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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

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Abstract

US Environmental Protection Agency (EPA) Method 331 determines perchlorate in drinking water using non-suppressed ion chromatography with tandem mass spectrometry. This study reports the results of calibration and recovery studies in reagent water, as well as of a recovery study in simulated drinking water (i.e., total dissolved solids are 500 mg/mL each of chloride, sulfate, and bicarbonate). The perchlorate concentrations in the study ranged from 0.05 to 64 ng/mL. At 95% confidence, the Hubaux-Vos detection limit (H-V DL) was 0.04 ng/mL for the calibration study and the simulated-drinking-water recovery study, and 0.03 ng/mL for the reagent-water recovery study. The lowest concentration minimum reporting level was 0.03 ng/mL for reagent water and 0.07 ng/mL for simulated drinking water, again at 95% confidence.

Keywords: Perchlorate; Tandem mass spectrometry; Non-suppressed ion chromatography; Lowest concentration minimum reporting level; Uncertainty interval; Detection limit

1. Introduction

Perchlorate is an anion that is commonly found as its ammonium, potassium, and sodium salts. Perchlorate is known to interfere with iodine uptake in the thyroid gland. Since iodine is an essential component of thyroid hormones, perchlorate has the ability to disrupt thyroid functions. Because perchlorate is highly water soluble and stable in aqueous matrices, it is a contaminate of concern in drinking water. Based on these considerations, US Environmental Protection Agency (EPA) Method 331 for perchlorate in drinking water was developed to support the proposed monitoring in EPA's Unregulated Contaminant Monitoring Rule 2 (UCMR2). Data obtained from the UCMR2 will be used in the regulatory decision-making process. Because of this use, it is important that the method be able to generate high-quality data that meet the data-quality objectives (DQOs) of UCMR2.

At the time of method development, existing liquid chromatography–tandem mass spectrometry (LC–MS/MS) methods for perchlorate required pretreatment of samples with cleanup cartridges to remove interfering matrix components [1,2]. Additionally, these methods generally required that standard addition be employed to compensate for matrix suppression or enhancement [2]. Because of the aforementioned DQOs, several goals were established for Method 331. The first objective was to eliminate the need for pretreatment of samples that were in the dissolved-solids range (2–400 mg/L) of normal drinking waters. This goal was accomplished by judicious choice of a chromatographic system that would provide adequate separation between perchlorate and common matrix interferences, while being compatible with LC–MS/MS. The second objective was to include an appropriate internal standard to compensate for matrix suppression or enhancement, or instrument drift. The third objective was to achieve statistically sound (i.e., based on

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prediction intervals for statistically designed regression curves), sub-ng/mL detection limits and reporting limits.

This paper presents detailed calibration and recovery studies for EPA Method 331. Thorough statistical analysis of the data is included, along with the calculation of detection and reporting limits.

2. Experimental

2.1. Materials

Methylamine (40%, w/w, Aldrich, Milwaukee, WI, USA) was used to prepare the 200 mM mobile phase. Sodium perchlorate (99+%, Aldrich) was used to make all solutions of standards and fortified samples. The internal standard (I.S.) ($NaCl^{18}O_4$) was custom synthesized by Isotec (Miamisburg, OH, USA). To prepare the matrix with high total dissolved solids (TDS), reagent-grade sodium chloride, sodium sulfate, and sodium bicarbonate were obtained from Aldrich; the high-TDS water was made to contain 500 mg/mL of each of the anions. Reagent water was purified using a Millipore ELIX-3 (Millipore, Bedford, MA, USA), followed by a Millipore Gradient A10 system; this water was used to prepare all solutions and the mobile phase.

2.2. Apparatus and columns

All experiments were conducted on a Waters 2690 liquid chromatograph (Waters, Milford, MA, USA) combined with a Micromass Quattro Micro triple-quadrupole mass spectrometer (Waters). The analytical column was a Dionex IonPac AS21 (250 mm × 2 mm) (Dionex, Sunnyvale, CA, USA). The mobile phase was 200 mM methylamine at a flow rate of 0.35 mL/min, the column temperature was 30 °C, and the injection volume was 100 μL. Mass-spectrometer settings are given in Table 1. Instrument control and data acquisition were performed via a personal computer and Micromass MassLynx v. 3.5 software. JMP 5.1 software (SAS Institute, Cary, NC, USA) was used for statistical analysis of the data.

2.3. Preparation of standards

All solutions were prepared in new Class A volumetric flasks (100-mL capacity) and stored in new high-density polyethylene bottles (125-mL capacity) (both from Nalge Nunc, Rochester, NY, USA). Rainin (Oakland, CA, USA) pipettes (2500, 250, and 100 μL, each with the appropriate tip) were used to deliver standard solutions for dilution. The concentration of the internal standard was 1.0 ng/mL in all standards and fortifications, and was introduced by adding 25 μL of a 77-ng/mL I.S. solution to 1.9 mL of sample solution.

The following working concentrations were prepared, both in reagent water and in high-TDS water: a blank; 0.05, 0.1, 0.5, 1, 2, 4, 8, 16, 32, and 64 ng/mL. Each set was prepared at the start of the study and used throughout. Both sets of standards were analyzed in random order over a 48 h period to generate eight replicate analyses for each fortification level. Standards were stored at room temperature throughout the study.

Table 1
Mass-spectrometer settings used for this research

Parameter	LC/MRM
Ion source	Electrospray
Polarity	Negative Ion
Capillary voltage	0.58 kV
Cone voltage	40 V
Extractor	3 V
RF lens	0.3 V
Source temperature	120 °C
Desolvation temperature	320 °C
Cone gas flow	60 L/h
Desolvation gas flow	N_2 800 L/h
LM 1 resolution	15
HM 1 resolution	15
Ion energy	0.6 V
Entrance	1
Collision	24
Exit	1
LM 2 resolution	14
HM 2 resolution	14
Ion energy	1 V
Multiplier	650 V
Gas cell pressure	Ar 3.54×10^{-3} mbar
Function dwell time	300 ms
Data smoothing (mean)	Window/Smoothes, 3/2

All masses were determined using a Mettler AT-200 Balance (Mettler-Toledo, Columbus, OH, USA) and were recorded to four decimal places. Dilution errors in the daily working standards were estimated by conducting a Monte Carlo simulation. This exercise was based on the upper bound (0.0001 g) on the magnitude of weighing error for the balance. In the simulation, weighing errors were randomly drawn from a Normal distribution with mean equal to zero and standard deviation equal to the upper bound. The distribution of these relative concentration errors was found never to exceed 0.1% relative error, which was considered negligible.

3. Results and discussion

3.1. Initial considerations

The predominant challenge in the development of the method was to achieve good chromatographic separation between perchlorate and the common anions often present in samples, using a volatile mobile phase. Sulfate, which elutes prior to perchlorate on most columns, was the main interference problem. When existing column chemistries were found to be inadequate, alternate column chemistries were investigated. The results of these efforts resulted in the development of the Dionex AS21 analytical column [3]. In separations performed using this column, perchlorate is well resolved from even high levels of chloride, sulfate, and bicarbonate, all of which elute well before the analyte (see Fig. 1).

To compensate for drift and variability of the detector signal, an isotopically labeled internal standard was used. For MS

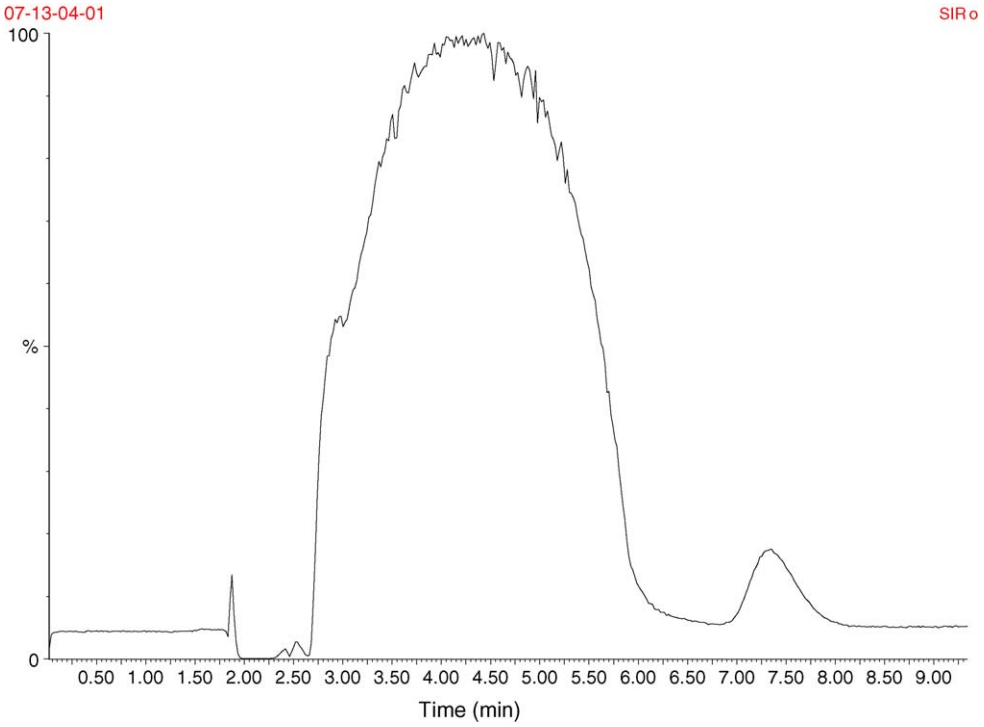

Fig. 1. Total-ion chromatogram (*m/z* 99, 101, 107) of high-TDS water (1000 mg/L each of chloride, sulfate, and bicarbonate) fortified with 0.5 ng/mL perchlorate.

work, the ideal I.S. is absent from the sample itself, and gives a characteristic and strong *m/z* response that is different from that of the analyte. The I.S. also should display a signal with tight precision and mimic the behavior of the analyte [4]. A stable isotope of the analyte typically meets these criteria extremely well. Thus, $Na^{35}Cl^{18}O_4$ was used.

Two product ions were monitored in the quantitation of perchlorate (see Table 2). The peak areas from the *m/z* 83 ions were used for calculations; these values were scaled by the peak areas of the *m/z* 89 ions from the internal standard. Additionally, the *m/z* 83/85 ratio was used for the qualitative confirmation of perchlorate. Ion chromatograms are shown in Fig. 2.

3.2. Calibration design and regression diagnostics

The calibration design outlined in Section 2.3 was chosen to accomplish several goals [5]. First, concentrations of less than one ng/mL were included to achieve a low Hubaux-Vos detection limit (H-V DL), as well as to detect non-linear-response behavior that might be exhibited in that region of concentrations. Second, levels out to 64 ng/mL were included to cover the concentration range expected from typical samples. Third, eight replicates were analyzed to give sufficient data for modeling of the standard deviations of the responses, as well as for determination of the appropriateness of the chosen model. Based on previous work with perchlorate's *m/z* 83 scaled responses, a straight-line (SL) model with ordinary-least-squares (OLS; a fitting technique that minimizes the sum of squares of the residuals) fitting was proposed to fit the data.

Table 2
Ions and retention times

Analyte	Parent ion (*m/z*)	Product ion (*m/z*)	Retention time (min)
$^{35}ClO_3$	99	83	8.68
$^{35}Cl^{18}O_3$	107	89	8.65
$^{37}ClO_3$	101	85	8.68

See Section 3.1 for details.

After the calibration study had been designed and conducted, the perchlorate data were scaled by the internal-standard responses and plotted versus true concentration. These regression data were diagnosed statistically [6–8] to see if the proposed model and fitting technique were appropriate for the calibration curve. It was found that weighted least squares (WLS; ordinary least squares, except the responses are numerically weighted to reflect non-uniform levels of variation in the responses) was needed for the fitting technique. However, no single model could be found to fit the entire concentration range adequately. Consequently, the data were divided into a low set (from the blank through 4 ng/mL) and a high set (from 4 to 64 ng/mL); the concentration of 4 ng/mL was included in both sets to act as a bridge between the two curves. Regression diagnostics showed that a quadratic model with WLS fitting provided an adequate fit for both the low- and high-concentration data. The resulting statistics (at 95% confidence) are shown in Table 3. It should be noted that at 4 ng/mL, the half-width of the prediction interval ([9]; the prediction interval is a range of values defined by a pair of limits that bracket uncertainty, in this case, uncertainty in one future measurement) is essentially the same for both the high- and low-concentration curves. Also, when the calibration-curve equations were applied to the calibration data (i.e., to the scaled peak areas), the predicted values for the

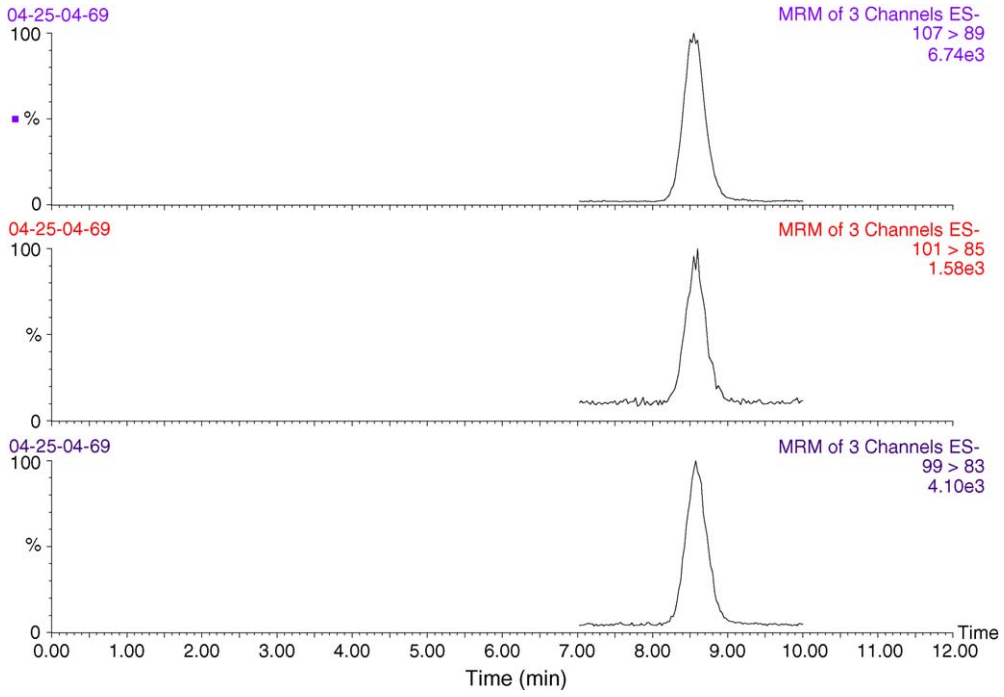

Fig. 2. Monitoring of the internal standard's *m/z* 89 (top tracing), and perchlorate's *m/z* 85 (center tracing) and *m/z* 83 (bottom tracing). Concentration of internal standard is 1.0 ng/mL; concentration of perchlorate is 0.5 ng/mL.

4-ng/mL standards were within 0.14 and 0.13 ng/mL of true for the low-and high-concentration curves, respectively. Thus, this concentration was deemed to be an appropriate bridge between the two plots. Furthermore, for both curves, the half-width of the respective prediction intervals was considered to be sufficiently small for this method.

The Hubaux-Vos detection limit can be determined graphically from a plot of the lower and upper prediction limits (LPL and UPL, respectively) for the regression line in question [10] (here, for the low-concentration curve; see Fig. 3.) At the 95% confidence level, the value was found to be 0.04 ng/mL.

3.3. Recovery study

3.3.1. Regression diagnostics

Because the goals of the recovery study were the same as for the calibration work, the same design was used. To determine the overall recovery of perchlorate in both reagent water and in the high-TDS matrix, the scaled data first were segmented into the same low- and high-concentration data sets as were the calibration-curve responses. Recovered concentrations were determined using the corresponding calibration curves. For each data set, calculated concentrations were plotted versus true concentrations. Via regression diagnostics (as referenced in Section 3.2), an appropriate model and fitting technique were chosen for each plot; for each curve, the prediction-interval half-width (at 95% confidence) and the prediction formula were determined (see Table 4). All prediction-interval half-widths were deemed acceptable for this method.

It should be noted that with the high-TDS matrix, the quadratic model displayed lack of fit for the high-concentration range. A cubic model was found to be adequate, but was rejected

Table 3
Prediction intervals for the calibration curves generated from the scaled *m/z* = 83 data (confidence level = 95%)

Statistic	Value (ng/mL) for *m/z* = 83 data
Prediction interval at 4 ng/mL (low-concentration curve)	±0.14
Prediction interval at 4 ng/mL (high-concentration curve)	±0.15
Prediction interval at 64 ng/mL (high-concentration curve)	±2.7

See Section 3.2 for details.

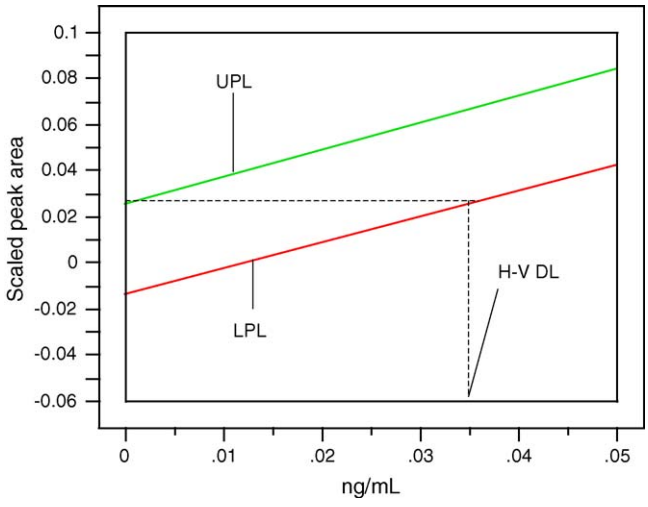

Fig. 3. Graphical determination of the H-V DL from the low-concentration calibration curve for perchlorate. See Section 3.2 for details.

Table 4
Statistics for the recovery curves for both reagent water and for high-TDS water (confidence level = 95%)

Water matrix	Concentration range	Model, fitting technique	Prediction-interval half-width (±ng/mL at ng/mL)	Equation for recovery curve; recovered x[a] =
Reagent	Low	Quadratic, WLS	±0.02 at 0.05; ±0.2 at 4	$-0.001 + 0.956x + 0.015x^2$
Reagent	High	Straight line, OLS	±0.8 at all	$0.073 + 0.978x$
High-TDS	Low	Quadratic, WLS	±0.02 at 0.05; ±0.2 at 4	$-0.016 + 0.977x + 0.017x^2$
High-TDS	High	Quadratic, OLS	Between 1.1 and 1.8 at all	$0.838 + 0.886x + 0.002x^2$

See Section 3.3.1 for details.
[a] x = ng/mL.

since a cubic equation is difficult to invert and is not available in all software packages. The prediction-interval calculations were modified to incorporate the bias that the quadratic model exhibited [11].

3.3.2. Determination of the lowest concentration minimum reporting level (LCMRL)

The LCMRL is the lowest true concentration that will guarantee a recovery between any two percentages, at the desired statistical-confidence level [12]. Both the percentage range and the confidence level are chosen by the user (for the UCMR2, 50 to 150%, at the 99% confidence level). The LCMRL is determined using the prediction intervals (as determined in Section 3.3.1) associated with a given recovery graph, thereby making the level statistically sound [9]. In addition to these lines, the lower percent-recovery line and the upper percent-recovery line are plotted. To be certain of being within the desired prediction interval, a calculated concentration must fall inside the interval's pair of limits. To be certain of being within the desired percent-recovery range, a calculated concentration must fall inside the lines that represent the two extremes of this range. For the LCMRL, the goal is to find the lowest concentration that will fall inside both of the above sets of lines. The percent-recovery lines typically intersect the prediction-interval envelope at two points, although it is possible to have only one intersection or none at all. When there are two intersections, the higher concentration is the LCMRL, since at the lower concentration, a result can fall outside one of the prediction limits.

For this research, the standard LCMRL procedure was used to calculate LCMRL values, with two exceptions. First, an improved protocol [6] for modeling the standard deviation is proposed, and has been used here in the regression and prediction-interval calculations. Second, the choice of a model for the recovery curve has been expanded to include a quadratic. Additionally, a recommendation to revise the definition of the LCMRL is proposed. This update would modify the definition to read, "The LCMRL is the lowest true concentration that will guarantee a recovery between any two percentages, at greater than or equal to the desired statistical-confidence level" (see below for an explanation).

Because the LCMRL is determined from low-level data, the low-concentration recovery plot was used for each matrix. A confidence level of 95% and a recovery range of 50–150% were used in this research (the standard LCMRL procedure allows flexibility in these choices). As can be seen in Fig. 4, the LCMRL is very similar for both the reagent-water matrix (0.03 ng/mL) and for the high-TDS matrix (0.07 ng/mL). Both values are roughly equal to the lowest non-zero concentration used in the recovery studies. In both plots, the 150% line falls above the upper prediction limit (UPL). To obtain the 150%-UPL intersection, the confidence level associated with the UPL will have to be increased. Thus, the revised definition (see preceding paragraph) of the LCMRL has been proposed.

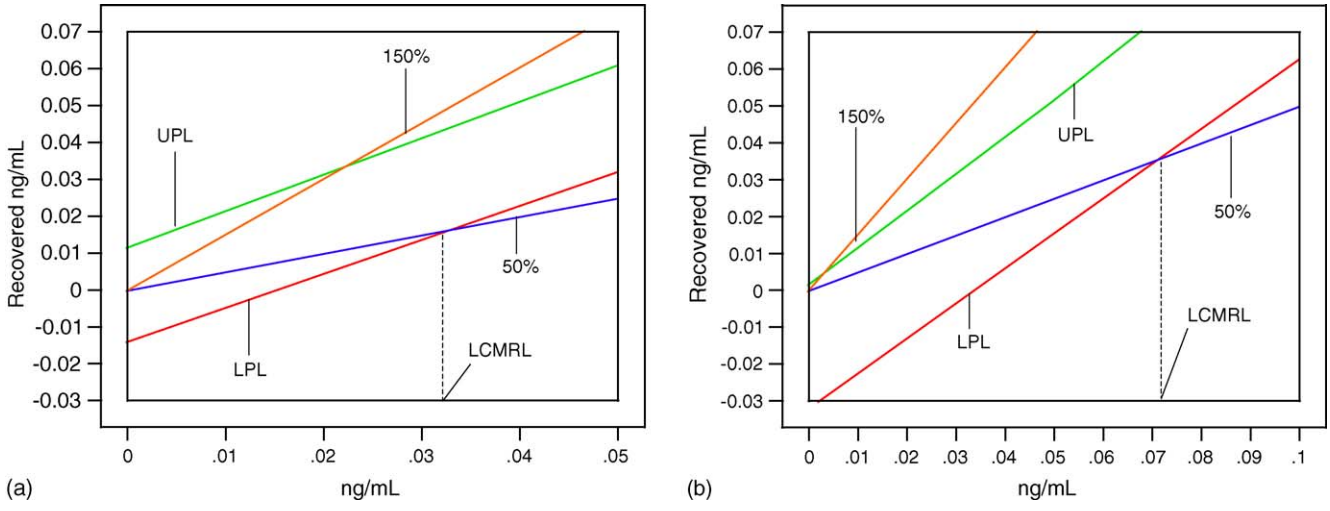

Fig. 4. Graphical determination of the LCMRL for perchlorate in: (a) reagent water and (b) high-TDS water. See Section 3.3.2 for details.

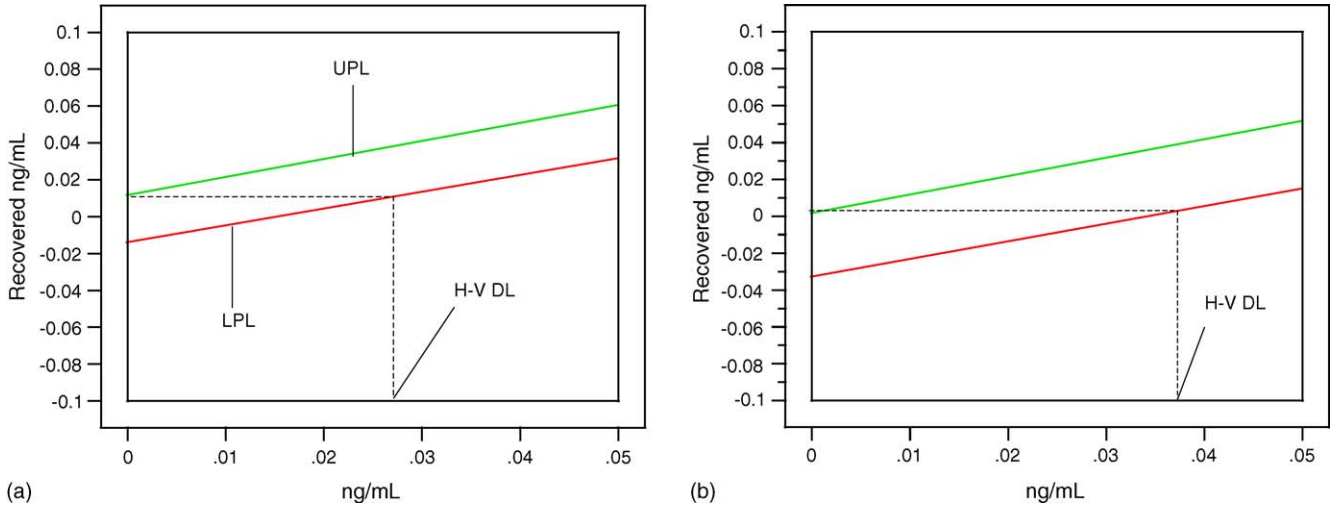

Fig. 5. Graphical determination of the H-V DL for perchlorate in: (a) reagent water and (b) high-TDS water. See Section 3.3.3 for details.

3.3.3. Determining Hubaux-Vos detection limits

H-V DLs were determined graphically from the plots of the prediction limits for the low-concentration recovery curves (see Fig. 5). These limits were 0.03 ng/mL for the reagent-water data and 0.04 ng/mL for the high-TDS data (both at 95% confidence).

4. Conclusions

This research has shown that EPA Method 331 is capable of generating statistically sound detection and reporting limits at sub-ng/mL levels. It should be pointed out that if the overall recovery for a procedure is 100% and if the lower recovery limit is 50%, the LCMRL will be the same value as the H-V DL. This equivalence is due to the fact that, at the H-V DL, the relative uncertainty is ±50%, assuming the false-negative and false-positive rates are the same. It is up to the user to decide if a reporting limit should be as low as a detection limit. If this situation is unacceptable, the allowed range of the recovery limits should be tightened, at least at the lower extreme.

It is possible that no LCMRL can be achieved for the allowed recovery range. This phenomenon may occur if the slope of the recovery line itself is less than the lower allowed recovery limit. Additionally, within the concentration range for the recovery study, the lower recovery limit may not intersect the lower prediction limit. This situation is possible if the *y*-intercept for the recovery line is significantly above zero. In such cases, the user may want to investigate the chemistry to identify and eliminate the source of the bias.

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References

[1] C.J. Koester, H.R. Bellar, R.U. Halden, Environ. Sci. Technol. 34 (2000) 1862.
[2] P. Winkler, M. Minteer, J. Willey, Anal. Chem. 76 (2004) 473.
[3] Product Manual - IonPac AS21 Column, Doc. 065043, Rev. 01, Dionex, Sunnyvale, CA, 2004.
[4] D. Coleman, L. Vanatta, Am. Lab. (December 2005) 23, available at www.iscpubs.com.
[5] D. Coleman, L. Vanatta, Am. Lab. (June 2003) 30, available at www.iscpubs.com.
[6] D. Coleman, L. Vanatta, Am. Lab. (November 2003) 40, available at www.iscpubs.com.
[7] D. Coleman, L. Vanatta, Am. Lab. (February 2004) 64, available at www.iscpubs.com.
[8] D. Coleman, L. Vanatta, Am. Lab. (March 2004) 46, available at www.iscpubs.com.
[9] D. Coleman, L. Vanatta, Am. Lab. (March 2003) 60, available at www.iscpubs.com.
[10] L.E. Vanatta, D.E. Coleman, J. Chromatogr. A 770 (1997) 105.
[11] D. Coleman, L. Vanatta, Am. Lab. (May 2005) 37, available at www.iscpubs.com.
[12] EPA Document 815-R-05-006, US Environmental Protection Agency, 2004.