United States Environmental Protection Agency Perchlorate Method 332.0. Statistically Sound Recovery Studies in Simulated Drinking Water

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Abstract

This research is a continuation of an earlier work, which evaluated the United States Environmental Protection Agency's Perchlorate Method 332.0, in which standards were prepared in deionized water over an extended concentration range (i.e., to a maximum of 200 µg/L). This current paper investigates the performance of the same method in which standards were made in simulated drinking water. A microbore format with a 15-µL injection volume was employed to conduct a recovery study and generate recovery curves (which hold the key to a statistically sound assessment of method performance in more complex matrices). The maximum analyte concentration range was 1 to 200 µg/L. For various subset concentration ranges, recovery evaluations were made using both raw peak-area data and analyte responses scaled by the internal standard (ISTD). The results indicate that in complicated matrices such as drinking water, ISTDs may not provide simultaneously high precision and recovery.

Introduction

The United States Environmental Protection Agency's Perchlorate Method 332.0 (1) uses the techniques of ion chromatography (IC) and electrospray mass spectrometry (ESI-MS); analyte quantitation involves the use of internal standards (ISTDs). The procedure is typically used for samples with low-µg/L concentrations of the analyte; the highest calibration standard mentioned in the method is only 10 µg/L. However, actual samples sometimes contain higher levels of perchlorate. Thus, a reliable procedure for extended concentration ranges would be desirable.

In a previous paper (2), broader concentration ranges were analyzed statistically, using standards prepared in deionized water (DIW), the simplest matrix possible. Two calibration curves were evaluated, one using the raw peak areas (PAs) of the analyte and the other using the ratios obtained by scaling the raw data by the PAs of the ISTD. Concentrations as high as 200 µg/L were investigated. Results indicated that acceptable precision may be possible without the use of ISTDs, especially if the ionic load into the mass spectrometer is restricted.

Because typical samples are more complicated than deionized water (e.g., raw and finished drinking water), this current research was conducted to further the work in the original paper (2). When matrices grow in complexity, statistically sound

recovery studies are virtually mandatory. Thus, the goals of this research were: (*i*) to perform an extended-range recovery study in simulated drinking water; and (*ii*) to generate statistically sound recovery curves to assess the overall performance of the method. Because most laboratories utilize microbore instruments when quantifying perchlorate, this format was used here. Because reduced ionic loads showed promise in the previous deionized-water studies, a 15-µL injection volume was installed. Both peak-area and ratio response data were analyzed; in all statistical work, the confidence level was 95%. (See the "Results and Discussion: Introduction" section for an explanation of recovery curves, and their importance and advantages.)

Experimental

Instrumentation

The ion chromatograph was a Dionex ICS 3000 (Dionex Corp., Sunnyvale, CA) that included an analytical pump (DP1), a post-column solvent pump (DP2), an eluent generator, a conductivity detector (CD), an autosampler, and a column compartment. A grounding adaptor was in-line to eliminate the buildup of voltage between the conductivity and the ESI-MS detectors. As is mentioned in Method 332, a three-way valve was placed between the CD detector cell and the ES inlet; this configuration allows the matrix ions to be sent to waste rather than into the ES interface. The API 2000 triple-quadruple mass spectrometer (ABI-Sciex, Toronto, Quebec, Canada) had a pneumatically and thermally-assisted ESI source with a moveable electrospray probe. Chromeleon DCMSLink for Analyst Software Version 2.0 (Dionex) was used for instrument control and data collection. JMP 8.0 (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 employed; the ASRS 300 (2-mm format) was used for eluent suppression. 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 the standards and ISTDs, respectively. Acetonitrile (Burdick and Jackson, Muskegon, MI) was used for post-suppressor solvent addition to the mass spectrometer.

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Standards and matrix

A stock solution of sodium perchlorate was prepared at a 10-mg/L concentration. Working standards of perchlorate were prepared in simulated drinking water and in deionized water. With both matrices, the concentrations were 1, 5, 10, 25, 50, 100, 150, and 200 µg/L; a blank was also prepared.

For each matrix, two sets of working standards were made. The first set contained an ISTD at 5 µg/L, and the second set did not. Every time a set of standards was analyzed, a standard without an ISTD was run immediately after the corresponding solution with an ISTD.

When the 1-mg/L stock ISTD was added to the native standard, the addition was made using a calibrated Eppendorf (Westbury, New York) pipette. Native standards were produced by weighing. These standards were injected in quintuplicate on each day a data set was collected; each matrix type was analyzed on a different day. Within each replicate, the concentrations were analyzed in random order.

Simulated drinking water was prepared by dissolving in deionized water the following anions (as sodium salts): (*i*) chloride at 200 mg/L; (*ii*) sulfate at 200 mg/L; (*iii*) carbonate at 100 mg/L; and (*iv*) nitrate at 10 mg/L.

IC–MS–MS conditions

Conditions for the IC-system are provided in Table I. The position of the ESI probe was optimized. The conditions for the mass spectrometer were as follows: (*i*) probe temperature = 475°C; (*ii*) probe voltage = –4.2 kV, (*iii*) curtain gas = 20; (*iv*) collision gas = 4; and (*v*) gas 1/gas 2 = 50/50 psi. Details for the multiple reaction monitoring are given in Table II.

Results and Discussion

Introduction*

With analytical methods that involve regression, a calibration curve is typically the type of plot that is generated. The *y*-values are the raw responses (e.g., PAs) that the standards produce; the *x*-values are the true concentrations. The standards themselves may have been prepared in pure solvent (e.g., deionized water) or in a matrix that typifies the samples that will be tested via the method.

A second type of plot, called a recovery curve, can be constructed if two types of standards are prepared and analyzed [i.e., solutions are made in: (*i*) pure solvent and (*ii*) blank matrix]. In such circumstances, a calibration curve is first generated, using the solvent-based data. Next, the recovered concentrations of the matrix-based standards are estimated by subjecting the matrix-based raw responses to the calibration curve. Finally, these estimates become new *y*-values that are plotted versus the true concentrations; the result is a recovery curve.

This recovery plot has distinct advantages over matrix-based calibration curves. First, as with any regression curve, a prediction interval can be plotted as well. Thus, the half-width of the interval (at the user-chosen level of confidence) provides the precision of the overall method. Second, the intercept and slope of

the curve estimate the method's bias and proportional recovery, respectively. If recoveries are deemed to be low, then the recovered concentrations can be adjusted via the slope and intercept data (assuming such adjustments are allowed by the standard operating procedures for the laboratory). Additional details on recovery curves are available (3,4).

In this research, the pure solvent was deionized water, and the matrix was simulated drinking water.

Preliminary analysis of matrix data

Matrix-based check standards were analyzed periodically throughout the sequence of DIW standards; one injection for each of the five matrix-based standards (1, 5, 10, 25, and 50 µg/L) was made. With the exception of the 1-µg/L solution, the responses from the check standards showed no statistically significant difference versus corresponding responses obtained during the matrix-based study. Thus, it was deemed appropriate to compare responses between days.

To determine the maximum concentration range that would be feasible for constructing recovery curves, the matrix responses (both PAs and ratios) were plotted versus true concentration. A level of 200 µg/L was found to be unrealistic, as PAs were excessively noisy at that level. Furthermore, for the ratio data, the curve showed excessive curvature, to the point that the plot doubled back on itself above approximately 100 µg/L. Thus, recovery evaluations were restricted to ranges up to a maximum of only 100 µg/L.

Evaluation of recovery curves

With one exception (i.e., the ratio-based curve from 1–100 µg/L), a straight line was appropriate for all concentration ranges and response types, although a slight bias again remained on occasion. Weighted least squares was needed as the fitting technique for both of the 1–100-µg/L plots, as well as for the 1–25-µg/L curve based on the PA-based data; ordinary least squares was appropriate for the other three curves. Precision results (as given by the half-width of the prediction interval) and recoveries are given in Table III.

Table I. Chromatography Conditions

Column:	IonPac AS20, 250- × 2-mm i.d.
Eluent:	45 mM KOH
Analytical flow rate:	0.3 mL/min
Suppressor:	ASRS 300, 2-mm
Suppressor Current:	50 mA
Post-suppressor solvent:	Acetonitrile
Solvent flow rate:	0.3 mL/min

Table II. Conditions for Multiple Reaction Monitoring (MRM)*

MRM	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

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

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*Regression diagnostics mentioned in the following are detailed in Reference 2, or otherwise referenced as needed.

The precision was better (especially at the high end of each plot) for the ratio-based work, although at the low end of the 1–25-µg/L range, the uncertainty was virtually the same when the ratio and PA results were compared. The intercept was not significantly offset in any case, with the worst case's being a 2.6-µg/L intersection for the PA-based version of the 1–100-µg/L curve. However, for this widest range, the overall recovery (as measured by the slope of the line) was low for both types of response data.

If internal standards do indeed compensate for the drift, noise, etc. in electrospray sources, why was recovery generally low (see the "Slope" column in Table III) for two of the three ratio-based plots? A probable contributing factor could be found in the behavior of the ISTD, which when added, was always at a concentration of 5 µg/L (see the "Experimental: Standards and matrix" section). This level was selected to keep the ISTD's concentration in the lower third of the working range, thereby minimizing any effect on the signal of the analyte itself (also, using higher ISTD concentrations becomes extremely expensive). The tradeoff is that using a low level of ISTD increases the possibility of ISTD-signal suppression when the native concentration is relatively high.

Details of the ISTD behavior are as follows: first, for both sets of data (i.e., solvent and the matrix), the responses of the ISTD were plotted versus the concentration of the native perchlorate. A statistically significant downward trend was found only for the range of 1–100 µg/L and only with the pure-solvent data. As was discussed in the previous paper, this phenomenon necessarily skews the ratios as the analyte concentration increases. Thus, the effect on this range's overall recovery is not surprising.

Second, for each matrix, all concentration pairs of ISTD PAs were compared statistically, using the Tukey-Kramer HSD (honestly significant difference) test. For deionized water, only one significant difference was found (i.e., when the data from the 1-µg/L standard were compared with the values from the 100-µg/L solution). Thus, the drop in ISTD PAs for the 100-µg/L standard drives the significant downward trend mentioned in the previous paragraph. However, for the simulated drinking water, many differences were found for the 1–100-µg/L and 1–50-µg/L ranges. For the 1–25-µg/L range, the 5-µg/L standard was different from all the others; given that there were only four levels in this range, these differences may have contributed to the lowering of the slope. Interestingly, the multiple differences for the 1–50-µg/L range did not result in a low slope; the fact that the ISTD PAs did not trend downward with increasing concentration may have offset the pair-comparison differences.

It should be noted that for both matrices, the influence of the ISTD's presence on the responses of the native analyte was also evaluated. Data sets with ISTD were compared with sets without the second component, using the Student's t-test. Only one concentration (50 µg/L) in only the DIW matrix showed a significant difference between the with-ISTD and without-ISTD solutions. This finding did not affect the recovery for the 1–50-µg/L range, although it may have contributed to the low recoveries seen for the 1–100-µg/L range.

Conclusions

In general, precision for each matrix tended to be higher for the ratio-based curves, but recovery was better for PA-based plots for two of the three ranges.

Also, precision and recovery were not as high for the range of 1–100 µg/L as for the other two ranges. Thus, for simulated drinking water, the extended range of Method 332.0 probably should be kept to a maximum that is somewhere between 50 and 100 µg/L.

Based on the above recovery findings, it was concluded that in a fairly complicated matrix such as drinking water, the use of ISTDs probably will involve tradeoffs. Results here show that the ISTDs may not behave as consistently as hoped. Thus, the use of ratios may decrease the data's noise, but may simultaneously introduce a bias that lowers recoveries.

A general recommendation is as follows: for any electrospray-based method that will involve matrices other than pure solvent, a recovery study should be conducted and the behavior of the ISTD responses examined statistically. Next, the accompanying recovery curves should be analyzed statistically, thereby allowing the user to decide if: (*i*) the results meet the quality objectives of the project and (*ii*) a pure-solvent-based calibration curve (which is required in Method 332.0) can be used as is.

Table III. Summary of Precision and Recovery Results (for Both Peak-Area and Ratio-Related Curves)*

| Conc. range (µg/L) | Response (PA or Ratio) | Precision Data | | Recovery data | |
		Prediction-interval half-width at 1 µg/L[†]	Prediction-interval half-width at highest µg/L[†]	Intercept	Slope
1–25	PA	1[‡]	11[‡]	–0.07	1.00
	Ratio	0.6	0.6	0.17	0.88
1–50	PA	10.7	10.7	< 0.01	1.00
	Ratio	4.2	4.2	< 0.01	1.00
1–100	PA	3[‡]	69[‡]	2.6	0.73
	Ratio	1.4[‡]	10[‡]	0.23	0.84[§]

* See the "Results and Discussion: Evaluation of recovery curves" Section for details.
[†] Confidence level = 95%; units = µg/L.
[‡] Model exhibited bias.
[§] A quadratic model was used. The slope was estimated by regressing a straight line through the data.

References

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