

Improved Determination of Trace Concentrations of Perchlorate in Drinking Water Using Preconcentration with Two-Dimensional Ion Chromatography and Suppressed Conductivity Detection

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Introduction

The multiple pathways from which perchlorate may be ingested into the body and its associated health risks has increased the interest in the determination of low concentrations of perchlorate. Perchlorate inhibits the normal uptake of iodide by the thyroid gland, which results in reduced thyroid hormone production. Low thyroid hormone production results in improper metabolic regulation and can potentially lead to the development of thyroid tumors in adults.^{1,2} The fetuses of pregnant women with hypothyroidism are particularly at higher risk because reduced thyroid hormone production can cause impaired mental development, and in some cases birth defects.^{1,3} In 2005, the National Academy of Sciences recommended a reference dose of 0.7 µg/kg/day from all available sources, which it believes should not threaten the health of even the most sensitive populations.¹

Perchlorate has been detected at nearly 400 sites across the United States where most contamination appears to be confined to the western and southwestern regions.⁴ It is estimated that over 11 million people have perchlorate in their drinking water supplies at a concentration of 4 µg/L (ppb) or greater.¹ Evidence also suggests that perchlorate can be taken up by plants through contaminated irrigation water and soil.⁵ In addition, a recent study reported the detection of perchlorate in food items, such as milk and lettuce.^{4,6}

Currently, there are no federal drinking water regulations for perchlorate. However, several states have adopted their own advisory levels that range in concentration from 1 to 18 ppb perchlorate. In 2004, the California Office of Environmental Hazard Assessment established a public health goal of 6 ppb perchlorate.⁷ While Massachusetts, Maryland, and New Mexico have established lower

perchlorate advisory levels of 1 ppb.⁸ The U.S. Environmental Protection Agency (EPA) identified perchlorate as a contaminant of potential concern with its Contaminant Candidate List publication in 1998. Following this publication, the EPA proposed the Unregulated Contaminant Monitoring Rule.² EPA Method 314.0 was developed in conjunction with this publication to determine trace concentrations of perchlorate in drinking water.⁹ This method describes the use of a 4 mm Thermo Scientific™ Dionex™ IonPac™ AS16 column and a 1 mL direct injection with suppressed conductivity detection to determine perchlorate at concentrations of 4 ppb or greater. Although significant improvements have been made to reduce the method reporting limit (MRL) from 4 to 1 ppb, the determination of trace perchlorate in high-ionic-strength matrices is still a challenging problem.¹⁰ Typically, this requires the use of sample pretreatment cartridges to remove the common anions chloride, sulfate, and carbonate from the matrix. This sample treatment procedure can be a very time consuming and laborious process.

Recently, the EPA published Method 314.1 as an update to Method 314.0 to improve the sensitivity for perchlorate in high-ionic-strength matrices. This method requires the concentration of a 2 mL sample on a Dionex IonPac Cryptand C1 preconcentration column followed by matrix elimination with 1 mL of 10 mM sodium hydroxide.^{11,12} Perchlorate is then separated by using a 2 mm Dionex IonPac AS16 column in the primary method. If perchlorate is positively identified with this method, then the sample must be reanalyzed on a confirmatory column, the Dionex IonPac AS20, to verify the presence of perchlorate and thereby reduce the likelihood of a false positive.

Alternatively, a two-dimensional ion chromatographic approach can be used to resolve perchlorate from high concentrations of common matrix ions.¹³ The first dimension uses a 4 mm Dionex IonPac AS20 column to divert the matrix ions while 5 mL of the suppressed effluent containing perchlorate is trapped on a Dionex IonPac TAC-ULP1 Trace Anion Concentrator Column and then separated on a 2 mm Dionex IonPac AS16 column in the second dimension for quantitative analysis. This method provides several advantages, such as the ability to inject large sample volumes, the ability to focus the perchlorate that is partially resolved in the first dimension onto a concentrator column and separate it in the second dimension, and the ability to combine two different column chemistries to enhance the selectivity and reduce the possibility of a false positive. This application note demonstrates this approach for determining trace concentrations of perchlorate in environmental waters using the same criteria specified in Method 314.1.

Equipment

- Thermo Scientific™ Dionex™ ICS-3000 Reagent-Free™ Ion Chromatography (RFIC™) System:
 - DP Dual Pump module
 - EG Eluent Generator module
 - DC Detector/Chromatography module (single or dual temperature zone configuration)
 - AS Autosampler with a 5 mL syringe (P/N 053915), 8.2 mL sampling needle assembly (P/N 061267), and sequential injection option (P/N 063294)
- Two Thermo Scientific™ Dionex™ EluGen™ EGC II KOH cartridges (P/N 058900)
- Two Thermo Scientific™ Dionex™ CR-ATC Continuously Regenerated Anion Trap Columns (P/N 060477)
- Thermo Scientific™ Dionex™ Carbonate Removal Device (CRD), 2 mm (P/N 062986) and 4 mm (P/N 062983)
- Four 4 L plastic bottle assemblies for external water mode of operation
- Thermo Scientific™ Dionex™ Chromeleon™ 6.7 Chromatography Management Software

Reagents and Standards

- Deionized water, Type I reagent grade, 18 MΩ-cm resistivity or better
- Sodium perchlorate, NaClO₄
- Sodium chloride, NaCl
- Sodium sulfate, Na₂SO₄
- Sodium bicarbonate, NaHCO₃

Conditions

First Dimension Conditions

Columns:	Dionex IonPac AS20 Analytical, 4 × 250 mm (P/N 063148) Dionex IonPac AG20 Guard, 4 × 50 mm (P/N 063154)
Eluent:	35 mM potassium hydroxide 0–30 min, step to 60 mM at 30.1 min*, 60 mM 30.1–40 min, step to 35 mM at 40.1 min, 35 mM 40.1–45 min
Eluent Source:	Dionex ICS-3000 EG
Flow Rate:	1 mL/min
Temperature:	30 °C (lower compartment) 30 °C (upper compartment)
Inj. Volume:	4000 µL
Detection:	Suppressed conductivity, Thermo Scientific™ Dionex™ ASRS™ ULTRA II Suppressor, 4 mm, auto-suppression, external water mode (flow rate: 3–5 mL/min); Power setting – 150 mA
CRD:	4 mm format (P/N 062983)
System Backpressure:	~2500 psi
Background Conductance:	~0.2–0.3 µS
Noise:	~1–2 nS/min peak-to-peak
Run Time:	45 min
*The step change described here should occur after the valve on system #2 has switched from the load to inject position.	

Second Dimension Conditions

Columns:	Dionex IonPac AS16 Analytical, 2 × 250 mm (P/N 055378) Dionex IonPac AG16 Guard, 2 × 50 mm (P/N 055379)
Eluent:	65 mM potassium hydroxide
Eluent Source:	Dionex ICS-3000 EG
Flow Rate:	0.25 mL/min
Temperature:	30 °C (lower compartment) 30 °C (upper compartment)
Inj. Volume:	5 mL (on the concentrator column from first dimension)
Concentrator:	Dionex TAC-ULP1, 5 × 23 mm (P/N 061400)
Detection:	Suppressed conductivity, Dionex ASRS ULTRA II Suppressor, 2 mm, auto-suppression, external water mode (flow rate: 1–3 mL/min); Power setting – 41 mA
CRD:	2 mm format (P/N 062986)
System Backpressure:	~2500 psi
Background Conductance:	~0.7–0.8 µS
Noise:	~1–2 nS/min peak-to-peak
Run Time:	45 min

Preparation of Solutions and Standards

Stock Perchlorate Standard Solution

Dissolve 0.1231 g sodium perchlorate in 100 mL of deionized water for a 1000 mg/L standard solution. When stored in an opaque, plastic storage bottle, this stock solution may be stable for up to one year.

Perchlorate Primary Dilution Standard

Prepare 10 mg/L perchlorate solution by adding 1 mL of the 1000 mg/L stock standard in a 100 mL volumetric flask and dilute to volume with deionized water. When stored in an opaque, plastic storage bottle, the resulting solution is stable for at least one month.

Perchlorate Secondary Dilution Standard

Prepare a 1 mg/L perchlorate solution by adding 10 mL of the primary dilution solution to a 100 mL volumetric flask and dilute to volume with deionized water. When stored in an opaque, plastic storage bottle, the resulting solution is stable for at least one month.

Perchlorate Calibration Standards

Prepare perchlorate calibration standards at 0.3, 0.5, 1, 3, 5, and 10 µg/L by adding the appropriate volumes of the perchlorate secondary dilution solution to separate 100 mL volumetric flasks.

Common Anion Stock Solution

Prepare 25 mg/mL (25,000 mg/L) each of chloride, sulfate, and bicarbonate. Dissolve 4.121 g sodium chloride in deionized water and dilute to 100 mL. Dissolve 3.696 g sodium sulfate in deionized water and dilute to 100 mL. Dissolve 3.442 g sodium bicarbonate in deionized water and dilute to 100 mL.

Sample Preparation

All samples must be sterile filtered with a 0.2 µm syringe filter (26 mm surfactant-free cellulose acetate) to remove any potential microorganisms. Perchlorate is susceptible to microbiological degradation by anaerobic bacteria.⁷ A disposable sterile syringe (Henke Sass Wolf, 20 mL Luer-Lok™, [14-817-33]) is used to draw up ~20 mL of the sample followed by attaching a sterile syringe filter. Discard the first 3–5 mL of the sample, and then filter the remaining sample in a 125 mL sterile sample container (high density polyethylene, HDPE, I-Chem [N411-0125]). Discard the syringe and filter after each use.

System Preparation and Setup

Install and configure the EG module by first installing backpressure tubing in place of the columns on both system channels to produce a total backpressure of ~2000–2500 psi at a flow rate of 1 mL/min. Install a Dionex EGC II KOH cartridge for each system channel. Condition the cartridges by setting the KOH concentration to 50 mM at 1 mL/min for 30 min. After completing the conditioning process, disconnect the backpressure tubing temporarily installed in place of the column set. Install a Dionex CR-ATC Continuously Regenerated Anion Trap Column between the Dionex EGC II KOH cartridge and the EGC degas. Hydrate the Dionex CR-ATC column prior to use by following the instructions

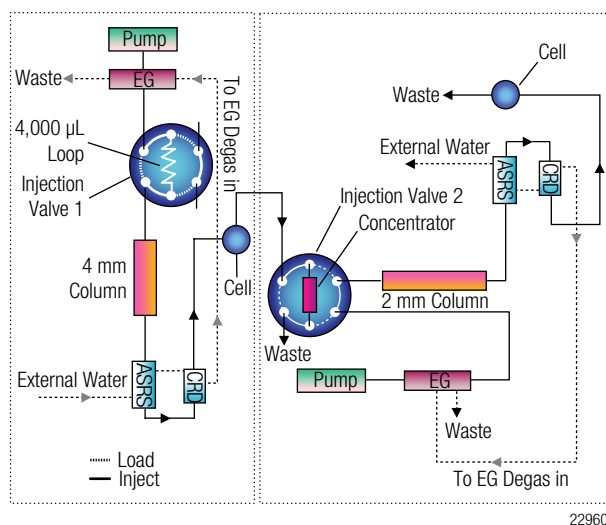


Figure 1. Schematic diagram of two-dimensional system for the determination of trace concentrations of perchlorate.

outlined in the Dionex EluGen Cartridge Quickstart Guide (Document No. 065037-02). Figure 1 shows a schematic diagram of the system setup.

Install and configure the AS autosampler. The most accurate and precise sample injections with the AS autosampler are made with a calibrated sample loop, flushed with about four to five times the loop volume. Because this application requires large sample injection volumes, a minimum sample syringe size of 5 mL (P/N 053915) should be installed. To accommodate the larger volume, an 8.2 mL sampling needle assembly (P/N 061267) is also required for operation. To inject 4000 µL, select the normal mode from the front panel of the autosampler and set the injection loop size to 4000 µL. Prepare a 4000 µL sample loop by measuring approximately 345.5 in. of 0.030 in. i.d. tubing. Verify the volume of the loop by first weighing the empty tubing, fill the tube with deionized water, then reweigh the filled tube and calculate the volume. The total sample volume should be ~4000 µL ± 5%. Install the sample loop on injection valve 1 of the DC module. To allow independent control of the DC module injection valves, the DC settings in the Chromeleon software system configuration must be changed. To modify this configuration, go to the DC high pressure valves tab in the system configuration, double-click InjectValve_2, and change controlled by AS to DC.

Install the Dionex IonPac AG20 (4 × 50 mm) and the IonPac AS20 (4 × 250 mm) columns on system #1 in the lower compartment of the DC module. Install the Dionex IonPac AG16 (2 × 50 mm) and the IonPac AS16 (2 × 250 mm) columns on system #2. Connect a piece of 0.01 in. i.d. tubing from the cell out on system #1 to the sample inlet port on injection valve #2. The length of this tubing should be kept to a minimum. Install a Dionex IonPac TAC-ULP1 Trace Anion Concentrator Column (5 × 23 mm) in place of the sample loop on system #2. The direction of sample loading should be in the opposite direction of the analytical flow. Make sure the pressure for

both systems is ~2200–2500 psi using the operating conditions described earlier to allow the degas assembly to effectively remove electrolysis gases from the eluent. If necessary, install additional backpressure tubing between the degas assembly and the injection valve to achieve the recommended pressure setting. Monitor the pressure periodically as it can gradually rise over time. To reduce pressure, trim the backpressure tubing.

Hydrate the Dionex ASRS ULTRA II Suppressor prior to installation by using a disposable plastic syringe and push ~3 mL of degassed deionized water through the Eluent Out port and ~5 mL of degassed deionized water through the Regen In port. Allow the suppressor to stand for ~20 min to fully hydrate the suppressor screens and membranes. Hydrate the CRD according to the instructions in the operating manual. Prior to installing the suppressor, rinse the analytical column with 65 mM KOH while diverting to waste. Install the Dionex ASRS ULTRA II Suppressor for use in the external water mode by connecting the Regen Out of the suppressor to the Regen In of the CRD and connect the Regen In of the suppressor to the external water source. The Regen Out of the CRD is connected to the Regen In of the Dionex CR-ATC column, while the Regen Out of the CR-ATC connects to the Regen In of the EG degasser.

Equilibrate the Dionex IonPac AS20 column with 35 mM KOH and the AS16 column with 65 mM KOH at their respective flow rates shown in the conditions section for approximately 60 min. Analyze a matrix blank by injecting deionized water. An equilibrated system has a background conductance of <0.3 μS and <0.8 μS for the Dionex IonPac AS20 and AS16 columns, respectively. Determine the cut time (preconcentration time) for the second dimension, as described in the next section, before analyzing perchlorate.

Determining the Cut Time for the Second Dimension

Because there may be slight variations in system plumbing, column capacity, and tubing lengths, individual laboratories should first determine the optimum cut time (from the first dimension) before determining perchlorate in the second dimension. The injection of 4000 μL of sample or standard will increase the retention time of perchlorate on the Dionex IonPac AS20 column compared to the provided quality assurance report of the column. Therefore, we recommend performing duplicate 4000 μL injections of 5 ppb perchlorate to determine the average perchlorate retention time on the Dionex IonPac AS20 column. *It is important to verify the retention time of perchlorate on the Dionex IonPac AS20 column weekly to ensure good trapping efficiency on the Dionex IonPac TAC-ULP1 concentrator column.* In our experiments, the perchlorate retention time (t_{ClO_4}) was approximately 26 min. Therefore, valve #2 in the second dimension was placed in the load position at 22 min ($t_{\text{ClO}_4} - 4$ min) and then switched to the inject position at 27 min ($t_{\text{ClO}_4} + 1$ min). In this configuration, perchlorate eluted in ~41 min from the Dionex IonPac AS16 column. Figure 2 shows example chromatograms of 5 ppb perchlorate separated on the Dionex IonPac AS20 and AS16 columns.

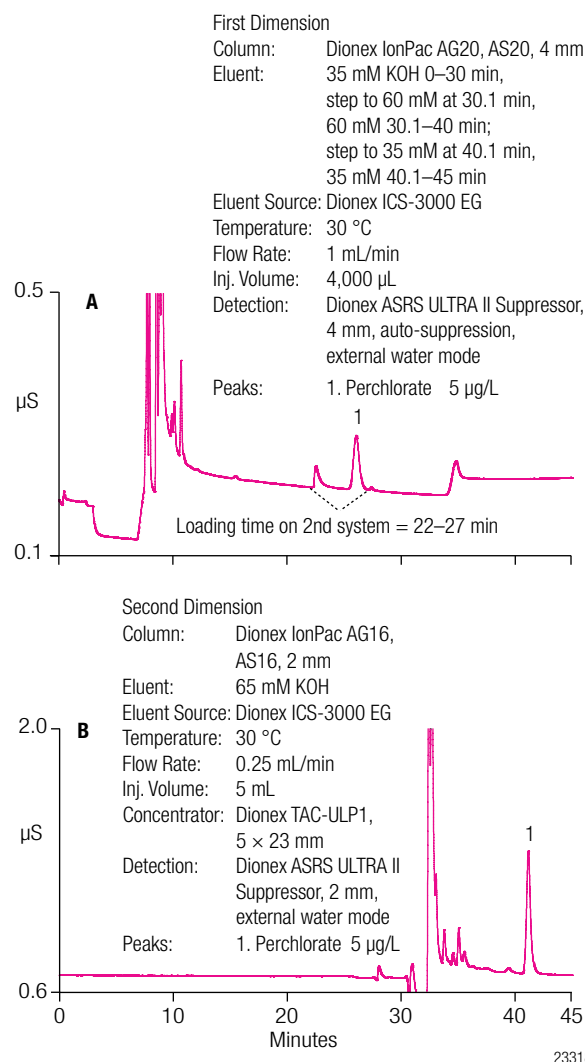


Figure 2. Separation of a 5 $\mu\text{g/L}$ perchlorate standard with the (A) Dionex IonPac AS20 column in the first dimension and (B) Dionex IonPac AS16 column in the second dimension.

Results and Discussion

The second system was calibrated by injecting one blank and duplicate injections of six calibration standards to cover the desired concentration range. Because this two-dimensional (2-D) approach was found to be slightly more sensitive than Method 314.1, the system was calibrated from 0.3 $\mu\text{g/L}$ instead of 0.5 $\mu\text{g/L}$.¹² However, the minimum reporting level (MRL) remained at 0.5 $\mu\text{g/L}$ for the 2-D method to compare with data generated by Method 314.1. The peak area response generated by the calibration standards was tabulated against the perchlorate concentration using a quadratic regression curve. Table 1 summarizes the calibration data obtained from injecting standards in the range of 0.3–10 $\mu\text{g/L}$ perchlorate. This calibration curve produced a correlation coefficient of 0.9998 with the 2 mm Dionex IonPac AS16 column in the second dimension. We verified the accuracy of the calibration curve by injecting a 5 ppb perchlorate standard from a second source. This produced a calculated recovery of 103.3%, well within the $\pm 25\%$ required by Method 314.1.

Table 1. Calibration data and method detection limits for perchlorate.

Analyte	Range (µg/L)	Linearity (r ²)*	MDL Std (µg/L)	SD (µg/L)	Calculated MDL (µg/L)
Perchlorate	0.3–10	0.9998	0.06	0.005	0.016

*Quadratic fit

Section 9.2.7 of Method 314.1 states that the determination of the detection limit is not a specific requirement of this method. However, some laboratories may require this determination due to the various regulatory bodies associated with compliance monitoring. The limit of detection (LOD) was determined for perchlorate using the 2-D method by performing seven replicate injections of deionized water fortified with 0.06 ppb perchlorate. The LOD was calculated using the following equation:

$$\text{LOD} = St_{(n-1, 1-\alpha = 0.99)}$$

where:

$t_{(n-1, 1-\alpha = 0.99)}$ = students' t-value for a 99% confidence level with n-1 (t = 3.14 for seven replicate injections)

n = number of replicates

S = standard deviation of replicate analyses

The results from this equation produced a calculated LOD of 16 ng/L, slightly less than the 23–26 ng/L previously determined with Method 314.1. Table 1 summarizes the results of this calculation using the 2-D method.

To confirm that 0.5 µg/L perchlorate is an appropriate MRL, seven replicates at this concentration were analyzed. The mean and standard deviation of the replicate analyses were then calculated. Section 9.2.4 of Method 314.1 describes equations used to determine the upper and lower limits for the Prediction Interval of Results (PIR). The results of these equations produced lower and upper limits for the PIR at 86.9% and 107.5%, respectively. These recovery limits are well within the ±50% requirement of Method 314.1. Therefore, 0.5 µg/L perchlorate is an acceptable MRL for this application. Figure 3 shows a chromatogram of 0.5 µg/L perchlorate standard separated on the Dionex IonPac AS16 column.

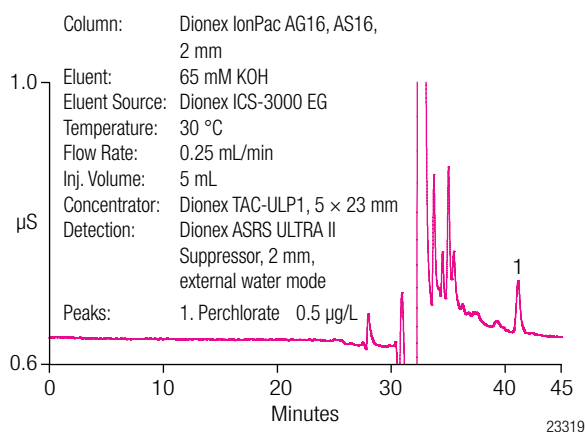


Figure 3. Chromatogram of a 0.5 µg/L perchlorate standard with the Dionex IonPac AS16 column in the second dimension.

Samples containing high concentrations of the common anions chloride, sulfate, and carbonate influence the integrity of perchlorate and dramatically reduce the likelihood of obtaining meaningful results. To overcome this challenge, Method 314.0 required the use of Thermo Scientific™ Dionex™ OnGuard™ sample preparation cartridges. However, this time-consuming and laborious procedure still may not yield the desired results depending on the ionic strength of the sample being analyzed. This procedure was overcome with the development of Method 314.1 that allowed the direct analysis of high-ionic-strength matrices with lower LOD. The performance of the 2-D method described in this application note was evaluated by preparing the Laboratory Fortified Sample Matrices (LFSM) used in Method 314.1. This was accomplished by adding known quantities of perchlorate to each matrix and calculating the percent recovery. We evaluated the recovery of perchlorate by analyzing six matrices, including reagent water, a synthetic high-ionic-strength inorganic water (HIW), and four drinking waters from different sources. Each sample was fortified with 0.5 and 5 µg/L perchlorate. To ensure the accuracy of the calibration curve, quality control standards prepared at 0.5, 5, and 10 µg/L perchlorate were analyzed at the beginning, middle, and end of each sample analysis batch.

Table 2 summarizes the performance of the method for determining low concentrations of perchlorate using a 2-D ion chromatography method. Calculated recoveries for samples fortified with 0.5 µg/L perchlorate were in the range of 96–102%, well within the ±50% specification of Method 314.1. Similarly, samples fortified with 5 µg/L perchlorate produced recoveries from 99–101% which were within ±25% requirement. Figure 4 shows chromatograms of unfortified and fortified drinking water D using the combined Dionex IonPac AS20/AS16 2-D approach. As shown, perchlorate is well resolved from any potential matrix interference, and therefore produces an excellent recovery of 97% when fortified with 0.5 µg/L perchlorate. Previously, an unknown interferent was observed that coeluted with perchlorate in this sample using the Dionex IonPac AS20 with Method 314.1.¹² Consequently, this interferent is eliminated by first separating perchlorate on the Dionex IonPac AS20 and then trapping a 5 mL portion of the effluent containing the perchlorate peak on a Dionex IonPac TAC-ULP1 concentrator column and, finally, separating the perchlorate on a Dionex IonPac AS16 column. By using this 2-D approach, no interfering peaks were observed in the sample matrices examined in this study. Also, some samples may contain significantly higher concentrations of the common anions typically found in most drinking water samples. To demonstrate applicability of these sample types, a synthetic high inorganic water (HIW) was prepared and analyzed by this method. Figure 5 shows an example of a synthetic HIW sample fortified with 0.5 µg/L perchlorate. As shown, nearly the entire sample matrix is eliminated and, therefore resulted in excellent recovery of the perchlorate peak.

Table 2. Perchlorate recoveries from laboratory fortified sample matrices.

Matrix	Amount Found (µg/L)	Amount Added (µg/L)	Replicates	Peak Area Precision (%RSD)	Recovery (%)
Reagent water	—	0.5	7	2.66	97.1
		5.0	7	0.79	101.0
HIW ¹	—	0.5	7	2.08	95.8
		5.0	7	0.22	99.7
Drinking water A	0.060	0.5	7	1.41	95.9
		5.0	7	0.76	98.9
Drinking water B	0.085	0.5	7	1.80	102.0
		5.0	7	0.97	99.0
Drinking water C	0.055	0.5	7	1.56	100.8
		5.0	7	1.00	100.9
Drinking water D	<MDL ²	0.5	7	1.53	97.1
		5.0	7	0.99	100.9

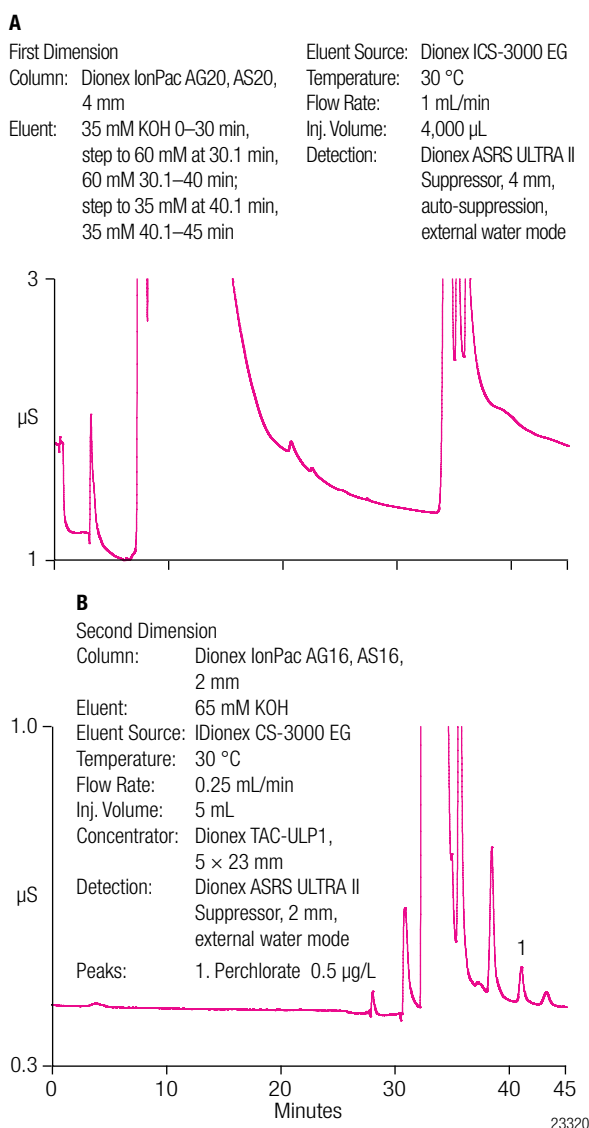
¹HIW = high inorganic water contains 1000 mg/L each of chloride, sulfate, and bicarbonate²<MDL = less than the method detection limit

Figure 4. Chromatogram of drinking water D fortified with 0.5 µg/L perchlorate in the (A) first dimension and (B) second dimension.

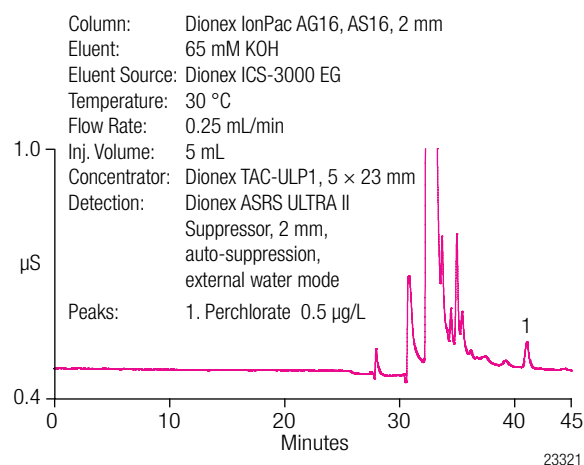


Figure 5. Chromatogram of synthetic high inorganic water fortified with 0.5 µg/L perchlorate in the second dimension.

Conclusion

This application note describes a two-dimensional system for determining trace concentrations of perchlorate in environmental waters. The method resulted in an improvement to the existing EPA Methods 314.0 and 314.1 by providing lower detection limits and improved precision and recovery of perchlorate fortified in different sample matrices. In addition, samples can be injected directly without the need for sample preparation, a sample rinse step with sodium hydroxide, and the addition of matrix ions to the standards and samples. These characteristics enhance the method's ease-of-use and can provide improved results between analysts and laboratories. Also, the method is further expanded by combining two different analytical columns with slightly different selectivities to allow for the determination of low concentrations of perchlorate in a wide range of sample matrices.

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