Environmental Water Applications Notebook

Perchlorate • Cyanide

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Introduction to Environmental Water Analysis

Everyone in the global community is impacted by the quality of water resources. The water we drink must be free from harmful chemicals to ensure good health. The purity of ground and surface waters in our environment is critical to ensuring sustainable use. The water discharged by municipal wastewater treatment plants and industrial facilities must be monitored to ensure strict compliance with environmental guidelines. Process waters must be kept clean from contaminants to ensure product quality and acceptable exposure levels.

Thermo Fisher Scientific is committed to enhancing the quality of our global water resources. As innovation leaders in ion and liquid chromatography, our analytical instruments are used by government and industry to provide solutions for environmental water testing for a wide range of regulated and emerging inorganic elements and organic compounds.

As pioneers of suppression technology, we started a revolution in ion chromatography (IC) that increased the sensitivity and accuracy of ion determination. As constant innovators, we developed Reagent-Free™ (RFIC™) systems that set a new benchmark for ion analysis. Today, RFIC systems with eluent generation and eluent regeneration provide the ultimate in sensitivity and ease of use.

We also have a full high-performance liquid chromatography (HPLC) product line for the analysis of organic contaminants, from nano- to preparative-scale separation capabilities, including ultra HPLC (UHPLC).

In fact, we are the only separations science company that provides instrumentation, columns, and applications perfectly suited for both inorganic and organic contaminants.

THERMO SCIENTIFIC AND DIONEX INTEGRATED SYSTEMS

Dionex Products are now a part of the Thermo Scientific brand, creating exciting new possibilities for scientific analysis. Now, leading capabilities in LC, IC, and sample preparation are together in one portfolio with those in mass spectrometry (MS). Combining Dionex's leadership in chromatography with Thermo Scientific's leadership position in mass spec, a new range of powerful and simplified workflow solutions now becomes possible.

For more information on how the new line-up of Thermo Scientific products can expand your capabilities and provide the tools for new possibilities, choose one of our integrated solutions:

- Ion Chromatography and Mass Spectrometry
- Liquid Chromatography and Mass Spectrometry
- Sample Preparation and Mass Spectrometry

GROUND AND SURFACE WATER

Surface water is the largest source of fresh water used for human consumption. The U.S. Geological Survey implemented the National Water-Quality Assessment (NAWQA) Program in 1991 to develop long-term data on streams, rivers, groundwater, and aquatic systems. The data support national, regional, state, and local policies and decisions related to water-quality management. The NAWQA program is designed to answer the following questions:

- What is the condition of our nation's streams, rivers, and groundwater?
- How are these conditions changing over time?
- How do natural features and human activities affect these conditions, and where are those effects most pronounced?

Thermo Scientific has codeveloped several methods with the U.S. EPA Office of Ground Water and Drinking Water. This collaboration has strengthened with the development of unique technology, including electrolytic suppression and RFIC with eluent generation or regeneration.

DRINKING AND BOTTLED WATER

Currently, less than 1% of the planet's water is available for human consumption—making this valuable resource even more important. With surface water contamination and groundwater resources overexploited, the need for effective water analysis and monitoring has never been higher.

Regulatory agencies around the world have developed standards for water analysis and have provided guidance on water disinfection to assure drinking water quality. Thermo Scientific provides a variety of solutions for inorganic and organic drinking water contaminants.

WASTEWATER

Wastewater includes liquid waste from residences, industry, and agriculture, comprising a wide range of potential contaminants and concentrations. Industries discharge a variety of pollutants in their wastewater, including heavy metals, organic toxins, oils, nutrients, and solids, all of which endanger ecosystems and pose a threat to human health. In some areas, treated wastewater is recycled for irrigation purposes and even as drinking water. This reuse of water is gaining closer scrutiny as demand increases for water resources.

Treating and recycling wastewater requires careful analysis and monitoring, including the determination of low-level contaminants such as pharmaceuticals and personal care products (PCPs). Dionex HPLC and IC instruments are well suited to determine a wide range of nonpolar, polar, and ionic contaminants.

FAST WATER ANALYSIS

High-Throughput Solutions for Inorganic and Organic Contaminant Analyses

The Challenge:

Emerging contaminants, stricter regulations, growing municipalities and industries—all increase analytical laboratories' workloads, requiring processing of more samples and performing more tests in less and less time.

We have developed new technologies and methods to help labs and businesses increase their productivity and throughput for the analysis of inorganic and organic contaminants in a variety of water matrices.

Columns

Thermo Scientific Dionex IonPac Fast IC columns for anions, organic acids, oxyhalides, cations, and amines use the same proven chemistry in shorter column formats, decreasing run times by as much as three times while still retaining sufficient resolution.

Thermo Scientific Acclaim columns for organic contaminants use smaller particles that allow higher flow rates at standard pressures and compatibility with higher pressure systems. When used with the Thermo Scientific Dionex UltiMate 3000 rapid separation LC (RSLC) systems, these columns provide separation times as much as 30 times faster than standard columns and systems.

Inorganic Contaminants

The Thermo Scientific Dionex ICS-5000 capillary RFIC system provides IC on demand, reducing equilibration times and calibration requirements that save labor and increase throughput. The innovative Thermo Scientific Dionex IC Cube module, with half the connections of a standard IC configuration, makes plumbing and reconfiguring the system easier. Capillary Fast IC and monolith columns combine the speed of Fast IC with the convenience of IC whenever you need it—on demand. The simultaneous injection, sample, and standard preparation features of the Thermo Scientific Dionex AS-AP Autosampler, along with its AutoDilution capability, increase throughput, reduce manual labor, and decrease delays from out-of-range samples.

Organic Contaminants

UltiMate™ 3000 HPLC and RSLC systems are all UHPLC+ focused, enabling faster separations at standard HPLC system prices. From the economical Basic Automated system to the ×2 Dual RSLC system for high throughput, automated sample preparation, sample concentration, and matrix elimination, Thermo Scientific has the system to fit your needs and budget.

Thermo Scientific Dionex Chromeleon Chromatography Data System software version 7.1 streamlines your path from samples to results. eWorkflows guide the operator through a minimal number of choices needed to run that workflow, making configuration of even the most complex multidimensional analysis easy. Data analysis tools help users process chromatograms with minimal effort, report templates and audit trails, and help ensure regulatory compliance, and System Wellness tools increase up time.

Thermo Scientific is committed to enhancing the quality of our global water resources. Our analytical instruments are used by government and industry labs globally to provide services for environmental water testing for a wide range of regulated and emerging inorganic elements and organic compounds.

Analysis of Perchlorate

Environmental Water Applications Notebook

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

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 thyroide 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. EPA identified perchlorate as a contaminant of potential concern with its Contaminant Candidate List (CCL) publication in 1998. Following this publication, the EPA proposed the Unregulated Contaminant Monitoring Rule (UCMR).[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 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 US EPA published Method 314.1 as an update to 314.0 to improve the sensitivity for perchlorate in high ionic strength matrices. This method requires the concentration of a 2-mL sample on an IonPac Cryptand C1 preconcentration column followed by matrix elimination with 1 mL of 10 mM sodium hydroxide.[11,12] Perchlorate is then separated using a 2-mm 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 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 IonPac AS20 column to divert the matrix ions while 5 mL of the suppressed effluent containing perchlorate is trapped on a TAC-ULP1 concentrator column and then separated on a 2-mm 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 EPA Method 314.1.

EQUIPMENT

Dionex® ICS-3000 Reagent-Free™ Ion Chromatography (RFIC™) system consisting of:

- 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 EluGen® EGC II KOH cartridges (P/N 058900)

Two Continuously-Regenerated Anion Trap Columns, CR-ATC (P/N 060477)

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

Chromeleon® 6.7 Chromatography Management Software

REAGENTS AND STANDARDS

Deionized water, Type I reagent grade, 18 MΩ-cm resistivity or better

Sodium Perchlorate, $NaClO_4$ (Aldrich 41,024-1)

Sodium Chloride, NaCl (J.T. Baker; VWR P/N JT3625-1)

Sodium Sulfate, Na_2SO_4 (Aldrich 29,931-3)

Sodium Bicarbonate, $NaHCO_3$ (EM Science SX0320-1)

CONDITIONS

First Dimension

Columns:	IonPac AS20 Analytical, 4 × 250 mm (P/N 063148) 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:	ICS-3000 EG
Flow Rate:	1 mL/min
Temperature:	30 ˚C (lower compartment) 30 ˚C (upper compartment)
Inj. Volume:	4000 µL
Detection:	Suppressed conductivity, ASRS® ULTRA II (4 mm), Autosuppression 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

Columns:	IonPac AS16 Analytical, 2 × 250 mm (P/N 055378) IonPac AG16 Guard, 2 × 50 mm (P/N 055379)
Eluent:	65 mM potassium hydroxide
Eluent Source:	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	TAC-ULP1, 5 x 23 mm (P/N 061400)
Detection:	Suppressed conductivity, ASRS ULTRA II (2 mm), Autosuppression 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 (Corning 26-mm surfactant-free cellulose acetate, Fisher 09-754-13) 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 lock, Fisher 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 sample, and then filter the remaining sample in a 125–mL sterile sample container (high density polyethylene, HDPE, I-Chem, Fisher N411-0125). Discard the syringe and filter after each use.

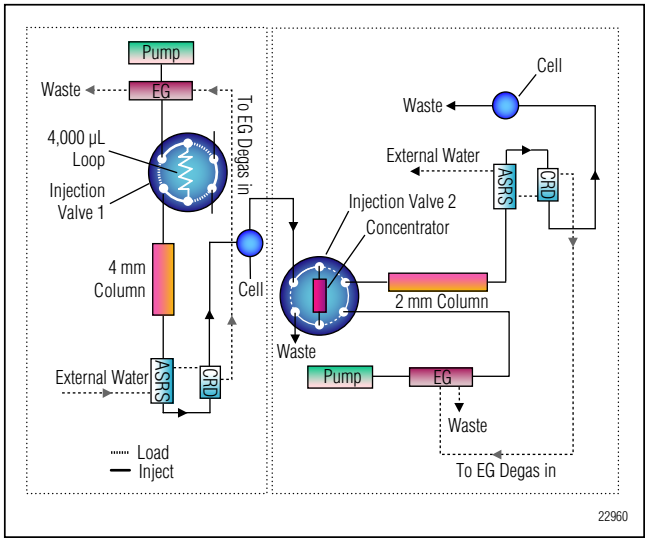

Figure 1. Schematic diagram of two-dimensional system for the determination of trace concentrations of perchlorate.

SYSTEM PREPARATION AND SETUP

Install and configure the EG 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 an 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 CR-ATC between the EGC II KOH cartridge and the EGC degas. Hydrate the CR-ATC prior to use by following the instructions outlined in the 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 DI 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-3000. To allow independent control of the DC-3000 injection valves, the DC settings in the Chromeleon 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 IonPac AG20 (4 × 50 mm) and the IonPac AS20 (4 × 250 mm) columns on system #1 in the lower compartment of the DC. Install the IonPac AG16 (2 × 50 mm) and the IonPac AS16 (2 x 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 TAC-ULP1 (5 × 23 mm) concentrator 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 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 ASRS ULTRA II 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 CR-ATC, while the Regen Out of the CR-ATC connects to the Regen In of the EG degasser.

Equilibrate the AS20 with 35 mM KOH and the AS16 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 AS20 and AS16 columns, respectively. Determine the cut time (preconcentration time) for the second dimension, as described in the next section, before analyzing perchlorate.

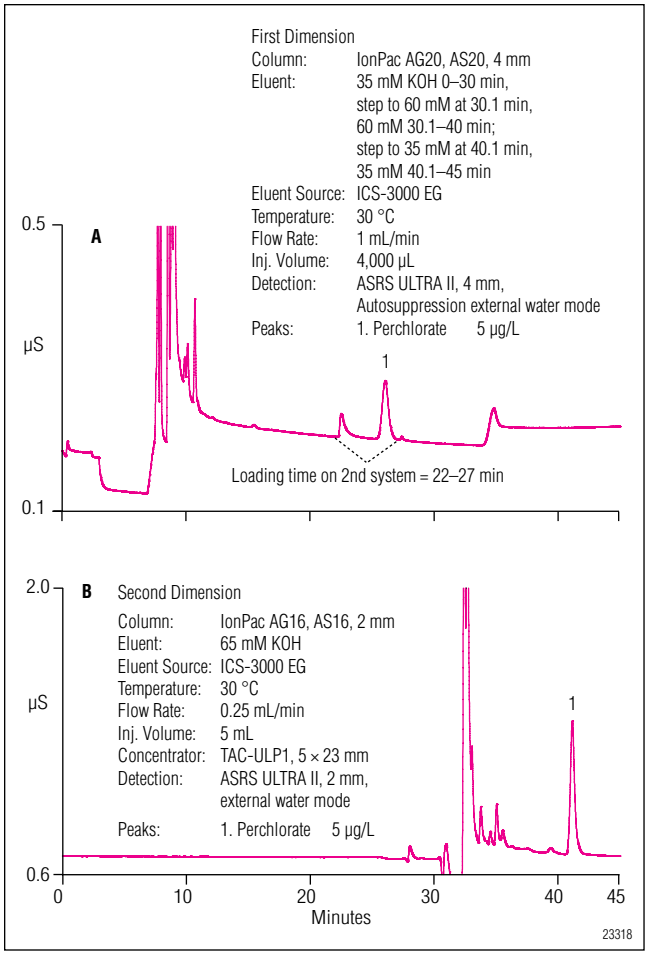

Figure 2. Separation of a 5 µg/L perchlorate standard with the (A) IonPac AS20 column in the first dimension and (B) IonPac AS16 column in the second dimension.

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 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 AS20 column. *It is important to verify the retention time of perchlorate on the AS20 column weekly to ensure good trapping efficiency on the TAC-ULP1 concentrator.* In our experiments, the perchlorate retention time (t_{ClO4}) was approximately 26 min. Therefore, valve #2 in the second dimension was placed in the load position at 22 min (t_{ClO4} – 4 min) and then switched to the inject position at 27 min (t_{ClO4} + 1 min). In this configuration, perchlorate eluted in ~41 min from the AS16 column. Figure 2 shows example chromatograms of 5 ppb perchlorate separated on the AS20 and AS16 columns.

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 µg/L instead of 0.5 µg/L.[12] However, the minimum reporting level (MRL) remained at 0.5 µg/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 µg/L perchlorate. This calibration curve produced a correlation coefficient of 0.9998 with the 2-mm 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 ±25% required by Method 314.1.

Table 1. Calibration Data and Method Detection Limits for Perchlorate

Analyte	Range (µg/L)	Linearity (r^2)*	MDL Standard (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:

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

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 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 AS16 column.

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 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 limits of detection. 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.

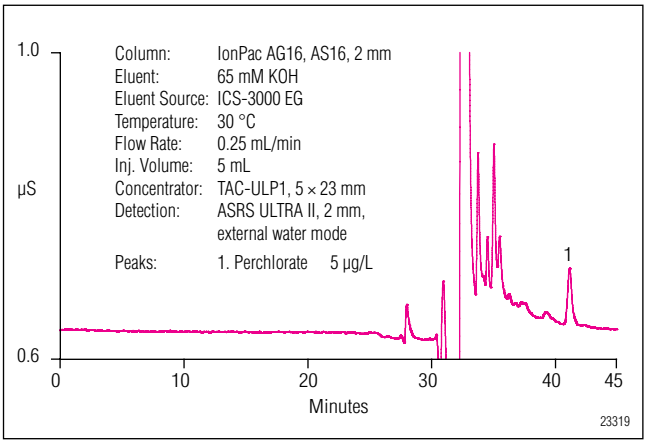

Figure 3. Chromatogram of a 0.5 µg/L perchlorate standard with the IonPac AS16 column in the second dimension.

Table 2. Perchlorate Recoveries from Laboratory Fortified Sample Matrices (LFSM)					
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

[1]HIW = high inorganic water contains 1000 mg/L each of chloride, sulfate, and bicarbonate

[2]<MDL = less than the method detection limit

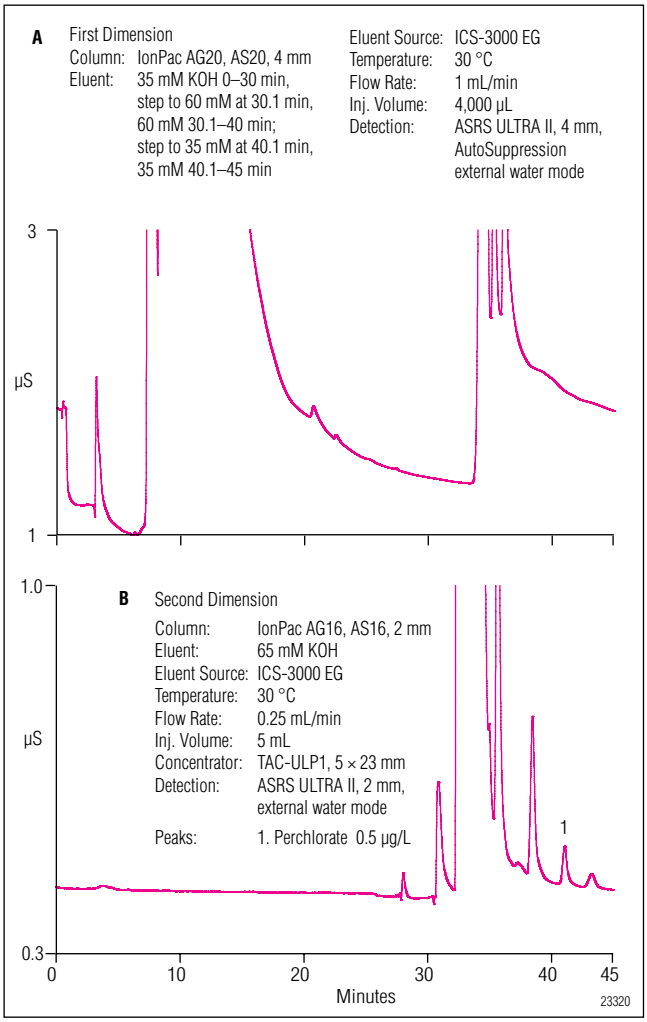

Figure 4. Chromatogram of drinking water D fortified with 0.5 µg/L perchlorate in the (A) first dimension and (B) second dimension.

Table 2 summarizes the performance of the method for determining low concentrations of perchlorate using a two-dimensional 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 IonPac AS20/AS16 two-dimensional 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 IonPac AS20 with EPA Method 314.1.[12] Consequently, this interferent is eliminated by first separating perchlorate on the IonPac AS20 and then trapping a 5-mL portion of the effluent containing the perchlorate peak on a TAC-ULP1 concentrator and, finally, separating the perchlorate on an IonPac AS16 column. By using this two-dimensional 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.

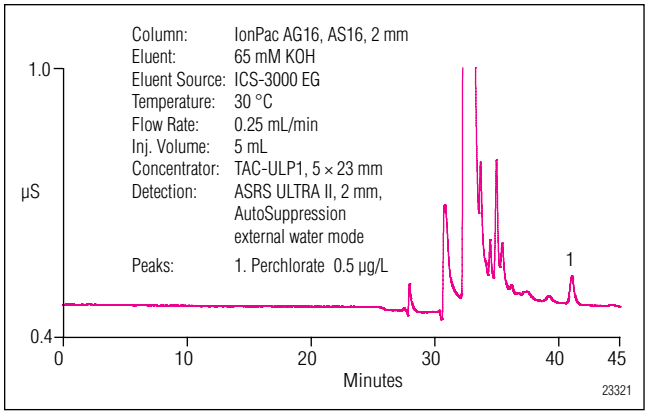

Figure 5. Chromatogram of synthetic high inorganic water fortified with 0.5 µg/L perchlorate in the second dimension.

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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Determining Sub-ppb Perchlorate in Drinking Water Using Preconcentration/Matrix Elimination Ion Chromatography with Suppressed Conductivity Detection by U.S. EPA Method 314.1

INTRODUCTION

Perchlorate is a well-known environmental contaminant. It has most often been associated with military defense and aerospace activities where it is used in the manufacture and testing of solid rocket propellants and missiles. Perchlorate has also been used in the private sector for the manufacture and development of pyrotechnics, air bag inflators, safety flares, and commercial explosives.[1,2] Industrial wastes resulting from the manufacture and disposal of this highly mobile and soluble anion have contaminated soils, surface waters, and groundwaters, where the contamination may persist for decades. Perchlorate has been found at nearly 400 sites across the United States, although most contaminated sites appear to be confined to the western states such as California, Arizona, New Mexico, and Nevada. Perchlorate has also been found in food products such as milk and lettuce.[3] Health studies have shown that perchlorate targets primarily the thyroid gland, which is responsible for extracting iodide from blood and converting it into organic iodide in the form of hormones that regulate metabolism.[4] The fetuses of expectant mothers may be particularly sensitive to the ingestion of perchlorate. The concentrations at which the perchlorate will demonstrate negative effects on the developing fetus, however, are still unknown.[3]

In 1998, the U.S. Environmental Protection Agency (EPA) placed perchlorate on its Candidate Contaminant List for drinking water. Although there are currently no federal regulations for perchlorate, several states have adopted their own advisory levels. Concentrations established at the state level range from 1 ppb (µg/L) to 18 ppb perchlorate. In 2004, the California Office of Environmental Health Hazard Assessment established a public health goal (PHG) of 6 ppb perchlorate.[5] However, a few states such as Maryland, Massachusetts, and New Mexico have set advisory levels for perchlorate at 1 ppb.

Perchlorate has commonly been determined using ion chromatography (IC) with suppressed conductivity detection as described in U.S. EPA Method 314.0. This method reports a method detection limit (MDL) of 0.53 ppb and a minimum reporting limit (MRL) of 4 ppb.[6] However, minor modifications to Method 314.0, such as the use of an improved suppressor that generates lower baseline noise and therefore a higher signal-to-noise ratio, coupled with the use of an electrolytically-generated potassium hydroxide eluent, results in a lower MRL of 1 ppb.[7] Regardless, Method 314.0 is subject to interferences and loss of sensitivity caused by the presence of high concentrations of the common matrix ions chloride, sulfate, and carbonate. In addition, some anionic compounds, such as chloro-benzene sulfonates, are known to elute at a similar

retention time as perchlorate, and can therefore lead to a false positive. To avoid these complications, the EPA and Dionex Corporation collaboratively developed EPA Method 314.1, an improved IC method that uses preconcentration/matrix elimination and suppressed conductivity detection.[8] This method uses an IonPac® Cryptand C1 preconcentration column to trap perchlorate from the matrix followed by matrix elimination with 10 mM sodium hydroxide. Perchlorate is then separated using a 2-mm IonPac AS16 as the primary column. To minimize the identification of a false positive peak, a second analytical column, the IonPac AS20, is used as the confirmatory column to verify the presence of perchlorate in the sample. The AS20 has a different selectivity than the AS16, and therefore provides an improved separation of perchlorate and potentially interfering anions such as chloro-benzene sulfonates. In this application note, we demonstrate the application of this method to the determination of trace (< 1 ppb) perchlorate in drinking water samples and a simulated high-ionic-strength matrix.

Although the method described herein is effective and should be used for compliance with EPA Method 314.1, Dionex recommends the method described in Application Note 178[9] for the determination of trace concentrations of perchlorate in high ionic strength matricies. The method in AN 178 was developed to support EPA Method 314.2, which had not been released at the time this note was published.

EQUIPMENT

Dionex ICS-3000 Reagent-Free™ Ion Chromatography (RFIC™) system consisting of:

- DP Dual Pump module
- EG Eluent Generator module
- DC Detector/Chromatography module (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 EluGen® EGC II NaOH cartridges (P/N 058908)

Two Continuously Regenerated Anion Trap Columns, CR-ATC (P/N 060477)

Four 4-L plastic bottle assemblies for external water mode of operation

Chromeleon® 6.7 Chromatography Workstation

REAGENTS AND STANDARDS

Deionized water, Type I reagent grade, 18.2 MΩ-cm resistivity or better

Sodium Perchlorate ($NaClO_4$) (Aldrich 41,024-1)

Sodium Chloride (NaCl) (J.T. Baker, VWR P/N JT3625-1)

Sodium Sulfate (Na_2SO_4) (Aldrich, 29,931-3)

Sodium Bicarbonate ($NaHCO_3$) (EM Science, SX0320-1)

Sodium Hydroxide, 50% w/w (NaOH) (Fisher Scientific, SS254-1)

CONDITIONS

Columns: (A) Primary Method
IonPac AS16 Analytical, 2 x 250 mm (P/N 055378)
IonPac AG16 Guard, 2 x 50 mm (P/N 055379)
(B) Confirmatory Method
IonPac AS20 Analytical, 2 x 250 mm (P/N 063065)
IonPac AG20 Guard, 2 x 50 mm (P/N 063066)

Eluent: 0.5 mM sodium hydroxide for 0–12 min, step at 12 min to 65 mM, 65 mM for 12–28 min, step at 28 min to 100 mM, 100 mM for 28–35 min*

Eluent Source: ICS-3000 EG

Flow Rate: 0.25 mL/min

Temperature: 35 ˚C (lower compartment)
30 ˚C (upper compartment)

Inj. Volume: 2 mL

Rinse Volume: 1 mL (10 mM sodium hydroxide)

Concentrator: IonPac Cryptand C1, 4 x 35 mm (P/N 062893)

Detection: Suppressed conductivity, ASRS® ULTRA II (2 mm), AutoSuppression® external water mode
Power setting—100 mA

SYSTEM:

Backpressure: Adjust to ~2400 psi

Background Conductance: 1–2 µS

Noise: 1–2 nS/min peak-to-peak

Run Time: 35 min

*The columns should be allowed to equilibrate at 0.5 mM NaOH for 6 min prior to injection.

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 0.5, 1, 3, 5, and 10 µg/L by adding the appropriate volumes of the perchlorate secondary dilution solution to separate 100-mL volumetric flasks. Then add 2 mL of the primary common anion solution to each flask and dilute to volume with deionized water.

Important: For the Cryptand C1 to effectively trap the perchlorate from the matrix, each standard must contain 100 mg/L each of chloride, sulfate, and bicarbonate by adding 2 mL of the primary common anion solution.

Common Anion Stock Solutions

Prepare 25 mg/mL (25,000 mg/L) each of chloride, sulfate, and bicarbonate as follows: 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.

Primary Common Anion Solution

Prepare a combined common anion solution consisting of 5,000 mg/L each of chloride, sulfate, and bicarbonate by adding 20 mL of each common anion stock solution to a 100-mL volumetric flask and diluting to volume with deionized water.

Concentrator Rinse Solution

Prepare 10 mM NaOH by adding 0.8 g of 50% w/w NaOH in ~800 mL of degassed deionized water in a 1-L volumetric flask and dilute to volume with deionized water.

Important: Store this solution under helium in a pressurized vessel at all times when not in use to avoid the accumulation of carbonate. A rinse solution that is one week or older should be discarded.

Sample Preparation

All samples must be sterile-filtered with a 0.2-µm syringe filter (Corning 26 mm, surfactant-free cellulose acetate, Fisher 09-754-13) 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 lock, Fisher 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 sample, and then filter the remaining sample into a 125-mL sterile sample container (high density polyethylene, HDPE, I-Chem, Fisher N411-0125). Discard the syringe and filter after each use.

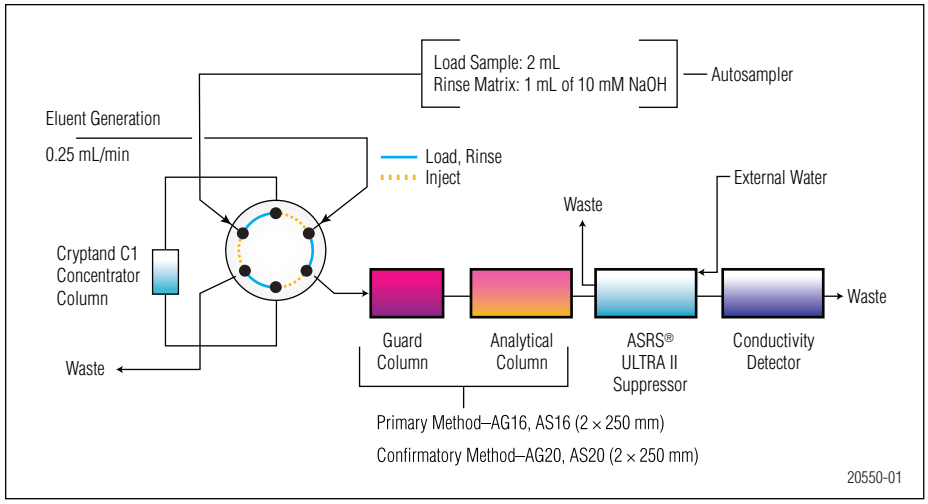

Figure 1. Schematic diagram of system configuration for U.S. EPA Method 314.1.

SYSTEM PREPARATION AND SETUP

Install 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 an EGC II NaOH cartridge for each system channel. Condition the cartridges by setting the NaOH concentration to 50 mM at 1 mL/min for 30 min. After completing the condition process, disconnect this temporarily installed backpressure tubing. Install a CR-ATC between the EGC II NaOH cartridge and the EGC degas. Hydrate the CR-ATC prior to use by following the instructions outlined in the 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 AS autosampler must be configured with the sequential injection option (P/N 063294) to simultaneously determine perchlorate on the primary and confirmatory columns. The AS concentration option allows the AS to deliver sample to a low-pressure concentrator at a maximum pressure of 100 psi. Therefore, a sample syringe speed no greater than 2 should be selected for this application. Because this application requires large sample injection volumes, a 5-mL sample syringe (P/N 053915) should be installed. To accommodate the larger volumes, an 8.2-mL sampling needle assembly (P/N 061267) is also required for operation.

To operate the AS autosampler in the concentrate + sequential injection mode, the injection port volume (including the needle seal tubing and the tubing from the diverter valve to the sample injection valve) must be calibrated to accurately deliver the designated volumes to the concentrator. Connect the AS injection port tubing to the diverter valve and connect the diverter valve tubing to each injection valve. The diverter valve tubing must be the same length for each system. Calibrate the tubing volume for system #1 by performing the following procedure: 1) disconnect the diverter valve tubing from the sample injection valve of system #1; 2) press Menu, #5, #5, and select the SEQ + CONC sample mode; 3) press Menu, #8, #5 to go to the liquid control screen; 4) with the cursor in the Vial # field, press Select to change to INJ and press Enter; 5) select the following options (from: FLUSH, SYRINGE SPEED: 5, DIV VLV: 1 SYRINGE: SAMPLE, ASPIRATE: 1000 µL, DISPENSE: 500 µL); 6) change ACTION to ASPIRATE and press Enter to aspirate 1000 µL into the sample syringe; 7) change ACTION to DISPENSE and press Enter to remove any potential air in the tubing; 8) select the following options (ASPIRATE: 500 µL, from: NEEDLE); 9) change ACTION to ASPIRATE to remove all liquid from the tubing; 10) change VIAL to FLU and press Enter and change ACTION to EMPTY and press Enter; 11) select the following options (ASPIRATE: 2000 µL, from: FLUSH, ACTION: ASPIRATE); 12) change VIAL to INJ and press Enter. Initially, a low DISPENSE volume should be selected (i.e., 50 µL) and the SYRINGE SPEED should be set to 1. Change the ACTION to DISPENSE and press Enter.

Closely watch the end of the diverter tubing to observe if a small drop of liquid appears. If liquid appears from the tubing then the injection port volume must be recalibrated from the beginning. Otherwise, change DISPENSE to 1 µL and press Enter. Set the cursor on ACTION and continue to press Enter until a small liquid drop appears.

Important: Be aware of the total volume dispensed during this time (i.e., 50 µL + the number of 1-µL dispensings).

After observing and verifying that a liquid is present at the end of the diverter tubing, record the injection port volume by going to menu, #5, #5. After completing this procedure, verify that the injection port volume for diverter valve 2 is approximately the same as diverter valve 1. The difference in volume between the diverter valves should be <2 µL.

This application requires a matrix elimination step using 10 mM NaOH. There are two possible procedures for accomplishing this task:

- The best method for performing the rinse step is to use the sample prep syringe of the AS autosampler with a 5-mL syringe installed. In this setup, the hydroxide solution is always kept in a pressurized bottle under helium during sample analyses. However, the use of the sample prep syringe for rinsing the concentrator will require an additional 10 min per injection.
- An alternative is to fill a sample vial with 10 mM hydroxide and direct the autosampler to aspirate 1 mL from this vial for each injection. Because this vial is not stored under helium, it is strongly advised to change the hydroxide solution in the vial every day to maintain optimum performance of the method. In addition, separate rinse vials should be used for different standards and samples to minimize the potential for contamination of the rinse solution.

The advantage of using an AS vial for the rinse step is the reduced analysis time between injections compared to using the sample prep syringe. However, the disadvantages include potential carbonate contamination of the solution, the need for different rinse vials for different solutions, and the occupation of additional space in the autosampler tray.

Install an IonPac AG16 (2 x 50 mm) and an IonPac AS16 (2 x 250 mm) column on system #1 in the lower compartment of the DC. Install an IonPac AG20 (2 x 50 mm) and an IonPac AS20 (2 x 250 mm) column on system #2 in the lower compartment of the DC. Install an IonPac Cryptand C1 (4 x 35 mm) concentrator in place of the sample loop on the injection valves of each system. *Important: The sample loading and rinse steps must use the same direction of flow as the analytical column to allow perchlorate to refocus at the head of the guard column during the NaOH gradient.*

Make sure the pressure for both systems is approximately 2400 psi when 65 mM NaOH is delivered at 0.25 mL/min at a column temperature of 35 °C 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. Because the pressure can gradually rise over time, monitor the pressure periodically. To reduce pressure, trim the backpressure tubing.

Hydrate the 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. To fully hydrate the suppressor screens and membranes, allow the suppressor to stand for approximately 20 min. Install the ASRS ULTRA II for use in the external water mode by connecting the Regen Out of the suppressor to the Regen In of the CR-ATC and the Regen In of the suppressor to the external water source. The Regen Out of the CR-ATC should be connected to the Regen In of the EG degasser.

Equilibrate the columns with 65 mM NaOH at 0.25 mL/min for at least 60 min prior to performing any injections. Analyze a matrix blank by injecting a solution consisting of 100 mg/L each of chloride, sulfate, and bicarbonate. There should be no peaks eluting within the same retention time window as perchlorate. Verify that the method is being performed correctly by approximating the signal height of the matrix peaks that typically begin to elute around 18 min. The maximum height of these peaks should be ~30–50 µS. Inject a 5 µg/L perchlorate standard. The peak area of this standard should be >0.080 µS·min. Inject this standard on the system at least twice to verify that the peak areas and retention times are approximately the same. Inject a 0.5 µg/L perchlorate standard. The response of this standard should be easily observed.

CHROMELEON PROGRAM

Specific Chromeleon commands are required to allow the AS autosampler to concentrate the sample and rinse the matrix from the concentrator column. Therefore, an example program for Method 314.1 using the AS autosampler is shown in Appendix A.

SYSTEM CALIBRATION

Calibrate the system by injecting one blank and five calibration standards to cover the desired concentration range. All blanks and standard solutions should contain 100 mg/L each of chloride, sulfate, and bicarbonate. The lowest concentration standard should be at or below the target minimum reporting level (MRL). The target MRL for this application was established at 0.5 µg/L perchlorate. Tabulate the peak area response against the perchlorate concentration using the appropriate regression curve. EPA Method 314.1 allows the use of quadratic calibration curves in addition to the standard linear curve used for most applications.[7] The measured concentration for each calibration point, except the MRL, should be between 75 and 125% of its true value. The MRL should calculate to between 50 and 150% of its true value.

RESULTS AND DISCUSSION

Table 1 summarizes the calibration data obtained by injecting calibration standards at 0.5, 1, 3, 5, and 10 µg/L. A quadratic regression curve was used for both systems resulting in a correlation coefficient of 0.9999 for the primary and confirmatory columns. The accuracy of the calibration curve was verified by injecting a 5 µg/L perchlorate standard. The calculated recoveries for this standard were 104.5 and 99.8% for the primary and confirmatory columns, respectively. These recoveries are well within the 75–125% specifications of the method. Figure 2 shows a chromatogram of 5 µg/L perchlorate for the AS16 and AS20 columns obtained using the described method parameters.

The 0.5 µg/L perchlorate MRL was confirmed by analyzing seven replicate injections of the standard. The mean concentration and standard deviation of the replicate analyses were then calculated for the replicates. The Half Range for the prediction interval of the results was calculated according to the equation in Section 9.2.4.1 in Method 314.1. This calculation produced results of 0.099 and 0.103 µg/L for the AS16 and AS20 columns, respectively. These values were then used to determine the lower and upper limits for the Prediction Interval of Results (PIR) using the equations described in Section 9.2.4.2. The results from these calculations should produce values that are ±50%. The lower limit PIR for the primary and confirmatory column was 77% and 73%, respectively. The upper limit PIR was 117% and 115% for the primary and confirmatory columns, respectively. Therefore, an MRL of 0.5 µg/L was determined acceptable for this application because the

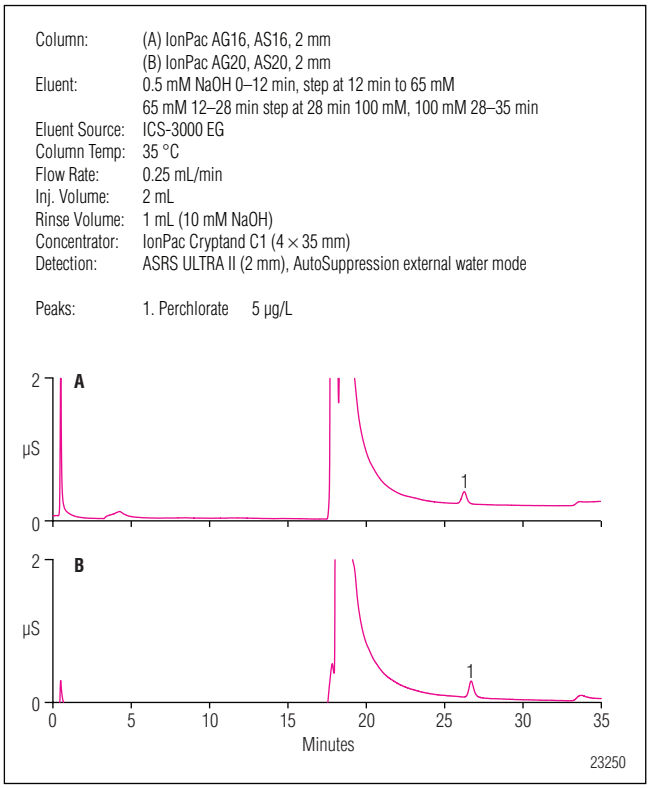

Figure 2. Chromatograms of a 5 µg/L perchlorate standard separated on (A) IonPac AS16 and (B) IonPac AS20 columns.

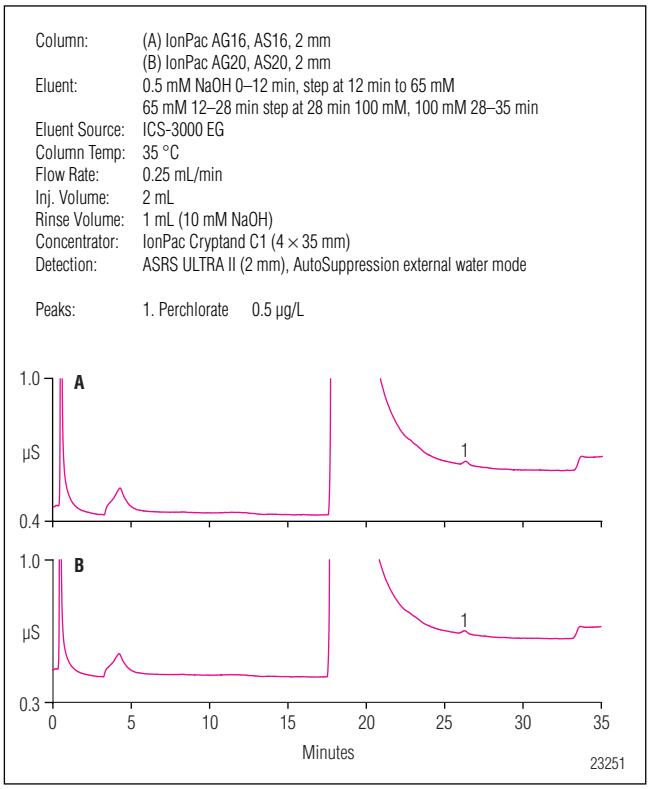

Figure 3. Chromatograms of a 0.5 µg/L perchlorate standard separated on (A) IonPac AS16 and (B) IonPac AS20 columns.

lower and upper limit PIR were within the specifications of the method. Figure 3 shows a chromatogram of 0.5 µg/L perchlorate for the primary and confirmatory methods of EPA 314.1.

To demonstrate the accuracy and precision of the method, seven replicate injections of 5 µg/L pechlorate (midpoint calibration standard) were analyzed. The precision and recovery of the replicate injections were calculated as part of the initial demonstration of capability. Sections 9.2.2 and 9.2.3 specify that the %RSD and recovery of the replicate values should be ≤ 20% and ± 25% of the true values, respectively. The IonPac AS16 primary column produced a %RSD of 5.4% and an average recovery of 100.5%. The IonPac AS20 confirmatory column had a %RSD of 4.2% and a recovery of 91.2% for seven replicate injections of 5 µg/L perchlorate. The calculated values for both columns were well within the specifications of the method.

Although the determination of the detection limit of perchlorate is not a specific requirement of Method 314.1, some individual laboratories that are governed by various regulatory bodies may require this determination for compliance monitoring. Therefore, the perchlorate detection limit was determined for this application by fortifying deionized water with 0.1 µg/L perchlorate and performing seven replicate injections. For the Cryptand C1 concentrator to effectively trap perchlorate from the matrix, 100 mg/L each of chloride, sulfate, and bicarbonate must be included in the standard. The detection limits for the primary and confirmatory columns were calculated using the equation provided in Section 9.2.7. The calculated detection limits using Method 314.1 were comparable to those determined by IC-MS and about four times lower than demonstrated in Application Update 148.[2,8] Table 1 summarizes the results of this calculation for each column.

Table 1. Calibration Data and Method Detection Limits for Perchlorate

Method	Analyte	Range (µg/L)	Linearity[a] (r^2)	MDL Standard (µg/L)	SD (µg/L)	Calculated MDL[b] (µg/L)
Primary	Perchlorate	0.5–10	0.9999	0.1	0.007	0.023
Confirmatory	Perchlorate	0.5–10	0.9999	0.1	0.008	0.026

[a] Quadratic regression curve
[b] MDL = σts_{99} where tx_{99} = 3.14 for n = 7

A final demonstration of initial laboratory performance for EPA Method 314.1 is an MRL confirmation in a matrix consisting of 1000 mg/L each of chloride, sulfate, and bicarbonate. We fortified this matrix with 0.5 µg/L perchlorate and analyzed seven replicates using the primary and confirmatory methods. The criteria described in Section 9.2.4.2 were applied to this series of replicates. The results further demonstrated that 0.5 µg/L is an acceptable MRL to use for Method 314.1.

Samples containing high concentrations of common anions, in particular chloride, sulfate, and carbonate, are known to interfere with the determination of low concentrations of perchlorate. In EPA Method 314.0, the determination of the matrix conductivity threshold (MCT) was required to assess the maximum concentration of common anions that the column could tolerate before observing significant loss of sensitivity for perchlorate.[6] For samples that exceeded the MCT, a dilution or sample pretreatment using OnGuard® cartridges was required. However, sample dilution raises the MRL by an equivalent proportion whereas sample pretreatment can be a very time-consuming and tedious process. EPA Method 314.1 eliminates these procedures by using a Cryptand C1 concentrator column that retains perchlorate while most of the matrix ions are diverted to waste. Consequently, this allows the injection of larger sample volumes in high-ionic-strength matrices without loss of sensitivity for perchlorate. Figure 4 compares the injection of a 5 µg/L perchlorate standard with and without a rinse step using 10 mM NaOH. This comparison demonstrates

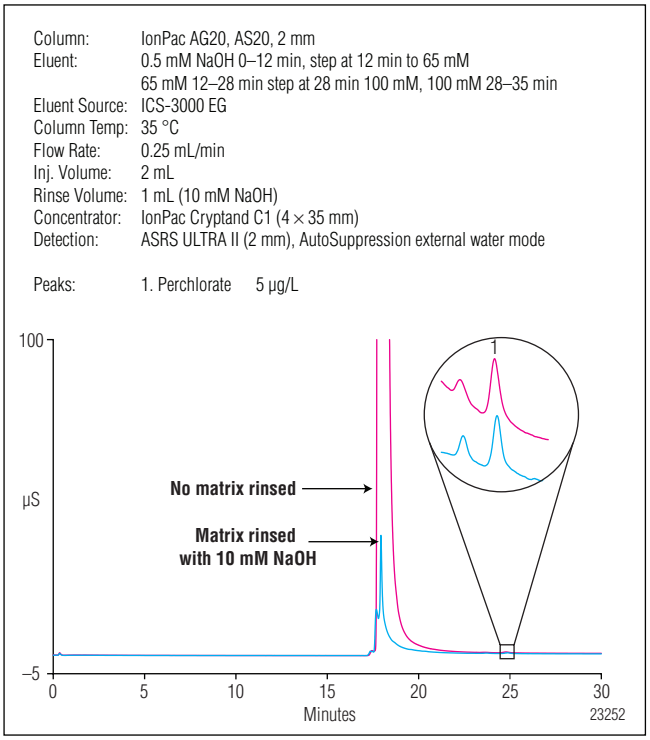

Figure 4. Comparison of a 5 µg/L perchlorate sample with and without a matrix rinse step.

that >90% of the 100 mg/L each of chloride, sulfate, and bicarbonate in the standard are diverted from the concentrator to waste, while the perchlorate is retained.

The ionic strength of drinking water matrices can vary considerably and therefore influence the integrity of the perchlorate results. To assess the performance of Method 314.1 for the determination of trace perchlorate, prepare and analyze a laboratory fortified sample matrix (LFSM). This is accomplished by adding a known quantity of perchlorate to the matrix and calculating the percent recovery of the amount added. This will determine whether the sample matrix contributes bias to the analytical results. For samples that produce a positive result for perchlorate on the primary column at or above the MRL should be verified with the confirmatory column. The recovery of perchlorate was assessed in six matrices: reagent water, four drinking waters from various sources, and a synthetic high-ionic-strength (HIW) matrix. Samples were fortified with 0.5 and 5 µg/L perchlorate. Some samples may require correction for native concentrations of perchlorate <MRL when the samples are fortified at or near the MRL.This is the only permitted use of analyte results <MRL according to Section 12.2.1 in Method 314.1. In addition, continuing calibration check standards were analyzed throughout each group of sample matrices to verify the accuracy of the calibration curve and integrity of the concentrator column during the field sample analyses. Low, mid, and high-level calibration check standards consisting of perchlorate concentrations of 0.5, 5, and 10 µg/L perchlorate, respectively, in 100 mg/L and 1000 mg/L each of chloride, sulfate, and bicarbonate were used.

Tables 2 and 3 summarize the performance of the method for determining trace concentrations of perchlorate in various matrices using the primary and confirmatory methods. For samples fortified with 0.5 µg/L perchlorate, recoveries ranged from 93 to 110% and 94 to 104% using the primary and confirmatory methods, respectively. These recoveries are well within the ±50% acceptance criteria of Method 314.1. For samples fortified with 5 µg/L perchlorate, recoveries ranged from 100 to 112% and 91 to 120%, respectively, which were within the ±25% criteria. The highest native perchlorate concentration was detected in drinking water C that contained approximately 1.9 µg/L perchlorate. However, this concentration was still about three times lower than the California Department of Health Service's PHG of 6 µg/L. Because this concentration is well above our 0.5 µg/L MRL, confirmation of the perchlorate result was required according to Section 11.3.4.2 in Method 314.1. The confirmatory IonPac AS20 column produced approximately the same perchlorate concentration. Fortification of this sample with 5 µg/L perchlorate resulted in calculated recoveries of 112.5% and 108.7% using the

Table 2. Perchlorate Recoveries from Laboratory Fortified Sample Matrices (LFSM) Using the Primary Method

Matrix	Amount Found (µg/L)	Amount Added (µg/L)	Replicates	Peak Area Precision (% RSD)	Average Recovery (%)
Reagent water	—	0.5	7	5.15	97.0
		5.0	7	5.40	100.5
HIW	—	0.5	7	4.24	93.3
		5.0	7	2.22	106.4
Drinking water A	0.096	0.5	7	3.30	106.6
		5.0	7	1.42	97.4
Drinking water B	0.289	0.5	7	3.40	98.8
		5.0	7	2.60	108.8
Drinking water C	1.87	0.5	7	0.76	110.0
		5.0	7	1.81	112.5
Drinking water D	<MDL	0.5	7	4.00	107.8
		5.0	7	2.24	112.0

Table 3. Perchlorate Recoveries from Laboratory Fortified Sample Matrices (LFSM) Using the Confirmatory Method

Matrix	Amount Found (µg/L)	Amount Added (µg/L)	Replicates	Peak Area Precision (% RSD)	Average Recovery (%)
Reagent water	—	0.5	7	5.64	94.2
		5.0	7	4.21	91.2
HIW	—	0.5	7	4.10	95.7
		5.0	7	2.22	101.8
Drinking water A	NA[1]	0.5	7	NA	NA
		5.0	7	5.96	120.0
Drinking water B	0.182	0.5	7	2.28	103.8
		5.0	7	2.21	108.7
Drinking water C	1.92	0.5	7	1.43	95.2
		5.0	7	2.10	108.7
Drinking water D	NA	0.5	7	NA	NA
		5.0	7	4.95	107.3

[1] NA = not available due to a coeluting peak.

IonPac AS16 and AS20 columns, respectively. Figure 5 shows chromatograms of the unfortified and fortified drinking water C using the IonPac AS16 primary column.

The use of two analytical columns with different selectivities is extremely beneficial in determining whether perchlorate is truly present in the sample. Two of our drinking water samples highlighted the need for two column selectivities. Figure 6A shows a chromatogram of 0.5 µg/L perchlorate fortified in drinking water A using the IonPac AS16 column. As shown, no additional peaks elute within the same retention time window as perchlorate. However, Figure 6B shows the same separation on the IonPac AS20. In this chromatogram, an unknown peak elutes at almost exactly the same time as perchlorate. A false positive result may have been reported if the separation on the IonPac AS16 had not confirmed the absence of perchlorate in the sample.

Figure 7 shows a second example of a potentially false positive perchlorate identification. This chromatogram shows a separation of drinking water D fortified with 0.5 µg/L perchlorate using the primary column. As shown, there are several unidentified peaks observed. However, perchlorate is still well resolved from the unknown peaks. Figure 8A shows the chromatogram of the unfortifed drinking water D. Figure 8B shows the chromatogram of the same sample fortified with 5 µg/L perchlorate separated on the IonPac AS20. As the first chromatogram illustrates, several unknown peaks elute before the expected retention time of perchlorate and one unidentified peak elutes at approximately the same retention time as perchlorate. After fortification of the sample, the peak is split into two separate peaks that verify that the unknown peak is not perchlorate. This conclusion was further confirmed based on the previous results with the IonPac AS16 column (Figure 7).

The performance of this method can be improved by removing carbonate prior to detection using a Carbonate Removal Device (CRD). The CRD can produce lower baseline conductivity, reduce the slope from the matrix signal and, therefore, increase the sensitivity of the method for perchlorate. Although a CRD was not implemented for the data shown in Tables 2 and 3, we evaluated the benefits of this device for selected drinking water matrices. Figure 9 compares the separation of 0.5 µg/L fortified in drinking water D with and without a CRD. As this figure illustrates, the baseline is dramatically reduced when the CRD is implemented into the system. In addition, a 10% increase in the perchlorate signal was observed with the CRD.

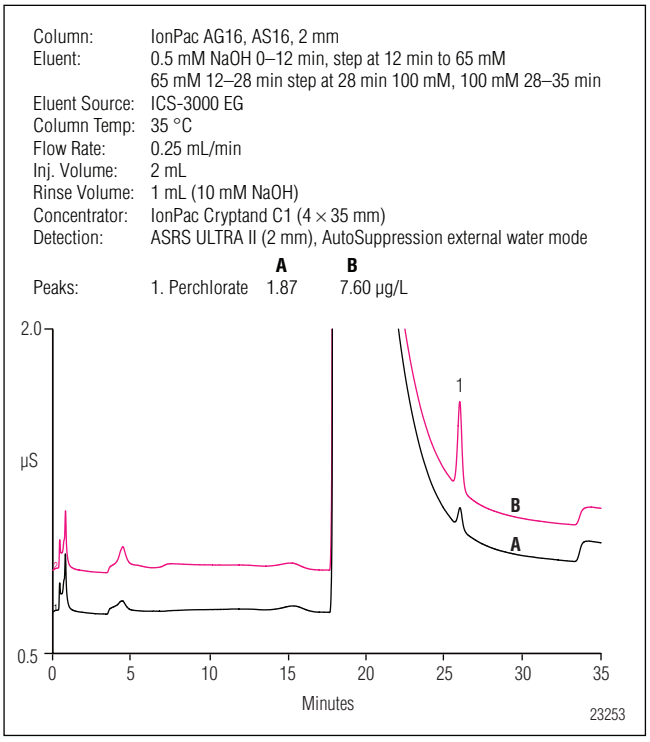

Figure 5. Chromatograms of (A) unfortified and (B) fortified drinking water C with 5 µg/L perchlorate using the IonPac AS16 column.

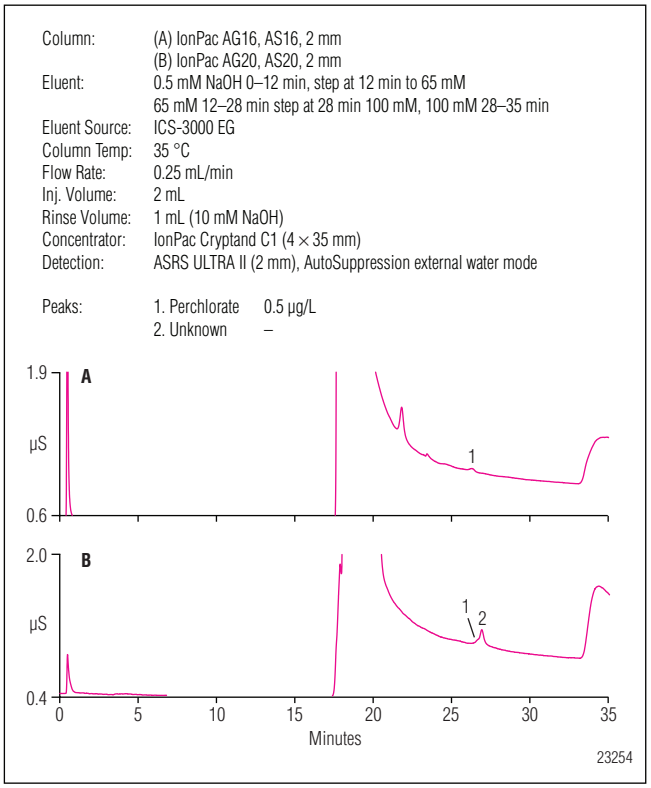

Figure 6. Chromatograms of drinking water A fortified with 0.5 µg/L perchlorate using the (A) primary method and (B) confirmatory method.

CONCLUSION

This application note demonstrates the ability to concentrate 2 mL of sample and eliminate most of the matrix ions using a dilute sodium hydroxide solution for trace perchlorate determinations as described in EPA Method 314.1. This method is a significant improvement to Method 314.0 for determining perchlorate in various drinking water matrices. The injection of larger sample volumes and elimination of the matrix ions resulted in an improved perchlorate detection limit of ~0.02 µg/L and a lower minimum reporting level of 0.5 µg/L. In addition, Method 314.1 can tolerate higher-ionic-strength samples without sample dilution or pretreatment compared to Method 314.0. This can further simplify the sample analysis for most laboratories that are required to determine perchlorate for compliance monitoring. The sequential + concentrate feature of the AS autosampler automates all the sample loading and rinsing steps for both the primary and confirmatory methods. The eluent generator further simplifies the method by automatically producing the required NaOH eluent concentrations required for the method. The results presented in this application note meet all performance requirements specified in EPA Method 314.1.

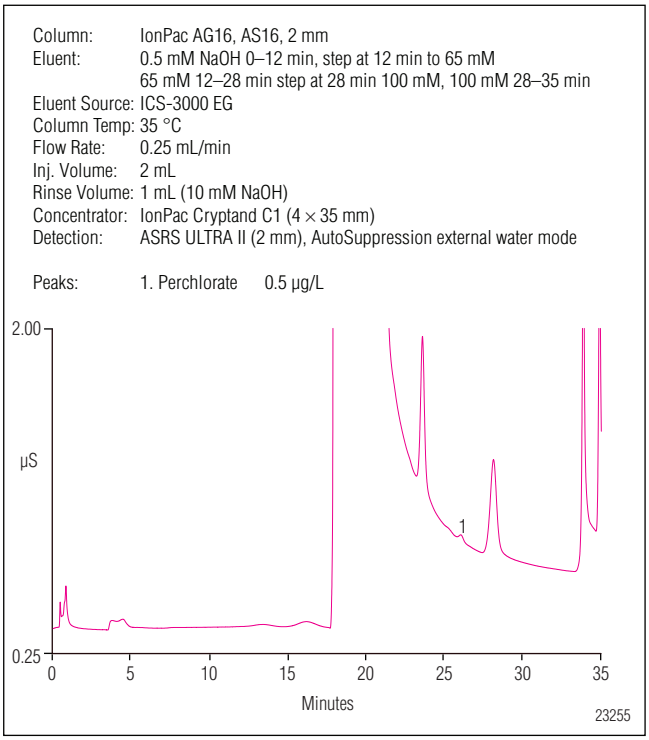

Figure 7. Chromatogram of drinking water D fortified with 0.5 µg/L perchlorate using the primary method.

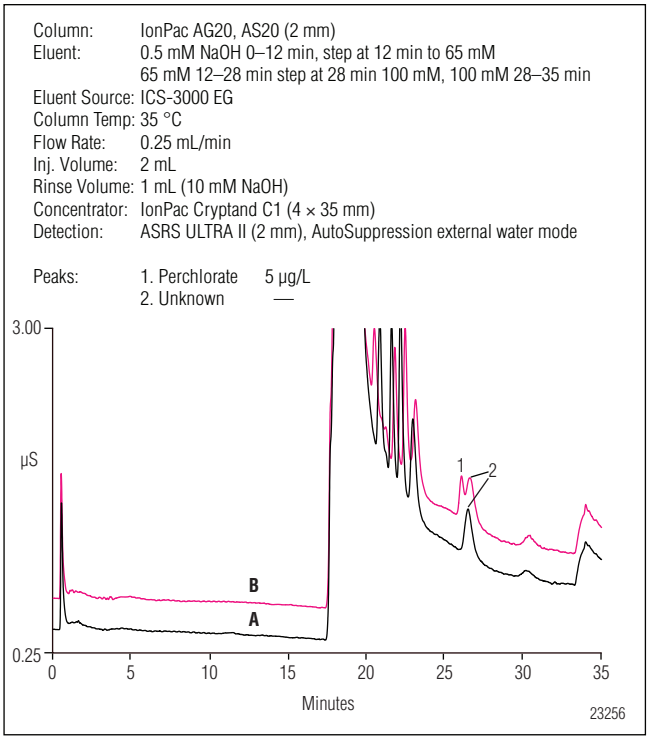

Figure 8. Chromatograms of (A) unfortified drinking water D and (B) fortified drinking water D using the confirmatory method.

REFERENCES

1. Jackson, P. E., Gokhale, S., Streib, T., Rohrer, J. S., Pohl, C. A. Improved Method for the Determination of Trace Perchlorate in Ground and Drinking Waters by Ion Chromatography. *J. Chromatogr. A,* **2000**, *888*, 151.
2. Hedrick, E., Munch, D. Measurement of Perchlorate in Water by Use of an ^{18}O-Enriched Isotopic Standard and Ion Chromatography with Mass Spectrum Detection. *J. Chromatogr. A*, **2004**, *1039*, 83.
3. *Perchlorate: A System to Track Sampling and Cleanup Results is Needed.* Document GAO-05-462, United States Government Accountability Office; Washington, D.C., May **2005**.
4. Urbansky, E. T. Review and Discussion of Perchlorate Chemistry as Related to Analysis and Remediation. *Biorem. J.* **1998**, *2*, 81.
5. *Perchlorate in California Drinking Water: Overview and Links.* Updated January, **2006**, California Department of Health Services. www.dhs.ca.gov/ps/ddwem/chemicals/perchl/perchlindex.htm.
6. *Determination of Perchlorate in Drinking Water Using Ion Chromatography.* Method 314.0; U.S. Environmental Protection Agency, Cincinnati, Ohio, **1999**.

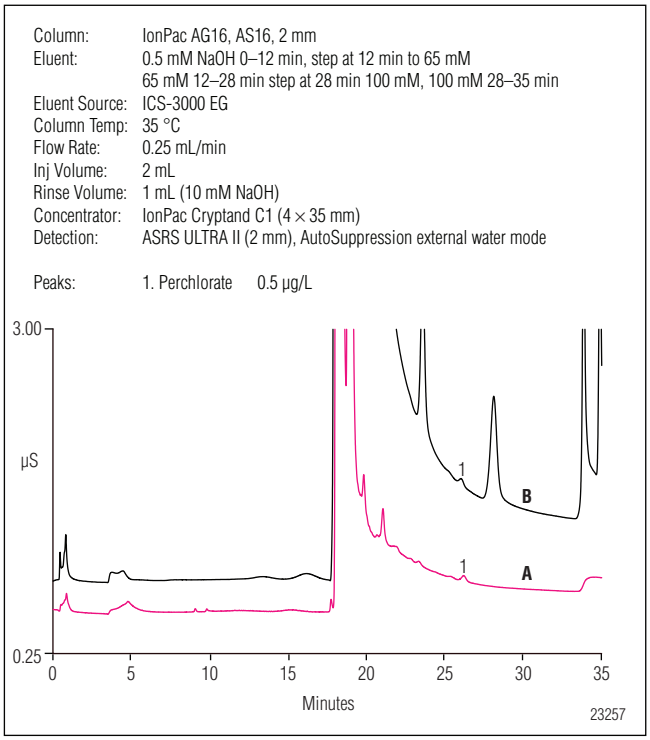

Figure 9. Comparison of drinking water D fortified with 0.5 µg/L perchlorate (A) with the CRD and (B) without the CRD using the primary method.

7. *Determination of Perchlorate in Drinking Water Using Reagent-Free Ion Chromatography*. Application Update 148, Dionex Corporation, Sunnyvale, CA.
8. *Determination of Perchlorate in Drinking Water Using In-Line Column Concentration/Matrix Elimination Ion Chromatography with Suppressed Conductivity Detection: Method 314.1.*; U.S. Environmental Protection Agency, Cincinnati, Ohio, **2005.**
9. *Improved Determination of Trace Concentrations of Perchlorate in Drinking Water Using Preconcentration with Two-Dimensional Ion Chromatography and Suppressed Conductivity Detection*. Application Note 178, Dionex Corporation, Sunnyvale, CA.

APPENDIX A

Example Chromeleon Program

Sampler.AcquireExclusiveAccess

Sampler_DiverterValve.Position_1

Column_TC.AcquireExclusiveAccess

Compartment_TC.AcquireExclusiveAccess

Pressure.LowerLimit = 200 [psi]

Pressure.UpperLimit = 3000 [psi]

MaximumFlowRamp = 6.00 [ml/min²]

%A.Equate = "%A"

%B.Equate = "%B"

%C.Equate = "%C"

%D.Equate = "%D"

CR_TC = On

Flush Volume = 4999

Wait FlushState

NeedleHeight = 2 [mm]

CutSegmentVolume = 0 [µl]

SyringeSpeed = 2

CycleTime = 0 [min]

WaitForTemperature = False

Data_Collection_Rate = 5.0 [Hz]

Temperature_Compensation = 1.7 [%/°C]

CellHeater.Mode= On

CellHeater.TemperatureSet = 35.00 [°C]

Column_TC.Mode = On

Column_TC.TemperatureSet = 35.00 [°C]

Compartment_TC.Mode = On

Compartment_TC.TemperatureSet = 30.00 [°C]

Suppressor1.Type = ASRS_2mm

CurrentSet = 100 [mA]

; Suppressor1.Carbonate = 0.0

; Suppressor1.Bicarbonate = 0.0

; Suppressor1.Hydroxide = 100.0

; Suppressor1.Tetraborate = 0.0

; Suppressor1.Other eluent = 0.0

; Suppressor1.Recommended Current = 62

Note: the following commands are used to concentrate the sample and rinse the matrix from the concentrator using the AS sample prep syringe (Option 1).

Concentrate ValvePosition = LoadPosition

ReagentPrime Volume = 20000.0, SourceReservoir = Reservoir_C, ValvePosition = NoChange

ReagentFlush Volume = 1000.0, SourceVial = Reservoir_C, ValvePosition = NoChange

Note: the following commands are an alternative to Option 1 above by using an AS autosampler vial containing 10 mM NaOH to perform the rinse step (Option 2). However, different programs will be required for different "Sourcevials" (i.e., AS autosampler vials).

Concentrate ValvePosition = LoadPosition

ReagentFlush Volume = 1000.0, SourceVial = 37, ValvePosition = NoChange

```
Wait      SampleReady
Flow =  0.250 [ml/min]
%B =    0.0 [%]
%C =    0.0 [%]
%D =    0.0 [%]
Pump_1.Curve = 5
-6.100   Concentration =  100.00 [mM]
      EGC_1.Curve =       5
-6.000   Concentration =  100.00 [mM]
      EGC_1.Curve =       5
      Concentration =     0.50 [mM]
      EGC_1.Curve =       5

 0.000   Wait CycleTimeState
      Sampler_InjectValve.InjectPosition
      CD_1.AcqOn
      CD_1_Total.AcqOn
 0.100   Home
 1.000   BeginOverlap
      Sampler.ReleaseExclusiveAccess
12.000   Concentration =  0.50 [mM]
      EGC_1.Curve =       5
      Concentration =     65.00 [mM]
      EGC_1.Curve =
28.000   Concentration =  65.00 [mM]
      EGC_1.Curve =       5
      Concentration =     100.00 [mM]
      EGC_1.Curve =       5
35.000   CD_1.AcqOff
      CD_1_Total.AcqOff
      Concentration =     100.00 [mM]
      EGC_1.Curve =       5
      Compartment_TC.ReleaseExclusiveAccess
      Column_TC.ReleaseExclusiveAccess
```

Determination of Perchlorate in Environmental Waters by Ion Chromatography Coupled with Electrospray Mass Spectrometry (IC-MS)

INTRODUCTION

Perchlorate has been used as an oxidizer in rockets, munitions, and fireworks since the 1950s, and has been found to cause thyroid dysfunction in humans. The California Department of Health Services first reported the determination of perchlorate in drinking water in 1997.[1] The EPA has stated that defense facilities from Los Angeles to Cape Cod have discharged large amounts of perchlorate onto the ground, contaminating groundwater in many places. In 2001, it was reported that perchlorate (ClO_4^-) was migrating into groundwater in California from a landfill site for civilian and military explosives. In 2002, the EPA recommended a maximum containment level (MCL) for perchlorate of 1 ppb in drinking water. Some states have set their own limits ranging from 4–18 ppb. For example, California's current "action level" is 6 ppb.[1]

The ion chromatography (IC) method with conductivity detection for the determination of perchlorate can quantify perchlorate at 2 µg/L (ppb) using the 4-mm i.d. IonPac® AS16 column, large-loop injection, eluent generator and suppressor.[2–4] The IC-MS method uses a similar method, substituting a 2-mm column format, a stable-labeled internal standard, a matrix diversion valve to eliminate the need for off-line sample pretreatment, and a mass spectrometer with an electrospray interface as the detector. The mass spectrometer is a more selective detector than conductivity in that it monitors the mass/charge ratio (*m/z*) of the analyte.[5] The *m/z* ratio provides peak identification information for perchlorate at both 99 and 101 *m/z* due to the relative isotopic abundance of ^{35}Cl and ^{37}Cl .

MS can provide lower detection limits in high-ionic-strength matrices than conductivity. The selectivity of the mass spectrometer allows the quantification of perchlorate at 99/101 *m/z* in high-ionic-strength matrices at well below the California 6 ppb action level. Recoveries must be determined in the appropriate matrices.

This application note describes the use of IC-MS to determine perchlorate in environmental waters. The described method includes in-line diversion of the sample matrix to waste and the use of an internal standard. Quantification using 99 *m/z* as compared to the perchlorate isotope (101 *m/z*) is also shown.

EQUIPMENT

ICS 2500 Ion Chromatography System:
- GS50 Gradient Pump
- EG50 Eluent Generator
- CD25A Conductivity Detector, cell with shield or DS3
- LC30 Chromatography Oven or equivalent
- AS50 Autosampler, with or without injection valve, 100 µL or 250 µL loop

Rear-loading PEEK Rheodyne valve (Rheodyne 9126, for matrix diversion)

Grounding Adaptor, P/N 059066

MSQ™ ELMO mass spectrometer, P/N 060045

AXP-MS, auxiliary low-flow pump, P/N 060684

External water kit, P/N 038018

Static mixing tee, Upchurch, P/N U-466

Chromeleon® 6.5 or higher software

CONSUMABLES

Nitrogen source, 70-80 psi regulated

IonPac AS16, 250 × 2-mm i.d., P/N 055378

IonPac AG16, 50 × 2-mm i.d., P/N 055379

ASRS® ULTRA II, 2 mm, P/N 061562

EGC II KOH cartridge, P/N 058900

CR-ATC, P/N 060477

REAGENTS AND STANDARDS

Deionized water (DI H_2O), Type I reagent-grade, 17.8 megohm-cm resistance or better

Sodium perchlorate, 99% ACS reagent-grade or better (Aldrich)

Sodium perchlorate, Internal Standard, 1 mg/L, P/N 062923

ACS reagent-grade sodium salts for interference studies

SUMMARY OF CONDITIONS

Column:	IonPac AS16, 250 × 2-mm i.d.
Suppressor:	ASRS ULTRA II, 2 mm, external water, 70 mA
GS50 Eluent:	45 mM KOH
AXP-MS Eluent:	50/50 v/v acetonitrile/water
Flow Rate:	0.3 mL/min, GS50 and AXP-MS
Temperature:	28 °C
Matrix Diversion Time:	2–9 min
Injection Volume:	100 µL
Detection:	1. Suppressed Conductivity
	2. MS
MS Conditions:	mode, –ESI
	cone voltage, 70 V
	probe voltage, –3 kV
	probe temp., 450 °C
	SIM channels, 99, 101, and 107 *m/z*
	SIM parameters, span 0.3 amu, dwell time 1 s
Run Time:	13 min
Expected System Backpressure:	2100–2350 psi
Expected Background Conductance:	<1.5 µS

SETUP

The information in this section is provided as a general discussion of the main connections required for this method. See Figure 1. The individual modules have their own installation guides available on the Dionex Reference CD-ROM.[6]

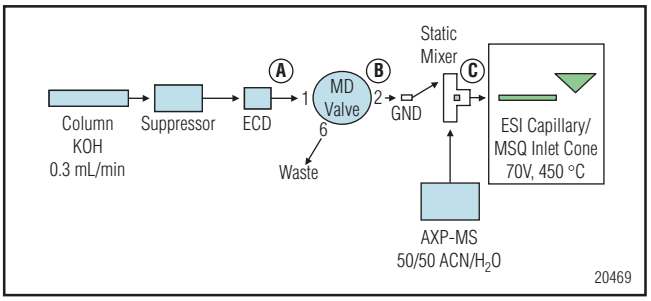

Figure 1. *Flow diagram showing IC-MS with matrix diversion.*

The ion chromatograph is set up in the standard configuration, from left to right, of pump/detector, eluent generator, chromatography module, and mass spectrometer. The MSQ is installed according to the installation guide (P/N 031869). It is assumed that the MSQ has pumped down and reached vacuum for several hours or overnight. The CR-ATC is installed between the pump and the eluent generator according to the CR-ATC manual. The eluent generator and cartridge are purged and operated according to the eluent generator installation guide for low-flow rate operation. The backpressure through the system should be about 2200 psi for optimal performance of the eluent generator. See the EG50 manual (P/N 031908) for complete instructions. The column and suppressor are plumbed in the normal configuration, with pressurized external water used for the suppressor, CR-ATC and EG50. The air pressure provided to the external water bottle is normally about 10–15 psi. Chemical regeneration of the suppressor is not recommended because higher background signal is seen in the MS. For example, sulfate from sulfuric acid regenerant has an isotopic peak at 99 *m/z*, the SIM mass for perchlorate.[3] Note that the backpressure downstream of the suppressor must be <100 psi. The tubing A, B, and C in Figure 1 can be black PEEK (0.010-in. i.d.) to obtain the necessary low backpressure.

The "Matrix Diversion" valve is plumbed as shown and kept in the divert position, with flow from the AXP-MS pump flowing to the MSQ, until the background conductivity is below 1.5 µS. The AXP-MS pump delivers 50/50 acetonitrile water to the MSQ throughout the method. The addition of solvent through the static mixer improves the electrospray process and provides higher area counts than a 100% water-based method.

The connection to the mass spectrometer is not made until the background conductivity is below 1.5 µS.

Trigger for the MSQ Start

A connection is made between the User I/O of the MSQ (rear panel, large green connector) and Relay 1 on the pump or detector module. This relay connection is necessary to supply the trigger that synchronizes the detector start times with the MSQ. See the MSQ Installation Guide for complete instructions.

Grounding the Detectors

The grounding adaptor is placed as shown in Figure 1.

Nitrogen Nebulization Gas

The nitrogen can be supplied by a nitrogen generator, cylinders of compressed gas, or liquid nitrogen Dewar cylinders. In standard 10-h operation, a 180-L liquid nitrogen Dewar will replace about 11–13 nitrogen cylinders and last about 2 weeks.

HOW TO SET UP AN INTERNAL STANDARD METHOD USING SODIUM PERCHLORATE OXYGEN–18 INTERNAL STANDARD (ISTD)

Use of a stable-labeled internal standard is a well-accepted methodology for accurate, long-term quantification in chromatography mass spectrometry methods. Because the internal standard and analyte are chemically indistinguishable, the two species have the same behavior in the analytical method and are affected in the same way by chemical and instrumental variations. Because the analyte and the internal standard coelute, two selected ion monitoring (SIM) channels are used in the mass spectrometer for selective detection. A ratio of the response for the internal standard and the analyte can give very accurate and sensitive quantification. In this method, 99 or 101 *m/z* is used to detect the indigeneous perchlorate and 107 *m/z* is used as the unique mass for the internal standard.

The Sodium Perchlorate ISTD is enriched with ^{18}O and the base mass peak is 107 *m/z*. The relative abundance of 99 and 101 *m/z* in the ISTD is less than 0.1%. For the quantification of trace-level perchlorate, the recommended concentration in each standard and sample is 1 µg/L, as indicated below.

Chromeleon software can provide the quantification using separate SIM channels for the analyte perchlorate and the internal standard. Quantification can be performed using 99 or 101 *m/z*, as desired. Figure 4 shows the QNT page from Chromeleon that can be set up to perform this calculation.

Standards

- perchlorate ISTD: Stable Label Sodium Perchlorate, 107/109 *m/z*, 1 mg/L in water
- perchlorate STD: $NaClO_4$, 99%, A.C.S. reagent, Aldrich cat. no. 41,024-1

Method of Use

1. Prepare 10 mL each of:
   - Water blank (deionized or suitable ionic composition)
   - 1 ppb perchlorate STD in water (no ISTD)
   - 125 ng/L, 250 ng/L, 500 ng/L, 1000 ng/L, 2500 ppt ng/L, and 5000 ng/L perchlorate STD in water
2. Add 10 µL 1 mg/L perchlorate ISTD to each 10-mL standard and a water blank

CHROMELEON PGM FOR MANUAL INJECTION

The PGM in Figure 2 controls: a pump, AS50 Autosampler, conductivity detector, eluent generator, ASRS ULTRA II suppressor, and MSQ mass spectrometer. A moderate amount of smoothing is performed on the MS data for on-line viewing of the data during acquisition. The injection valve is kept in the inject position throughout the run for better reproducibility. Note that the Chromeleon configuration has four items: a pump, a conductivity detector, the MS, and autosampler.

```
Pressure.LowerLimit =          200
Pressure.UpperLimit =          3000
%A.Equate =                    "%A"
%B.Equate =                    "%B"
%C.Equate =                    "%C"
%D.Equate =                    "%D"
FlushVolume =                   100
Wait                           FlushState
NeedleHeight =                 2
CutSegmentVolume =             0
SyringeSpeed =                 5
CycleTime =                    0
Data_Collection_Rate =         5.0
Temperature_Compensation =     1.7
DS3_Temperature =              Off
Suppressor_Type =              ASRS_2mm
Suppressor_Current =           70
Range =                        6
Smoothing =                    boxcar
SmoothingPoints =              3

;Matrix Diversion Valve:      Sample to MSQ
Pump_ColumnValve.State         Col_B
Sampler_InjectValve.State      LoadPosition
Concentration =                45.00
EluentGenerator.Curve =        5
Flow =                         0.3
%B =                           0.0
%C =                           100.0
%D =                           0.0
Pump.Curve =                   5
WaitForTemperature =           False
Wait                           SampleReady
Run =                          1
AuxFlow =                      0.30

-0.100                         ; this negative step is for command traffic.

 0.000                         ECD.Autozero
                               ECD_1.AcqOn
                               Sampler.Load
                               Wait                          Sampler.CycleTimeState
                               Sampler.Inject
                               Wait                          InjectState
                               ;MSQ Start
                               Pump_Relay_1.Closed           Duration = 2.00
                               Sampler_InjectValve.Inject    PositionDuration = 2.00

;Matrix Diversion Valve:      Sample Matrix to Waste for 7 minutes

2.000                          Pump_ColumnValve.State =      Col_A
                               Col_A                          Duration=420.00
11.700                         Log                           ECD_1.Signal.Value
13.0                           ECD_1.AcqOff
                               Pump_ColumnValve.State =      Col_A
                               Wait                          EluentGenerator.Ready
                               End

                                                                          20470
```

Figure 2. *Chromeleon PGM command page for perchlorate method using matrix diversion and the AS50 with internal injection valve.*

Figure 3 is a screen capture of the Chromeleon MSQ detector page in the PGM. The electrospray probe is set to 450 °C. Three selected ion monitoring (SIM) channels are set at 99, 101, and 107 *m/z*. The ionization mode is electrospray (ESI) and the polarity is negative (–ve) with a source voltage of 70 V. MS data is collected over the time range of 9–13 min during the 13-min run. The span as shown is set for 0.3 *m/z*. Data is collected for SIM 1 in the range of 98.85–99.15 *m/z* and SIM 2 in the range of 100.85–101.15 *m/z* and SIM 3 in the range of 106.85–107.15 *m/z*. This range can be narrowed for better signal-to-noise, if necessary.

Common matrix ions elute in the 0–5 min time range. The data file size is kept to a minimum by only collecting data as shown. But if desired, data can be collected over the entire run by changing the time range for MS data collection to 0–12 min. Also, a full scan can be run simultaneously with the SIM mode by opening the full-scan section of the setup screen and filling in the information. For a full scan, the mass range is usually set to cover from 15 *m/z* up to several hundred *m/z*. The "Additional Information Section" at the end of this document provides masses for some commonly occurring anions.

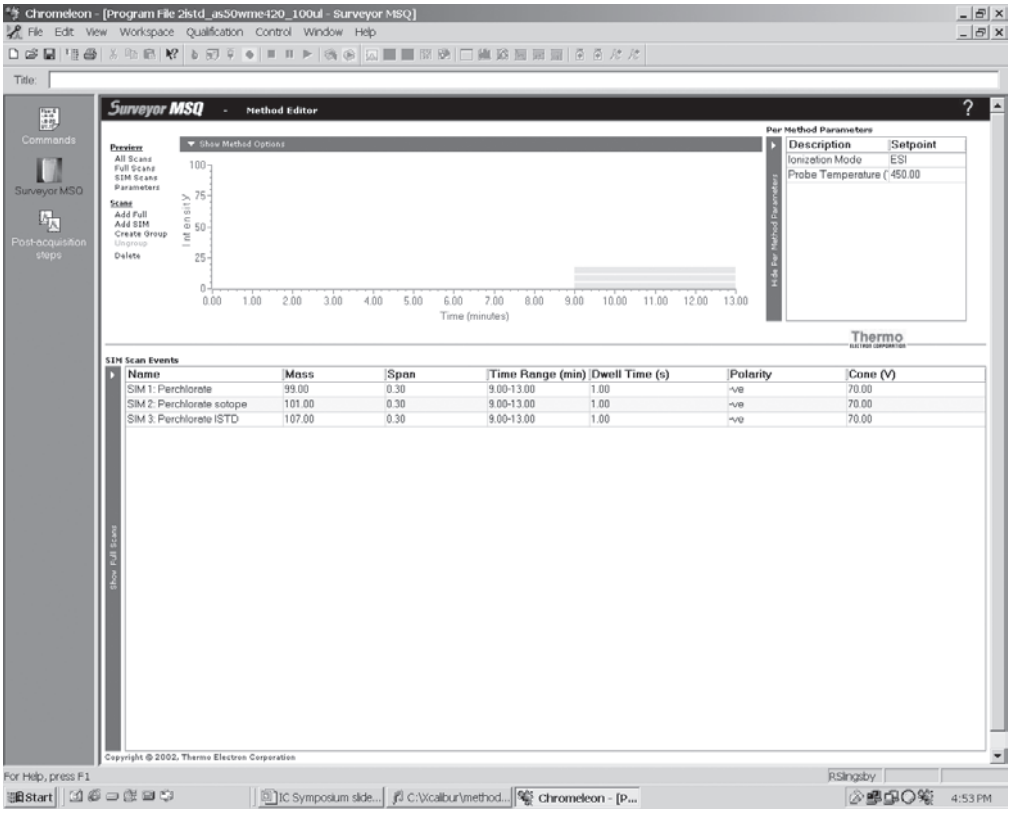

Figure 3. *Chromeleon MS page.*

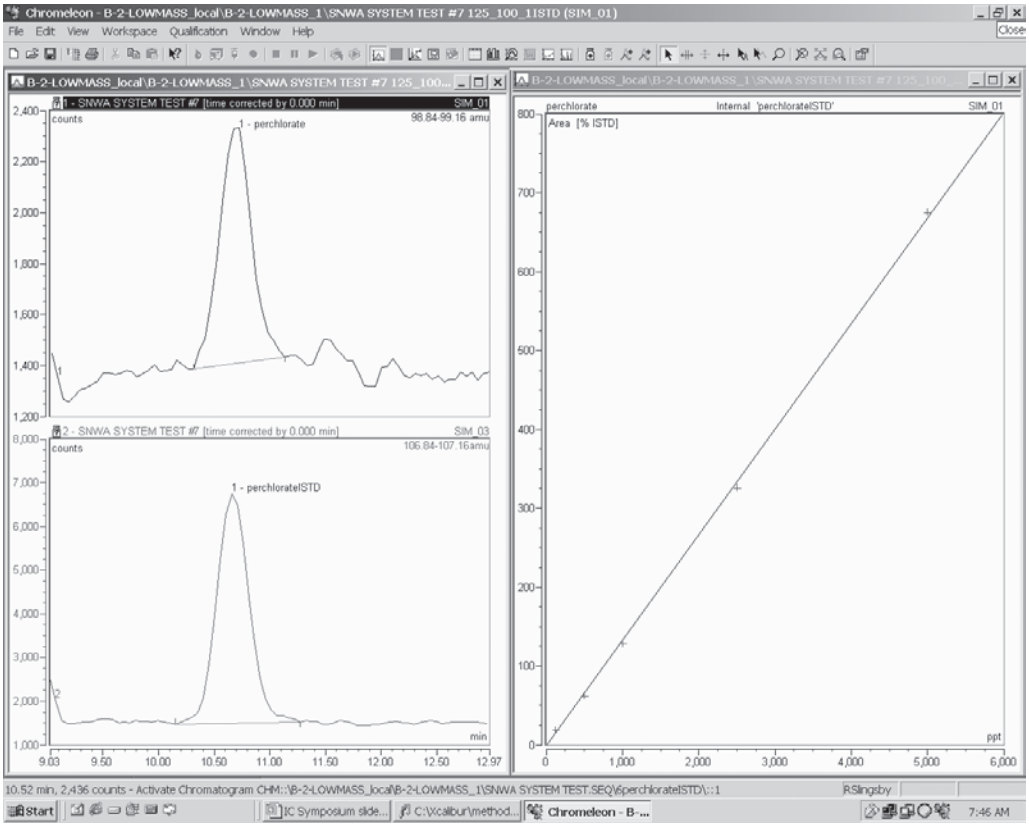

Figure 4. *Quantification of perchlorate with stable-labeled internal standard.*

DAILY START-UP FOR AN IC-MS SYSTEM

The following section can be used as a checklist for daily start-up of the IC-MS system. *The matrix diversion valve is set to divert the analytical stream to waste.* The end of the PEEK tube from port 6 of the MD valve can be placed into a waste container.

1. Add fresh deionized water to the instrument reservoir and turn on the external water to the suppressor and eluent generator.
2. Turn on the heater/voltage and nitrogen for the MS from the TUNE page of the MSQ software, making sure that the probe temperature is as expected at 450 °C.
3. Check the nitrogen source and confirm that the pressure is at 70–80 psi for electrospray operation.
4. Turn on the water in the analytical pump to start flow through the system to waste.
5. Turn on the AXP-MS pump to start flow to the MS.
6. Set the eluent generator concentration and suppressor current manually from the Chromeleon panel for IC-MS. Observe that the conductivity will begin high but drop rapidly to below 3 µS.
7. When the conductivity is below 1.5 µS, switch the matrix diversion valve to send the analytical flow to the MS. This operation can be accomplished from the panel.
8. Observe the MS background in –ESI mode on the TUNE page of the MSQ.
9. Equilibrate this system for at least 30 min. If working near the detection limit, at least an hour is needed for reproducible results.
10. Faster morning start-up can be accomplished by recycling the conductivity cell effluent back through the suppressor "Regen In" port overnight with the eluent concentration set to 5 mM and the suppressor current set to 10 mA.

DAILY SHUTDOWN OF THE IC-MS (WITHOUT RECYCLE)

1. Disconnect the PEEK tube and fitting from the inlet of the MSQ and place in a waste container.
2. Turn off the eluent flow (which also turns off the eluent generator and the suppressor current).
3. Turn off the external water source.
4. Turn off the heater and voltages from the TUNE page of the MSQ or from the Chromeleon panel.
5. Turn off the nitrogen once the temperature shown on the TUNE page is below 200 °C.

Note: If a salty or otherwise dirty liquid was flowing through the ESI probe at the time of shutdown, the probe should be flushed with water or 50/50 acetonitrile/water prior to shutdown. This flushing is conveniently accomplished using the AXP-MS pump. Be careful not to flow solvent through the analytical column and suppressor if solvent was not part of the method. A clogged ESI probe will cause poor signal.

RESULTS

Figure 5 shows an expected result from the injection of 1000 CCS high salt matrix (1000 mg/L each chloride, carbonate, and sulfate) spiked with 125 ng/L perchlorate in an IC-MS system. The system was configured as described in this application note, including the use of matrix diversion and the internal standard.

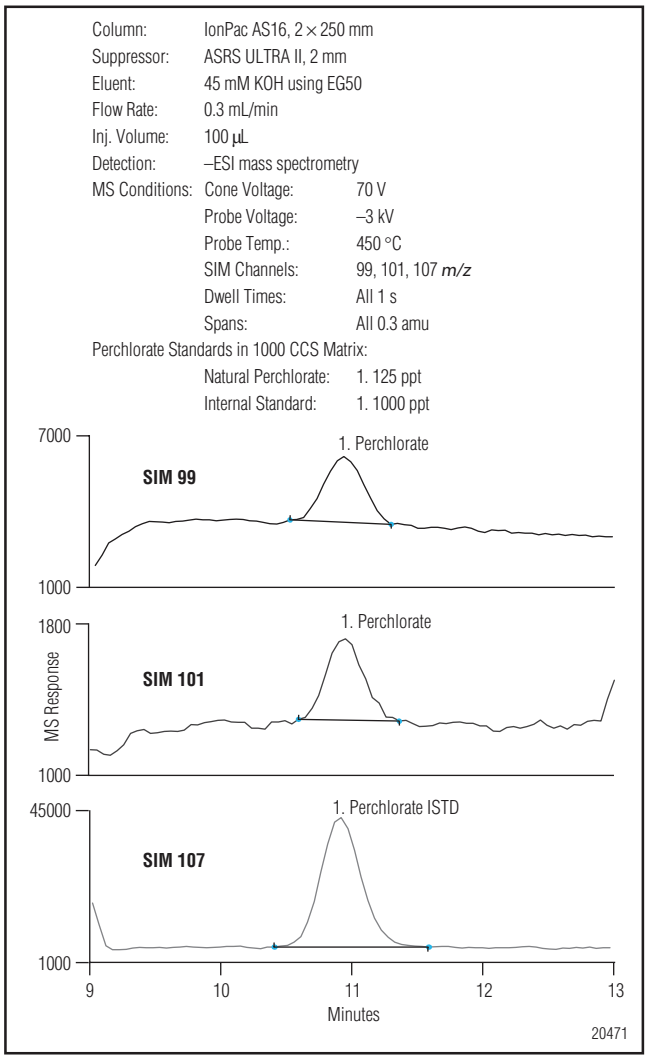

Figure 5. *High ionic matrix spiked with 125 ppt perchlorate and internal standard.*

Table 1 shows the results from seven replicate injections of 500 ng/L perchlorate in the 1000 CCS matrix. The amount values were calculated from an internal standard calibration curve. MDL values in deionized water matrix are 15–30 ng/L.

Table 1. MDL Calculation Using 500 ng/L Perchlorate and 1000 ng/L Internal Standard in 1000 CCS Matrix

Replicate	Amount 99 *m/z* (ng/L)	Amount 101 *m/z* (ng/L)
1	511	527
2	472	507
3	466	529
4	500	421
5	511	481
6	528	461
7	463	570
Average	493	499
Standard Deviation	25.7	49.3
% RSD	5.2	9.9
MDL (3.14 × s.d.)	80	155

Figure 6 shows the linear calibration curves for perchlorate in 1000 CCS matrix at 99 *m/z* and 101 *m/z* over the range of 125–5000 ng/L, using the internal standard method. The sodium chloride used to prepare the matrix contributed a small perchlorate peak to the matrix, as seen in the y-intercept. These data show that quantification can be performed at either mass with good linearity and sensitivity.

Figure 7 shows the recoveries in raw area counts and amount calculated by internal standard method over a sequence of 100 injections. The raw area counts have recoveries at all concentrations greater than 75%. The amount values calculated with the internal standard have recoveries in excess of 95% at all levels. The peak integrations were uncorrected in Chromeleon.

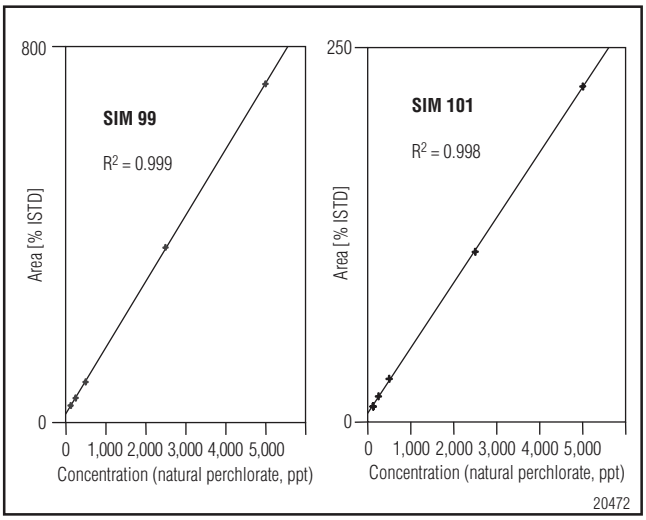

Figure 6. *Linear calibration of perchlorate spiked into 1000 CCS matrix using –ESI/MS and internal standard* $Cl^{18}O_4^-$.

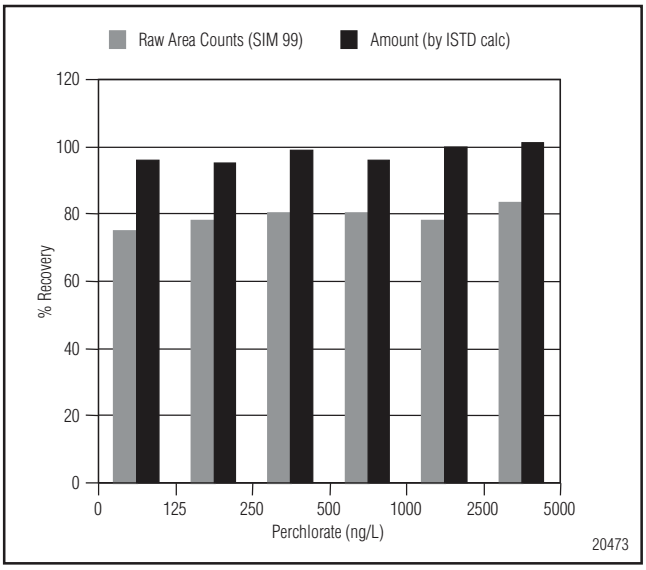

Figure 7. *IC-MS determination of perchlorate in a 1000 CCS matrix-%recovery for 100 injections.*

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3. Dionex Corporation. *Determination of Perchlorate in Drinking Water by Ion Chromatography*; Application Update 145; Sunnyvale, CA.
4. Dionex Corporation. *Determination of Perchlorate in Drinking Water Using Reagent-Free Ion Chromatography*; Application Update 148; Sunnyvale, CA.
5. Roehl, R.; Slingsby, R.; Avdalovic, N.; Jackson, P. E. *J. Chromatogr. A* ***2002,*** *956,* 245–254.
6. Dionex Reference Library CD-ROM, August 2002 or later.

ADDITIONAL INFORMATION

Table 2 on the next page provides a listing of other anions that are detectable by IC-MS, with the appropriate SIM masses for each. The last column in this table gives the isotopes, which most commonly are derived from the presence of S, Cl, and Br. The isotopic peaks provide confirmatory evidence for the presence of these elements in the analyte. Reference 2 provides retention time data for many of these anions for the AS16 column.

Table 2. Anion SIM Masses for IC-MS							
SIM Mass	**Anion**	**Formula**	**Major Isotopes**	**SIM Mass**	**Anion**	**Formula**	**Major Isotopes**
19	fluoride	F^-		113	thiosulfate	$HS_2O_3^-$	113/115
35	chloride	Cl^-		113	trifluoroacetate	$F_3CCO_2^-$	
45	formate	HCO_2^-		115	maleate	$HOOCCHCHCO_2^-$	
46	nitrite	NO_2^-		115	fumarate	$HOOCCHCHCO_2^-$	
58	thiocyanate	SCN^-	58/60	117	chromate	$HCrO_4^-$	
59	acetate	$CH_3CO_2^-$		117	succinate	$HOOCCH_2CH_2CO_2^-$	
61	bicarbonate	HCO_3^-		122.9	arsenate	ASO_3^-	
62	nitrate	NO_3^-		127	bromate	BrO_3^-	127/129
67	chlorite	ClO_2^-	67/69	127	dichloroacetate	$Cl_2CHCO_2^-$	127/129
73	glyoxylate	$CHOCOO^-$		127	iodide	I^-	
73	propionate	$CH_3CH_2CO_2^-$		128.9	selenite	SeO_3^-	128/126
75	glycolate	$HOCH_2CO_2^-$		129	bromate	BrO_3^-	127/129
79	bromide	Br^-	79/81	131	glutarate	$HOOC(CH_2)_3CO_2^-$	
83	chlorate	ClO_3^-	83/85	133	malate	$HOOCCH_2CHOHCO_2^-$	
87	butyrate	$CH_3CH_2CH_2CO_2^-$		133	tartrate	$HOOC(CHOH)_2CO_2^-$	
87	pyruvate	$CH_3COCO_2^-$		139	bromoacetate	$BrCH_2CO_2^-$	
89	methoxyacetate	$CH_3OCH_2CO_2^-$		141	arsenate	$ASO_3 \bullet H_2O$	
89	lactate	$CH_3CHOHCO_2^-$		144.9	selenate	SeO_4^-	144/142
89	oxalate	$CO_2HCO_2^-$		145	adipate	$HOOC(CH_2)_4CO_2^-$	
93	chloroacetate	$ClCH_2CO_2^-$	93/95	173	bromochloroacetate	$BrClCHCO_2^-$	mixed
95	methanesulfonate	$CH_3SO_3^-$	95/97	183	styrenesulfonate	$CH_2CHC_6H_4SO_3^-$	
96	sulfamate	$NH_2SO_3^-$	96/98	191	citrate	$HOOCCH_2COHCO_2HCH_2CO_2^-$	
97	sulfate	HSO_4^-	97/99	191	quinate	$C_7H_{12}O_6^-$	
97	phosphate	H_2PO_4		191	isocitrate	$HOOCCH_2COHCO_2HCH_2CO_2^-$	
99	perchlorate	ClO_4^-	99/101	207	dichlorobromoacetate	$Cl_2BrCCO_2^-$	mixed
101	valerate	$CH_3CH_2CH_2CH_2CO_2^-$		207	dibromochloroacetate	$Br_2ClCCO_2^-$	mixed
103	hydroxybutyrate	$CH_3CHOHCH_2CO_2^-$		217	dibromoacetate	$Br_2CHCO_2^-$	mixed
103	malonate	$HOOCCH_2CO_2^-$		248.8	tungstate	HWO_4^-	mixed
				251	tribromoacetate	$Br_3CCO_2^-$	mixed

Determination of Perchlorate in High Ionic Strength Fertilizer Extracts By Ion Chromatography

INTRODUCTION

Perchlorate anion (ClO_4^-) is a water soluble, mobile, and persistent environmental contaminant most clearly linked with the disposal of ammonium perchlorate used in rocket propellants.[1] Perchlorate may cause hypothyroidism by interfering with the uptake of iodide (a similarly polarizable anion) needed by the thyroid to produce thyroid hormones. The prescribed method for determination of perchlorate in drinking and ground waters, as required in the assessment phase of the Unregulated Contaminant Monitoring Rule, is U.S. Environmental Protection Agency (EPA) Method 314.0.[2] Perchlorate determination in water at the low-µg/L level is described in Dionex Application Note 134.[3]

Beyond the need to monitor drinking and ground water for perchlorate, there is also interest in identifying other potential sources of perchlorate contamination of the environment. This will demand analysis of more complex matrices with higher ionic strength than drinking water. For example, Chilean caliche (sodium nitrate) may contain naturally occurring perchlorate. Products derived from Chilean caliche currently represent only about 0.14% of present U.S. fertilizer use, but were more widely used in the past. Among the materials surveyed for perchlorate in recent reports were fertilizers and fertilizer feed stocks. One survey confirmed the occurrence of perchlorate in Chilean caliche, but did not identify perchlorate in any of the other materials tested.[4]

This Application Note provides a procedure for preparing aqueous leachates of fertilizers, inorganic salts, and other materials. The perchlorate anion in these extracts or similar high ionic strength matrices can be determined in 15 min by using an IonPac® AS16 column, EG40-generated hydroxide eluent, a 500-µL injection, and suppressed conductivity detection.

The IonPac AS16 is a high capacity, hydroxide-selective anion exchange column with ultra low hydrophobicity designed for the isocratic separation of polarizable anions including perchlorate, iodide, thiocyanate, and thiosulfate. The AS16 column's capacity of approximately 170 µeq/column allows large loop injections without column overloading. The higher capacity extends the method's linear range and improves its performance for trace-level determinations of perchlorate in high ionic strength matrices.

EQUIPMENT

A Dionex DX-600 chromatography system consisting of:
- GP50 Gradient Pump with Vacuum Degas Option
- EG40 Eluent Generator Module
- EluGen® Hydroxide Cartridge (EGC-KOH) (DIONEX P/N 053921)
- ED50 Conductivity Detector with DS3
- SRS Gas-Assisted Regeneration Kit (DIONEX P/N 56886)
- AS50 Automated Sampler with Thermal Compartment
- 1.0 mL sample syringe for AS50 (DIONEX P/N 55066)
- PeakNet® 6 Chromatography Workstation

Syringe filters (Gelman IC Acrodisc 0.2-µm, PN 4483)
Orbital Shaker (LAB-LINE 3520, Melrose Park, IL)
Centrifuge (Beckman GS-6R, Palo Alto, CA)

CONDITIONS

Columns:	IonPac AS16 Analytical 4 x 250 mm (DIONEX P/N 55376) IonPac AG16 4 mm Guard 4 x 50 mm (DIONEX P/N 55377)
Eluent:	65 mM potassium hydroxide (KOH)
Eluent Source:	EG40
Flow Rate:	1.2 mL/min
Temperature:	30 °C
Injection:	500 µL partial loop w/10 µL cut volume from a 1000-µL sample loop
Detection:	Suppressed conductivity, ASRS®-ULTRA (4 mm), gas assisted external-water mode, current setting—300 mA DS3 Cell (P/N 044130), 35 °C, 1.7% /°C
Background:	1–3 µS
Noise:	5–10 nS peak-to-peak
Backpressure:	Adjust to ~2500 psi
Run Time:	15 min

REAGENTS AND STANDARDS

Ammonium perchlorate (NH_4ClO_4) (Alpha Aesar 11658, ACS-grade)

PREPARATION OF SOLUTIONS AND STANDARDS

Reagent Water

Type I Reagent Grade distilled or deionized water with a specific resistance of 17.8 MΩ-cm or greater, filtered through a 0.2-µm filter immediately before use.

Eluent Solution

Generate the 65 mM KOH eluent on-line by pumping reagent water through the EG40. PeakNet software tracks the amount of KOH used and calculates the remaining lifetime of the EGC-KOH cartridge.

Alternatively, prepare 65 mM NaOH by pipetting 5.2 g of 50% (w/w) aqueous NaOH from the middle portion of the reagent bottle into a 1.00-L volumetric flask containing about 500 mL of degassed reagent water. Do not shake the 50% (w/w) NaOH bottle or pipette from the top of the solution where sodium carbonate may have formed. Dispense the aliquot of NaOH below the surface of the water to avoid the introduction of carbon dioxide from the air into the eluent. Bring to volume with degassed reagent water, mix and degas by sparging with helium or sonicating under vacuum for 10 min. Atmospheric carbon dioxide readily dissolves in dilute basic solutions, forming carbonate. Carbonate contamination of eluents can affect the retention times of the analytes, resulting in performance that may not be equivalent to that achieved with the EG40 eluent generator. Store the eluent in plastic labware. Maintain an inert helium atmosphere of 3–5 psi in the eluent reservoir to minimize carbonate contamination.

Stock Standard Solutions

Prepare a 1000 mg/L stock standard solution of perchlorate anion by dissolving 0.1181 g of ammonium perchlorate in reagent water and diluting to 100 mL. Store in glass, high density polyethylene or polypropylene bottles at 4 °C. This stock standard is stable for at least one month.

Working Standard Solutions

Prepare working standards at lower concentrations by diluting appropriate volumes of the 1000 mg/L stock standard with reagent water. Prepare working standards daily if they contain less than 100 mg/L of perchlorate.

SAMPLE PREPARATION

Dilute liquid samples as necessary and filter through a 0.2-µm IC syringe filter. Use a hydrophilic polypropylene or polyethersulfone filter; do not use polyvinylidene fluoride (PVDF). Discard the first 300 µL of filtrate and filter the remainder directly into a clean plastic autosampler vial. Qualify filters by analyzing a reagent water blank and a 10-µg/L perchlorate standard that has been passed through the filter. The blank should be free of peaks within the retention time window of perchlorate, and the recovery of the 10-µg/L standard should fall between 80% and 120%.

Use standard sampling practices to obtain a representative sample of the matrix to be analyzed, e.g., a fertilizer or soil sample.[5] Extract perchlorate anion from solid samples by combining 4.00 g solid with 40.0 mL reagent water in a 100-mL glass, high-density polyethylene or polypropylene bottle. Place the capped bottle on an orbital shaker and vigorously mix at 250 rpm for 8–15 h. Allow suspended material to settle, then decant into a 50-mL polypropylene centrifuge tube and centrifuge the supernatant at 6000 rpm for 10 min or until clear. Filter supernatant through a 0.2-µm IC syringe filter as above and inject.

In this study, high chloride samples (e.g., KCl) were analyzed after simply diluting and filtering the extract. Alternatively, reduce the concentration of chloride by treating the sample with the OnGuard® Ag cartridge (PN 39637) or the OnGuard II Ag 1 cc (PN 057089) followed by an OnGuard H cartridge (PN 39596) or OnGuard II H 1 cc (PN 057085). Prepare the OnGuard cartridges by passing 10 mL of reagent water through the cartridge at 2 mL/min. After preparing the cartridge, pass 5 mL of the undiluted sample through the cartridge. Discard the first 3 mL and collect the remainder for dilution or direct injection. (For details, consult Dionex Application Note 134 and the OnGuard cartridge manual, Document No. 032943, or the Installation Instructions and Troubleshooting Guide for the OnGuard II Cartridges, Document No. 031688.)

SYSTEM PREPARATION AND SETUP

Prepare the ASRS-ULTRA for use by hydrating the eluent chamber. Use a disposable plastic syringe to slowly push approximately 3 mL of 200 mN H_2SO_4 through the ELUENT OUT port and 5 mL of 200 mN H_2SO_4 through the REGEN IN port. Allow the suppressor to sit for approximately 20 min to fully hydrate the suppressor screens and membranes. Install the ASRS-ULTRA for use in the gas-assisted external water mode by following the Installation and Troubleshooting Instructions for the ASRS-ULTRA, Document No. 31367 and the ASRS-ULTRA Gas Assisted Regeneration Kit, Document No. 31665. Adjust the head pressure on the external water reservoir to deliver a flow rate of 5–10 mL/min (~ 10–15 psi). Use an ASRS-ULTRA current of 300 mA.

Install the EG40 and configure it with the PeakNet chromatography data system. Make sure that the high pressure degas tubing assembly (Degas Assembly, P/N AAA-053721) is installed to remove the electrolysis gas from the freshly generated eluent. Condition the EluGen KOH cartridge as directed in the EG40 manual by running a gradient from 1 to 60 mN KOH in 20 min, then 60 mN for 40 min at 1 mL/min. (For instructions on EG40 installation and use, see the OPERATOR'S MANUAL for the EG40 ELUENT GENERATOR SYSTEM, Document No. 031373).

Install a 4 mm x 50 mm IonPac AG16 and a 4 mm x 250 mm IonPac AS16 column. Make sure that the system pressure displayed by the pump is at least 2000 psi when 65 mM KOH is delivered at 1.2 mL/min, so that the Degas Assembly can effectively remove hydrolysis gas from the eluent. If necessary, install backpressure coils supplied with the EG40 ship kit to bring the system pressure to between 2000 and 2800 psi. Because the system pressure can rise over time, trim the backpressure coil as necessary to maintain system pressure under 3000 psi. Do not exceed 3000 psi or the Degas Assembly tubing may rupture.

The storage solution that the AS16 is shipped with is 35 mM NaOH. Equilibrate the column with 65 mM KOH eluent for 60 min; then analyze a system blank of reagent water. An equilibrated system has a background signal of less than 3 µS and peak-to-peak noise of less than 10 nS. There should be no peaks eluting within the retention time window of the perchlorate anion.

This application uses a partial-loop injection with a 1000-µL sample loop for a 500-µL injection. If injecting a different volume in the partial-loop injection mode, use a sample loop that is at least 2X the volume to be injected. Refer to the AutoSelect AS50 Autosampler Operator's Manual (Document No. 31169) for a complete discussion of the different injection modes.

Install a 1 mL Sample Syringe and set the Syringe Speed to 4 or 5 to make faster large-loop injections. Enter the correct sample Loop Size and Sample Syringe Volume in the AS50 Plumbing Configuration Screen. Inject a 100-µg/L standard. The column is equilibrated when two consecutive injections of the standard produce the same retention time for perchlorate. Confirm that the resulting chromatogram resembles the chromatogram of the 100-µg/L standard shown in Figure 1.

Results and Discussion

Figure 2 summarizes the calibration data for perchlorate anion obtained by using the partial-loop injection mode with a 10-µL cut volume to make duplicate 500-µL injections of calibration standards at 3, 5, 10, 15, 20, 30, 50, 100, 125, 150, 175, and 200 µg/L. The calibration curve is linear over two orders of magnitude because of the high capacity of the AS16 column and the good peak shape for the perchlorate anion.

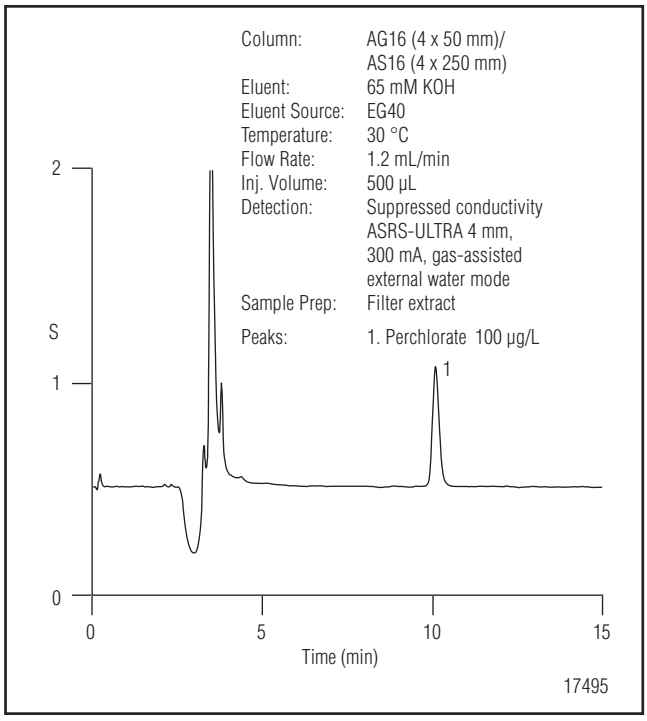

Figure 1. Perchlorate standard at 100 µg/L.

The method detection limit (MDL) is a measure of the precision of replicate injections of a low-level solution and is defined as the minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.[6] The MDL for perchlorate was determined by making seven injections of reagent water fortified with perchlorate at a concentration of 3 µg/L (five times the estimated instrument detection limit). Using the concentration values calculated from the calibration curve, the MDL is calculated as:

$$\begin{aligned} MDL &= (t) \times (S) \\ &= (3.14) \times (0.136) \\ &= 0.43 \text{ µg/L} \end{aligned}$$

where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates].

S = the standard deviation of the replicate analyses.

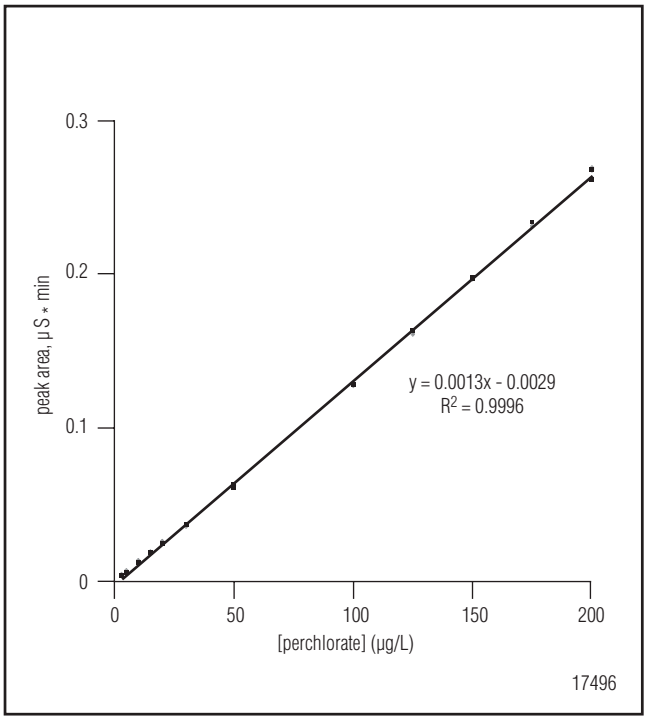

Figure 2. Calibration curve for perchlorate. See text for details.

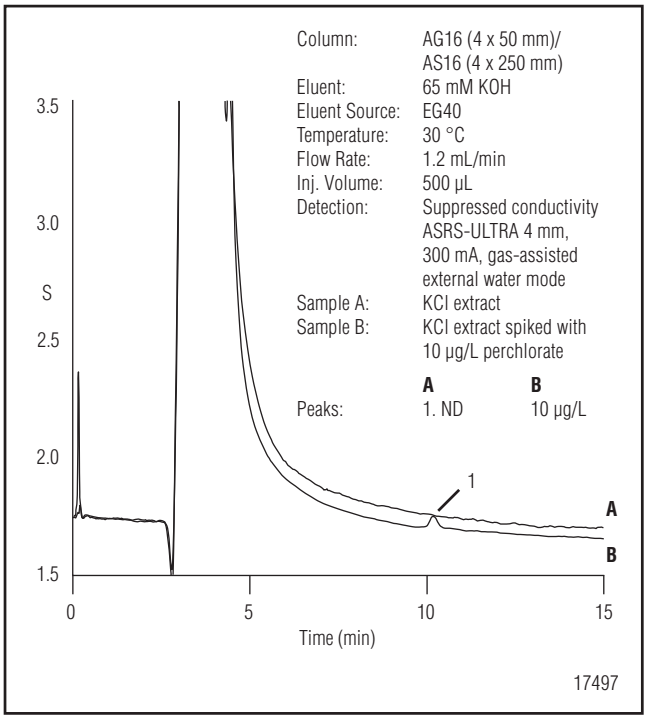

Figure 3. Recovery of a 10 µg/L spike from a perchlorate-free KCl extract.

The MDL of 0.43 µg/L obtained by this method, which uses a 500-µL sample injection and an ASRS in gas-assisted external water mode with suppressed conductivity detection, is similar to the MDL reported in U.S. EPA Method 314.0, which uses a 1000-µL sample injection and an ASRS in external water mode. The gas-assisted recycle mode consumes less water than does the external water mode, which may benefit some labs.

The performance of this method was evaluated by analyzing fortified samples to confirm retention time and assess recovery. First, the level of perchlorate was determined in various fertilizer extracts that were diluted 10-fold, filtered, and analyzed.

Samples with no detectable perchlorate peak were fortified with 10 µg/L perchlorate and reanalyzed. If the perchlorate spike was not quantitatively recovered (80–120%), the sample was diluted 100-fold, fortified with 10 µg/L perchlorate and reanalyzed. This was repeated at increasing dilutions until the perchlorate spike could be quantitatively recovered. The final dilution factor was multiplied by the MDL to calculate the reported detection limit for each sample. Figure 3 shows the chromatogram (A) of a 10-fold dilution of a KCl fertilizer extract with no detectable perchlorate compared with (B) the same sample spiked with 10 µg/L of perchlorate. The calculated recovery of the perchlorate spike in this case is 100%.

Samples observed to have a detectable peak within the perchlorate retention time window were diluted to bring the peak area within the linear range of the calibration curve. The diluted sample was fortified with perchlorate at a level 50–100% of the observed level and reanalyzed. Only if the spiked sample showed a single perchlorate peak with quantitative recovery of the spike was the sample reported as containing perchlorate.

Table 1 summarizes the spike recovery of perchlorate from various extracts analyzed as part of a recent U.S. EPA survey of fertilizers and related materials for perchlorate.[4] Generally, the higher the ionic strength of the original extract, the greater the dilution required to achieve acceptable spike recovery. The highest ionic strength samples, salts such as KCl or $NaNO_3$, could be successfully analyzed with a 100- or 1000-fold dilution. (A 100-fold dilution of a KCL sample prepared according to this method has a Cl^- concentration of about 500 mg/L). After an appropriate dilution, this method, provides acceptable recovery (i.e., 80-120%) of the perchlorate anion from each of these matrices.

Figure 4 shows the chromatogram (A) of a 2000-fold diluted extract of Chilean sodium nitrate fertilizer (Chile saltpeter) [4] containing perchlorate compared with (B) the same sample spiked with 100 µg/L perchlorate. The identity of the perchlorate peak is confirmed and the spike recovery is 106%.

Table 1. Spike Recovery of Perchlorate

Item [a]	Materials surveyed for perchlorate [b] (NPK ratio)	Dilution [c]	$[ClO_4^-]$ found, µg/g	$[ClO_4^-]$ [d] added, µg/L	Spike Recovery
4	Potassium chloride	100	ND	10	103%
7	Potassium magnesium sulfate	100	ND	10	96%
9	Osmocote (18-6-12)	100	ND	10	98%
10	Lawn fertilizer (36-6-6)	100	ND	10	96%
16	Ammonium sulfate	100	ND	10	106%
23	Ammonium dihydrogen phosphate	100	ND	10	106%
29	Urea	100	ND	10	110%
33d	Potassium sodium nitrate	5000	3850	100	101%
34d	Ammonium nitrate	100	ND	10	102%
35	Potassium nitrate	2000	2240	100	106%
36	Sodium nitrate	2000	1920	100	103%
38	Granular triplesuperphosphate (GTSP)	1000	ND	10	98%
41	Limestone	100	ND	10	99%
47	Ammonium monohydrogen phosphate	100	ND	10	100%
49	Potassium chloride + 6.8 mg/g ClO_4^-	10000	6140	50	95%
50	Soluble plant food + 6.2 mg/g ClO_4^-	10000	5430	40	99%
51	GTSP + 2.7 mg/g ClO_4^-	10000	2300	20	105%
52	Urea + 1.8 mg/g ClO_4^-	10000	1810	20	102%
55	Chilean sodium nitrate (Chile saltpeter)	2000	1700	100	102%

[a] Item numbers are consistent with Table 2.1 in Reference [4]; see [4] for definitions and sources.
[b] See text for sample preparation and analysis details.
[c] Refers to the final dilution of the original sample for liquids, or of the extract for solids.
[d] Perchlorate was added to the final dilution to augment the native perchlorate concentration by this amount.

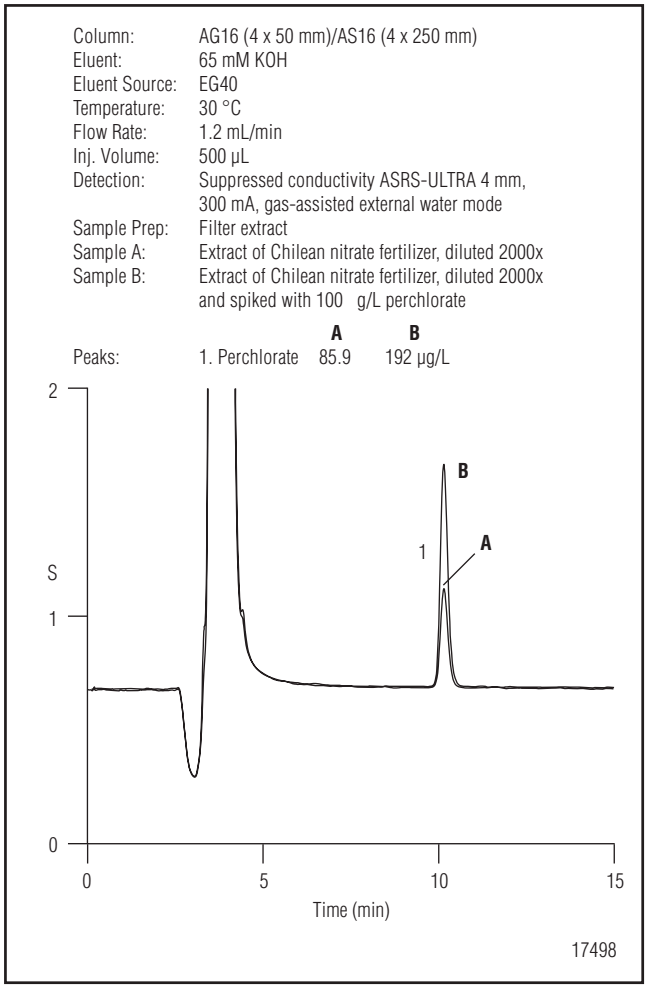

Figure 4. Recovery of a 100 µg/L spike from a perchlorate-containing Chilean nitrate fertilizer extract.

INTERFERENCES

Dionex Application Note 134 lists 22 potentially interfering anions that are eluted well before perchlorate by using a 65 mM KOH eluent. Once again, with the 65 mM KOH eluent used in this study, most of the potential interferences, including chloride, nitrate, sulfate and phosphate elute earlier and do not interfere with the determination of perchlorate.

The potential for interference from some additional polyvalent analytes that might be found in phosphate-containing fertilizers was also investigated. KOH concentrations ranging from 35–65 mM were used to elute dipolyphosphate ($P_2O_7^{4-}$, also called pyrophosphate), tripolyphosphate ($P_3O_{10}^{5-}$), and perchlorate anion. Depending on the eluent composition, column temperature, and sample matrix, either pyrophosphate or tripolyphosphate can coelute with perchlorate.

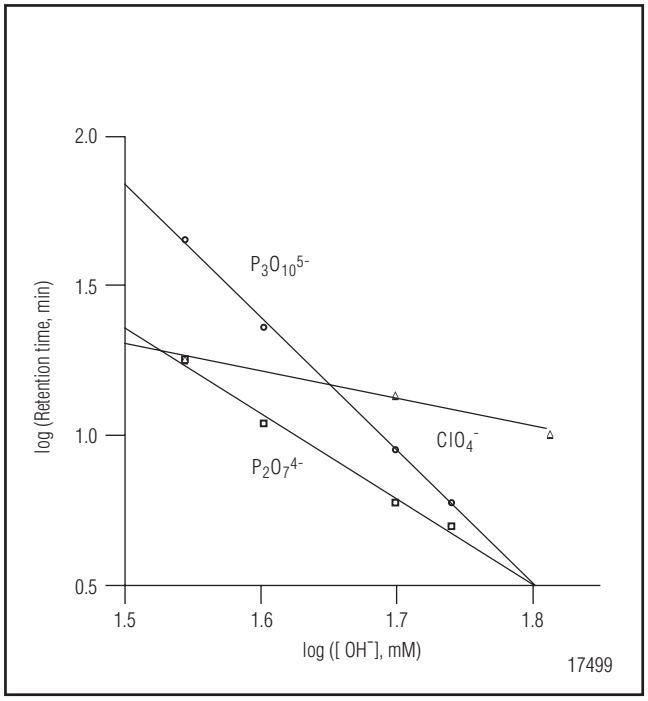

Figure 5. Effect of eluent concentration on retention of perchlorate and potential interfering anions.

The results are plotted in Figure 5 as the logarithm of the retention time versus the logarithm of the eluent concentration. As the concentration of KOH increases, the retention time of the polyvalent phosphates decreases faster than that of monovalent perchlorate. Theory predicts that for a hydroxide eluent, the slope of the line for each anion should be equal to the charge on that anion.[7] Figure 5 shows that perchlorate and pyrophosphate coelute at around 35 mM KOH (1.54 on the log scale), while perchlorate and tripolyphosphate coelute at around 45 mM KOH (1.65 on the log scale). At 65 mM KOH , pyrophosphate and tripolyphosphate should not interfere with the determination of perchlorate. However, because other unidentified polyvalent analytes could behave similarly, we recommend reanalyzing any sample that shows a peak within the retention time window of perchlorate, after fortifying it with perchlorate at a level 50–100% of that seen in the sample. The calculated recovery of the added perchlorate should be between 80% and 120%. There should be no indication of a peak coeluting with perchlorate in the chromatogram of the fortified sample. If necessary, reanalyze the spiked sample at 70 mM KOH. It is unlikely that an interfering anion will coelute with perchlorate at both 65 mM KOH and 70 mM KOH.

PRECAUTIONS

Peak area precision and accuracy depend on autosampler performance. Replace the water in the flush reservoir daily with freshly filtered and degassed water. Inspect the AS50 daily for bubbles in the sample syringe or its tubing. Purge to remove any bubbles by following the instructions in the AS50 manual.

Strongly retained compounds from injected samples can accumulate on the column and degrade its performance. Signs of a fouled column include loss of capacity, loss of resolution, shortened retention times, higher noise and background, spurious peaks, and peak tailing. The AS16 column can be flushed with up to 100% acetonitrile to help remove contaminants from the column. (For more information on column troubleshooting and cleanup, see the Installation Instructions and Troubleshooting Guide for the IonPac AS16 Analytical Column, Document No. 031475).

Some fertilizer extracts contain soluble aluminosilicates (clays) or potassium magnesium sulfate (langbeinite) that will plug the column and increase the backpressure despite careful centrifugation and filtering. Use a guard column to protect the analytical column; change the guard column if such a sample causes a sudden increase in total backpressure to greater than 3000 psi.

SUMMARY

This Application Note describes an EG40-based IC system for the determination of perchlorate in high-ionic strength matrices such as fertilizer extracts. It provides guidelines for system setup and sample preparation. Expected operating conditions for well performing systems are given as an aid to troubleshooting. The system uses an IonPac AS16 analytical column, 65 mM KOH eluent electrolytically generated by the EG40, and suppressed conductivity detection.

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7. Madden, J. E., Avdalovic, N., Jackson, P.E. and Haddad, P.R., *J. Chromatogr. A.* 837 (1999) 65.

SUPPLIERS

VWR Scientific Products, 3745 Bayshore Blvd., Brisbane, CA 94005, USA, 1-800-932-5000. www.vwrsp.com.

www.pall.com/gelman

Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178 USA, 1-800-325-3010, www.aldrich.sial.com.

LAB-LINE, Melrose Park, IL

Beckman Coulter, Inc., P.O. Box 3100, Fullerton, CA 92834-3100 USA, 800-233-4685 www.beckman.com

Alpha Aesar, 30 Bond Street, Ward Hill, MA 01835-8099 USA, 1-800-343-0660. www.alfa.com

Determination of Low Concentrations of Perchlorate in Drinking and Groundwaters Using Ion Chromatography

INTRODUCTION

Ammonium perchlorate is a key ingredient in solid rocket propellants. Perchlorate has recently been found in drinking water wells in regions of the U.S. where aerospace material, munitions, or fireworks were developed, tested, or manufactured.[1] Perchlorate poses a human health concern because it can interfere with the thyroid gland's ability to utilize iodine to produce thyroid hormones. Current data suggest that 4 to 18 µg/L (ppb) is an acceptable exposure level.[1] The State of California requires remedial action for drinking water sources containing more than 18 µg/L perchlorate.

The determination of perchlorate at trace levels is a difficult analytical task and ion chromatography represents the only viable means for the quantification of such low concentrations of perchlorate. In 1997, the California Department of Health Services (CDHS) developed an IC method to support the California action level of 18 µg/L.[2] The CDHS method uses an IonPac® AS5 column and an eluent of 120 mM sodium hydroxide containing 2 mM p-cyanophenol, which is added to minimize hydrophobic interactions with the resin.[3] In 1998, Dionex developed an updated method for determining low perchlorate concentrations using an IonPac AS11 column with an eluent of 100 mM sodium hydroxide and suppressed conductivity detection.[4,5]

This application note describes an improved method to quantify low levels of perchlorate. This method uses an IonPac AS16 column to separate perchlorate from the other anions typically found in drinking and groundwaters. The IonPac AS16 is a high-capacity, very hydrophilic, hydroxide-selective column designed for the fast separation of polarizable anions (e.g., thiosulfate, iodide, and perchlorate). Compareö to other anion-exchange columns, the polarizable anions are eluted with higher efficiency and improved peak shape, without the addition of organic solvents. The IonPac AS16 column is the column specified in U.S. EPA Method 314.0, which is the analytical method to be prescribed for the analysis of perchlorate in the assessment phase of the Unregulated Contaminant Monitoring Rule.[6]

Because perchlorate is well separated from other inorganic anions using the IonPac AS16 column at a lower hydroxide eluent concentration than needed for the IonPac AS11, the EG40 Eluent Generator can be used. This application note shows that perchlorate can be quantified at the 2-µg/L level using an IonPac AS16 column, EG40-generated hydroxide eluents, a large-loop injection, and suppressed conductivity detection.

EQUIPMENT

Dionex DX-500 IC system consisting of:

- GP50 Gradient Pump
- CD20 Conductivity Detector
- AS40 Automated Sampler
- LC30 Chromatography Oven
- EG40 Eluent Generator with an EluGen® Hydroxide Cartridge

Two 4-L plastic bottle assemblies (for external water mode suppression)

PeakNet® Chromatography Workstation

REAGENTS AND STANDARDS

Deionized water (DI H_2O), Type I reagent-grade, 18 MΩ-cm resistance or better

Sodium perchlorate, 99% ACS reagent-grade or better (Sigma-Aldrich)

ACS reagent-grade sodium salts (Fisher, Sigma-Aldrich, Fluka, EM Science) were used to make standards of other anions for interference studies.

CONDITIONS

Columns:	IonPac AS16 Analytical 4 x 250 mm (P/N 055376) IonPac AG16 Guard 4 x 250 mm (P/N 055377)
Eluent:	65 mM potassium hydroxide
Eluent Source:	EG40
Flow Rate:	1.2 mL/min
Temperature:	30 °C
Sample Volume:	1000 µL
Detection:	Suppressed conductivity, ASRS® ULTRA, 4 mm, AutoSuppression® external water mode; Power setting—300 mA
System Backpressure:	2600 psi
Background Conductance:	1–4 µS
Run Time:	12 min

PREPARATION OF SOLUTIONS AND REAGENTS

Stock Perchlorate Standard Solution

Dissolve 1.4120 g of sodium perchlorate monohydrate in 1000 mL of deionized water to prepare a 1000-mg/L standard solution. This standard is stable for at least one month when stored at 4 °C.

Working Standard Solutions

Appropriate dilutions of the 1000-mg/L perchlorate standard solution were made for studies of method linearity and the method detection limit (MDL). Method linearity was determined by diluting 2, 10, 20, 50, and 100 µL of the 1000-mg/L perchlorate standard to 1 L to prepare working standard solutions at 2, 10, 20, 50, and 100 µg/L and making two injections of each working standard. Seven injections of the 2-µg/L standard were made for the MDL study.

INTERFERENCE STUDIES

To determine if other anions interfere with perchlorate determinations, 1-mL samples containing 100 ppb of the chosen anion and 20 ppb of perchlorate were injected. Arsenate, arsenite, bromate, bromide, carbonate, chlorate, chloride, chromate, cyanide, humic acid, iodate, iodide, molybdate, nitrate, nitrite, phosphate, phthalate, selenate, sulfate, sulfite, thiocyanate, and thiosulfate were tested as possible interferences.

To ascertain the effect of high levels of common anions on perchlorate recovery, solutions containing 50, 200, 600, and 1000 mg/L carbonate, chloride, or sulfate and 20-µg/L perchlorate were prepared. The effect of sulfate on perchlorate recovery was further investigated by preparing solutions containing 50, 200, 600, and 1000 mg/L sulfate and either 2- or 200-µg/L perchlorate. One-milliliter aliquots of each of these samples were analyzed. To determine the effect of very high chloride concentrations, a sample was prepared that contained 10,000 mg/L chloride and 100 µg/L perchlorate. An aliquot of this sample was treated with an OnGuard® Ag cartridge (P/N 39637) followed by an OnGuard H cartridge (P/N 39596). Prepare the OnGuard cartridges by passing 10 mL of deionized water though them at 2 mL/min. (For details on cartridge preparation, refer to the OnGuard cartridge manual, P/N 032943.) After the cartridges have been prepared, pass 5 mL of the undiluted sample through the cartridge. Discard the first 3 mL and collect the remainder for injection.

SYSTEM PREPARATION AND SETUP

For determinations of target anions at trace concentrations, it is essential to have low baseline noise. To ensure a quiet baseline, the following steps must be taken during the system setup. The ASRS ULTRA is operated in the external water mode rather than the recycle mode. A 1000 psi backpressure coil must be added to the degas module on the eluent generator. Refer to the EG40 manual (P/N 031373) for details on adding backpressure to the degas module. The final

system backpressure should be in the range of 2400–2600 psi when using the EG40 Eluent Generator. Prior to sample analysis, determine a system blank by analyzing 1 mL of deionized water using the method described above. An equilibrated system has a background conductance between 1 and 4 µS with the peak-to-peak noise should not exceed ions 5–10 nS, and no peaks eluting with the same retention time as perchlorate (9.6 ± 0.2 min).

RESULTS AND DISCUSSIONS

Figure 1 shows a chromatogram of a 20-µg/L perchlorate standard. Perchlorate elutes at 9.6 min. The method linearity range was determined to ensure accurate quantification of perchlorate. Figure 2 shows that the method is linear from 2 to 100 µg/L, a concentration range appropriate for this application. This method is also linear in a larger concentration range (2–100 ppm, $r^2 = 0.9999$). The excellent linearity over a wide concentration range is a result of the high capacity for perchlorate and its symmetrical peak shape using the IonPac AS16 column. The method detection limit was established by making seven replicate injections of a 2-µg/L perchlorate standard. Table 1 shows the results of this study. The MDL calculated using the method described in U.S. EPA Method 300.0 is 151.4 ng/L.[7] Figure 3 shows a chromatogram of a 2-µg/L perchlorate standard.

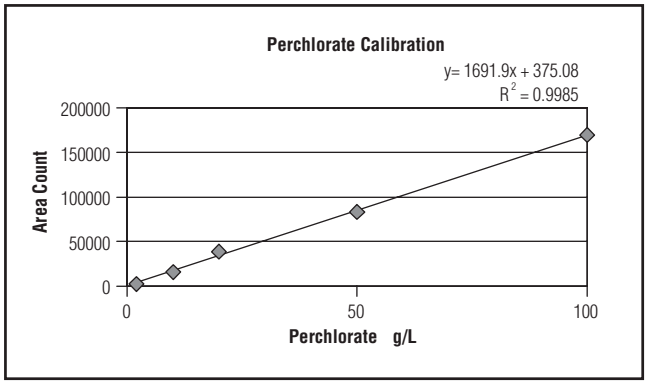

Figure 2. *Perchlorate calibration.*

Table 1. Determination of the Method Detection Limit for Perchlorate

Injection #	Peak Area	Retention Time (min)
1	2416	9.82
2	2314	9.82
3	2313	9.83
4	2323	9.85
5	2414	9.73
6	2317	9.82
7	2384	9.72
Average	2354	9.80
SD	48.22	0.05
RSD	2.05	0.53
MDL*	151.4 ng/L	

MDL = SD$t_{s,99}$ where $t_{s,99}$ = 3.14 for n = 7

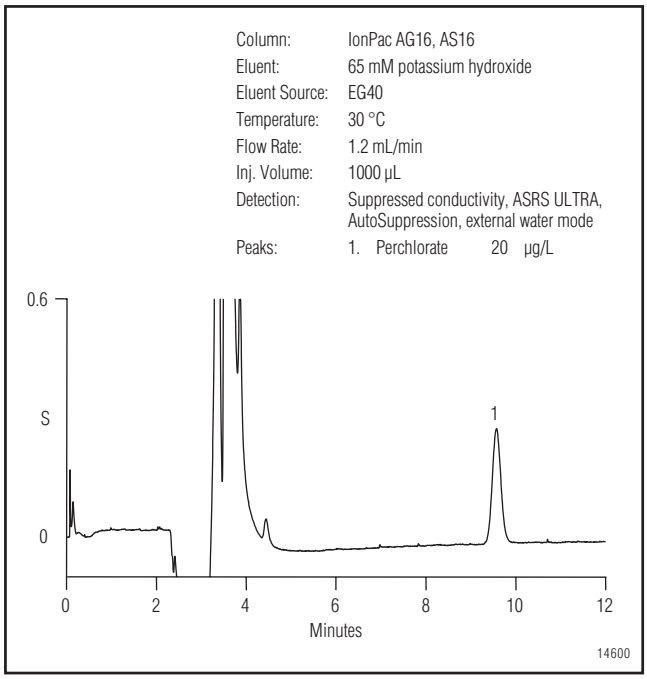

Figure 1. *Perchlorate standard at 20 µg/L.*

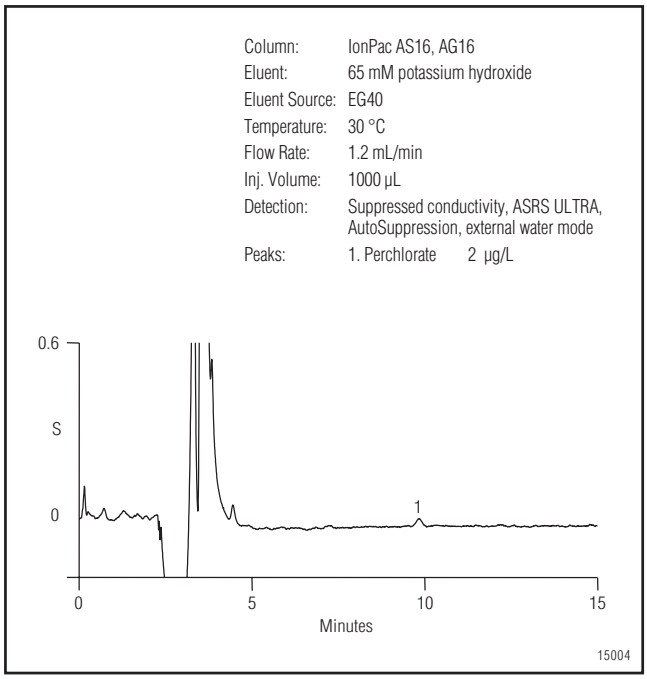

Figure 3. *Perchlorate standard at 2 µg/L.*

INTERFERENCE STUDIES

Common Anions

Twenty-two anions were injected using the conditions described in this Application Note to study whether they interfere with the determination of perchlorate. Included in these 22 anions were polarizable anions that are typically well retained on anion-exchange columns. The results of this study are shown in Table 2. All 22 anions elute well before perchlorate, most in less than 4 minutes, and therefore do not interfere with the determination of perchlorate.

Groundwater samples may contain high concentrations of common anions, particularly carbonate, chloride, or sulfate. The method outlined in this Application Note can be used to determine low concentrations of perchlorate in the presence of high concentrations of these common anions. The effect of mg/L levels of these anions on perchlorate recovery was investigated by injecting solutions of 20µg/L perchlorate in the presence of 50, 200, 600, and 1000 mg/L carbonate, chloride, or sulfate. Quantitative recoveries were obtained for perchlorate at the 20-µg/L level in all cases, as shown in Table 3.

Because sulfate is the most likely interference in groundwaters, the effect of sulfate on perchlorate recovery was further investigated. Perchlorate (200 µg/L) was determined in the presence of 50-, 200, 600, and 1000 mg/L sulfate. The recovery of perchlorate from these samples was 78, 89, 77, and 90%, respectively. The same study was also done with 2 µg/L perchlorate. For that experiment the recoveries were 115, 107, 109, and 110%, respectively. Figure 4 shows an overlay of chromatograms of 200 g/L perchlorate in the presence of 50 to 1000 mg/L sulfate, demonstrating that high concentrations of sulfate do not significantly affect the retention time or peak shape for perchlorate.

Extreme Chloride Matrices

Low concentrations of perchlorate are sometimes found in matrices containing a very high chloride concentration (e.g., brines). The sample used for this study had a chloride concentration of 10,000 mg/L and a perchlorate concentration of 100 µg/L. One approach

Table 2. Comparison of the Retention Times of 22 Anions and Perchlorate on the IonPac AS16 Column (1000 µL injected)*

Anion	Anion Retention Time (min)	Perchlorate Retention Time (min)
Arsenate	<4	9.78
Arsenite	<4	9.75
Bromate	<4	9.72
Bromide	<4	9.73
Carbonate	<4	9.72
Chlorate	<4	9.72
Chloride	<4	9.68
Chromate	<4	9.68
Cyanide	<4	9.65
Humic acid	<4	9.67
Iodate	<4	9.65
Iodide	5.28	9.65
Molybdate	<4	9.63
Nitrate	<4	9.65
Nitrite	<4	9.63
Phosphate	<4	9.63
Phthalate	<4	9.62
Selenate	<4	9.60
Sulfate	<4	9.60
Sulfite	<4	9.60
Thiocyanate	7.72	9.60
Thiosulfate	<4	9.58

* An eluent of 50 mM hydroxide at 1.5 mL/min was used for this study.

Table 3. Effect of mg/L Levels of Common Anions on Perchlorate Recovery (20 µg/L) on the IonPac AS16 Column (1000 µL injected)*

Anion	Anion Concentration (mg/L)	Perchlorate Recovery
Carbonate	50	97.6%
Carbonate	200	94.4%
Carbonate	600	95.4%
Carbonate	1000	93.5%
Chloride	50	96.1%
Chloride	200	96.7%
Chloride	600	109.6%
Chloride	1000	97.4%
Sulfate	50	94.4%
Sulfate	200	96.3%
Sulfate	600	94.7%
Sulfate	1000	95.5%

* An eluent of 50 mM hydroxide at 1.5 mL/min was used for this study.

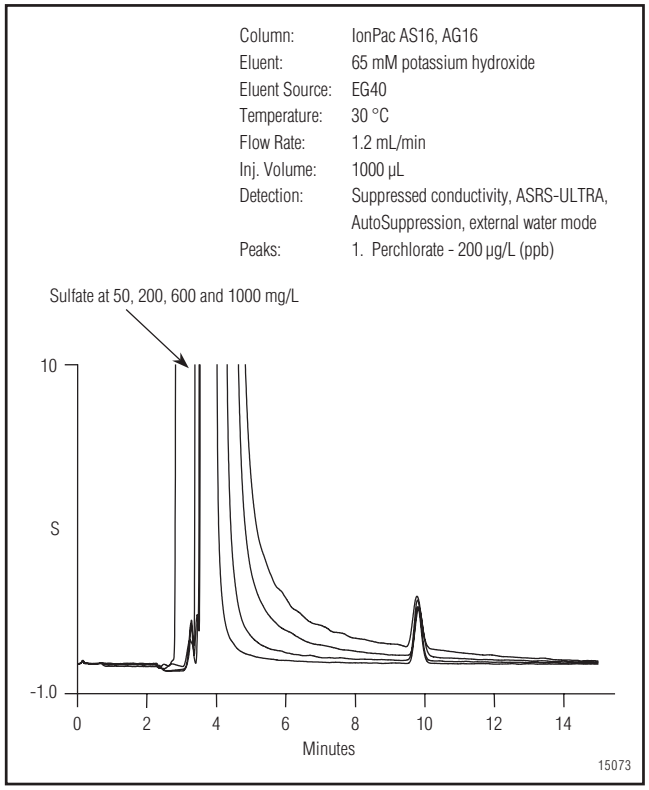

Figure 4. *Effect of sulfate on perchlorate recovery on the IonPac AS16 column.*

for determining perchlorate in an extreme chloride matrix is to reduce the sample's chloride concentration. This can be achieved by pretreating the sample with an OnGuard Ag cartridge. The cartridge packing is a high-capacity, strong acid, cation-exchange resin in the Ag^+ form that is designed to remove chloride from the sample by precipitating it as silver chloride. Figure 5B shows an analysis of the sample (10,000 mg/L chloride and 100 µg/L perchlorate) after treatment with an OnGuard Ag cartridge. This treatment allows perchlorate to be quantified with good recovery (92.6%). Analysis of the untreated sample is shown in Figure 5A.

Another approach for determining perchlorate in an extreme chloride matrix is to dilute the sample and/or reduce the eluent concentration. The same sample containing chloride at 10,000 mg/L and perchlorate at 100 µg/L was diluted 10-fold and the diluted solution was analyzed using two different eluent strengths, 35 mM and 65 mM KOH. Figure 6A shows that when using 65 mM KOH, perchlorate is difficult to quantify because it elutes on the tail of the large chloride peak. When the weaker eluent (35 mM KOH) is used, perchlorate elutes at 14 min and is easier to quantify (Figure 6B).

When choosing an approach for analyzing perchlorate in samples containing high concentrations of chloride, the perchlorate concentration must be considered. For a sample containing low levels of perchlorate (<40 µg/L), use the OnGuard Ag cartridge. In samples where the concentration of perchlorate is higher, sample dilution and a 35 mM KOH eluent is recommended.

CONCLUSION

The method described in this application note can be used to determine low-µg/L concentrations of perchlorate in drinking and ground waters. The use of IC with the AS5 or AS11 columns has previously been shown to provide an interference-free method for the analysis of perchlorate in modest-ionic-strength drinking water and groundwater samples[8]; the AS16 column provides similar results. The AS16 also is compatible with the EG40 and its higher capacity makes it most appropriate for the analysis of perchlorate in higher-ionic-strength samples.

SUPPLIERS

EM Science, P.O. Box 70, 480 South Democrat Road, Gibbstown, NJ 08027 USA, Tel: 800-222-0342, www.emscience.com.

Fisher Scientific, 2000 Park Lane, Pittsburgh, PA 15275-1126 USA, Tel: 800-766-7000, www.fishersci.com.

Fluka Chemika-BioChemika, Fluka Chemie AG, Industriestrasse 25, CH-9471, Buchs, Switzerland, Tel: +81 755 25 11, www.sigma-aldrich.com.

Sigma-Aldrich Chemical Company, P.O. Box 14508, St. Louis, MO 63178 USA, Tel: 1-800-325-3010, www.sigmaaldrich.com.

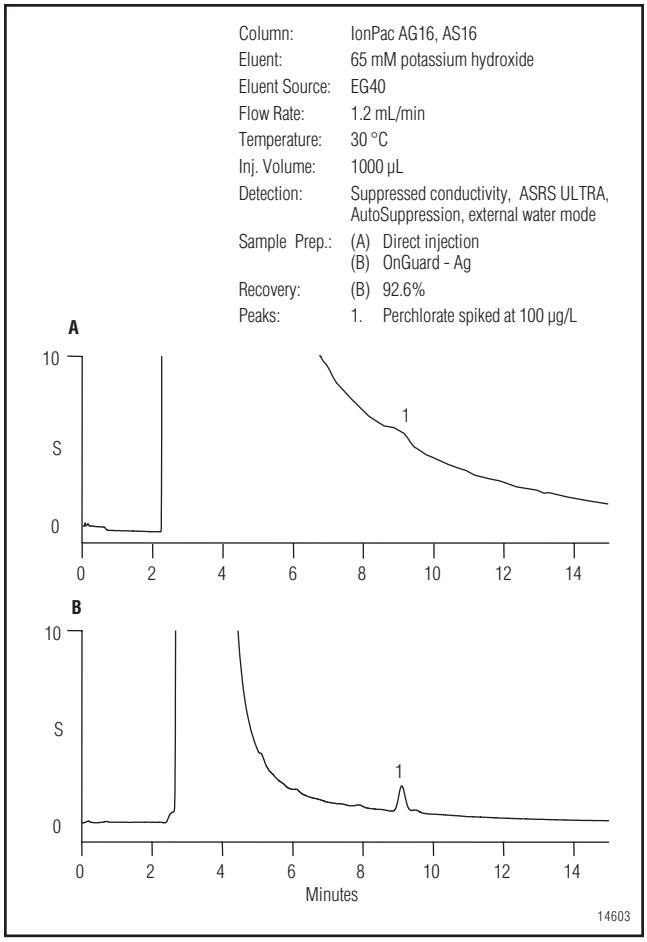

***Figure 5**. Determination of perchlorate in high chloride (10,000 mg/L) matrices.*

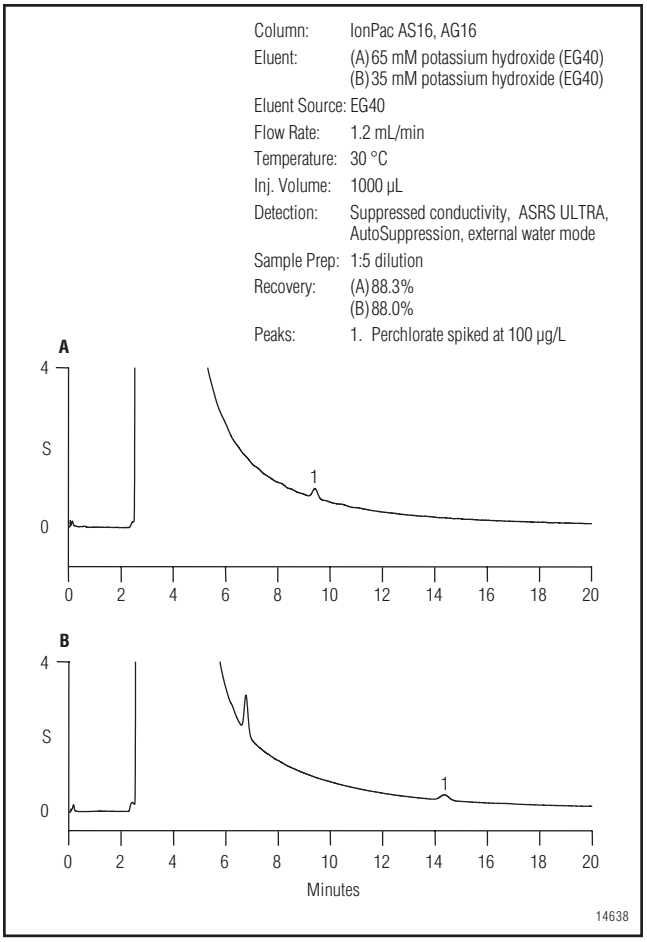

***Figure 6.** Determination of perchlorate in high chloride (10,000 mg/L) matrices.*

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Analysis of Low Concentrations of Perchlorate in Drinking Water and Ground Water by Ion Chromatography

INTRODUCTION

Perchlorate (as ammonium perchlorate), which is widely used in solid rocket propellants, has recently been found in drinking water wells in areas where aerospace materials and munitions were manufactured and tested.[1] Perchlorate is a health concern because it interferes with the production of thyroid hormones. Current data suggest that an exposure level range of 4 to 18 µg/L (ppb) is acceptable.[2] Although perchlorate is not yet regulated in the U.S. under the Federal Safe Drinking Water Act, the State of California requires remedial action for drinking water sources containing greater than 18 µg/L of perchlorate.

This Application Note details a new method developed to quantify low levels of perchlorate. A large loop injection (1000 µL) is used with an IonPac® AS11 column and suppressed conductivity detection to quantify perchlorate in drinking water down to approximately 2.5 µg/L.

EQUIPMENT

Dionex DX-500 Ion Chromatography system consisting of:
- GP40 Gradient Pump
- CD20 Conductivity Detector
- AS40 Automated Sampler
- LC20 Chromatography Enclosure with a rear-loading valve

4-L Plastic bottle assemblies (two for external water mode)

PeakNet® Chromatography Workstation

REAGENTS AND STANDARDS

Deionized water (DI H_2O), Type I reagent grade, 18 MΩ-cm resistance or better

Sodium hydroxide, 50% (w/w) aqueous solution (Fisher Scientific or other)

Sodium perchlorate, 99% ACS reagent grade or better (Aldrich or other)

Potassium sulfate, 1000 mg/L aqueous solution (Ultra Scientific or other)

CONDITIONS

Columns:	IonPac AS11 Analytical, 4 x 250 mm (P/N 044076) IonPac AG11 Guard, 4 x 50 mm (P/N 044078)
Eluent:	100 mM Sodium hydroxide
Run Time:	12 min
Flow Rate:	1.0 mL/min
Sample Volume:	1000 µL
Detection:	Suppressed conductivity, ASRS® (4 mm), AutoSuppression® external water mode
System Backpressure:	600–900 psi (3.95–5.93 MPa)
Background Conductance:	2–5 µS

PREPARATION OF SOLUTIONS AND REAGENTS

Standard Solution

Stock Perchlorate Standard Solution (1000 mg/L)

Dissolve 1.231 g of sodium perchlorate in 1000 mL of deionized water to prepare a 1000 mg/L standard. Standard is stable for at least one month when stored at 4 °C.

Working Standard Solutions

Dilute 1000 mg/L standard solution as required with deionized water to prepare the appropriate working standards.

Eluent Solution

100.0 mM Sodium Hydroxide

Weigh 992.0 g of deionized water into an eluent bottle. Degas water for approximately 5 minutes. Carefully add 8.0 g of 50% sodium hydroxide directly to the bottle. Mix then quickly transfer the eluent bottle to the instrument and pressurize the bottle with helium at 8 psi (0.055 MPa).

RESULTS AND DISCUSSION

For the best perfomance at low-ppb levels, it is critical that baseline noise be kept to a minimum. To minimize baseline noise, it is necessary to use the ASRS in external water mode rather than the recycle mode. An equilibrated system will produce a background conductance from 2–5 µS. Peak-to-peak noise is typically 10 nS and system backpressure is 600–900 psi (3.95–5.93 MPa). A system blank is determined by using deionized water as a sample. This blank establishes the baseline and confirms the lack of contamination in the system. The linear concentration range was determined to ensure accurate quantification of perchlorate in the 2.5–100 µg/L range. Figure 1 shows the results of a linearity study.

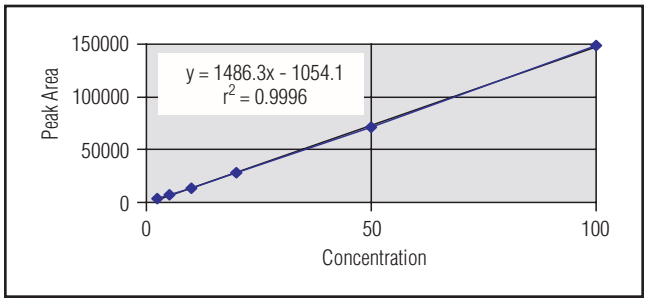

Figure 1. Perchlorate calibration.

This plot demonstrates that calibration of perchlorate is linear in the low-ppb range. Figure 2 shows a typical chromatogram of a 20 µg/L perchlorate standard. To determine the method detection limit (MDL), seven injections of the 2.5 µg/L perchlorate standard were made. Table 1 shows the results of a method detection limit study. The 1000-µL injection is large enough to achieve the desired detection limit without overloading the column. Note that this method is not intended for use with high (ppm) levels of perchlorate. The calculated MDL equals 880 ng/L (ppt).

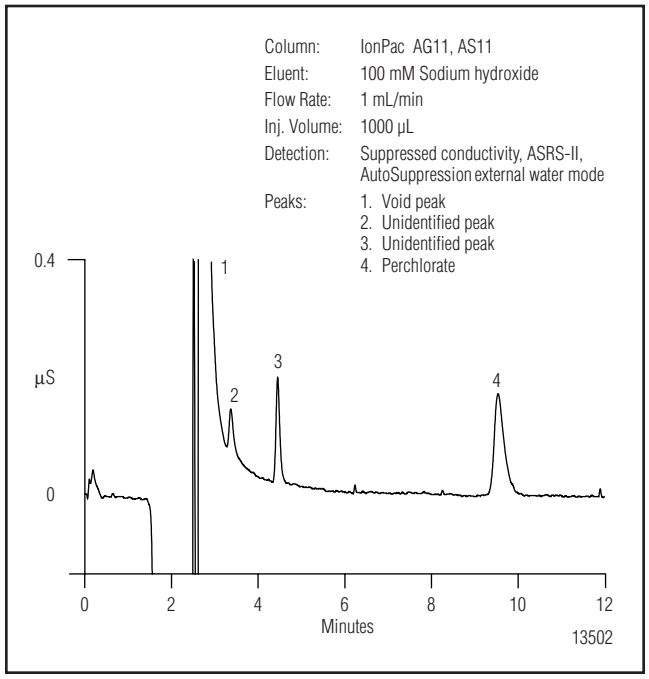

Figure 2. 20 µg/L perchlorate standard.

Table 1 MDL for Perchlorate Based on a 1000-µL Injection Volume

Injection #	Area counts	Retention time (min)
1	3391	9.48
2	3405	9.57
3	3504	9.50
4	3503	9.45
5	3435	9.47
6	3301	9.52
7	3315	9.43
Average	3408	9.49
SD	81	0.05
RSD	2.38	0.49

MDL=880 ng/L (ppt), MDL=SD*$t_{s,99}$ where $t_{s,99}$=3.14 for n=7

Figures 3 through 5 show chromatograms obtained for 2.5 µg/L perchlorate in three different matrices. Figure 3 shows the chromatogram of 2.5 µg/L perchlorate in deionized water. Figure 4 shows 2.5 µg/L perchlorate in tap water. Note that all other anions present in tap water elute in the void volume and do not interfere with perchlorate determination. Some environmental samples may contain low levels of perchlorate in the presence of a large amount of sulfate. Figure 5 shows the determination of 2.5 µg/L perchlorate in the presence of 700 mg/L sulfate. The high concentration of sulfate does not affect perchlorate recovery or the detection limit.

SUMMARY

The method outlined in this Application Note allows the determination of low-µg/L (ppb) levels of perchlorate. A linear concentration range has been established to accurately quantify perchlorate in drinking water and ground water samples.

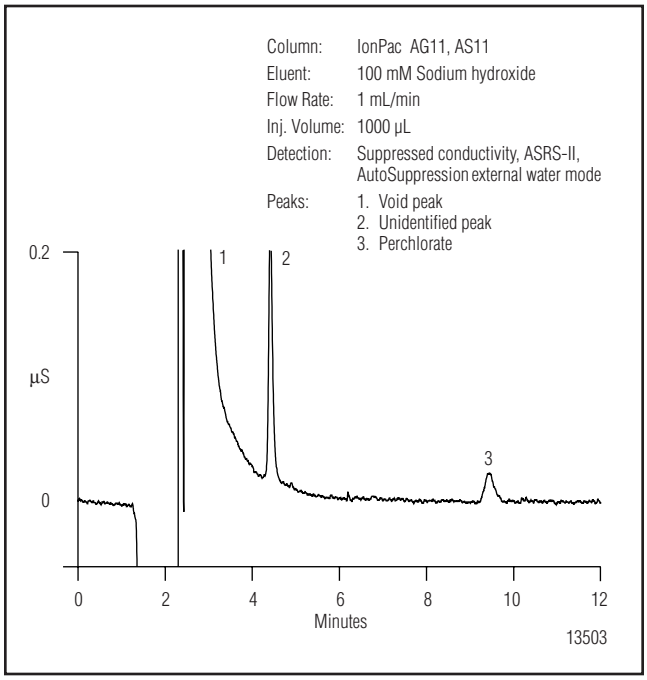

Figure 3. 2.5 µg/L perchlorate standard.

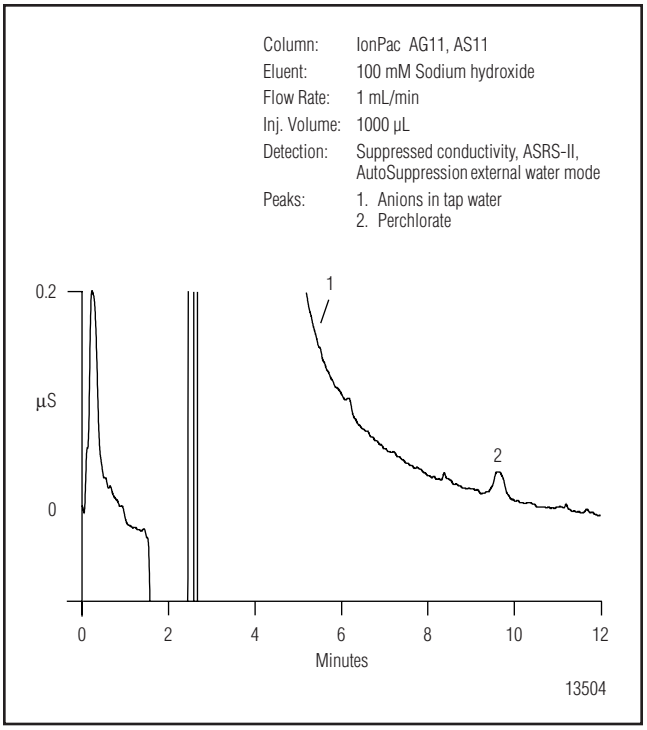

Figure 4. 2.5 µg/L perchlorate in tap water.

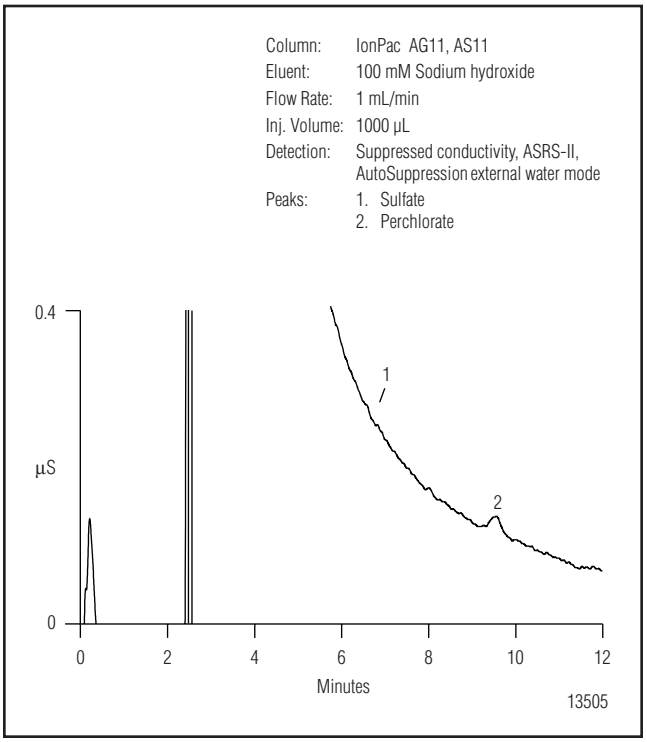

Figure 5. 2.5 µg/L perchlorate and 700 mg/L sulfate.

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2. Correspondence from Joan S. Dollarhide, National Center for Environmental Assessment, Office of Research and Development, to Mike Girrard, Chairman, Perchlorate Study Group, U.S. EPA, 1995.

SUPPLIERS

Aldrich Chemical Company, Inc., 1001 West Saint Paul Avenue, P.O. Box 355, Milwaukee, Wisconsin, 53233, USA. Tel: 1-800-558-9160.

Fisher Scientific, 711 Forbes Ave., Pittsburgh, Pennsylvania, 15219-4785, USA. Tel: 1-800-766-7000.

Ultra Scientific, 250 Smith Street, North Kingstown, Rhode Island, 02852, USA. Tel: 401-294-9400.

Determination of Perchlorate in Drinking Water Using Reagent-Free™ Ion Chromatography

INTRODUCTION

Perchlorate (ClO_4^-) is an environmental contaminant and has been found in drinking, ground, and surface waters in several states in the U.S.[1] However, most contaminated sites appear to be geographically confined, particularly in the western U.S., and linked to identifiable sources, such as military installations and manufacturing sites.[2] Because perchlorate targets the thyroid gland at sufficiently high concentrations,[3] in 1998 the EPA's Office of Groundwater and Drinking Water placed this anion on its Contaminant Candidate List (CCL) for drinking water. Currently, the EPA has not established any enforceable health regulations for perchlorate in drinking water or related matrices, although some states have set individual action levels. For example, the California Department of Health Services (CDHS) has adopted an action level of 4 µg/L perchlorate.[4] If concentrations are detected above this level, then the CDHS recommends that utilities remove the drinking water source from service for proper treatment.

Dionex Application Note 134 describes the determination of perchlorate in environmental waters to 2 µg/L using a large-loop injection with an IonPac® AS16 column and suppressed conductivity detection with an ASRS® ULTRA operating in external water mode.[5] However, the U.S. EPA is currently investigating different analytical approaches that can improve the sensitivity and selectivity for perchlorate. The sensitivity for perchlorate can be improved by coupling a mass spectrometer (MS) to an ion chromatograph or by using a 2-mm IonPac AS16 column.[6,7]

Further improvements to the existing chromatographic conditions for determining perchlorate as outlined in U.S. EPA Method 314.0 can be accomplished by decreasing the baseline noise. In this application update, we describe a minor modification to EPA Method 314.0 that replaces the standard ASRS ULTRA with an improved ASRS ULTRA II suppressor. The ASRS ULTRA II can routinely produce peak-to-peak noise in the range of 1–2 nS when operated in external water mode, compared to 9–10 nS for the ASRS ULTRA. Nearly an order of magnitude decrease in baseline noise significantly enhances the detection of perchlorate to 1 µg/L or less. As an additional benefit, the ASRS ULTRA II does not require chemical regenerates, such as the sulfuric acid required for the AMMS III suppressor as described in AU 145.[7] This application update describes the determination of perchlorate using the procedure outlined in EPA Method 314.0.[8] This application used an integrated ion chromatography system with a 4-mm IonPac AS16 column, an EGC II KOH cartridge, a 1000-µL injection, and suppressed conductivity detection with an ASRS ULTRA II operated in external water mode. This application update evaluates and describes the linear range, initial demonstration of capability (EPA Method 314.0,[9] Section 9.2), matrix conductivity threshold (MCT) (EPA Method 314.0, Section 9.2.8), method detection limits (MDLs) (EPA Method 314.0 Section 9.2.6), and recovery of perchlorate in typical environmental matrices (EPA Method 314.0, Section 9.4).

EQUIPMENT

A Dionex ICS-2000 Reagent-Free Ion Chromatography (RFIC) System was used in this work. The ICS-2000 is an integrated ion chromatograph that includes:

- Eluent Generator
- Column Heater
- Pump Degas
- EluGen® EGC II KOH Cartridge (Dionex P/N 058900)
- CR-ATC (Dionex P/N 060477)

AS50 Autosampler

Chromeleon® 6.5 Chromatography Workstation

Suppressor External Regen Installation Kit for External Water Mode (P/N 038018)

Conductivity Meter (Thermo Orion, Model 105)

This application update is also applicable to other RFIC systems.

REAGENTS AND STANDARDS

Deionized water, Type I reagent-grade, 18 MΩ-cm resistivity or better

Sodium Perchlorate ($NaClO_4$) (Aldrich 41,024-1)

Sodium Chloride (NaCl)
(J. T. Baker; VWR P/N JT3625-1)

Sodium Sulfate (Na_2SO_4) (Aldrich 29,931-3)

Sodium Carbonate Monohydrate ($Na_2CO_3 \cdot H_2O$)
(Fisher S262-3)

CONDITIONS

Columns:	IonPac AS16 Analytical, 4 x 250 mm (P/N 055376) IonPac AG16 Guard, 4 x 50 mm (P/N 055377)
Eluent:	65 mM potassium hydroxide
Eluent Source:	ICS-2000 EG with CR-ATC
Flow Rate:	1.2 mL/min
Temperature:	30 °C
Injection:	1000 µL (with 10-µL cut volume from a 1100-µL sample loop)
Detection:	Suppressed conductivity, ASRS ULTRA II (4 mm), Autosuppression external water mode Power setting, 193 mA
System Backpressure:	~2500 psi
Background Conductance:	~1–2 µS
Noise:	~1–2 nS/min peak-to-peak
Run Time:	15 min

PREPARATION OF SOLUTIONS AND REAGENTS

Stock Perchlorate Standard Solution

Dissolve 0.1231 g of sodium perchlorate in 100 mL of deionized water for a 1000-mg/L standard solution. This stock standard is stable for at least one month when stored at 4 °C.

Working Standard Solutions

Prepare working standards at lower concentrations by diluting the appropriate volumes of the 1000-mg/L stock standard with deionized water. In this application, calibration standards were prepared at 1, 2, 10, 25, 50, and 100 µg/L perchlorate, with each standard injected in duplicate.

Mixed Common Anion Stock Solutions (EPA Method 314.0, Section 7.4.1)

Prepare 25 mg/mL (25,000 mg/L) each of chloride, sulfate, and carbonate. Dissolve 4.1213 g of sodium chloride in deionized water and dilute to 100 mL. Dissolve 3.6965 g of sodium sulfate in deionized water and dilute to 100 mL. Dissolve 5.1658 g of sodium carbonate monohydrate (4.416 g of sodium carbonate) in deionized water and dilute to 100 mL. These solutions were used to prepare 50, 100, 200, 400, 600, 800, and 1000 mg/L (ppm) of mixed anion (MA) standards of chloride, sulfate, and carbonate. These standards were used to determine the matrix conductivity threshold (MCT) (EPA Method 314.0, Section 9.2.8) and the MDLs (Section 9.2.6[8]).

Sample Preparation

Measure the conductance of the samples with a calibrated conductivity meter that has a minimum measuring range of 1–10,000 µS/cm. Verify the conductivity meter calibration by measuring the conductance of a commercially available reference solution or a prepared 745-mg/L KCl standard (EPA Method 314.0 Section 7.5) with a conductance of 1410 µS/cm at 25 °C. The conductivity meter must yield a value between 1380 and 1440 µS/cm to be considered calibrated. Compare the sample conductivity to the MCT

determined in your laboratory, as explained in EPA Method 314.0, Section 11. Filter all samples with a 0.2- m syringe filter. Use a hydrophilic polypropylene or polyethersulfonate filter; do not use polyvinylidene fluoride (PVDF). Discard the first 300 µL of the filtrate and filter the remainder directly into a clean plastic autosampler vial. Qualify filters by analyzing a deionized water blank and a 10-µg/L perchlorate standard that has been passed through the filter. The blank should be free of peaks within the retention time window of perchlorate, and the recovery of the 10-µg/L standard should fall within 80–120%.

Samples that exceed the MCT can often be analyzed after an appropriate dilution followed by filtration with a 0.2-µm filter. EPA Method 314.0 Section 11.1.3 explains how to determine the sample's dilution factor based on the MCT. For diluted samples, the minimum reporting level (MRL) **must** be raised by a proportion equivalent to the dilution.

If sample dilution does not yield the desired results—or to avoid diluting samples—the concentration of the matrix ions can be reduced by treating the sample with Dionex OnGuard® cartridges. This procedure is explained in further detail in EPA Method 314.0, Section 11.1.4 and in Dionex Application Update 145.[7] In this application, no pretreatment or dilution was required for the samples analyzed.

SYSTEM PREPARATION AND SETUP

Install backpressure tubing in place of the column set to produce a total system pressure between 2000 and 2500 psi at a flow rate of 1 mL/min. Install an EGC II KOH cartridge (Dionex P/N 058900). Condition the cartridge as directed in the *EGC II Cartridge Quickstart Guide* (Document No. 031909) by setting the KOH concentration to 50 mM at 1 mL/min for 30 min. After completing the cartridge conditioning process, disconnect the backpressure tubing that was temporarily installed in place of the column set. Install a CR-ATC between the EGC II KOH cartridge and EGC degas. For more information on installing the CR-ATC, consult the *EGC II Cartridge Quickstart Guide.*

Install and configure the AS50 Autosampler. The precision and accuracy of the autosampler will vary depending on the injection mode. The most accurate and precise injections are made with a calibrated sample loop, flushed with about five times the loop volume. The largest full-loop injection possible with the AS50 is 300 µL. To inject 1000 µL, use the partial-loop injection mode with an 1100 µL sample loop, and a programmed "Sample Loop Volume" of 1100 µL and a "Cut Volume" of 10 µL. This injection procedure should provide peak area precision of <1% RSD. Install a 1-mL sample syringe and set the syringe speed to 4 or 5 to make faster large-loop injections. Enter the correct "Sample Loop Size" and "Sample Syringe Volume" in the AS50 Plumbing Configuration Screen. Refer to the *Autoselect AS50 Autosampler Operator's Manual* (Document No. 31169) for details.

Install a 4 x 50 mm IonPac AG16 and a 4 x 250 mm IonPac AS16 column in the column oven. Make sure the system pressure is 2300 – 200 psi when 65 mM KOH is delivered at 1.2 mL/min 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 adjust the system pressure to 2100–2500 psi. Do not allow the pressure to reach 3000 psi. Therefore, monitor the pressure periodically because pressure can gradually rise over time. To reduce pressure, trim the backpressure tubing.

Unlike the ASRS ULTRA suppressor, the ASRS ULTRA II does not require any *Quick Start* using acid regenerants, and the suppressor can be installed after hydration with deionized water. Configure the suppressor for external water mode according to the directions provided in the *ASRS ULTRA II Operator's Manual* (Document No. 031956).

The storage solution of the AS16 column is 35 mM NaOH; equilibrate the column with 65 mM KOH eluent at 1.2 mL/min for approximately 60 min, then analyze a system blank of deionized water. An equilibrated system has a background signal of less than 2 S and peak-to-peak noise of less than 2 nS. No peaks should elute within the same retention time window as perchlorate. Inject a 25-µg/L perchlorate standard. The column is equilibrated when two consecutive injections of the standard produce the same retention time for perchlorate.

RESULTS AND DISCUSSION

U.S. EPA Method 314.0 specifies the use of an IonPac AS16 column with an eluent of 50 mM NaOH at a flow rate 1.5 mL/min, followed by suppressed conductivity detection with an ASRS ULTRA operated in the external water mode and a 1000-µL large-loop injection. However, Section 6.1.3 of the method states that "An equivalent suppressor device may be utilized provided that comparable conductivity detection limits are achieved and adequate baseline stability is attained as measured by a combined baseline drift/noise of no more than 5 nS per minute over the background." Section 9.4.3 further states that, "In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options, such as the use of different columns (which meet the criteria in Section 6.1.2.2), injection volumes, and/or eluents, to improve the separations or lower the cost of measurements." Therefore, a different eluent concentration, flow rate, and suppressor may be used for U.S. EPA Method 314.0, provided that the quality control parameters are met. We replaced the ASRS ULTRA specified in the method with an improved ASRS ULTRA II suppressor. The ASRS ULTRA II provides significantly lower noise of 2 nS/min or less, and therefore improves the detection limits for perchlorate. In addition, the KOH concentration was increased from 50 mM to 65 mM, and the flow rate was proportionally adjusted to 1.2 mL/min, as specified in Dionex Application Note 134.

Calibrate the system by injecting one blank and at least five standards to cover two orders of magnitude concentration range. Section 10.2.2 of the method states that the linear calibration range "should not extend over more than two orders of magnitude in concentration." Tabulate the peak area response against the perchlorate concentration injected using a linear regression fit. Table 1 summarizes the calibration data from duplicate injections of 1, 2, 10, 25, 50, and 100 µg/L perchlorate standards. The calibration curve is linear over two orders of magnitude with a correlation coefficient of 0.9998.

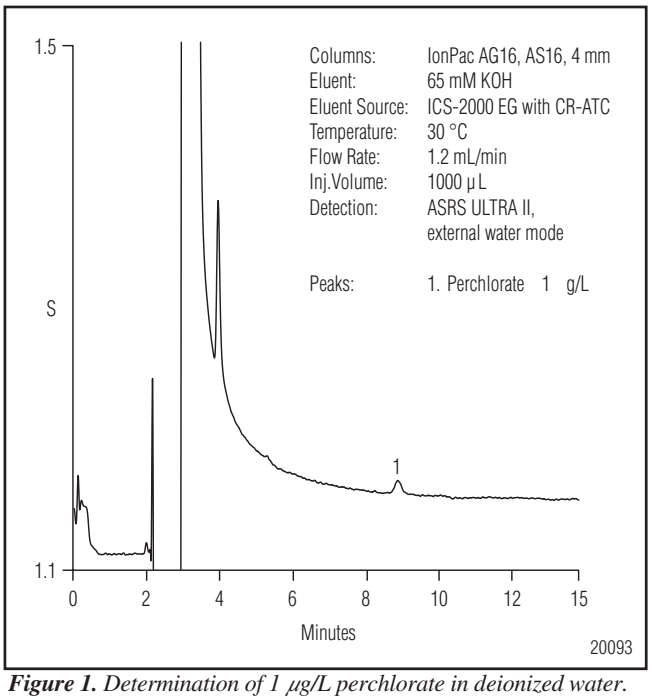

Figure 1. *Determination of 1 µg/L perchlorate in deionized water.*

Figure 1 shows a chromatogram of a 1-µg/L perchlorate standard using the conditions described in this application update. After establishing the calibration curve, a quality control standard (QCS) of 50 µg/L perchlorate was analyzed resulting in a recovery of 103.8%. This recovery meets the criteria outlined in Section 9.2.5 of the method that states the recovery of the QCS **must** be within ±10% of the stated value.

U.S. EPA Method 314.0 requires an initial demonstration of capability (IDC), as described in Section 9.2. The IDC is used to characterize the instrument and laboratory performance prior to performing any sample analyses by the method. This performance is determined by demonstrating an initial demonstration of accuracy (IDA) and an initial demonstration of precision (IDP) by performing seven replicate injections of a laboratory fortified blank (LFB) fortified with 25 µg/L perchlorate. To meet the requirements of the IDA and IDP, the recovery **must** be within ±10% and the percent RSD **must** be less than 10%, respectively. As shown in Table

Table 1:
Calibration Data for Perchlorate

Analyte	Range (µg/L)[a]	Linearity
Perchlorate	1–100	0.9998

[a] Calibration standards were 1, 2, 5, 10, 25, 50, and 100 g/L, each injected in duplicate

Table 2:
Initial Demonstration of Capability for Perchlorate

	EPA Method 314.0 Performance Requirements			Experimental Values	
Requirement	**Reference**	**Recovery (%)**	**RSD (%)**	**Recovery (%)**	**RSD (%)**
IDA	9.2.3	90–110		103.1	
IDP	9.2.4		<10		0.5

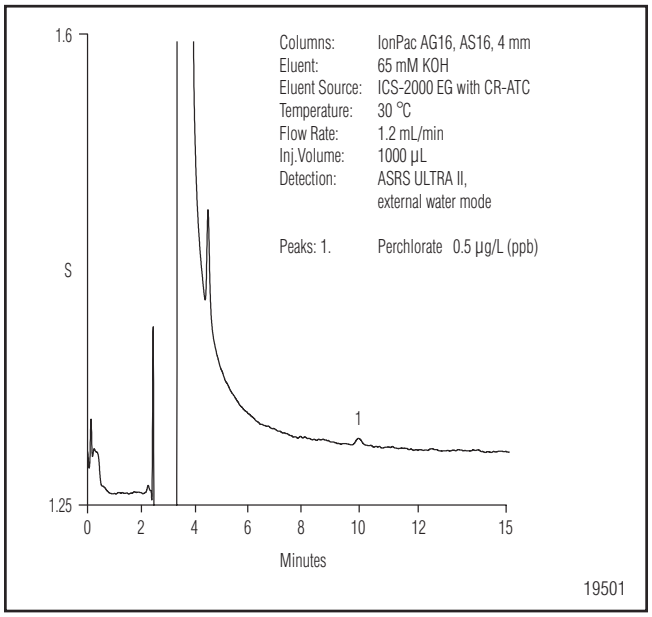

Figure 2. *Determination of 0.5 µg/L perchlorate in deionized water.*

Table 3: Perchlorate MDLs

Matrix	MDL Standard (µg/L)	Retention Time RSD (%)	Calculated MDL[a] (µg/L)
Deionized Water	0.5	0.10	0.10
MA(50)[b]	0.5	0.20	0.10
MA(100)	0.5	0.05	0.13
MA(200)	1.0	0.27	0.24
MA(400)	2.0	0.07	0.18
MA(600)	5.0	0.07	0.24

[a]The MDLs were calculated as MDL = *(t) .x (SD)* where *t* = Student's *t* value for a 99% confidence level and a standard deviation estimate with n – 1 degrees of freedom (*t* = 3.14 for seven replicate injections for the MDL standard) and SD = standard deviation of the replicate analyses.
[b]MA indicates a mixed common anion solution of chloride, sulfate, and carbonate included in the sample matrix at the parenthetical mg/L concentration for each anion.

2, our results for the IDA and IDP met the requirements described in Sections 9.2.3 and 9.2.4.

We determined the method detection limit (MDL), as described in Section 9.2.6, by performing seven replicate injections of deionized water fortified with perchlorate at a concentration of three to five times the estimated instrument detection limit. The concentration values determined from the calibration curve were used to calculate the MDL. Figure 2 shows a chromatogram of a 0.5 µg/L perchlorate MDL standard fortified in deionized water. This MDL value is only valid for perchlorate in a "clean" matrix, such as deionized water. The MDL is expected to change as the ionic strength of the sample increases. Therefore, in addition to deionized water, we determined the MDL in MA(50), MA(100), MA(200), MA(400), and MA(600), where MA indicates a mixed common anion solution of chloride, sulfate, and carbonate included in the sample matrix at the parenthetical mg/L concentration for each anion. Table 3 summarizes the results of this study. Because the MDL is based on precision and not accuracy, the determined MDL value for high-ionic-strength matrices, such as MA(400) and MA(600), do not meet the requirement of a fortified perchlorate concentration of three to five times the estimated instrument detection limits. Meeting this condition would typically require the analyst to repeat the MDL at a lower fortified perchlorate concentration. However, determining the MDL using lower perchlorate concentrations is not feasible because high concentrations of common anions interfere with the determination of perchlorate.

Section 9.2.8 describes the matrix conductivity threshold (MCT) as "an individual laboratory defined value" determined by preparing a series of sequentially increasing concentrations of chloride, sulfate, and carbonate fortified with a constant perchlorate concentration. Deionized water fortified with a recommended perchlorate concentration of 25 µg/L must be initially analyzed and followed by a series of increasing anionic solutions of chloride, sulfate, and carbonate, each containing 25 µg/L perchlorate. The recommended 25 µg/L perchlorate assumes that the MRL has been set between 3 µg/L and 5 µg/L. However, if an MRL of 1 µg/L is required, then the MCT should be determined at a perchlorate concentration of 5 µg/L. We determined the MCT using 5 µg/L and 25 µg/L perchlorate. To determine the MCT with 25 µg/L perchlorate, a standard was prepared in deionized water and injected in triplicate. Next, standards containing MA(50), MA(100), MA(200), MA(400), MA(600), MA(800), and MA(1000) were prepared by adding 0.2, 0.4, 0.8, 1.6, 2.4, 3.2, and 4 mL of each common anion from the stock solution (see the section "Preparation of Solutions and Reagents") to separate 120-mL polypropylene bottles. Then, 2.5 mL of perchlorate was added from a 1-mg/L secondary stock dilution standard to each MA solution and dilute each standard to a final volume of 100 mL. A calibrated conductivity meter measured and recorded the conductance for each of these prepared solutions. Section 9.2.8.5 states that the MA(400) solution "should display a conductance of between 3200 µS/cm and 3700 µS/cm."

Table 4 shows the results from this study. Based on multiple determinations, our laboratory determined the MCT with 25 µg/L perchlorate was a value varying from ~4500 µS/cm to 5330 µS/cm. However, individual results may vary within or between laboratories and analysts. The same procedure also determined the MCT using 5 µg/L perchlorate. In this study, the mixed anion solution did not exceed MA(600) because of a significant increase in the percent difference (PD) in the area to height (A/H) ratio. Table 5 shows the results for the MCT study using 5 µg/L perchlorate. Figure 3A and 3B show chromatograms of 5 µg/L and 25 µg/L perchlorate fortified in MA(200), respectively.

Table 4:
MCT Study—Perchlorate Fortified at 25 g/L

Sample	Conductivty (µS/cm)	Measured ClO_4^- (µg/L)	Percent Recovery	Peak Area	Peak Height	A/H Ratio	PD(A/H) (%)
LFB	<1	25.84	102.6	0.0511	0.223	0.229	0.00
MA(50)	568	25.21	99.9	0.0493	0.211	0.234	2.01
MA(100)	1089	25.25	100.4	0.0494	0.207	0.239	4.25
MA(200)	1979	24.93	99.6	0.0487	0.196	0.249	8.71
MA(400)	3590	24.85	100.2	0.0486	0.182	0.268	16.8
MA(600)	4890	24.30	96.8	0.0475	0.170	0.279	22.0
MA(800)	6070	23.96	95.9	0.0456	0.158	0.288	25.7
MA(1000)	7380	22.76	91.5	0.0454	0.148	0.306	33.6

Table 5:
MCT Study—Perchlorate Fortified at 5 g/L

Sample	Conductivty (µS/cm)	Measured ClO_4^- (µg/L)	Percent Recovery	Peak Area	Peak Height	A/H Ratio	PD(A/H) (%)
LFB	<1	5.09	101.3	0.0102	0.046	0.222	0.00
MA(50)	588	5.03	100.8	0.0101	0.045	0.224	1.22
MA(100)	1116	5.02	99.1	0.0101	0.045	0.226	1.90
MA(200)	1977	4.76	96.1	0.0094	0.040	0.233	4.93
MA(400)	3660	4.57	90.8	0.0090	0.035	0.259	16.6
MA(600)	4900	3.97	78.1	0.0075	0.026	0.284	28.1

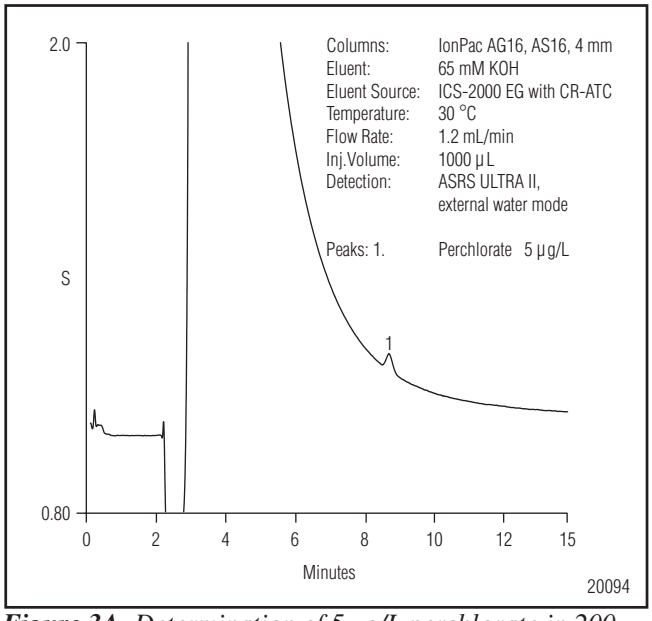

Figure 3A. *Determination of 5 µg/L perchlorate in 200 mg/L each of chloride, sulfate, and carbonate.*

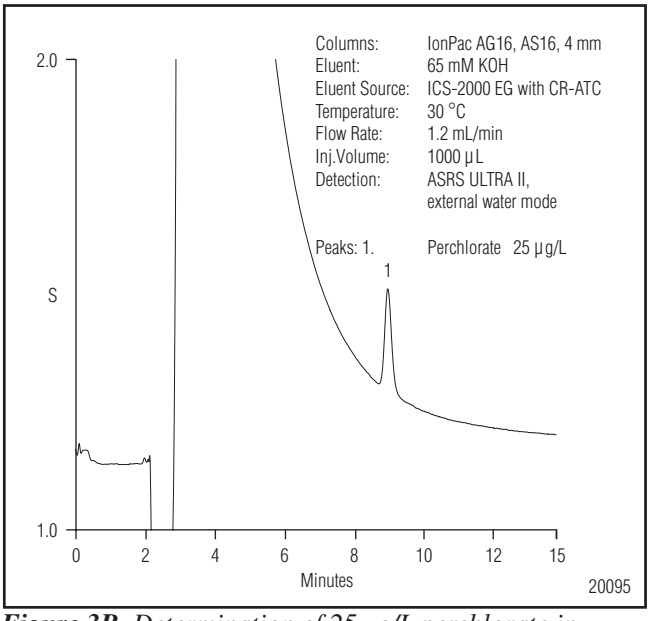

Figure 3B. *Determination of 25 µg/L perchlorate in 200 mg/L each of chloride, sulfate, and carbonate.*

Chromatographic performance of perchlorate can deteriorate at high ionic concentrations, primarily due to the presence of high concentrations of chloride, sulfate, and carbonate. Before samples are analyzed, the conductance **must** be determined. If the conductance is greater than the determined MCT, the samples should either be appropriately diluted or pretreated to reduce the common anion concentrations. One way to assess matrix effects is to prepare a laboratory fortified matrix (LFM). An LFM is accomplished by spiking the sample with a known amount of analyte and then determining the percent recovery from the amount added. This application analyzed four matrices: deionized water, drinking water, raw (untreated) drinking water, and surface water. Each matrix was spiked with 1 or 2 µg/L perchlorate and the recoveries were calculated with the equation provided in Method 314.0, Section 9.4.1.3. Table 6 shows the results of this study. The calculated perchlorate recoveries were ~97–108%, which was well within the 80–120% (Section 9.4.1.4) range specified by the method. Figure 4 shows a chromatogram of surface water spiked with 1 µg/L perchlorate.

Table 6:
Perchlorate Recoveries from Laboratory Fortified Matrices (LFM)

Matrix	Conductivty (µS/cm)	Amount Added (µg/L)	Number of Replicates	Precision (% RSD)	Recovery (%)
Deionized water	<1	1.0	7	2.76	99.3
Drinking water	130	1.0	8	10.0	106.8
Raw (untreated) Drinking water	467	2.0	8	5.26	97.6
Surface water	670	1.0	8	12.6	108.2

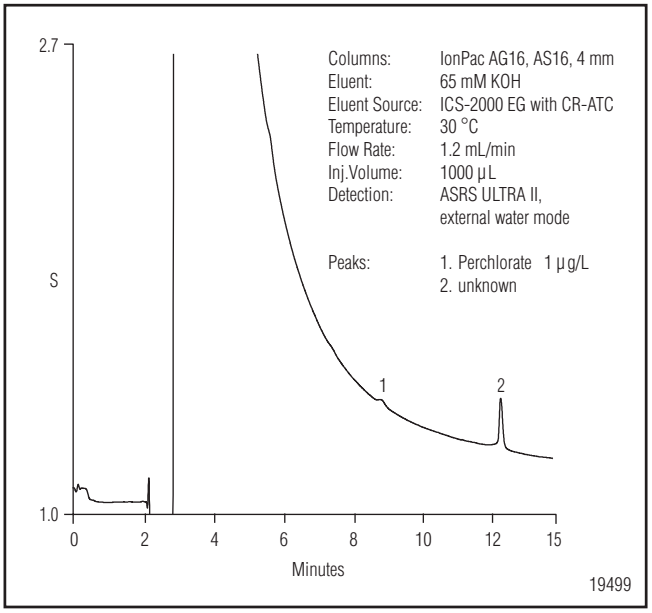

Figure 4. *Trace-level perchlorate spiked into surface water.*

CONCLUSION

This application update demonstrates an approved approach compared to Dionex Application Note 134 for the determination of perchlorate in environmental samples using U.S. EPA Method 314.0. The lower baseline noise from an ASRS ULTRA II compared to the ULTRA I suppressor improved the limit of detection and quantification of perchlorate resulting in a calculated MDL of 0.1 µg/L in deionized water. The MDLs in high-ionic-strength matrices containing up to 600 ppm each of chloride, sulfate, and carbonate ranged from 0.1 µg/L to 0.24 µg/L perchlorate. Calibration is linear over the range of 1–100 µg/L in deionized water, and acceptable recoveries were obtained for perchlorate spiked at 1–2 µg/L in typical environmental samples. The MCT determined in our lab using 5 µg/L perchlorate was ~3900 µS/cm and the MCT ranged from ~4500 to 5300 µS/cm using 25 µg/L perchlorate. However, results from individual laboratories or analysts may vary. The results presented in this application update meet or exceed the performance requirements specified in U.S. EPA Method 314.0.

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Analysis of Cyanide

Environmental Water Applications Notebook

Determination of Total Cyanide in Municipal Wastewater and Drinking Water Using Ion-Exclusion Chromatography with Pulsed Amperometric Detection (ICE-PAD)

INTRODUCTION

Cyanide is a well known acute toxin that prevents cellular respiration by irreversibly binding with the iron in cytochrome C oxidase.[1,2] In addition, thiocyanate, which is metabolized from cyanide, interferes with iodine uptake by the thyroid gland, causing goiters and other long-term iodine deficiency diseases.[1] Cyanide is regulated as an environmental contaminant by the US EPA for drinking water, surface water, and wastewater due to these health concerns.[3–5]

Total cyanide is defined by the US EPA as free cyanide ion and complex cyanides that are converted to hydrocyanic acid (HCN) during strong acid digestion.[6] More recently, total cyanide also includes ferrocyanide and ferricyanide due to free cyanide formed by exposure to light.[7] For drinking and surface waters, the US EPA has established a maximum contamination level (MCL) of 200 µg/L free cyanide determined by a total cyanide assay.[3] To determine total cyanide, the sample is digested with sulfuric acid to convert the cyanide to hydrogen cyanide gas, aspirated into a strong caustic solution, then assayed.

In wastewater, the EPA specifies cyanide discharge limits by industry and size of the facility (<38,000 or >38,000 liters per day).[8] The typical sources of cyanide contamination are industrial waste from plating and mining industries, burning coal and plastics, and effluent from publicly owned treatment works (POTW).[1,2] The EPA specifies 5.2 µg/L total cyanide continuous discharge limits for POTW and 22 µg/L maximum discharges into fresh water.[4,5] For salt water bodies, the continuous and maximum discharges are 1 µg/L total cyanide. The EPA defines these continuous (4 d) and maximum (1 h average) limits to ensure that aquatic life is unharmed.

Ninety percent of the cyanide in POTW influent and flow-through are attributed to the metal finishing and organic chemical industries.[2] However, many POTWs report that cyanide concentrations in wastewater effluents are higher than those from the influent levels.[9] Cyanide concentrations as high as 60 µg/L have been reported at discharge sites.[10] This cyanide generation is associated with chlorination and chloramination processes used for waste disinfection.[9,10] Nitrate formed from chlorination of ammonium creates unstable intermediates that degrade to cyanide during the harsh acid and temperature conditions typically used for acid-distillation in total cyanide determinations.

In EPA methods 335.2, 335.3, and 335.4, samples are individually acid- or UV-digested to convert all cyanide compounds to hydrogen cyanide gas which is distilled into sodium hydroxide (pH 13). Total cyanide is then determined spectrophotometrically or by titration.[6,11,12] These methods are complicated, often requiring multiple distillation apparatuses, and they are subject to interference from high-pH solutions, oxidizers, and sulfur-containing compounds.[13] Chromatography methods, such as ion-exchange (IE) and ion-exclusion (ICE) can eliminate some of these interferences by separation. With IE chromatography, cyanide is not fully

resolved from chloride and sulfide concentrations at mg/L levels. ICE is preferred because strong acid anions such as chloride and sulfate are excluded from the column, and cyanide is resolved from sulfide. Electrochemical detection by direct current (DC) amperometric, or pulsed amperometric detection (PAD), is sensitive, selective, and suitable for direct determinations of cyanide.[14,15] PAD is preferred over DC amperometry because in PAD the working electrode is cycled through three or four voltage potentials every second, resulting in an electrode surface which is continually cleaned, whereas in DC amperometry, the working electrode can foul over time, leading to a loss in peak response.[13] In the previous PAD methods used to detect cyanide, the silver working electrode also detected chloride and was incompatible with samples containing mg/L concentrations of sulfide.[15] Using PAD with a Pt working electrode, chloride is not detected, and the Pt working electrode is stable with mg/L sulfide concentrations. None of the previous ICE-PAD methods were used to determine cyanide.[15–17] With this method, the authors combine the advantages of ICE with the sensitivity, selectivity, and stability of PAD using a Pt working electrode to directly detect cyanide without interferences from chloride and sulfide.

In this Application Note, the authors describe a method with PAD using a Pt disposable working electrode and a waveform optimized for determination of total cyanide in drinking and wastewater. Prior to analyses, the samples are acid distilled, trapped in 1 M NaOH, and diluted to 250 mM NaOH using the EPA-approved MICRO DIST™ sample preparation system. This ICE-PAD method has the advantages of eluting cyanide before sulfide ($R_s > 3$) and excluding chloride and sulfate, which typically interfere in ion exchange methods. This ICE-PAD method provides a fast, reliable, sensitive, and selective method to directly determine µg/L to sub- µg/L concentrations of total cyanide in wastewater. The authors also demonstrate linearity, detection limits, accuracy, and precision for determination of total cyanide in drinking water and wastewater samples using the MICRO DIST system and ICE-PAD.

EXPERIMENTAL

Equipment

Dionex ICS-3000 Ion Chromatography system consisting of:

Single Gradient Pump (SP) module with degas option

Detector and Chromatography Module (DC) with single or dual heating zone, and 6-port injection valve

Electrochemical Detector ED (P/N 061718)

AS Autosampler with Sample Tray Temperature Controlling option, and 10 mL sample tray

An electrochemical cell containing a combination pH–Ag/AgCl reference electrode (cell and reference electrode, P/N AAA-061756) and a disposable (Pt) working electrode (P/N 064440 package of six)

Chromeleon® 6.8 Chromatography Workstation

Vial Kit, 10 mL polystyrene with caps and septa (P/N 055058)

Knitted reaction coil, 375 µL, (P/N 043700) with two PEEK™ unions (¼-28 thread female to 10-32 thread female, P/N 042806)

MICRO DIST System for sample distillation (Lachat Instruments/Hach Company, P/N MDD001) with user filled tube kit (Hach Company, P/N A17117 package of 100), heating block, protective gloves, test tube racks, and a small mechanical press.

Filter unit for vacuum filtration, 0.2 µm nylon (Nalgene® Media-Plus with 90 mm filter, Nalge Nunc International, P/N 164-0020) or equivalent nylon filter.

Vacuum pump

Syringe filter (Pall Life Sciences, GHP Acrodisc® 25 mm with 0.45 µm GHP membrane, P/N 4560T) or filter unit for sample filtration, 0.45 µm nylon (Nalgene Media-Plus with 50 mm filter, Nalge Nunc International, P/N 153-0045) or equivalent nylon filter

PEEK Tubing:

Red (0.127 mm or 0.005 in i.d., P/N 052310 for 5 ft) tubing used for liquid line connections from injection valve to the guard and analytical columns, and cell.

Yellow (0.76 mm or 0.003 in i.d., P/N 052301 for 5 ft) tubing used for system backpressure loop.

50 µL PEEK sample loop (P/N 042950)

REAGENTS AND STANDARDS

Reagents

Deionized water, Type 1 reagent grade, 18.2 MΩ-cm resistivity, freshly degassed by ultrasonic agitation and applied vacuum.

Use only ACS reagent grade chemicals for all reagents and standards.

Magnesium chloride, hexahydrate (VWR, P/N JT2444-1)

Methanesulfonic acid (Aldrich, P/N 64280; Dionex, P/N 033478)

pH 7 (yellow) buffer solution (VWR International, P/N BDH5046)

pH 4 (red) buffer solution (VWR International, BDH5018)

Sodium cyanide, anhydrous (Aldrich, P/N 20,522-2)

Sodium hydroxide, 50% (w/w) (Fisher Chemicals, P/N SS254-500)

Sulfuric acid (VWR, P/N JT9681-33)

For Interference Experiments

Ammonium chloride (Aldrich, P/N 213330, FW 53.49)

Sodium cyanate (Aldrich, P/N 185086, FW 65.01)

Sodium sulfide, nonahydrate, > 99.99% (Aldrich, P/N 431648, FW 240.18)

Sodium thiocyanate, (Aldrich, P/N 251410, FW 81.07)

Sodium nitrate (Aldrich, P/N SS506, FW 84.99)

Sodium sulfate (Aldrich, P/N 239313, FW 142.04)

Samples

Certified Wastewater Standard for cyanide, 40 µg/L total cyanide (20 µg/L free cyanide from potassium cyanide and 20 µg/L complexed cyanide from potassium ferricyanide in 0.5% potassium hydroxide) (High-Purity Standards, P/N CWW-CN-D).

Municipal wastewater effluent samples were collected at the same time and location. Sodium hydroxide was added to one of the samples immediately after collection.

A municipal drinking water sample stabilized with 2 g of 50% sodium hydroxide per 250 mL of sample.

CONDITIONS

Column:	IonPac® ICE-AG1 Guard, 4 × 50 mm (P/N 067842) IonPac ICE-AS1 Analytical, 4 × 250 mm (P/N 064198)
Flow Rate:	0.2 mL/min
Eluent:	50 mM Methanesulfonic acid
Column Temperature:	30 °C
Tray Temperature:	10 °C
Inj. Volume:	50 µL
Detection:	Pulsed Amperometric Detection (PAD)
Waveform:	See Table 1.
Reference Electrode:	pH-Ag/AgCl electrode (P/N 061879) in AgCl mode
Working Electrode:	Disposable Platinum
Typical Background:	70–120 nC
Typical System Backpressure:	2200 psi
Noise:	20–30 pC
Typical pH:	1.2–1.3
Run Time:	30 min

Table 1: Cyanide Detection Waveform Optimized for Acid Eluents[17]

Time (sec)	Potential vs. Ag/AgCl (V)	Gain Region[a]	Integration	Ramp[a]
0.00	+ 0.30	Off	Off	Ramp
0.31	+ 0.30	On	Off	Ramp
0.32	+ 1.15	On	Off	Ramp
0.64	+ 1.15	On	On (Start)	Ramp
0.66	+ 1.15	On	Off (End)	Ramp
0.67	– 0.30	On	Off	Ramp
1.06	–0.30	Off	Off	Ramp
1.07	+ 0.30	Off	Off	Ramp

[a]The gain and ramp are instrument settings for the ICS-3000 IC electrochemical detector.

PREPARATION OF SOLUTIONS AND REAGENTS

When preparing eluents, it is essential to use high quality, Type 1 water (18.2 MΩ-cm resistivity or better) that contains as little dissolved gas as possible. Dissolved gases can cause higher noise levels. Degas the deionized water before eluent preparation using a Nalgene filter flask (P/N 164-0020) with 0.2 µm nylon filter with applied vacuum. Prepare 1 L of degassed Type 1 water weekly for the AS Autosampler flush solution.

Preparation of Eluent

To prepare 2 L of 50 mM methanesulfonic acid (MSA) eluent, pipette 4.5 mL (9.6 g) MSA (FW 96.10) into a 2 L glass eluent bottle containing 1993 g of Type 1 degassed, deionized water. Immediately cap the bottle, connect it to the Eluent A line, and place the eluent under ~4–5 psi of helium or other inert gas. Thoroughly mix the eluent solution and prime the pump with the new eluent.

Preparation of Standards

Warning: Cyanide is a poison by inhalation, contact, and ingestion. Solutions containing cyanide can generate hydrogen cyanide gas at neutral or acidic pH, and must be stabilized with base. Read and follow the material safety data sheet (MSDS) instructions for personnel handling, exposure, and disposal information. Consult local safety personnel for regulations concerning the proper disposal of cyanide. Add 100 mL of 50% NaOH into the system waste container. Hydrogen cyanide gas is created during the acid digestion of cyanide-containing samples. Conduct the acid digestion sample preparation in a well-ventilated hood.

Use high quality, 50% (w/w) sodium hydroxide solution for diluent preparation. Sodium hydroxide pellets are coated with sodium carbonate and cannot be used for this application.

Preparation of 100 mM Sodium Hydroxide Diluent

To prepare 250 mL of 100 mM sodium hydroxide (NaOH) diluent, pipette 1.3 mL (2.0 g) of 50% NaOH into a 250 mL HDPE bottle containing 248.7 g degassed Type 1 deionized water. Swirl the bottle gently to thoroughly mix the solution. Use this solution as the diluent for all cyanide standards. Prepare a fresh solution daily or as needed.

Cyanide Standards

To prepare a 1000 mg/L stock solution, weigh 0.189 g of reagent grade sodium cyanide into a 100 mL polyethylene bottle and dissolve thoroughly in 100 g of 100 mM NaOH diluent. Prepare an intermediate 1.0 mg/L cyanide standard by pipetting 50 µL of the 1000 mg/L stock solution into a 50 mL polyethylene bottle and diluting with 100 mM NaOH to a final weight of 50.00 g.

To prepare 1.0, 2.0, 5.0, 10, and 25 µg/L cyanide working standards from the 1.0 mg/L intermediate standard, pipette 20, 40, 100, 200, and 500 µL respectively, of the intermediate standard into 20 mL polyethylene bottles. Dilute these working standards with 100 mM NaOH to 20.00 g total weight. The stock solution and the intermediate standard are stable for more than a month when refrigerated. The working standards should be prepared daily.

Standards for Interference Experiments

As a test for positive interferences of cyanide methods, the ASTM D19.06 Cyanide Task Group devised an ASTM Challenge Matrix stock solution,[18] containing 17.8 mM ammonium chloride (FW 53.49), 17.8 mM potassium nitrate (FW 101.10), 49.4 mM sodium sulfate (FW 142.04), 5.95 mM potassium cyanate (FW 81.12), 2.6 mM potassium thiocyanate (FW 97.18), and 12 mM NaOH (1 mL of 12 M NaOH in 1 L). The Challenge Matrix working solution is a 10-fold dilution of the stock solution.

Individual interference stock solutions (Table 2) were prepared at 10 times the concentration of the ASTM Challenge Matrix Stock to facilitate preparation of individual interference solutions. Sulfide causes a negative interference with cyanide determinations in some methods, and was therefore added to the interference testing solution. Sulfide was prepared at the same molar concentration (17.8 mM) as nitrate, ammonium, and chloride. To prepare individual stock solutions (ammonium, chloride, cyanate, nitrate, sulfate, sulfide, thiocyanate), add the amount of reagent grade compound (Table 2) to a 100 mL polyethylene bottle and dilute with 100 g of deionized water.

Table 2. Amount of Compound Used to Prepare 100 mL of Individual Stock Solutions			
Ion	**Compound**	**Mass (g)**	**Concentration mg/L (mM)**
Ammonium	Ammonium chloride (NH_4Cl, FW 53.49)	0.954	3220 (178)
Chloride			6320 (178)
Cyanate	Sodium cyanate (NaOCN, FW 65.01)	0.387	2500 (59.5)
Nitrate	Sodium nitrate ($NaNO_3$, FW 84.99)	1.51	11,000 (178)
Sulfate	Sodium sulfate (Na_2SO_4, FW 142.04)	7.03	4750 (494)
Sulfide	Sodium sulfide, nonahydrate ($Na_2S \bullet 9H_2O$, FW 240.18)	4.28	1900 (178)
Thiocyanate	Sodium thiocyanate (NaSCN, FW 81.07)	0.209	1500 (26)

To prepare separate or combined interference standards, dilute the stock solutions 100-fold with 100 mM NaOH by pipetting 200 µL of the stock solutions into 19.8 g of 100 mM NaOH. Prepare the combined 5 µg/L cyanide/19 mg/L sulfide interference standard by pipetting 100 µL of the 1 mg/L cyanide intermediate standard and 200 µL of the 1900 mg/L sulfide stock solution into 19.7 g of 100 mM NaOH.

Sodium sulfide solutions degrade quickly upon exposure to air. Prepare sulfide solutions from a new bottle of sodium sulfide nonahydrate solid and store at 4 °C, as degradation accelerates as temperature increases. The 1900 mg/L sulfide stock solution must be prepared every 2 weeks when stored at 4 °C. Sulfide solutions at concentrations <1 mg/L should be prepared every two days. With the 1900 mg/L sulfide stock solution, long-term stability can only be achieved by freezing at -10 °C.

Sample Preparation

Free cyanide is reactive and unstable, and therefore water samples should be stabilized at the time of collection. Oxidizing agents decompose free cyanide and any free cyanide present at neutral pH will volatilize to hydrogen cyanide. Sodium hydroxide solution (2 g of 50% (w/w)) was added to ~250 g of municipal drinking water samples for preservation. The cyanide certified wastewater (CWW) sample was prepared according to the instructions then diluted 10-fold by combining 10 mL of the prepared CWW sample with 90 mL 100 mM NaOH diluent. The municipal wastewater effluent samples were filtered with 0.45 µm syringe filters prior to sample digestion to remove particulate matter and bacteria. Control samples of 100 mM NaOH blank and 5 µg/L cyanide standard samples were prepared in the same manner. To filter samples >50 mL, the authors used the 150 mL Nalgene filter apparatus (0.45 µm, nylon).

Separate 1 µg/L cyanide spike recovery samples were prepared from the municipal drinking water samples by pipetting 20 µL of 1.0 mg/L cyanide standard into separate 20 mL polyethylene bottles containing 20 g of base-treated water sample. The 5 µg/L cyanide spiked samples of municipal wastewater effluent and the 10-fold dilution of the cyanide CWW samples were prepared similarly with 100 µL of 1.0 mg/L cyanide standard added into 20 g of sample.

Acid Digestion

The MICRO DIST sample preparation system uses a three-part tube (Figure 1) and a digestion block designed to hold 21 assembled tubes. The tube includes a polypropylene sample tube, hydrophobic membrane, and a polypropylene collector tube that contains the trapping solution and functions as a measuring tube. The membrane separates the sample tube from the collector tube and allows only the gaseous sample to pass into the trapping solution. During the initial experiments, both the prefilled (assembled with the trapping solution) and user-filled (unassembled without the trapping solution) tubes were tested. The user-filled collector tubes were used for the final development of this Application Note. During digestion at 120 °C, hydrogen cyanide gas is generated in the sample tube from the reaction of cyanide in the sample with 7.11 M sulfuric acid and 0.75 M magnesium chloride solution. Hydrogen cyanide gas passes through the sample membrane in the collector tube and is dissolved as cyanide in the 1 M NaOH trapping solution. After the 20 min digestion time, the tubes are removed from the heating block, the sample tube is discarded, and the collector tube is inverted to cool for 10 min. The condensate is collected off the walls of the collector tube by the trapping solution. To prepare the sample for dilution and analysis, the collector tube is broken at the breakaway point to yield a measuring tube (M). The distillation (D) half of the collector tube is discarded. The sample in M tube is diluted to 6 mL with deionized water for a final concentration of 250 mM NaOH.

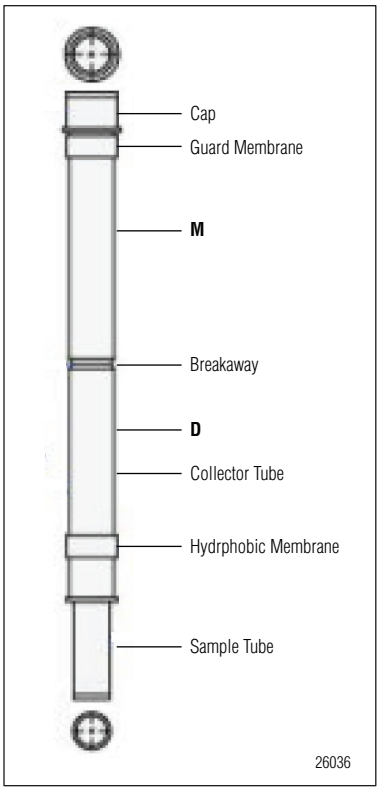

Figure 1. MICRO DIST tube assembly[14]

MICRO DIST Solutions

Prepare the 7.11 M sulfuric acid/0.75 M magnesium chloride digestion and 1 M NaOH trapping solutions according to the MICRO DIST Cyanide-1 Method, 10-204-00-1-X[19] instructions. Caution: Carefully prepare the sulfuric acid/ magnesium chloride solution in the exhaust hood with the hood sash positioned between you and the acid. Concentrated sulfuric acid reacts exothermically with water, and at this concentration, the solution can exceed the boiling point of water and violently boil over and splatter. To minimize isolated hot spots and violent flashbacks, add the concentrated sulfuric acid (MW 98.08, 95.7%) slowly, in 50 mL increments, pouring down the side of the flask and mixing gently between additions. Cool to room temperature and dilute to the 1 L mark.

MICRO DIST Acid Digestion

The cyanide samples, 100 mM NaOH blanks, and cyanide control standards were digested according to MICRO DIST Cyanide-1, Method 10-204-00-1-X. Each digestion experiment should include duplicate 100 mM NaOH blanks, control cyanide standards, and samples.

Use the following procedure to digest the samples:

- Place the heater block in the exhaust hood, turn it on, and set the temperature to 120 °C. Allow at least 40 min for the heating block to stabilize.
- Rinse the MICRO DIST user-filled collector tubes (Figure 1) on both sides of the D side membrane with 1 mL each of acid and base solutions prior to use to minimize contamination.
- To assemble the collector tubes, first add 1.5 mL of 1 M NaOH trapping solution to the M side of the collector tubes, then cap the collector tube (M side) with the cap and a filter membrane. The cap must be securely attached and the filter must completely cover the top of the tube. The cap and filter are responsible for containing the final solution in the collector tube.
- Label the collector tubes on each side of the breakaway point.
- Place the collector tubes in the test tube rack with the M side up.
- Label the sample tubes, weigh 6.0 g sample into each tube, and place in the test tube rack.

The next three steps must be performed quickly;

- Add 0.6 mL of the 7.11 M sulfuric acid/0.75 M magnesium chloride to one sample tube and immediately place the assembled collector tube over the sample.
- To press-fit the tubes, place the tubes in the press (D side down), support the tubes around the breakaway mark, and pull the press lever down to smoothly press-fit the collector tube into place over the sample tube.
- Using the heat-protective gloves, place the fully assembled tube in the pre-stabilized heating block (D side down), and digest at 120 °C for 30 min. Repeat with the other samples, blanks, and controls. The manufacturer recommends adding the tubes to the heat block within one minute.
- After the 30 min digestion, quickly remove the tube from the heating block using heat-protective gloves, remove the sample tube within 4 s, and quickly invert the collector tube (D side up). Discard the sample tube and the solution from the sample tube according to safety regulations. Remove the other tubes in the same manner.
- Allow to cool for 10 min.

- To rinse the condensate off the collector tube walls, gently tip the collector tube and the trapping solution until all of the condensate is collected. Tap the collector tube to collect any droplets clinging to the membrane.
- To break off the D side of the collector tube, firmly place both hands on both sides of the breakaway point and break the tube by pushing away. Place the M side of the collector tube into test tube rack. Discard the D side of the tube.
- Dilute to the 6 mL mark with deionized water. Swirl the sample to mix.
- Transfer the samples to the AS Autosampler sample vials.

As noted in the instructions, the digestion temperature and time, the condensation time, quick removal of the sample vial after digestion, and efficient rinsing of the condensate off the collector tube walls are critical to achieving good sample recovery.

SYSTEM PREPARATION AND SETUP

The IonPac ICE-AS1 column should not exceed backpressure >1000 psi. Do not remove or install the ED module while the DC module is turned on, as power surges can cause internal damage to the ED module

Configuring Virtual Channel to Monitor pH

It is useful to monitor and record the pH during sample analyses. To continuously record the pH during sample determinations, create a Virtual Channel in the Server Configuration program according to the instructions in AN 188.[15] (The pH virtual channel becomes one of the available signal channels.) More information on Virtual Channels can be found in the Chromeleon "help" program.

Plumbing the Chromatography System

CAUTION: Cyanide is converted to hydrogen cyanide, a toxic gas, at pH < 9. Add concentrated NaOH to the waste container prior to starting the system to maintain the pH of the waste stream and to prevent evolution of gaseous hydrogen cyanide. Add 100 mL of 50% NaOH for each 5 gallons of waste. This will yield 5 gallons of NaOH at ~1–2x the MSA eluent concentration.

Use red PEEK (0.127 mm or 0.005 in i.d.) tubing for all eluent lines from after the injection valve to the cell inlet. Black PEEK (0.25 mm or 0.010 in i.d.) tubing can be used from the pump to injection valve. Install the IonPac ICE-AS1 column set according to the product manual.[20] Column pressure is typically ~850 psi, which is sufficiently below the recommended operating pressure limit of 1000 psi for this column. A 1000 psi backpressure loop can be installed between the pump and injection valve to further reduce system noise. Install the 375 µL knitted reaction coil between the IonPac ICE-AS1 column and the electrochemical cell as described in AN 188.

Assemble the Electrochemical Cell

Assemble the electrochemical cell according the instructions in AN 188. In this application, the working electrode is a disposable platinum electrode. Typically, the background will stabilize within 10 min. However, a longer equilibration may be required when initially setting up the system.

RESULTS AND DISCUSSION

Sample Preparation

Initial experiments with the MICRO DIST sample preparation system found total cyanide (1–2 µg/L) when 100 mM NaOH was used as a sample (blank) with either the prefilled collector tubes or as received user-filled tubes with lab prepared 1 M NaOH trapping solution. The source of the contamination was unknown but it is likely a non-cyanide contaminant related to the hydrophobic membrane in the collector tubes. As discussed previously, cyanide can be generated when nitrate and nitrite are present under acid-digestion conditions. This problem was eliminated for the user-filled collector tubes by pre-rinsing the sides of the tubes labled D and M with 1 mL of the 7.11 M sulfuric acid/0.75 M magnesium chloride solution and 1 M NaOH solution. These experiments illustrate the importance of control samples and standards in the acid-digestion sample preparation.

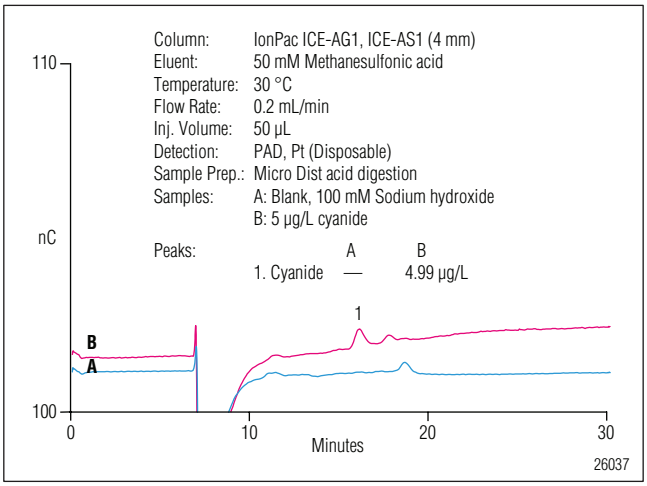

Figure 2. Comparison of A) Blank and B) 5 µg/L cyanide standard.

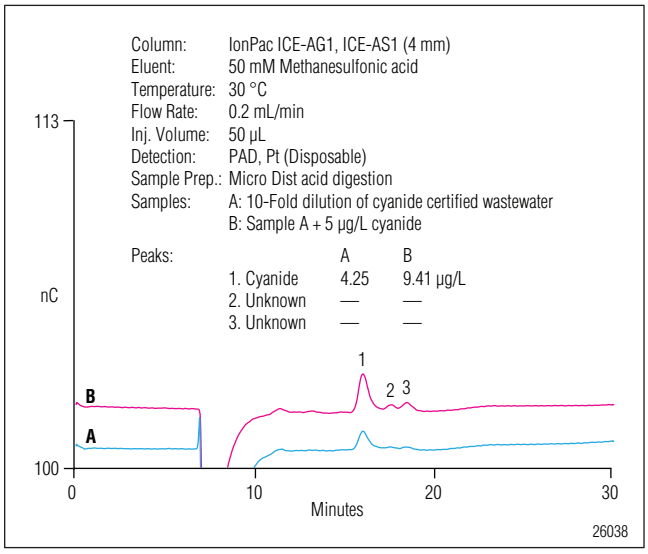

Figure 3. Comparison of A) 10-fold dilution of cyanide certified wastewater sample, and B) Sample A with 5 µg/L cyanide added.

Separation

ICE achieves better separation of cyanide from other anions in the sample compared to ion-exchange chromatography. In ICE (also known as Donnan exclusion), the fully sulfonated ion-exchange resin acts as a semipermeable membrane with separating molecular species rather than ions.[21,22] Strong acid anions, such as chloride and sulfate, are excluded by Donnan exclusion on the stationary phase and pass quickly through the column.[22] While weak acid anions, such as cyanide and sulfide, are protonated by the strong acid eluent to neutral compounds. These neutral compounds are not excluded but instead partition in the aqueous phases within and between the resin beads and separate in the order of increasing pKas.[21]

With this method, cyanide was separated by ICE using an IonPac ICE-AS1, 4 × 250 mm column using 50 mM MSA at a flow rate of 0.2 mL/min and detected by PAD using a Pt disposable working electrode with an amperometric waveform optimized for acid eluents. Figure 2 shows the separation of 5 µg/L cyanide standard prepared in 100 mM NaOH. The cyanide peak is symmetrical (A_s = 1.1 (EP)) and elutes in 16 min.

Method Qualification

The authors determined the linearity and estimated limit of detection (LOD) to qualify the method. To determine the LOD, the peak to peak noise was determined per min in three consecutive runs of deionized water for 60 min each, resulting in an average noise of 19.8 pC. The LOD of 0.27 µg/L was determined by multiplying the Student's *t*-test value of 3.14 for 99% confidence limits and the standard deviation (0.0085) of seven replicate injections of 0.50 µg/L cyanide standard. The linearity of cyanide detection was determined by calibrating with triplicate injections of five standards from 1.0 to 25 µg/L cyanide and comparing the peak area response to concentration (r^2 = 0.9999).

Samples

The authors applied the method to acid-digested samples of CWW, municipal drinking water, and municipal wastewater effluent. To determine total cyanide in the CWW sample, the sample was diluted 10-fold to a certified concentration of 4 µg/L total cyanide. Recovery was 4.25 ± 0.07 µg/L cyanide, 6.3% higher than the total cyanide certified value (Figure 3, chromatogram A). The cyanide peak has similar peak shape as in the prepared standard in Figure 2 with two small unknown peaks eluting at approximately 17–18 min. Determination of total cyanide was also evaluated in the municipal drinking water and wastewater effluent samples. In this study, 0.67 ± 0.02 µg/L (n = 6) total cyanide was detected in the municipal drinking water after acid digestion (Figure 4, chromatogram A). Because municipal wastewater effluent samples are known to have high

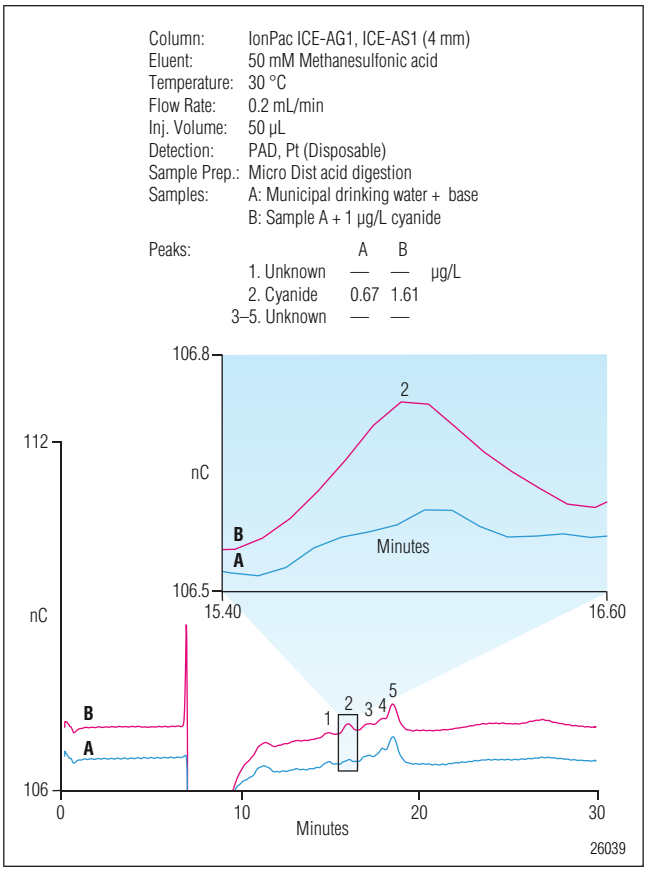

Figure 4. Comparison of A) Municipal drinking water, and B) Sample A with 1 µg/L cyanide added.

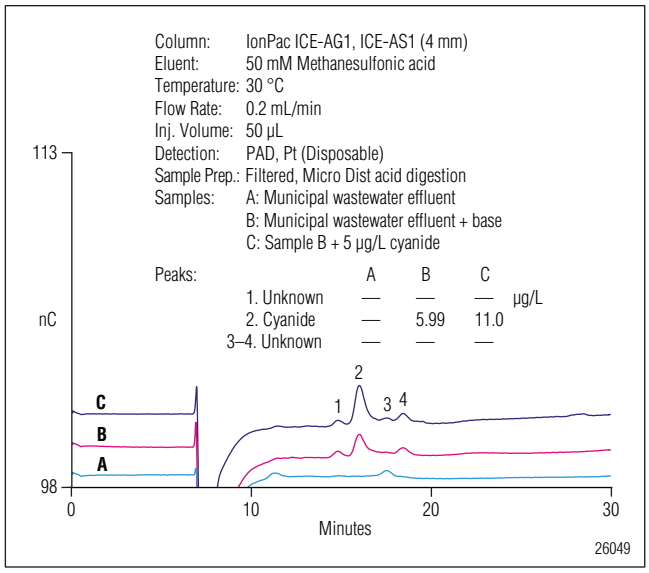

Figure 5. Comparison of A) Municipal wastewater effluent, B) second sample of A with base added, and C) Sample B with 5.0 µg/L cyanide added.

levels of bacteria and other particulates, both samples of the municipal wastewater effluent (with and without NaOH added) were filtered prior to acid digestion. Solutions of 100 mM NaOH and 5 µg/L cyanide prepared in 100 mM NaOH were also filtered as controls. The municipal wastewater effluent samples with and without base added during collection showed 5.99 ± 0.09 µg/L cyanide and no detectable cyanide (Figure 5), respectively. These results agree with previous reports that chloramine and chlorine disinfectant treatments used in POTWs generate unstable cyanide intermediates and that NaOH may stabilize these intermediate compounds.[9,10]

To determine the method precision, six replicate injections were performed using a 5 µg/L cyanide standard, a 10-fold dilution of CWW sample, and the same sample spiked with 5 µg/L cyanide. The calculated RSDs ranged from 0.57 to 2.9%. The accuracy of the method was evaluated over three days by adding known concentrations of cyanide to the samples prior to acid digestion (Figures 3, 4, 5, chromatograms A, B, and C, respectively). Table 3 summarizes the results of this study.

Table 3. Average Cyanide Determinations Over Three Days

Sample	Amount Found (µg/L)[a]	Amount Added (µg/L)	Average Recovery[a] (%)
100 mM sodium hydroxide	<LOD	1.06	110 ± 6.4
Filtered 100 mM sodium hydroxide	<LOD	5.02	102 ± 1.0
10-fold dilution of certified cyanide wastewater sample (4.0 µg/L total cyanide)	4.25 ± 0.07	4.99	102 ± 0.9
Municipal drinking water	0.67 ± 0.02	0.99	97.4 ± 2.0
Filtered municipal wastewater effluent without base	<LOD	Not Tested	—
Filtered municipal wastewater effluent with base	5.99 ± 0.09	4.97	99.5 ± 1.0

[a]n = 6

As shown, the method demonstrated good accuracy with average recoveries of 97.4–102%.

Interferences

The ASTM Cyanide Task Group researched the effect of ions that can cause false positives for total cyanide and therefore developed a challenge matrix to evaluate results for various analytical methods.[18] The challenge matrix contains 95.4 mg/L ammonium chloride, 25 mg/L cyanate, 15 mg/L thiocyanate, 110 mg/L nitrate, and

Table 4. Effect of Potential Interferences on Total Cyanide Determinations	
Sample[a]	**Average Cyanide Found (µg/L)**
100 mM Sodium hydroxide blank	<LOD
5 µg/L cyanide	5.03
ASTM challenge matrix	32.32
Ammonium chloride, cyanate, thiocyanate, and nitrate	36.20
Ammonium chloride, cyanate, and thiocyanate	21.29
Ammonium chloride, cyanate	26.31
Ammonium chloride, thiocyanate	0.44
Ammonium chloride	<LOD
Cyanate	16.21
Thiocyanate	<LOD

n = 2
[a]Interfering ions are the same molar concentrations as in the ASTM challenge matrix: 32.2 mg/L ammonium, 63.2 mg/L chloride, 25 mg/L cyanate, 110 mg/L nitrate, 47.5 mg/L sulfate, and 15 mg/L thiocyanate.[13]
ND is not detected.

475 mg/L sulfate. To determine the potential for false positives from the challenge matrix, the authors analyzed the challenge matrix samples for total cyanide and an undigested 25 µg/L cyanate sample for free cyanide. The experiments showed that the cyanide was generated from the acid digestion of ASTM challenge matrix primarily from cyanate (Table 4). No free cyanide was found in the undigested cyanate standards. Total cyanide concentrations increased when nitrate or thiocyanate was added to cyanate-containing samples then acid-digested. These results agree with the false positives previously reported in the literature and associated with acid-digestion and oxidation of thiocyanate and cyanate by nitrate to cyanide.[9,10] In wastewater treatment plants, nitrate is formed from chlorination of ammonium which reacts with other unstable intermediates to degrade to cyanide during acid digestion.

Sulfide is a known interferent with cyanide determinations and its presence in samples can yield poor recoveries. Sulfide concentrations at mg/L concentrations can foul the silver working electrode used in electrochemical methods and cause falsely high results in flow injection methods.[13] To determine whether sulfide interfered with accurate cyanide determinations, the authors analyzed a 19 mg/L sulfide sample spiked

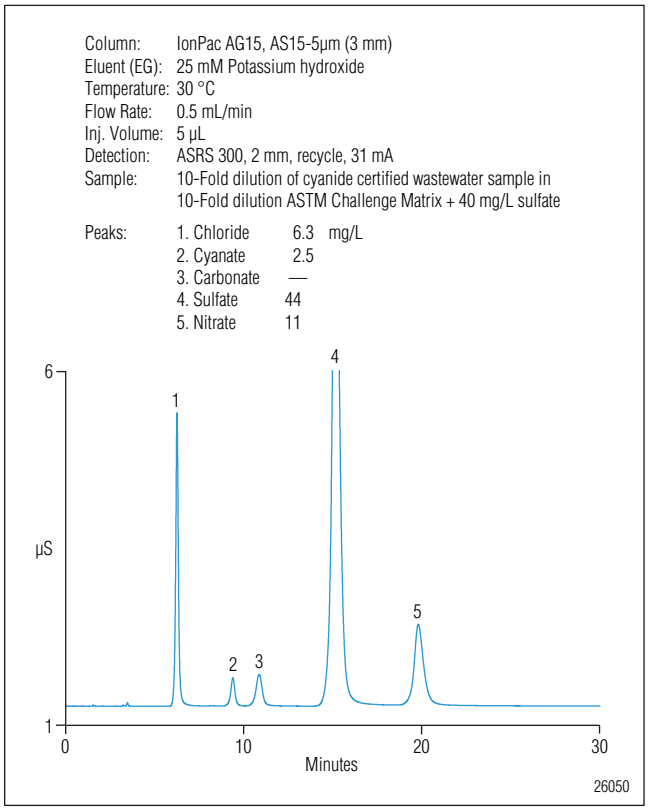

Figure 6. Determination of cyanate using a Reagent-Free IC system.

with 5.0 µg/L cyanide. Cyanide was fully resolved from sulfide, despite a cyanide-to-sulfide concentration ratio of 1:3800 (not shown), and the cyanide recovery was 99.2%. The results show that, unlike methods which use a silver working electrode or flow injection methods, sulfide does not interfere with accurate determinations of cyanide using the technique described in this application.

The effect of nitrate, nitrite, cyanate, and thiocyanate interferences and other oxidizing agents can be minimized by pre-treating the samples with sulfamic acid or sodium arsenite prior to adding base for preservation and acid digestion.[23] The presence of interfering anions can be determined by IC with suppressed conductivity detection using AN 154[24] for nitrite and nitrate, AN 138[25] for thiocyanate, and AN 200[26] for cyanate determinations. Figure 6 shows the determination of 2.5 mg/L cyanate by the conditions in AN 200 in a 10-fold dilution of the certified cyanide wastewater standard and ASTM matrix plus 40 mg/L of additional sulfate.

Table 5. Results of Robustness Experiments					
Parameter	**Value**	**Retention Time[a] (min)**	**Difference (%)**	**Peak Area[a] (nC-min)**	**Difference (%)**
Eluent Concentration (mM MSA)	47.5	15.92 ± 0.04	-0.3	0.377± 0.005	-0.8
	50	15.96 ± 0.02	—	0.380 ± 0.004	—
	52.5	15.91 ± 0.03	-0.3	0.380 ± 0.009	—
Column Temperature (°C)	28	16.01 ± 0.04	+0.3	0.385 ± 0.014	-1.3
	30	15.96 ± 0.02	—	0.380 ± 0.004	—
	32	15.89 ± 0.01	-0.4	0.373 ± 0.017	-1.8
Working Electrode	Conventional	15.96 ± 0.04	—	0.384 ± 0.006	+1.1
	Disposable Lot 080917	15.96 ± 0.02	—	0.380 ± 0.002	—
	Disposable Lot 080917	15.99 ± 0.03	+0.2	0.376 ± 0.005	-1.1
Column (Lot)	008-05-003	15.96 ± 0.02	—	0.380 ± 0.004	—
	008-05-092	16.68 ± 0.03	+4.5	0.385 ± 0.007	+1.3

[a]n = 6

Robustness

To determine the robustness of the method, the authors evaluated the effects of Pt working electrodes (conventional and disposable electrodes within the same lot), eluent concentration, column temperature, and lot-to-lot column variation on 5.0 µg/L cyanide peak responses and retention times (Table 5). The results demonstrated that slight variations in eluent concentration, column temperature, and different working electrodes had little effect on the retention times of cyanide (<0.5%). Using a column from a different lot showed the greatest effect on retention time (+4.5%). In terms of the cyanide peak area; only nominal effects (<1.5%) were observed for the variables investigated in this study.

CONCLUSION

This Application Note describes an ICE-PAD method using the EPA-approved Lachat MICRO DIST acid digestion system to determine µg/L concentrations of total cyanide in municipal drinking water and municipal wastewater effluent. The method provides low detection limits and improvement of cyanide recoveries due to exclusion of chloride and resolution from sulfide. False positives from cyanate and thiocyanate in the presence of nitrate in POTW wastewater effluent are related to the POTW chloramination processes and the acid digestion conditions during sample preparation. The effect of these false positive interferences can be minimized by identifying the presence of nitrate and nitrite, thiocyanate, and cyanate by methods described in AN 154, AN 138, and AN 200, respectively, followed by pretreatment with sulfamic acid.

SUPPLIERS

Fisher Scientific International Inc., Liberty Lane, Hampton, NH, USA 03842. 1-800-766-7000 www.fisherscientific.com

Hach Company (Lachat Instruments), PO Box 389, Loveland, CO, USA 80539. 1-800-227-4224 www.hach.com

High-Purity Standards, P.O. Box 41727, Charleston, SC, USA 29423. 1-843-767-7900 www.highpuritystandards.com

Sigma-Aldrich Corp., St. Louis, MO, USA. 1-800-325-3010 www.sigmaaldrich.com

VWR International, Inc., Goshen Corporate Park West, 1310 Goshen Parkway, West Chester, PA, USA 19380 1-800-932-5000 www.vwrsp.com

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Direct Determination of Cyanide in Drinking Water by Ion Chromatography with Pulsed Amperometric Detection (PAD)

INTRODUCTION

The toxicity of cyanide is well known. Cyanide occurs naturally in many foods (cassava, sorghum, African lima beans, bamboo shoots, bitter almonds, and apricot, cherry, and peach pits) and is naturally generated by microorganisms.[1] Cyanide is used in many industries (e.g., plating and mining) and it can be released into the air from burning coal and plastics. In the U.S., drinking water contamination with cyanide is typically from an industrial source or leached from waste sites.

The U.S. government classifies cyanide as a regulated inorganic contaminant in drinking water (U.S. National Primary Drinking Water Regulations, 40CFR 141.62).[2] These regulations are enforced by the U.S. EPA and state EPA agencies. Bottled water is classified separately as a food, and is regulated by the FDA Center for Food Safety and Applied Nutrition (CFSAN) division. For community water systems, non-transient non-community water systems (defined as a temporary water system for ≥25 people used for less than six months), and drinking water, the maximum contaminant level (MCL) is 200 µg/L cyanide as free cyanide. Typical free cyanide levels are much lower. A 1978 U.S. EPA survey showed that only 7% of drinking water had cyanide concentrations >10 µg/L.[3]

Cyanide is determined as total cyanide (EPA 335.2),[4] disassociated cyanide, and free (amenable) cyanide. Total cyanide is determined by distillation with acid and an oxidizing agent to generate hydrogen cyanide gas that is captured in a pH 13 sodium hydroxide solution, and then determined by a colorimetric or titration method.

The EPA approved free cyanide methods use spectophotometry (335.1),[5] colorimetry (335.3),[6] and ion-selective electrode detection (Standard Methods SM-4500-CN-F).[7] The colorimetric and spectrophotometric methods require distillation and have many interferences, including difficulty with high-pH solutions, oxidizers, and sulfur-bearing compounds. The ion-selective electrode method does not require distillation, but it is very matrix sensitive. Ion chromatography (IC) methods for cyanide use DC amperometric detection[8] or pulsed amperometric detection (PAD).[9–11] The DC amperometry method exhibits electrode fouling problems over time. The reported PAD detection based methods did not determine cyanide in drinking water samples.

This Application Note demonstrates fast, accurate determinations of free cyanide in drinking water samples using IC-PAD with a waveform optimized for cyanide, and use with a disposable silver working electrodes. This method is compatible with the basic solutions used to preserve drinking water samples for cyanide analysis and is unaffected by other compounds typically found in drinking water.

EXPERIMENTAL EQUIPMENT

Dionex ICS-3000 system consisting of:

- Single Gradient Pump (SP) or Dual Gradient Pump (DP) module with degas option and gradient mixer (Dionex GM-4, P/N 049135)
- Detector and Chromatography Module (DC) with a single temperature zone and one injection valve
- Electrochemical Detector ED (P/N 061718) with an electrochemical cell containing a combination pH-Ag/AgCl reference electrode (cell and reference electrode, P/N AAA-061756, reference electrode P/N 071879) and a Certified Disposable Silver (Ag) working electrode (Package of 6 electrodes, P/N 063003)
- AS Autosampler with Sample Tray Temperature Controlling option and 1.5 mL sample tray
- Chromeleon® Chromatography Workstation with Chromeleon 6.7
- Filter unit, 0.2-µm nylon (Nalgene Media-Plus with 90-mm filter, Nalge Nunc International, P/N 164-0020) or equivalent nylon filter
- Vacuum pump
- 1.5-mL polypropylene sample vials, with caps and slit septa (Dionex vial kit, P/N 061696)
- Disposable polystyrene 10-mL and 25-mL graduated pipettes
- Micropipettor and tips for preparing samples, standards, and pipetting samples into vials
- Dionex OnGuard® II H cartridges (2.5 cc, package of 48, P/N 057086)
- Black PEEK (0.254-mm or 0.010-in. i.d.) tubing, used for eluent connections to cell, Pump 1, and columns (5 ft, P/N 052306)
- Red PEEK (0.127-mm or 0.005-in. i.d.) tubing, installed in DC heat exchanger (5 ft, P/N 052310)
- Green PEEK (0.76-mm or 0.030-in. i.d.) tubing, installed in AS Autosampler (5 ft, P/N 052305)

REAGENTS AND STANDARDS

Use only ACS reagent grade chemicals for all reagents and standards.

Deionized water, Type 1 reagent-grade, 18.2 MΩ-cm resistivity or better, freshly degassed by vacuum filtration

Sodium cyanide, anhydrous (Aldrich, P/N 20,522-2)

Sodium hydroxide, 50% (w/w) (Fisher Chemicals, P/N SS254-500)

pH 7 (yellow) and pH 10 (blue) buffer solutions (VWR International, P/N 34170-130, 34170-133)

Used for experiments that determined retention times and possible interferences:

Copper reference standard, Certified 1000 ppm ±1% (Fisher Chemical, P/N SC194-100)

Iron reference standard, Certified 1000 ppm ±1% (Fisher Chemical, P/N SI124-100)

Nickel reference standard, Certified 1000 ppm ±1% (Fisher Chemical, P/N SN70-100)

Sodium bromide, anhydrous (Aldrich, P/N 310506)

Sodium iodide, anhydrous (Aldrich, P/N 383112)

Sodium sulfide, nonahydrate, >99.99% (Aldrich, P/N 431648)

Sodium sulfite, anhydrous (Aldrich, P/N 239321)

Sodium thiocyanate, (Aldrich, P/N 251410)

Sodium thiosulfate, pentahydrate (Aldrich, P/N 2929)

SAMPLES:

City of Sunnyvale (sampled on multiple days), City of San Jose, and Twain Harte Valley, CA drinking water samples

The sources (flumes) of Twain Harte Valley (an old gold mining region) drinking water and Alamitos Creek in Almaden region (an old mercury mining region) of San Jose, CA

ELECTROCHEMICAL DETECTOR (ED)

The ICS-3000 electrochemical detector is composed of an ED module with the electronics and an amperometric cell containing working, reference, and counter electrodes. The ED is a "plug and play" module and easily installs into the ICS-3000 Detector/Chromatography (DC) upper chamber and the cell mounts on the ED.

In this application, the working electrode is a disposable silver working electrode. When used with a recommended waveform, the disposable silver working electrodes have a background specification of -45 to +55 nC against the reference electrode in AgCl mode. Typically, the background will rise or fall to the equilibrium background within 10 min. The waveform was optimized for cyanide but it can also detect sulfide, bromide, and thiosulfate (Table 1).[11]

Calibration, handling, and installation tips for the reference electrode and Certified Disposable Silver working electrodes are thoroughly described in the System Preparation and Setup section of this application note, the Dionex ICS-3000 Operator's Manual,[12] and the Dionex Product Manual for Gold and Silver Disposable Electrodes.[13]

Table 1. Cyanide Waveform				
Time (sec)	Potential vs Ag/AgCl (V)	Gain Region	Integration	Ramp
0.00	–0.10	Off	Off	On
0.20	–0.10	On	On (Start)	On
0.90	–0.10	On	Off (End)	On
0.91	–1.00	On	Off	On
0.93	–0.30	Off	Off	On
1.00	–0.30	Off	Off	On

CONDITIONS

Columns: IonPac® AS15 Analytical, 2 × 250 mm (P/N 053941)
IonPac AG15 Guard, 2 × 50 mm (P/N 053943)

Flow Rate: 0.25 mL/min

Eluent: 63 mM Sodium hydroxide (31.5% Eluent B, 200 mM sodium hydroxide)

Column Temp: 30 ºC

Tray Temp: 10 ºC

Inj. Volume: 10 µL (PEEK sample loop, P/N 042949), full-loop injection

Detection: Pulsed Amperometric Detection (PAD)

Waveform: See Table 1

Electrodes: Reference: pH-Ag/AgCl electrode (P/N 061879) in AgCl mode

Working: Certified disposable Ag working electrode

Background: 3–13 nC versus Ag/AgCl[a]

Backpressure: ~1100 psi

Noise: <7 pC

Run Time: 25 min

Syringe Speed: 4

Flush Volume: 250 µL

[a] The disposable silver electrodes have a background specification of -45 to + 55 nC versus Ag/AgCl with a recommended waveform.

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent Preparation

It is essential to use high quality Type 1 water (>18.2 MΩ-cm) containing as little dissolved carbon dioxide as possible. Degas the deionized water before eluent preparation. It is also essential to use high quality 50% (w/w) sodium hydroxide solution for eluent and diluent preparation. Sodium hydroxide pellets are coated with sodium carbonate and, therefore, are not acceptable for this application. Eluent preparation is thoroughly discussed in the AminoPac® PA10 and *AAA-Direct*™ Product Manuals.[14]

Eluent A (degassed deionized water)

To prepare degassed deionized Type 1 water (Eluent A), degas 2-L of Type 1 deionized water using ultrasonic agitation and applied vacuum to aid in removing the gas bubbles. Pour the degassed deionized water into a 2-L precleaned eluent bottle. Connect the eluent bottle to the Eluent A line from the pump and place the eluent bottle under ~4–5 psi of helium or other inert gas. Prime the pump with the new eluent.

Eluent B (200 mM Sodium Hydroxide)

Add 2000.0 g of degassed Type 1 deionized water into a 2-L precleaned eluent bottle. This is measured on a top loader balance that is accurate to ±0.01 g. Rinse a 25-mL graduated plastic pipette several times with deionized water and shake out the excess water. Using the pipette, remove 21.0 g of deionized water from the 2-L eluent bottle and discard it. Shake out or blow out with a pipette bulb the last remaining drops in the pipette. Using the same pipette, add 32.0 g (~21.0 mL) of 50% (w/w) sodium hydroxide solution into the 2-L eluent bottle. Connect the eluent bottle to the Eluent B line from the pump and place the eluent bottle under ~4–5 psi of helium or other inert gas. Swirl the eluent bottle to thoroughly mix the eluent. Prime the pump with the new eluent.

100 mM Sodium Hydroxide Diluent Solution

All of the cyanide standards were prepared gravimetrically in 100 mM sodium hydroxide diluent. To prepare the 100 mM sodium hydroxide solution, add 1000.0 g of degassed deionized water into a 2-L precleaned eluent bottle. Rinse a 10-mL graduated, plastic pipette several times with deionized water and shake

out the excess water. Using the pipette, remove 5.2 g of deionized water from the 2-L eluent bottle and discard it. Shake out or blow out with a pipette bulb the last remaining drops in the pipette. Using the same 10-mL pipette, add 8.0 g (~5.25 mL) of 50% (w/w) sodium hydroxide solution into the 2-L eluent bottle. Place the eluent bottle under ~4–5 psi of helium or other inert gas. Swirl the eluent bottle to thoroughly mix the diluent.

AS Autosampler Flush Solution

Prepare the degassed deionized water in the same manner described in Eluent A.

STANDARD PREPARATION

Warning: Cyanide is a poison by inhalation, contact, and ingestion. It generates the poisonous hydrogen cyanide gas at neutral or acidic pH. Solutions containing cyanide must be stabilized with base. Read and follow the material safety data sheet (MSDS) instructions for personnel handling, exposure, and disposal information. Also consult local safety personnel for regulations concerning the proper disposal of cyanide.

Cyanide Standards

To prepare a 1000 mg/L stock solution, weigh 0.0377 g of reagent grade, sodium cyanide into a 20-mL polyethylene bottle. Add 100 mM sodium hydroxide diluent to a total weight of 20.00 g. Prepare an intermediate standard solution of 1.0 mg/L cyanide by pipetting 20 µL of the 1000 mg/L stock solution into a 20-mL polyethylene bottle and dilute with 100 mM sodium hydroxide to a final weight of 20.00 g.

To prepare 2.0, 3.0, 5.0, 10.0, 50.0, 100 µg/L working standards of cyanide from the 1.0 mg/L intermediate standard, pipette 40, 60, 100, 200, 1000, and 2000 µL, respectively, of the intermediate standard into 20-mL polyethylene bottles. Dilute these working standards with 100 mM sodium hydroxide to 20.00 g total weight. The stock solution and the intermediate standard are stable for more than a month when refrigerated. The working standards should be prepared daily.

Table 2. Amount of Compound Used to Prepare 20.00 g (~ 20 mL) of Individual 1000 mg/L Stock Solutions

Anion	Compound	Mass (g)
Bromide	Sodium bromide (NaBr)	0.0258
Iodide	Sodium iodide (NaI)	0.0362
Sulfide	Sodium sulfide, nonahydrate ($Na_2S \cdot 9H_2O$)	0.1498
Sulfite	Sodium sulfite (Na_2SO_3)	0.0315
Thiocyanate	Sodium thiocyanate (NaSCN)	0.0279
Thiosulfate	Sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$)	0.0440

Standards for Interference and Retention Time Determination Experiments

To prepare individual 1000 mg/L stock solutions of the ions (bromide, iodide, sulfide, sulfite, thiocyanate, and thiosulfate) for the interference experiments, dissolve the amount of reagent grade compound (Table 2) in deionized water in a 20-mL polyethylene bottle and dilute to 20.00 g with deionized water. These stock standards will be diluted to 1.0 mg/L concentration for interference and retention time determination experiments.

Prepare separate intermediate standards of 1.0 mg/L from each of the 1000 mg/L stock solutions. Pipette 20 µL of the individual stock solution into a 20-mL polyethylene bottle and dilute with deionized water to 20.00 g total weight. To prepare 10 µg/L individual standards of bromide, iodide, sulfide, sulfite, thiocyanate, and thiosulfate, pipette 200 µL of the intermediate standard into a separate 20-mL polyethylene bottle and dilute with 100 mM sodium hydroxide to 20.00 g total weight. These standards were used to determine anion retention times.

To prepare 5 µg/L and 10 µg/L of separate cyanide standards spiked with 10 µg/L of the individual interference ion (bromide, iodide, sulfide, sulfite, thiocyanate, or thiosulfate), pipette 100 µL and 200 µL, respectively, of the 1.0 mg/L cyanide intermediate standard into individual 20-mL polyethylene bottles. Pipette 200 µL of the 1.0 mg/L intermediate standard of the interference ion (bromide, iodide, sulfide, sulfite, thiocyanate, or thiosulfate) into the 20-mL polyethylene bottle and dilute with 100 mM sodium hydroxide to 20.00 g total weight. These standards were used to evaluate possible interferences with cyanide determinations.

Note: Sodium sulfide solutions degrade quickly. They should only be prepared from a new bottle of

sodium sulfide, nonahydrate solid. Once exposed to air, sulfide rapidly breaks down to sulfite. All the sulfide solutions, including the 1000 mg/L, are unstable even when refrigerated. Low-level sulfide standards must be prepared every two days and tested at 10 ºC. Intermediate sulfide standards must be prepared every 2 weeks. The 1000 mg/L sulfide solution showed long term stability only when it was preserved by freezing at -10 ºC.

STANDARDS FOR METAL INTERFERENCE EXPERIMENTS

To prepare 650 µg/L iron, and separate 300 µg/L of copper and nickel working standards from the 1000 mg/L iron, copper, and nickel reference standards, pipette 65 µL of the iron reference standard, and 30 µL each of the copper, and nickel reference standards, into individual 125-mL polypropylene bottles. Dilute these working standards with deionized water to 100.00 g total weight. These standards were used to evaluate the interference of dissolved metals on free cyanide determinations.

PROCEDURE TO REMOVE DISSOLVED METALS

To remove the dissolved metals, treat the dissolved metal solutions with 2.5-cc capacity, Dionex OnGuard II H cartridges per the product manual instructions.[15] These cartridges are designed to remove alkali and alkaline earth metals, and cationic transition metals. Fill a 5-mL disposable syringe with the dissolved metal solution, attach a new 2.5-cc OnGuard II H cartridge, and dispense the solution through the cartridge, at ~1 mL/min, and into a 10-mL graduated cylinder. Fill the syringe again and dispense through the same cartridge until the graduated cylinder contains 6 mL of filtrate. Discard this initial filtrate. Tare a 10-mL sample vial and dispense the filtrate into the sample vial. Continue to refill the syringe and dispense the filtrate until 7.80 g of filtrate is dispensed in the vial. Add ~2 drops of 50% sodium hydroxide to the filtrate. Mix thoroughly, and spike the solution with 100 µL of 1.0 mg/L of cyanide, resulting in 10 µg/L cyanide in ~100 mM sodium hydroxide. It is critical that the sodium hydroxide is added and mixed into the solution prior to adding the cyanide. As a control, we treated 10 µg/L of cyanide in 100 mM sodium hydroxide with an OnGuard II cartridge in the same manner as the dissolved metal solutions, except that no additional sodium hydroxide or cyanide was added. A new cartridge was used for each dissolved copper, iron, and nickel solution.

To prepare 10 mL each of the combined cyanide and metal solutions, spike the metal solutions with ~2 drops of 50% sodium hydroxide, mix thoroughly, and spike with 100-µL of 1.0 mg/L of cyanide to a final concentration of 10 µg/L cyanide. These samples were tested immediately.

SAMPLE PREPARATION

Cyanide is reactive and unstable, therefore drinking water samples should be stabilized as soon as possible and free cyanide determined as soon as possible. Oxidizing agents decompose cyanide. Also any free cyanide present at neutral pH will volatilize to hydrogen cyanide.

Because of these issues, the drinking and surface water samples were stabilized as soon as practical. Sunnyvale and San Jose municipal drinking water samples were stabilized within one hour of sampling by addition of a 50% (w/w) sodium hydroxide solution. The procedure is as follows: rinse the 10-mL graduated plastic pipette several times with deionized water and shake out the remaining drops. Using the same pipette, add 2.00 g (~1.5 mL) of 50% (w/w) sodium hydroxide solution into a 250-mL polypropylene bottle, add the municipal drinking water to 250.00 g total weight, then mix thoroughly.

Alamitos Creek (in the Almaden mining area of San Jose) and Twain Harte Valley were located farther away from the laboratory so sampling kits were prepared for the Alamitos Creek surface water, and the Twain Harte Valley drinking and surface water samples. The kit contained disposable vinyl gloves for chemical handling, 100-mL wide-mouth specimen containers to collect the samples, and the 250-mL sampling bottles to store and stabilize the samples, all placed into a large resealable bag. The 250-mL sampling bottles were prepared in a similar manner as those for the municipal drinking samples. At the sampling site, 250 mL of the drinking and surface water samples were collected and measured with the 100-mL specimen containers, transferred to the 250-mL sampling bottles, and then thoroughly mixed with 50% sodium hydroxide, as described above.

Spike recovery samples of 5 and 10 µg/L cyanide from both municipal drinking water samples and the Alamitos Creek surface water were prepared by pipetting 100 µL and 200 µL, respectively, of 1.0 mg/L cyanide standard into 20-mL polyethylene bottles, and diluting with the base-treated water sample to a total weight of 20.00 g.

To remove potentially interfering dissolved metals, portions of both municipal drinking waters, and Twain Harte drinking and surface water samples were treated with the OnGuard II H cartridges. These samples were prepared in the same manner as the dissolved metals samples, though without further sodium hydroxide additions. To prepare 5 and 10 µg/L cyanide spike recovery samples of the drinking and surface water samples and the cartridge-treated drinking and surface water samples, pipette 50 µL and 100 µL, respectively, of 1.0 mg/L cyanide in individual 20-mL polyethylene bottles, and dilute with water sample or treated water sample to 10.00 g total weight.

SYSTEM PREPARATION AND SETUP

The setup for the individual modules, components, and system is thoroughly described in the ICS-3000 Operator's Manual,[16] and ICS-3000 Installation Manual,[17] and the Chromeleon "Help" menus.

PLUMBING THE CHROMATOGRAPHY SYSTEM

Connect black PEEK (0.254-mm or 0.010-in. i.d.) tubing from Pump 1 to the gradient mixer (GM-4) and from the gradient mixer to position "P" on Injection Valve 1 inside the DC module. Connect the red PEEK (0.127-mm or 0.005-in. i.d.) tubing from Injection Valve 1, position "C" to the heat exchanger. Install the IonPac AS15 column set according to the IonPac AS15 Product Manual.[18] Connect the red PEEK tubing exiting the System 1 heat exchanger to the column set. The free end will be installed into the ED cell. Install a 10-µL loop in DC Injection Valve 1 in both "L" positions. Connect the AS Autosampler injection port tubing and the green PEEK (0.76-mm or 0.030-in. i.d.) tubing waste line to DC Injection Valve 1 positions "S" and "W", respectively.

CONFIGURING THE AS AUTOSAMPLER

Configure the AS Autosampler and connect the sample prep and sample syringes according the AS Autosampler Operator's Manual.[19] Enter the loop size (10 µL) in loop size V1, on the AS front panel, under Menu and Plumbing Configuration. Select the syringe sizes of the sample prep and the sample syringe from the pull down menus, under Menu and System Parameters. Also select "Normal" sample mode and "Enable" Wait function, under Menu and System Parameters.

CONFIGURING THE SYSTEM

With all the power on and the Chromeleon monitor program running, open the Chromeleon Server Configuration program. To configure the system, first create a cyanide timebase and then add the devices: ICS-3000 DP or SP pump module, the DC module, and AS Autosampler. If a SP single pump is used, assign the Pump 1 to the cyanide timebase, (right click on SP module, select properties, select Devices tab, and select the cyanide timebase on pull-down menu for the pump). If a DP dual pump is used, select Devices tab, click off the share boxes ("share eluent bottles", and "share waste bottle"). Also insert a "pump off" command in the program for Pump 2. Verify that the AS device has the same options (e.g., sample preparation, temperature tray control, etc.,) as listed on the AS Autosampler module (Installed Options under Module Setup Menu). Save and check the configuration before leaving the program.

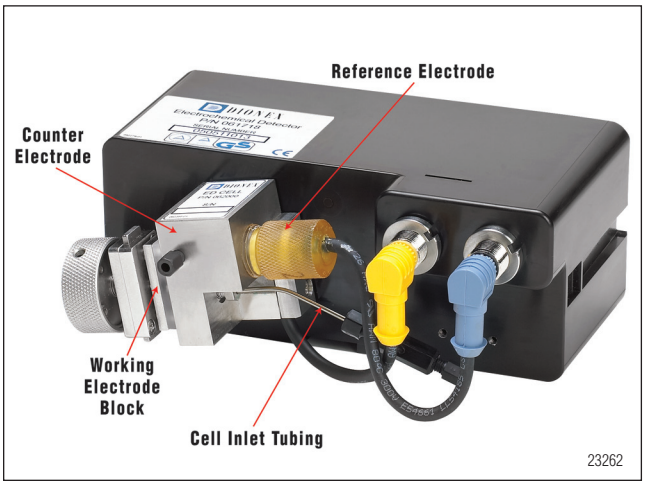

Figure 1. Amperometry cell.

ELECTROCHEMICAL DETECTOR AND AMPEROMETRY CELL

Install the electrochemical detector ED and amperometry cell (Figure 1)[20] and calibrate the combination pH-Ag/AgCl reference electrode. Install the ED module in the middle DC chamber, above Injection Valve 1. Remove the storage cap from the reference electrode but leave the storage cap O-ring in place on top of the reference electrode. The storage cap O-ring will be used again when the reference electrode is removed someday and sealed into the storage cap. It does not interfere with the installation of the reference electrode. Rinse the

KCl storage solution off the reference electrode, pat dry, and place the reference electrode in pH 7 buffer. Open Chromeleon and connect to the cyanide timebase. Click on the Chromeleon Panel icon, expand the cyanide timebase panel, and select the EC Detector tab. Connect the blue lead of the reference electrode to the ED black port. Check the cell on/off button to ensure that the cell is turned off. The pH electrode remains active regardless of the cell power. Click on the "Calibration" button which opens the ED Wellness Panel. Follow the calibration instructions in the "instructions" button or in the ICS-3000 Operator's Manual. Wait for the pH reading to stabilize, then press the "pH Offset Cal" button and wait while it calculates the pH offset. After the reference electrode is finished reading, remove, rinse, and pat it dry. Place the reference electrode in pH 10 buffer and wait until the reading is stable. Enter the "10.00" in the pH slope buffer value, press the "pH slope Cal." button, and wait while it calculates the slope intercept. When the slope intercept is calculated, save, upload the new calibration values, and close the ED Wellness Panel.

Assemble the electrochemical cell. Check that the reference electrode O-ring on the bottom of the reference electrode is in place (install one if it is missing or damaged). Gently screw the reference electrode into the electrochemical cell body. Tighten to a snug fit (finger-tight, do not use tools). Install the Certified Disposable Silver Electrode in the electrochemical cell, according to the Disposable Electrode Ag Installation Guide[21] received with the electrodes. Install the electrochemical cell into the ED. Connect the "yellow" lead on the cell to the "yellow" port on the ED and connect the "blue" lead to the "black" port on the ED. Connect the red PEEK tubing exiting the columns to the cell inlet and direct the cell outlet tubing to waste.

RESULTS AND DISCUSSION

The Cyanide Waveform

The cyanide waveform is a three-potential waveform using E_1, E_2, and E_3. These voltages are applied at the designated times during a 1-sec waveform. E_1, detection and integration potential, is -0.10 V vs Ag/AgCl and maintained from 0.00 to 0.90 sec. E_2 is -1.0 V vs Ag/AgCl at 0.91 sec, and E_3 is -0.30 V vs Ag/AgCl from 0.93 to 1.00 sec. E_2 and E_3 clean and restore the working electrode (Table 1). The waveform is applied continuously when the amperometric cell is turned on.

Chromatography

In the publication that reported the above waveform, the recommended eluent concentration was 62.5 mM sodium hydroxide.[11] In this experiment, the eluent concentration used was 63 mM sodium hydroxide, however, a 200 mM sodium hydroxide solution was prepared because it is easier to prepare consistently and requires less frequent preparation. The pump was programmed to proportion this prepared 200 mM sodium hydroxide solution to create the desired eluent concentration. Cyanide elutes at 5.8 min. Figure 2 shows both 5 and 10 µg/L cyanide standards in 100 mM sodium hydroxide.

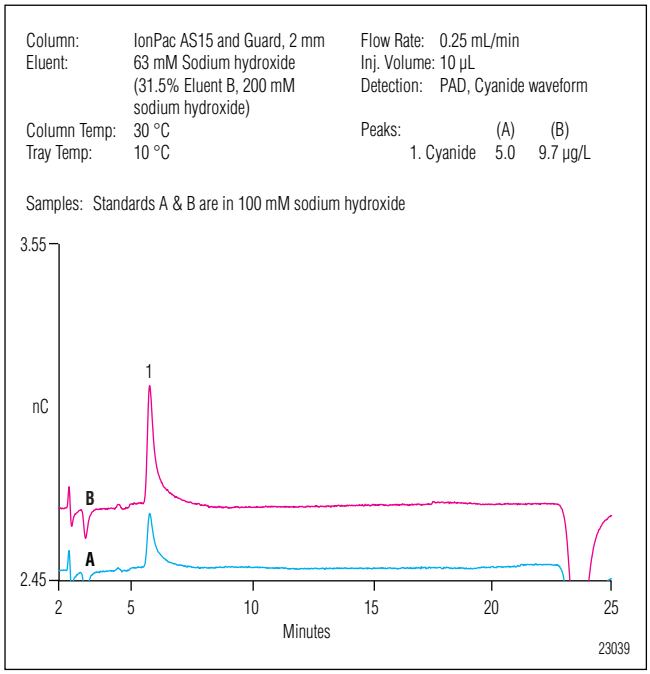

Figure 2. Cyanide standards in 100 mM sodium hydroxide.

INTERFERENCES

In the EPA methods, sulfide and sulfide-generating compounds are cited most often as potential interferences. Sulfide complexes with free cyanide to form thiocyanate. Other interferences cited are nitrate, nitrite, and chlorine. Copper and other transition metals complex with cyanide, preventing the measurement of free cyanide. Copper (II) rapidly oxidizes cyanide to cyanogen gas and copper (I) cyanide precipitates. Acidification will volatilize cyanide to the poisonous hydro-

Table 3. Effect of Bromide, Iodide, Sulfide, Sulfite, Thiocyanate, and Thiosulfate on Cyanide Recovery

Anion[a]	Recovery of 10 µg/L Cyanide
None	102.9 ± 2.3%
Bromide	99.9 ± 2.6%
Iodide	99.9 ± 2.9%
Sulfide	95.9 ± 1.7%
Sulfite	97.7 ± 2.5%
Thiocyanate	110.1 ± 2.6%
Thiosulfate	99.9 ± 2.7%

n=10 for each experiment.

[a] The concentration of each anion was 20 µg/L.

gen cyanide gas, thus preventing it from being measured as free cyanide. In this application, electroactive anions (iodide, thiosulfate, bromide, thiocyanate, and sulfide) are potential inferences, that is, anions detected using a silver working electrode and this waveform.

Possible Anionic Interferences

We determined the interference effects of the non-oxidized and partially oxidized sulfur-containing anions (sulfide, thiosulfate, thiocyanate, and sulfite), bromide, and iodide by analyzing solutions of 10 µg/L cyanide and 20 µg/L of each potential interfering anion. Table 3 shows that the free cyanide concentrations were not significantly affected by any of the anions. The free cyanide concentration did show a small decrease with sulfide and a small increase with thiocyanate. Sulfide and thiocyanate are not expected at high concentrations in drinking water. Because sulfide is not desirable in drinking water due to its disagreeable odor and taste, it is typically removed from municipal water systems by oxidation during the sanitation process. Noise levels always increase during the thiosulfate experiments because thiosulfate is a reducing agent and interacts with the working electrode. These experiments confirmed that the cyanide waveform detects thiosulfate, sulfide, and bromide under these conditions. Although sulfite, thiocyanate, and iodide are also potential interfering anions to cyanide, they were not detected by this waveform (sulfite and thiocyanate) or elute under these conditions (iodide). For applications requiring resolution of cyanide from sulfide, the IonPac AS7[22] column set should be selected.[9,10]

Metal Interferences

We determined the effects of dissolved iron, copper, and nickel on free cyanide determinations. We treated a 10 µg/L cyanide standard with each of the dissolved metal solutions and compared these three solutions to an untreated standard. We selected the iron concentration (600 µg/L) based on the expected levels in drinking water.[23] We arbitrarily set the copper and nickel concentrations for this experiment to 300 µg/L, 50% of the iron levels. We also treated the metal solutions with OnGuard II H cartridges to remove the metals and then added these solutions to cyanide standards.

The results show that copper and nickel reduce free cyanide concentrations. In the iron solution, the free cyanide concentration loss was comparable to the control, 7–10% over 3 days (Figure 3). After 92 h, only 28% of free cyanide remained in the copper solution (Figure 4). In the nickel solution, the free cyanide decreased to 75% within 20 h and then stabilized for the remainder of the 3-day experiment (Figure 5).

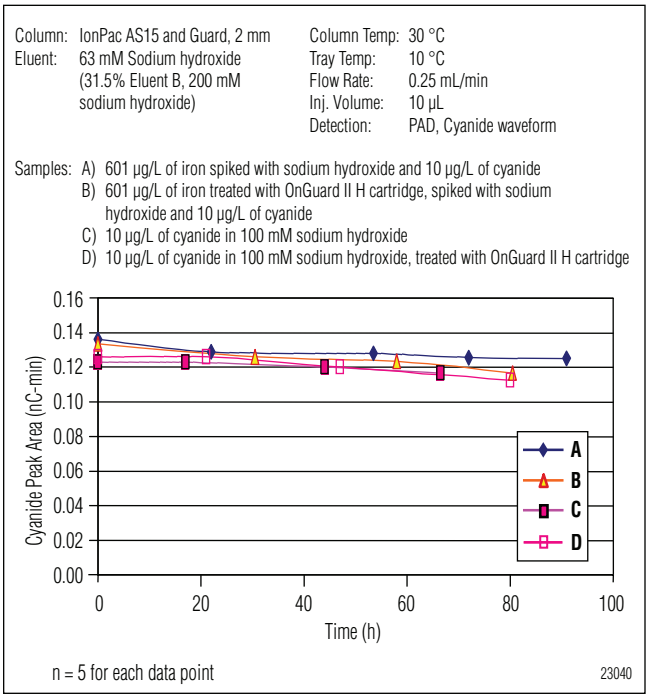

Figure 3. Effect of dissolved iron on free cyanide (10 µg/L).

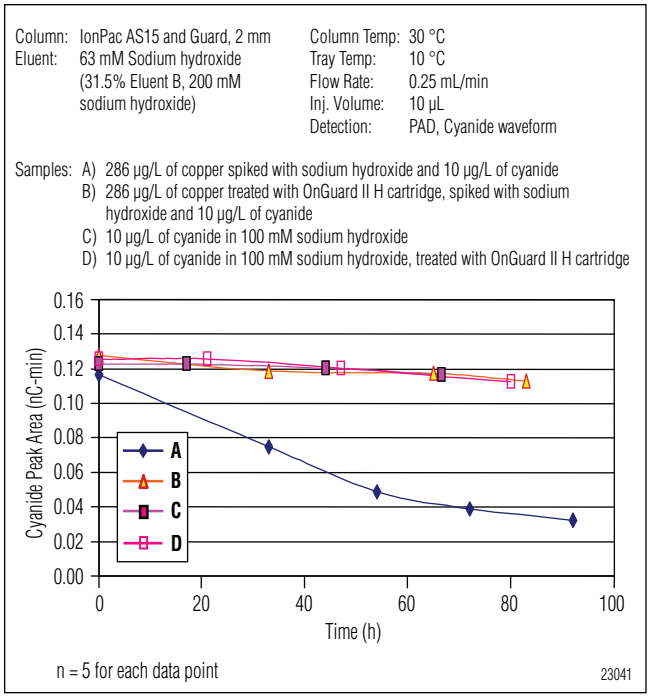

Figure 4. Effect of dissolved copper on free cyanide (10 µg/L).

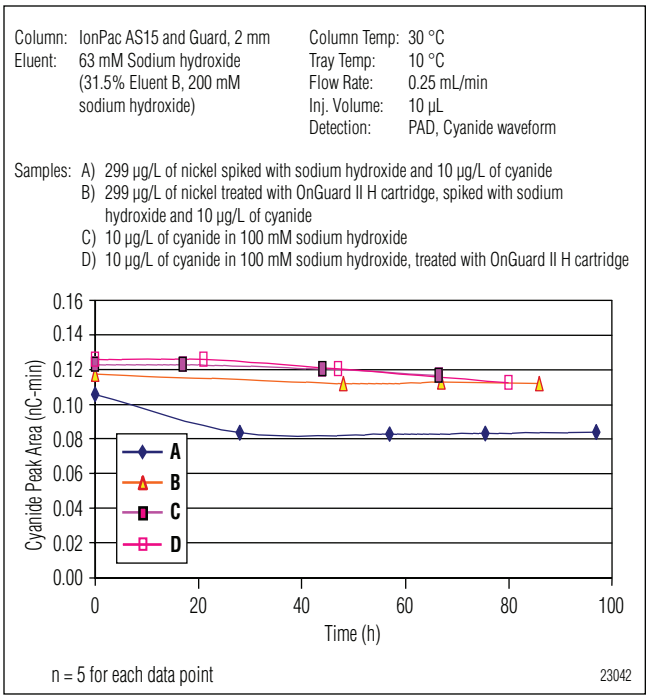

Figure 5. Effect of dissolved nickel on free cyanide (10 µg/L).

The cyanide control sample was not affected (102% ± 1% recovery) by the OnGuard II H treatment. Most importantly, the free cyanide concentration is as stable as the untreated standard when the metal-containing solutions are treated with the OnGuard II H cartridges (7–10% loss over three days) and, therefore, the cartridges effectively remove the dissolved metals. The results also confirm that the free cyanide concentration declines over three days and that the samples should be analyzed as soon as possible.

METHOD QUALIFICATION

The cyanide method was qualified prior to determining cyanide in real drinking water samples by determining the linearity over a 50-fold concentration range, typical noise, the method detection limit (MDL), reproducibility, and ruggedness. The linearity of cyanide response was determined by measuring cyanide in six replicates each of six standards (2.0, 3.0, 5.0, 10.0, 50.0, and 100 µg/L). The calibration results showed good linearity over this concentration range ($r^2 > 0.999$).

For each of the five disposable electrodes, the noise was determined over two 60-min runs, when no sample was injected, by measuring the noise in 1-min intervals from 5 to 60 min. The noise value determined by this experiment was 7.0 ± 1.8 pC (n = 10). The method detection limit (MDL) was defined as the peak in a standard with a peak height that is three times the noise level. For this application, the MDL was 1.0 µg/L. The signal to noise ratio of the 2.0 µg/L cyanide standard was 16.3 ± 4.8 (n = 10).

The reproducibility and ruggedness of the cyanide method was determined over 140 injections, ~62 h. During this study, cyanide in 10 µg/L cyanide standards was measured and the same standards spiked in copper, iron, and nickel metal solutions pretreated with OnGuard II H. Deionized water injections were inserted between the sample groups. The results (Figures 6 and 7) showed that retention time and peak areas were stable over 62 h of the experiment. The retention time and peak area reproducibilities were 5.78 ± 0.027 min and 0.1232 ± 0.0016 nC-min, respectively.

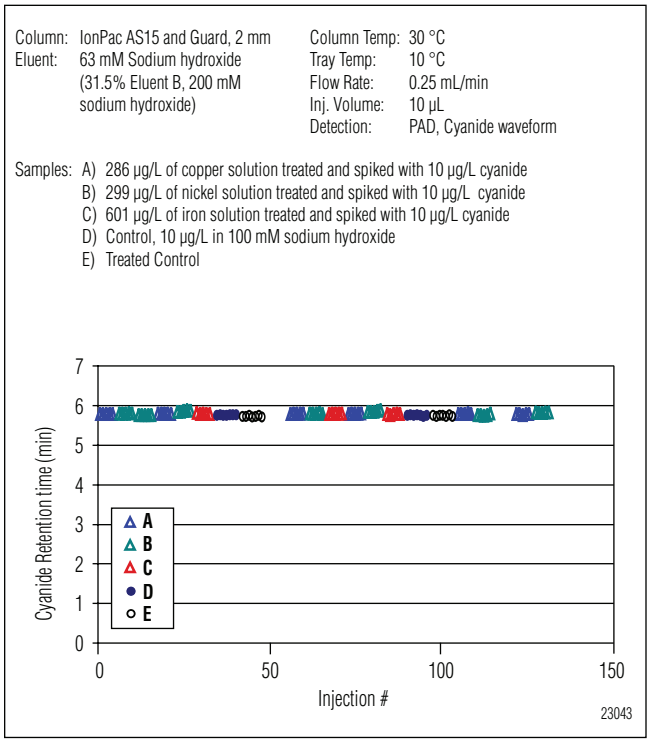

Figure 6. Retention time stability of 10 µg/L cyanide.

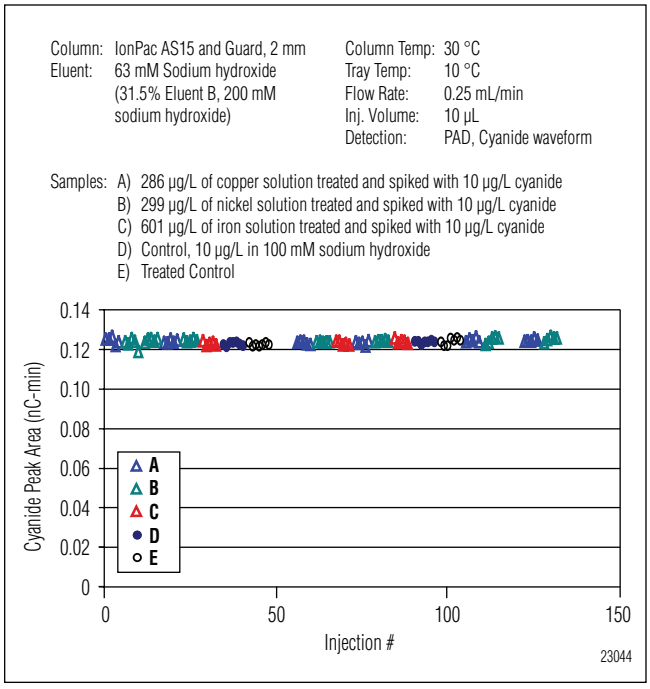

Figure 7. Peak area stability of 10 µg/L cyanide.

DETERMINATION OF CYANIDE IN DRINKING WATER AND SURFACE WATER

The characteristics of most drinking water change with the season. Free cyanide concentrations and spike recoveries of 5 and 10 µg/L cyanide in the City of Sunnyvale water were determined throughout the course of the application experiments and some changes were observed.

Sunnyvale drinking water sampled during the summer showed good spike recovery of cyanide for 5 µg/L and 10 µg/L of spiked cyanide (91.5 % ± 1.0% [n=10] and 98.2 % ± 1.7% [n=10], respectively). Cyanide also showed good recovery when 10 µg/L of sulfide was added (91.9% ± 1.7%). Spiking cyanide into 100 mM sodium hydroxide yielded similar recoveries. No free cyanide was measured in the unspiked samples of City of Sunnyvale drinking water (Figure 8).

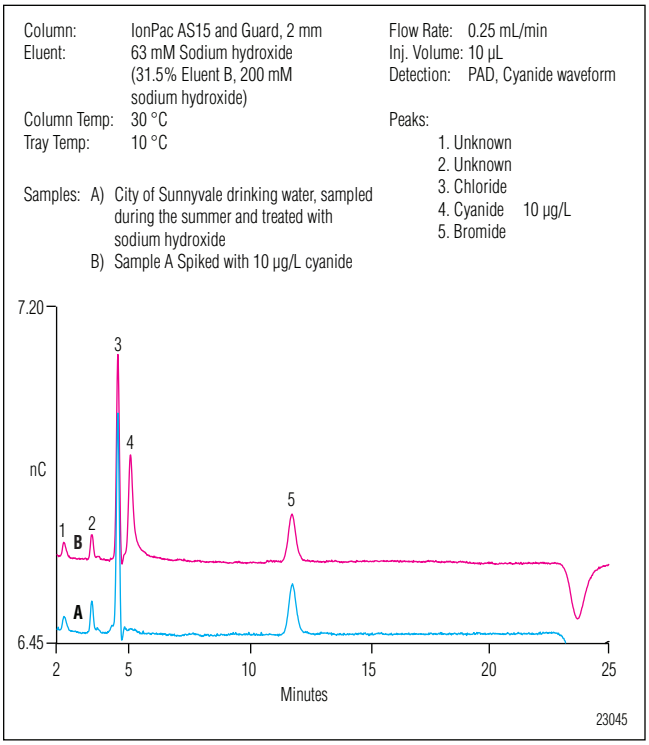

Figure 8. City of Sunnyvale drinking water with and without spiked cyanide.

Table 4. Recovery of Cyanide from Untreated Drinking Water and Surface Water Samples[a]

Concentration of Cyanide Spike (µg/L)	City of Sunnyvale Drinking Water[a]	City of San Jose Drinking Water	Alamitos Creek in Almaden
5	9.6 ± 3.0%	74.3 ± 11.8%	102.0 ± 1.3%
10	55.5 ± 2.8%	99.6 ± 0.5%	97.3 ± 2.4%

n=5 for each sample.
*Sampled during the fall months.

Table 5. Recovery of Cyanide in Treated Water Samples[a] (OnGuard II H Cartridges)

Concentration of Cyanide Spike (µg/L)	City of Sunnyvale Drinking Water	City of San Jose Drinking Water	Twain Harte Valley Drinking Water	Twain Harte Valley Flume
5	80.6 ± 5.5%	87.3 ± 6.4%	95.9 ± 2.5%	81.1 ± 3.2%
10	99.5 ± 2.8%	99.4 ± 2.5%	96.8 ± 3.1%	93.1 ± 1.5%

n=5 for each sample.
*Sampled during the fall months.

The method described in this document was used to measure free cyanide and the recovery of cyanide from two drinking water samples sampled in the fall (City of San Jose and City of Sunnyvale) and one surface-water sample (Alamitos Creek in the old Almaden mining region of San Jose) that were collected in the fall. The results showed no initial concentrations of free cyanide and variable recovery of cyanide spikes (Table 4). Only the Alamitos Creek surface water sample (Figure 9) exhibited acceptable recovery. The City of Sunnyvale drinking water had poor recovery at both spike levels (9.6% ± 3.0% and 55.5% ± 2.8%, for 5 µg/L and 10 µg/L cyanide, respectively). The City of San Jose drinking water samples had mixed results (74.3% ± 11.8% recovery of 5 µg/L and 99.6% ± 0.5% for 10 µg/L cyanide). These cyanide recovery results for the City of Sunnyvale sample were contradictory to the initial results (Figure 8). It is possible that City of Sunnyvale drinking water changed since the initial sampling. The cyanide recovery from the City of San Jose drinking water over time showed a trend similar to those observed with metal interferences (Figure 10). Therefore, the samples were treated with OnGuard II H cartridges and the recovery experiments repeated. Drinking and surface water samples were also analyzed from Twain Harte Valley, an old gold mining region. The results (Table 5) show good recovery for all samples (Figure 11–13) and good stability (> 84% of the initial peak response) for 31 h (not shown). No free cyanide was measured in any of the drinking or surface water samples.

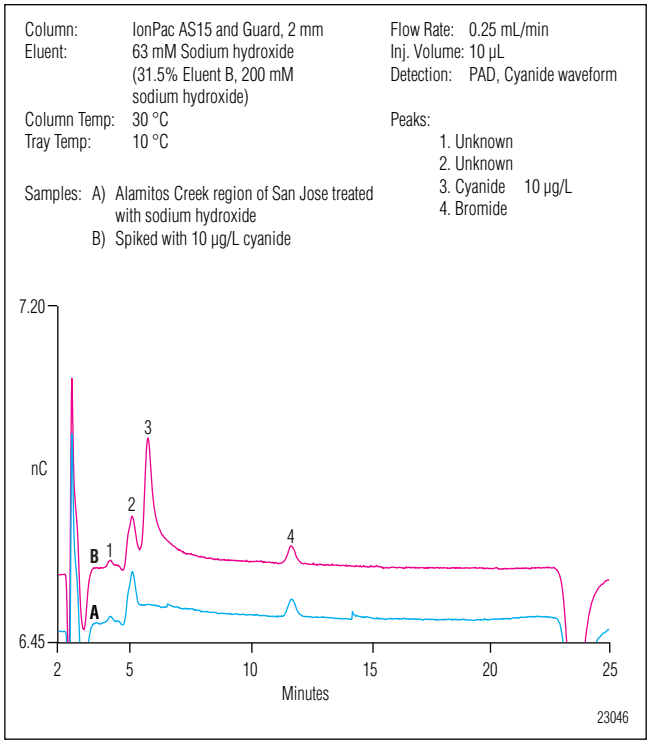

Figure 9. Alamitos Creek surface water sample with and without cyanide.

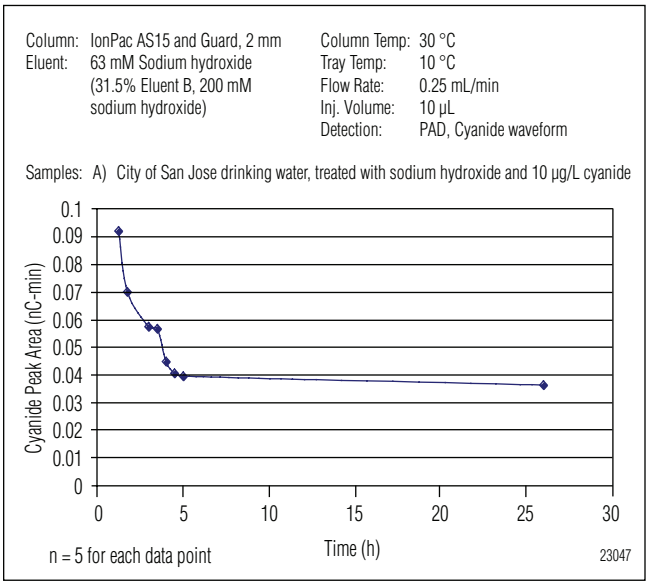

Figure 10. Degradation of cyanide spiked into the city of San Jose drinking water.

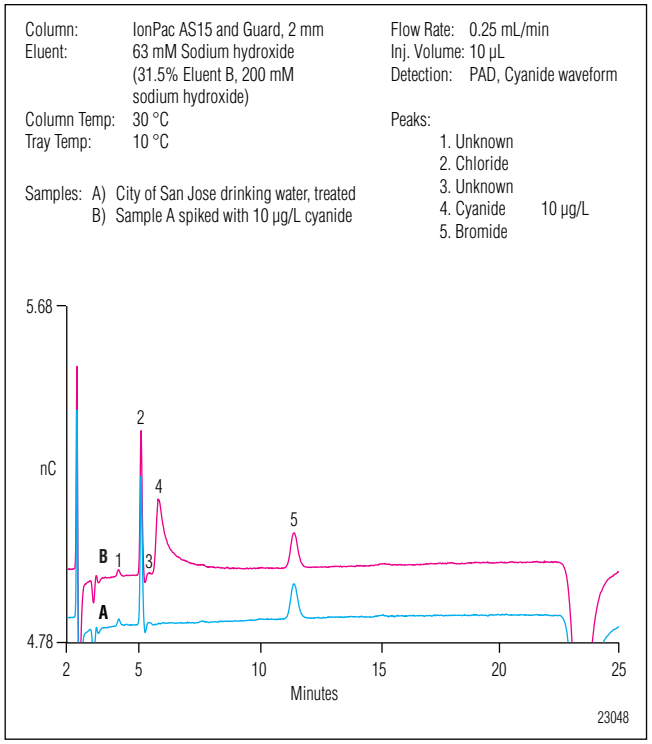

Figure 11. Treated city of San Jose drinking water with and without 10 µg/L of cyanide.

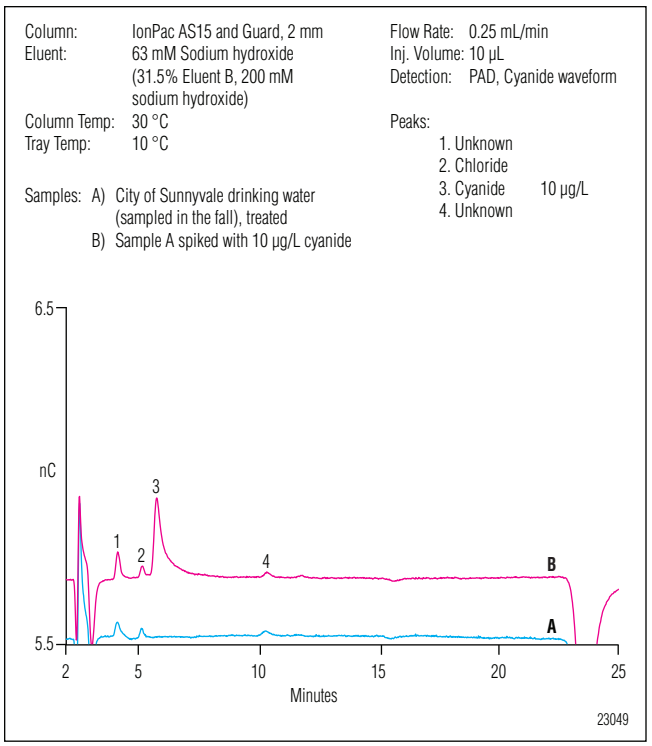

Figure 12. Treated city of Sunnyvale drinking water with and without 10 µg/L of cyanide.

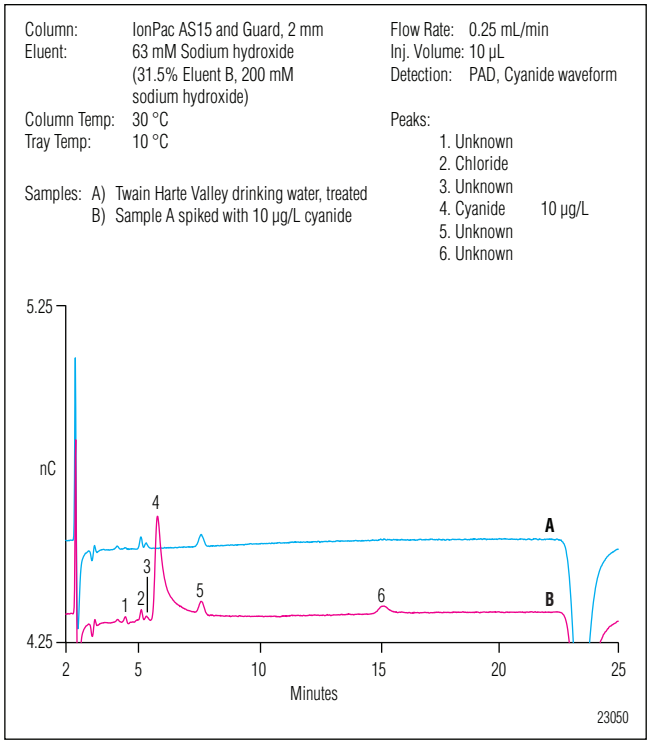

Figure 13. Treated city of Twain Harte Valley drinking water with and without 10 µg/L of cyanide.

DISPOSABLE SILVER WORKING ELECTRODES

In a published application using a waveform to determine iodide, the disposable silver working electrodes exhibited comparable or better reproducibility, linearity, and sensitivity than the conventional working silver electrodes.[24] The authors reported that they also saved time by discarding the disposable silver working electrodes at 80% of the peak response rather than re-polishing the conventional working electrode. In this study, the lifetimes of five disposable silver working electrodes were evaluated during the interference experiments, method qualification, and the testing of the municipal drinking water samples. Each electrode was installed, tested, and removed after two weeks of continuous use.

The average peak areas of 10 µg/L cyanide in 100 mM sodium hydroxide were compared over the five electrodes. The average peak area was 0.1206 ± 0.0038 nC-min, less than 1% variation. All five of the disposable silver working electrodes exceeded the 14-day lifetime specification (>80% of the peak response). Three of the five disposable silver working electrodes were only removed after three weeks so that another electrode could be tested. The other two electrodes showed >10 pC of noise during the last few days of operation.

CONCLUSION

Free cyanide can be determined in drinking water by IC-PAD. This method exhibits good sensitivity (MDL of 1 µg/L) and recovery, and exhibits linearity from 2 to 100 µg/L. This method can tolerate basic pH solutions, therefore it is believed that this method can determine cyanide in samples prepared for total cyanide determinations without dilution or neutralization of pH 13 distillation samples.

Transition metals can interfere with free cyanide determinations in drinking water. Dissolved transition metals are often present in drinking water and, therefore, it is a prudent to eliminate this possible interference by treating the water samples with OnGuard II H cartridges prior to analysis. Cyanide determinations in drinking water should always include spike recovery to ensure accurate determinations.

PRECAUTIONS

Warning: Cyanide is a poison. Never add cyanide to any solutions that have not been stabilized with base to a pH >9. Read and follow all safety precautions, handling, and waste disposal information prior to handling or using cyanide. Cyanide solutions cannot be poured into the water system without treatment. Consult your local safety representative for waste handling.

The Eluent Generator is not recommended as the eluent source for this application because of undesirably high noise levels.

Drinking and surface water samples should be stabilized immediately with sodium hydroxide. Cyanide solutions in 100 mM sodium hydroxide are stable for about one week. In drinking water samples without metal or other cation interferences, cyanide is stable for about three days.

Sodium hydroxide will etch glass and will foul the silver electrode, therefore, use only plastic pipettes, vials, and bottles for this application.

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SUPPLIERS

Fisher Scientific International Inc., Liberty Lane, Hampton, NH 03842 USA 1-800-766-7000. www.fisherscientific.com

Sigma-Aldrich Corp., St. Louis, MO, USA. 1-800-325-3010. www.sigmaaldrich.com

U.S. Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852 179, USA. 1-800-227-8772. www.usp.org

VWR International, Inc., Goshen Corporate Park West, 1310 Goshen Parkway, West Chester, PA 19380 USA 1-800-932-5000. www.vwrsp.com

Determination of Metal Cyanide Complexes by Ion Chromatography with On-Line Sample Preconcentration and UV Absorbance Detection

INTRODUCTION

Metal cyanides are negatively charged ionic complexes represented by the general formula $[M(CN)_b]^{x-}$, where several cyanide ions are bound to a single transition metal cation such as Ag^+, Au^+, or Fe^{2+}. Metal cyanides can dissociate to release highly toxic HCN into the environment. The toxicity of metal cyanides varies widely from one species to another. Weak metal cyanides that readily dissociate, such as $[Ag(CN)_2]^-$, pose a significant threat to health, whereas strong metal cyanide complexes that dissociate only under strongly acidic conditions, such as $[Fe(CN)_6]^{4-}$, pose a lesser risk.

Several methods measure free cyanide, but rely on some operational definition to distinguish between weak and strong cyanide complexes. Examples include methods that measure "cyanides amenable to chlorination",[1] "weak acid dissociable cyanides",[2] and "total cyanide"[3] by subjecting the sample to increasingly harsh conditions to dissociate some fraction of the cyanide complexes and liberate free cyanide. The definitions are imprecise and highly dependent on the matrix and procedure used. These methods also require time-consuming sample pretreatment, such as distillation to remove interferences, and even the distillate must be treated to remove interference from sulfides, chlorine, and thiosulfate.

Ion chromatography (IC) resolves each individual metal cyanide complex during an automated, 30-min separation. IC thus allows a precise differentiation of complexes of limited toxicity from those of greater toxicity.

Approval of a standard method based on IC will be of immediate benefit to those engaged in compliance monitoring or risk assessment of cyanide in the environment. To this end, we present an improved IC method that was subjected to a joint ASTM/EPA interlaboratory collaborative study to validate the use of IC for the determination of metal cyanide complexes in environmental waters. The metal cyanide complexes of silver, gold, copper, nickel, iron, and cobalt ($[Ag(CN)_2]^-$, $[Au(CN)_2]^-$, $[Cu(CN)_3]^{2-}$, $[Ni(CN)_4]^{2-}$, $Fe(CN)_6]^{4-}$, and $[Co(CN)_6]^{3-}$) are separated on an anion-exchange column and quantified by measuring their absorbance at 215 nm. Sensitivity for most of the metal cyanide complexes is improved by over two orders of magnitude, compared to a direct injection,[4] by preconcentrating metal cyanide complexes from a large sample volume onto a trap column before separation. The method was evaluated for reproducibility, linearity, accuracy, precision, and spike recovery from various environmental water matrices.[5]

EQUIPMENT

Dionex ICS-2500 IC system consisting of:

- GS50 Gradient Pump
- AD25 Absorbance Detector
- AS50 AutoSelect, PEEK, with Chromatography Compartment and Chemistry Switching Option
- AS50 Dual-Valve Needle Assembly (P/N 061267-01)
- Sample PREP Syringe, 10-mL (P/N 055068)

Chromeleon® Chromatography Workstation

DQP-1 Sample/Reagent Pump (P/N 035250)

Consumables

Syringe filters (Gelman IC Acrodisk® 0.2-µm, PN 4483)

Storage bottles, amber HDPE (VWR IRN301-0125 or 16172-144)

Trap Columns, Metal-Free MFC-1, 2 each (P/N 037017)

Vial Kit 10-mL polystyrene (P/N 055058)

CONDITIONS

Columns:	IonPac® AS11 Analytical, 2 × 250 mm (P/N 44076)
	IonPac AG11 Guard, 2 × 50 mm, 2 each (P/N 44078)
	IonPac ATC-3 (P/N 059660)
Temperature:	30 °C
Injection:	5 mL
Detection:	Absorbance at 215 nm
Expected System	
Backpressure:	850 psi
Noise:	1–5 mAU
Run Time:	32 min
Flow Rate:	0.25 mL/min
Eluent A:	20 mM sodium hydroxide/150 mM sodium cyanide
Eluent B:	20 mM sodium hydroxide/300 mM sodium perchlorate
Eluent C:	20 mM sodium hydroxide

Program:

0.000	Pressure.LowerLimit =	80
	Pressure.UpperLimit =	3000
	%A.Equate =	"Eluent A 150 mM NaCN"
	%B.Equate =	"Eluent B 300 mM $NaClO_4$"
	%C.Equate =	"Eluent C 20 mM NaOH"
	%D.Equate =	"%D"
	Sampler.Prime	Volume=2000, PrimeReservoir= Flush_Reservoir, PrimeSyringe=Sample
	SyringeSpeed =	2
	NeedleHeight =	2
	TrayTemperature =	Off
	Data_Collection_Rate =	5.00
	Rise_Time =	2.0
	Wavelength =	215
	UV_Lamp =	On
	Visible_Lamp =	Off
	WaitForTemperature =	False
	Col_B	
	Flow =	0.25
	%B =	10.0
	%C =	80.0
	%D =	0.0
	Curve =	5
	Load	
	Inject	
10.000	Col_A	
	Flow =	0.25
	%B =	10.0
	%C =	80.0
	%D =	0.0
	Curve =	5
10.200	Autozero	
	UV_VIS_1.AcqOn	
	Flow =	0.25
	%B =	10.0
	%C =	80.0
	%D =	0.0
	Curve =	5

```
28.200 Flow =          0.25
       %B =            45.0
       %C =            45.0
       %D =            0.0
       Curve =         5

32.200 UV_VIS_1.AcqOff
       Flow =          0.25
       %B =            45.0
       %C =            45.0
       %D =            0.0
       Curve =         5
       End
```

REAGENTS AND STANDARDS

Copper cyanide (AlfaAesar 12135)

Potassium dicyanoargentate (I) (AlfaAesar 12551)

Potassium dicyanoaurate (I) (AlfaAesar 12552)

Potassium ferrocyanide (II) trihydrate (Aldrich 22,768-4)

Potassium hexacyanocobaltate (III) (AlfaAesar 23126)

Potassium tetracyanonickelate (II) hydrate (Strem 93-2836)

Sodium cyanide, 99.99% (Aldrich 43,159-1)

Sodium hydroxide solution 50% w/w (Fisher SS254)

Sodium perchlorate monohydrate, HPLC-grade (Fisher S490)

PREPARATION OF SOLUTIONS AND REAGENTS

Caution: Sodium cyanide and some of the metal cyanide complexes are very toxic. Avoid contact with water or acid. Clean up and properly dispose of any spills.

Prepare all solutions from analytical reagent-grade chemicals. Use ASTM Type I reagent-grade water with a specific resistance of 18.0 MΩ-cm or greater. Filter the water through a 0.2-µm filter immediately before use and degas by sonicating under vacuum or sparging with helium for 10–15 min.

Always prepare sodium hydroxide eluents with 50% (w/w) sodium hydroxide solution. (Do not use sodium hydroxide pellets; they are covered with a thin layer of sodium carbonate that will cause irreproducible results). Keep all eluents blanketed under helium at 34–55 kPa (5–8 psi) after preparation. Properly dispose of old eluent and prepare fresh after one week.

Eluent Preparation

Eluent A (20 mM Sodium Hydroxide/150 mM Sodium Cyanide)

Place 14.70 g sodium cyanide into a 2-L volumetric flask containing 1.9 L of degassed reagent water. Use a plastic pipette to deliver 2.08 mL (or 3.2 g) of 50% (w/w) sodium hydroxide. Bring to volume with degassed reagent water. Cap and invert the volumetric flask eight times to mix. (Note: Do not excessively mix, as this will increase the carbonate ion in the solution by trapping carbon dioxide from the air). Blanket with helium as described above.

Eluent B (20 mM Sodium Hydroxide/300 mM Sodium Perchlorate)

Place 84.20 g HPLC-grade sodium perchlorate monohydrate ($NaClO_4 \cdot H_2O$) into a 2-L container containing 1.9 L of reagent water and degas for 20 min by sonicating under vacuum. Transfer to a 2-L volumetric flask. Use a plastic pipette to deliver 2.08 mL (or 3.2 g) of 50% (w/w) sodium hydroxide. Bring to volume with degassed reagent water. Cap and invert the volumetric flask eight times to mix. Blanket with helium as described above.

Eluent C (20 mM Sodium Hydroxide)

Add 2.08 mL (or 3.2 g) of 50% (w/w) sodium hydroxide to a 2-L volumetric flask containing 1.9 L of degassed reagent water. Bring to volume with degassed reagent water. Cap and invert the volumetric flask eight times to mix. Blanket with helium as described above.

Standard Preparation

Store the metal cyanide reagent salts in a dessicator protected from the light. Prepare 1000-mg/L stock standards of each metal cyanide complex by consulting Table 1. Weigh the reagent salt into a 100-mL volumetric flask. Add enough 20 mM sodium hydroxide solution to dissolve, bring to volume with the 20 mM sodium hydroxide solution, mix, and transfer to an amber HDPE bottle. Store at 4–6 °C. The individual stock standards are stable under these conditions for the periods shown in Table 1.

Table 1. Preparation of Metal Cyanide Stock Standards

Anion	Compound	Mass (g)	Stability** (days)
$[Ag(CN)_2]^-$	$KAg(CN)_2$	0.1244	1
$[Au(CN)_2]^-$	$KAu(CN)_2$	0.1157	30
$[Cu(CN)_3]^{2-}$	Cu(CN) and NaCN	0.0632*	1
$[Ni(CN)_4]^{2-}$	$K_2Ni(CN)_4 \bullet H_2O$	0.1591***	1
$[Fe(CN)_6]^{4-}$	$K_4Fe(CN)_6 \bullet 3H_2O$	0.1993	30
$[Co(CN)_6]^{3-}$	$K_3Co(CN)_6$	0.1546	30

* Dissolve the CuCN with 0.138 g sodium cyanide in a 100-mL volumetric flask containing 50 mL 20 mM sodium hydroxide solution. Bring to volume with 20 mM sodium hydroxide solution. Stir or sonicate for 1 h or until dissolved.

** Stability in number of days when stored in amber HDPE at 4–6 °C. Prepare fresh stock standards as needed according to this table.

*** Dissolve $(1.4806 + 0.1107 \times n)$ g of potassium nickel cyanide mono- or polyhydrate, $[K_2Ni(CN)_4] \bullet nH_2O$, where n = number of water molecules of hydration.

Prepare calibration standards spanning the linear calibration range of each analyte by diluting the 1000-mg/L stock standards with 20 mM sodium hydroxide solution. To prepare mixed standards, measure appropriate volumes of the 1000-mg/L standards into 100-mL volumetric flasks, bring to volume with the 20 mM sodium hydroxide solution, mix, and transfer to an amber HDPE bottle. These mixed calibration standards should be prepared fresh on the day of use.

Table 2 shows the concentration of calibration standards prepared in this way for the ASTM study.

SAMPLE PREPARATION

This section briefly summarizes the sample preparation procedure outlined in the ASTM/EPA draft method followed in the study. The ASTM study organizers provided six matrices and concentrated spiking solutions with instructions to spike each matrix at six levels. The six levels consisted of three pairs of closely spaced concentrations (Youden Pairs). The six matrices included reagent water, drinking water, groundwater, groundwater from a manufactured gas plant (MGP) site, surface water, and wastewater.

Upon collection, the samples were treated, if necessary, with powdered lead carbonate to remove sulfide interferences, and with sodium thiosulfate to remove interfering oxidants, in accordance with *Standard Methods*.[6] The samples were then adjusted with sodium hydroxide to pH = 12.5 and stored in amber bottles at 4–6 °C. Samples preserved in this manner must be analyzed within 14 days. On the day of analysis, the samples were brought to room temperature, spiked with the spiking solutions, and then filtered through 0.22-µm IC syringe filters into the autosampler vials.

Table 2. Metal Cyanide Calibration Standards

Anion	Concentration (µg/L)				
	Level 1	Level 2	Level 3	Level 4	Level 5
$[Ag(CN)_2]^-$	125	62.5	31.2	15.6	7.81
$[Au(CN)_2]^-$	100	50.0	25.0	12.5	6.25
$[Cu(CN)_3]^{2-}$	5.00	2.50	1.25	0.63	0.31
$[Ni(CN)_4]^{2-}$	100	50.0	25.0	12.5	6.25
$[Fe(CN)_6]^{4-}$	20.0	10.0	5.00	2.50	1.25
$[Co(CN)_6]^{3-}$	200	100	50.0	25.0	12.5

SYSTEM PREPARATION AND SETUP

Verify that the GS50 pump flow rate is within specifications and recalibrate if necessary. A GS50 should deliver water at 1.0 ± 0.005 mL/min against a constant backpressure of 2000 psi. The DQP-1 used for sample preconcentration performs best with moderate headpressure on its inlet side and backpressure on its outlet side. Pressurize the reagent water reservoir to 34–55 kPa (5–8 psi) with compressed air, nitrogen or helium. Plumb a 5–10 foot long piece of red 0.005-in. i.d. PEEK tubing between the DQP-1 and MFC-1 trap column, before the column valve. Adjust the length of the tubing to provide 100–200 psi of backpressure. Then adjust the DQP-1 pump to deliver a flow rate of 1 mL/min against the backpressure that results during the preconcentration step. The DQP-1 should be left on continuously during a day's run; refill the reagent water reservoir as needed. Verify that the UV-Vis absorbance detector wavelength accuracy is within specifications and recalibrate if necessary. Consult the pump or detector manuals for procedural details. Keep a record of the AD25 reference cell current and the UV lamp's elapsed time. This information may prove useful for troubleshooting.

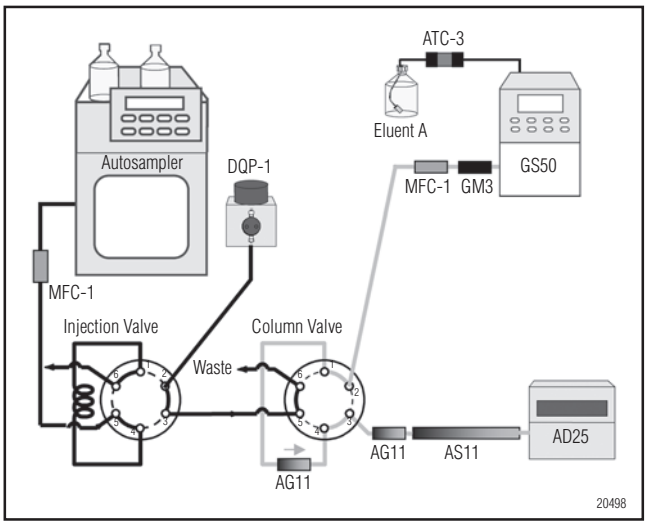

Figure 1. *Diagram of the system used for determination of metal cyanide complexes by IC with on-line sample preconcentration.*

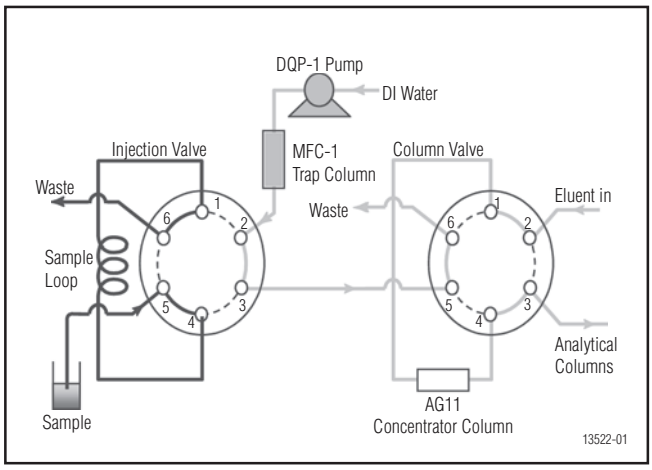

Figure 2. *Schematic of an IC system during loading of the sample loop.*

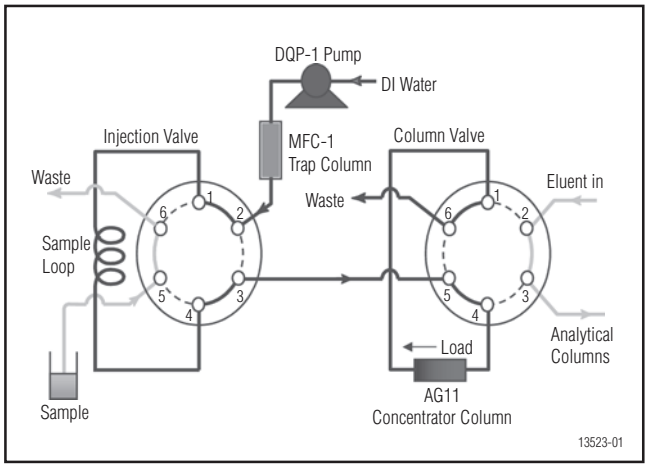

Figure 3. *Schematic of an IC system during preconcentration of the sample on the AG11 column.*

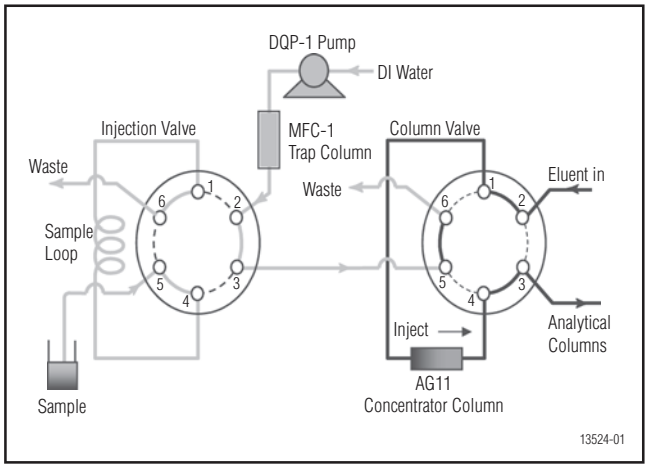

Figure 4. *Schematic of an IC system during separation of the metal cyanide complexes.*

Prepare the eluents and set up the system as depicted in Figure 1. Using this system, the AS50 uses 8 mL of sample to flush and fill the 5-mL sample loop. At Time = 0, the inject valve moves to the inject position, allowing the DQP-1 pump to preconcentrate the 5 mL of sample onto the AG11 concentrator column at 1 mL/min for 10 min. At Time = 10 min, the column valve switches to "Column A" to place the preconcentrator column in-line with the AG11/AS11 separatory columns. At Time = 10.2 min, the eluent gradient begins and the metal cyanide complexes are separated. See Figures 2–4 for details of the valve switching process.

Install an IonPac ATC-3 between eluent reservoir A and the pump inlet. The ATC-3 removes metal cyanide impurities present in the sodium cyanide solution that would otherwise cause elevated background noise. Regenerate the ATC-3 as needed by using a Trap Column/Suppressor Cleanup Kit (P/N 059659) according to the installation and instruction manual (Document No. 031835)

Install and configure the autosampler with the AS50 dual-valve needle assembly (P/N 061267). Install a 10-mL sample syringe and enter a "Sample Syringe Volume" of 1 mL in the AS50 Plumbing Configuration Screen. Install a 5-mL sample loop between ports 1 and 4 of the injection valve and enter a "Sample Loop Size" of 200 µL in the AS50 Plumbing Configuration Screen.

Table 3. Linear Ranges and MDLs for Metal Cyanide Complexes

Analyte	Range (µg/L)	MDL Standard (µg/L)	r^2	MDL* (µg/L)
$[Ag(CN)_2]^-$	8–125	15	0.99611	1.08
$[Au(CN)_2]^-$	6–100	10	0.99931	1.92
$[Cu(CN)_3]^{2-}$	0.3–5	0.8	0.98538	0.41
$[Ni(CN)_4]^{2-}$	6–100	50	0.99523	4.11
$[Fe(CN)_6]^{4-}$	1–20	1.0	0.99995	0.17
$[Co(CN)_6]^{3-}$	12–200	10	0.99999	2.20

* MDL = (t) × (S) Where t = Student's t value for a 99% confidence level and a standard deviation estimate with n – 1 degrees of freedom (t = 3.14 for seven replicates of the MDL Standard), and S = standard deviation of the replicate analysis.

When setting up the sequence, enter an "Injection Volume" of 200 µL. The AS50 will then draw 8 mL of sample and use it to fill the 5-mL sample loop. Set the "Syringe Speed" to 3. (Important: setting the syringe speed too high may cause the inject port to leak during loading of the sample loop.) Install the 2-mm AG11 preconcentrator column between ports 1 and 4 of the column valve. Refer to the operator's manual for the AutoSelect AS50 (Document No. 31169) and the AS50 large-volume sampling needle assembly instructions for details.

Install a 2 × 50 mm IonPac AG11 and 2 × 250 mm IonPac AS11 column. Rinse the column with the ending eluent composition (10:45:45) for 30 min. Equilibrate the column with the initial eluent composition (10:10:80) for 10 min before analyzing a system blank of deionized water. In an equilibrated system, the background shift during the gradient run should be less than 100 mAU. The peak-to-peak noise and drift should not exceed 5 mAU/min. There should be no significant peaks eluting within the retention time windows of the metal cyanide analyte anions.

Inject a mid-level standard, such as the Level 2 standard in Table 2. The column is equilibrated when two consecutive injections of the standard produce the same retention time for the metal cyanide complex anions. Confirm that the resulting chromatogram resembles the chromatogram of the standard shown in Figure 5B.

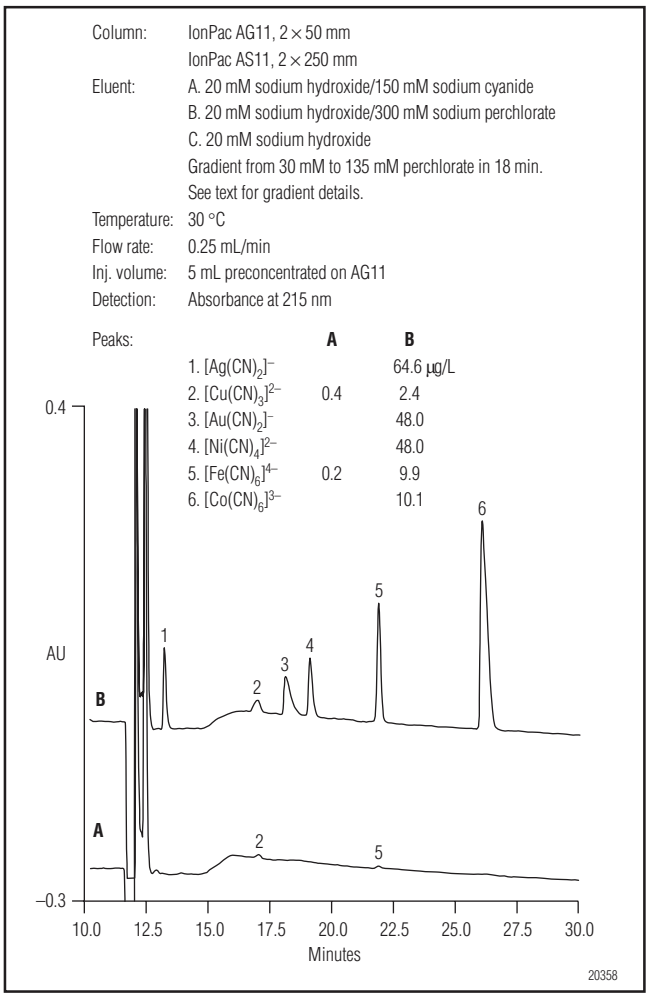

Figure 5. *Anion-exchange separation with preconcentration and absorbance detection at 215 nm of metal cyanide complexes in reagent water. Reagent water matrix blank (A) and matrix spiked with metal cyanide complexes as shown (B).*

RESULTS AND DISCUSSION

Calibrate the system by injecting one blank and at least five standards for every two decades of the calibration range. Plot the peak area for each metal cyanide complex versus the concentration injected, and use a linear regression to fit the data. Table 3 summarizes the calibration data for a typical calibration curve obtained by injecting calibration standards covering the ranges shown. The calibration curve is linear over about one and one-half orders of magnitude for each of the complexes. On the AS11, the copper cyanide, gold cyanide, and nickel cyanide complexes begin to coelute at higher concentrations. When running samples that contain a high concentration of either of these metal cyanide complexes, dilute the samples as needed to resolve these peaks. If necessary, modify the eluent gradient program to optimize the separation for a particular analysis.

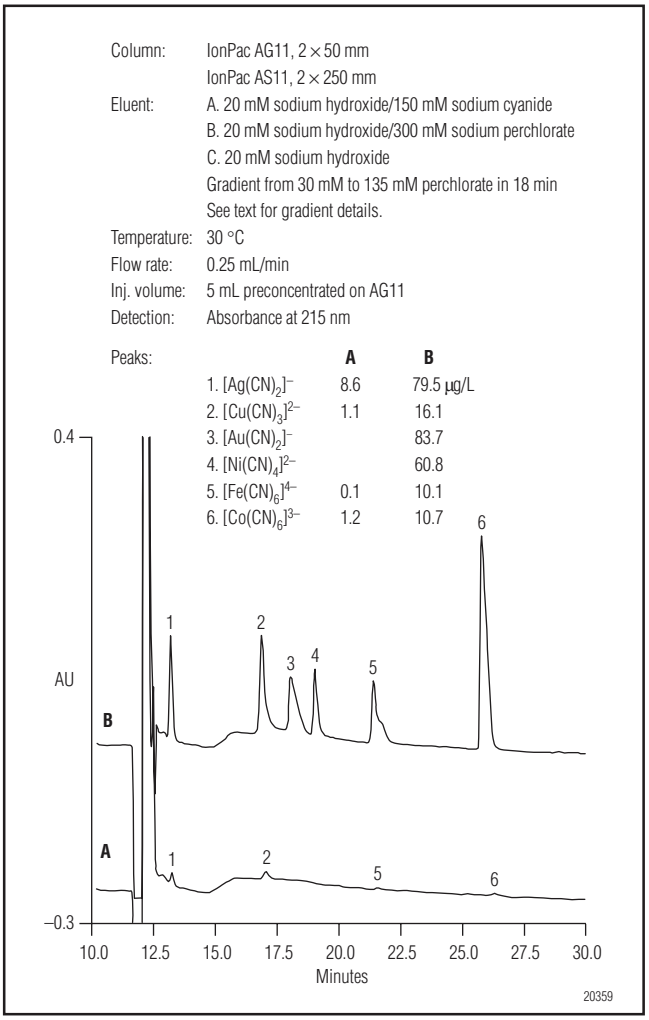

Figure 6. *Anion-exchange separation with preconcentration and absorbance detection at 215 nm of metal cyanide complexes in drinking water from a municipal well. Drinking water matrix blank (A) and matrix spiked with metal cyanide complexes (B) as shown.*

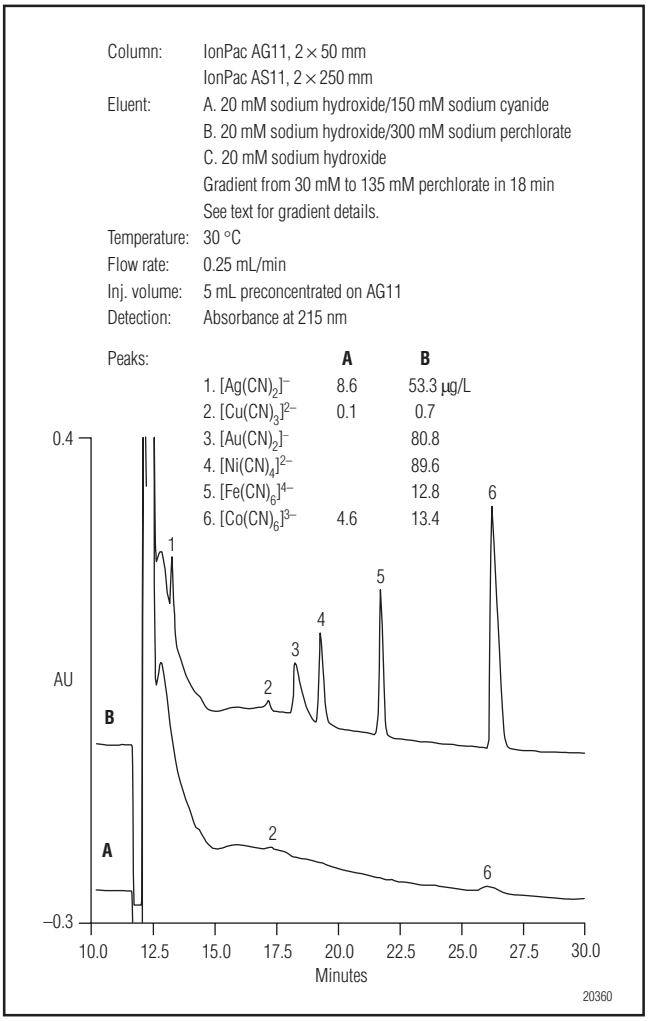

Figure 7. *Anion-exchange separation with preconcentration and absorbance detection at 215 nm of metal cyanide complexes in surface water from an industrial site. Surface water matrix blank (A) and matrix spiked with metal cyanide complexes (B) as shown.*

To determine method detection limits (MDLs) for this method, make seven injections of reagent water fortified with metal cyanide complexes at concentrations yielding peaks approximately five times higher than the background noise. In column 3 of Table 1, we list the concentrations of six metal cyanides analyzed for this application note, and the resulting MDLs. Note that the concentrations of the silver cyanide and nickel cyanide complexes were higher than recommended above—because the concentrations used were specified by the collaborative study organizers. (The formula given below Table 1 was used to calculate the MDL for each analyte.) MDLs are in the low-µg/L range for the metal cyanide complexes in reagent water, varying mostly because of differences in molar absorptivity at 215 nm among the metal cyanide complexes. At the low concentrations analyzed in this application note, a small interfering peak was observed to coelute with copper cyanide $[Cu(CN)_3]^{2-}$. This interferent increases the MDL for copper cyanide. Careful attention to regenerating the MFC-1 columns when necessary should keep this peak to a minimum.

Figures 5–8 are typical chromatograms obtained for each of the matrices analyzed in the ASTM/EPA collaborative study. Each figure displays the matrix blank along with the matrix spiked with metal cyanide complexes at concentrations approximating a mid-level standard. The actual concentrations vary somewhat because they were adjusted as needed to suit each matrix. Table 4 summarizes the spike concentrations and spike recovery data for each of the matrices. A few of the chromatograms are discussed below.

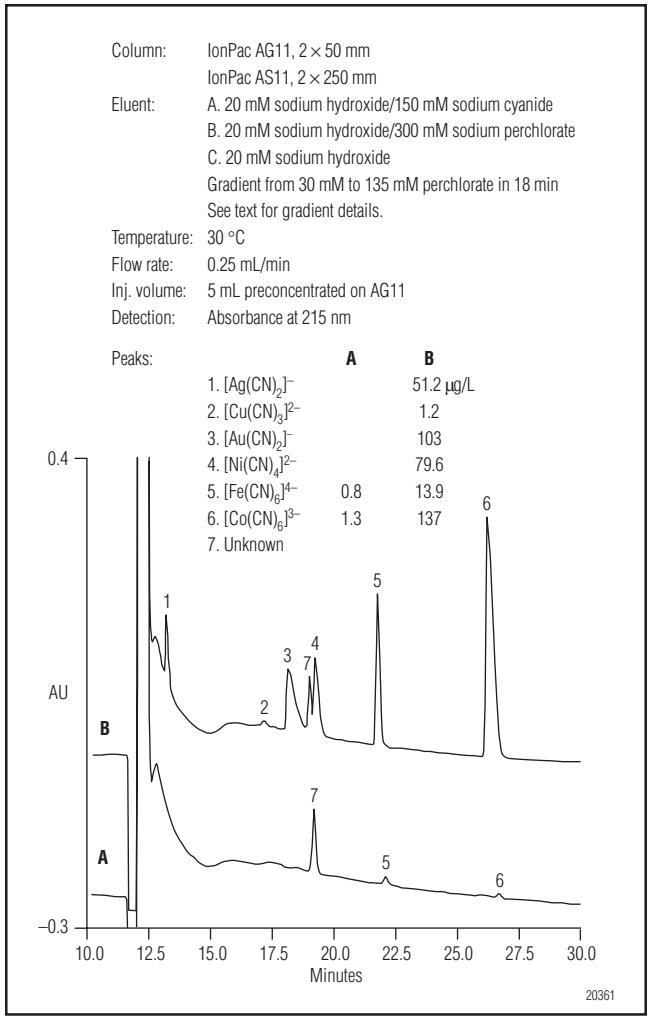

Figure 8. *Anion-exchange separation with preconcentration and absorbance detection at 215 nm of metal cyanide complexes in groundwater from a manufactured gas plant (MGP) site. Groundwater matrix blank (A) and matrix spiked with metal cyanide complexes (B) as shown.*

Figure 5A is the chromatogram of a reagent water blank and Figure 5B is reagent water fortified with metal cyanides. Table 4 summarizes the percent recovery of metal cyanides spiked into this matrix.

Figure 6A is the chromatogram of drinking water from a municipal well. The matrix blank shows some traces of the silver, copper, iron, and cobalt cyanides. The spiked matrix yielded good recoveries for all the analytes, except copper cyanide, which was biased high. A matrix interferent is present just after the iron cyanide peak in Figure 6B.

Figure 7A is the chromatogram of a surface water sample from an industrial waste site. The matrix ions elute as a broad, tailing peak in this sample. This matrix may contain significant concentrations of bicarbonate or

Table 4. Recovery of Metal Cyanide Complexes from Environmental Waters

Anion	% Recovery				
	Deionized Water	Drinking Water	Surface Water	Ground-water	Waste-water
$[Ag(CN)_2]^-$	63.6	82.3	54.3	51.1	30.84
$[Au(CN)_2]^-$	107	110	82.4	120	92.6
$[Cu(CN)_3]^{2-}$	121	408	41.9	18.0	38.0
$[Ni(CN)_4]^{2-}$	99.4	76.3	88.2	104	2.52
$[Fe(CN)_6]^{4-}$	101	99.9	100	99.5	97.2
$[Co(CN)_6]^{3-}$	99.0	105	105	100	99.9

UV-absorbing organic substances. Traces of copper and cobalt cyanide complexes are observed in the matrix blank. In the spiked sample of Figure 7B, the matrix peak adversely affects the recovery of silver cyanide that elutes on the matrix peak's tailing baseline, and copper cyanide is biased low, as discussed below. The other metal cyanide complexes are unaffected and show good recoveries in Table 4.

Figure 8A is the chromatogram of a groundwater sample from a manufactured gas plant (MGP) site. This chromatogram exhibits tailing of the initial matrix ion peak similar to that observed in the surface water sample. Traces of the iron and cobalt cyanide complexes are seen in the matrix blank, along with a prominent unknown that elutes at 19–20 min. This peak has been observed in many samples taken from old MGP sites, and has been tentatively identified as a different iron cyanide complex.

Interferences

Exposure to light causes photodecomposition of some metal cyanide complexes and reduces their concentration. Protect samples and standards from UV light by storing them in amber HDPE containers.

This method assumes that the alkaline conditions used will reduce iron (III) cyanide (ferricyanide) to iron (II) cyanide (ferrocyanide), resulting in a single peak for $[Fe(CN)_6]^{4-}$. Calibration is based on the iron (II) cyanide complex. Report results as mg/L of $[Fe(CN)_6]^{3-/4-}$ so as to represent the sum of ferrocyanide and ferricyanide. If unreduced $[Fe(CN)_6]^{3-}$ is present, it elutes as a tail on the iron ferrocyanide peak.

The sodium cyanide used to prepare Eluent A may contain metal cyanide complex impurities. Reduce the level of these impurities by using 99.99% sodium cyanide to prepare the eluent, and by installing an ATC-3 anion trap column as described in this application note.

Precautions

Prepare all cyanide-containing solutions within a ventilation hood. Wear gloves, avoid inhalation, and avoid skin or eye contact. Do not let acid contact any of the cyanide-containing samples, standards, or eluents. Such contact will liberate hydrogen cyanide gas, an extremely toxic substance. Dispose of the eluent waste in accordance with applicable laws.

Strongly retained compounds from injected samples can accumulate on the column and degrade its performance. Signs of a fouled column include loss of capacity, loss of resolution, shortened retention times, higher noise and background, spurious peaks, and peak tailing. The AS11 column can be flushed with up to 100% acetonitrile to help remove contaminants from the column. For more information on column troubleshooting and cleanup, see the *Installation Instructions and Troubleshooting Guide for the IonPac AS11 Analytical Column* (Document No. 034791).

Some samples contain particulates that will plug the column and increase the backpressure. Use a guard column to protect the analytical column; change the guard column if such a sample causes a sudden increase in total backpressure to greater than 3000 psi.

CONCLUSION

This method of on-line preconcentration allows determination of metal cyanide complexes at µg/L concentrations in a variety of environmental water matrices. This method provides good recoveries for the gold, iron, and cobalt cyanide complexes in all matrices studied, and for the nickel cyanide complex in all matrices except wastewater. This method shows increased bias for the silver and copper cyanide complexes, especially in higher-ionic-strength matrices. The low recoveries for the early-eluting silver cyanide complex result from interference by the matrix ions peak, and possibly some loss of the silver cyanide complex from the concentrator column during preconcentration. Low recovery of the copper cyanide complex may result from dissociation of the complex in high-ionic-strength matrices; an unknown coeluting species may impair determination of low concentrations of the copper cyanide complex.

REFERENCES

1. *Standard Methods for the Examination of Water and Wastewater.* 17th Edition, 1989. APHA-AWWA-WPCF. 4500-CN B., pp 4–34.
2. Ibid, p 38.
3. Ibid, p 28.
4. Dionex Corporation. Application Update 147; Sunnyvale, CA.
5. *WK2791 Standard Test Method for Determination of Metal Cyanide Complexes in Wastewater, Surface Water, Groundwater and Drinking Water using Anion Exchange Chromatography with UV Detection;* ASTM International; West Conshohocken, PA.
6. *Standard Methods for the Examination of Water and Wastewater.* 17th Edition, 1989. APHA-AWWA-WPCF. 4500-CN B., pp 4–25.

LIST OF SUPPLIERS

Aldrich Chemical Company, Inc., 1001 West Saint Paul Avenue, P.O. Box 355, Milwaukee, WI 53233 USA, Tel: 800-558-9160, www.aldrich.sial.com.

Alfa Aesar, 30 Bond St., Ward Hill, MA 01835 USA, Tel: 800-343-0660, www.alfa.com.

Fisher Scientific, 2000 Park Lane, Pittsburgh, PA 15275-1126 USA, Tel: 800-766-7000, www.fishersci.com.

Strem Chemical, 7 Mulliken Way, Newburyport, MA 01950-4098 USA, Tel.: 1-800-647-8736, www.strem.com.

VWR Scientific Products, 1310 Goshen Parkway, West Chester, PA 19380 USA, Tel: 800-932-5000, www.vwr.com.

Determination of Metal Cyanides

INTRODUCTION

Transition metal cyanides typically exist in solution as the anionic cyanometallates, $M(CN)_x^{n-}$. Since these complexes are very stable (formation constants being as high as 10^{35} for $Fe(CN)_6^{4-}$), they may be separated by anion exchange chromatography.

Cyano complexes of most of the transition metals absorb low-wavelength ultraviolet light, making detection at ca 215 nm a convenient method for determination.

STANDARDS

The standards for this method should be sodium or potassium salts of the cyanometallates. The table below lists available potassium salts as well as possible suppliers and amounts necessary to prepare 100 mL aqueous standards with metal concentrations of 1000 ppm. No cyanocuprate is available, although copper(I) cyanide (CuCN) will slowly dissolve in water containing sodium cyanide. Atomic absorption standards are not recommended for use as cyanometallate standards.

Reagent	Mass per 100 mL Solution	Supplier
$KAg(CN)_2$	0.185 gram	Aesar (800-343-1990)
$KAu(CN)_2$	0.146	Aesar
CuCN	0.136*	Alfa (800-343-0660)
$K_2Ni(CN)_4$	0.411	Strem (617-462-3191)
$K_4Fe(CN)_6 \cdot 3H_2O$	0.756	Alfa
$K_2Pd(CN)_4 \cdot 3H_2O$	0.322	Alfa
$K_3Co(CN)_6$	0.564	Alfa
$K_2Pt(CN)_4 \cdot 3H_2O$	0.221	Aesar

*Dissolve the CuCN with 0.300 g sodium cyanide in 50 mL of water, then dilute the solution to 100 mL. All other reagents should be dissolved in water and diluted to 100 mL.

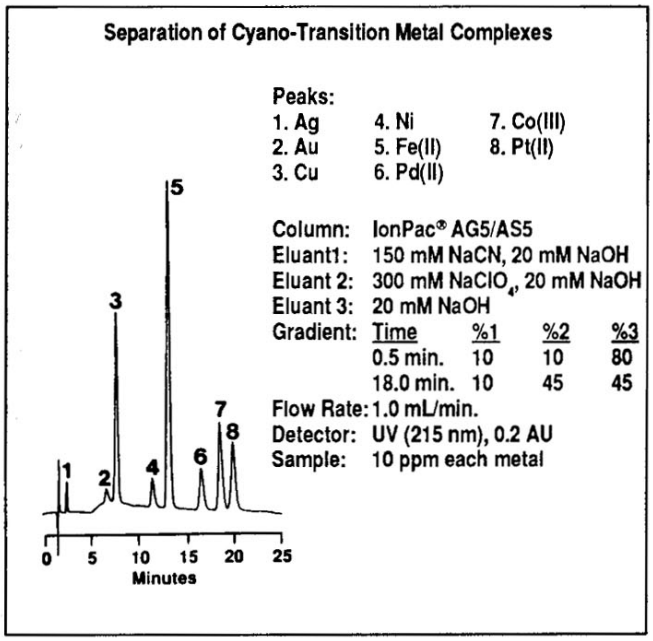

Figure 1

ANALYTES

$Ag(CN)_2^-$, $Au(CN)_{2n}^-$, $Cu(CN)_3^{2-}$, $Ni(CN)_4^{2-}$, $Fe(CN)_6^{4-}$, $Pd(CN)_4^{2-}$, $Co(CN)_6^{3-}$ and $Pt(CN)_4^{2-}$.

DISCUSSION OF METHOD

The anionic cyanometallates are separated on the IonPac® AS5 column using an 18-minute gradient of 30 to 135 mM perchlorate (Figure 1). Hydroxide and cyanide concentrations are constant at 20 mM and 15 mM, respectively, and are present to maintain the integrity of the complexes.

Form of metal anion The silver, gold, copper and nickel peaks are measures of total metal. Silver is present as $Ag(I)(CN)^-_2$. If present, Cu(II) would be reduced to Cu(I) under the chromatographic conditions. $Au(I)(CN)_2^-$ and $Au(III)(CN)_4^-$ display identical chromatograms, so one is probably converted to the other. The cyanocuprate ion behaves as the dianion, $Cu(CN)_3^{2-}$. Nickle is present as $Ni(II)(CN)_4^{2-}$.

$Fe(III)(CN)_6^{3-}$ is slowly reduced to $Fe(II)(CN)_6^{4-}$ by the high pH of metal cyanide samples, hence iron will usually be in the reduced form exclusively. $Fe(III)(CN)_6^{3-}$, if present, elutes as a tail of the Fe(II) peak.

Cobalt, on the other hand, is present in the oxidized form in fresh samples and remains as $Co(III)(CN)_6^{3-}$ for months before slowly reducing to a Co(II) complex. $Pd(II)(CN)_4^{2-}$ and $Pt(II)(CN)_4^{2-}$ are very inert dianions, which do not interconvert with the more oxidized $Pd(IV)(CN)_6^{2-}$ and $Pt(IV)(CN)_6^{2-}$. Furthermore, the Pd(IV) and Pt(IV) complexes do not strongly absorb ultraviolet light so they cannot be detected by this method.

It is possible to alter the gradient to suit individual requirements. For example, if better separation of Ag, Au and Cu is needed, starting with weaker perchlorate or delaying the start of the gradient will accomplish that. If only strongly retained complexes such as Pd or Pt are of interest, isocratic conditions at about 120 mM perchlorate may be more desirable. In any case, it is important to elute all complexes after each analysis to avoid loss of column capacity. This will usually involve a gradient to remove Fe and Co. The standard injection loop size is 50 µL; however, injections of 500 µL may be made with equal success.

RECOMMENDED EQUIPMENT

Dionex Series 4500i or 4000i Ion Chromatograph with a VDM-I, VDM-II or UDM.

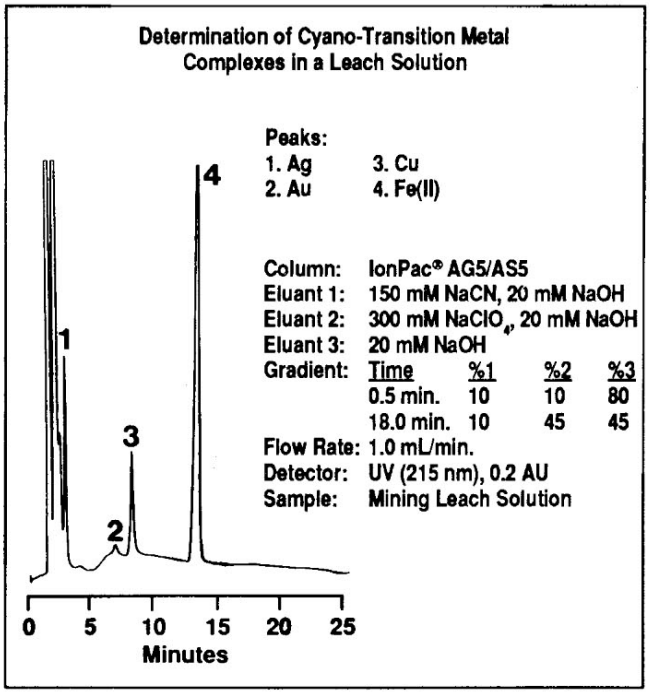

Figure 2

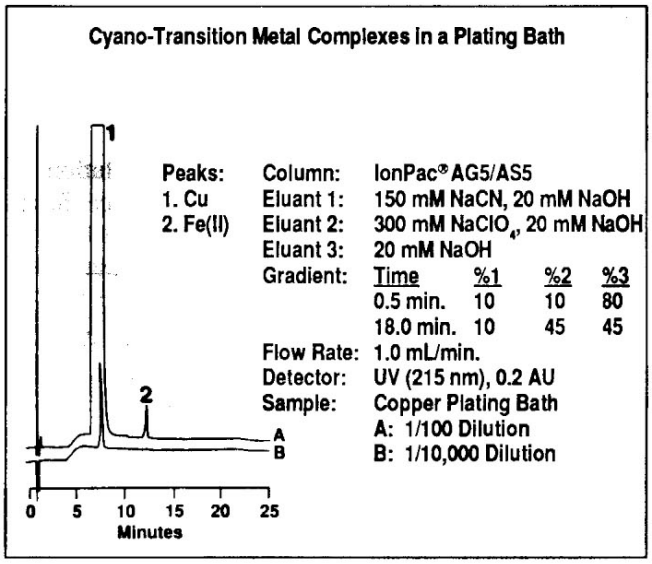

Figure 3

PRECAUTIONS

It is recommended that gloves be worn when working with any cyanide. Under no circumstance should acid contact cyanide; this would generate hydrogen cyanide gas, an extremely toxic substance. All work should be done in a well-ventilated area. It is advisable to add sodium hypochlorite (household bleach) to the eluant waste container in order to destroy cyanide.

CONDITIONS

Sample Loop Volume:	50 µL
Guard Column:	IonPac AG5
Separator Column:	IonPac AS5
Eluant 1:	20 mM NaOH, 150 mM NaCN
Eluant 2:	20 mM NaOH, 300 mM $NaClO_4$
Eluant 3:	20 mM NaOH
Eluant Purifier (Eluant 1):	1.0 cm × 10 cm low pressure column body filled with Bio-Rad® AG®2-X8 or equivalent
Flow Rate:	1.0 mL/min.
Expected Pressure:	700–900 psi
Detector Wavelength:	215 nm

Gradient Program:

Time min.	Eluant 1	Eluant 2	Eluant 3
0.0	10%	10%	80%
0.5	10	10	80
18.0	10	45	45

Reset the GPM to initial conditions after the analysis is complete. Hold at initial conditions for 10 minutes before the next injection is made.

SOLUTIONS AND REAGENTS

Eluant 1: 20 mM NaOH, 150 mM NaCN
Dilute 1.6 g of sodium hydroxide solution (50%, low carbonate) and dissolve 7.35 g of sodium cyanide (ACS reagent grade) in enough 18 M-ohm deionized water to make 1.0 L of solution.

Eluant 2: 20 mM NaOH, 300 mM NaCN
Dilute 1.6 g of sodium hydroxide solution (50%, low carbonate) and dissolve 36.7 g of sodium perchlorate (Fisher HPLC grade) in enough 18 M-ohm deionized water to make 1.0 L of solution.

Eluant 3: 20 mM NaOH
Dilute 1.6 g of sodium hydroxide solution (50%, low carbonate) in enough 18 M-ohm deionized water to make 1.0 L of solution.

Commercial sodium cyanide contains some cyanometallate impurities which interfere with the analysis. These may be removed by placing a low pressure anion exchange column between Eluant 1 and the GPM. Use the Iron Guard Column Kit (P/N 042021) filled with a high-capacity anion exchange resin (Bio-Rad AG2-X8 or equivalent). After filling the column casing with dry resin (ca 6 g), the resin is conditioned by passing 400 mL of 500 mM NaOH followed by 300 mL of Eluant 1 through the column. A column conditioned in this manner will purify at least 3 L of Eluant 1.

SAMPLE PREPARATION

This analytical method is most suited to alkaline samples containing excess cyanide, e.g., mining and metal finishing samples. Often, the samples need only be filtered through a 1.0 μ or smaller filter. If high concentrations of metals are present, the sample may have to be diluted with deionized (18 M-ohm) water. No further pretreatment of the sample should be necessary. Figures 2 and 3 are typical sample chromatograms.

Determination of Metal Cyanide Complexes in Solid Wastes by Anion-Exchange Chromatography with UV Absorbance Detection

Metal cyanides are negatively charged ionic complexes represented by the general formula $[M(CN)_b]^{x-}$, where one or several cyanide ions are bound to a single transition metal cation such as Ag^+, Au^+, or Fe^{2+}. The environmental impact of metal cyanides varies widely from one species to another depending on how readily they release free cyanide (HCN/CN^-) into the environment. Several indirect methods are available for determining free and complexed cyanide.[1] However, anion-exchange separation of these complexes is the only method available that specifically identifies and quantifies each metal cyanide complex in a sample. Previous Dionex application documents describe the determination of metal cyanide complexes in metal finishing and mining wastes[2] and environmental waters at mg/L[3] or µg/L concentrations.[4]

This application update describes the determination of the metal cyanide complexes of iron, cobalt, silver, gold, copper, and nickel in solid wastes by anion-exchange chromatography with UV absorbance detection. Metal cyanide complexes are solubilized and recovered by an alkaline extraction procedure (SW846 Method 9013)[5] prior to chromatographic analysis. Two analytical approaches are available depending on the concentration of metal cyanides expected in the leachate; the two methods differ only in how the sample is injected:

- Metal cyanide complex concentrations between 0.20–200 mg/L are determined by direct injection of the sample. This range is approximate and depends on the detection sensitivity, which varies among the analytes. Concentrations exceeding the linear calibration range may be determined after appropriate dilution.
- Metal cyanide complex concentrations less than 0.20 mg/L are determined by on-line sample preconcentration. This application update uses a simpler and more direct approach to preconcentration than that described in Dionex Application Note 161.[4] The sample is directly applied to the concentrator column by the AS50 sample syringe, eliminating the sample loop, column valve, and extra pump required by Application 161.

This application update also summarizes expected method performance as determined during participation in an interlaboratory collaborative study to validate the use of anion-exchange chromatography for the determination of metal cyanide complexes in solid wastes. The method was evaluated for sensitivity, linearity, accuracy, precision, and spike recovery from various matrices.[6]

EQUIPMENT

Dionex ICS-2500 IC system consisting of:
- GS50 Gradient Pump
- AD25 Absorbance Detector

AutoSelect™ AS50 Autosampler (USB)

AS50 Dual-Valve Needle Assembly (P/N 061267)

Sample PREP Syringe, 10-mL (P/N 055068)

Chromeleon® Chromatography Workstation (Version 6.6 or higher)

CONSUMABLES

Syringe filters (Gelman IC Acrodisk® 0.2-µm, PN 4483)

Storage bottles, amber HDPE (VWR IRN301-0125 or 16172-144)

Vial Kit 10-mL polystyrene (Dionex P/N 055058)

GM-4 Gradient Mixer (Dionex P/N 49135)

CONDITIONS

Columns:	Analytical: IonPac® AS11 2 x 250 mm (P/N 44077)
	Guard: IonPac AG11 2 x 50 mm (P/N 44079)
	Concentrator: IonPac AG11 Guard 4 x 50 mm (P/N 44078)
	Trap: IonPac ATC-3 (P/N 059660)
	Trap: MFC-1 (P/N 37017), 2
Temperature:	35 °C (or ambient)
Injection:	100 µL (or 5 mL concentrated on 4-mm AG11 column)
Detection:	Absorbance at 215 nm
System Pressure:	~850 psi
Noise:	1–5 mAU
Run Time:	32 min
Flow Rate:	0.25 mL/min
Eluent A:	20 mM sodium hydroxide/150 mM sodium cyanide
Eluent B:	20 mM sodium hydroxide /300 mM sodium perchlorate
Eluent C:	20 mM sodium hydroxide

Pump Program:

Time (min)	*%A*	*%B*	*%C*
Init.	10	10	80
0.0	10	10	80
18.0	10	45	45
22.0	10	45	45
25.0	10	10	80
35.0	10	10	80

REAGENTS AND STANDARDS

Copper cyanide (AlfaAesar 12135)

Potassium dicyanoargentate (I) (AlfaAesar 12551)

Potassium dicyanoaurate (I) (AlfaAesar 12552)

Potassium ferrocyanide (II) trihydrate (Aldrich 22,768-4)

Potassium hexacyanocobaltate (III) (AlfaAesar 23126)

Potassium tetracyanonickelate (II) hydrate (Strem 93-2836)

Sodium cyanide, 99.99 % (Aldrich 43,159-1)

Sodium hydroxide solution, 50% (w/w) (Fisher SS254)

Sodium perchlorate monohydrate, HPLC-grade (Fisher S490)

PREPARATION OF SOLUTIONS AND REAGENTS

Precaution: Sodium cyanide and some of the metal cyanide complexes are very toxic. Avoid contact with water or acid. Clean up and properly dispose of any spills.

Prepare all solutions from analytical reagent-grade chemicals. Use ASTM Type I reagent-grade water with a specific resistance of 18.0 MΩ-cm or greater. Filter the water through a 0.2-µm filter immediately before use and degas by sonicating under vacuum or sparging with helium for 10–15 min.

Always prepare sodium hydroxide eluents with 50% (w/w) sodium hydroxide solution. (Do not use sodium hydroxide pellets; they are covered with a thin layer of sodium carbonate that will cause irreproducible results). Keep all eluents blanketed under helium at 34–55 kPa (5–8 psi) after preparation. Properly dispose of old eluent and prepare fresh after one week.

Eluent Preparation

Eluent A (20 mM Sodium Hydroxide/150 mM Sodium Cyanide)

Place 14.70 g sodium cyanide into a 2-L volumetric flask containing 1.9 L of degassed reagent water. Use a plastic pipette to deliver 2.08 mL (or 3.2 g) of 50% (w/w) sodium hydroxide. Bring to volume with degassed reagent water. Cap and invert the volumetric flask eight times to mix.

Note: Do not mix excessively, as this will increase the carbonate ion in the solution by trapping carbon dioxide from the air.

Eluent B (20 mM Sodium Hydroxide/300 mM Sodium Perchlorate)

Place 84.20 g HPLC-grade sodium perchlorate monohydrate ($NaClO_4 \bullet H_2O$) into an implosion-proof container that contains 1.9 L of reagent water and degas for 20 min by sonicating under vacuum. Transfer to a 2-L volumetric flask. Use a plastic pipette to deliver 2.08 mL (or 3.2 g) of 50% (w/w) sodium hydroxide. Bring to volume with degassed reagent water. Cap and invert the volumetric flask eight times to mix. Blanket with helium as described above.

Eluent C (20 mM Sodium Hydroxide)

Add 2.08 mL (or 3.2 g) of 50% (w/w) sodium hydroxide to a 2-L volumetric flask containing 1.9 L of degassed reagent water. Bring to volume with degassed reagent water. Cap and invert the volumetric flask eight times to mix. Blanket with helium as described above.

Table 1. Preparation of Metal Cyanide Stock Standards

Anion	Compound	Mass (g)	Stability** (Days)
$[Ag(CN)_2]^-$	$KAg(CN)_2$	0.1244	1
$[Au(CN)_2]^-$	$KAu(CN)_2$	0.1157	30
$[Cu(CN)_3]^{2-}$	Cu(CN) and NaCN	0.0632*	1
$[Ni(CN)_4]^{2-}$	$K_2Ni(CN)_4 \bullet H_2O$	0.1591***	1
$[Fe(CN)_6]^{4-}$	$K_4Fe(CN)_6$	0.1993	30
$[Co(CN)_6]^{3-}$	$K_3Co(CN)_6$	0.1546	30

*Dissolve the CuCN with 0.138 g sodium cyanide in a 100-mL volumetric flask containing a 50-mL 20 mM sodium hydroxide solution. Bring to volume with 20 mM sodium hydroxide solution.

**Stability in number of days when stored in amber HDPE at 4–6 C. Prepare fresh stock standards as needed according to this table.

***Dissolve (1.4806 + 0.1107 n) g of potassium nickel cyanide mono- or polyhydrate, $[K_2Ni(CN)_4 \bullet nH_2O$, where n = number of water molecules of hydration.

Standard Preparation

Store the metal cyanide reagent salts in a dessicator protected from the light. Prepare 1000 mg/L stock standards of each metal cyanide complex by consulting Table 1. Weigh the reagent salt into a 100-mL volumetric flask. Add enough 20 mM sodium hydroxide solution to dissolve, bring to volume with 20 mM sodium hydroxide solution, mix, and transfer to an amber HDPE bottle. Store at 4–6 °C. The individual stock standards are stable under these conditions for the periods shown in Table 1.

Prepare calibration standards spanning the range of interest for each analyte by diluting the 1000-mg/L stock standards with 20 mM sodium hydroxide solution. To prepare mixed standards, measure appropriate volumes of the 1000-mg/L standards into 100-mL volumetric flasks, bring to volume with a 20 mM sodium hydroxide solution, mix, and transfer to an amber HDPE bottle. These mixed calibration standards should be prepared fresh on the day of use.

Tables 2 and 3 show the concentrations of calibration standards used for this document.

Table 2. Linear Ranges and MDLs for Metal Cyanide Complexes Determined by Preconcentration of 5 mL

Anion	Concentration (µg/L)							
	Level 1	Level 2	Level 3	Level 4	Level 5	r^2	MDL Standard (g/L)	MDL* (g/L) (n = 5)
$[Ag(CN)_2]^-$	125	62.5	31.25	15.63	7.81	0.99979	15	1.92
$[Cu(CN)_3]^{2-}$	5.0	2.50	1.25	0.63	0.31	0.99893	0.8	0.74
$[Au(CN)_2]^-$	100	50.0	25.0	12.5	6.25	0.99999	10	2.19
$[Ni(CN)_4]^{2-}$	100	50.0	25.0	12.5	6.25	0.99995	50	4.84
$[Fe(CN)_6]^{4-}$	20	10.0	5.0	2.50	1.25	0.99999	1.0	0.23
$[Co(CN)_6]^{3-}$	200	100	50.0	25.0	12.5	0.99991	10	0.85

*MDL = *(t)* x (S) where *t* = Student's *t* value for a 99% confidence level and a standard deviation estimate with n – 1 degrees of freedom (*t* = 3.75 for five replicates of the MDL Standard), and S = standard deviation of the replicate analysis.

Table 3. Linear Ranges and MDLs for Metal Cyanide Complexes Determined by Direct Injection of 100 L

Anion	Concentration (mg/L)							
	Level 1	Level 2	Level 3	Level 4	Level 5	r^2	MDL Standard (mg/L)	MDL* (mg/L) (n = 5)
$[Ag(CN)_2]^-$	100	25.0	6.25	2.5	0.50	0.99998	1	0.14
$[Cu(CN)_3]^{2-}$	2.00	0.50	0.12	0.05	0.01	0.99997	0.2	0.048
$[Au(CN)_2]^-$	50.0	12.5	3.12	1.25	0.25	0.99997	1	0.16
$[Ni(CN)_4]^{2-}$	200	50.0	12.5	5.00	1.00	0.99997	1	0.34
$[Fe(CN)_6]^{4-}$	20.0	5.0	1.25	0.50	0.10	0.99999	0.5	0.10
$[Co(CN)_6]^{3-}$	100	25.0	6.25	2.50	0.50	1.00000	1	0.064

*MDL = *(t)* x (S) where *t* = Student's *t* value for a 99% confidence level and a standard deviation estimate with n – 1 degrees of freedom (*t* = 3.75 for five replicates of the MDL Standard), and S = standard deviation of the replicate analysis.

SAMPLE PREPARATION

This section gives a brief summary of the sample preparation procedure followed in the EPA OSW study. The study organizers provided extracts from various matrices. The four matrices analyzed for the high-level study (mg/L concentrations by direct injection) were labeled, "Clean Ottawa Sand Leachate", "Manufactured Gas Plant Soil Leachate" (two samples), and "Aluminum Reduction Plant Soil Leachate". The single matrix analyzed for the low-level study (µg/L concentrations by sample preconcentration) was labeled, "Clean Ottawa Sand Leachate".

The samples were extracted in accordance with SW 846 Method 9013.[5] Briefly, up to 25 g of the solid sample are combined with 500 mL of water plus 5 mL of 50% (w/w) NaOH in a bottle and extracted by tumbling continuously for 16 h. The pH is maintained above 10 throughout the extraction step. The extract is filtered and the filtrate (leachate) stored in amber bottles at 4–6 °C until the time of analysis, which should be within 14 days.

On the day of analysis, the leachate samples were brought to room temperature and spiked with the concentrated spiking solutions provided by the study organizers. Each matrix was spiked at six levels. The six levels consisted of three pairs of closely spaced concentrations (Youden Pairs). After spiking, the samples were filtered through 0.22-µm IC syringe filters into the autosampler vials.

SYSTEM PREPARATION AND SETUP

Assemble and configure the ICS-2500 system modules. Verify that the pump flow rate is within specifications and recalibrate if necessary. Verify that the UV-Vis Absorbance Detector wavelength accuracy is within specifications and recalibrate if necessary. (Both the pump flow rate and detector wavelength accuracy can be verified by performing the Instrument OQ/PQ per Document No. 031726). To aid in troubleshooting, it is good practice to periodically record the visible lamp output (i.e., the reference cell current in nA) and elapsed time. Consult the pump or detector manuals for procedural details.

Install an IonPac ATC-3 between "Eluent Reservoir B" and the pump inlet. The ATC-3 removes metal cyanide impurities present in the sodium cyanide solution that would otherwise cause elevated background noise. Regenerate the ATC-3 as needed by using a Trap Column/Suppressor Cleanup Kit (P/N 059659) according to the *Installation and Instruction Manual* (Document No. 031835). Install a GM-4 gradient mixer and an MFC-1 column between the pump outlet and injection valve. The GM-4 reduces background noise by ensuring an adequate mixing of eluents. The MFC-1 traps metal cations that could combine with the eluent to create interferences. If reagent water blanks indicate contamination by the eluent, regenerate the MFC-1 according the *Installation and Instruction Manual* (Document No. 034990). Prepare the eluents and prime the pump, eluent lines, and trap columns with eluent.

For samples containing metal cyanide complexes above 0.2 mg/L install a 100-µL sample loop between ports 1 and 4 of the injection valve and enter a "Sample Loop Size" of 100 µL in the AS50 Plumbing Configuration Screen. When a sample volume of 100 µL is entered into the sequence editor, the AS50 makes a 100-µL full-loop injection.

For samples containing metal cyanide complexes below 0.2 mg/L, install and configure the autosampler with the AS50 dual-valve needle assembly (P/N 061267). Install a 10-mL sample syringe and enter a "Sample Syringe Volume" of 10 mL in the AS50 Plumbing Configuration Screen. Set the "Syringe Speed" to 2. (Important—setting the Syringe Speed too high may cause the inject port to leak during loading of the sample loop.)

Install a 4-mm AG11 concentrator column between ports 1 and 4 of the injection valve and enter a "Sample Loop Size" of 5 mL in the AS50 Plumbing Configuration Screen. When a sample volume of 5000 µL is entered into the sequence editor, the AS50 directly concentrates 5 mL of sample onto the AG11 concentrator column. Refer to the *AutoSelect AS50 Autosampler (USB) Operator's Manual* (Document No. 031935) for details.

Install a 2 x 50 mm IonPac AG11 and a 2 x 250 mm IonPac AS11 column. Rinse the column with the ending eluent composition (10:45:45) for 30 min. Equilibrate the column with the initial eluent composition (10:10:80) for 10 min before analyzing a system blank of deionized water. In an equilibrated system, the background shift during the gradient run should be less than 100 mAU. The peak-to-peak noise and drift should not exceed 5 mAU/min. There should be no significant peaks eluting within the retention time windows of the metal cyanide analyte anions.

Inject a mid-level standard. The column is equilibrated when two consecutive injections of the standard produce the same retention time (± 0.2 min) for the analyte anions. Confirm that the resulting chromatogram resembles the chromatogram of the standard shown in Figure 1.

Calibrate the system by injecting one blank and at least five standards for every two decades of the calibration range. Plot the peak area for each metal cyanide complex versus the concentration injected and use a linear regression to fit the data.

RESULTS AND DISCUSSION

Table 2 summarizes the calibration data obtained by preconcentrating 5 mL of standards in the µg/L concentration range. Table 3 summarizes the calibration data obtained by directly injecting 100 µL of standards in the mg/L concentration range. Both calibration curves are linear over about one and one-half orders of magnitude for each of the complexes. On the AS11, the copper cyanide, gold cyanide, and nickel cyanide complexes begin to coelute at higher concentrations. Samples that contain higher concentrations of these metal cyanide complexes can be diluted with 20 mM NaOH as needed to resolve these peaks. If necessary, experienced chromatographers can modify the eluent gradient program to optimize the separation for a particular analysis.

Figure 1 shows the high-level (mg/L) method detection limit (MDL) standard analyzed by directly injecting 100 µL of the standard. Four replicates of this standard were analyzed and the resulting concentration statistics were used to calculate the MDLs given in Table 2. Figure 2 shows the low-level (µg/L) MDL standard analyzed by preconcentrating 5 mL of the standard. Five replicates of this standard were analyzed and the resulting concentration statistics were used to calculate the MDLs given in Table 3. The MDL standards were prepared by diluting a 100x limit of detection (LOD) stock standard provided by the collaborative study organizers. In some cases, the resulting concentrations were higher than the usual recommendation of 3–5 times above the expected MDL.

Figures 3–5 are typical chromatograms obtained for several of the matrices analyzed in the EPA OSW collaborative study. Each figure displays the matrix blank along with the matrix spiked with metal cyanide complexes at concentrations approximating a mid-level standard. The samples analyzed by direct injection were spiked at mg/L concentrations whereas the samples

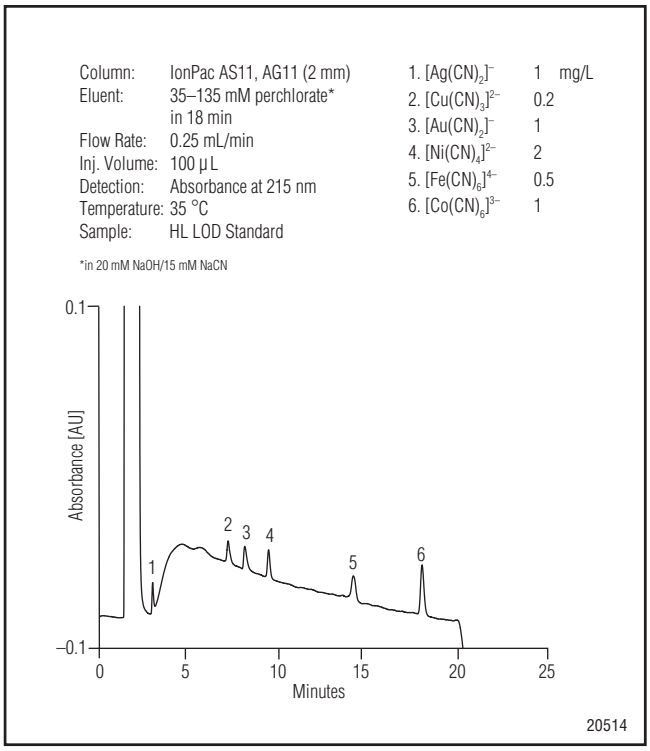

***Figure 1**. Anion-exchange separation with absorbance detection at 215 nm of metal cyanide complexes in reagent water. High-level (mg/L) limit of detection (LOD) standard analyzed by directly injecting 100 µL of the standard.*

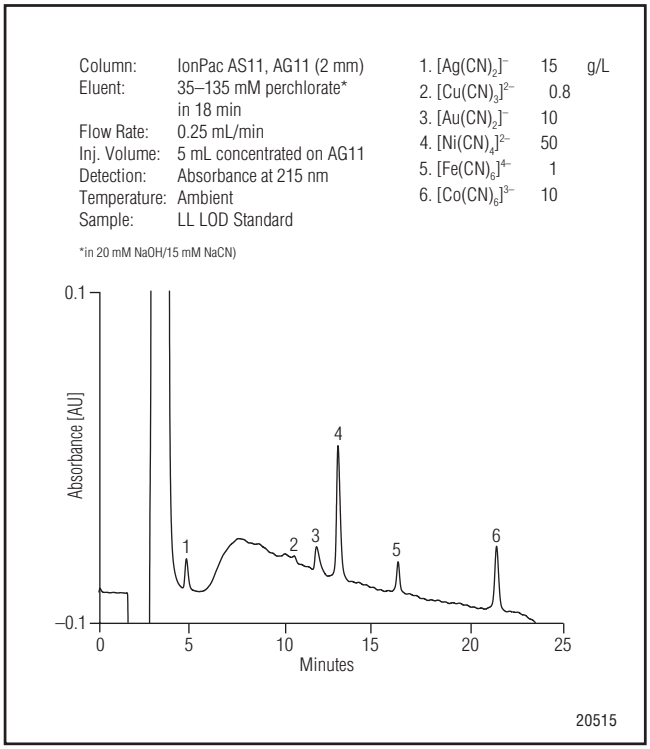

***Figure 2**. Anion-exchange separation with absorbance detection at 215 nm of metal cyanide complexes in reagent water. Low-level (µg/L) LOD standard analyzed by preconcentrating 5 mL of the standard.*

analyzed by preconcentration were spiked at µg/L concentrations. A few of the chromatograms are discussed below.

Figure 3A is the chromatogram of a manufactured gas plant (MGP) soil leachate blank and Figure 3B is MGP soil leachate fortified with metal cyanides. The matrix blank shows traces (<0.04 mg/L) of copper and iron cyanide complexes. The metal cyanides spiked into this matrix were all quantitatively recovered, as summarized in Table 4 for all of the matrices evaluated.

Figure 4A is the chromatogram of an aluminum reduction plant (ARP) soil leachate blank and Figure 4B is ARP soil leachate fortified with metal cyanides. The matrix blank is free of metal cyanide complexes. The metal cyanides spiked into this matrix were all quantitatively recovered, as summarized in Table 4.

Figure 5 shows the result of an Ottawa sand leachate analyzed by preconcentrating 5 mL of sample on the AG11 column. Figure 5A is the matrix blank and 5B is the matrix spiked with µg/L concentrations of metal cyanide complexes. Although the silver cyanide complex shows evidence of additional band broadening during the preconcentration step (compared to Figure 2), the peak is well resolved from the matrix ions peak and—along with the other metal cyanide complexes—quantitatively recovered from the matrix. The resolution and recovery of the silver cyanide complex should be carefully monitored in leachates from other sample types for evidence of matrix effects.

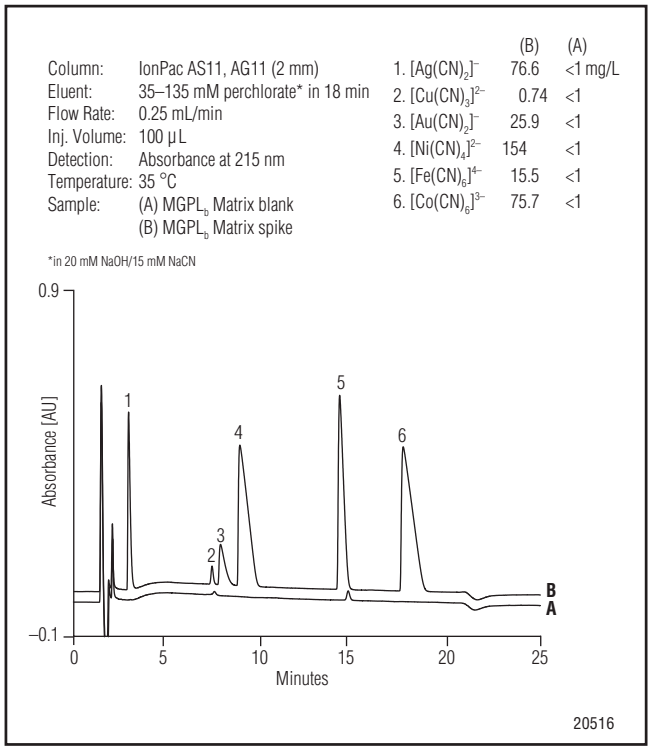

***Figure 3**. Anion-exchange separation with absorbance detection at 215 nm of metal cyanide complexes in a manufactured gas plant soil leachate (MGPL). (A) MGPL$_b$ matrix blank and (B) matrix spiked with metal cyanide complexes as shown.*

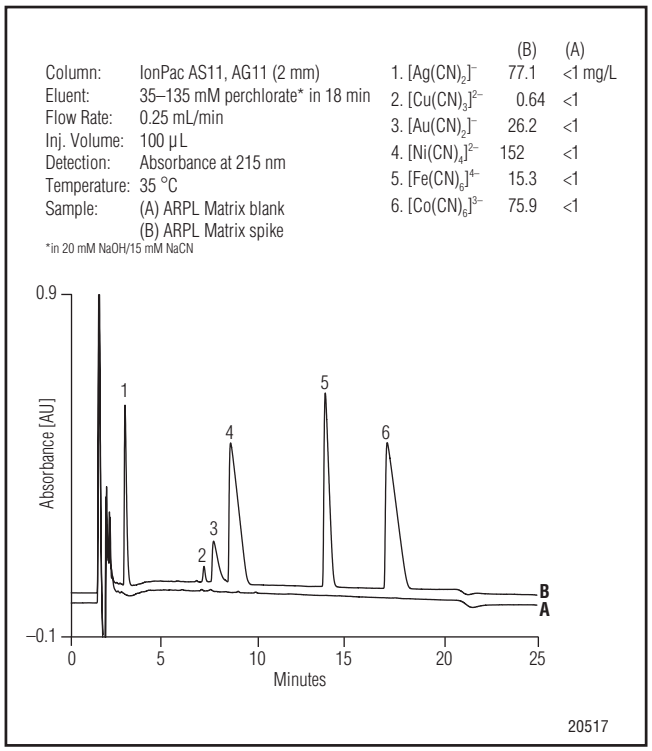

***Figure 4**. Anion-exchange separation with absorbance detection at 215 nm of metal cyanide complexes in an aluminum reduction plant soil leachate (ARPL). (A) ARPL soil leachate matrix blank and (B) matrix spiked with metal cyanide complexes as shown.*

Interferences

Before analyzing a matrix that has not been previously characterized, perform an evaluation of spike recovery and precision to rule out matrix effects.

This method assumes that the alkaline conditions used will reduce iron (III) cyanide (ferricyanide) to iron (II) cyanide (ferrocyanide), resulting in a single peak for $[Fe(CN)_6]^{4-}$. Calibration is based on the iron (II) cyanide complex. To represent the sum of ferrocyanide and ferricyanide, report results as mg/L of $[Fe(CN)_6]^{3-/4-}$. If unreduced $[Fe(CN)_6]^{3-}$ is present, it will elute as a tail on the iron ferrocyanide peak.

The sodium cyanide used to prepare Eluent A may contain metal cyanide complex impurities. Reduce the level of these impurities by using 99.99% sodium cyanide to prepare the eluent, and by installing an ATC-3 anion trap column as described in this application update.

Precautions

Some metal cyanide complexes will decompose when exposed to light. Protect samples and standards from UV light by storing them in amber HDPE containers.

Prepare all cyanide-containing solutions within a ventilation hood. Wear gloves, avoid inhalation, and avoid skin or eye contact. Do not let acid contact any of the cyanide-containing samples, standards, or eluents. Such contact will liberate hydrogen cyanide gas, an extremely toxic substance. Dispose of the eluent waste in accordance with applicable laws.

Strongly retained compounds from injected samples can accumulate on the column and degrade its performance. Signs of a fouled column include loss of capacity, loss of resolution, shortened retention times, higher noise and background, spurious peaks, and peak tailing. The AS11 column can be flushed with up to 100% acetonitrile to help remove contaminants from the column. For more information on column troubleshooting and cleanup, see the *Installation Instructions and Troubleshooting Guide for the IonPac AS11 Analytical Column* (Document No. 034791).

Some samples contain particulates that will plug the column and increase the backpressure. Use a guard column to protect the analytical column; change the guard column if such a sample causes a sudden increase in total backpressure greater than 3000 psi.

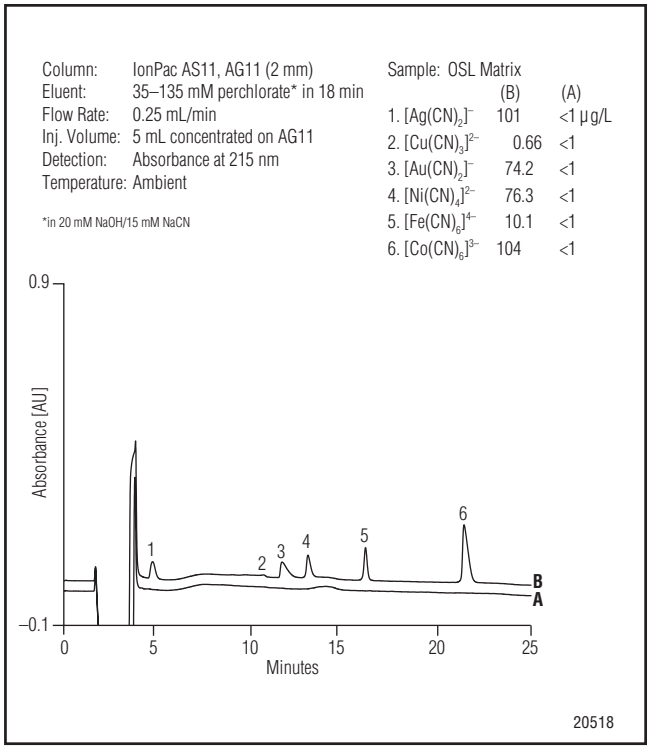

Figure 5*. Anion-exchange separation with preconcentration and absorbance detection at 215 nm of metal cyanide complexes in an Ottawa sand (OS) leachate. (A) OS leachate matrix blank and (B) matrix spiked with metal cyanide complexes as shown.*

Table 4. Recovery of Metal Cyanide Complexes from Solid Waste Leachates

Anion	% Recovery				
	Ottawa Sand 21–90 mg/L	Manufactured Gas Plant Soil$_a$ 21–90 mg/L	Manufactured Gas Plant Soil$_b$ Plant Soil 21–90 mg/L	Aluminum Reduction Plant Soil 21–90 mg/L	Ottawa Sand 20–115 g/L
$[Ag(CN)_2]^-$	106.5	104	102.9	102.2	100.9
$[Cu(CN)_3]^{2-}$	108	106.1	128	112.6	109.9
$[Au(CN)_2]^-$	106.1	102.8	103.7	102.3	100
$[Ni(CN)_4]^{2-}$	103.2	102.5	101.8	100.2	91.1
$[Fe(CN)_6]^{4-}$	102.2	101.5	103.8	99.5	102.7
$[Co(CN)_6]^{3-}$	104.6	103.3	102.5	101.7	103.3

REFERENCES

1. *Standard Methods for the Examination of Water and Wastewater*. 17th Edition 1989; APHA-AWWA-WPCF; 4500-CN B; pp. 4–38.
2. Dionex Corporation. Application Note 55; Sunnyvale, CA.
3. Dionex Corporation. Application Update 147; Sunnyvale, CA.
4. Dionex Corporation. Application Note 161; Sunnyvale, CA.
5. *Cyanide Extraction Procedure for Solids and Soils: Test Methods for Evaluating Solid Wastes SW-846, Third Edition*; EPA Method 9013; United States Environmental Protection Agency, Washington, DC; 1986.
6. EPA Inter-Laboratory Collaborative for Office of Solid Waste, Sharon Drop, Task Group Chairperson.

LIST OF SUPPLIERS

Alfa Aesar, 30 Bond St., Ward Hill, MA 01835 USA, Tel: 800-343-0660, www.alfa.com.

Fisher Scientific, 2000 Park Lane, Pittsburgh, PA 15275-1126 USA, Tel: 800-766-7000, www.fishersci.com.

Sigma-Aldrich Chemical Company, P.O. Box 14508, St. Louis, MO 63178 USA, Tel: 1-800-325-3010, www.sigmaaldrich.com.

Strem Chemical, 7 Mulliken Way, Newburyport, MA 01950-4098 USA, Tel: 1-800-647-8736, www.strem.com.

VWR International, 1310 Goshen Parkway, West Chester, PA 19380 USA, Tel: 800-932-5000, www.vwr.com.

Direct Determination of Metal Cyanides by Ion Chromatography with UV Absorbance Detection

INTRODUCTION

Metal cyanide complexes are negatively charged ionic complexes consisting of one or more cyanide ions (CN^-) bound to a single transition metal cation. These complexes have the general formula $[M(CN)_b]^{x-}$, where M represents a transition metal cation (such as Ag^+, Au^+, Cu^+, Ni^{2+}, Fe^{2+}, Co^{3+}, etc.), b is the number of bound cyanide ions, and x is the total anionic charge of the complex. Metal cyanide complexes are of environmental concern because they release cyanide upon dissociation. In environmental waters below pH 9.3, the cyanide ion converts to HCN, an extremely toxic substance. Metal cyanide complexes are also of interest in the mining and reclamation of precious metals, and in the metal finishing industry.

Many metal cyanide complexes are relatively stable and require moderate to strongly acidic conditions to liberate cyanide. The stability of metal cyanide complexes with respect to dissociation is expressed by their formation constants, and shown in Table 1. As indicated in Table 1, metal cyanide complexes with formation constants greater than about 10^{35} are often termed "strong metal cyanide complexes". The distinction between weak and strong complexes is environmentally relevant because strong complexes such as iron (II) cyanide dissociate only under strongly acidic conditions (pH < 2) and are considered relatively stable and nontoxic, whereas weak metal cyanide complexes dissociate under mildly acidic conditions (pH >3 but <6) to liberate cyanide.

Previous methods that assess the potential toxicity due to cyanide directly measure only free cyanide, typically by a colorimetric reaction with a pyridine-barbituric acid reagent. These methods then rely on an operational definition to distinguish between weak and strong cyanide complexes, by subjecting the sample to increasingly harsh conditions to dissociate some fraction of the cyanide complexes and liberate free cyanide. Examples include methods that measure "cyanides amenable to chlorination",[1] "weak acid dissociable cyanides",[2] "available cyanide",[3] and "total cyanide".[4] The results are highly dependant on the matrix, the type of metal cyanide complexes present, and the procedure used. These methods also require time-consuming sample pretreatment or distillation to reduce interference from sulfides, chlorine, thiosulfate, and other compounds.

TABLE 1. STABILITY OF METAL CYANIDE COMPLEXES[8, 9]

Metal Cyanide Complex	Stability Constant (log K at 25 °C)	Type of Complex
$[Co(CN)_6]^{3-}$	64	Strong
$[Fe(CN)_6]^{3-}$	43.6	Strong
$[Pd(CN)_4]^{2-}$	42.4	Strong
$[Pt(CN)_4]^{2-}$	40.0	Strong
$[Hg(CN)_4]^{2-}$	39.0	Strong
$[Au(CN)_2]^-$	37	Strong
$[Fe(CN)_6]^{4-}$	35.4	Strong
$[Ni(CN)_4]^{2-}$	30.2	Weak
$[Cu(CN)_4]^{3-}$	23.1	Weak
$[Cu(CN)_3]^{2-}$	N.A.*	Weak
$[Ag(CN)_2]^-$	20.5	Weak
$[Zn(CN)_4]^{2-}$	19.6	Weak
$[Cd(CN)_4]^{2-}$	17.9	Weak

* N.A. Data not available

Ion chromatography (IC) is superior to the aforementioned methods because it resolves each individual metal cyanide complex into a discrete chromatographic peak. IC thus allows a precise differentiation of complexes of lesser toxicity from those of greater toxicity. This application update presents modifications to the IC method described in Dionex Application Note 55.[5] The metal cyanide complexes of silver, gold, copper, nickel, iron, and cobalt ($[Ag(CN)_2]^-$, $[Au(CN)_2]^-$, $[Cu(CN)_3]^{2-}$, $[Ni(CN)_4]^{2-}$, $[Fe(CN)_6]^{4-}$, $[Co(CN)_6]^{3-}$) are separated on a 2-mm IonPac® AS11 column and quantified by measuring their absorbance at 215 nm. Use of the 2-mm AG11/AS11 column set provides different selectivity, lower eluent use, and better solvent compatibility than the AG5/AS5 column set featured in Application Note 55. This application update also presents a thorough comparison of the performance expected from the different column sets, with data obtained during participation in a joint ASTM/EPA interlaboratory collaborative study to validate the use of IC for the determination of metal cyanide complexes in environmental waters. The method was evaluated for reproducibility, linearity, accuracy, precision, and spike recovery from various matrices.[6]

EQUIPMENT

Dionex ICS-2500 IC system consisting of:

GP50 Gradient Pump

AD25 Absorbance Detector

AS50 Autosampler with Thermal Compartment

Chromeleon® Chromatography Workstation

Consumables

Syringe filters (Gelman IC Acrodisk® 0.2-µm, PN 4483)

Storage bottles, amber HDPE (VWR IRN301-0125 or 16172-144)

CONDITIONS

Columns:	IonPac AS11 Analytical 2 x 250 mm (P/N 44076) IonPac AG11 Guard 2 x 50 mm (P/N 44078) IonPac ATC-3 (PN 059660) (2 each)
Temperature:	30 °C
Injection:	25 µL
Detection:	Absorbance at 215 nm
Expected System Backpressure:	900 psi
Noise:	1–5 mAU
Run Time:	35 min
Flow Rate:	0.25 mL/min
Eluent A:	20 mM NaOH/150 mM NaCN
Eluent B:	20 mM NaOH/300 mM $NaClO_4$
Eluent C:	20 mM NaOH

Pump Program:

Time (min)	*%A*	*%B*	*%C*
Init.	10	10	80
0.0	10	10	80
18.0	10	45	45
22.0	10	45	45
25.0	10	10	80
35.0	10	10	80

REAGENTS AND STANDARDS

Sodium hydroxide solution 50% w/w (Fisher SS254)

Sodium cyanide, 99.99 % (Aldrich 43,159-1)

Sodium perchlorate monohydrate, HPLC-grade (Fisher S490)

Copper cyanide (AlfaAesar 12135)

Potassium ferrocyanide(II) trihydrate (Aldrich 22,768-4)

Potassium dicyanoargentate(I) (AlfaAesar 12551)

Potassium dicyanoaurate(I) (AlfaAesar 12552)

Potassium hexacyanocobaltate(III) (AlfaAesar 23126)

Potassium tetracyanonickelate(II) monohydrate (Strem 93-2836)

PREPARATION OF SOLUTIONS AND REAGENTS

Prepare all solutions from analytical reagent-grade chemicals and Type I reagent-grade deionized water with a specific resistance of 18.0 MΩ-cm or greater, filtered through a 0.2-µm filter immediately before use.

Eluent Preparation

Eluent 1 (20 mM Sodium Hydroxide/150 mM Sodium Cyanide)

Use high-quality water of high resistivity (18.0 MΩ-cm or better) that contains as little dissolved carbon dioxide as possible. Biological contamination should be absent. Sodium hydroxide eluent should be prepared with 50% (w/w) sodium hydroxide solution. Sodium hydroxide pellets are covered with a thin layer of sodium carbonate and should not be used under any circumstances. Keep all eluents blanketed under helium at 34–55 kPa (5–8 psi) after preparation. If maintained under helium, this and the following eluents can be used for approximately one week.

To prepare 2 L of 20 mM NaOH/150 mM NaCN, place 14.70 g sodium cyanide into a 2-L volumetric flask containing 1.9 L of deionized water and degas for 20 min by sonicating under vacuum. Use a plastic pipette to deliver 2.08 mL (or 3.2 g) of 50% (w/w) sodium hydroxide. Bring to volume with degassed deionized water. Cap and invert the volumetric flask eight times to mix. *Note: Do not excessively mix, because this action will increase the carbonate ion in the solution by trapping carbon dioxide from the air.*

Eluent 2 (20 mM Sodium Hydroxide/300 mM Sodium Perchlorate)

Place 84.20 g HPLC-grade sodium perchlorate monohydrate ($NaClO_4 \cdot H_2O$) into a 2-L volumetric flask containing 1.9 L of deionized water and degas for 20 min by sonicating under vacuum. Use a plastic pipette to deliver 2.08 mL (or 3.2 g) of 50% (w/w) sodium hydroxide. Bring to volume with degassed deionized water. Cap and invert the volumetric flask eight times to mix. Continue degassing for 10 min and then blanket with helium as described above.

Eluent 3 (20 mM Sodium Hydroxide)

Add 2.08 mL (or 3.2 g) of 50% (w/w) sodium hydroxide to a 2-L volumetric flask containing 1.9 L of degassed deionized water. Bring to volume with degassed deionized water. Cap and invert the volumetric flask eight times to mix. Continue degassing for 10 min and then blanket with helium as described above.

Diluent (10 mM Sodium Hydroxide)

Add 1.04 mL (1.6 g) of 50% (w/w) sodium hydroxide to a 2-L volumetric flask containing 1.9 L of degassed deionized water. Bring to volume with degassed deionized water. Cap and invert the volumetric flask eight times to mix. Continue degassing for 10 min.

Standard Preparation

Store the metal cyanide reagent salts in a dessicator protected from the light. To prepare stock standards (1000 mg/L) for each metal cyanide species, weigh the amount of the appropriate compound given in Table 2 into a 100-mL volumetric flask. Add enough 10 mM sodium hydroxide solution to dissolve the cyanide complex, bring to volume with 10 mM sodium hydroxide solution, mix, and transfer to an amber HDPE bottle. Store at 4–6 °C. The individual stock standards are stable under these conditions for the periods shown in Table 2.

TABLE 2. PREPARATION OF METAL CYANIDE STOCK STANDARDS

Anion	Compound	Mass (g)	Stability** (days)
$[Ag(CN)_2]^-$	$KAg(CN)_2$	0.1244	1
$[Au(CN)_2]^-$	$KAu(CN)_2$	0.1157	30
$[Cu(CN)_3]^{2-}$	Cu(CN) and NaCN	0.0632*	1
$[Ni(CN)_4]^{2-}$	$K_2Ni(CN)_4 \cdot H_2O$	0.1591***	1
$[Fe(CN)_6]^{4-}$	$K_4Fe(CN)_6 \cdot 3\ H_2O$	0.1993	30
$[Co(CN)_6]^{3-}$	$K_3Co(CN)_6$	0.1546	30

* Dissolve the CuCN with 0.1384 g sodium cyanide in a 100-mL volumetric flask containing 50 mL 10 mM sodium hydroxide solution. Bring to volume with 10 mM sodium hydroxide solution. Stir or sonicate for 1 h or until dissolved.

** Stability in number of days when stored in amber HDPE at 4–6 °C. Prepare fresh stock standards as needed according to this table.

*** Dissolve (1.4806 + 0.1107 n) g of potassium nickle cyanide mono- or polyhydrate, $K_2[Ni(CN)_4] \cdot n\ H_2O$, where n = number of water molecules of hydration.

Precaution: Some of the metal cyanide complexes are very toxic. Avoid contact with water or acid. Clean up and appropriately dispose of any spills.

Prepare calibration standards spanning the linear calibration range of each analyte by diluting the 1000-mg/L stock standards with 10 mM sodium hydroxide solution. To prepare mixed standards, measure appropriate volumes of the 1000-mg/L standards into 100-mL volumetric flasks, bring to volume with 10 mM sodium hydroxide solution, mix, and transfer to an amber HDPE bottle. These mixed calibration standards should be prepared fresh on the day of use.

Table 3 shows the concentration of calibration standards prepared in this way for the ASTM study.

TABLE 3. CONCENTRATIONS OF METAL CYANIDE CALIBRATION STANDARDS

Anion	Concentration (mg/L)				
	Level 1	Level 2	Level 3	Level 4	Level 5
$[Ag(CN)_2]^-$	100	25	6.250	1.562	0.391
$[Au(CN)_2]^-$	50	12.50	3.125	0.781	0.195
$[Cu(CN)_3]^{2-}$	2.0	0.50	0.125	0.031	0.008
$[Ni(CN)_4]^{2-}$	200	50	12.50	3.125	0.781
$[Fe(CN)_6]^{4-}$	20	5.0	1.250	0.312	0.078
$[Co(CN)_6]^{3-}$	100	25	6.250	1.562	0.391

SAMPLE PREPARATION

This section briefly summarizes the sample preparation procedure outlined in the ASTM/EPA draft method followed in the study. The ASTM study organizers provided six matrices and concentrated spiking solutions with instructions to spike each matrix at six levels. The six levels consisted of three pairs of closely spaced concentrations (Youden Pairs). The six matrices were deionized water, drinking water, groundwater, surface water, wastewater, and ocean water.

Upon collection, the samples were treated, if necessary, with powdered lead carbonate to remove sulfide interferences, and with sodium thiosulfate to remove interfering oxidants, in accordance with *Standard Methods*.[7] The samples were then adjusted with sodium hydroxide to pH = 12.5 and stored in amber bottles at 4–6 °C. Samples preserved in this way must be analyzed within 14 days. On the day of analysis, the samples were brought to room temperature, spiked with the spiking solutions, and then filtered through 0.22-µm nylon filters into the autosampler vials.

SYSTEM PREPARATION AND SETUP

Verify that the pump flow rate is within specifications and recalibrate if necessary. A GP50 should deliver water at 1.0 ± 0.005 mL/min against a constant backpressure of 2000 psi. Verify that the UV/Vis absorbance detector wavelength accuracy is within specifications and recalibrate if necessary. It is a good practice to periodically record the visible lamp output (i.e., the reference cell current in nA) and elapsed time to aid in troubleshooting. Consult the pump or detector manuals for procedural details.

Install an IonPac ATC-3 between the eluent reservoir containing sodium cyanide and the pump inlet. The ATC-3 removes metal cyanide impurities present in the sodium cyanide solution that would otherwise cause elevated background noise. Regenerate the ATC-3 as needed by using a trap column/suppressor cleanup kit (059659) according to the installation and instruction manual (Document No. 031835).

Install and configure the autosampler and thermal compartment with a 25-µL sample loop. The most accurate and precise injections are made with a calibrated sample loop flushed with about four loop volumes of sample before injection. Install a 250-µL sample syringe and set the "Syringe Speed" to 4 or 5. Enter the correct sample "Loop Size" and "Sample Syringe Volume" in the AS50 Plumbing Configuration Screen. Refer to the AutoSelect™ *AS50 Autosampler Operator's Manual* (Document No. 31169) for details. Install a gradient mixer and an IonPac ATC-3 between the pump and injection valve.

Install a 2 x 50 mm IonPac AG11 and a 2 x 250 mm IonPac AS11 column. Rinse the column with the ending eluent composition (10:45:45) for 30 min. Equilibrate the column with the initial eluent composition (10:10:80) for 10 min before analyzing a system blank of deionized water. In an equilibrated system, the background shift during the gradient run should be less than 100 mAU. The peak-to-peak noise and drift should not exceed 5 mAU/min. There should be no significant peaks eluting within the retention time windows of the metal cyanide analyte anions.

Inject a mid-level standard. The column is equilibrated when two consecutive injections of the standard produce the same retention time for the metal cyanide complex anions. Confirm that the resulting chromatogram resembles the chromatogram of the standard shown in Figure 1B.

RESULTS AND DISCUSSION

Calibrate the system by injecting one blank and at least five standards for every two decades of the calibration range. Plot the peak area for each metal cyanide complex versus the concentration injected and use a linear regression to fit the data. Table 4 summarizes the calibration data for a typical calibration curve obtained by injecting calibration standards covering the ranges shown. The calibration curve is linear over two orders of magnitude for the silver, nickel, iron, and cobalt cyanide complexes. Copper cyanide and gold cyanide were calibrated over a smaller interval. On the AS11, the copper cyanide, gold cyanide, and nickel cyanide complexes begin to coelute at higher concentrations, requiring the analyst to judge where to split the peaks. The problem is less severe for nickel cyanide, the later-eluting peak. Any samples containing high levels of copper or gold cyanide should be diluted to a level where coelution does not occur. Note that because of differences in molar absorptivity at 215 nm among the metal cyanide complexes, the linear calibration range is significantly different from one complex to another.

Figure 1 compares the chromatograms of a calibration standard obtained by using either the AS5 or AS11 columns. Both chromatograms were obtained using the same eluent gradient program. Under these conditions, the AS5 column (1A) provides better separation of the gold, copper, and nickel cyanide complexes, whereas the AS11 column (1B) provides better separation between the nickel, iron, and cobalt cyanide complexes. These differences in selectivity can be exploited to improve certain analyses. For example, the AS11 may better determine low levels of iron cyanide in the presence of high levels of nickel cyanide, because there is less likelihood of a large nickel cyanide peak obscuring a smaller iron cyanide peak. Note that the elution order of copper and gold is reversed on the two columns. Therefore, the AS5 may better determine trace levels of gold cyanide in the presence of higher levels of copper cyanide. If necessary, experienced chromatographers can modify the eluent gradient program to optimize the separation for a particular analysis. One noteworthy feature of the 2-mm AS11 is that it consumes 1/4 the eluent required by the 4-mm AS5—an important advantage that can lower reagent and waste disposal costs.

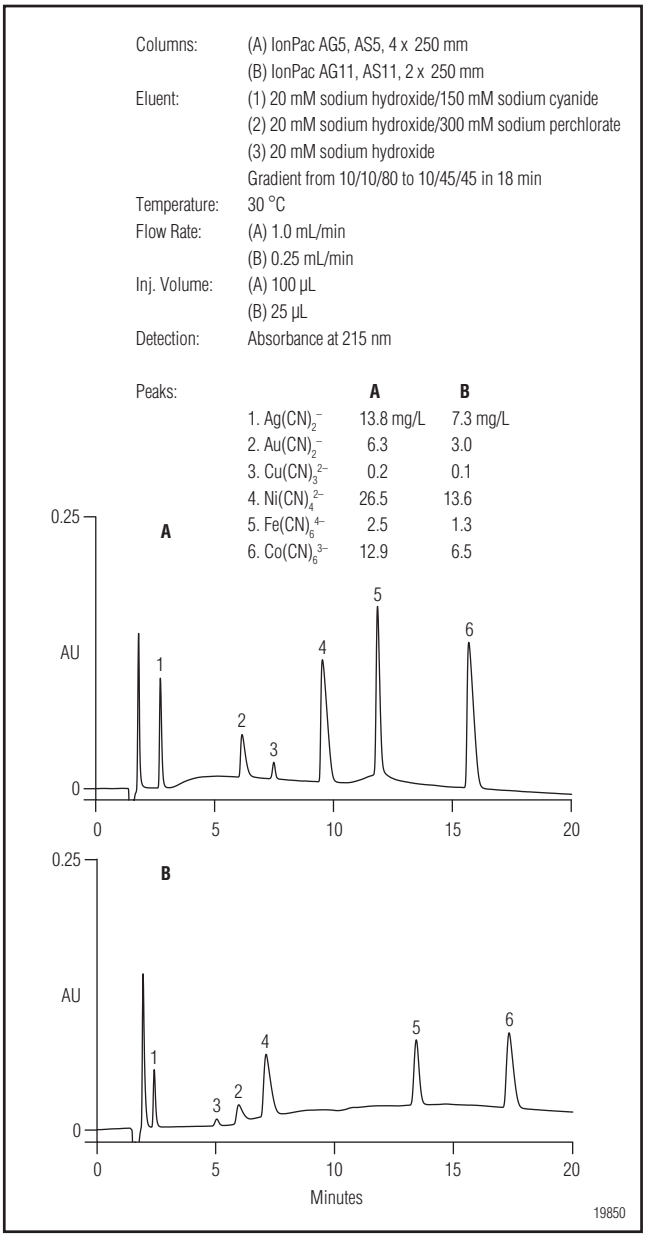

Figure 1. *Anion-exchange separation with absorbance detection at 215 nm of metal cyanide complex standard on the (A) AS5 column and (B) AS11 column.*

The limits of detection of this method were determined by making seven injections of deionized water, fortified with the analytes at levels yielding peaks approximately five times higher than the background noise. Column 3 of Table 4 shows the actual concentrations of the six metal cyanides analyzed. The formula given below Table 4 was used to calculate the minimum detection limit (MDL) for each analyte. Table 4 compares the calculated MDLs obtained on both the AS5 and AS11 columns.

Both columns provide minimum detection limits in the low-mg/L range for the tested metal cyanide complexes. Silver cyanide $[Ag(CN)_2]^-$ exhibits a significantly higher MDL on the AS11 column because the complex elutes considerably earlier, in an area that has interference from the tail of the matrix peak. This interference makes integration of the silver cyanide peak less reliable, and the precision of the peak area measurement is reduced. At the low concentrations analyzed in this application update, both columns also exhibit some interference with iron cyanide $[Fe(CN)_6]^{4-}$. On the AS5 column, the iron peak shows considerable tailing, whereas on the AS11 column, a small interfering peak is seen to coelute with iron. In both cases, the MDL for iron cyanide increases somewhat because of the interfering peak. It is possible that the interfering peak is from the unreduced iron (III) cyanide complex. If so, the AS11 might succeed in resolving these two complexes with a minor change to the gradient program.

The next several chromatograms illustrate typical real-world results. Figure 2 compares the AS5 and AS11 when analyzing a groundwater sample. In this fairly low-ionic-strength matrix, the two columns provide comparable results, with the AS5 (Figure 2A) better resolving the gold, copper, and nickel complexes, whereas the AS11 (Figure 2B) exhibits less tailing of the iron cyanide peak and a better baseline in that region.

Figure 3 compares the AS5 and AS11 columns when analyzing a wastewater sample. In this high-ionic-strength matrix, the matrix ions elute as a very broad, tailing peak that poses a significant problem for recovery of silver cyanide. The AS5 (Figure 3A) shows significantly better recovery for the silver cyanide complex because it is resolved slightly better from the matrix ions than on the AS11 (Figure 3B). The peaks for the copper, gold, and nickel cyanide complexes are also more adversely affected on the AS11, though to a lesser extent than the silver cyanide complex.

Interferences

Exposure to light will cause photodecomposition of some metal cyanide complexes and reduce their concentration. Protect samples and standards from UV light by storing them in amber HDPE containers.

This method assumes that the alkaline conditions used will reduce iron (III) cyanide (ferricyanide) to iron (II) cyanide (ferrocyanide) resulting in a single peak for $[Fe(CN)_6]^{4-}$. Calibration is based on the iron (II) cyanide complex. Report results as mg/L of $[Fe(CN)_6]^{3-/4-}$ to represent the sum of ferrocyanide and ferricyanide. If unreduced $[Fe(CN)_6]^{3-}$ is present, it will elute as a tail on the iron ferrocyanide peak.

The sodium cyanide used to prepare eluent 1 may contain metal cyanide complex impurities. To reduce the level of these impurities, use 99.99% sodium cyanide to prepare the eluent, and install an ATC-3 anion trap column as described in this application update.

TABLE 4. LINEAR RANGES AND MDLS FOR METAL CYANIDE COMPLEXES

Analyte	Range (mg/L)	MDL Standard (mg/L)	r^2 AS5	r^2 AS11	MDL* AS5 (mg/L)	MDL*AS11 (mg/L)
$[Ag(CN)_2]^-$	1–100	5.0	0.99936	0.99921	0.18	1.48
$[Au(CN)_2]^-$	1–50	5.0	0.99994	0.99999	0.64	0.28
$[Cu(CN)_3]^{2-}$	0.1–2	0.1	0.99985	0.99993	0.08	0.06
$[Ni(CN)_4]^{2-}$	1–200	5.0	0.99992	0.99991	1.10	0.77
$[Fe(CN)_6]^{4-}$	0.1–20	0.5	0.99998	0.99999	0.12	0.09
$[Co(CN)_6]^{3-}$	1–100	5.0	0.99998	0.99996	0.54	0.50

* The MDLs were calculated as MDL = *(t)* x (S) Where *t* = Student's *t* value for a 99% confidence level and a standard deviation estimate with n – 1 degrees of freedom (*t* = 3.14 for seven replicates of the MDL Standard), and S = standard deviation of the replicate analysis.

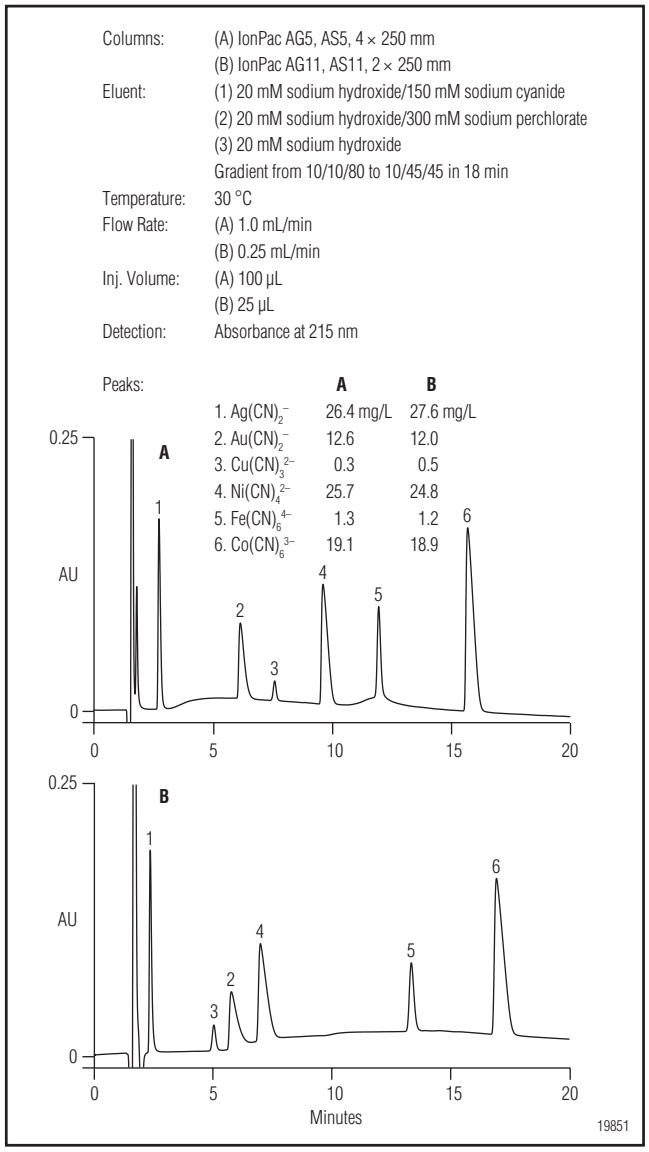

Figure 2. *Determination of metal cyanide complexes in groundwater. Injection onto the (A) AS5 column and (B) AS11 column.*

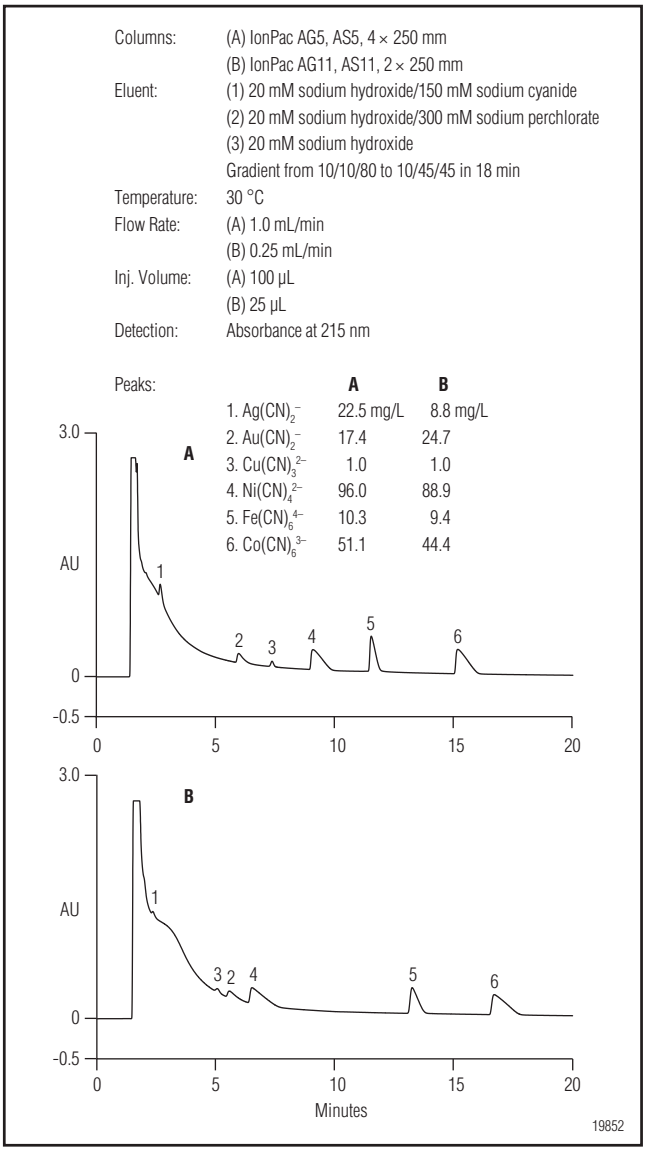

Figure 3. *Determination of metal cyanide complexes in wastewater from a former manufactured gas plant. Injection onto the (A) AS5 column and (B) AS11 column.*

Precautions

Prepare all cyanide-containing solutions within a ventilation hood. Wear gloves, and avoid inhalation and skin or eye contact. Do not let acid contact any of the cyanide-containing samples, standards, or eluents. This contact would liberate hydrogen cyanide gas, an extremely toxic substance. Properly dispose of the old standards and eluent waste in accordance with applicable laws.

Strongly retained compounds from injected samples can accumulate on the column and degrade its performance. Signs of a fouled column include loss of capacity, loss of resolution, shortened retention times, higher noise and background, spurious peaks, and peak tailing. The AS11 column can be flushed with up to 100% acetonitrile to help remove contaminants from the column. The AS5 cannot tolerate more than 5% organic solvent in the eluent. For more information on column troubleshooting and cleanup, see the *Installation Instructions and Troubleshooting Guide for the IonPac AS11 Analytical Column* (Document No. 034791).

Some groundwater samples contain particulates that will plug the column and increase the backpressure. Use a guard column to protect the analytical column; change the guard column if such a sample causes a sudden increase in total backpressure to greater than 3000 psi.

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LIST OF SUPPLIERS

Aldrich Chemical Company, Inc., 1001 West Saint Paul Avenue, P.O. Box 355, Milwaukee, WI 53233 USA, Tel: 800-558-9160, www.aldrich.sial.com.

Alfa Aesar, 30 Bond St., Ward Hill, MA 01835 USA, Tel.: 800-343-0660, www.alfa.com.

Fisher Scientific, 2000 Park Lane, Pittsburgh, PA 15275-1126 USA, Tel: 800-766-7000, www.fishersci.com.

VWR Scientific Products, 1310 Goshen Parkway, West Chester, PA 19380 USA, Tel: 800-932-5000, www.vwr.com (for Burdick and Jackson, EM Science, Fluka, and Gelman).

Direct Determination of Cyanide in Strongly Alkaline Solutions

Applicable to distillation methods for determination of total cyanide in water

PERFORMANCE

The minimum detection limit for a 200-µL sample injection is 10 ppb. The recommended working range for this volume injected is 30–1000 ppb.

APPLICATION AREAS

- Industrial waste water
- Hazardous waste
- Plating and metal finishing baths
- Ground water and drinking water

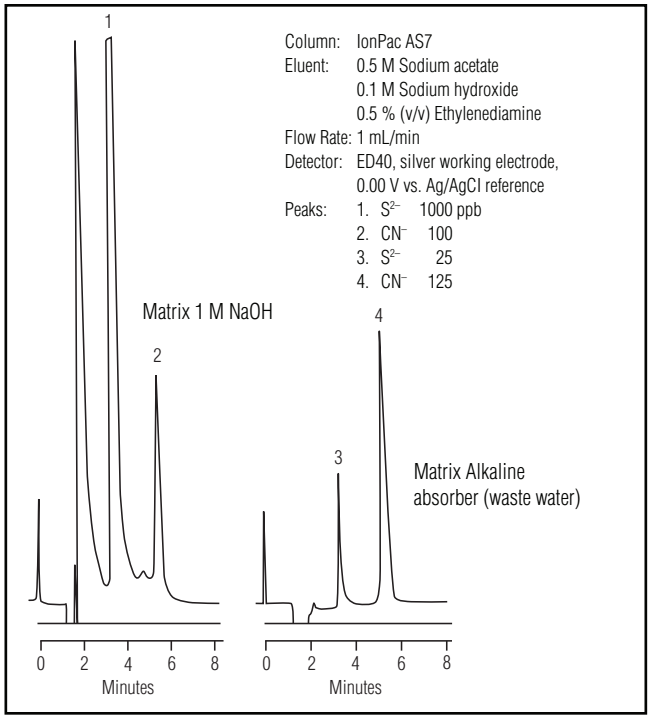

Figure 1 *Cyanide in strongly alkaline solutions.*

CONDITIONS

Column: IonPac® AS7
Eluent: 0.5 M Sodium acetate
0.1 M Sodium hydroxide
0.5% (v/v) Ethylenediamine
Flow Rate: 1 mL/min
Detector: ED40, silver working electrode,
0.00 V vs. Ag/AgCl reference

COMMENTS

Determination of total cyanide in water is usually done by refluxing the sample in an acid digest and trapping the liberated HCN gas in a strongly alkaline-absorbing solution. Most methods for analyzing the trapping solution have an upper pH limit of about 12.5 to 13. This direct injection method can determine cyanide in solutions ranging up to pH 14. This allows absorbing solutions (such as the 1.25 M NaOH solution specified in U.S. EPA method 335.2) to be quickly analyzed without dilution or other pretreatment. In addition, the technique is not subject to as many interferences as titrimetric or spectrophotometric methods, and it is well suited to automated analysis.

RECOMMENDED EQUIPMENT

Dionex DX-500 Ion Chromatograph equipped with an ED40 detector.

Column Selection Guide

Environmental Water Applications Notebook

Column Selection Guide

Silica Columns

			Reversed-Phase (RP)						Mixed-Mode			HILIC		Application-Specific						
			Acclaim 120 C18	Acclaim 120 C8	Acclaim 300 C18	Acclaim Polar Advantage (PA)	Acclaim Polar Advantage II (PA2)	Acclaim Phenyl-1	Acclaim Trinity P1	Acclaim Mixed-Mode WAX-1	Acclaim Mixed-Mode WCX-1	Acclaim Mixed-Mode HILIC-1	Acclaim HILIC-10	Acclaim Organic Acid	Acclaim Surfactant	Acclaim Explosives E1	Acclaim Explosives E2	Acclaim Carbamate	Example Applications
General Applications	*Neutral Molecules*	High hydrophobicity	√	√	√	√	√	√	√	√	√	√	√						*Fat-soluble vitamins, PAHs, glycerides*
		Intermediate hydrophobicity	√	√	√	√	√	√	√	√	√	√							*Steroids, phthalates, phenolics*
		Low hydrophobicity	√			√	√					√	√						*Acetaminophen, urea, polyethylene glycols*
	Anionic Molecules	High hydrophobicity	√	√	√	√	√	√	√	√	√	√							*NSAIDs, phospholipids*
		Intermediate hydrophobicity	√	√	√	√	√	√	√	√		√							*Asprin, alkyl acids, aromatic acids*
		Low hydrophobicity				√			√	√		√	√						*Small organic acids, e.g. acetic acids*
	Cationic Molecules	High hydrophobicity	√	√	√	√	√	√		√	√	√							*Antidepressants*
		Intermediate hydrophobicity	√	√	√	√	√	√	√		√	√							*Beta blockers, benzidines, alkaloids*
		Low hydrophobicity	√			√			√		√	√	√						*Antacids, pseudoephedrine, amino sugars*
	Amphoteric/ Zwitterionic Molecules	High hydrophobicity	√	√	√	√	√	√	√	√	√	√							*Phospholipids*
		Intermediate hydrophobicity	√	√	√	√	√	√			√								*Amphoteric surfactants, peptides*
		Low hydrophobicity				√	√		√	√	√	√	√						*Amino acids, aspartame, small peptides*
	Mixtures of Neutral, Anionic, Cationic Molecules	Neutrals and acids	√			√	√		√	√									*Artificial sweeteners*
		Neutrals and bases	√			√	√		√		√								*Cough syrup*
		Acids and bases				√			√										*Drug active ingredient with counterion*
		Neutrals, acids, and bases				√			√										*Combination pain relievers*
Specific Applications	*Surfactants*	Anionic	√	√	√	√	√								√				*SDS, LAS, laureth sulfates*
		Cationic													√				*Quats, benzylalkonium in medicines*
		Nonionic	√	√	√	√	√					√			√				*Triton X-100 in washing tank*
		Amphoteric	√	√	√	√	√								√				*Cocoamidopropyl betaine*
		Hydrotropes													√				*Xylenesulfonates in handsoap*
		Surfactant blends													√				*Noionic and anionic surfactants*
	Organic Acids	Hydrophobic							√	√				√					*Aromatic acids, fatty acids*
		Hydrophilic							√	√				√					*Organic acids in soft drinks, pharmaceuticals*
	Environmental Contaminants	Explosives														√	√		*U.S. EPA Method 8330, 8330B*
		Carbonyl compounds															√		*U.S. EPA 1667, 555, OT-11; CA CARB 1004*
		Phenols	√			√													*Compounds regulated by U.S. EPA 604*
		Chlorinated/Phenoxy acids				√													*U.S. EPA Method 555*
		Triazines	√			√													*Compounds regulated by U.S. EPA 619*
		Nitrosamines				√													*Compounds regulated by U.S. EPA 8270*
		Benzidines	√			√													*U.S. EPA Method 605*
		Perfluorinated acids				√													*Dionex TN73*
		Microcystins	√																*ISO 20179*
		Isocyanates					√					√							*U.S. OSHA Methods 42, 47*
		Carbamate insecticides																√	*U.S. EPA Method 531.2*
	Vitamins	Water-soluble vitamins				√	√		√										*Vitamins in dietary supplements*
		Fat-soluble vitamins	√	√	√	√	√	√		√									*Vitamin pills*
	Pharmaceutical Counterions	Anions							√	√									*Inorgaic anions and organic acids in drugs*
		Cations							√		√								*Inorgaic cations and organic bases in drugs*
		Mixture of Anions and Cations							√										*Screening of pharmaceutical counterions*
		API and counterions							√										*Naproxen Na^+ salt, metformin Cl^- salt, etc.*

Polymer Columns

		IonPac AS23	IonPac AS22	IonPac AS22-Fast	IonPac AS14/A	IonPac AS12A	IonPac AS9/HC/SC	IonPac AS4A/SC	IonSwift MAX-100	IonPac AS24	IonPac AS21	IonPac AS20	IonPac AS19	IonPac AS18	IonPac AS18-Fast	IonPac AS17-C	IonPac AS16	IonPac AS15	IonPac AS11(-HC)	IonPac AS10	IonPac AS7	IonPac AS5	IonPac Fast Anion IIIA	OmniPac PAX-100	OmniPac PAX-500	IonPac CS18	IonPac CS17	IonPac CS16	IonPac CS15	IonPac CS14	IonPac CS12A	IonPac CS11	IonPac CS10	IonPac CS5A	OmniPac PCX-100	OmniPac PCX-500	AminoPac PA10	AminoPac PA1	CarboPac PA200	CarboPac PA100	CarboPac PA20	CarboPac PA10	CarboPac PA1	CarboPac MA1	DNAPac PA200	DNAPac PA100	ProPac WAX/SAX	ProPac WCX/SCX	ProPac IMAC	ProPac HIC	ProPac PA1	ProSwift	IonPac ICE-AS6	IonPac ICE-AS1	IonPac ICE-Borate	IonPac NS1
ANIONS	Inorganic Anions	√	√	√	√	√	√	√	√	√		√	√	√	√	√		√	√	√																																							
	Oxyhalides	√				√	√			√			√																																											
	Bromate	√					√			√			√																																											
	Perchlorate										√	√					√																																							
	Organic Acids								√							√		√	√	√																																					
	Phosphoric/Citric Acids																						√																																	
	Poly/High-Valence Anions								√			√					√		√		√	√																																		
	Hydrophobic Anions								√			√					√		√																																					
	Hydrophobic/Halogenated Anions								√			√							√					√																															
	Anionic Neutral Molecules									√	√	√	√												√																														
CATIONS	Inorganic Cations																									√	√	√	√	√	√	√	√																						
	Sodium/Ammonium																											√	√		√																							
	Amines/Polyvalent Amines																									√	√																											
	Aliphatic/Aromatic Amines																										√			√	√																						
	Alkanol/Ethhanolamines																									√	√		√																									
	Biogenic Amines																									√	√																											
	Transition/Lanthanide Metals																																	√																				
	Hydrophobic Cations																									√	√			√					√																		
	Cationic Neutral Molecules																																			√																		
BIO-MOLECULES	Amino Acids																																				√	√																	
	Phosphorylated Amino Acids																																				√																	
	Amino Sugars																																				√		√	√	√	√											
	Oligoscharides																																						√	√			√	√									
	Mono-/Di-Saccharides																																						√	√	√	√									
	Glycoproteins																																								√	√	√									
	Alditols/Aldoses mono/di Saccharides																																						√	√	√	√									
	ds Nucleic Acids																																											√	√							
	Single-Stranded Oligonucleotides																																											√	√							√
	Peptides																																													√	√	√	√				
	Proteins																																												√	√	√	√	√				
	Metal-binding Proteins																																												√								
	Monoclonal antibodies																																							√	√			√			√	√	√	√	√	√	√	√
ORGANIC MOLECULES	Aliphatic Organic Acids																																																				√	√		√
	Alcohols																																																				√	√		
	Borate																																																						√		
	Large Molecules, Anions																																																						√
	Small Molecules																																																						
	Small Molecules/LC-MS																																																						
	Polar/Non-Polar Small Molecules																																																					
	Hydrophobic/Aliphatic Organic Acids																																																				
	Surfactant Formulations																																																				√
	Explosives/EPA 8330																																																				
MODE	Anion Exchange / Carbonate	√	√	√	√	√	√	√																																													
	Anion Exchange / Hydroxide								√	√	√	√	√	√	√	√	√	√	√	√	√	√	√			√	√	√	√	√	√	√	√					√	√	√	√	√	√											
	Cation Exchange																									√	√	√	√	√	√	√	√	√												√			√	√					
	Multi-Mode																								√	√										√	√																	
	Affinity																																													√								
	Ion Exclusion																																																			√	√	√	
	Reversed Phase																																																			√	√	√	√
	Anion Exchange/Other																																			√	√														√	√	√	

Column Specifications

IC Anion Columns

Column	Format	Primary Eluent	Application	Particle Diameter	Substrate Crosslinking	Latex Diameter	Latex Crosslinking	Capacity (per column)	Functional Group	Hydrophobicity
IonPac AS24	2 × 250 mm	Hydroxide	Recommended column for haloacetic acids prior to MS or MS/MS detection	7 µm	55%	-	-	140 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS23	2 × 250 mm 4 × 250 mm	Carbonate	Recommended column for inorganic anions and oxyhalides. Trace bromate in drinking water.	6 µm	55%	-	-	80 µeq 320 µeq	Alkyl quaternary ammonium	Ultralow
IonPac AS22	2 × 250 mm 4 × 250 mm	Carbonate	Recommended column for fast analysis of common inorganic anions.	6.5 µm	55%	-	-	52.5 µeq 210 µeq	Alkyl quaternary ammonium	Ultralow
IonPac AS21	2 × 250 mm	Hydroxide	Recommended column for trace perchlorate prior to MS or MS/MS detection	7.0 µm	55%	-	-	45 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS20	2 × 250 mm 4 × 250 mm	Hydroxide	Recommended column for trace perchlorate prior to suppressed conductivity detection.	7.5 µm	55%	-	-	77.5 µeq 310 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS19	2 × 250 mm 4 × 250 mm	Hydroxide	Recommended column for inorganic anions and oxyhalides. Trace bromate in drinking water.	7.5 µm	55%	-	-	60 µeq 350 µeq	Alkanol quaternary ammonium	Low
IonPac AS18	2 × 250 mm 4 × 250 mm	Hydroxide	Recommended column for the analysis of common inorganic anions.	7.5 µm	55%	65 nm	8%	75 µeq 285 µeq	Alkanol quaternary ammonium	Low
IonPac AS17-C	2 × 250 mm 4 × 250 mm	Hydroxide	Trace anions in HPW matrices. Carboxylated resin, no sulfate blank. Low capacity for fast analysis of common inorganic anions using gradient elution with the Eluent Generator.	10.5 µm	55%	75 nm	6%	7.5 µeq 30 µeq	Alkanol quaternary ammonium	Low
IonPac AS16	2 × 250 mm 4 × 250 mm	Hydroxide	High capacity for hydrophobic anions including iodide, thiocyanate, thiosulfate, and perchlorate. Polyvalent anions including: polyphosphates and polycarboxylates	9 µm	55%	80 nm	1%	42.5 µeq 170 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS15	2 × 250 mm 4 × 250 mm	Hydroxide	High capacity for trace analysis of inorganic anions and low molecular weight organic acids in high purity water matrices.	9 µm	55%	-	-	56.25 µeq 225 µeq	Alkanol quaternary ammonium	Medium-High
IonPac AS15-5mm	3 × 150 mm	Hydroxide	Fast run, high capacity for trace analysis of inorganic anions and low molecular weight organic acids in high purity water matrices.	5 µm	55%	-	-	70 µeq	Alkanol quaternary ammonium	Medium-High
IonPac AS14A-5 µm	3 × 150 mm	Carbonate	Recommended column for fast analysis of common inorganic anions.	5 µm	55%	-	-	40 ueq	Alkyl quaternary ammonium	Medium
IonPac AS14A	4 × 250 mm	Carbonate	For analysis of common inorganic anions.	7 µm	55%	-	-	120 µeq	Alkyl quaternary ammonium	Medium
IonPac AS14	2 × 250 mm 4 × 250 mm	Carbonate	Moderate capacity for fast analysis of common inorganic anions.	9 µm	55%	-	-	16 µeq 65 µeq	Alkyl quaternary ammonium	Medium-High

Column	Format	Primary Eluent	Application	Particle Diameter	Substrate Crosslinking	Latex Diameter	Latex Crosslinking	Capacity (per column)	Functional Group	Hydrophobicity
IonPac AS12A	2 × 200 mm 4 × 200 mm	Carbonate	Moderate capacity for analysis of inorganic anions and oxyhalides. Trace chloride and sulfate in high carbonate matrices.	9 µm	55%	140 nm	0.20%	13 µeq 52 µeq	Alkyl quaternary ammonium	Medium
IonPac AS11-HC	2 × 250 mm 4 × 250 mm	Hydroxide	High capacity for the determination of organic acids and inorganic anions in uncharacterized samples.	9 µm	55%	70 nm	6%	72.5 µeq 290 µeq	Alkanol quaternary ammonium	Medium-Low
IonPac AS11	2 × 250 mm 4 × 250 mm	Hydroxide	Low capacity for fast profiling of organic acids and inorganic anions in well-characterized samples.	13 µm	55%	85 nm	6%	11 µeq 45 µeq	Alkanol quaternary ammonium	Very Low
IonPac AS10	2 × 250 mm 4 × 250 mm	Hydroxide	High capacity for the analysis of inorganic anions and organic acids in high nitrate samples.	8.5 µm	55%	65 nm	5%	42.5 µeq 170 µeq	Alkyl quaternary ammonium	Low
IonPac AS9-HC	2 × 250 mm 4 × 250 mm	Carbonate	High-capacity column for inorganic anions and oxyhalides. Trace bromate in drinking water.	9 µm	55%	90 nm	18%	48 µeq 190 µeq	Alkyl quaternary ammonium	Medium-Low
IonPac AS9-SC	4 × 250 mm	Carbonate	Low capacity for fast analysis of inorganic anions and oxyhalides. Specified column in US EPA Method 300.0 (B).	13 µm	55%	110 nm	20%	30-35 µeq	Alkyl quaternary ammonium	Medium-Low
IonPac AS4A-SC	2 × 250 mm 4 × 250 mm	Carbonate	Low capacity for fast analysis of common inorganic anions. Specified column in U.S. EPA Method 300.0 (A).	13 µm	55%	160 nm	0.50%	5 µeq 20 µeq	Alkanol quaternary ammonium	Medium-Low
IonPac Fast Anion IIIA	3 × 250 mm	Hydroxide	Recommended column for phosphoric and citric acids in cola soft drinks.	7.5 µm	55%	-	-	55 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS7	4 × 250 mm	Specialty Eluents	Polyvalent anions including chelating agents, polyphosphates and polyphosphonates. Cyanide, sulfide, hexavalent chromium, and arsenic speciation.	10 µm	2%	530 nm	5%	100 µeq	Alkyl quaternary ammonium	Medium-High
IonPac AS5A	4 × 150 mm	Hydroxide	Low capacity for fast profiling of organic acids and inorganic anions in well-characterized samples.	5 µm	2%	60 nm	4%	35 µeq	Alkanol quaternary ammonium	Low
IonPac AS5	4 × 250 mm	Hydroxide	Metal-EDTA complexes, metal-cyanide complexes, and oxyanions.	15 µm	2%	120 nm	1%	20 µeq	Alkanol quaternary ammonium	Low

IC Cation Columns

Column	Format	Primary Eluent	Application	Particle Diameter	Substrate Crosslinking	Latex Diameter	Latex Crosslinking	Capacity (per column)	Functional Group	Hydrophobicity
IonPac CS18	2 × 250 mm	MSA	Recommended column for polar amines (alkanolamines and methylamines) and moderately hydrophobic and polyvalent amines (biogenic and diamines). Nonsuppressed mode when extended calibration linearity for ammonium and weak bases is required	6 µm	55%	-	-	0.29 µeq	Carboxylic acid	Medium
IonPac CS17	2 × 250 mm 4 × 250 mm	MSA	Recommended column for hydrophobic and polyvalent amines (biogenic amines and diamines)	7 µm	55%	-	-	0.363 µeq 1.45 µeq	Carboxylic acid	Very Low
IonPac CS16	3 × 250 mm 5 × 250 mm	MSA	Recommended column for disparate concentration ratios of adjacent-eluting cations such as sodium and ammonium. Can be used for alkylamines and alkanolamines.	5 µm	55%	-	-	3.0 µeq 8.4 µeq	Carboxylic acid	Medium
IonPac CS15	2 × 250 mm 4 × 250 mm	MSA	Disparate concentration ratios of ammonium and sodium. Trace ethanolamine in high-ammonium or high- potassium concentrations. Alkanolamines.	8.5 µm	55%	-	-	0.7 µeq 2.8 µeq	Carboxylic acid/ phosphonic acid/ crown ether	Medium
IonPac CS14	2 × 250 mm 4 × 250 mm	MSA	Aliphatic amines, aromatic amines, and polyamines plus mono- and divalent cations.	8.5 µm	55%	-	-	0.325 µeq 1.3 µeq	Carboxylic acid	Low
IonPac CS12A-MS	2 × 100 mm	MSA	IC-MS screening column for fast elution and low flow rates required for interfacing with IC-MS	8.5 µm	55%	-	-	0.28 µeq	Carboxylic acid/ phosphonic acid	Medium
IonPac CS12A-5 µm	3 × 150 mm	MSA	Recommended column for high efficiency and fast analysis (3 min) of mono- and divalent cations.	5 µm	55%	-	-	0.94 µeq	Carboxylic acid/ phosphonic acid	Medium
IonPac CS12A	2 × 250 mm 4 × 250 mm	MSA	Recommended column for the separation of mono- and divalent cations. Manganese morpholine, alkylamines, and aromatic amines.	8.5 µm	55%	-	-	0.7 µeq 2.8 µeq	Carboxylic acid/ phosphonic acid	Medium
IonPac CS11	2 × 250 mm	HCl + DAP	Separation of mono- and divalent cations. Ethanolamines if divalent cations are not present.	8 µm	55%	200 nm	5%	0.035 µeq	Sulfonic acid	Medium
IonPac CS10	4 × 250 mm	HCl + DAP	Separation of mono- and divalent cations.	8.5 µm	55%	200 nm	5%	0.08 µeq	Sulfonic acid	Medium
IonPac CS5A	2 × 250 mm 4 × 250 mm	Pyridine dicarboxylic acid	Recommended column for transition and lanthanide metals analysis. Aluminum analysis.	9 µm	55%	140 nm 75 nm	10% 20%	0.02 µeq/ 0.005 µeq 0.04 µeq/ 0.01 µeq	Sulfonic acid/ alkanol quaternary ammonium	-

Transferring HPLC Methods to UHPLC

Environmental Water Applications Notebook

Easy Method Transfer from HPLC to RSLC with the Dionex Method Speed-Up Calculator

INTRODUCTION

The goal of every chromatographic optimization is a method that sufficiently resolves all peaks of interest in as short a time as possible. The evolution of packing materials and instrument performance has extended chromatographic separations to new limits: ultrahigh-performance liquid chromatography (UHPLC).

The new Dionex UltiMate® 3000 Rapid Separation LC (RSLC) system is ideal for ultrafast, high-resolution LC. The RSLC system was designed for ultrafast separations with flow rates up to 5 mL/min at pressures up to 800 bar (11,600 psi) for the entire flow-rate range. This industry-leading flow-pressure footprint ensures the highest flexibility possible; from conventional to ultrahigh-resolution to ultrahigh-speed methods. The RSLC system, with autosampler cycle times of only 15 seconds, oven temperatures up to 110 °C, and data collection rates up to 100 Hz (even when acquiring UV-Vis spectra), sets the standard for UHPLC performance. Acclaim® RSLC columns with a 2.2 µm particle size complete the RSLC dimension.

A successful transfer from an HPLC method to an RSLC method requires recalculation of the chromatographic parameters. Underlying chromatographic principles have to be considered to find the appropriate parameters for a method transfer. With the Method Speed-up Calculator, Dionex offers an electronic tool that streamlines the process of optimum method transfer. This technical note describes the theory behind the Method Speed-Up Calculator and the application of this interactive, multi-language tool, illustrated with an exemplary method transfer from a conventional LC separation to an RSLC separation. You may obtain a copy of this calculator from your Dionex representative.

METHOD SPEED-UP STRATEGY

The purpose of method speed-up is to achieve sufficient resolution in the shortest possible time. The strategy is to maintain the resolving power of the application by using shorter columns packed with smaller particles. The theory for this approach is based on chromatographic mechanisms, found in almost every chromatography text book. The following fundamental chromatographic equations are applied by the Method Speed-Up Calculator for the method transfer from conventional to ultrafast methods.

The separation efficiency of a method is stated by the peak capacity *P*, which describes the number of peaks that can be resolved in a given time period. The peak capacity is defined by the run time divided by the average peak width. Hence, a small peak width is essential for a fast method with high separation efficiency. The peak width is proportional to the inverse square root of the number of theoretical plates N generated by the column. Taking into account the length of the column, its efficiency can also be expressed by the height equivalent to a theoretical plate *H*. The relationship between plate height H and plate number *N* of a column with the length L is given by Formula 1.

Formula 1: $$N = \frac{L}{H}$$

Low height equivalents will therefore generate a high number of theoretical plates, and hence small peak width for high peak capacity is gained. Which factors define *H*? For an answer, the processes inside the column have to be considered, which are expressed by the Van Deemter equation (Formula 2).

Formula 2: $$H = A + \frac{B}{u} + C \cdot u$$

The Eddy diffusion *A* describes the mobile phase movement along different random paths through the stationary phase, resulting in broadening of the analyte band. The longitudinal diffusion of the analyte against the flow rate is expressed by the term *B*. Term *C* describes the resistance of the analyte to mass transfer into the pores of the stationary phase. This results in higher band broadening with increasing velocity of the mobile phase. The well-known Van Deemter plots of plate height *H* against the linear velocity of the mobile phase are useful in determining the optimum mobile phase flow rate for highest column efficiency with lowest plate heights. A simplification of the Van Deemter equation, according to Halász[1] (Formula 3), describes the relationship between column efficiency (expressed in plate height *H*), particle size d_p (in µm) and velocity of mobile phase *u* (in mm/s):

Formula 3: $$H = 2 \cdot d_p + \frac{6}{u} + \frac{d_p^2 \cdot u}{20}$$

The plots of plate height H against velocity u depending on the particle sizes dp of the stationary phase (see Figure 1, top) demonstrate visually the key function of small particle sizes in the method speed-up strategy: The smaller the particles, the smaller the plate height and therefore the better the separation efficiency. An efficiency equivalent to larger particle columns can be achieved by using shorter columns and therefore shorter run times.

Another benefit with use of smaller particles is shown for the 2 µm particles in Figure 1: Due to improved mass transfer with small particle packings, further acceleration of mobile phases beyond the optimal flow rate with minimal change in the plate height is possible.

Optimum flow rates and minimum achievable plate heights can be calculated by setting the first derivative of the Halász equation to zero. The optimal linear velocity (in mm/s) is then calculated by Formula 4.

Formula 4: $$u_{opt} = \sqrt{\frac{B}{C}} = \frac{10.95}{d_p}$$

The minimum achievable plate height as a function of particle size is calculated by insertion of Formula 4 in Formula 3, resulting in Formula 5.

Formula 5: $$H_{min} \approx 3 \cdot d_p$$

Chromatographers typically prefer resolution over theoretical plates as a measure of the separation quality. The achievable resolution *R* of a method is directly proportional to the square root of the theoretical plate number as can be seen in Formula 6. *k* is the retention factor of the analyte and *k* the selectivity.

Formula 6: $$R = \frac{1}{4} \cdot \sqrt{N} \cdot \frac{k_2}{1 + k_2} \cdot \frac{\alpha - 1}{\alpha}$$

If the column length is kept constant and the particle size is decreased, the resolution of the analytes improves. Figure 1, bottom, demonstrates this effect using 5 µm and 2 µm particles.

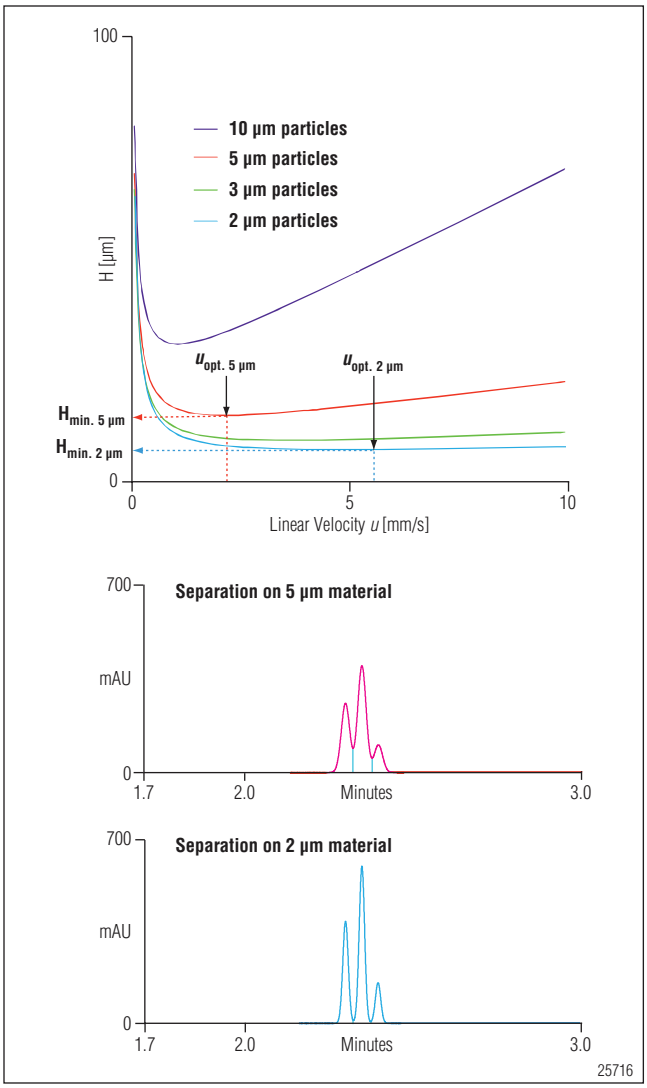

Figure 1. Smaller particles provide more theoretical plates and more resolution, demonstrated by the improved separation of three peaks (bottom) and smaller minimum plate heights H *in the Van Deemter plot (top). At linear velocities higher than uopt,* H *increases more slowly when using smaller particles, allowing higher flow rates and therefore faster separations while keeping separation efficiency almost constant. The speed-up potential of small particles is revealed by the Van Deemter plots (top) of plate height* H *against linear velocity u of mobile phase: Reducing the particle size allows higher flow rates and shorter columns because of the decreased minimum plate height and increased optimum velocity. Consequently, smaller peak width and improved resolution are the result (bottom).*

When transferring a gradient method, the scaling of the gradient profile to the new column format and flow rate has to be considered to maintain the separation performance. The theoretical background was introduced by L. Snyder[2] and is known as the gradient volume principle. The gradient volume is defined as the mobile phase volume that flows through the column at a defined gradient time t_G. Analytes are considered to elute at constant eluent composition. Keeping the ratio between the gradient volume and the column volume constant therefore results in a correct gradient transfer to a different column format.

Taking into account the changed flow rates F and column volume (with diameter d_c and length L), the gradient time intervals t_G of the new methods are calculated with Formula 7.

Formula 7: $$t_{G,new} = t_{G,old} \cdot \frac{F_{old}}{F_{new}} \cdot \frac{L_{new}}{L_{old}} \cdot \left(\frac{d_{c,new}}{d_{c,old}} \right)^2$$

An easy transfer of method parameters can be achieved by using the Dionex Method Speed-Up Calculator (Figure 2), which incorporates all the overwhelming theory and makes manual calculations unnecessary. This technical note describes the easy method transfer of an example separation applying the calculator. Just some prerequisites described in the following section have to be taken into account.

PREREQUISITES

The Method Speed-Up Calculator is a universal tool and not specific for Dionex products. Nevertheless, some prerequisites have to be considered for a successful method transfer, which is demonstrated in this technical note by the separation of seven soft drink additives.

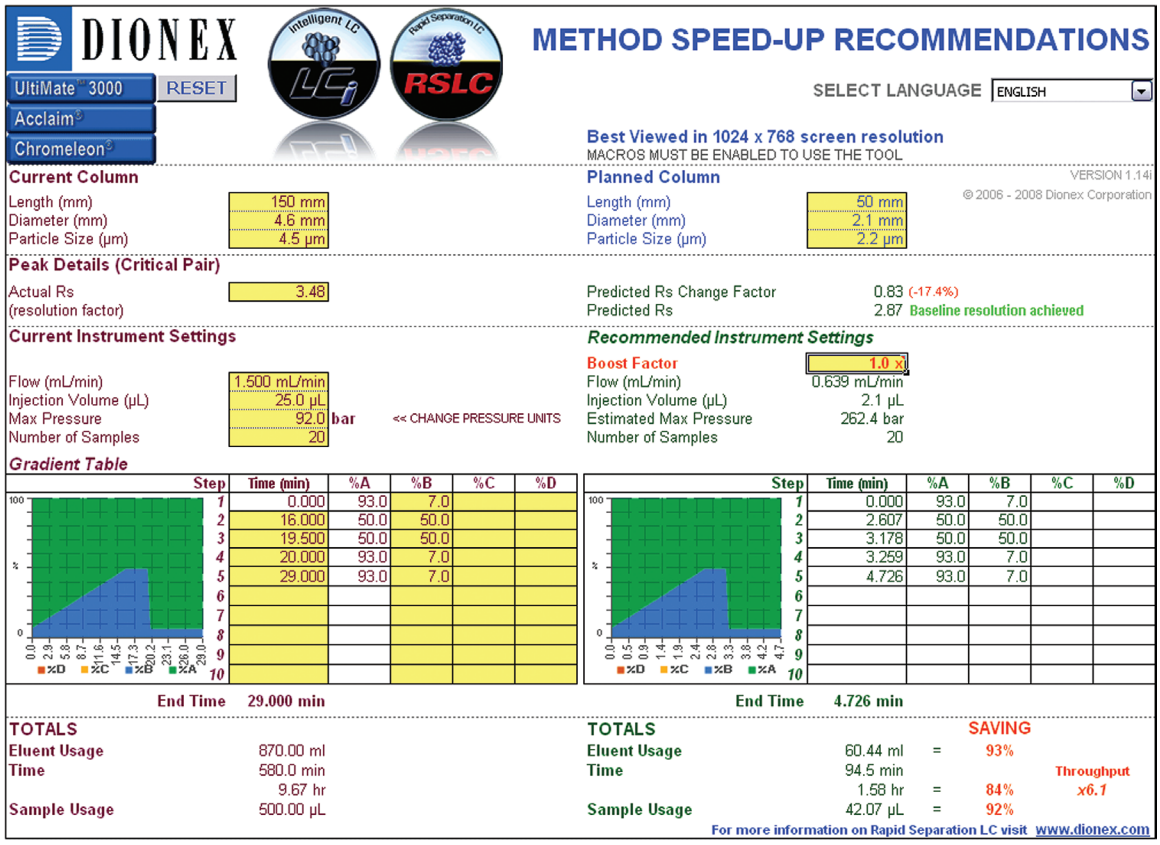

Figure 2. The Dionex Method Speed-Up Calculator transfers a conventional (current) HPLC method to a new (planned) RSLC method.

Column Dimension

First, the transfer of a conventional method to an RSLC method requires the selection of an adequate column filled with smaller particles. The RSLC method is predicted best if the selectivity of the stationary phase is maintained. Therefore, a column from the same manufacturer and with nominally identical surface modification is favoured for an exact method transfer. If this is not possible, a column with the same nominal stationary phase is the best choice. The separation is made faster by using shorter columns, but the column should still offer sufficient column efficiency to allow at least a baseline separation of analytes. Table 1 gives an overview of the theoretical plates expected by different column length and particle diameter size combinations using Dionex Acclaim column particle sizes. Note that column manufacturers typically fill columns designated 5 µm with particle sizes 4–5 µm. Dionex Acclaim 5 µm columns are actually filled with 4.5 µm particles. This is reflected in the table.

Table 1. Theoretical Plates Depending on Column Length and Particle Diameter (Calculated Using Formula 5)

	Theoretical Plates N		
Particle size	4.5 µm	3 µm	2.2 µm
Column length: 250 mm	18518	27778	37879
150 mm	11111	16667	22727
100 mm	7407	11111	15152
75 mm	5555	8333	11364
50 mm	3703	5556	7576

If the resolution of the original separation is higher than required, columns can be shortened. Keeping the column length constant while using smaller particles improves the resolution. Reducing the column diameter does not shorten the analysis time but decreases mobile phase consumption and sample volume. Taking into account an elevated temperature, smaller column inner diameters reduce the risk of thermal mismatch.

System Requirements

Smaller particles generate higher backpressure. The linear velocity of the mobile phase has to be increased while decreasing the particle size to work within the Van Deemter optimum. The UltiMate 3000 RSLC system perfectly supports this approach with its high maximum operation pressure of 800 bar (11,600 psi). This maximum pressure is constant over the entire flow rate range of up to 5 mL/min, providing additional potential to speed up applications even further by increasing the flow rate.

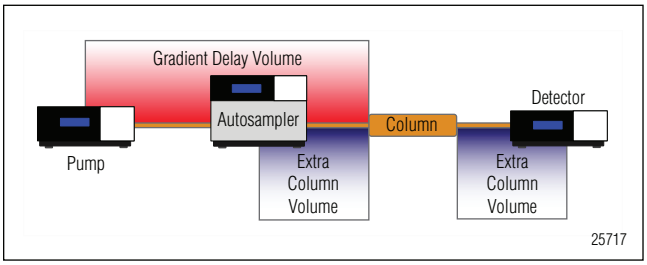

Figure 3. Gradient delay volume and extra column volume of an HPLC system. Both play an important role in method speed-up.

For fast gradient methods, the gradient delay volume (GDV) plays a crucial role. The GDV is defined as the volume between the first point of mixing and the head of the column. The GDV becomes increasingly important with fast, steep gradients and low flow rate applications as it affects the time taken for the gradient to reach the head of the column. The larger the GDV, the longer the initial isocratic hold at the beginning of the separation. Typically, this leads to later peak elution times than calculated. Early eluting peaks are affected most. In addition, the GDV increases the time needed for the equilibration time at the end of a sample and therefore increases the total cycle time. A general rule is to keep the gradient steepness and the ratio of GDV to column volume constant when transferring a standard method into a fast LC method. This will maintain the selectivity of the original method.[3]

The GDV can be adjusted to the column volume by installing appropriate mixer kits to the RSLC pump (see Table 2), which contributes most to the GDV. Typically, 100 µL or 200 µL mixers are good starting points when operating a small volume column in an RSLC system.

Another option is to switch the sample loop of the split-loop autosampler out of the flow path. The GDV is then reduced by the sample loop volume in the so-called bypass mode. The GDV of a standard sample loop of the RSLC autosampler is 150 µL, the micro injection loop has a 50 µL GDV.

Table 2. Mixer Kits Available for UltiMate 3000 RSLC System to Adapt GDV of Pump

Mixer Kit	GDV pump
Mixer kit 6040.5000	35 µL
Static mixer kit 6040.5100	100 µL
Static mixer kit 6040.5150	200 µL

Besides the gradient delay volume, the extra column volume is an important parameter for fast LC methods. The extra column volume is the volume in the system through which the sample passes and hence contributes to the band broadening of the analyte peak (Figure 3). The extra column volume of an optimized LC system should be below $^{1}/_{10}$th of the peak volume. Therefore the length and inner diameter of the tubing connections from injector to column and column to detector should be as small as possible. Special care has to be taken while installing the fittings to avoid dead volumes. In addition, the volume of the flow cell has to be adapted to the peak volumes eluting from the RSLC column. If possible, the flow cell detection volume should not exceed $^{1}/_{10}$th of the peak volume.

Detector Settings

When transferring a conventional method to an RSLC method, the detector settings have a significant impact on the detector performance. The data collection rate and time constant have to be adapted to the narrower peak shapes. In general, each peak should be defined by at least 30 data points. The data collection rate and time constant settings are typically interrelated to optimize the amount of data points per peak and reduce short-term noise while still maintaining peak height, symmetry, and resolution.

The Chromeleon® Chromatography Management Software has a wizard to automatically calculate the best settings, based on the input of the minimum peak width at half height of the chromatogram. This width is best determined by running the application once at maximum data rate and shortest time constant. The obtained peak width may then be entered into the wizard for optimization of the detection settings. Refer to the detector operation manual for further details.

METHOD SPEED-UP USING THE CALCULATOR

Separation Example

Separation was performed on an UltiMate 3000 RSLC system consisting of a HPG-3200RS Binary Rapid Separation Pump, a WPS-3000RS Rapid Separation Well Plate Sampler with analytical sample loop (100 µL), a TCC-3000RS Rapid Separation Thermostatted Column Compartment with precolumn heater (2 µL), and a VWD-3400RS Variable Wavelength Detector with semi-micro flow cell (2.5 µL). Chromeleon Chromatography Management Software (version 6.80, SR5) was used for both controlling the instrument and reporting the data. The modules were connected with stainless steel micro capillaries, 0.01" ID, $^{1}/_{16}$" OD when applying the conventional LC method, 0.007" and 0.005" ID, $^{1}/_{16}$" OD when applying the RSLC methods. A standard mixture of seven common soft drink additives was separated by gradient elution at 45 °C on two different columns:

- Conventional HPLC Column: Acclaim 120, C18, 5 µm, 4.6 × 150 mm column, (P/N 059148)
- Rapid Separation Column: Acclaim RSLC 120, C18, 2.2 µm, 2.1 × 50 mm column (P/N 068981).

The UV absorbance wavelength at 210 nm was recorded at 5 Hz using the 4.6 × 150 mm column and at 25 Hz and 50 Hz using the 2.1 × 50 mm column. Further method details such as flow rate, injection volume, and gradient table of conventional and RSLC methods are described in the following section. The parameters for the method transfer were calculated with the Dionex Method Speed-Up Calculator (version 1.14i).

The conventional separation of seven soft drink additives is shown in Figure 4A. With the Method Speed-Up Calculator, the method was transferred successfully to RSLC methods (Figure 4B and C) at two different flow rates. The easy method transfer with this universal tool is described below.

Column Selection for Appropriate Resolution

The column for method speed-up must provide sufficient efficiency to resolve the most critical pairs. In this example, separating peaks 5 and 6 is most challenging. A first selection of the planned column dimensions can be made by considering the theoretical plates according to Table 1. The 4.6 × 150 mm, 5 µm column is actually filled with 4.5 µm particles. Therefore, it provides 11,111 theoretical plates. On this column, the

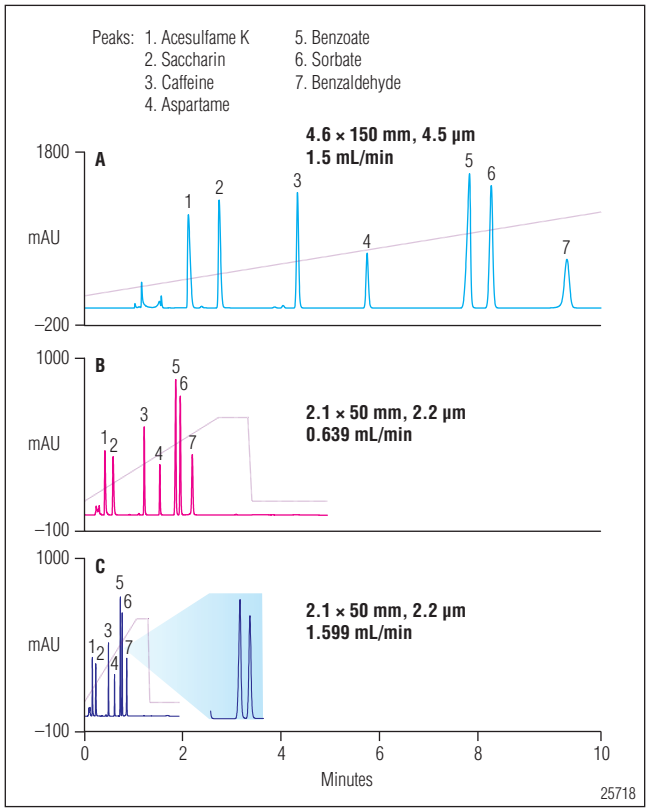

Figure 4. Method transfer with the Method Speed-Up Calculator from A) a conventional LC separation on an Acclaim 5 µm particle column, to B) and C) RSLC separations on an Acclaim 2.2 µm particle column.

resolution is $R_{(5,6)}$=3.48. This resolution is sufficiently high to select a fast LC column with fewer theoretical plates for the speed up. Therefore, a 2.1 × 50 mm, 2.2 µm column with 7579 plates was selected.

The first values to be entered into the yellow field of the Method Speed-Up Calculator are the current column dimension, planned column dimension, and the resolution of the critical pair. To obtain the most accurate method transfer, use the particle sizes listed in the manufacturer's column specifications sheet instead of the nominal size, which may be different. Dionex Acclaim columns with a nominal particle size of 5 µm are actually filled with 4.5 µm particles, and this value should be used to achieve a precise method transfer calculation. This has a positive impact on the performance and pressure predictions for the planned column. Based on the assumption of unchanged stationary phase chemistry, the calculator then predicts the resolution provided by the new method (Figure 5).

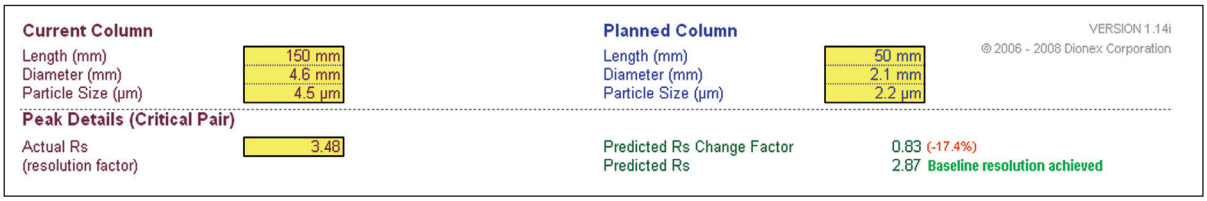

Figure 5. Column selection considering the resolution of the critical pair.

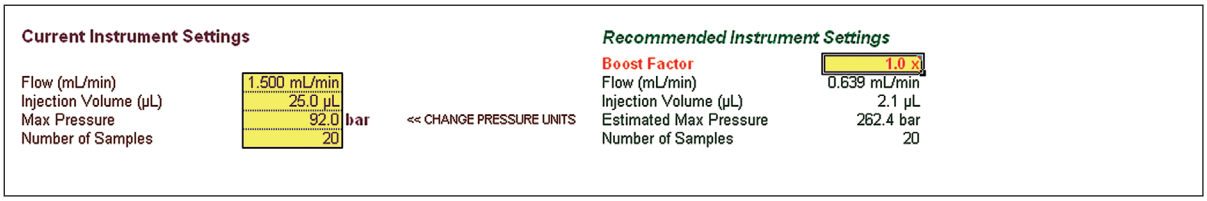

Figure 6. The flow rate, injection volume and backpressure of the current method are scaled to the new column dimension.

In the example in Figure 5, the predicted resolution between benzoate and sorbate is 2.87. With a resolution of $R \geq 1.5$, the message "Baseline resolution achieved" pops up. This indicates that a successful method transfer with enough resolution is possible with the planned column. If R is smaller than 1.5, the red warning "Baseline is not resolved" appears. Note that the resolution calculation is performed only if the boost factor BF is 1, otherwise it is disabled. The function of the boost factor is described in the Adjust Flow Rate section.

Instrument Settings

The next section of the Method Speed-Up Calculator considers basic instrument settings. These are flow rate, injection volume, and system backpressure of the current method (Figure 6). In addition to these values, the detector settings have to be considered as described in the earlier section "Detector Settings". Furthermore, the throughput gain with the new method can be calculated if the number of samples to be run is entered.

Adjust Flow Rate

As explained by Van Deemter theory, smaller particle phases need higher linear velocities to provide optimal separation efficiency. Consequently, the Dionex Method Speed-Up Calculator automatically optimizes the linear velocity by the ratio of particle sizes of the current and planned method. In addition, the new flow rate is scaled to the change of column cross section if the column inner diameter changed. This keeps the linear velocity of the mobile phase constant. A boost factor (*BF*) can be entered to multiply the flow rate for a further decrease in separation time. If the calculated resolution with $BF=1$ predicts sufficient separation, the method can be accelerated by increasing the boost factor and therefore increasing the flow rate. Figure 1 shows that applying linear velocities beyond the optimum is no problem with smaller particle phases, as they do not significantly loose plates in this region. Note that the resolution calculation of the Method Speed-Up Calculator is disabled for $BF \neq 1$.

For the separation at hand, the flow rate is scaled from 1.5 mL/min to 0.639 mL/min when changing from an Acclaim 4.6 × 150 mm, 4.5 µm column to a 2.1 × 50 mm, 2.2 µm column (see Figure 6), adapting the linear velocity to the column dimensions and the particle size. The predicted resolution between peak 5 and 6 for the planned column is $R=2.87$. The actual resolution achieved is $R=2.91$, almost as calculated (chromatogram B in Figure 4).

A Boost Factor of 2.5 was entered for further acceleration of the method (Figure 7). The method was then performed with a flow rate of 1.599 mL/min, and resolution of the critical pair was still sufficient at $R=2.56$ (see zoom in chromatogram C in Figure 4).

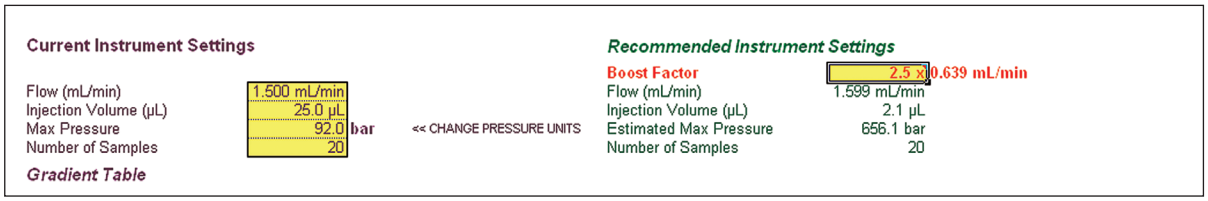

Figure 7. The new flow rate is further accelerated by applying the Boost Factor of 2.5.

Scale Injection Volume

The injection volume has to be adapted to the new column dimension to achieve similar peak heights by equivalent mass loading. Therefore the injection plug has to be scaled to the change of column cross section. In addition, shorter columns with smaller particles cause a reduced zone dilution. Consequently, sharper peaks compared to longer columns are expected. The new injection volume $V_{inj,new}$ is then calculated by Formula 8, taking a changed cross section and reduced band broadening by changed particle diameter into account.

Formula 8: $$V_{inj,new} = V_{inj,old} \cdot \left(\frac{d_{c,new}}{d_{c,old}}\right)^2 \cdot \sqrt{\frac{L_{new} \cdot d_{p,new}}{L_{old} \cdot d_{p,old}}}$$

Generally, it is recommended that a smaller flow cell be used with the RSLC method to minimize the extra column volume. Also, the difference in path length of different flow cell sizes has to be taken into account while scaling the injection volume. In the example of the soft drink analysis, the injection volume is scaled from 25 µL to 2.1 µL when replacing the Acclaim 4.6 × 150 mm, 4.5 µm column with a 2.1 × 50 mm, 2.2 µm column (see Figure 6).

Predicted Backpressure

Speeding-up the current method by decreasing particle size and column diameter and increasing flow rate means elevating the maximum generated backpressure. The pressure drop across a column can be approximated by the Kozeny-Carman formula.[4] The pressure drop of the new method is predicted by the calculator considering changes in column cross section, flow rate, and particle size and is multiplied by the boost factor. The viscosity of mobile phase is considered constant during method transfer. The calculated pressure is only an approximation and does not take into account nominal and actual particle size distribution depending on column manufacturer. If the predicted maximum pressure is above 800 bar (11,600 psi) the warning "Exceeds pressure limit RSLC" is shown, indicating the upper pressure limit of the UltiMate 3000 RSLC system. However, in the case the method is transferred to a third party system, its pressure specification has to be considered.

In the example of the soft drink analysis, the actual pressure increases from 92 bar to 182 bar with *BF*=1 on the 2.1 × 50 mm column, and to 460 bar for the RSLC method with *BF*=2.5. The pressures predicted by the Method Speed-Up Calculator are 262 bar and 656 bar, respectively. The pressure calculation takes into account the change of the size of the column packing material. In a speed up situation, the pressure is also influenced by other factors such as particle size distribution, system fluidics pressure, change of flow cell, etc. When multiplication factors such as the boost factor are used, the difference between calculated and real pressure is pronounced. The pressure calculation is meant to give an orientation, what flow rates might be feasible on the planned column. However, it should be confirmed by applying the flow on the column.

Adapt Gradient Table

The gradient profile has to be adapted to the changed column dimensions and flow rate following the gradient-volume principle. The gradient steps of the current method are entered into the yellow fields of the gradient table. The calculator then scales the gradient step intervals appropriately and creates the gradient table of the new method.

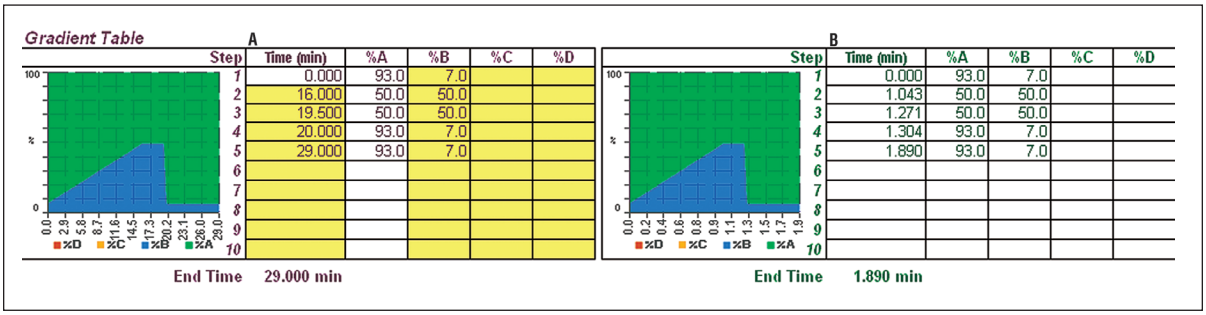

Figure 8. The gradient table of the current method (A) is adapted to the boosted method (B) according to the gradient-volume principle.

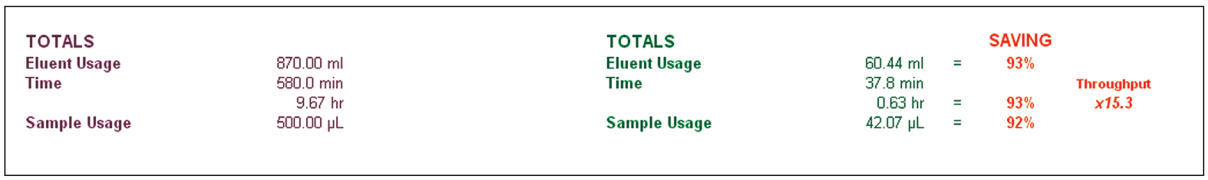

Figure 9. The absolute values for analysis time, eluent usage, and sample usage of the current (purple) and planned (green) method are calculated by the Method Speed-Up Calculator. The savings of eluent, sample, and time due to the method transfer are highlighted.

The adapted gradient table for the soft drink analysis while using a boost factor *BF*=1 is shown in Figure 8. According to the gradient-volume principle, the total run time is reduced from 29.0 min to 4.95 min by taking into account the changed column volume from a 4.6 × 150 mm, 5 µm (4.5 µm particles entered) to a 2.1 × 50 mm, 2.2 µm column and the flow rate reduction from 1.5 mL/min to 0.639 mL/min. The separation time was further reduced to 1.89 min by using boost factor *BF*=2.5. Gradient time steps were adapted accordingly. The comparison of the peak elution order displayed in Figure 4 shows that the separation performance of the gradient was maintained during method transfer.

Consumption and Savings

Why speed-up methods? To separate analyte peaks faster and at the same time reduce the mobile phase and sample volume consumption. Those three advantages of a method speed-up are indicated in the Method Speed-Up Calculator sheet right below the gradient table. The absolute values for the time, eluent, and sample usage are calculated taking the numbers of samples entered into the current instrument settings section of the calculation sheet into account (see Figure 6).

Regarding the soft drink analysis example, geometrical scaling of the method from the conventional column to the RSLC method means saving 93% of eluent and 92% of sample. The sample throughput increases 6.1-fold using *BF*=1. The higher flow rate at *BF*=2.5 results in a 15.3-fold increased throughput compared to the conventional LC method (Figure 9).

CONCLUSION

Fast method development or increased sample throughput are major challenges of most analytical laboratories. A systematic method speed-up is accomplished by reducing the particle size, shortening the column length, and increasing the linear velocity of the mobile phase. The Dionex Method Speed-Up Calculator automatically applies these rules and scales the conventional LC parameters to the conditions of the RSLC method. The interactive electronic tool is universally applicable. New instrument settings are predicted and gradient tables are adapted for optimum performance for the new method. The benefit of the method transfer is summarized by the integrated calculation of savings in time, eluent and sample. In addition, users can benefit from getting results earlier and thereby reducing the time to market. The Dionex Method Speed-Up Calculator is part of Dionex's total RSLC solution, which further consists of the industry leading UltiMate 3000 RSLC system, powerful Chromeleon Chromatography Management Software, and high-efficiency Acclaim RSLC columns.

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