0.03	0.06	0.04
50		0.14	0.006	0.01	0.02
75		0.08	0.003	0.007	0.013
100		0.05	0.002	0.004	0.010
150		0.02	0.0008	0.002	0.007
200		0.01	0.0004	0.001	0.005

Table 4B. Ratio					
ppb	Microbore 100 µL (Day 1)	Microbore 100 µL (Day 2)	Capillary 4 µL Day 1	Capillary 4 µL Day 2	1/x
0	0.60	2.76	2.44	2.37	Infinity
0.25	0.63	2.61	2.37	2.32	4.00
0.5	0.65	2.46	2.31	2.26	2.00
1	0.70	2.21	2.19	2.16	1.00
5	1.42	1.09	1.51	1.54	0.20
10	6056	0.58	1.02	1.08	0.10
25	1.10	0.18	0.44	0.48	0.04
50	0.10	0.06	0.18	0.20	0.02
75	0.03	0.03	1.10	0.11	0.013
100	0.02	0.02	0.06	0.07	0.010
150	0.007	0.008	0.03	0.03	0.007
200	0.004	0.004	0.02	0.02	0.005

Conclusions

- Across formats and days, statistically significant differences were observed. The analyst must decide if these differences are practically important.
- In microbore format, the analyte suppresses the ISTD signal as analyte concentration increases.
- In both formats, useful calibration curves (from 0.25–200 ppb) can be generated using either PAs or ratios. If there is time to calibrate daily (including at least a few replicates of several concentrations), then an ISTD is not required; this observation is especially in the capillary format, where lower DLs are achievable using PAs.
- The data should be allowed to “speak” for themselves. WLS should be used if needed (usually the case). If there is curvature and/or trends in data noisiness, then a non-SL model should be used. If neither a SL or quadratic model is adequate, the bias-corrected prediction interval likely will not change drastically.
- Although most chromatography programs do not include all of the statistical techniques used in this study, statistical-software packages do. Analysts are advised to consult a statistician for help with regression diagnostics.
- Capillary format can function with or without internal standards over a wide concentration range.
- Overall capillary format allows smaller prediction intervals and lower detection limits than microbore format, over a wide calibration range.

Glossary of acronyms

ASRS – Anion Self-Regenerating Suppressor	IC-MS/MS – ion chromatograph-mass spectrometer/ mass spectrometer
CE – Collision Energy	ID – internal diameter
CXP – Cell Exit Potential	ISTD – internal standard
DI – Deionized	LC-MS – liquid chromatograph-mass spectrometer
DL – Detection limit	MRM – Multiple Reaction Monitoring
DP – Declustering Potential	PA-IS – peak area of the internal standard
EP – Entrance Potential	SD – standard deviation
FP – Focusing Potential	SL – straight line
ESI-MS/MS – Electrospray ionization-mass spectrometer/mass spectrometer	s/n – signal-to-noise ratio
IC-MS – Ion chromatograph-mass spectrometer	US EPA – United States Environmental Protection Agency

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