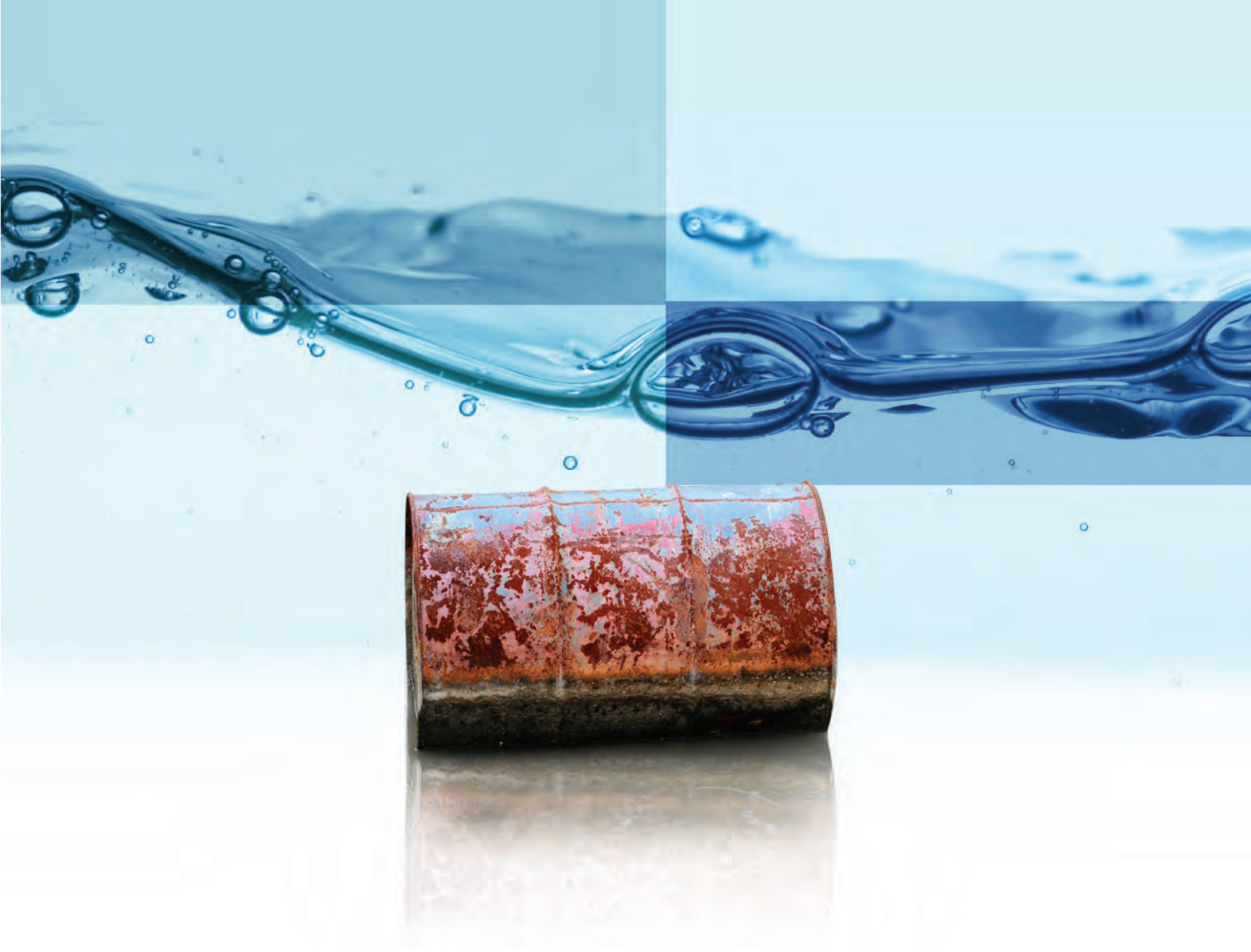

Environmental Water Applications Notebook

Hexavalent Chromium • Metals

Thermo SCIENTIFIC

Table of Contents

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 Hexavalent Chromium

Environmental Water Applications Notebook

Sensitive Determination of Hexavalent Chromium in Drinking Water

INTRODUCTION

Chromates are oxyanions (e.g., CrO_4^{2-}, $Cr_2O_7^{2-}$) of chromium in oxidation state +6. All hexavalent chromium Cr(VI) compounds are strong oxidizing agents and considered toxic and potentially carcinogenic. Hence, chromates are regulated in the environment and are a primary drinking water contaminant in the United States (US).[1] For example, in 1999, the state of California established a public health goal (PHG) of 0.2 µg/L (ppb) for Cr(VI) and 2.5 µg/L for total chromium.[2] The PHG is based on an estimated one-in-one-million lifetime cancer risk level.

Drinking water standards are regularly re-evaluated by the US Environmental Protection Agency (EPA). In 2008, the agency conducted a comprehensive review of the health effects of chromate based on toxicity studies done by the US National Toxicology Program.[3] In 2009, the Office of Environmental Health Hazard Assessment (OEHHA) at the California EPA proposed to lower the PHG for Cr(VI) to 0.06 ppb.[4] In September 2010, the EPA released the Toxicological Review of Hexavalent Chromium.[5] Based on this review, OEHHA in the state of California recently issued a new PHG for chromate at 0.02 ppb in drinking water.[6]

Currently, dissolved hexavalent chromium is measured as chromate according to a modified version of US EPA Method 218.6.[7-9] This method is based on anion-exchange chromatography on a Thermo Scientific Dionex IonPac™ AS7 column (4 × 250 mm) and detection after postcolumn reaction with diphenylcarbazide, which yields a compound with visible absorbance at 530 nm. This permits a method detection limit (MDL) for chromate at 0.02 µg/L and can support a reporting limit of 0.06 µg/L.[7]

However, the current method does not allow sufficient sensitivity for routine analysis at the proposed California PHG level of 0.02 µg/L. The work shown here describes modification of the conditions described in EPA Method 218.6, including use of the column in the 2 mm format and a smaller reaction coil to increase method sensitivity. The modified method uses a Dionex IonPac AG7 guard (2 × 50 mm) and Dionex IonPac AS7 analytical columns (2 × 250 mm), an eluent of 250 mM ammonium sulfate/100 mM ammonium hydroxide at a flow rate of 0.36 mL/min, a 1000 µL injection volume, and postcolumn reaction with 2 mM diphenylcarbazide/10% methanol/1 N sulfuric acid (using a 125 µL reaction coil) followed by visible absorbance detection at 530 nm. This modified method permits an MDL for chromate of 0.001 µg/L. This results in a quantitation limit of 0.003 µg/L, which is more than sufficient for analysis at the proposed California PHG level.

EQUIPMENT

Thermo Scientific Dionex ICS-2100, ICS-1600, ICS-1100,* ICS-3000, or ICS-5000 system including:

SP Single Pump or DP Dual Pump module**

DC Detector/Chromatography module**

Injection loop, 1000 µL

Reaction coil, 125 µL (P/N 053640), 375 µL (P/N 043700)

Sample syringe, 5 mL

ICS Series VWD UV-vis Absorbance Detector (P/N 069117, 4 wavelength or P/N 069116, single wavelength) with PEEK™ semi-micro flow cell, 2.5 µL, 7 mm (Victrex P/N 6074-0300) or PEEK standard flow cell, 11 µL, 10 mm (Victrex P/N (6074.0200)

Postcolumn Delivery Configuration:

DP** or PC10 Postcolumn Pneumatic Delivery Package or AXP (P/N 063973) or the AXP-MS Metering Pump (P/N 060684)

AS Autosampler

Thermo Scientific Dionex Chromeleon™ Chromatography Data System (CDS) software

Eluent Organizer, including 2 L plastic bottles (P/N 072057) and pressure regulator (P/N 038201)

Polypropylene injection vials with caps (0.3 mL vial kit, P/N 055428)

Nalgene™ 125 mL HDPE narrow mouth bottles (VWR P/N 16057-062)

Nalgene 250 mL HDPE narrow mouth bottles (VWR P/N 16057-109)

Nalgene 250 mL 0.2 µm nylon filter units (VWR P/N 28199-371)

Nalgene 1000 mL 0.2 µm nylon filter units (VWR P/N 28198-514)

*With addition of the optional column heater
**For the Dionex ICS-3000 or ICS-5000

REAGENTS AND STANDARDS

Reagents

Prepare all solutions from analytical reagent-grade chemicals (when commercially available). Note: There is a possibility of the presence of trace levels of chromate in some commercially available chemicals.

Deionized (DI) water, 18 MΩ or better

Ammonium sulfate (Mallinckrodt General P/N AR 7725)

Ammonium hydroxide (Sigma P/N A6899)

Sulfuric acid, 95-98% (JT Baker Instra-Analyzed P/N 9673)

Methanol, HPLC grade (Fisher Optima P/N A454-4)

Potassium dichromate (JT Baker P/N 4765-01)

Sodium and potassium salts, ACS reagent-grade, for preparing the anion standards

CONDITIONS

Method

Columns:	Dionex IonPac AG7 Guard 2 × 50 mm (PN 063099), Dionex IonPac AS7 Analytical 2 × 250 mm (PN 063097)
Eluent:	250 mM Ammonium sulfate and 100 mM ammonium hydroxide
Eluent Flow Rate:	0.36 mL/min
Inj. Volume:	1000 µL (Full loop)
Temperature:	30 °C
Back Pressure:	1700–2000 psi

Postcolumn Reagent (PCR):

	2 mM diphenylcarbazide, 10 % methanol, 1 N sulfuric acid
PCR Flow Rate:	0.12 mL/min
Detection:	Visible absorbance, 530 nm
Noise:	6–8 µAU
Run Time:	10 min

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent

250 mM Ammonium sulfate

100 mM Ammonium hydroxide

Dissolve 66 g of ammonium sulfate in ~1 L of DI water and add 13 mL of 29% ammonium hydroxide solution. Dilute to 2.0 L with DI water.

Sample Adjustment Buffer

250 mM Ammonium sulfate

1000 mM Ammonium hydroxide

Dissolve 3.3 g of ammonium sulfate in ~75 mL of DI water and add 6.5 mL of 29% ammonium hydroxide. Dilute to 100 mL with DI water.

Postcolumn Reagent

2 mM Diphenylcarbazide

10% Methanol

1 N Sulfuric acid

Add 28 mL of 98% sulfuric acid to ~500 mL of DI water in a 1.0 L volumetric flask (caution: this mixture may get hot). Mix and allow to cool. Add 0.5 g of 1,5-diphenylcarbazide to ~75 mL of HPLC-grade methanol in a 100 mL volumetric flask and sonicate to dissolve. Bring to volume with methanol, mix, and add to the cooled sulfuric acid solution. Dilute to 1.0 L with DI water, mix, and transfer to the pressurized PCR container. The PCR is stable for 3–4 days. Prepare fresh as needed.

Standard Solutions

Add 0.283 g of potassium dichromate (dried at 100 °C to a constant weight) to ~50 mL of DI water in a 100 mL volumetric flask. Dissolve and bring to volume with DI water. Store the stock standard at 4 °C. Prepare working standards fresh daily. Adjust the pH to 9.0–9.5 by adding 1 mL of sample adjustment buffer per 100 mL of final volume before bringing to final volume.

High-Ionic Strength Water

High ionic strength water (HIW) is defined in EPA Method 300.1[10] as simulated drinking water prepared from DI water fortified with chloride (100 mg/L), nitrate (10 mg/L as N), phosphate (10 mg/L as P), sulfate (100 mg/L), and carbonate (100 mg/L). In the current work, HIW was prepared from DI water and fortified with fluoride (1 mg/L), nitrite (0.1 mg/L), and bromide (0.02 mg/L), in addition to the ions mentioned in EPA Method 300.1. This HIW was prepared by diluting appropriate volumes of the 1000 mg/L stock standards with DI water.

SAMPLE PREPARATION

Clean all sample collection equipment and containers with concentrated HNO_3 diluted 1:1 with DI water and rinse well with DI water before use. Collect samples in amber glass bottles with plastic lined caps. Do not filter the samples at the time of collection, but immediately add the sample adjustment buffer dropwise until the sample pH falls in the range of 9.0–9.5. Be careful not to contaminate the sample while measuring the pH. Most drinking water samples can be adjusted to pH 9.0–9.5 by adding 1 mL or less of the adjustment buffer per 100 mL of sample, which introduces an acceptable 1% dilution error.

For more difficult samples, start with a known amount of sample and accurately measure the amount of buffer added so that the amount of Cr(VI) as CrO_4^{2-} determined by ion chromatography (IC) can be corrected for dilution. Cool to 4 °C and hold at 4 °C during transport and storage. Analyze samples within 24 h of collection to minimize the potential loss of Cr(VI) through chemical reduction.

System Preparation and Configuration

The VWD UV-vis absorbance detector can be equipped with either a standard (PEEK) or a semi-micro (PEEK) flow cell when the DP or AXP pumps are used to deliver the postcolumn reagent. If the PC10 is used for postcolumn reagent delivery, the standard (PEEK) flow cell must be used. An end line filter (P/N 045987) can be used for the eluent and postcolumn reagent lines to reduce noise, though none were used for the data shown in this application update.

Alternatives for Postcolumn Delivery

DP Pump Module

The postcolumn reagent can be delivered via the second pump of the DP module, the PC10, or the AXP pump. Operate the DP pump at a backpressure of 1400–1600 psi. Use green PEEK tubing (P/N 044777) between the pump and backpressure tubing (connect using union P/N 042627) to reduce pump noise.

AXP Pump

Configure the AXP pump as described in the AXP/AXP-MS Manual and operate at a typical backpressure of 1400–1600 psi.[11] Use green PEEK tubing (P/N 044777) between the AXP pump and backpressure tubing (connect using union P/N 042627) to reduce pump noise. An equilibrated system has peak-to-peak noise of less than 10 µAU.

PC10

Configure the IC and the PCR system as shown in Figure 3 of Dionex Technical Note 26,[9] and as described in the PC10 Postcolumn Delivery System installation instructions.[12] A standard (PEEK) UV cell is recommended with the PC10. Pump the eluent at 0.36 mL/min and set the PC10 pneumatic pressure to ~40 psi.

To measure the PCR flow rate, collect the effluent from the detector (i.e., the total flow from the IC pump and the PCR module) in a 10 mL graduated cylinder for 10 min. The PCR flow rate is the difference between the total flow rate and that of the IC pump. Adjust the air pressure of the postcolumn delivery module (PC10) and remeasure the flow rate until the correct PCR flow rate of 0.12 mL/min is established. Variations in the PCR flow rate affect the postcolumn reaction time, pH, dilution, mixing rate, and ratio of the reactants. Stable day-to-day results depend on a well-controlled PCR flow rate.

Confirm this flow rate on a daily basis or whenever detector response for a calibration check standard deviates beyond quality control acceptance criteria. Once the flow rate has been established, the PCR flow rate can be monitored by observing the absorbance at 280 nm (obtained from a 4-channel VWD). The absorbance at 280 nm should remain at the same level during the full series of injections.

Column Equilibration

The storage solution that the Dionex IonPac AS7 column is shipped with is 30 mM nitric acid. After equilibrating the column with eluent for 60 min, analyze a system blank of 1000 µL of DI water. An equilibrated system has a background signal of less than 200 mAU and peak-to-peak noise of less than 10 µAU. No peaks should elute within the retention time window of the chromate peak. The column is equilibrated when two consecutive injections of a standard produce the same retention time for chromate.

Instrument Operational Considerations

After running a sequence of injections, if the system will not be running this application for a few days, it is recommended to run the pumps used for eluent and PCR delivery with DI water for >2 h. The UV-vis lamps should be turned off to extend their life. When the system is idle for short periods (1–2 weeks), the pump can be run with DI water at a reduced flow rate to achieve rapid startup.

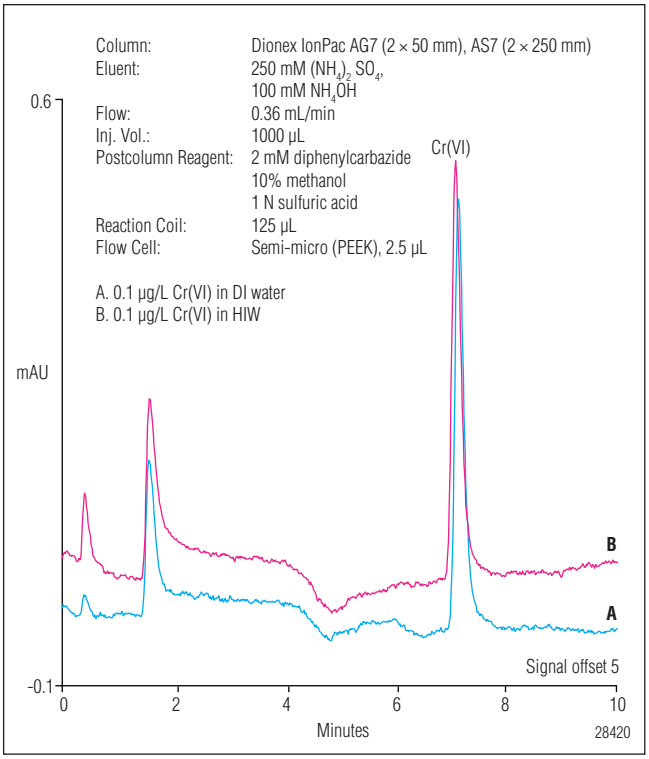

Figure 1. Determination of chromate (0.1 µg/L) in A) DI water and B) HIW on a Dionex ICS-3000 system. Postcolumn reagent delivered by a DP. Flow cell: semi-micro (PEEK).

RESULTS AND DISCUSSION

ICS-3000 System

Figure 1 shows a chromatogram of a 0.1 µg/L Cr(VI) as CrO_4^{2-} standard in DI water and in HIW using a DP pump for PCR delivery and a semi-micro flow cell. The elution time for chromate was about 7 min. A slight shift (0.05 min) in the retention time for chromate in the HIW matrix was observed. However, the peak shape and the peak area response are similar to standards in DI water. In the concentration range 0.005–1 µg/L, the peak response recovery ranges from 89–103% in the presence of 100 mg/L chloride, sulfate, and carbonate.

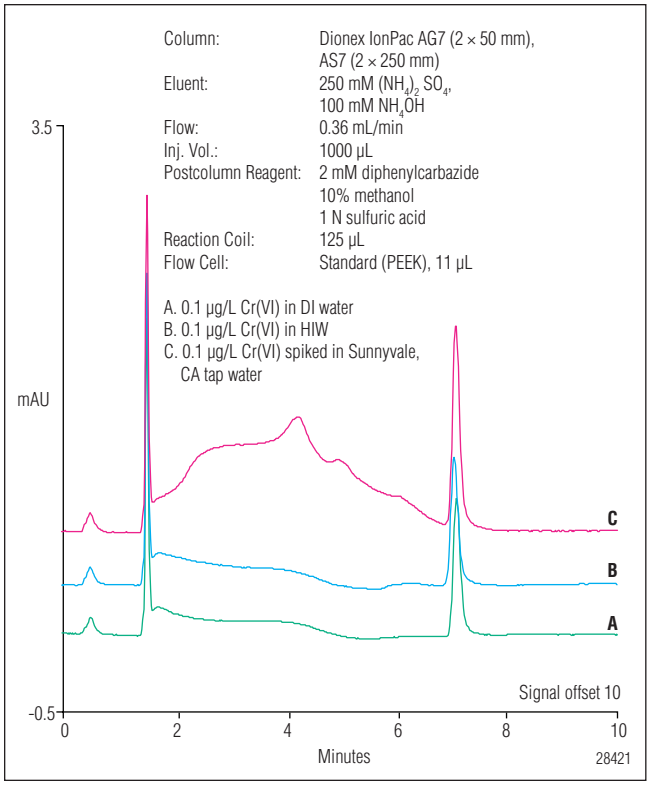

Figure 2. Determination of chromate in A) DI water, B) HIW, and C) Sunnyvale, CA tap water using a Dionex ICS-3000 system. Sunnyvale, CA tap water blank has a background Cr(VI) level of 0.05 µg/L chromate. Postcolumn reagent delivered by an AXP pump. Flow cell: standard (PEEK).

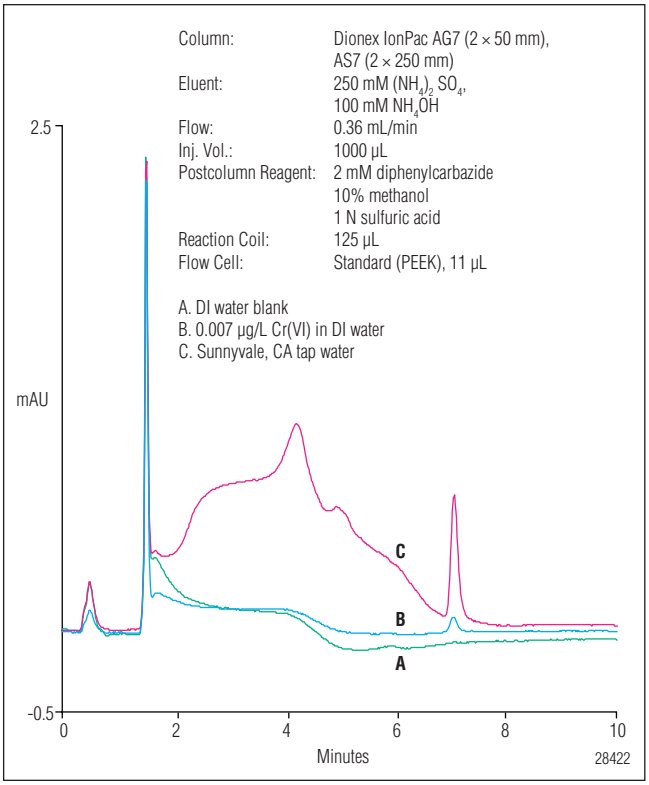

Figure 3. Determination of chromate in A) DI water blank, B) 0.007 µg/L, and C) Sunnyvale, CA tap water blank on a Dionex ICS-3000 system. Postcolumn reagent delivered by the second pump of DP. Flow cell: standard (PEEK).

Figure 2 shows the chromatograms obtained for DI water, HIW, and Sunnyvale, CA tap water spiked with Cr(VI) at 0.1 µg/L using an AXP pump for the PCR delivery and a standard flow cell. The baseline (2–6 min) signal in the tap water sample did not affect the chromate peak eluting at ~7 min. The tap water blank has a background level of 0.05 µg/L chromate (Figure 3, C). The calibration curve is linear over the calibration range 0.01–0.2 µg/L for Cr(VI) as CrO_4^{2-}, with a coefficient of determination of 0.994.

Table 1. Method Detection Limits for Chromate in HIW Based on a 1000 µL Injection

Chromate Conc. (µg/L)	Std. Dev. (µg/L)	RSD (%)	MDL (µg/L)
0.001	0.0003	10.03	0.0009
0.005	0.0004	6.62	0.0013

MDL for chromate for 1000 µL n = 7 injections

The MDLs for Cr(VI) as CrO_4^{2-} are summarized in Table 1. MDL is a measure of the precision of replicate injections of a low-level standard 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.

In this application, the MDL for chromate Cr(VI) as CrO_4^{2-} was determined by analyzing seven replicate injections of HIW fortified with Cr(VI) as CrO_4^{2-} at two concentration levels of 0.001 and 0.005 µg/L (i.e., approximately 3–5× the estimated instrument detection limit). Both levels produced a calculated MDL value of 0.001 µg/L. This will enable a minimum quantitation limit of 0.003 µg/L for Cr(VI) as CrO_4^{2-}, which will be adequate for routine analysis at the proposed California PHG of 0.02 µg/L.

Comparable results were obtained for seven replicate injections of DI water fortified with Cr(VI) as CrO_4^{2-}. Figure 3 shows a 0.007 µg/L Cr(VI) as CrO_4^{2-} standard in DI water B), a DI water blank A), and a Sunnyvale, CA tap water sample C) with a measured concentration of 0.05 µg/L Cr(VI).

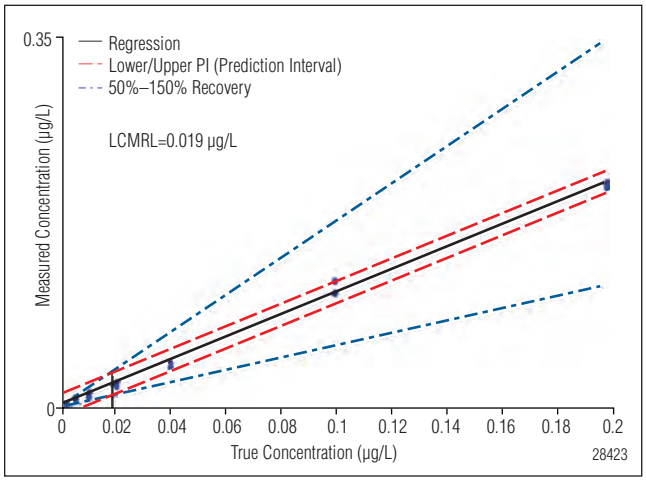

Figure 4. Determination of the LCMRL using LCMRL calculator.

Lowest Concentration Minimum Reporting Limit

Lowest concentration minimum reporting limit (LCMRL) is defined as the lowest spiking concentration such that the probability of spike recovery in the 50–150% range is at least 99%. The LCMRL calculated using EPA's LCRML calculator for chromate in HIW was 0.019 µg/L (Figure 4).[13]

ICS-2100 System

Figure 5 shows a 0.1 µg/L Cr(VI) as CrO_4^{2-} standard in DI water A) and in HIW B), by using the AXP pump for PCR delivery and a semi-micro flow cell. The two configurations (ICS-3000 and ICS-2100) provided similar results (Figures 2 and 5).

The reaction coil is not temperature controlled in an ICS-2100 system. This may result in nonsystematic baseline noise. This will not interfere with chromate detection because the nonsystematic noise is less than 5% of the chromate signal at 0.005 µg/L.

Reaction Coil

The current method can be configured with a 125 µL or a 375 µL reaction coil. The data shown here was generated with a 125 µL reaction coil. Increased peak area response was obtained with a 375 µL reaction coil in the concentration range 0.02–0.2 µg/L. However, there was no significant difference in the MDL when measured using either the 125 µL or the 375 µL reaction coil.

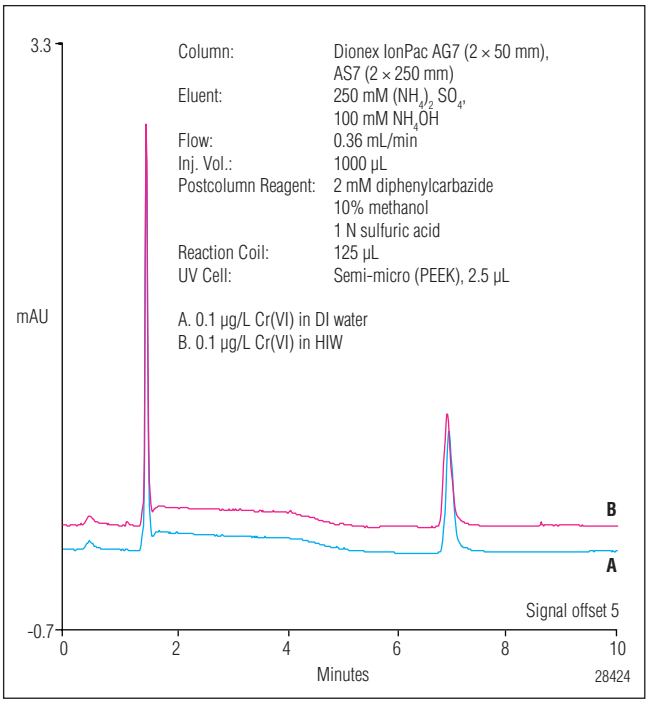

Figure 5. Determination of chromate (0.1 µg/L) in A) DI water and B) HIW on an ICS-2100 system. Postcolumn reagent delivered by an AXP pump. Flow cell: semi-micro (PEEK).

Although the 375 µL reaction coil ensures greater reaction efficiency, it also causes greater peak dilution. At lower concentrations (<0.02 µg/L), there is less difference in reaction efficiency and any gain from the 375 µL reaction coil is lost due to peak dilution. Hence, both coils yield the same lower detection limits.

CONCLUSION

This testing presents modifications to the existing US EPA Method 218.6 to allow sufficient sensitivity for determining hexavalent chromium (i.e., Cr(VI) as CrO_4^{2-}) at the proposed California PHG level of 0.02 µg/L. This includes the use of a Dionex IonPac AG7 guard (2 × 50 mm) and Dionex IonPac AS7 analytical (2 × 250 mm) columns while appropriately reducing the flow rates and reaction coil volume. Postcolumn reagent delivery can be configured three ways. The resulting MDL for Cr(VI) as CrO_4^{2-} at 0.001 µg/L will allow a minimum quantitation limit of 0.003 µg/L, which is more than sufficient for the proposed California PHG of 0.02 µg/L.

SUPPLIERS

VWR, 1310 Goshen Parkway, West Chester, PA 19380, U.S.A. Tel: 800-932-5000.

Sigma-Aldrich Chemical Co., P.O. Box 2060, Milwaukee, WI 53201, U.S.A. Tel: 800-558-9160.

Fisher Scientific, 711 Forbes Avenue, Pittsburgh, PA 15219, U.S.A. Tel: 800-766-7000

Mallinckrodt Baker, 222 Red School Lane, Phillipsburg, NJ 08665, U.S.A. Tel: 800-582-2537

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Separation of Chromium (III) and Chromium (VI) by Ion Chromatography

INTRODUCTION

Chromium in the environment exists primarily in two oxidation states: Cr(III) and Cr(VI). While the trivalent Cr(III) is only toxic at high concentrations, hexavalent Cr(VI), a strong oxidizer, is considered toxic to humans and the environment at µg/L concentrations. Because of this toxicity, many countries and states strongly regulate the concentration of Cr(VI) in drinking and wastewaters and require that its concentration is measured and reported. The state of California, for example, has established a public health goal (PHG) of 0.2 µg/L Cr(VI) in drinking water. Dissolved hexavalent chromium can be measured as the chromate ion following USEPA Method 218.6. This method uses ion chromatography and the IonPac® AS7 column with detection by postcolumn reaction to yield a compound measured by visible absorbance. In Application Update 144, Dionex updated Method 218.6 and Dionex Application Note 80 (Determination of Dissolved Hexavalent Chromium in Drinking Water, Groundwater, and Industrial Wastewater Effluents by Ion Chromatography) to allow the method to be used to meet the California PHG of 0.2 µg/L Cr(VI).[1-2] Dionex Technical Note 26 shows application of Method 218.6 for determination of Cr(VI) in wastewater and solid waste extracts.[3]

Reliable determination of Cr(III) in the presence of Cr(VI) is complicated by the aqueous chemistry of each oxidation state, as Cr(III) exists primarily as a cation in solution, and Cr(VI) exists primarily as an anion, although these states are dependent on solution pH. Approaching neutral pH, Cr(III) will form a hydroxo-Cr(III) species; at alkaline pH it forms a hydroxide precipitate. As pH increases, Cr(III) can oxidize to form Cr(VI) (chromate). Efforts to preserve Cr(III) (e.g. lowering solution pH) can lead to the loss of Cr(VI). When designing sample preparation and analysis methods for determining the Cr(III) and Cr(VI) contents of soil, wastewater, or other samples, the biggest challenge is to ensure that the sample preparation and analysis procedures do not change the distribution of oxidation states in the sample.

In 2002, Ščančar, and Milačič designed a method for speciating airborne chromium.[4] They separated Cr(III) and Cr(VI) on a monolithic disc modified with weak anion-exchange groups. Cr(III) was not bound, and Cr(VI) (as chromate) was eluted using an ammonium nitrate solution buffered between pH 4 and 12 to match the pH of the sample. The authors noted that as pH increased, some Cr(III) was partially retained due to the formation of the hydroxo-Cr(III) species partially bound to the disc. Chromium was measured in the unretained and bound fractions using electrothermal atomic absorption spectrometry (EAAS). The authors then applied this method to determinations of Cr(VI) in soils and cement.[5-6]

European Union Directive 2003/53/EC restricts the use of cement and cement products to those that contain <2 mg/kg Cr(VI) when hydrated. For determination of Cr(VI) in cement, the authors also used the IonPac CS5A with 350 mM ammonium nitrate eluent prior to EAAS, and noted that the Cr(III) remained bound to the column. One major theme pertaining to both of these studies was measurement of Cr(VI) in a variety of samples, while taking care not to convert Cr(III) to Cr(VI).

Dionex Technical Note 24 describes a method for simultaneous determination of Cr(III) and Cr(VI) (Method A).[7] TN24 shows separation of a mixed Cr(III)/(VI) standard using the IonPac CS5 column, and discusses the steps required for preparing a sample containing Cr(III) and Cr(VI) for chromatography. In this application update, the authors demonstrate separation of Cr(III) and Cr(VI) on the IonPac CS5A, the replacement column for the CS5. A revised sample preparation method for stabilizing both Cr(III) and Cr(VI) during sample preparation, and discussion on the feasibility of using this method for soil and wastewater analyses are also provided.

EQUIPMENT

Dionex ICS-3000 consisting of:

- DP Dual-Gradient Pump*
- DC Detector/Chromatography module with 6-port injection valve
- VWD Variable Wavelength Detector with PEEK™ flow cell, 11 µL, 10 mm (Dionex P/N 6074.0200)

PC10 Postcolumn Delivery System

Chromeleon® 6.8 Chromatography Management Software

* This application can also be run using an Isocratic or Gradient Pump module.

REAGENTS AND STANDARDS

Deionized water (DI), Type I reagent grade, 18 MΩ-cm resistivity.

Pyridine-2,6-dicarboxylic acid (PDCA) (Dionex P/N 039671)

Disodium hydrogen phosphate anhydrous (Na_2HPO_4)

Sodium iodide (NaI)

Ammonium acetate ($NH_4CH_3CO_2$)

Lithium hydroxide (LiOH)

1,5-diphenylcarbohydrazide (DPC)

Nitric acid (HNO_3)

Methanol (CH_3OH)

Sulfuric acid (H_2SO_4) (96%; spectrophotometric grade)

Chromium (III) nitrate ($Cr(NO_3)_3 \cdot 9H_2O$)

Disodium chromate (Na_2CrO_4)

All compounds should be ACS reagent grade or better, purchased from reliable sources.

CONDITIONS

Guard column:	IonPac CG5A, 4 × 50 mm (Dionex P/N 046100)
Column:	IonPac CS5A, 4 × 250 mm (Dionex P/N 046104)
Eluent:	2 mM PDCA, 2 mM Na_2HPO_4 10 mM NaI, 50 mM CH_3CO_2 NH_4 2.8 mM LiOH
Flow Rate:	1.0 mL/min
Injection Volume:	50 µL
Expected System Pressure:	1500 psi
Detector Wavelength:	0.0–4.7 min, 335 nm 4.7–8.0 min, 530 nm
Postcolumn Reagent:	2 mM DPC 10% CH_3OH 0.9 N H_2SO_4
Postcolumn Flow Rate:	0.5 mL/min*
Reaction Coil:	375 µL (Dionex P/N 043700)

*The flow rate of the eluent and postcolumn reagent are critical to this analysis. The combined flow rate should be 1.5 mL/min, as the eluent flow rate is 1.0 mL/min and the postcolumn reagent flow rate is 0.5 mL/min. After measuring the flow rate at the waste line, adjust the PC10 pressure regulator and measure to obtain the total flow rate of 1.5 mL/min.

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent Stock: (10X concentrate)

To prepare 1 L, dissolve the following reagents in deionized water: 3.34 g pyridine-2,6-dicarboxylic acid (PDCA), 5.36 g disodium hydrogen phosphate heptahydrate, 15 g sodium iodide, 38.5 g ammonium acetate, and 1.1 g lithium hydroxide monohydrate. PDCA is slow to dissolve. Heating the solution before adding the reagents increases the rate of dissolution.

ELUENT

2 mM PDCA/2 mM Na_2HPO_4/10 mM NaI/50 mM CH_3CO_2 NH_4/ 2.8 mM LiOH

To prepare 1 L, add 100 mL of the eluent stock to a 1 L volumetric flask and bring to volume with deionized water. The pH of this solution should be between 6.70 and 6.80.

POSTCOLUMN REAGENT

2 mM DPC/10% CH_3OH/0.9 N H_2SO_4

To prepare 1L, dissolve 0.5 g 1,5-diphenylcarbohydrazide (DPC) in 100 mL HPLC grade methanol in a 1 L volumetric flask. Add approximately 500 mL deionized water containing 25 mL of 96% sulfuric acid. Bring to volume with DI H_2O.

STANDARD SOLUTIONS

Stock Standards

Cr (III) and Cr (VI) standards were prepared in 50 mM nitric acid.

50 mM Nitric Acid

To prepare 1 L, add approximately 100 mL DI H_2O to a 1 L volumetric flask, weigh 3.15 g concentrated nitric acid into a 50 mL beaker and transfer to the volumetric flask. Bring to volume with DI H_2O.

1000 mg/L Chromium Standards

To prepare 1000 mg/L Cr(III) and Cr(VI) stock standards, dissolve the weights of the appropriate chromium salt, listed below, in 100 mL of a 50 mM nitric acid solution.

Standard	Salt	Weight (g)
Cr(III)	$Cr(NO_3)_3 \cdot 9H_2O$	0.769
Cr(VI)	Na_2CrO_4	0.312

Standard Preparation

Standards are prepared for chromatography in the same manner as samples; preparation of a 0.75 mg/L Cr(VI) standard is shown below.

1. Add 1.5 mL 50 mg/L Cr(VI) in 50 mM nitric acid to a 100 mL Pyrex® beaker.
2. Add 10 mL eluent stock (10× concentration of the eluent) and 10 µL concentrated nitric acid.
3. Mix thoroughly, place the container (uncovered) in a boiling water bath for 30 min.
4. Cool the standard to room temperature; transfer to a 100 mL volumetric flask and bring to volume with DI H_2O.

Cr(III) and mixed Cr(III)/Cr(VI) standards are prepared in the same manner.

Sample Preparation

Soil and wastewater samples were used to show the feasibility of determining Cr(III) and Cr(VI) using the chromatography method described in this application update. Sample preparation for analysis is shown below.

1. Add approximately 0.1 g soil sample or 5 mL wastewater sample to a 100 mL Pyrex beaker.
2. Add 5 mL 50 mM nitric acid, 10 mL eluent stock, and 10 µL concentrated nitric acid.
3. Mix thoroughly, place the container (uncovered) in a boiling water bath for 30 min.
4. Cool the sample to room temperature; transfer to a 100 mL volumetric flask, and bring to volume with DI water.
5. Filter sample with qualitative 2, 110 mm filter paper (Whatman, Catalog No. 1002 110) and treat with an OnGuard® II P sample pretreatment cartridge (Dionex P/N 057087)

For the feasibility demonstration, the authors used a simple qualitative filtration. To obtain the optimum column performance and prolong the life of the column, the authors recommend using a 0.45 µm filter.

Spike Sample Preparation

Standards of 1000 mg/L Cr(III) and 50 mg/L Cr(VI) were used to spike the soil and wastewater samples. An example of preparing a spiked sample with a final concentration of 10 mg/L Cr(III) and 0.5 mg/L Cr(VI) is presented below.

1. Add approximately 0.1 g soil sample or 5 mL wastewater sample to a 100 mL Pyrex beaker.
2. Add 1 mL 1000 mg/L Cr(III) and 1 mL 50 mg/L Cr(VI).
3. Add 5 mL 50 mM nitric acid, 10 mL eluent stock, and 10 µL concentrated nitric acid.
4. Mix thoroughly, place the container (uncovered) in a boiling water bath for 30 min.
5. Cool the sample to room temperature; transfer to a 100 mL volumetric flask, and bring to volume with DI H_2O.
6. Filter sample, as above, and pretreat using an OnGuard II P sample pretreatment cartridge.

RESULTS AND DISCUSSION

Discussion of the Chromatography Method

Cr(III) and Cr(VI) species are separated on the CS5A column, which is packed with resin containing both cation- and anion-exchange functional groups. The CS5A was designed to replace the CS5 for improved transition metal analysis using both PDCA and oxalic acid eluents. The resin in the CS5A column has lower capacity and a different ratio of anion-to-cation functionality than the resin used in the CS5 column (0.5:1 for the CS5, and to 2:1 for the CS5A.) Both these changes affect the Cr(III)/Cr(VI) separation. Using the PDCA-based eluent system, trivalent chromium is separated as the $Cr(PDCA)^{2-}$ complex, while the hexavalent chromium is separated as the chromate ion (CrO_4^{2-}). Chromate does not form a complex with PDCA. Due to the slow kinetics of ligand exchange for Cr(III), a precolumn derivatization with PDCA is used to form the Cr(III)-PDCA complex in the sample.

After separation, the Cr(III)-PDCA complex is detected by absorbance at 335 nm. This value is 30 nm less than that used in TN 24, but was found to be the wavelength maximum using a photodiode array detector. Chromate is subjected to postcolumn derivatization with DPC and detected at 530 nm as shown in AU 144. The Cr(III)-PDCA complex does not react with DPC but can be detected at 530 nm. While not ideal, the product of the Cr(VI) and DPC reaction can also be detected at 335 nm. Figure 1 shows Cr(III) and Cr(VI) detected at both 335 and 530 nm. To maximize sensitivity for each species, wavelengths were set at 335 nm from 0.0 min to 4.7 min for Cr(III), and 530 nm from 4.7 min to 8.0 min for Cr(VI).

Figure 2 shows an overlay of three concentrations of a mixed standard. The retention times of Cr(III) and Cr(VI) are 3.4 and 6.0 min, respectively. The baseline dip at 4.7 min is due to changing the detector wavelength. Note the sensitivity for the Cr(III)-PDCA complex at 335 nm is lower than for Cr(VI) at 530 nm after reaction with DPC. This method uses a 50 µL injection volume rather than a 250 µL injection volume loop used in TN24.

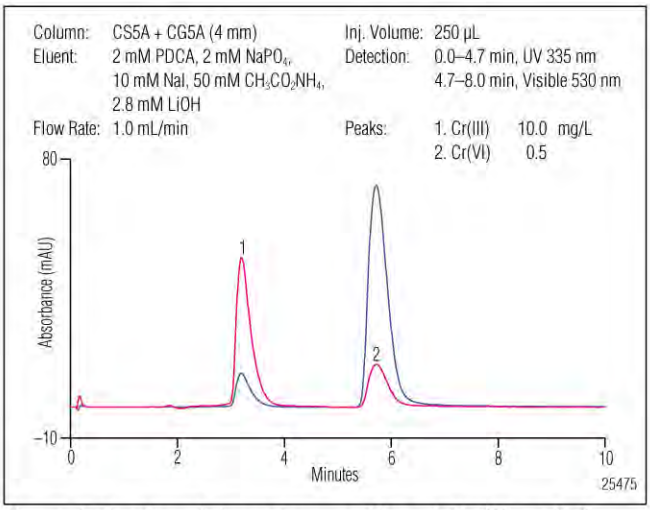

Figure 1. Overlay of chromatograms 10 mg/L Cr(III) and 0.5 mg/L Cr(VI) at 335 nm (pink) and 530 nm (blue).

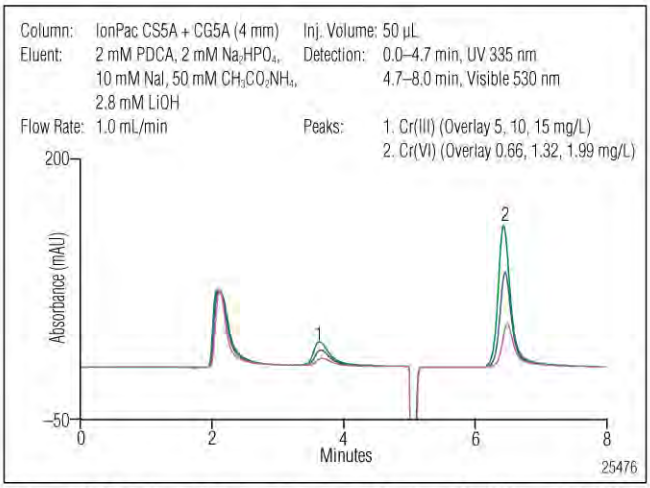

Figure 2. Overlay of chromatograms of mixed Cr(III) and Cr(VI) standards.

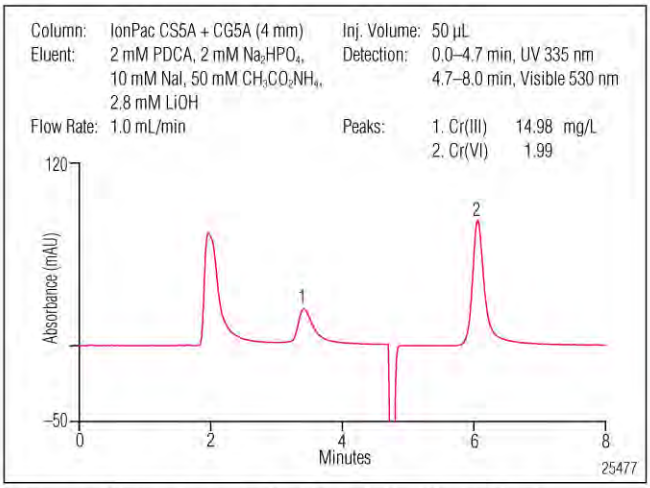

Figure 3. Chromatogram of a 15 mg/L Cr(III) standard.

A 250 µL injection volume loop overloaded the CS5A column using the sample preparation method below. This may be a result of the reduced anion-exchange capacity of the CS5A column compared to the CS5, and/or the change in the sample preparation procedure. It may be possible to use injection volumes between 50 and 250 µL without overloading the column, but such volumes were not tested. When evaluating samples use a Cr(VI) standard spiked into a prepared sample to evaluate recovery (i.e., whether the sample is overloading the column.)

Sample Preparation to Produce the Cr(III)-PDCA Complex

To produce the Cr(III)-PDCA complex, the sample must be treated with the PDCA eluent prior to injection. The PDCA eluent is buffered at pH 6.8. In a heated solution containing dissolved oxygen, Cr(III) can oxidize to Cr(VI) at neutral and higher pH values. The formation of the Cr(III)-PDCA complex is inhibited at pH values above 6, but as samples can contain both Cr(III) and Cr(VI), the sample preparation must also preserve Cr(VI). At pH values below 6, chromate can convert to dichromate, which can be harmful to the column and reduce the measurable Cr(VI). Dichromate can also oxidize the Cr(III). TN24 Figure 2 shows the effect of sample pH on the peak heights of Cr(III) and Cr(VI). The sample preparation procedure has been developed to maximize the formation of the Cr(III)-PDCA complex while minimizing the oxidation of Cr(III) to Cr(VI) and the conversion of chromate to dichromate.

A sample preparation method to maximize Cr(III) and Cr(VI) recovery in a sample was empirically determined using standards. Ten mL of the 10× PDCA and 10 µL concentrated nitric acid were added to 1.5 mL of standard prepared in 50 mM nitric acid and the sample was boiled. The addition of the nitric acid lowers the sample pH to approximately 5.9. Experimentation showed that the addition of nitric acid preserves Cr(III) while having no effect on the amount of Cr(VI). Figure 3 shows the analysis of a 15 mg/L Cr(III) standard. Under these conditions, 8-9% of the Cr(III) was converted to Cr(VI) irrespective of Cr(III) concentration in the range of 5 to 15 mg/L. Higher amounts of nitric acid (e.g. 50 µL) resulted in greater preservation of Cr(III) but caused a loss of Cr(VI). Reducing the heating time from 30 min may reduce Cr(III) loss. A one min heating time was inadequate for Cr(III)-PDCA complex formation, but for

this application, no other times <30 min were examined.

Prior to sample analysis, the system was calibrated to account for approximately 8-9% conversion of Cr(III) to Cr(VI). The system was first calibrated with Cr(VI) using concentrations of 0.25, 0.5, and 0.75 mg/L, which yielded a correlation coefficient of 0.9993. Figure 4 shows the chromatography of the Cr(VI) standards. This calibration was used to measure the amount of Cr(VI) formed from duplicate preparations of a 15 mg/L Cr(III) standard. As shown in Table 1, 1.24 mg/L Cr(VI) was produced from 15 mg/L Cr(III), which represents an 8.27% conversion of Cr(III). Three mixed standards were then prepared with the concentrations listed in Table 2; their chromatography is also shown in Figure 2. The third row of Table 2, (Cr(VI) Total), reflects the predicted amount of Cr(VI) from the amount added and the amount derived from Cr(III). Calibrations were linear for both oxidation states with correlation coefficients of 0.9999 and 0.9998, respectively. To ascertain the accuracy of this approach, a mixed standard was analyzed with added concentrations of Cr(III) and Cr(VI) of 15 and 0.5 mg/L respectively. Due to the expected conversion of some of the Cr(III) to yield an additional 1.24 mg/L, the authors expected a total of 1.99 mg/L Cr(VI). The measured values for Cr(III) and Cr(VI) were 15.0 mg/L and 1.99 mg/L.

Determination of Cr(III) and Cr(VI) in Soil and Wastewater

To determine the feasibility of this method for speciation of chromium in soil and wastewater samples, the authors spiked known quantities of a mixed standard into samples that contained no measurable amounts of chromium. Samples spiked with standards and unspiked samples were treated with 50 mM nitric acid to approximate the manner in which the standards were treated, and followed by immediate addition of the 10× eluent concentrate buffered at pH 6.8. In reference 5, the authors extracted soil using phosphate buffer at the pH of the suspended soil sample to efficiently extract Cr(VI) from the soil. In this method, the authors did not attempt this type of extraction, as pH values significantly different from 6.8 may interfere with production of the Cr(III)-PDCA complex.

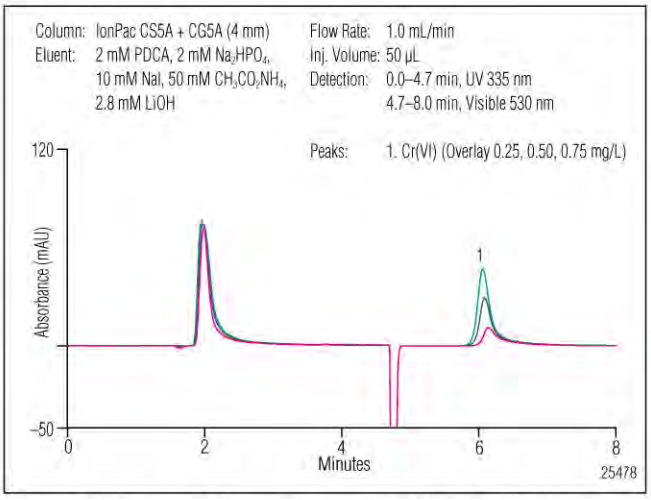

Figure 4. Overlay of chromatograms of Cr(VI) standards: 0.25, 0.5 and 0.75 mg/L, respectively.

Table 1. Determination of the Amount of Cr(VI) in the 15 mg/L Cr(III) Standard

Number	Area mAU/min	Height mAU	Amount Cr(VI) (mg/L)
1	16.98	81.69	1.24
2	16.97	81.55	1.24
Average:	16.97	81.62	1.24

Table 2. Mixed Standard Concentrations

Analyte	Concentration (mg/L)		
	Level 1	Level 2	Level 3
Cr(III)	5.0	10.0	15.0
Cr(VI)	0.25	0.5	0.75
Cr(VI) Total*	0.66	1.32	1.99

*This value reflects the amount of Cr(VI) added and the amount expected from Cr(III) oxidation.

After treatment with PDCA buffer and acid, the samples were filtered through a 0.45 µm filter, then through an OnGuard II P cartridge. This cartridge contains a polyvinylpyrrolidone (PVP) polymer with a high selectivity for phenolics, azo-containing compounds, aromatic carboxylic acids, and aromatic aldehydes. The OnGuard II P was used to remove the humic acids from soil and wastewater samples to avoid contaminating the CS5A column. Figures 5 and 6 each show an overlay of chromatograms of the soil or wastewater sample and the same sample spiked with a mixed Cr(III)/Cr(VI) standard. Both samples contain no measurable chromium. Tables 3 and 4 show recovery of Cr(III) and Cr(VI) from both samples. The recovery calculations assume an 8.27% loss of Cr(III) and concomitant production of Cr(VI) during sample preparation, as measured in the experiments described earlier. This method allows an estimation of the Cr(III) and Cr(VI) amounts in each sample in a single injection. For an accurate measure of the sample concentration of Cr(VI), the authors recommend the method described in Dionex Application Update 144. For a measure of total chromium, samples can be oxidized with ammonium persulfate then measured using the method in AU144. Cr(III) concentration can be derived from the difference of Cr(VI) and total chromium.

This application update demonstrates separation and detection of Cr(III) and Cr(VI) using an IonPac CS5A and absorbance detection. This method also describes a sample preparation technique that can be used to estimate the concentrations of Cr(III) and Cr(VI) in soil and wastewater samples.

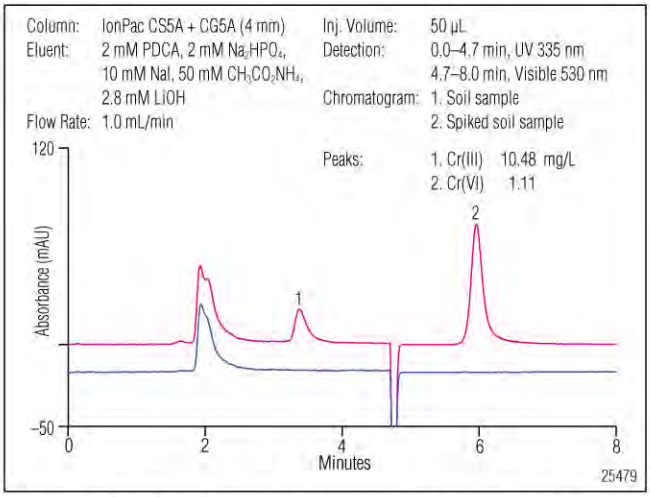

Figure 5. Overlay of the chromatograms of the soil sample and the same sample spiked with 10 mg/L Cr(III) and 0.5 mg/L Cr(VI).

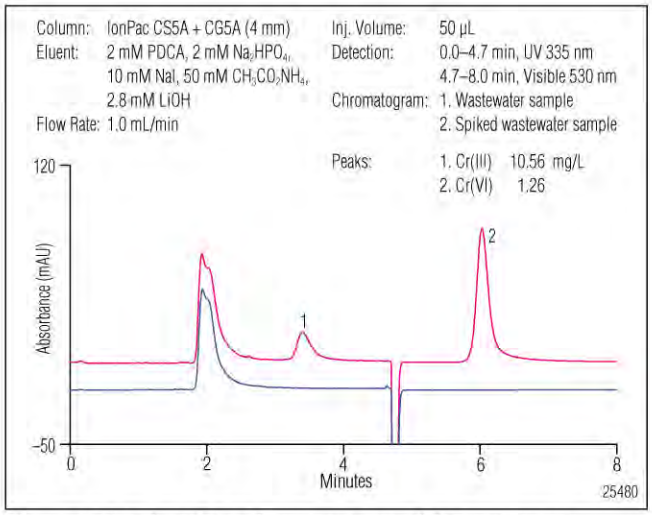

Figure 6. Overlay of the chromatograms of the wastewater sample and the sampled spiked with 10 mg/L Cr(III) and 0.5 mg/L Cr(VI).

Table 3. Recovery of 10 mg/L Cr(III) and 0.5 mg/L Cr(VI) in a Soil Sample

Sample No.	Amount (mg/L)	
	Cr(III)	Cr(VI)
1	10.49	1.11
2	10.46	1.11
3	10.49	1.11
Average	10.48	1.11
Spike	10	0.5
Total Cr*	10	1.33
% Recovery	**104.8**	**83.5**

*This value reflects the amount of Cr(VI) added and the amount expected from Cr(III) oxidation.

Table 4. Recovery of 10 mg/L Cr(III) and 0.5 mg/L Cr(VI) in a Wastewater Sample

Sample No.	Amount (mg/L)	
	Cr(III)	Cr(VI)
1	10.70	1.26
2	10.40	1.26
3	10.58	1.26
Average	10.56	1.26
Spike	10	0.5
Total Cr*	10	1.33
% Recovery	**105.6**	**94.7**

*This value reflects the amount of Cr(VI) added and the amount expected from Cr(III) oxidation.

REFERENCES:

1. Dionex Application Update 144
2. Dionex Application Note 80
3. Dionex Technical Note 26
4. Ščančar, J., and Milačič, R. The Analyst, 127, **2002**, 629-633
5. Ščančar, J., Zupančič, M., and Milačič, R., Water Air Soil Pollut. 185, **2007**, 121-129.
6. Ščančar, J., Milačič, R., Séby, F, and Donard, O.F.X. J. Anal. At. Spectrom. 20, **2005**, 871-875

Determination of Hexavalent Chromium in Drinking Water Using Ion Chromatography

INTRODUCTION

Hexavalent chromium, Cr(VI), is the most toxic form of the metal chromium, a primary drinking water contaminant in the U.S. Dissolved hexavalent chromium can be determined as chromate (CrO_4^{2-}) by ion chromatography in drinking water, groundwater, and industrial wastewater effluents as described in U.S. EPA Method 218.6 and Dionex Technical Note 26.[1,2] Dionex Technical Note 26 uses a 250-µL injection onto a high-capacity IonPac® AS7 anion exchange column to separate Cr(III) from Cr(VI) in four minutes. The product of the postcolumn reaction between Cr(VI) and diphenylcarbazide is detected by absorbance at 530 nm, yielding a method detection limit of 0.4 µg/L in reagent water.

The California Department of Health Services (DHS) recently issued a new Public Health Goal (PHG) of 2.5 µg/L for total chromium and 0.2 µg/L for Cr(VI). In January 2001, California DHS added Cr(VI) to the list of unregulated chemicals that must be monitored. As a result of this regulation, public water systems are now monitoring for Cr(VI) in drinking water.[3]

EPA Method 218.6 does not allow sufficient sensitivity for analysis at the California PHG level of 0.2 µg/L. This application update describes modifications to Method 218.6 that significantly increase sensitivity over the existing method. The modifications include lower eluent and postcolumn reagent (PCR) flow rates, a larger reaction coil, and a larger injection volume. The resulting MDL for Cr(VI) as CrO_4^{2-} of 0.02 µg/L is more than sufficient for determinations at the California PHG level.

EQUIPMENT

A Dionex DX-600 chromatography system consisting of:

- GS50 Gradient Pump with Vacuum Degas Option
- AS50 Automated Sampler with Chromatography Compartment
- AD25 UV/Visible Absorbance Detector
- 1.0 mL sample syringe for AS50 (Dionex PN 55066)
- PC10 Postcolumn Pneumatic Delivery Package, 4-mm (Dionex PN 50601)
- 750- L knitted reaction coil (Dionex PN 42631)
- PeakNet® 6.1 Chromatography Workstation
- Syringe filters (Gelman IC Acrodisc 0.2-µm, PN 4483)

REAGENTS AND STANDARDS

Prepare all solutions from analytical reagent grade chemicals (when available).

Deionized water, 17.8 MΩ-cm or better
Ammonium sulfate, (Mallinckrodt Gen AR # 7725)
Ammonium hydroxide (Sigma A6899)
1,5-diphenylcarbazide (JT Baker K620-03)
Methanol, HPLC grade (Fisher Optima A454-4)
Sulfuric acid, 95–98% (JT Baker Instra-Analyzed #9673)
Potassium dichromate (JT Baker 4765-01)

CONDITIONS

Guard Column:	IonPac NG1 Guard 4 × 50 mm (Dionex PN 039567)
Analytical Column:	IonPac AS7 Analytical 4 × 250mm (Dionex PN 035393)
Eluent:	250 mM ammonium sulfate/ 100 mm ammonium hydroxide
Eluent Flow Rate:	1.0 mL/min
Sample Volume:	1000 L partial loop with 10 µL cut volume from 1100-µL loop
Postcolumn Reagent:	2 mM diphenylcarbazide, 10% methanol, 1 N sulfuric acid
PCR Flow rate:	0.33 mL/min
Detection:	UV/Vis absorbance, 530 nm
Noise:	25–50 µAU peak-to-peak
Backpressure:	1200–1300 psi
Run Time:	10 min (Retention time = 6–7 min)

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent:

250 mM ammonium sulfate
100 mM ammonium hydroxide

Dissolve 66.0 g of ammonium sulfate in about 1 L of reagent water and add 13.0 mL of 29% ammonium hydroxide. Dilute to 2.0 L with water.

Sample Adjustment Buffer:

250 mM ammonium sulfate
1000 mM ammonium hydroxide

Dissolve 3.3 g of ammonium sulfate in about 75 mL of reagent water and add 6.5 mL of 29% ammonium hydroxide. Dilute to 100 mL with water.

Postcolumn reagent:

2 mM diphenylcarbazide
10% methanol
1 N sulfuric acid

Add 28 mL of 98% sulfuric acid to about 500 mL of water in a 1.0 L volumetric flask. (Caution: may get hot.) Mix and allow to cool. Add 0.5 g of 1,5-diphenylcarbazide to about 75 mL of HPLC-grade methanol in a 100 mL volumetric flask, and sonicate to dissolve. Bring to volume with methanol, mix, and add to the cooled sulfuric acid solution. Dilute to 1.0 L with deionized water, mix, and transfer to the pressurized PCR reagent container. The PCR reagent is stable for several days. Prepare fresh as needed.

Standard

Add 0.283 g of potassium dichromate (dried at 100 C to a constant weight) to about 50 mL of deionized water in a 100 mL volumetric flask. Dissolve and bring to volume with deionized water. Or, prepare a 1000 mg/L stock solution of Cr(VI) as CrO_4^{2-} from a commercially available standard (J. T. Baker, Phillipsburg, NJ). Store the stock standard at 4 °C. Prepare working standards fresh daily. Adjust the pH to 9.0–9.5 by adding 1 mL of sample adjustment buffer per 100 mL of final volume before bringing to final volume.

SAMPLE PREPARATION

Clean all sample collection equipment and containers with 1:1 HNO_3 and rinse well with deionized water before use. Collect samples in amber glass bottles with plastic lined caps. Do not filter the samples at the time of collection, but immediately add the sample adjustment buffer dropwise until the sample pH falls in the range of 9.0–9.5. Be careful not to contaminate the sample while measuring the pH. Most drinking water samples can be adjusted to pH 9.0–9.5 by adding 1 mL or less of the adjustment buffer per 100 mL of sample, which introduces an acceptable 1% dilution error. For more difficult samples, start with a known amount of sample and accurately measure the amount of buffer added so that the amount of Cr(VI) as CrO_4^{2-} determined by IC can be corrected for dilution. Cool to 4 °C and hold at 4 °C during transport and storage. Analyze samples within 24 hr of collection to minimize the potential loss of Cr(VI) through chemical reduction.[4]

Filter drinking water samples through 0.2-µm Acrodisc IC syringe filters (Gelman, Ann Arbor, MI) just prior to injection. 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 Cr(VI) as CrO_4^{2-} standard that has been passed through the filter. The blank should be free of peaks within the retention time window of chromate, and the recovery of the 10-µg/L standard should fall between 80% and 120%.

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 as an aid in troubleshooting. Consult the pump or detector manuals for procedural details.

The precision and accuracy of the AS50 will vary depending on the mode of injection. The most accurate and precise injections are made with a calibrated sample loop in the full-loop injection mode, which aspirates a total of four times the sample volume to flush the sample loop and make the injection. 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 cut volume of 10 µL. This injection procedure should provide peak area precision of <1% RSD. 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 four or five to make faster large-loop injections. Enter the correct sample loop size and sample syringe volume in the AS50 Plumbing Configuration screen.

Configure the IC with the PCR system as depicted in Figure 3 of Dionex Technical Note 26, and as described in the PC10 Postcolumn Delivery System installation instructions. Pump the eluent at 1.0 mL/min and set the PC10 pneumatic pressure to 70 psi. To measure the PCR flow rate, collect the effluent from the detector *(i.e., the total flow from the IC pump and the PCR module)* in a 10-mL graduated cylinder for 5 min. The PCR flow rate is the difference between the total flow rate and that of the IC pump. Adjust the air pressure of the postcolumn delivery module (PC10) and remeasure the flow rate until the correct PCR flow rate of 0.33 mL/min is established.

Variations in the PCR flow rate affect the postcolumn reaction time, pH, dilution, mixing rate, and ratio of the reactants. Stable day-to-day results depend on a well-controlled PCR flow rate.

Confirm this flow rate on a daily basis or whenever detector response for a calibration check standard deviates beyond quality control acceptance criteria.

The storage solution that the AS7 is shipped with is 30 mM nitric acid. After equilibrating the column with eluent for 60 min, analyze a system blank of 1000 µL of reagent water. An equilibrated system has a background signal of less than 200 mAU and peak-to-peak noise of less than 50 µAU. There should be no peaks eluting within the retention time window of the chromate anion. The column is equilibrated when two consecutive injections of a standard produce the same retention time for chromate.

RESULTS AND DISCUSSION

Effect of Reaction Coil and Injection Volume

The 375-µL reaction coil and higher flow rates recommended in Dionex Technical Note 26 are adequate for the rapid determination of Cr(VI) as CrO_4^{2-} at the 1-µg/L level, but a standard 375-µL knitted reaction coil does not provide the maximum peak response for chromate.[5] To optimize the sensitivity of this method,

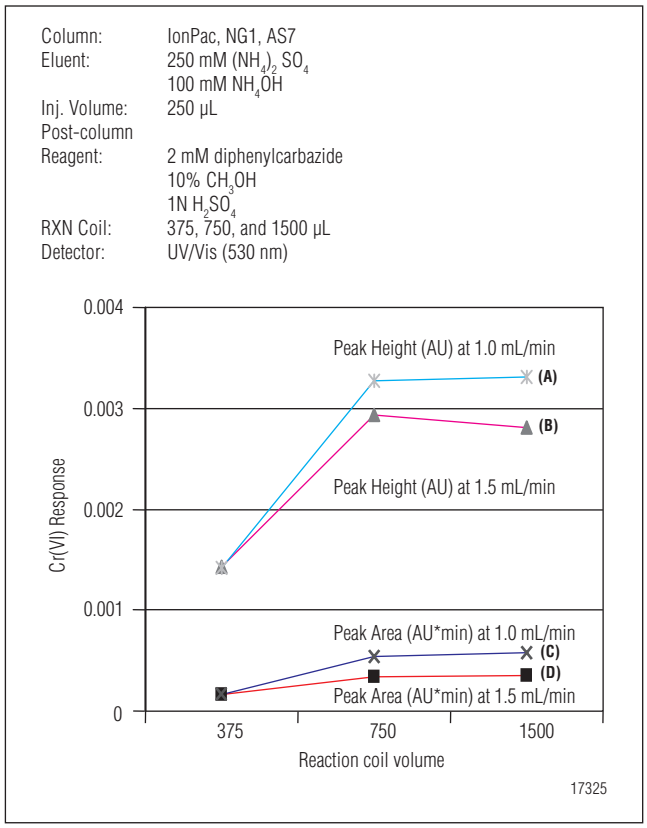

Figure 1. Effect of reaction coil volume on chromate peak response. Conditions: guard column, IonPac NG1; analytical column, IonPac AS7; eluent, 250 mM ammonium sulfate/100 mM ammonium hydroxide; flow rate, 1.0 (A and C) or 1.5 (B and D) mL/min; postcolumn reagent, 2 mM diphenylcarbazide / 10% methanol / 1.0 N sulfuric acid; reaction coil volume, 375–1500 µL as indicated; postcolumn flow rate, 0.33 (A and C) or 0.5 (B and D) mL/min; detection, UV/Vis at 530 nm; injection volume, 250 µL; peaks, chromate (10 µg/L).

the responses obtained with 375-µL, 750-µL, and 1500-µL reaction coils were compared . Figure 1 shows the effect of reaction coil volume on chromate peak height and area at two different flow rates. In both cases, the postcolumn reagent flow rate was adjusted to one-third the eluent flow rate by varying the applied pneumatic pressure. At an eluent flow rate of 1.0 mL/min (A and C) the postcolumn reagent flow rate was 0.33 mL/min. At an eluent flow rate of 1.5 mL/min (B and D) the postcolumn reagent flow rate was 0.50 mL/min.

Increasing the reaction coil volume from 375µL to 750 µL significantly increases the peak response, while the change from 750 µL to 1500 µL only marginally increases the response. Larger coil sizes require greater pneumatic pressure to deliver the necessary PCR flow rate. Also, higher eluent flow rates require greater pneumatic pressure to deliver the necessary PCR flow

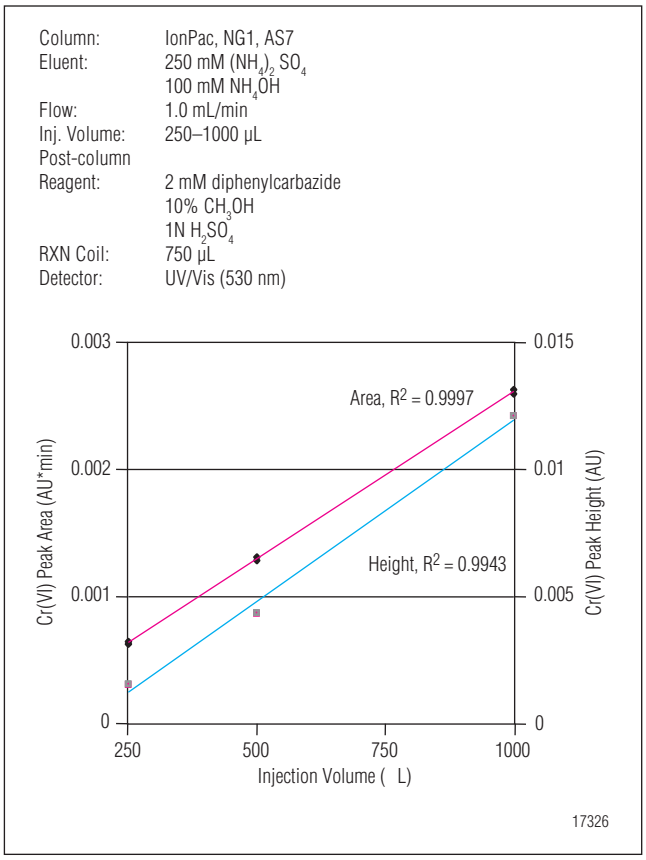

Figure 2. Effect of injection volume on chromate peak response. Conditions: as for Fig. 1, except; flow rate, 1.0 mL/min; postcolumn flow rate, 0.33 mL/min; reaction coil volume, 750 µL; injection volume, 250–1000 µL as indicated.

rate. For example, a pneumatic pressure of over 100 psi was required to deliver the PCR reagent at a flow rate of 0.5 mL/min against an eluent flow rate of 1.5 mL/min through a 1500-µL reaction coil. We recommend using a 750-µL reaction coil with an eluent flow rate of 1.0 mL/min and a PCR flow rate of 0.33 mL/min. This combination provides nearly the maximum peak response while requiring a modest pneumatic pressure of about 70 psi to deliver the postcolumn reagent at the necessary flow rate.

Greater sensitivity can be gained by increasing the volume of sample injected, but too large a sample can cause retention time shifts or loss of efficiency when column overloading leads to excessive peak distortion. The effect of injection volume on chromate peak response was studied using the conditions described above. Figure 2 shows a linear increase in both chromate peak height and area as the injection volume is increased from 250 µL to 1000 µL. We did not test larger injection volumes because a 1000 µL injection volume is the largest that can be made with the AS50 Autosampler.

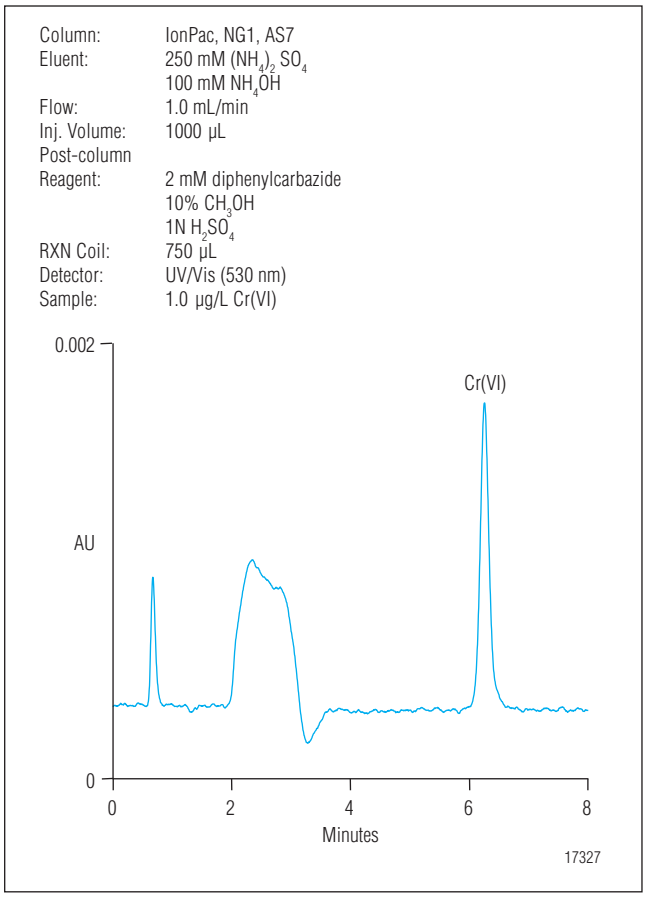

Figure 3. Determination of chromate using optimized EPA Method 218.6. Conditions: as for Fig. 2, except injection volume, 1000 µL; peaks, chromate (1.0 µg/L).

Sample Adjustment Buffer

Method 218.6 requires a solution consisting of 330 g/L ammonium sulfate and 65 mL/L ammonium hydroxide to adjust the sample pH. However, the large-loop injection used in this application update increases the possibility of overloading the analytical column if the sample ionic strength is too high. A solution consisting of 33 g/L ammonium sulfate and 65 mL/L ammonium hydroxide is a suitable substitute to adjust the sample pH and provides results comparable to those obtained by using the Method 218.6 buffer (data not shown).

Optimized Method Performance

By using a larger volume reaction coil, lower eluent flow rate and increased injection volume, this updated method provides greater than a 10-fold increase in the chromate peak area compared to the response obtained by using the standard conditions specified in Method 218.6. Figure 3 shows a chromatogram of a 1.0-µg/L

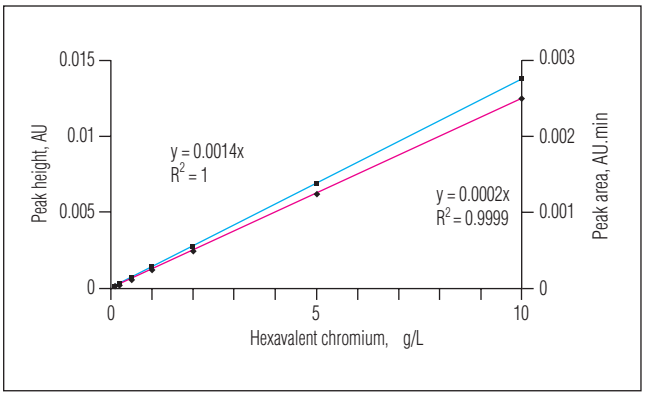

Figure 4. Calibration curve for Cr(VI) as chromate.

Cr(VI) as CrO_4^{2-} standard obtained by using the optimized conditions described above.

Figure 4 summarizes the calibration data for chromate anion obtained by using the partial-loop injection mode to make 1000-µL injections of Cr(VI) as CrO_4^{2-} standards at 0, 0.04, 0.1, 0.2, 0.5, 1, 2, 5, 10, and 20 g/L. The calibration curve is linear over the calibration range of 0.1–10 µg/L for Cr(VI) as CrO_4^{2-} with a coefficient of determination of 0.9999.

Table I. Method Detection Limits for Chromate Based on a 1000- µL Injection

Chromate Conc. (µg/L)	Std. Dev. (g/L)	RSD (%)	MDL* (µg/L)
0.1	0.0060	6.986	0.018
0.2	0.0056	3.193	0.018

* MDL = (Std. Dev.) x ($t_{s, 99}$), where $t_{s, 99}$ = 3.14 for n = 7.

Method Detection Limits (MDLs) for Cr(VI) as CrO_4^{2-} are summarized in Table 1. The 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] We determined the MDL for Cr(VI) as CrO_4^{2-} by analyzing seven replicates of reagent water fortified with Cr(VI) as CrO_4^{2-} at two concentrations levels of 0.1 and 0.2 µg/L (i.e., about 3–5 times the estimated instrument detection limit). Both levels produced a calculated MDL value of 0.018 µg/L. This permits a minimum limit (ML) for quantitation of 0.06 µg/L for Cr(VI) as CrO_4^{2-}, which is

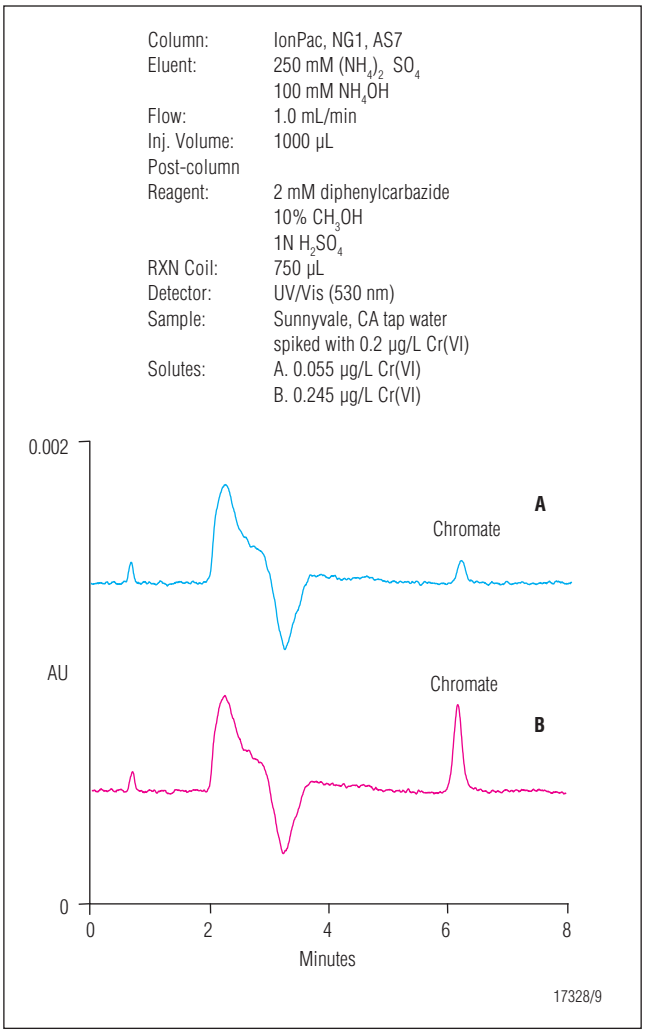

Figure 5. Determination of chromate in drinking water. Conditions: as for Fig. 3, except sample, buffered Sunnyvale, CA drinking water (A) and drinking water spiked with 0.2 µg/L chromate (B); peaks, (A) chromate (0.055 µg/L) and (B) chromate (0.245 µg/L)

adequate for routine analysis at the California PHG level of 0.2 µg/L.

Fig. 5 shows chromatograms obtained by using the optimized conditions of a Sunnyvale, CA, tap water blank (A) and tap water sample spiked with Cr(VI) as CrO_4^{2-} at the PHG level of 0.2 µg/L (B). In both cases, the sample was adjusted to pH 9 by using the solution consisting of 33 g/L ammonium sulfate and 65 mL/L ammonium hydroxide. The presence of the ammonium sulfate and ammonium hydroxide in the sample did not adversely affect the chromate peak shape and a recovery of 96% was obtained for the Cr(VI) as CrO_4^{2-} spike at this level. The tap water blank contained a background level of 0.06 µg/L chromate.

Although the IonPac AS7 column specified in Method 218.6 has a relatively high capacity of

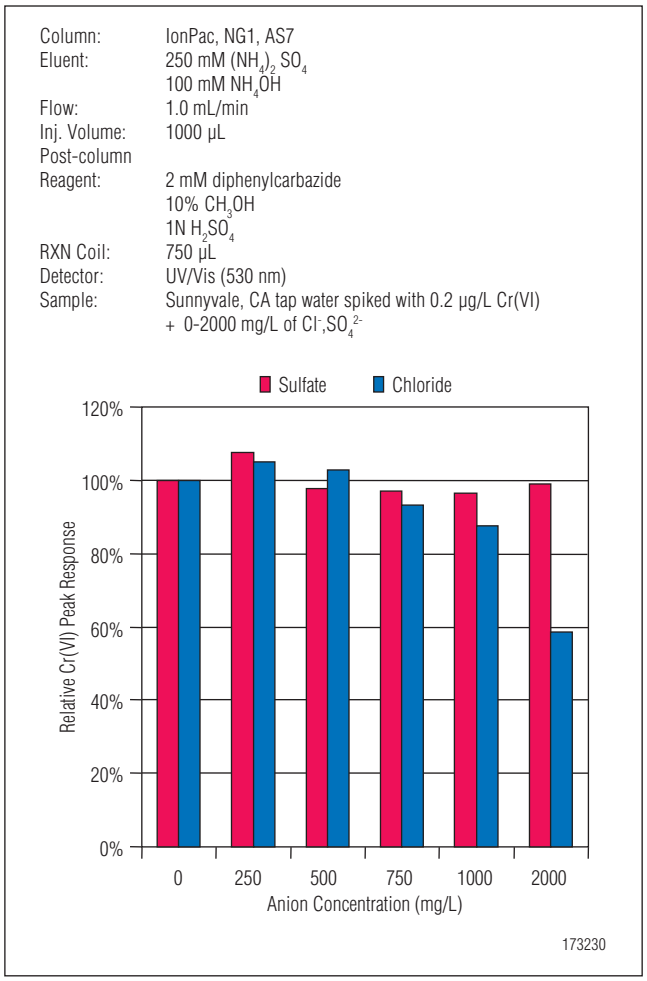

Figure 6. Effect of common anions on chromate peak response. Conditions: as for Fig.3, except sample, buffered Sunnyvale, CA drinking water spiked with 0.2 µg/L chromate to which 0–2000 mg/L of sulfate or chloride was added, as indicated.

100 µ equivalents/column, the large injection volume used in this application update increases the possibility of interference from other anions in the sample. Hence, the effect of chloride and sulfate on Cr(VI) as CrO_4^{2-} response was investigated, as some drinking and ground waters can contain elevated levels of these common anions. Increasing concentrations of sulfate or chloride were added to a series of tap water samples that had been adjusted to pH 9 with the recommended buffer and spiked with 0.2 µg/L Cr(VI) as CrO_4^{2-}. Figure 6 shows the effect of common anions on chromate peak response, relative to the peak area for 0.2 µg/L Cr(VI) as CrO_4^{2-} spiked in tap water containing no added sulfate or chloride. This method provides acceptable performance, for example greater than 80% recovery in the presence of up to 1000 mg/L chloride or 2000 mg/L sulfate. Typical ground and drinking waters are not expected to contain more than 200 mg/L chloride or 500 mg/L sulfate.

CONCLUSIONS

U.S. EPA Method 218.6, as published, does not allow sufficient sensitivity to determine hexavalent chromium (i.e., Cr(VI) as CrO_4^{2-}) at the California PHG level of 0.2 µg/L. Modifications to the method, including the use of a lower eluent flow rate and larger reaction coil (to increase reaction time) and a larger injection volume, significantly increase the sensitivity of Method 218.6, resulting in an MDL for Cr(VI) as CrO_4^{2-} of 0.02 µg/L. These modifications allow a minimum limit (ML) of quantitation for Cr(VI) as CrO_4^{2-} of 0.06 µg/L, which is more than sufficient for analysis at the California PHG level. Calibration was linear over the range of 0.1–10 µg/L, and quantitative recoveries were obtained for Cr(VI) as CrO_4^{2-} spiked at 0.2 µg/L in drinking water. The modified method provides acceptable performance, in terms of peak shape and recovery, in the presence of up to 1000 mg/L chloride or 2000 mg/L sulfate.

REFERENCES

1. *Determination of Dissolved Hexavalent Chromium in Drinking Water, Groundwater, and Industrial Wastewater Effluents by Ion Chromatography;* U.S. Environmental Protection Agency, Method 218.6; Cincinnati, OH (1991).
2. Dionex Corporation. Determination of Cr(VI) in Water, Waste Water, and Solid Waste Extracts; Technical Note 26; Sunnyvale, CA.
3. Governor Signs Bill Speeding Water Testing, *The Los Angeles Times*, Valley Ed., September 30, 2000, p. 1.
4. *Chromium-6 (Hexavalent Chromium) in Drinking Water,* California Department of Health Services, Update, August 22, 2001.
5. Raimund Roehl, California Department of Health Services, personal communication, May 2001.
6. *The Determination of Inorganic Anions in Water by Ion Chromatography;* U.S. Environmental Protection Agency, Method 300.0; Cincinnati, Ohio (1993).

SUPPLIERS

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

Fisher Scientific, 711 Forbes Ave., Pittsburgh, PA 15219 USA, Tel. 800-766-7000, www.fishersci.com.

Gelman Sciences, 600 S. Waygner Road, Ann Arbor, MI 48106-1448 USA, 800-521-1520 www.pall.com/gelman.

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

Mallinckrodt Baker, 222 Red School Lane, Phillipsburg, NJ 08665 USA, Tel. 800-582-2537, www.jtbaker.com.

Determination of Hexavalent Chromium in Dyes

INTRODUCTION

Hexavalent chromium (Cr(VI)) is the most toxic form of the metal chromium. In addition to regulations concerning its concentration in drinking water, many governments regulate Cr(VI) in consumer products. Leather products can be colored with dyes containing trivalent chromium, and sweat can transfer the chromium from the leather to the skin. It is possible that some of the trivalent chromium oxidizes to Cr(VI). For example, leather gloves were found to contain Cr(VI).[1] In this application brief (AB), we analyze two dyes for Cr(VI) using a sensitive ion chromatography (IC) method with postcolumn reaction to produce a colored complex detected by visible absorbance.[2] Figure 1 shows that Cr(VI) was not found in a 1:100 dilution of the Fast Red dye while 56.1 µg/L Cr(VI) was found in a 1:100 dilution of the Metal Complex Black. Spiking both diluted dye samples with 30 µg/L Cr(VI) yielded recoveries of 99.3 and 101% for the red and black dyes respectively, demonstrating method accuracy. This IC method delivers an easy, fast, sensitive, and accurate determination of Cr(VI) in dyes.

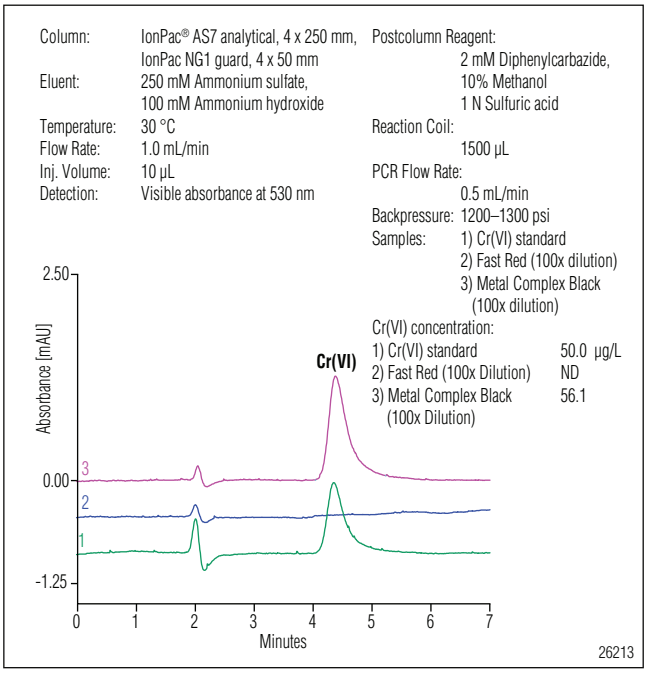

Figure 1. Chromium (Cr(VI)) in dyes.

CONDITIONS

System – ICS-3000 with a DP dual pump, VWD absorbance detector, and AS autosampler.

See Figure 1 for chromatography conditions.

Sample Preparation – Samples were diluted 1:100 with deionized water and then passed through an OnGuard® II P cartridge (Dionex, P/N 057087) prior to analysis.

REFERENCES

1. Peterson, J.; Murphy, B.; Perati, P.; Richter, B. *LCGC The Applications Notebook,* 2007, June, 28.
2. Dionex Application Update 144. January 2003. Sunnyvale, CA.

ACKNOWLEDGEMENT

Thanks to the Textile Testing Center, Thailand Textile Institute for kindly providing the samples.

Determination of Cr(VI) in Water, Waste Water, and Solid Waste Extracts

INTRODUCTION

Chromium, while not unique in its properties, is commonly used in various industries because of the characteristics of the metal and its compounds. The predominant use of chromium in industry unfortunately introduces an environmental concern. Chromium exists almost exclusively in the Cr(III) oxidation state or in the Cr(VI) oxidation state. In the environment Cr(III) is typically not a problem. The uncomplexed trivalent species is the chromic ion, Cr^{3+}, and while it is soluble in acidic solutions, it typically precipitates as the hydroxide in alkaline solutions. It shares the quality with all other metals of being toxic to biological systems at some level. Fortunately its relative toxicity is low. This is due to the slow ligand exchange kinetics of Cr(III), causing it to be fairly unreactive. Actually, Cr(III) is essential to mammalian systems, admittedly at low concentrations, for the maintenance of several metabolic pathways. In contrast, Cr(VI) seems to serve no useful biological purpose to living things.

The hexavalent species exists primarily as chromic acid (H_2CrO_4) and its salts, hydrogen chromate ion ($HCrO_4^-$) and chromate ion (CrO_4^{2-}), depending on the pH. The predominant species present, as a function of the pH, are H_2CrO_4 at pHs less than about 1, $HCrO_4^-$ at pHs between 1 and 6, and CrO_4^{2-} at pHs above about 6 (see Figure 1). The dichromate ion ($Cr_2O_7^{2-}$) is a dimer of $HCrO_4^-$, less a water molecule, which forms when the concentration of chromium exceeds approximately 1 g/L.

Cr(VI) is a strong oxidizer and therefore harmful in biological systems. This fact warrants its regulation in the environment. As is typical, the oxidizing power of Cr(VI) is a function of pH. As the pH becomes lower, Cr(VI) is more inclined to oxidize something. Fortunately, environmental samples are typically alkaline and, because the reduction potential of Cr(VI) decreases as the pH increases, Cr(VI) is less reactive at these higher pHs.

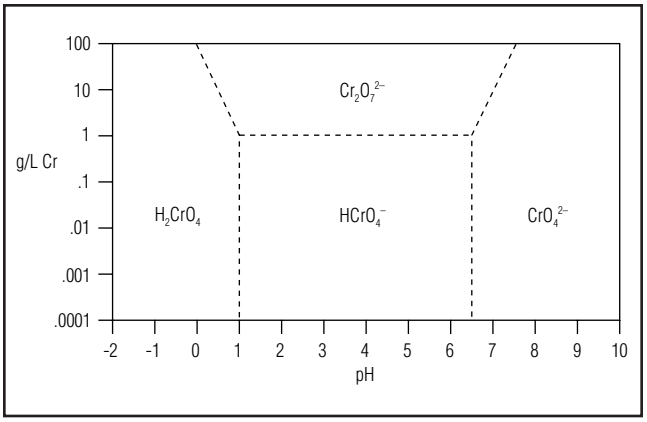

Figure 1. Relative distribution of Cr(VI) species in water as a function of pH and Cr(VI) concentration.

The contrast in qualities of Cr(III) and Cr(VI) has become the reason it is critical to differentiate between the two oxidation states when analyzing environmental or process samples. Various industries assert that environmental regulation efforts should be focused on Cr(VI) instead of on the relatively harmless Cr(III). This position has credence but, whether one wants or needs to determine one or both species in a sample, the analytical method must be capable of differentiating between the two. Speciation of various oxidation states of a metal in a sample is not always easy. Even after an analytical method has been developed, the question of whether or not the sample preparation procedure has altered the relative concentration of the species of interest still remains.

These issues combine to make a difficult analytical situation. To date, the study of sampling and sample preparation procedures for the speciation of chromium is an area of considerable activity. Existing sample preparation procedures (extraction, digestion, filtration) are undergoing critical review. They have proven to be imprecise, to be incomplete, and to alter the relative oxidation state concentrations.

The current trend is toward sample digestion and extraction procedures which give values for dissolved (free) Cr(VI) or total chromium as Cr(VI). Examples of these are the TCLP (Toxicity Characteristic Leaching Procedure) Extraction and Alkaline-Persulfate Digests, both of which generate high ionic strength matrices; not matrices of choice for most instrumental analytical methods.

Development of analytical methods is also ongoing, but the methods are dependent on the sample preparation procedures to determine the ultimate applicability of the techniques. Most spectroscopic and electrochemical methods are not specific enough. Because of these inadequacies, substantial sample work-up is required for useful analytical results.

The method presented in this Technical Note overcomes many analyte interference problems by separating the two chromium oxidation states and the other sample components and using a detection method specific for the Cr(VI) capable of handling the high ionic strength sample matrices generated in many leaching, impinging, and digestion procedures. This method is consistent with U.S. EPA method 218.6 and is described in several other publications [2–5].

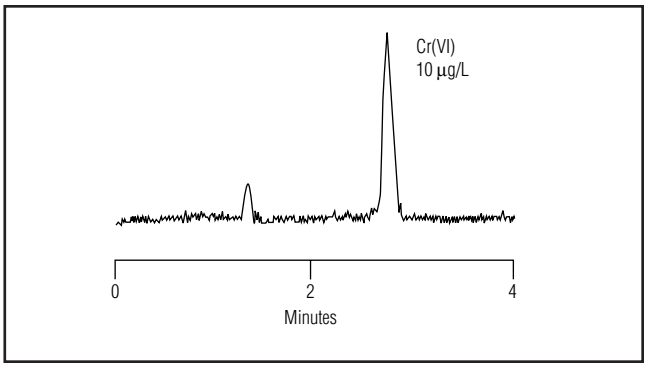

Figure 2. Determination of Cr(VI) in water, waste water, and solid waste extracts.

DISCUSSION OF METHOD

The method allows the detection of low-µg/L levels of Cr(VI) in typical high ionic strength matrices. As discussed, most analysts are concerned with free Cr(VI) only, or total chromium as Cr(VI), so the method is specific for Cr(VI).

Using this method, hexavalent chromium is chromatographed as the divalent CrO_4^{2-} anion on the IonPac® AS7 column using a well-buffered ammonium sulfate, ammonium hydroxide eluent (see Figure 2). After the

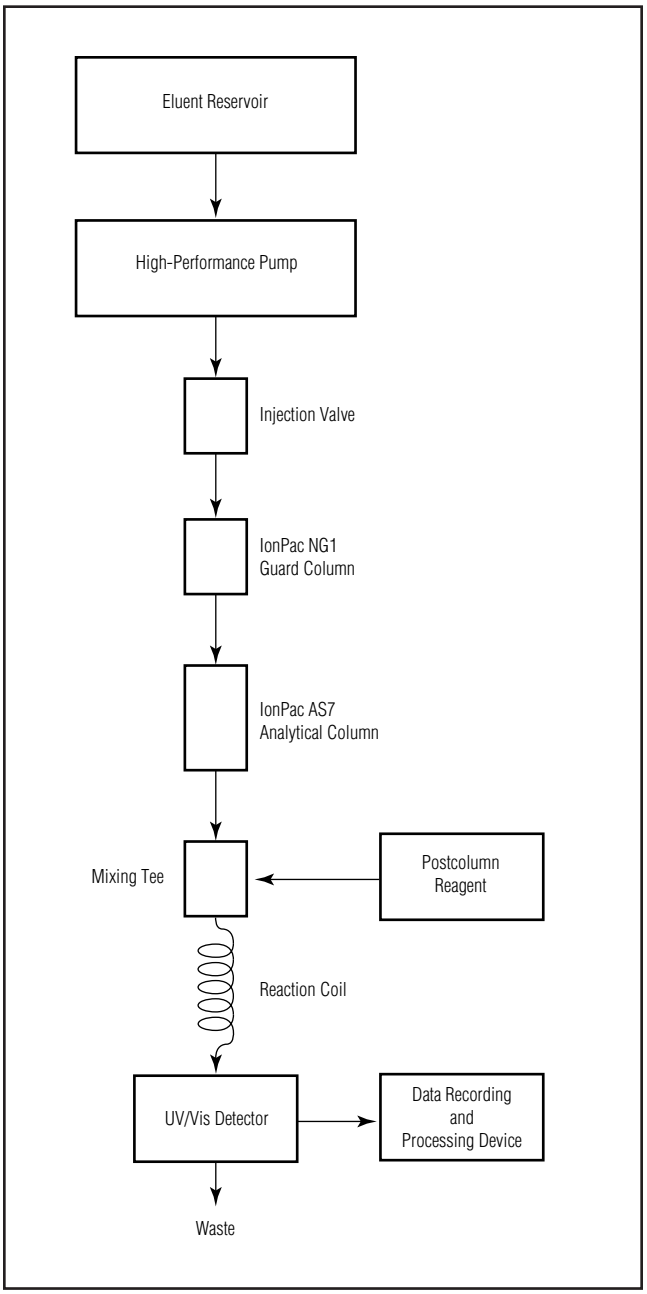

Figure 3. System flow diagram.

separation, Cr(VI) reacts with the color reagent diphenylcarbohydra-zide (DPC) in the following reaction:

$$2\ CrO_4^{2-} + 3\ H_4L + 8\ H^+ \rightarrow Cr(III)\ (HL)_2^+ + Cr^{3+} + H_2L + 8\ H_2O$$

where:

H_4L = diphenylcarbazide

H_2L = diphenylcarbazone

The reaction is apparently the simultaneous oxidation of diphenylcarbazide to diphenylcarbazone, reduction of Cr(VI) to Cr(III), and the chelation of Cr(III) by diphenylcarbazone. The actual structure of the chelate is not known, but it is detected by visible absorbance using a photometric detector at 520 to 530 nm. A diagram of the system flow path is shown in Figure 3.

The analysis time is about 5 minutes. The method has a linear detection response from the detection limit, which is about 50 pg or 1 µg/L using a 50-µL loop, up to around 0.5 µg or 10 mg/L using a 50-µL loop (Figure 4). Relative standard deviations from multiple injections of the same sample are 1% to 3% at concentrations above 10 µg/L.

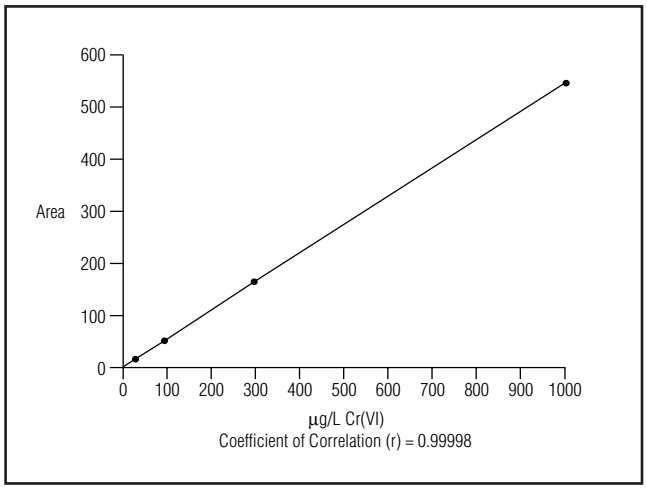

Figure 4. Area response for colorimetric chromium detection.

CONDITIONS

Guard Column:	IonPac NG1
Analytical Column:	IonPac AS7
Eluent:	250 mM Ammonium sulfate 100 mM Ammonium hydroxide
Eluent Flow Rate:	1.5 mL/min
Postcolumn Reagent:	2 mM DPC 10% Methanol, 1 N Sulfuric acid
Postcolumn Reagent Flow Rate:	0.5 mL/min
Detection Wavelength:	520 nm
Sample Volume:	50–100 µL

The method can handle samples of up to 5% sodium sulfate, 2% sodium chloride, 1 M acetate buffer, or 0.5 M carbonate buffer without adverse effects on the analysis. Figures 5A, 5B, and 5C illustrate the responses of 100-µg/L Cr(VI) spikes in various concentrations of these sample matrices. Increasing the matrix ionic strength, as illustrated in Figure 6 for the acetate buffer, eventually causes column overload and compromises the chromatography and detection.

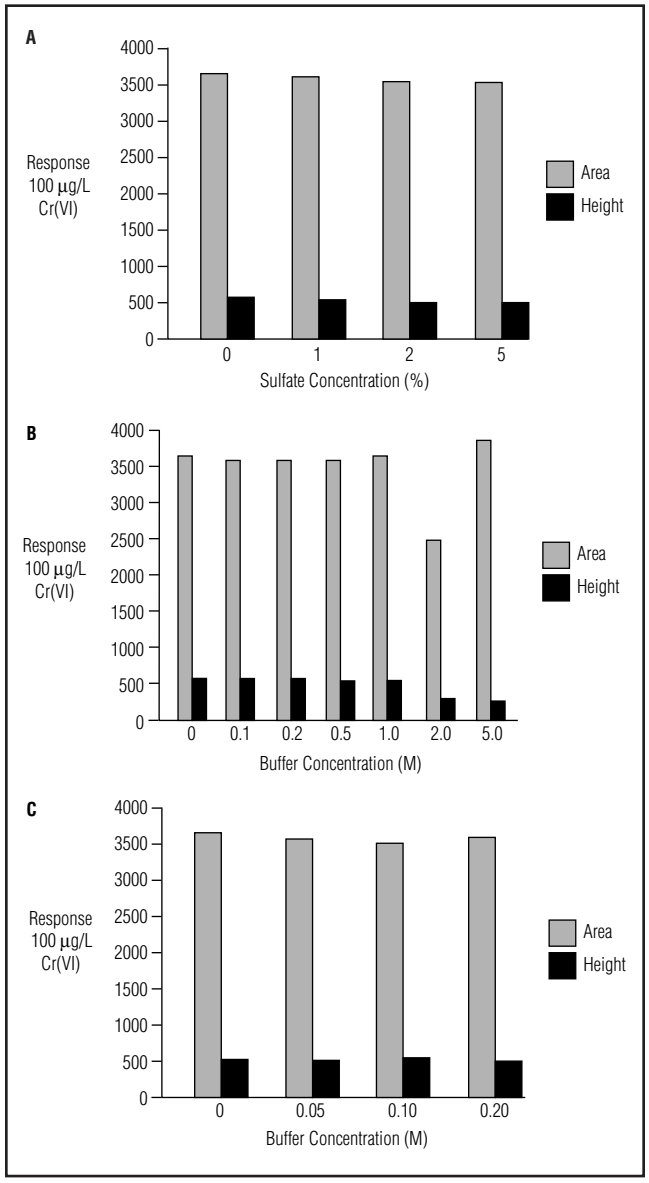

Figure 5. A) Alkaline sulfate samples. B) Acetate buffer samples. C) Carbonate buffer samples.

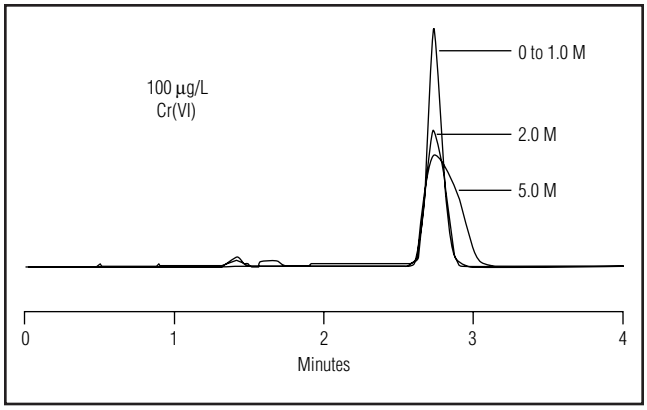

Figure 6. Cr(VI) in acetate buffer.

RECOMMENDED EQUIPMENT

DX-500 Chromatography System:

- GP40 Gradient Pump or IP20 Isocratic Pump
- AD20 Absorbance Detector
- PC10 Postcolumn Pneumatic Delivery Package
- OnGuard™-P Sample Pretreatment Cartridges

REAGENTS AND STANDARDS

All reagents are analytical reagent grade or better.

- Deionized water, 18 MΩ-cm
- Sulfuric acid, 96%
- Methanol, HPLC grade
- Ammonium hydroxide, 29%
- Ammonium sulfate
- 1,5-Diphenylcarbohydrazide
- Potassium dichromate

REAGENT PREPARATION

Eluent:

250 mM Ammonium sulfate
100 mM Ammonium hydroxide

Dissolve 33.0 g of ammonium sulfate in about 500 mL of water. Add 6.5 mL of 29% ammonium hydroxide. Mix well and dilute to 1.0 L in a volumetric flask. Transfer the solution to the eluent bottle. If you desire, you may multiply the weight and volumes listed for the eluent by an appropriate factor to prepare a larger volume of the eluent.

Postcolumn Reagent:

2.0 mM Diphenylcarbohydrazide
10% Methanol
1 N Sulfuric acid

Dissolve 0.5 g of 1,5-diphenylcarbohydrazide in 100 mL of HPLC-grade methanol. Add to about 500 mL of water containing 28 mL of 98% sulfuric acid. Dilute, with stirring, to 1.0 L in a volumetric flask. Transfer the solution to the pressurized reagent container. The solution is stable for several days but should only be prepared as it is used, one liter at a time.

STANDARDS PREPARATION

Chromium Stock:

1000 ppm Cr(VI)

Dissolve 0.283 g of potassium dichromate ($K_2Cr_2O_7$ dried at 100 °C for one hour) in water. Dilute to 100 mL in a volumetric flask.

Chromium Standard:

Standards are prepared by appropriate dilutions of the stock solution. As an example, for a 1-mg/L Cr(VI) standard, pipet 1.00 mL of the Chromium Stock solution into a 1.0 L volumetric flask. Dilute to volume with water.

SAMPLE PREPARATION

Collect samples in amber glass bottles with plastic lined caps. Clean the bottles with 1:1 HNO_3 and rinse well with deionized water before use.

Because Cr(VI) is an oxidizer, care must be taken in sampling and sample preparation procedures. Sampling and preservation procedures often involve changing the sample pH, which may result in changes in the relative concentrations of the oxidation states.

Refrigeration of the samples, minimal sample handling, and immediate analysis is suggested as the best protocol for maintaining the integrity of the samples. After collecting the samples, store at 4 °C to minimize chemical reactivity. Analyze within 24 hours.

Analyze drinking water, rain water, and air particulate extract solutions directly with no sample preparation (other than possible dilution). Filter ground water and waste water samples through 0.45-µm filters before injection.

Pass samples such as ground water, waste water, and solid waste extracts, which may contain high concentrations of organic contaminants, through OnGuard-P syringe cartridges before injection. This procedure is not absolute-ly necessary, but it helps prevent premature fouling of the column. Be sure to follow the instructions for the use of the cartridges, which are enclosed with the cartridge package.

TROUBLESHOOTING

This method is simple and rugged but this troubleshooting guide has been included to minimize any down time. The guide lists the symptoms of some of the problems you might experience as well as their likely causes and remedies.

If you continue to have any difficulties, please call your local Dionex Regional Office.

Symptom: No peak observed

- ■ Possible Cause: No sample injected.
- □ Remedy: Ensure that the pressurized gas used to switch the injection valve is on.
- □ Remedy: Ensure that the sample is loaded from the autosampler or syringe.
- ■ Possible Cause: Recording device not properly connected.
- □ Remedy: Check that the computer interface is turned on, or that the recording device is connected to the detector.
- ■ Possible Cause: No postcolumn reagent flow.
- □ Remedy: Check that the flow rate out of the cell is 2.0 mL/min and that the backpressure past the mixing tee is less than 50 psi.
- ■ Possible Cause: Wrong detector wavelength.
  Remedy: Check that the wavelength readout is 530 nm.

Symptom: Noisy baseline

- ■ Possible Cause: Air bubble in cell.
- □ Remedy: Disconnect line cell inlet, replace with a luer-type adapter, and flush the cell with a few mL of methanol or ispropanol.

Symptom: Low column pressure

- ■ Possible Cause: Air in pump head.
- □ Remedy: Prime the pump with eluent (see your pump instruction manual).
- ■ Possible Cause: Leak in system.
- □ Remedy: Tighten or replace leaking fitting.

Symptom: Excessive pressure on column

- ■ Possible Cause: Improper flow rate.
- □ Remedy: Check that the pump flow rate is 1.5 mL/min.
- ■ Possible Cause: Fitting is plugged.
- □ Remedy: With the pump off, remove the columns and reconnect the eluent lines. Turn on the pump and check that the system pressure is less than 100 psi when the valve is in either the LOAD or INJECT position.
- ■ Possible Cause: Column bed support plugged.
- □ Remedy: Replace the bed support on the column. See the column manual for instruction.

Symptom: Peak response too high or too low

- ■ Possible Cause: Incorrect detector range (applies when using analog output only).
- □ Remedy: Check that the detector range is correct.
- ■ Possible Cause: Incorrect sample loop size.
- □ Remedy: Ensure that the sample loop volume is 50 µL or 100 µL.
- ■ Possible Cause: Low postcolumn reagent flow rate.
- □ Remedy: Check that the flow rate from the detector waste line is 2.0 mL/min.

Symptom:

Poor peak shape — reasonable retention time

- ■ Possible Cause: Column is overloaded with a sample concentration that is too high.
- □ Remedy: Dilute the sample so that the peak response is below that expected for a 1-µg injection.

Symptom:

Poor peak shape — incorrect retention time

- ■ Possible Cause: The eluent was prepared incorrectly.
- □ Remedy: Prepare new eluent; the pH of the eluent should be approximately 8 to 9.
- ■ Possible Cause: The column is contaminated with strongly retained anions, metals, or organics.
- □ Remedy: Pump acetonitrile through ONLY the NG1 guard column for about 30 minutes, and then rinse with deionized water for about 15 minutes. Pump 1 N HCl through all of the columns for one hour, rinse with deionized water for 30 minutes, and reequilibrate with eluent for 30 minutes.

REFERENCES

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3. Arar, E.J.; Pfaff, J.D. J. Chromatogr. Sci. **1991**, 546, 335–340.
4. ASTM Method D5257-93: "Dissolved Hexavalent Chromium in Water by Ion Chromatography", June 1991, American Society for Testing and Materials (ASTM), Committee D-19, Philadelphia, PA 19103.
5. Proposed ASTM Method: "Collection and Analysis of Hexavalent Chromium in the Atmosphere", Nov. 1991, ASTM, Committee D-22, Philadelphia, PA 19103.

Analysis of Metals

Environmental Water Applications Notebook

Determination of Chelating Agents in Drinking Water and Wastewater Samples

INTRODUCTION

Aminopolycarboxylate chelating agents (Figure 1) are used extensively in many domestic products and industrial processes, with the most important applications in cleaning compounds, pulp and paper manufacturing, and agriculture.[1–5] Chelating agents form stable water-soluble complexes with alkali and transition metal ions, thus increasing metal solubility and preventing metal-catalyzed reactions. Therefore, chelating agents prevent metals from interfering with the detergent's ability to remove soils and stains from clothing, from degrading oxidizing and bleaching agents in paper and textile manufacturing, and from precipitating in fertilizers. Chelating agents are also used in soil remediation to remove heavy metal contamination.[6]

Because of their broad application range, chelating agents are typically produced and used in large quantities. In 2004, the global consumption of the most common aminopolycarboxylic acids, such as ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic

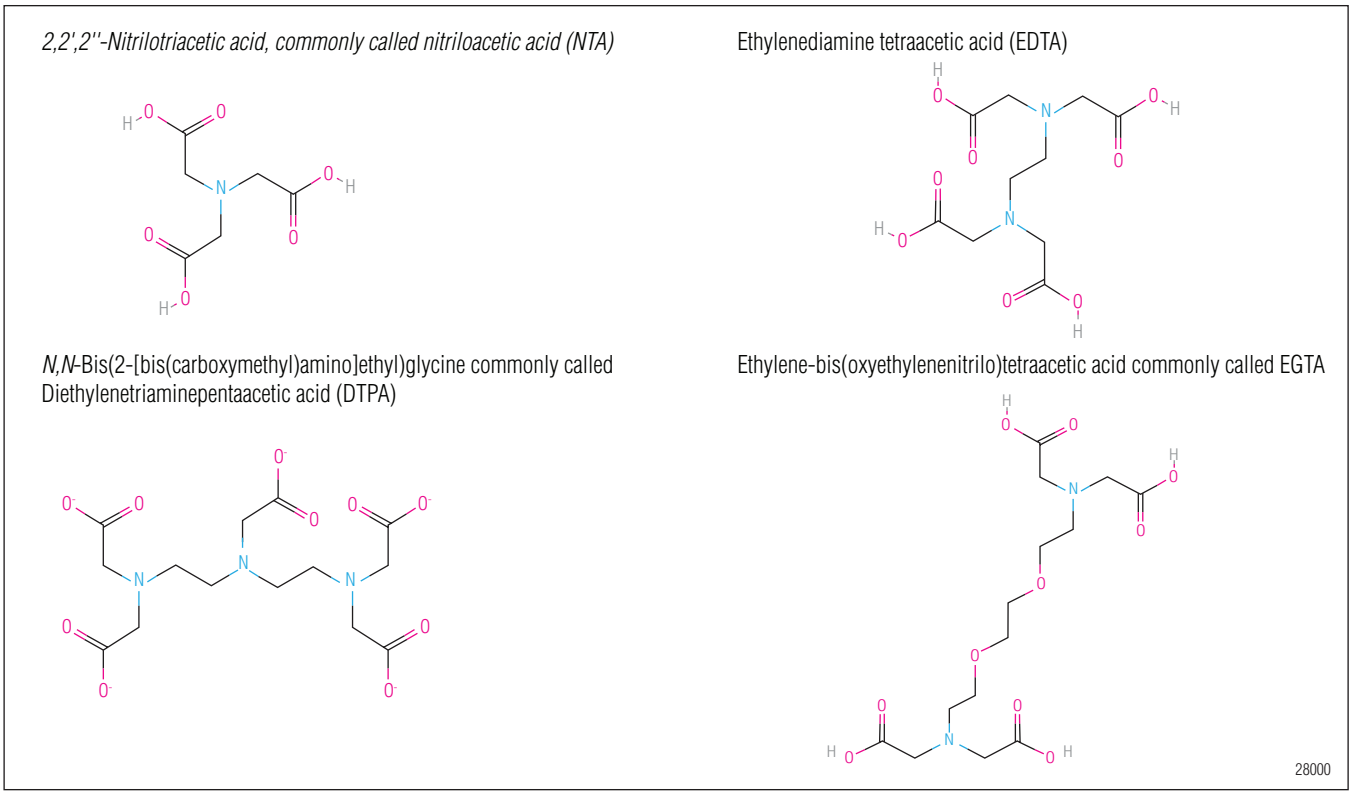

Figure 1. Aminopolycarboxylate chemical structures.

acid (NTA), diethylenetriaminepentaacetic acid (DTPA), and glycol-*bis* (2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA)) averaged over 200,000 tons per year.[7] Chelating agents that form low or moderately high stability constants, such as NTA, are readily biodegradable.[5] However, chelating agents that form stronger metal complexes, such as EDTA and DTPA, degrade slowly and therefore are persistent in the environment.[2,3–5,7] Although these chelating agents do not concentrate in the food chain, up to 800 µg/L of EDTA has been found in some U.S. industrial and municipal wastewater treatment plants and up to 12 mg/L in European bodies of water.[2,4,5]

In wastewater treatment plants, chelating agents can interfere with metal removal processes, allowing toxic metals to pass through untreated and contaminate the environment.[6,7] Some studies have suggested that >1 mg/L concentrations can interfere with biological processes.[7,8] Therefore, in 2003 the World Health Organization specified a 600 µg/L limit for EDTA in drinking water.[2,9] Based on additional studies, the European Aminocarboxylates Committee established the predicted no effect concentration (PNEC) limit of 2.2 mg/L in the aqueous environment.[3] (PNEC is defined as the predicted concentration that is not expected to cause adverse effects.) Therefore, sensitive analytical methods are needed to monitor surface water, municipal drinking water, and wastewater to meet regulatory compliance in Europe and to address increasing public concerns about the environmental fate of aminopolycarboxylate chelates in the environment.

This study describes the determination of µg/L concentrations of four aminopolycarboxylate chelates, EDTA, NTA, DTPA, and EGTA, in municipal drinking water and wastewater samples. The chelating agents are separated by ion chromatography (IC) on a high-capacity IonPac® AS7 anion-exchange column using methanesulfonic acid to elute the analytes in 16 min followed by pulsed amperometric detection (PAD) with a Pt working electrode. The IonPac AS7 column is designed for separating polyvalent anions and therefore is ideal for this application. Additionally, the column's high capacity minimizes column overload from the high salt matrix in wastewater samples. This method also uses the advantages of PAD to selectively quantify µg/L concentrations of chelating agents without interference from high concentrations of common anions commonly found in environmental samples. The qualification results and effect of the dissolved metals on the chromatography are also discussed. This method provides a selective and sensitive method to directly determine µg/L concentrations of chelating agents and therefore allows monitoring of these persistent contaminants in industrial wastewater samples.

EQUIPMENT

Dionex ICS-3000 or ICS-5000 system including:

SP Single Gradient Pump module with degas option

DC Detector/Chromatography Module (dual-temperature configuration)

AS Autosampler and 10 mL sample tray

ED Electrochemical Detector (P/N 061718)

Electrochemical cell (cell and reference electrode, P/N 061756)

Pt working electrode (P/N 064440 package of six)

Combination pH–Ag/AgCl reference electrode (P/N 061879)

Knitted reaction coil, 125 µL (P/N 053460)

25 µL PEEK™ sample loop (P/N 042857)

Chromeleon® Chromatography Data System (CDS) software

2 L glass eluent bottle, Type GL45 (P/N 045901)

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

Filter unit for vacuum filtration, 1 L, 0.20 µm nylon (Nalgene® Media-Plus with 90 mm filter, Nalge Nunc International P/N 164-0020) or equivalent nylon filter

Vacuum pump

Sample Preparation:

- Pall Corporation IC Acrodisc®, 25 mm syringe filters, 0.2 µm Supor® (PES) membrane, IC certified (P/N 4583T)
- Filter unit for vacuum filtration, 250 mL, 0.20 µm nylon (Nalgene Media-Plus with 90 mm filter, Nalge Nunc International P/N 153-0020) or equivalent nylon filter
- Hot plate
- pH meter
- 5 mL Disposable syringes
- Sodium hydroxide for pH adjustment

REAGENTS AND STANDARDS

Deionized water, Type 1 reagent-grade, 18.2 MΩ-cm resistivity

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

EDTA, disodium dihydrate (disodium ethylenediaminetetraacetate dihydrate, Sigma-Aldrich P/N E4884, FW 372.24)

NTA (nitrilotriacetic acid, Sigma-Aldrich P/N 398144, FW 191.14)

DTPA (diethylenetriaminepentaacetic acid, Sigma-Aldrich P/N 32319, FW 393.35)

EGTA (ethylene glycol tetraacetic acid, Sigma-Aldrich P/N 32319, FW 380.35)

Methanesulfonic acid, >99.5% (Fluka P/N 64280, FW 96.11)

Sodium hydroxide, 50% w/w certified (Fisher Scientific P/N SS254)

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

pH 4 (red) buffer solution (VWR International P/N BDH5018)

1000 mg/L single element standards used for metal-chelator experiments

- Copper (VWR International P/N JT5713-4)
- Cobalt (VWR International P/N JT5712-4)
- Iron (III) (VWR International P/N JT5764-4)
- Nickel (VWR International P/N JT5770-4)
- Zinc (VWR International P/N JT5791-4)

SAMPLES

Municipal wastewater effluent samples from Cities A and B

Town C surface water

Municipal drinking water from Cities A and B

CONDITIONS

Column:	IonPac AG7 Guard, 2 × 50 mm (P/N 063099)
	IonPac AS7 Analytical, 2 × 250 mm (P/N 63097)
Eluent:	A: Degassed deionized water
	B: 200 mM Methanesulfonic acid (MSA)
Gradient:	17.5% B (35 mM MSA) from -5 to 1 min, step to 50% B (100 mM MSA) at 1 min, 50% B from 1 to 12 min, step to 17.5% B at 12 min, 17.5% B from 12 to 16 min
Flow Rate:	0.3 mL/min
Trap Column:	IonPac CTC-1, 9 × 24 mm (P/N 040192)
Temperature:	30 °C
Inj. Volume:	25 µL
Detection:	PAD, Pt disposable WE, waveform (Table 1)
Data Collection Rate:	0.9 Hz
Typical Background:	130–180 nC
Typical Noise:	60–80 pC
Typical pH:	0.9–1.1
Run Time:	16 min

Table 1. Waveform[10]

Time (sec)	Potential vs Ag/AgCl (V)	Gain Region[a]	Integration	Ramp[a]
0.00	+ 0.30	Off	Off	Ramp
0.31	+ 0.30	Off	Off	Ramp
0.32	+ 1.15	Off	Off	Ramp
0.64	+ 1.15	On	On (Start)	Ramp
0.66	+ 1.15	On	Off (End)	Ramp
0.67	- 0.30	Off	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 (and ICS-5000) electrochemical detector.

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent A (Degassed Deionized Water)

When preparing eluents, use high quality, Type 1, 18.2 MΩ-cm resistivity deionized water that contains as little dissolved gas as possible, which can cause increased noise in electrochemical detection. To prepare 2 L of Eluent A, degas 2 L of deionized water by using vacuum filtration and ultrasonic agitation with applied vacuum for 10 to 20 min. Connect the eluent bottle to the Eluent A line, place the eluent bottle under ~4 to 5 psi of nitrogen or another inert gas, and prime the pump. Also, prepare 1 L of degassed Type 1 water weekly for the AS Autosampler flush solution.

Eluent B (200 mM MSA)

To prepare 2 L of eluent, degas 2 L of deionized water by using vacuum filtration and ultrasonic agitation with applied vacuum for 10 to 20 min, add 1974 ± 0.1 g of the degassed deionized water into a 2 L glass GL45 eluent bottle placed on a top loader balance, and add 38.4 ± 0.1 g (26 mL) of MSA. Connect the eluent bottle to Eluent B line, place the eluent bottle under ~4 to 5 psi of nitrogen or another inert gas, mix the eluent thoroughly, and prime the pump with the new eluent.

Stock Standard Solutions

To prepare 500 mg/L of individual EDTA and NTA stock solutions, transfer 64.1 ± 0.1 mg of the disodium EDTA dihydrate and 50 ± 0.1 mg of the NTA anhydrous reagent, respectively, into separate 100 mL Class A volumetric flasks. Add deionized water to the 100 mL mark. Mix the standard solutions thoroughly by inversion until the reagents are fully dissolved. EGTA and DTPA are slightly soluble in water, and therefore require additional mixing. To prepare the EGTA and DTPA stock solutions, add 50 mg of reagent plus 75 mL of deionized water and a stir bar into separate 100 mL Class A volumetric flasks. Stir for at least 3 h with a magnetic stirrer until the reagents are fully dissolved. Remove the magnetic stir bar, rinse the stir bar into the volumetric flask, and dilute to the 100 mL mark with deionized water. Mix the solutions thoroughly by inversion.

Intermediate and Working Standard Solutions

To prepare combined 10 mg/L EDTA, 20 mg/L NTA and DTPA, and 40 mg/L EGTA intermediate standards, pipet 400 µL of 500 mg/L EDTA, 800 µL of 500 mg/L NTA and DTPA, and 1600 µL of 500 mg/L EGTA stock standards into a 20 mL glass scintillation vial. Add deionized water to 20.000 g total weight and mix thoroughly. To prepare the five working standards, pipet 100, 200, 400, 1000, and 2000 µL of the combined intermediate standards into individual 20 mL glass scintillation vials placed on an analytical balance. Add deionized water to 20.000 g total weight and mix thoroughly. The first working standard, containing 50 µg/L EDTA, 100 µg/L NTA and DTPA, and 200 µg/L EGTA, was used to determine the limit of quantification (LOQ) and diluted 1:1 with water to determine the limit of detection (LOD). Prepare the working standards daily, the intermediate standard weekly, and the stock standard monthly. Store at 5 °C.

Sample Preparation

All surface, municipal, and wastewater effluent samples were filtered prior to analysis to remove particulates using an individual 250 mL Nalgene 0.2 µm filter flask. Syringe filters (Pall Corporation Acrodisc) were used during the initial evaluations and are also suitable. Additionally, the wastewater effluent samples were also degassed with ultrasonic agitation and applied vacuum for 30 min and diluted 1:10 to reduce the matrix effects. All samples were spiked with 200 µg/L EDTA, DTPA, and NTA, and 400 µg/L EGTA from the 100 mg/L stock solutions for accuracy determinations.

Metal-Chelate Solutions

To prepare the metal-chelate solutions, first make a 0.5 mg/L EDTA, NTA, and 1 mg/L DTPA, EGTA mixed standard solution by pipetting 20 µL each of the 500 mg/L EDTA and NTA, and 40 µL each of the 500 mg/L DTPA and EGTA stock solutions into a 20 mL scintillation vial. Add deionized water to a total weight of 20.000 g. Add 25 and 250 µL aliquots of the 1000 mg/L metal standard (Fe^{+3}, Cu^{+2}, Co^{+2}, Ni^{+2}, Zn^{+2}) to separate 5 mL aliquots of the mixed standard and then mix the solutions thoroughly.

The metal-chelate solutions were analyzed before and after treatments used to remove the metal from the chelating agent. All of the selected metal-chelate solutions were evaluated with Treatment 1, described below. The copper- and iron-chelate solutions were evaluated with all four treatments.

- *Treatment 1:* Withdraw 4 mL of solution with a 5 mL disposable syringe. Pass the solution through an OnGuard® M cation trap cartridge. Discard the first mL passing through cartridge and collect the remainder of sample after it passes through the cartridge. The OnGuard M cation trap cartridge is designed to remove divalent transition and alkali metals.
- *Treatment 2:* Adjust the sample to pH 11 with a dilute sodium hydroxide solution. Filter the solution with a Pall IC syringe filter.
- *Treatment 3:* Adjust the sample to pH 11 and heat the solution to 50 °C for 30 min. Cool to room temperature, adjust the volume to the starting volume, and then filter the solution with a Pall IC syringe filter.
- *Treatment 4*: Prepare the solution with Treatment 3 followed by Treatment 1.

PRECAUTIONS

Any solution treated with base must be filtered to remove particulates prior to injection. Acid eluents, such as the MSA used in this application, must only be stored in glass eluent bottles to reduce contamination introduced from the polymeric bottles.

SYSTEM PREPARATION AND CONFIGURATION

Preparing the System

To prepare the system, remove any previous metal contaminated components and conduct routine maintenance on the AS autosampler and the pumps. If the AS autosampler has not been maintained recently or has been exposed to high concentrations (mg/L) of metals, it may be necessary to install and calibrate a new AS injection port transfer line and install and align a new AS needle assembly.

Configuring the System

To configure the system, install the ED and the IonPac CTC-1 (9 × 24 mm) trap column after the pump and install ~61 cm yellow PEEK (0.076 mm i.d./0.003 in i.d.) tubing to the trap column outlet. The trap column removes any residual dissolved metals in the eluent. Install the free end of the yellow PEEK tubing, the 25 µL sample loop, and heat exchanger on the injection valve. Flush with 100 mM MSA eluent for 1 h at 1.0 mL/min to waste. Then install the IonPac AG7 guard and AS7 analytical column using red PEEK (0.127 mm i.d./ 0.005 in i.d.) tubing, which is temporarily directed to waste and flushed with deionized water for 10 min at 0.3 mL/min followed by 100 MSA eluent at the same flow rate overnight. Refer to the product manuals for more information.[11,12]

Assembling the Electrochemical Cell

To assemble the electrochemical cell, follow the instructions in AN 188, calibrate (from pH 7 to pH 4) and install the reference electrode and a Pt disposable working electrode.[13] Connect one free end of the 125 µL knitted reaction coil to the outlet of the analytical column and the other end to the cell inlet. Connect a small section (~4 to 6 in) of red PEEK tubing in the cell outlet as a backpressure loop. As a precaution, wait until after the pump is delivering eluent to the cell and the pH <2 before turning on the ED cell and loading the waveform. Equilibrate the system with the cell at least an hour to obtain a stable baseline and peak response. The working electrodes exhibited the minimum lifetime (two weeks) or greater, based on the peak response of >80% relative to the initial peak response of a newly installed and stabilized electrode.

Monitoring pH

Monitoring pH during sample analyses provides details on reference electrode drift and noise, and confirms proper eluent preparation. To monitor and record the pH, insert *Log* commands in the Chromeleon program by opening the instrument method, selecting *Script Editor*, highlighting a row, right click, insert a new time, enter the command *Log* and enter the value *pH*.

RESULTS AND DISCUSSION

To determine aminopolycarboxylate chelates, the IonPac AS7 (2 × 250 mm) was selected because it is a high-capacity anion-exchange column designed for the separation of polyvalent anions. The strong acid eluent partially protonates the analytes, thus reducing the effective charge, and allowing elution of the analytes from the column.[14–16] The IonPac AS7 column was previously used with PAD to separate the target chelating agents using 100 mM MSA eluent. The selectivity for these chelators was NTA < EDTA < DTPA < EGTA.[17] However, NTA and EDTA peaks were weakly retained and could co-elute with other less retained sample components. To improve this method, the eluent conditions from 25 to 150 mM MSA were evaluated to increase the resolution of EDTA and NTA from the void volume, while eluting DTPA and EGTA from the column within a reasonable time to improve sample throughput. This was accomplished by setting the initial eluent condition at 35 mM MSA for 1 min to elute EDTA and NTA and then stepping the concentration to 100 mM to elute EGTA and DTPA within 16 min. These conditions reverse the selectivity between NTA and EDTA, which indicates that NTA is very responsive to changes in eluent strength.

The analytes were detected by PAD using a Pt working electrode and a three-potential Pt waveform optimized at 100 mM MSA.[10]

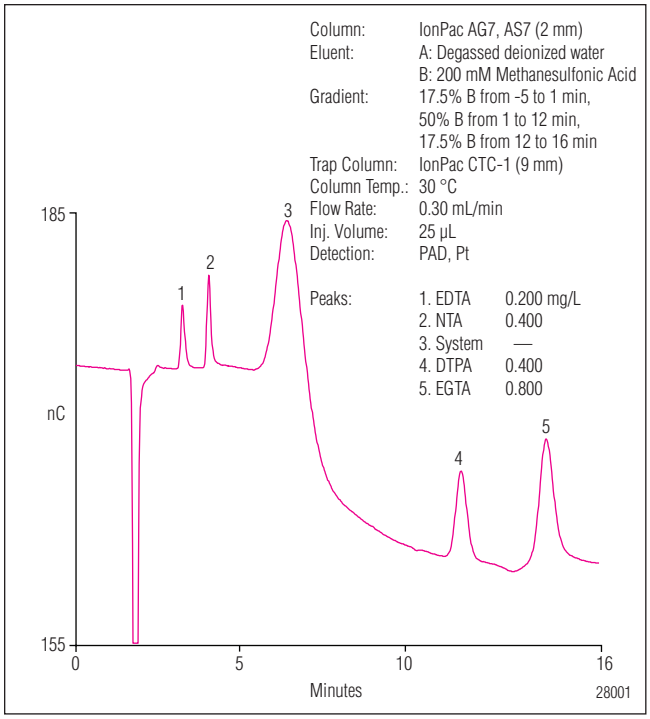

Figure 2. Mixed aminocarboxylate chelate standard separated and detected by anion-exchange chromatography with pulsed amperometric detection.

Figure 2 shows the separation of 200 µg/L EDTA, 400 µg/L NTA and DTPA, and 800 µg/L EGTA standards in deionized water. The peaks show good peak symmetry (A_s <1.2) except for NTA and EGTA (A_s = 1.4), efficiencies >4000 plates, and peak responses well above the baseline. The large peak at approximately 5 min is a baseline disturbance from the eluent step change at 1 min. The CTC-1 (9 × 24 mm) trap column adds a 5 min void time and therefore the change in baseline from the eluent step change occurs around 6 min in the chromatogram. The eluent concentration is reduced to the initial conditions at 12 min and held through the injection of the next sample, which produces an approximately 9 min total equilibration.

Table 2. LOD, LOQ, and Linearity Results

	Linear Range (µg/L)	Coefficient of Determination (r^2)	LOD (µg/L)	LOQ (µg/L)
EDTA	50–1000	0.9991	15	50
NTA	100–2000	0.9992	20	67
DTPA	100–2000	0.9992	30	100
EGTA	200–4000	0.9993	63	210

n = 7

LOD and LOQ are defined as 3× and 10× S/N.

Limit of Detection, Limit of Quantification, Linear Range, and Precision

To qualify the method, the estimated limit of detection (LOD), limit of quantification (LOQ), linear range, and precision were determined. The LOD and LOQ were determined by measuring the peak-to-peak noise in 1 min increments from 20 to 60 min in four replicate runs without a sample injection and found that the noise averaged 65 ± 12 pC. The estimated LODs and LOQs were calculated based on the peak response of the standards at 3× and 10× the signal-to-noise (S/N) (Table 2). The estimated LODs ranged from 15 to 63 µg/L and the LOQs ranged from 50 to 210 µg/L. To determine the method linearity, five combined calibration standards from 50 to 1000 µg/L EDTA, 100 to 2000 µg/L NTA and DTPA, and 200 to 4000 µg/L EGTA were injected in four replicates, which produced coefficients of determination (r^2) >0.999. The retention time precisions, based on seven replicate injections of 0.50 mg/L EDTA, 1.0 mg/L NTA and DTPA, and 2.0 mg/L EGTA combined standards, had RSDs of <0.3. NTA, EDTA, DTPA, and EGTA had peak area precisions of 2.35, 2.17, 3.37, and 2.48 RSDs, respectively.

Chelating Agents in Surface Water, Municipal Drinking Water, and Wastewater Samples

This method was applied to five municipal water samples that included surface water, drinking water, and wastewater effluent from two municipalities. All samples were filtered to remove particulates prior to analysis. Trace EGTA concentrations (0.13 to 0.18 mg/L) were detected in all samples. However, trace concentrations of EDTA (≤50 µg/L) were only detected in the wastewater samples. DTPA and NTA were not detected in any of the samples investigated in this study.

To determine the accuracy of the method, the samples were spiked with 0.200 mg/L EDTA, NTA, and DTPA, and 0.400 mg/L EGTA. Figure 3 Chromatogram B shows the chromatography of the unspiked surface water sample with only EGTA detected. Figure 3 Chromatogram C shows the same sample spiked with known concentrations of chelating agents. A water blank (Figure 3 Chromatogram A) is shown for comparison. As shown, EDTA was well resolved from a small baseline dip and from NTA. DTPA and EGTA eluted well after the baseline disturbance at ~7 min and a larger dip at 10 min.

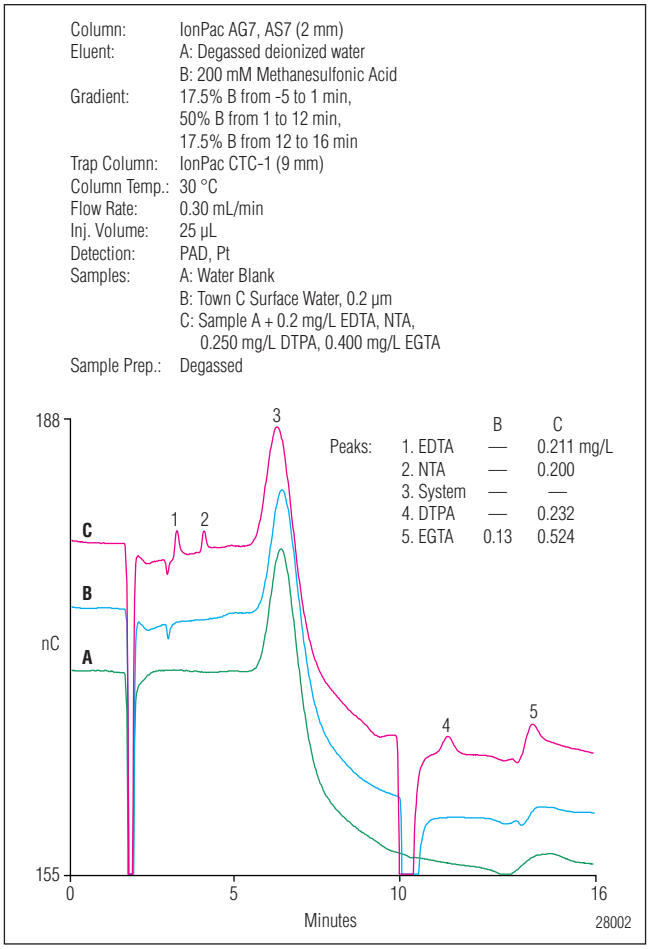

Figure 3. Comparison of Town C surface water B) without and C) with chelates added.

Table 3. Results and Recoveries in Drinking Water and Wastewater Samples						
Sample	**Analyte**	**Amount Found (mg/L)**	**RSD**	**Amount Added (mg/L)**	**RSD**	**Amount Recovered (%)**
Town C Surface Water[a]	EDTA	N.D.	—	0.198 ± 0.008	4.3	107 ± 0.7
	NTA	N.D.	—	0.198 ± 0.012	5.9	108 ± 0.6
	DTPA	N.D.	—	0.232 ± 0.013	5.8	107 ± 0.8
	EGTA	0.13 ± 0.02[c]	3.8	0.565 ± 0.030	5.2	110 ± 2.1
City A Drinking Water[a]	EDTA	N.D.	—	0.198 ± 0.008	4.3	103 ± 0.5
	NTA	N.D.	—	0.198 ± 0.012	5.9	106 ± 0.8
	DTPA	N.D.	—	0.232 ± 0.013	5.8	112 ± 0.9
	EGTA	0.18 ± 0.02[c]	4.6	0.565 ± 0.030	5.2	89.0 ± 3.2
City B Drinking Water[a]	EDTA	N.D.	—	0.198 ± 0.008	4.3	109 ± 1.8
	NTA	N.D.	—	0.198 ± 0.012	5.9	114 ± 0.7
	DTPA	N.D.	—	0.232 ± 0.013	5.8	89.5± 0.6
	EGTA	0.18 ± 0.02[c]	4.6	0.565 ± 0.030	5.2	109 ± 3.2
1:10 Dilution of City A Wastewater Effluent[b]	EDTA	0.054 ± 0.010	18	0.198 ± 0.008	4.3	95.2 ± 0.3
	NTA	N.D.	—	0.198 ± 0.012	5.9	95.1 ± 1.1
	DTPA	N.D.	—	0.232 ± 0.013	5.8	95.5 ± 1.0
	EGTA	0.18 ± 0.02	14	0.565 ± 0.030	5.2	101 ± 6.2
1:10 Dilution of City B Wastewater Effluent[b]	EDTA	0.024 ± 0.009	3.7	0.198 ± 0.008	4.3	101 ± 0.7
	NTA	N.D.	—	0.198 ± 0.012	5.9	107 ± 0.8
	DTPA	N.D.	—	0.232 ± 0.013	5.8	96.0 ± 2.8
	EGTA	0.14 ± 0.02[c]	4.5	0.565 ± 0.030	5.2	124 ± 8.8

n = 5

[a] Samples were filtered with 0.2 µm filter prior to determination

[b] Samples were filtered, degassed for 30 min with ultrasonic agitation and applied vacuum, and diluted 1:10 prior to determination

[c] Calculated value below calibration range

N.D.: Not Detected

The recovery of the chelating agents spiked in the sample ranged from 107 to 110% (Table 3). The municipal drinking water samples had similar chromatography and a slightly larger range of recoveries, compared to the surface water sample (89 to 112%). The initial evaluation of the municipal wastewater effluent samples showed a large baseline disturbance prior to the elution of EDTA, possibly from organic acids and carbonate, which may interfere with the accurate quantification of EDTA. To minimize the matrix effects of the wastewater, the samples were degassed with an applied vacuum and ultrasonic agitation, then diluted 1:10. Using these additional steps, detection of 0.024 and 0.054 mg/L EDTA was achieved in 1:10 dilution of degassed City A and City B wastewater effluent samples, and 95 and 101% recoveries, respectively.

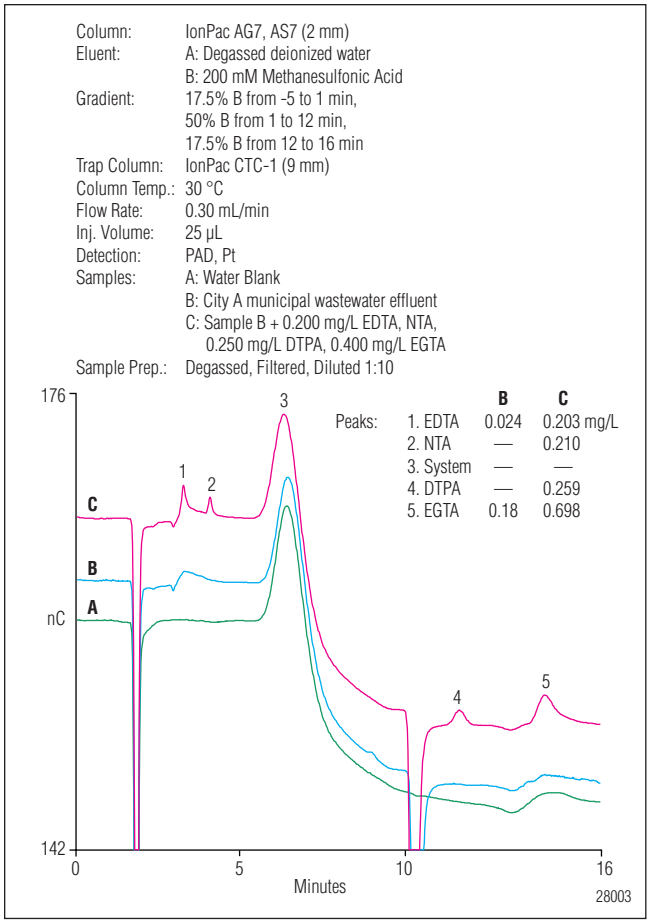

Figure 4. Comparison of 1:10 dilution of City A municipal wastewater effluent B) without and C) with chelates added.

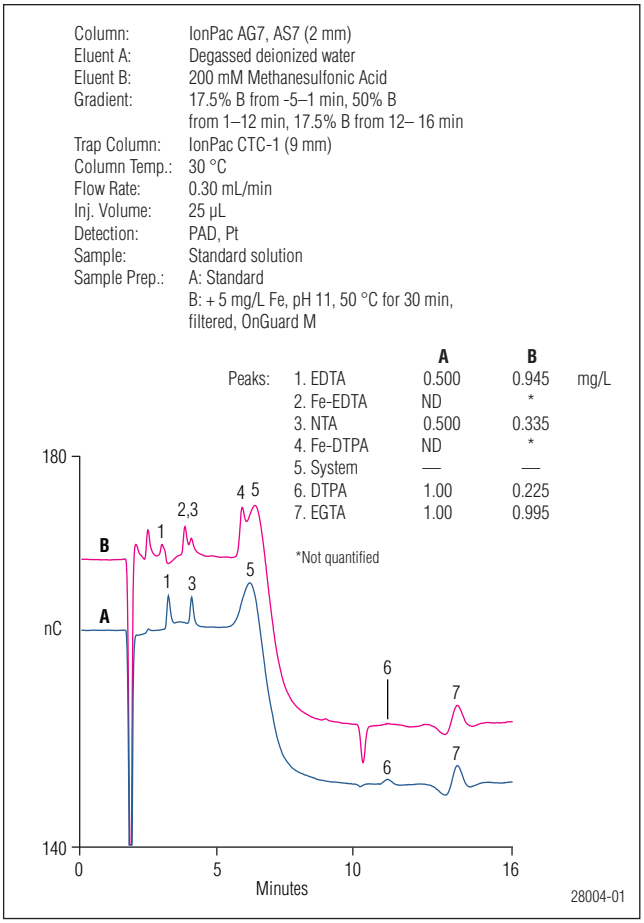

Figure 5. Effect of iron on chelating agents.

Figure 4 compares a water blank and City A municipal wastewater sample with and without 0.200 mg/L of EDTA, NTA, DTPA, and 0.400 mg/L EGTA added. After degassing and diluting the wastewater samples, the negative peak eluting near the EDTA peak was minimized, resulting in improved recovery of EDTA. While these additional sample treatments improved EDTA detection, the sensitivity for all analytes in the municipal wastewater samples was reduced by the dilution factor.

Effect of Metal Chelation

The effects of metal chelation on the separation of chelating agents has been discussed extensively.[2,4,16] Metal chelates reduce the ionic charge compared to the free chelate due to the binding of the metal, with the exception of NTA, which retains the same ionic charge.[2,4,16] To evaluate the effect of the change in charge distribution on the anionic separation, amounts of 5 and 50 mg/L of iron (III), copper, cobalt, nickel, and zinc were added to individual solutions of the mixed chelate standard (0.5 mg/L EDTA, NTA, 1 mg/L DTPA, EGTA). Some samples were treated with OnGuard M cartridge, which is a cation sample-preparation cartridge designed to remove free metals. The responses and retention times of DTPA, EDTA, and NTA were significantly affected by the presence of iron (Figure 5). The complexed Fe-DTPA eluted 4.5 min earlier at ~6.9 min relative to the uncomplexed DTPA, which eluted at ~11.4 min. In addition, the response of Fe-DTPA increased by ~400%. In contrast to Fe-DPTA, the Fe-EDTA retention time decreased only slightly by 0.5 min and partially co-eluted with NTA, resulting in a 42.5% decrease in peak area response relative to the uncomplexed EDTA. The NTA peak area response was significantly lower (73 to 74%) than the untreated sample. However, no shift in retention time was observed. No changes in responses or retention times were observed for EGTA from the presence of the investigated transition metals.

Previous studies demonstrated that the metal chelates were unstable at a pH <6 and >8.[15] These authors achieved partial success in recovering the chelate after treating the solutions with OnGuard M cartridges. To improve the recoveries of DTPA and EDTA, the effects of treating the solutions by adjusting the pH, heating and filtering the metal precipitates, and treating the complexed chelators with OnGuard M cartridges were evaluated. Metal-chelate sample solutions were diluted 1:10 and 1:100, then prepared by adding aliquots of 1000 mg/L metal standards in an acid matrix to produce a sample solution pH from 1 to 3. In the initial experiments, the iron-chelate test solutions were analyzed without treatment and after treatment with OnGuard M cartridges, but no changes in peak responses and retention times were observed. The metal-chelate sample solutions were also adjusted with dilute sodium hydroxide solution to pH 11 and filtered with a 0.2 µm syringe filter to remove any metal particulates.

Additionally, to improve the metal precipitate formation, aliquots of the iron-chelate solutions were heated at 50 °C for 30 min, then filtered with a 0.2 µm syringe filter. Some aliquots were also treated with OnGuard M cartridges. The best results were found by using Treatment 4, combining pH adjustment, heating, filtering the precipitate, and using an OnGuard M cartridge. As a result of the treatments, another peak appeared at the original EDTA retention time but at 35.7% of the original EDTA peak area (Table 4), along with the Fe-EDTA peak at 3.8 min but at a ~5% lower peak area. The combined peak areas of the two peaks were comparable (93%) to the peak area of EDTA prior to treatment with iron. However, no improvement in the recovery of DTPA was observed. These experiments show that iron binds strongly with DTPA and EDTA, interfering with the separation and quantification of the two chelating agents (Chromatogram C, Figure 5). However, treatment with base, elevated temperature, and OnGuard M cartridges improves the recoveries of EDTA, but the treatment does not completely release the metal from the complex.

Table 4: Recoveries of 0.5 mg/L Chelates in the Presence of Select Metals

Metal	Amount Metal Added (mg/L)	EDTA Recovery (%)	NTA Recovery (%)	DTPA Recovery (%)	EGTA Recovery (%)
Cobalt	5.0	98.7	101	95.6	99.6
	50	95.4	105	84.7	85.3
Copper	5.0	98.7	99.6	98.8	92.6
	50	87.0	95.4	84.8	101
Iron	5.0	~58.5[b]	72.6	--[d]	99.3
	5.0, Treated[a]	35.7[c] ~55.2[b]	83.5	--[d] < LOQ[e]	91.2
	50	~58.9[b]	73.5	--[d]	97.1
Nickel	5.0	90.3	98.8	88.9	92.7
	50	85.9	98.7	93.8	96.4
Zinc	5.0	98.5	97.7	98.9	96.3
	50	93.5	93.1	98.7	103

n = 2

[a] Adjusted to pH 11, heated to 50 °C for 30 min, filtered, and treated with OnGuard M cartridge.

[b] Estimated, based on peak area of Fe-EDTA peak eluting ~0.8 min later than the EDTA peak.

[c] EDTA peak appearing near original retention time.

[d] Fe-DTPA peak eluting near 6 min was not quantified.

[e] DTPA peak detected at original retention time.

CONCLUSION

This experimental study describes a direct, sensitive, and accurate method to determine µg/L concentrations of NTA, EDTA, EGTA, and DTPA in surface water, municipal drinking water, and wastewater samples.

This method takes advantage of the selectivity of the IonPac AS7 column to separate large hydrophobic anionic compounds, such as chelating agents, and the selectivity of PAD to determine low concentrations of these compounds without detecting common anions that are typically present at high concentrations in wastewater samples. Improved recoveries of EDTA in the presence of iron (III) can be achieved by treating the samples with base, elevated temperature, and OnGuard M cartridges. This experimental study determines concentrations of chelating agents needed to assess the contamination levels in water systems and provide adequate safety to the environment.

SUPPLIERS

Sigma-Aldrich, Inc., P.O. Box 951524, Dallas, TX 75395-1524, U.S.A. Tel: 1-800-325-3010. www.sigmaaldrich.com

VWR International, Inc., Goshen Corporate Park West, 1310 Goshen Parkway, West Chester, PA 19380, U.S.A. Tel: 1-800-932-5000. www.vwrsp.com

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Determination of Trace Sodium and Transition Metals in Power Industry Samples by Ion Chromatography with Nonsuppressed Conductivity Detection

INTRODUCTION

It is critical for power plants to continuously monitor corrosive ionic impurities in various plant water streams, such as cooling waters, boiler waters, feed waters, and steam condensates. The presence of impurities above a specified concentration can result in stress-corrosion cracking and other corrosion mechanisms, and therefore have a significant negative impact on power generation. Damage from corrosive ions can cause forced outages and component failures, costing millions of dollars in lost revenue.[1,2] The continuous monitoring of ionic species provides valuable information regarding the source of contamination, assists power plants in understanding corrosion mechanisms, allows recommendations for appropriate treatment to prevent corrosion impacts, and produces concentration trends for individual ions over a length of time. This information can be used to minimize corrosive damage.

Corrosive contaminants—particularly sodium, chloride, and sulfate—have been implicated as a major source of corrosion and deposition-related plant shutdowns in nuclear and fossil-fueled plants.[3] Ion chromatography (IC) can measure these contaminants and has been implemented in several U.S. power plant water chemistry monitoring programs. This methodology allows analysts to achieve parts per trillion (ppt) detection limits for individual anionic and cationic species and operate on-line to effectively measure and eliminate corrosive hideout, thereby reducing the plant's operating costs.

The measurement of ultratrace levels of sodium in boiler waters treated with amine additives, such as ethanolamine, is a particularly challenging analytical problem. The purpose of this all-volatile treatment (AVT) is to provide a high-pH and high-purity environment to minimize corrosion of metal surfaces. This environment is accomplished by adding the amine to the boiler water at concentrations typically in the range of 0.5–10 mg/L. In effect, the high amine concentration can preclude the separation and detection of sodium typically present in the low-ppt range. However, many of these challenges have been overcome by the development of efficient high-capacity cation-exchange columns capable of tolerating high-ionic-strength matrices.[4]

Other cationic species are also of interest to many power plants. For example, the determination of calcium and magnesium may be used to calculate water hardness and reveal the presence of condenser leaks and water polisher failures. These and other cationic species must be detected at the sub-µg/L concentrations in power plant samples. The lowest detection limits are achieved by suppressed conductivity detection.

An alternative to suppressed conductivity detection is analysis without the suppressor (i.e., nonsuppressed conductivity). In this detection mode, the column effluent flows directly into the conductivity cell. Consequently, lower-capacity columns using dilute acidic eluents are required to achieve a reasonable background signal. Weakly acidic complexing agents, such as tartaric acid, pyridine-2,6-dicarboxylic acid (PDCA), and oxalic acid, are also commonly used with nonsuppressed IC columns.

Determination of cations by IC with suppressed conductivity detection has demonstrated detection limits that are at least an order of magnitude (i.e., 10 times) lower than nonsuppressed systems.[5] This advantage has clearly made suppressed conductivity the preferred detection mode for the determination of cations, particularly low-ppt sodium determinations, in the power industry. Dionex Application Note 157 provides a general discussion of suppressed versus nonsuppressed conductivity detection.

Nuclear and fossil-fueled power plants also require iron and copper monitoring at the sub- to low-µg/L (ppb) concentrations at various locations in the steam cycle. These transition metals can accumulate or deposit in the steam generators as sludge. This accumulation can create areas where ultratrace ionic impurities present in the feed water can concentrate by several orders of magnitude, resulting in a highly corrosive environment. Additional metals, such as zinc, are also of interest to some power plants. Zinc is added to pressurized water reactors (PWRs) to control corrosion. Zinc additions to the PWRs can alleviate two key challenges for plant operators[6]: (1) reduce degradation of the coolant materials due to stress corrosion cracking, and (2) lower the shutdown radiation dose rates. The concentration of added zinc is typically in the range of 1–10 ppb.

IC provides a convenient and reliable methodology to separate and detect transition metals on-line at low- to sub-ppb concentrations. The separation of these metals requires the formation of a complex with a weak organic acid—such as citric acid, oxalic acid, tartaric acid, or PDCA—to reduce their effective positive charge. This complexation allows a change in selectivity of the metal ions that cannot be accomplished using only a monovalent eluent ion, such as hydronium.[7] The most common mode of detection used for this analysis involves derivatization of the column effluent with 4-(2-pyridylazo)resorcinol (PAR) and subsequent photometric detection at 520–530 nm. PAR provides a very broad selectivity and sensitivity for transition metals.

Although suppressed and nonsuppressed conductivity can be used for the detection of alkali and alkaline earth metals, only nonsuppressed conductivity is suitable for the detection of transition metals. The formation of insoluble metal hydroxides from the suppressor reaction precludes the use of a suppressor for this analysis. However, in comparison to photometric detection, nonsuppressed conductivity does not provide the required specificity for many transition metals in high-ionic-strength matrices because all cations are detected.

This application note compares suppressed to nonsuppressed conductivity detection for the determination of sub-ppb concentrations of sodium in simulated power plant matrices. Linearity, limits of detection, and recovery of sub-ppb sodium spiked into simulated samples containing high concentrations of ethanolamine are reported. In addition, this application note describes the determination of transition metals by nonsuppressed conductivity detection.

Note: *The equipment, analytical conditions, system preparation, and system setup for trace sodium determinations by suppressed conductivity detection and transition metals by postcolumn reaction with visible detection are available in Application Notes 152 and 131, respectively.*

EQUIPMENT

Nonsuppressed Cation System

Dionex ICS-1000, 1500, or 2000 Ion Chromatography System consisting of:
- Dual-piston pump
- Column heater
- Digital conductivity detector

Pressurized Sample Vessel (Dionex P/N 037460)*

Dionex DXP Single-Piston Pump (Dionex P/N 043047)*

Chromeleon® Chromatography Workstation

*This equipment was used for trace-level determinations

REAGENTS AND STANDARDS

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

Lithium standard, 1000 mg/L (Ultra Scientific; VWR P/N ULICC 104)

Sodium standard, 1000 mg/L (Ultra Scientific; VWR P/N ULICC 107)

Ammonium standard, 1000 mg/L (Ultra Scientific; VWR P/N ULICC 101)

Potassium standard, 1000 mg/L (Ultra Scientific; VWR P/N ULICC 106)

Magnesium standard, 1000 mg/L (Ultra Scientific; VWR P/N ULICC 105)

Calcium standard, 1000 mg/L (Ultra Scientific; VWR P/N 103)

Lithium chloride (LiCl; Fisher L-121-100)

Sodium chloride (NaCl; Fisher S-271)

Ammonium chloride (NH_4Cl; Fisher A-5666)

Potassium chloride (KCl; Sigma P-3911)

Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$; Aldrich Chemical Co. 24,696-4)

Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$; Fisher C79-500)

Ethanolamine, 99%, reagent-grade (Aldrich Chemical Co. 39,813-6))

Combined Six Cation Standard-II (Dionex P/N 046070)

Tartaric acid (Aldrich Chemical Co. 48,379-6)

Oxalic acid (Aldrich Chemical Co. 19,413-1)

Copper, atomic absorption grade, 1000 mg/L (EM Science; VWR P/N EM-CX1917-1)

Nickel, atomic absorption grade, 1000 mg/L (EM Science; VWR P/N EM-NX0290-1)

Zinc, atomic absorption grade, 1000 mg/L (EM Science; VWR P/N EM-ZX0007-1)

Cobalt, atomic absorption grade, 1000 mg/L (EM Science; VWR P/N EM-CX1767-1)

Manganese, atomic absorption grade, 1000 mg/L (EM Science; VWR P/N EM-MX0172-1)

Cadmium, atomic absorption grade, 1000 mg/L (EM Science; VWR P/N EM-CX0011-1)

CONDITIONS

Nonsuppressed Conductivity Detection of Trace Sodium

Columns:	IonPac® SCS 1 Analytical, 4 × 250 mm (Dionex P/N 079809)
	IonPac SCG 1 Guard, 4 × 50 mm (Dionex P/N 061523)
Eluent:	3 mM MSA
Flow Rate:	1 mL/min
Temperature:	30 °C
DXP Flow Rate:	2 mL/min
Sample Volume:	3 mL

Table 1. Retention Times (min) of Common Cations and Transition Metals on the IonPac SCG 1 and SCS 1 Columns

Eluent Concent. / Analyte	3 mM MSA	2 mM MSA 0.5 mM Oxalic Acid	4 mM Oxalic Acid	3 mM Oxalic Acid	3 mM Oxalic Acid/ 0.5 mM MSA	5 mM Tartaric Acid/ 0.16 mM PDCA	4mM Tartaric Acid/ 2mM Oxalic[b]
Lithium	6.70	6.80	7.10	8.60	7.40	9.10	7.40
Sodium	8.70	8.80	9.20	11.3	9.60	11.9	9.60
Ammonium	10.0	10.1	10.5	13.1	11.0	13.7	10.9
Potassium	13.9	13.8	14.4	18.1	15.0	18.7	15.0
Magnesium	25.6	27.5	27.4	45.5	31.7	58.8	32.2
Calcium	33.7	35.8	36.8	61.9	42.3	64.1	41.8
Copper	>60.0	7.50	2.98	—	3.40	—	3.90
Zinc	29.6	24.1	10.8	16.8	13.8	4.80	16.6
Cobalt	29.3	25.6	12.7	20.2	16.2	9.90	19.4
Manganese	—[a]	—	24.3	41.0	29.4	46.9	30.7
Cadmium	—	—	32.1	—	38.6	—	39.8

[a] Not determined

[b] See Figure 7

Concentrator

Column:	TCC-LP1, 4 × 35 mm (Dionex P/N 046027)
Detection:	Nonsuppressed conductivity
Background:	~1100 µS
Noise:	~5–10 nS peak-to-peak
Backpressure:	~2100 psi
Run Time:	15 min

Nonsuppressed Conductivity Detection for Transition Metals

Columns:	IonPac SCS 1 Analytical, 4 × 250 mm (Dionex P/N 079809)
	IonPac SCG 1 Guard, 4 × 50 mm (Dionex P/N 061523)
Eluent:	See Table 1
Flow Rate:	1 mL/min
Temperature:	30 °C
Injection:	25 µL
Detection:	Nonsuppressed conductivity
Noise:	~5–10 nS peak-to-peak
Backpressure:	~2100 psi

PREPARATION OF SOLUTIONS AND REAGENTS FOR TRACE SODIUM ANALYSIS

Eluent Solution

Prepare 1.0 N MSA stock solution by adding 96.10 g of methanesulfonic acid (MSA, >99%, Dionex P/N 033478) to a 1-L volumetric flask containing about 500 mL of deionized water. Dilute to the mark and mix thoroughly. Prepare 3 mM MSA by diluting 3 mL of the 1.0 N MSA stock solution to 1 L with deionized water. Degas the eluent by sonicating under vacuum for 10 min or by sparging with helium. Store the eluent in plastic labware. The eluent generator is not recommended for use with the nonsuppressed cation system because a significant increase in baseline noise is observed for this application.

Eluents containing complexing agents, such as tartaric acid, oxalic acid, or PDCA, can be prepared by weighing the appropriate amounts for the desired concentrations: (1) tartaric acid = 0.1501 g tartaric acid per mM for a 1 L solution, (2) oxalic acid = 0.0900 g oxalic acid per mM for a 1 L solution, (3) PDCA = 0.167 g PDCA per mM for a 1 L solution. PDCA does not dissolve readily in water; therefore, allow the solution to stir until the PDCA fully dissolves.

Standard Solutions

Certified stock solutions can be purchased or 1000-mg/L standards can be prepared for the cations of interest. Dissolve the appropriate amounts of the required analytes in deionized water in a 100-mL plastic volumetric container according to the amounts in Table 2. Dilute to volume with deionized water. Store in a plastic container at 4 °C. Working standards were prepared by serial dilutions from the 1000 mg/L (ppm) concentrate. For trace sodium analysis, working standards containing less than 1 ppm sodium were prepared every 2–3 days, standards containing less than 1 µg/L (ppb) were prepared daily and analyzed almost immediately after preparation.

1000 mg/L Ethanolamine Standard Solution

Dissolve 0.100 mL of ethanolamine in 100 mL of deionized water.

Matrix Preparation

In this study, two different matrices were prepared containing 3 and 5 ppm ethanolamine from the stock standard solution. Each matrix was spiked with ~0.250 ppb sodium.

SYSTEM PREPARATION AND SETUP

The ICS-1000, ICS-1500, or ICS-2000 integrated IC systems can be used for nonsuppressed cations analysis. This application note describes the proper setup and system preparation of the ICS-2000 system for nonsuppressed cations. Configure the IC system for trace sodium analysis as shown in Figure 1. Install the 4 × 50 mm

Table 2. Mass of Compound Required to Prepare 100 mL of a 1000-mg/L Standard Solution

Cation	Compound	Mass (g)
Li^+	Lithium (LiCl)	0.6108
Na^+	Sodium (NaCl)	0.2542
NH_4^+	Ammonium (NH_4Cl)	0.2965
K^+	Potassium (KCl)	0.1907
Mg^{2+}	Magnesium ($MgCl_2 \bullet 6H_2O$)	0.8365
Ca^{2+}	Calcium ($CaCl_2 \bullet 2H_2O$)	0.3668

IonPac SCG 1 and 4 × 250 mm IonPac SCS 1 columns in the column oven. Set the signal polarity by navigating to the dropdown menu on the LCD screen and press "Detector". In the conductivity polarity option, change the polarity to "Inverted". The conductivity polarity of the ICS-1000 must be changed using Chromeleon software because there is no LCD screen.

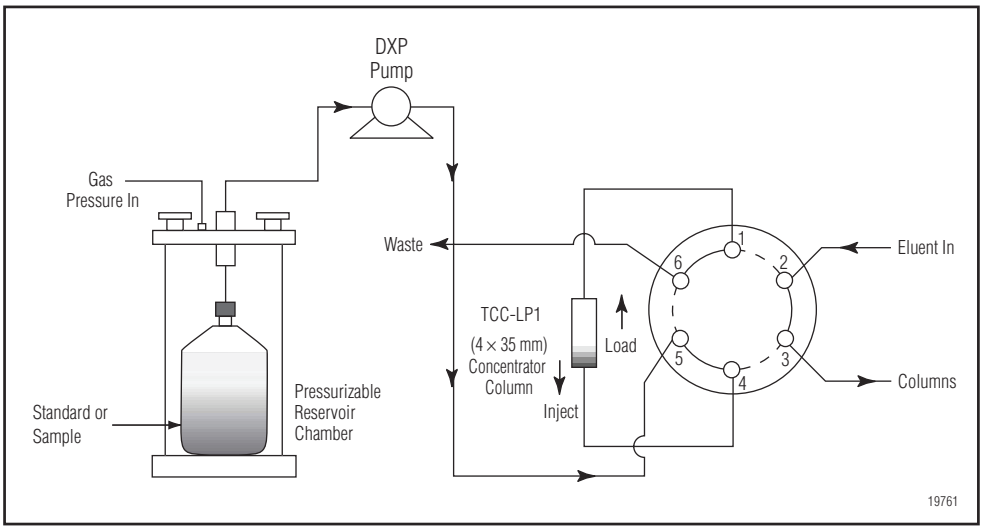

Figure 1. *Ion chromatiography preconcentration system (sample loading position shown).*

The eluent generator on the ICS-2000 system should be bypassed by placing a 10-32 in. union in place of the inlet and outlet fittings of the EluGen® cartridge because the eluent generator is not used. A separate union should be placed between the inlet and outlet fittings for the continuously regenerated trap column. Connect the conductivity detector cell outlet to the tubing labeled "Regen Out"; because a suppressor is not used in this work, the column effluent will be directed to waste. The Chromeleon program (*.pgm file) should have an MSA concentration of 0 mM and the suppressor should be set to "None".

Equilibrate the columns with 3 mM MSA at 1 mL/min for at least 60 min. Assess the stability of the baseline and peak-to-peak noise by measuring a representative section of the baseline where no peaks are eluting. An equilibrated system should have a background signal of <1100 µS and peak-to-peak noise of <10 nS. We strongly recommend equilibrating the system overnight for trace-level analysis with the analytical conditions described in this application note. For optimal system performance for trace analysis, we recommend continuously running the system.

RESULTS AND DISCUSSION

Trace Sodium Analysis

Special precautions must be taken when performing trace analysis. Use only the highest-quality deionized water to prepare eluents, standards, and dilutions. To achieve the low sodium concentrations specified in this application note, an autosampler should not be used. Application Note 152 provides a more detailed discussion of the necessary precautions required for the determination of trace sodium.

In the power industry, it is critical to determine trace amounts of sodium at sub-ppb concentrations in boiler water treated with amine additives, such as ethanolamine. However, the amine is often present at significantly higher concentrations than the target analyte. The preconcentration of a high-ionic-strength sample can cause the sample to act as an eluent and therefore prevent the retention of the analytes of interest on the concentrator column.[8] Because the resin in a concentrator column has a finite capacity (i.e., the column can only retain a given amount of ions from a sample matrix), the target analytes are no longer retained if the capacity of the column is exceeded. The volume of sample that just exceeds the capacity of the concentrator column is defined as the breakthrough volume. In this application note, trace levels of sodium were measured in the presence of high concentrations of ethanolamine. The high amine concentration in these samples limits the sample volume that can be concentrated. For a sample containing 0.500 ppb sodium in the presence of 5000 ppb ethanolamine, the breakthrough volume of the TCC-LP1 (4 × 35 mm) was determined to be ~4.5 mL. This volume is significantly less than the breakthrough volume of ~30 mL for 0.250 ppb sodium in a matrix of 5000 ppb ethanolamine using the higher-capacity CG16 (5 × 50 mm) as the concentrator column with suppressed conductivity detection.[4] Therefore, the suppressed conductivity system can detect significantly lower analyte concentrations in higher-ionic-strength matrices than the nonsuppressed system.

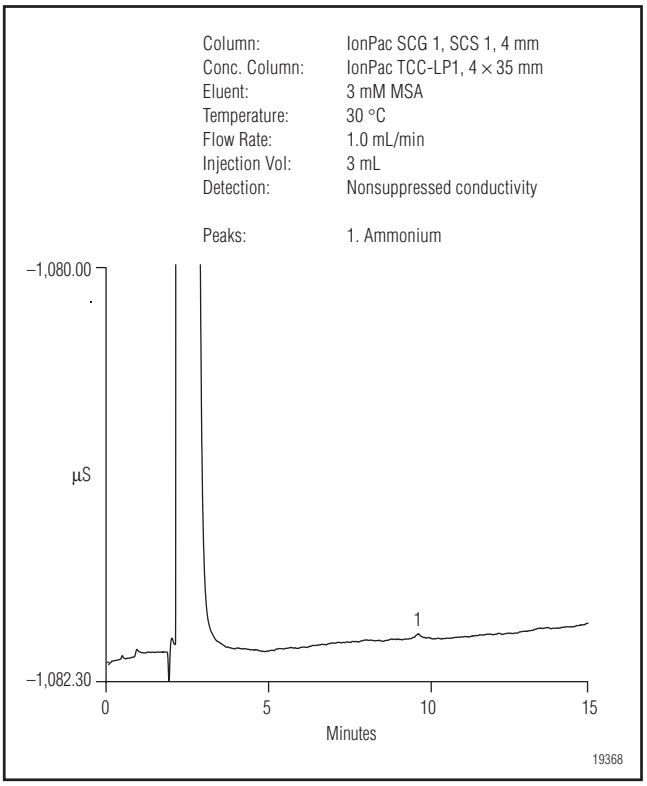

Figure 2. *A representative deionized water blank.*

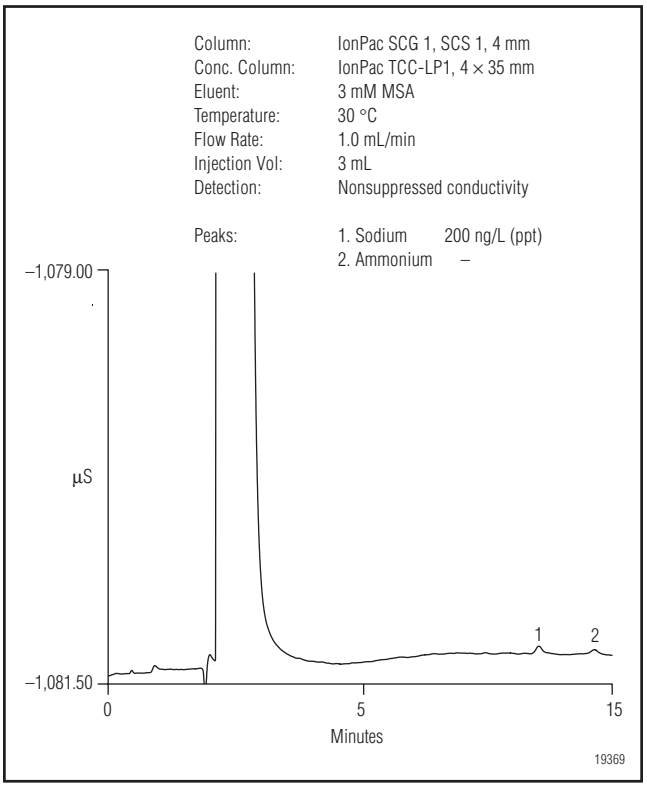

Figure 3. *Determination of 200 ng/L sodium spiked into deionized water using a 3-mL sample volume.*

In this application note, sub-ppb concentrations of sodium were determined in the presence of high concentrations of ethanolamine—a common additive found in boiler water—using nonsuppressed conductivity detection. This determination was accomplished by concentrating 3 mL (70% of the breakthrough volume) on a TCC-LP1. The TCC-LP1 is packed with a 20-µm carboxylate-functionalized resin, has high pH stability, and a cation-exchange capacity of 260 µeq/column. The TCC-LP1 is preferred for concentrating amine-treated samples because of the resin's higher pH stability and higher capacity compared to the 4 × 50 mm SCG 1 (63 µeq/column). However, eluents containing PDCA should not be used with this concentrator.

It is important to initially establish a blank and ensure the stability of the blank analyses over a period of several days. The quality of the blank depends on several factors, such as the cleanliness of the laboratory, purity of the water source, presence of contaminants in the IC system, and other factors that can contribute to high blank levels. In this study, no sodium was detected in the blank. Previous studies of our in-house deionized water system revealed sodium concentrations of ~5 ng/L (ppt),[4,9] significantly below the detection limit for the nonsuppressed system using a 3-mL sample volume. Figure 2 shows a representative blank chromatogram of deionized water with only a trace amount of ammonium detected. Because ammonia is ubiquitous, its concentration can vary from day to day.

The calibration curve for sodium was obtained by preparing standards in deionized water. Table 3 shows the calibration and method detection limits (MDLs) for sodium using nonsuppressed conductivity detection. The calculated MDL, based on seven replicate injections of a

Table 3. Calibration and MDL for Sodium Using Nonsuppressed Conductivity Detection with a 3-mL Sample Volume

Analyte	Range[a] (ng/L)	Linearity (r^2)	Calculated MDL[b] (ng/L)	MDL Standard (ng/L)
Sodium	100–500	0.9998	68	200

[a] Calibration levels were 100, 250, and 500 ng/L (each standard injected in duplicate)

[b] MDL = $t \times S$ 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

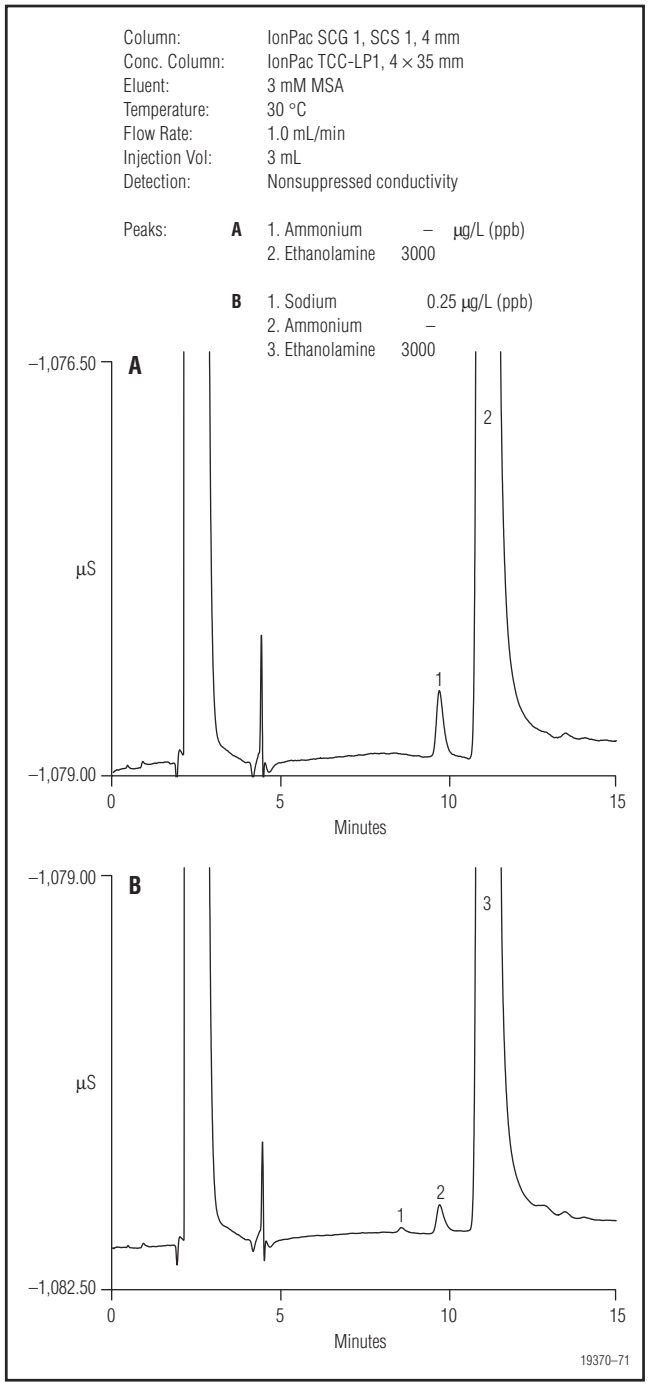

Figure 4. *A representative chromatogram of 3000 µg/L ethanolamine spiked with (A) no sodium, and (B) 0.25 µg/L sodium.*

200-ppt sodium standard in a 3-mL sample volume, was 68 ppt. The precision of the retention times, based on the same replicate injections, yielded an RSD of 0.02%. Figure 3 shows a representative chromatogram of 200 ppt sodium prepared in deionized water. In contrast, the MDL for suppressed conductivity detection using a 10-mL sample volume was ~3 ppt.[4,9] However, extrapolating the MDL from a 10-mL to 3-mL volume on the suppressed system results in a detection limit of ~10 ppt, a difference of nearly an order in magnitude compared to the nonsuppressed system.

The method performance for trace sodium analysis using nonsuppressed conductivity detection was evaluated by analyzing 3 mL of synthetic samples containing ethanolamine concentrations of 3 and 5 ppm that simulate samples encountered in the power industry. The detection of sub-ppb sodium in a matrix of 10 ppm ethanolamine cannot be performed by this method. This matrix is equivalent to concentrating 6 mL of 5 ppm ethanolamine, approximately 45% above the breakthrough volume of the TCC-LP1. Furthermore, concentrating less than 3 mL for this matrix would not allow the sodium concentration examined (i.e., 250 ppt) to be detected. As expected, only ammonium was detected in the 3-ppm ethanolamine matrix (Figure 4A). The most likely source of the ammonium is as a breakdown product of ethanolamine. Figure 4B shows the same matrix spiked with ~250 ppt sodium. The average spiked recoveries for 250 ppt sodium, based on triplicate injections, was 97.3%.

Table 4 summarizes the spiked recovery data for sodium in simulated ethanolamine-treated matrices. As shown, the method performed well for samples spiked with sub-ppb sodium in the presence of up to 5 ppm ethanolamine. In general, it is not recommended to spike these sample with <250 ppt sodium because of low signal-to-noise (S/N) ratio with the nonsuppressed system. Table 5 compares suppressed to nonsuppressed conductivity detection for trace sodium in ethanolamine-treated samples.

Table 4. Spiked Recovery Data for Sodium in the Presence of 3000 and 5000 µg/L Ethanolamine Using Nonsuppressed Conductivity Detection

Spiked Sodium Concentration (µg/L)	Ethanolamine Concentration (µg/L)	Average[a] Sodium Recovery (%)	RSD[a] (%)
0.242	3000	97.3	3.2
0.257	5000	92.7	9.7

[a] The average recovery and relative standard deviations were calculated from triplicate injections (n = 3)

Table 5. Comparison of Suppressed to Nonsuppressed Conductivity Determinations in Ethanolamine-Treated Matrices

Feature	Nonsuppressed Conductivity Detection	Suppressed Conductivity Detection[a]
Columns Compared	SCG 1, SCS 1, 4 mm TCC-LP1 as conc.	CG16, CS16, 3 mm CG16 (5 mm) as conc.
Max. Sample Volume[b] (based on sample containing 5000 ppb ethanolamine)	4.5 mL	30 mL
Sample Volume Used	3 mL[c]	10 mL[d]
Sodium Calibration Range	100–500 ppt	25–250 ppt
Sodium MDL	68 ppt	3.2 ppt
Estimated Volume Required to Achieve Single-Digit ppt MDLs for Sodium	~70 mL[e]	10 mL
ppb Sodium in High Ethanolamine Matrices	Yes	Yes
Low ppt Sodium in High Ethanolamine Matrices	No (capacity limited)	Yes

[a] Data from Dionex Application Note 152

[b] Breakthrough volume

[c] Volume represents ~70% of the breakthrough volume

[d] Volume represents ~30% of the breakthrough volume

[e] Estimated volume by extrapolating data using suppressed conductivity detection from 10 mL to 3 mL, where suppressed conductivity detection was calculated with a 7× lower MDL

As shown in Table 5, considerably more sample volume is required with nonsuppressed conductivity detection to achieve single-digit ppt sodium detection limits, because of the lower S/N obtained with this detection mode. Consequently, nonsuppressed detection cannot achieve the required sensitivity for sodium in these matrices.

In some power plants, the determination of trace concentrations of sodium in high concentrations of ammonium may be required. Unlike ethanolamine-treated samples, samples containing high concentrations of ammonium cause column overloading at much lower concentrations. For example, concentrating a 3-mL sample containing 1 ppm ammonium spiked with 250 ppt sodium causes severe broadening of the sodium peak and a recovery of ~60%. The lower capacity of the SCS 1 compared to the CS16 is a significant contributor to this result. Therefore, the concentration volume was decreased from 3 mL to 1.5 mL and the amount of sodium spiked was increased from 250 ppt to 1000 ppt. Figure 5 illustrates this separation and shows an excellent resolution between sodium and ammonium, as well as good sodium peak shape. The average spiked recoveries of sodium in this matrix was 99.9%, based on duplicate injections.

Separation of Transition Metals Using the IonPac SCS 1 Column

Nuclear and fossil-fueled power plants also require the monitoring of certain transition metals, particularly iron and copper, down to the low ppb concentrations. To achieve the best selectivity and sensitivity, the samples should be preconcentrated and optical detection after postcolumn reaction should be used.[10] However, an alternative detection mode is to use nonsuppressed conductivity detection. As with trace sodium analysis, the determination of trace transition metals is limited by the purity of the water source and the reagents. In addition, the use of PEEK metal-free flow paths is essential to maintain the integrity of the analytical system. In contrast to common cations, the separation of transition metals requires a complexing agent in the eluent to achieve the necessary selectivity.

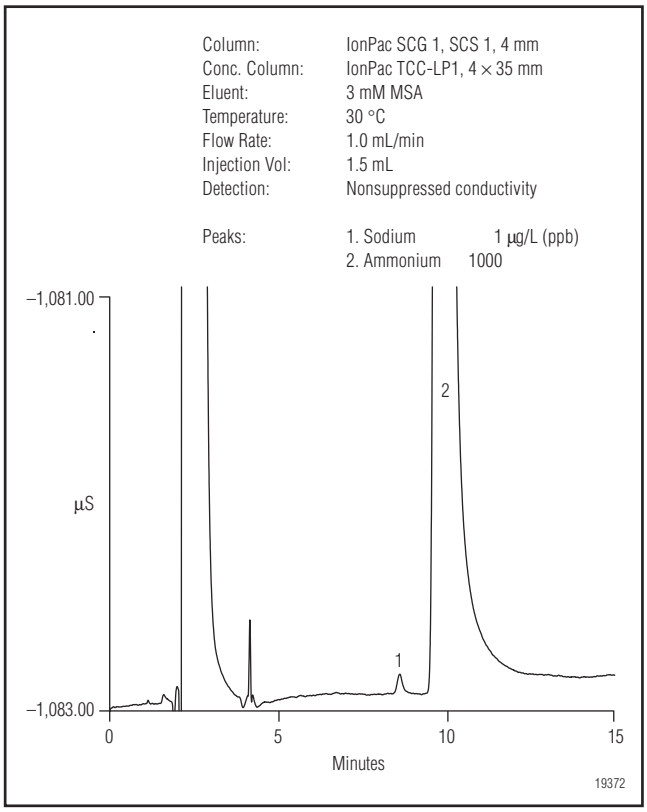

Figure 5. *Separation of trace sodium in a high concentration of ammonium.*

Table 1 lists the retention times of common cations and transition metals using various eluent components and concentrations, including MSA, oxalic acid, tartaric acid, and PDCA. Because PDCA forms very strong complexes with the metal ions, very low PDCA concentrations are required to elute the metals from the column. However, at low-PDCA concentrations, calcium and magnesium are more retained, resulting in longer run times. In addition, PDCA generally does not provide an optimum selectivity for resolving many transition metals from the common cations often present at much higher concentrations. Therefore, for most transition metal applications, PDCA is not recommended for use with a weak cation exchanger, such as the IonPac SCS 1 column. Eluents containing MSA and oxalic acid are best for separating common cations and most transition metals. The oxalic acid is primarily used to control the selectivity of the transition metals and alkaline earth metals, whereas the MSA contributes to the separation of alkaline earth metal ions and alkali metal ions.

Figure 6 shows a chromatogram of a 3-mL simulated feed water sample containing 7 ppm ethanolamine spiked with sub-ppm levels of common cations, diethanolamine, zinc, cobalt, and manganese. As this figure illustrates, most cations, including the transition metals, were well separated with the exception of magnesium and manganese.

Cation exchange with nonsuppressed conductivity detection can separate many transition metals at nominal concentrations, including copper, nickel, zinc, cobalt, manganese, and cadmium. Note that the separation of iron was not included in this study. However, high concentrations of other common cations can interfere with the separation of some transition metals. Figure 7 shows a separation of the six transition metals and common cations in a single analysis at ppm concentrations using a 25-µL injection.

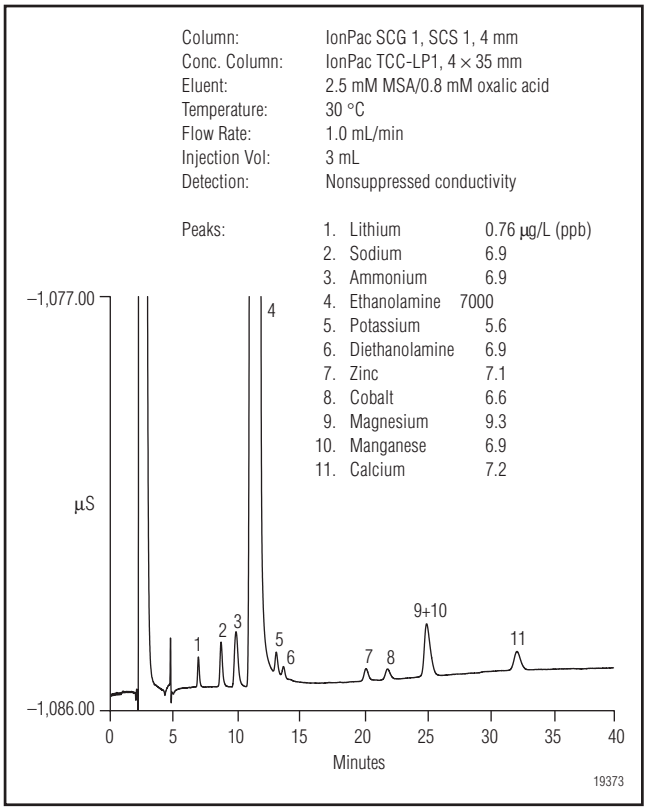

Figure 6. *Chromatogram of a simulated feed water sample.*

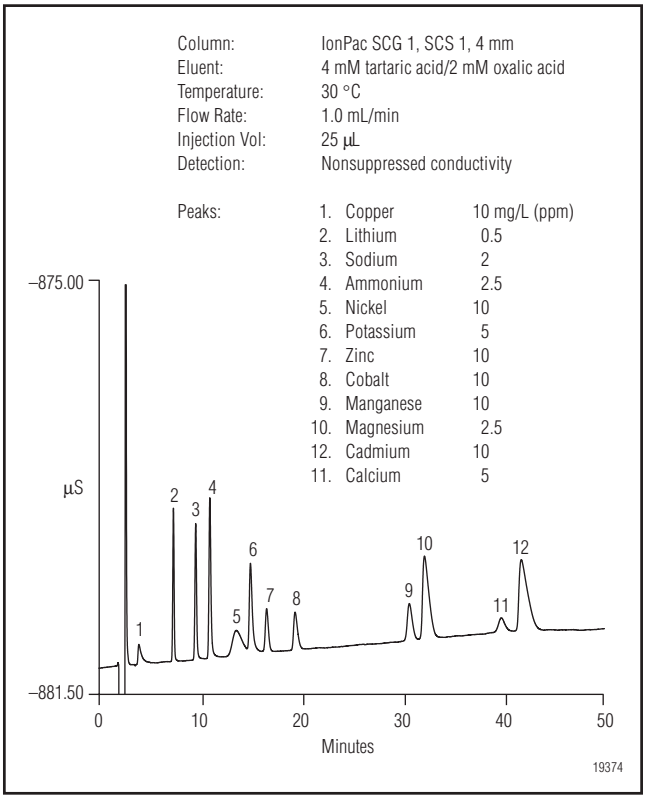

Figure 7. *Separation of common inorganic cations and transition metals.*

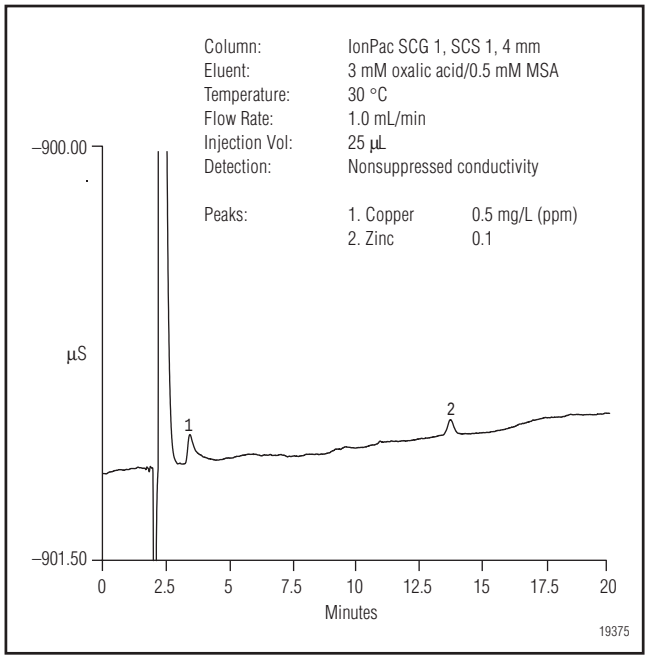

Figure 8. *Determination of low concentrations of copper and zinc on the IonPac SCS 1 column.*

Sensitivity of Copper and Zinc with a Standard-Loop Injection Using Nonsuppressed Conductivity Detection

Copper and zinc often require continuous monitoring in the power industry. For trace-level analysis of these transition metals, samples should be preconcentrated rather than injected directly. However, in this application note, it was only of interest to determine the linearity and MDLs of these metals using a standard-sized 25-µL loop injection. Table 6 shows the calibration linearity and MDLs for copper and zinc. The MDLs were determined by performing seven replicate injections of 125 ppb copper and 25 ppb zinc. These injections resulted in calculated MDLs of 33 and 6.7 ppb for copper and zinc, respectively. The retention time precisions, based on the replicate injections, were 0.85% and 0.13%, respectively. Figure 8 shows a separation of copper and zinc at sub-ppm concentrations.

Table 6. Calibration and MDL for Copper and Zinc Using Nonsuppressed Conductivity Detection

Analyte	Range[a] (mg/L)	Linearity (r^2)	Calculated MDL[b] (µg/L)	MDL Standard (µg/L)
Copper	0.125–1.00	0.9998	33	125
Zinc	0.025–0.50	0.9999	6.7	25

[a] 25-µL injection volume

[b] MDL = $t \times S$ 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

CONCLUSION

This application note demonstrates the capability of nonsuppressed conductivity detection for the determination of trace sodium in ethanolamine-treated samples and the determination of some transition metals. This method yielded good recoveries for sodium spiked at the sub-ppb concentrations in ethanolamine samples containing up to 5 ppm. However, higher ethanolamine concentrations will overload the concentrator and analytical columns, causing decreased peak response and increased peak broadening for sodium. In effect, the low breakthrough volume of the TCC-LP1 in combination with the IonPac SCS 1 column with nonsuppressed conductivity detection reduces the sensitivity that can be achieved by this method. This characteristic makes cation exchange with suppressed conductivity detection a more favorable approach because it does not suffer from these limitations. Suppressed conductivity allows the use of higher-capacity columns, such as the IonPac CS16, which tolerates high-ionic-strength matrices and higher samples volumes such as those required in the power industry. In addition, the improved sensitivity achieved by suppressed conductivity detection requires significantly lower samples volumes to obtain single-digit ppt detection limits of sodium compared to nonsuppressed conductivity detection. However, the IonPac SCS 1 nonsuppressed column offers good selectivity for many transition metals of interest that cannot be accomplished by columns used with suppressed conductivity.

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10. Dionex Corporation. Application Note 131; Sunnyvale, CA.

SUPPLIERS

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

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

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

Determination of Transition Metals at PPT Levels in High-Purity Water and SC2 (D-clean) Baths

INTRODUCTION

Metal atoms and conductive particle contaminants are undesirable and potentially damaging in semiconductor manufacturing processes. Oxidative cleaning baths and large volumes of ultrapure rinse water are used to remove metallic contamination from wafer surfaces.[1] For optimal cleaning efficiency, the concentrations of iron and other metals in cleaning solutions should be minimized.[2] To monitor metals in cleaning baths and rinse water, improved analytical methods are needed.

This Application Note describes a method for determining low ng/L amounts of transition metals in high purity water and semiconductor bath solutions. The IonPac® CS5A column is used for the separation of transition metals. This column has a unique bilayer latex structure consisting of both anion- and cation-exchange retention mechanisms. Transition metals can be separated using either anion or cation exchange chromatography, depending on the choice of the complexing agent used in the eluent. Pyridine-2,6-dicarboxylic acid (PDCA) is a very strong complexing agent that forms stable, anionic metal complexes. This results in very efficient chromatography. Another commonly used complexing agent is oxalic acid, a moderate chelating agent. Because the complexation is weaker, metals are separated either as free metal cations (Pb, Mn, Cd), as anionic complexes (Cu), or as a combination of the two (Co, Zn, Ni). Transition metals are detected using postcolumn derivitization with 4-(2-pyridilazo)resorcinol (PAR) with absorbance detection at 520–530 nm. The PAR displaces the PDCA and forms highly absorbing metal complexes. PAR exhibits broad selectivity for transition metals and provides a very sensitive detection method with low background.

EQUIPMENT

Dionex DX-500 Ion Chromatography system consisting of:

- GP40 Gradient Pump (microbore configuration)
- AD20 UV/Vis detector with 10 mm path length cell
- LC30 Chromatography Enclosure with rear-loading Rheodyne injection valve
- Concentrator Pump, DQP (P/N 35250)
- RP-1 Postcolumn reagent pump (P/N 041950) with pulse damper (2' of 0.020" i.d. tubing after the RP-1)
- Postcolumn reagent bottle (P/N 044411 with its O-ring replaced by a teflon encapsulated O-ring (P/N 043523))
- Knitted Reaction Coil (P/N 053640)
- Pressurizable Reservoir Chamber (P/N 37053)
- PeakNet Chromatography Workstation

REAGENTS AND STANDARDS

Deionized water (DI H_2O), Type 1 reagent grade, 18 MΩ-cm resistance or better

PDCA Eluent: 7.0 mM Pyridine-2,6-dicarboxylic acid (PDCA), 66 mM KOH, 5.6 mM K_2SO_4, 74 mM Formic acid (MetPac™ PDCA Eluent Concentrate P/N 046088).

Postcolumn reagent: 0.06 g of PAR (P/N 039672) in 1-L MetPac PAR postcolumn reagent diluent (P/N 046094). The formulation of the diluent is 1.0 M 2-Dimethylaminoethanol + 0.50 M Ammonium hydroxide + 0.30 M Sodium bicarbonate.

Hydrochloric acid, ultrapure reagent. ULTREX® II (J.T. Baker or equivalent)

Hydrogen peroxide, semiconductor grade (Aldrich, 99.999% solution in water)

1 g/L individual transition metal standards (VWR, atomic absorption grade)

CONDITIONS

Columns:	IonPac CS5A Analytical, 2 x 250 mm (P/N 052576) IonPac CG5A Guard, 2 x 50 mm (P/N 052836) TCC-2 Concentrator, 3 x 35 mm (P/N 43103)
Eluent:	PDCA
Eluent Flow Rate:	0.3 mL/min
LC-30 Temperature:	30.0 °C
Postcolumn Reagent:	0.06 g of PAR in 1 L MetPac PAR Postcolumn Diluent
Postcolumn Flow Rate:	0.15 mL/min
Concentrator Pump Flow Rate:	2.0 mL/min
Run Time:	15 min
Detection:	Visible, High setting, 530 nm
System Backpressure:	1700–2000 psi

PREPARATION OF SOLUTION AND REAGENTS

Transition Metals Standards

Appropriate concentrations of standards are prepared from 1g/L stock standards solutions. All standards were prepared in 2 mM HCl to ensure their stability and prevent the formation of insoluble oxides and hydroxides.

Eluent solution

PDCA Eluent

Add 200 mL of MetPac PDCA Eluent Concentrate to 800.0 mL of degassed water for a total volume of 1000.0 mL or 204.0 g of MetPac PDCA Eluent Concentrate to 800.0 g of degassed water for a total weight of 1004.0 g.

Postcolumn reagent

Dissolve 0.06 g of PAR in 1.0 L MetPac PAR postcolumn diluent.

Stock solution for sample and pH adjustment

1 M Hydrochloric acid

Weigh 909.70 g of deionized water (Type I reagent grade, 18 MΩ-cm resistance or better) into an eluent bottle. Tare the bottle and carefully add 90.3 mL of ultrapure reagent grade hydrochloric acid directly to the bottle.

Standard and sample preparation

Add 1.0 mL of 1 M hydrochloric acid to 499 g of sample or standard solution. The final concentration of hydrochloric acid is 2 mM.

Glassware cleanings

Prior to use, high density polyethylene (HDPE) containers used for samples and standards preparation were rinsed with DI water and an aliquot of the sample to reduce the amount of leachable transition metals from the bottle. To avoid contamination and pH errors when formulating the eluent and the PAR reagent, use the high purity reagents offered by Dionex.

SYSTEM OPERATION

System configuration and operation parameters for this application are outlined in a previously published document.[3]

To ensure efficient 2-mm column operation, 0.125-mm (0.005 in.) tubing must be used. Lengths of connecting tubing should be kept as short as possible to minimize system void volume. Carefully use a razor blade or plastic tubing cutter so that the ends of the tubing cuts are straight and smooth. Irregularity on the surface of a tubing end can result in unwanted dead volume.

Sample preconcentration is used to improve sensitivity and lower the detection limits. Samples with transition metal concentrations below 2 µg/L must be preconcentrated for accurate quantification. The sample is loaded onto the TCC-2 (Trace Cation Concentrator) with a pressurized reservoir or DQP concentrator pump. TCC-2 column stationary phase is surface-functionalized sulfonated resin. We used a flow rate of 2 mL/min and times of 5 and 15 minutes to concentrate 10 and 30 mL of sample. An RP-1 pump was used to deliver postcolumn reagent (PAR). Pneumatic delivery is also acceptable and either of these techniques can be used successfully in this method. Figure 1 shows the system configuration.

RESULTS AND DISCUSSION

Trace level analysis of transition metals is limited by the purity of water and the reagents. PEEK, metal-free flow paths are a very important factor in the integrity of the analytical system. Precautions must be taken at every step of sample and standard preparation to minimize contamination. All plastic containers and pipettes must be cleaned with highest purity reagents (soak in 10 mM HCl overnight and rinse thoroughly with water). Information about the content of leachable transition metals in these containers should be obtained from the supplier. The analytical system flow path, including tubing, pumps, postcolumn reagent, and sample must be thoroughly cleaned with 50% IPA/H_2O at start-up.

To perform analysis of trace levels less than 2 µg/L, samples must be preconcentrated rather than directly injected. Figure 2 shows the analysis of 30 mL of a 1 µg/L transition metals standard. All peaks are well separated from the void volume and from each other and are therefore easily quantified. Figures 3 and 4 show the analyses of 10 and 30 mL of high quality deionized water. These samples were concentrated at 2 mL/min for 5 and 15 min respectively. Iron, copper, and zinc are major contaminants. Trace analysis of real samples containing these analytes will depend on the levels of transition metals present in the water blank. The iron concentration in 30 mL of water is estimated to be 45 ng/L (ppt) based on the iron area count in the standard (30 mL of 1 µg/L (ppb) of each transition metal). The concentration of Fe^{3+} in 10 mL of water was also approximately 45 ng/L (ppt). Therefore, the concentration of the Fe^{3+} is due to the Fe^{3+} in the water and reagents and not the chromatography system. Because it is possible to quantify the amount of Fe in the 10 mL sample, if that amount was found in a 30-mL sample (less contaminated water), the minimum detection limit would be 15 ppt. Table 1 shows the result of the analysis of 30 mL of high quality deionized water on three consecutive days. The peak area and retention time RSDs were less than 2%.

Figure 5 shows the chromatogram of an SC2 bath. Concentrations of Fe^{+3}, Cu^{+2}, and Zn^{+2} were 80, 75, and 106 ng/L (ppt) respectively. Recovery of iron from the bath is higher than from water, indicating that chemicals used in bath preparation contain iron.

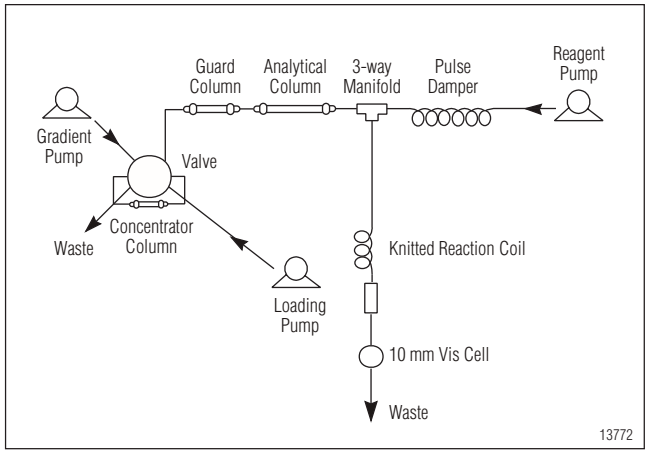

Figure 1 *System configuration for detection of transition metals*

SUMMARY

The method outlined in this Application Note describes the chromatographic analysis of trace levels of transition metals using a preconcentration technique.

REFERENCES

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3. Dionex 2-mm Transition Metal System with Postcolumn Delivery Installation and Troubleshooting Manual, P/N 031355

Table 1. Analysis of iron in 30 mL DI H_2O

Day #	Concentration ng/L (ppt)	Area count	Retention time
1	38.1	71123	6.13
2	37.4	69786	6.17
3	38.7	72075	6.10
RSD	1.71	1.62	0.57

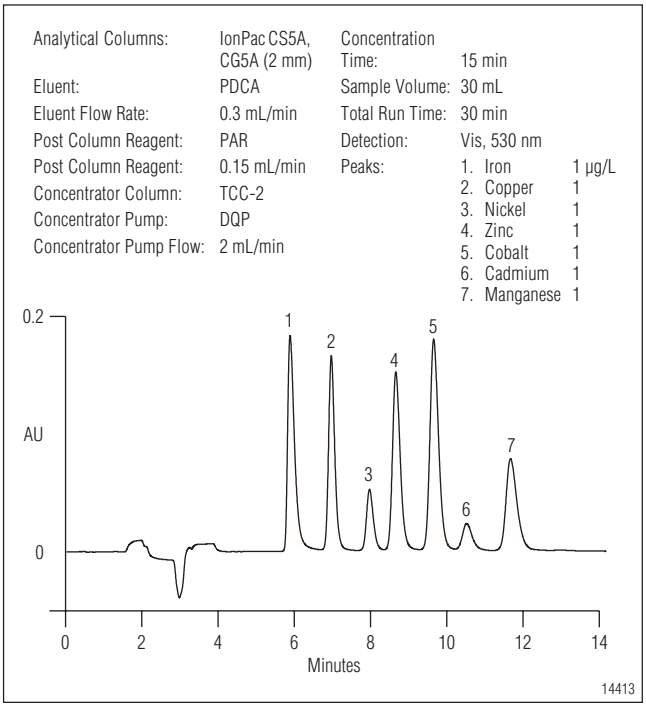

Figure 2 *1 µg/L (ppb) transition metals standard (30 mL concentrated)*

Analytical Columns:	IonPac CS5A, CG5A (2 mm)	Sample Volume:	10 mL
Eluent:	PDCA	Total Run Time:	20 min
Eluent Flow Rate:	0.3 mL/min	Detection:	VIS, 530 nm
Post Column Reagent:	PAR	Peaks:	1. Iron
Post Column Reagent:	0.15 mL/min		2. Copper
Concentrator Column:	TCC-2		3. Nickel
Concentrator Pump:	DQP		4. Zinc
Concentrator Pump Flow:	2 mL/min		
Concentration Time:	5 min		

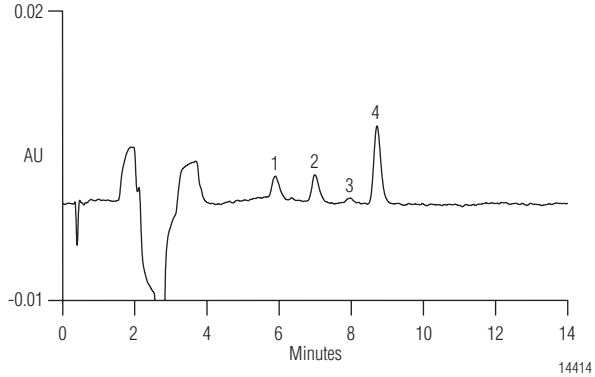

Figure 3 *Water blank (10 mL concentrated)*

Analytical Columns:	IonPac CS5A, CG5A (2 mm)	Sample Volume:	30 mL
Eluent:	PDCA	Total Run Time:	30 min
Eluent Flow Rate:	0.3 mL/min	Detection:	VIS, 530 nm
Post Column Reagent:	PAR	Peaks:	1. Iron
Post Column Reagent:	0.15 mL/min		2. Copper
Concentrator Column:	TCC-2		3. Nickel
Concentrator Pump:	DQP		4. Zinc
Concentrator Pump Flow:	2 mL/min		
Concentration Time:	15 min		

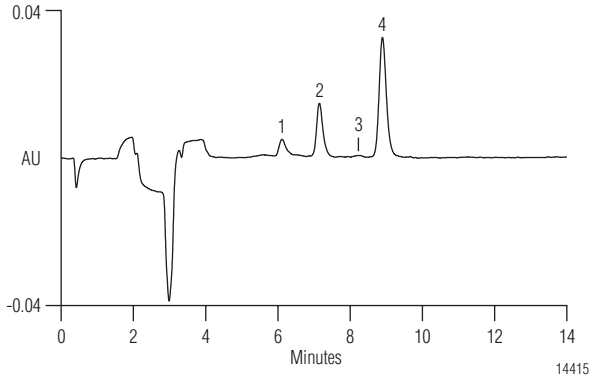

Figure 4 *Water blank (30 mL concentrated)*

Analytical Columns:	IonPac CS5A, CG5A (2 mm)	Concentration Time:	15 min	
Eluent:	PDCA	Sample Volume:	30 mL	
Eluent Flow Rate:	0.3 mL/min	Total Run Time:	30 min	
Post Column Reagent:	PAR	Detection:	VIS, 530 nm	
Post Column Reagent:	0.15 mL/min	Peaks:	1. Iron	80 ng/L
Concentrator Column:	TCC-2		2. Copper	75
Concentrator Pump:	DQP		3. Nickel	-
Concentrator Pump Flow:	2 mL/min		4. Zinc	106

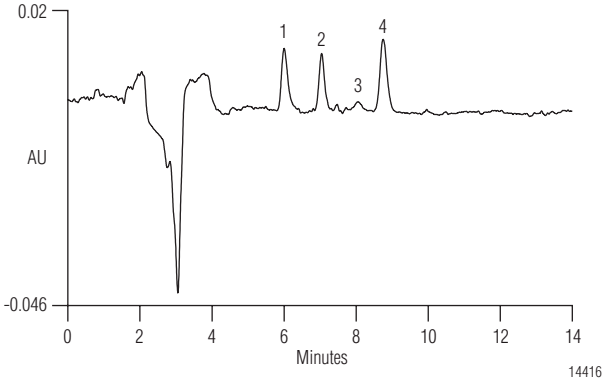

Figure 5 *SC2 batch (30 mL concentrated) containing 1 mL HCl / 5 mL H_2O_2 / 494 mL H_2O*

LIST OF SUPPLIERS

J.T. Baker Incorporated, 222 Red School Lane, Phillipsburg, NJ 08865, USA. Tel: 1-800-582-2537

VWR Scientific, P.O. Box 7900, San Francisco, CA 94120, USA. Tel: 1-800-932-5000

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

Determination of Transition Metals in Serum and Whole Blood by Ion Chromatography

INTRODUCTION

The determination of transition metals in physiological fluids is of considerable interest in clinical chemistry. In recent years several studies have linked the concentrations of specific transition metals to various diseases. Low serum copper level is used as a marker for Wilson's disease. Serum copper levels are elevated in a large number of chronic and acute illnesses such as Hodgkin's disease, leukemia, and many other malignancies.[1] Zinc is an important nutritive factor as well as a cofactor for many metalloenzymes. Zinc is necessary for the growth and division of cells, especially during the stages of life when growth rates are high. Zinc deficiency is associated with syndromes that cause short stature and dwarfism.[2] There is also interest in the biochemical relationship of copper and zinc.[3] Studies have linked an increase in plasma copper level with decreasing plasma zinc concentration in childhood lymphatic leukemia. Determination of iron in whole blood is used to monitor anemia.

Traditionally, atomic absorption spectrophotometric (AAS) techniques have been used by most clinical chemistry laboratories to determine transition metals in physio-logical fluids. These techniques have their limitations. Flame AAS has limited sensitivity for copper, and graphite atomic absorption spectrophotometry is susceptible to solute vaporization interferences such as depression of element signal, especially in physiological samples.[4–6] The protein content of the physiological fluid samples can cause absorption abnormalities. High sodium chloride content can hamper sensitivity, linearity, and cause burner clogging.

This application note describes an attractive alternative to traditional spectroscopic methods by using the principles of ion exchange. As a sample moves through the ion exchange column, bands of transition metals migrate through at differential rates determined largely by the relative affinities of the different metal-ligand complexes for the stationary ion exchange sites. A strong metal complex-ing colorimetric reagent is supplied pneumatically and mixed with the column effluent. The bands of transition metals are then determined at a visible wavelength using an absorbance detector.[7] Separation between individual metals can be enhanced or altered simply by changing eluents. Figure 1 illustrates the selectivity differences observed on an IonPac® CS5A column when using A) a pyridine-2,6-dicarboxylic acid eluent, and B) an oxalic acid eluent. This method is precise, sensitive, and requires minimum sample preparation.

RECOMMENDED EQUIPMENT

Dionex DX-500 system consisting of:
- GP40 Gradient Pump
- AD20 Absorbance Detector
- LC20 Chromatography Module

PC10 Postcolumn Pneumatic Controller

PC10 Automation Kit (optional)

PeakNet® Chromatography Workstation

REAGENTS AND STANDARDS

Reagents

Deionized water, 17.8 MΩ-cm resistance or higher

MetPac™ PDCA Eluent Concentrate and/or MetPac Oxalic Acid Eluent Concentrate

MetPac Postcolumn Diluent

4-(2-Pyridylazo)resorcinol, monosodium, monohydrate (P/N 039672)

Nitric acid, trace-metal grade (Fisher Scientific)

Sulfuric acid (Fisher Scientific)

Hydrogen peroxide, 30% (Fisher Scientific)

Trichloroacetic acid (Fluka Chemika-BioChemika)

Standards

Transition metal standards of 1000 mg/L are available from chemical supply companies (e.g. Aldrich, Sigma) for use with atomic absorption spectrometry. These are always dissolved in dilute acid solutions and can be used as IC standards.

CONDITIONS

Either of two analytical systems may be used for the chromatographic determination of transition metals. The most current method employs the IonPac CS5A, a highly efficient, solvent-compatible, mixed anion/cation exchange column. An older column, the IonPac CS5, may be used for these analyses, but efficiencies are superior and cadmium is better resolved on the CS5A. Refer to Table 1 for a complete listing of experimental conditions for both columns.

PREPARATION OF SOLUTIONS AND REAGENTS

Two eluent systems can be used for transition metal separations with the IonPac CS5A or CS5 column. The PDCA eluent is used for iron, copper, nickel, zinc, cobalt, cadmium, and manganese. The oxalic acid eluent is used for lead, copper, cobalt, zinc, and nickel. Cadmium and manganese coelute using the oxalic acid eluent.

MetPac PDCA Eluent

Dilute 200 mL of the MetPac PDCA Eluent Concentrate to 1.0 L with deionized water.

MetPac Oxalic Acid Eluent

Dilute 100 mL of the MetPac Oxalic Acid Eluent Concentrate to 1.0 L with deionized water.

Table 1 Conditions for Two Analytical Systems Used for the Chromatographic Determination of Transition Metals

Columns	IonPac CS5A analytical and IonPac CG5A guard	IonPac CS5 analytical and IonPac CG5 guard
Eluents	A) MetPac PDCA Eluent (Alternatively, 8.0 mM PDCA, 66 mM potassium hydroxide, 74 mM formic acid, and 5.6 mM potassium sulfate may be used.) B) MetPac Oxalic Acid Eluent (Alternatively, 8 mM oxalic acid, 50 mM potassium hydroxide, and 100 mM tetramethylammonium hydroxide may be used.)	A) 6.0 mM PDCA 40 mM Sodium hydroxide 90 mM Acetic acid B) 50 mM Oxalic acid 95 mM Lithium hydroxide
Flow Rate	1.2 mL/min	1.0 mL/min
Detection	Absorbance, 530 nm	Absorbance, 520 nm
Postcolumn Reagent	0.5 mM PAR, dissolved in MetPac Postcolumn Diluent. (Alternatively, 1.0 M 2-dimethylaminoethanol, 0.50 M ammonium hydroxide, and 0.30 M sodium bicarbonate may be used.)	0.4 mM PAR 1.0 M 2-dimethylaminoethanol 0.50 M Ammonium hydroxide 0.30 M Sodium bicarbonate
Postcolumn Reagent Flow Rate	0.7 mL/min	0.5 mL/min

PAR [4-(2-Pyridylazo)resorcinol] Postcolumn Reagent

Prepare the postcolumn reagent directly in the 1-L plastic reagent reservoir container. Add 0.15 g of 4-(2-pyridylazo)resorcinol, monosodium, monohydrate, to 1.0 L of the MetPac Postcolumn Diluent and ultrasonicate for five minutes. Add a stir bar and stir for several minutes to ensure that the PAR has completely dissolved. The color of the final solution should be yellow to yellow-orange. Place the reagent container in the reagent reservoir.

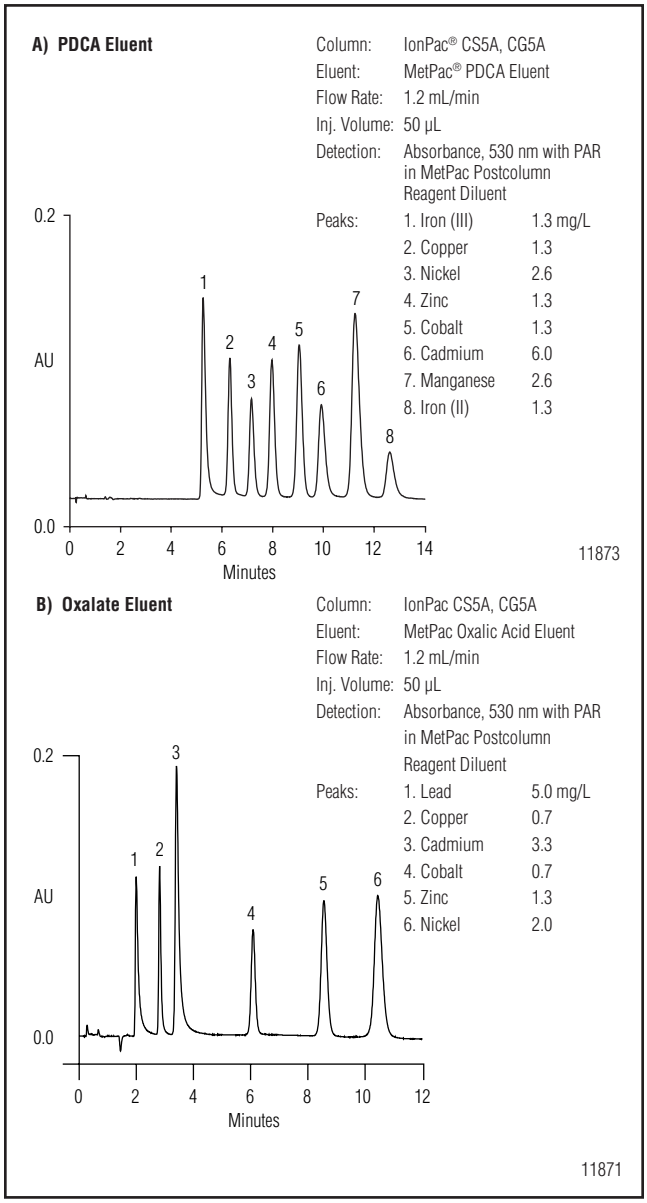

Figure 1. Separation of transition metals using A) PDCA Eluent and B) Oxalate Eluent (IonPac CS5A).

SAMPLE PREPARATION

Biological matrices contain higher concentrations of alkali and alkaline earth metals than transition metals. In such instances, a chelation concentration step can be used where the alkali and alkaline earth metals are removed from the matrix and the transition metals are selectively concentrated. For the determination of trace metals in physiological fluids or tissues, the sample must first be acid-digested to a single phase. In this application, the serum and whole blood samples were digested using the following procedure: to a 250-mL evaporation dish add 10–100 mL of sample. Next, add 5 mL of concentrated HNO_3 and 2 mL of 30% H_2O_2. Evaporate on a hot plate at medium heat to a volume of 15 to 20 mL. Cover with a watch glass to avoid sample loss by spattering. Transfer the concentrate and any precipitate to a 125 mL conical flask using 5 mL of concentrated HNO_3. Add 10 mL of concentrated H_2SO_4 and a few boiling chips or glass beads. Evaporate on a hot plate in a hood until dense white fumes of SO_3 appear. If the solution does not clear, add 10 mL of concentrated HNO_3 and repeat evaporation. Remove all HNO_3 before continuing treatment. (All HNO_3 is removed when the solution is clear and no brownish fumes are evident.[8]) Cool and dilute to about 50 mL with eluent.

Alternatively, samples can be digested using nitric acid. Add 40 g of concentrated nitric acid to approximately 75 g of sample. Add a 10-mL aliquot of the digested sample to 20 mL of 2 M ammonium acetate. The final pH of the sample should be 5.5.

A trichloroacetic acid deproteinization procedure is sometimes used for serum and plasma samples. Add 0.2 mL of 50% trichloroacetic acid to 0.4 mL of serum or plasma sample. Centrifuge the mixture for 5 minutes at 1500 x g. Inject an appropriate volume of the supernatant (e.g. 25–50 µL).

DISCUSSION AND RESULTS

The method outlined in this application note permits rapid separation of various transition metals. The separations are based on one of two different eluent systems. The first is a pyridine-2,6-dicarboxylic acid (PDCA) eluent, which is a strong complexing agent that separates the metal ion complexes by anion exchange.

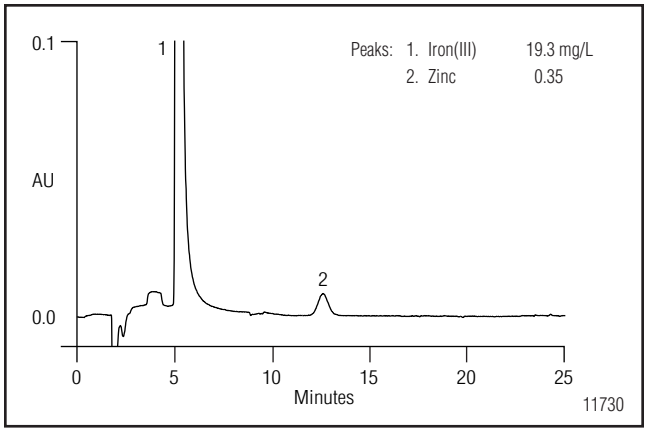

Figure 2. Determination of iron(III) and zinc in whole blood (IonPac CS5, PDCA eluent).

PDCA is best suited for iron(II) and iron(III), copper, nickel, zinc, cobalt, cadmium, and manganese (see Figure 2). This method allows one to speciate the oxidation states of iron, Fe(II) and Fe(III). However, since ferrous ion is easily oxidized to ferric, oxygen must be removed from the eluent by degassing. Oxygen should

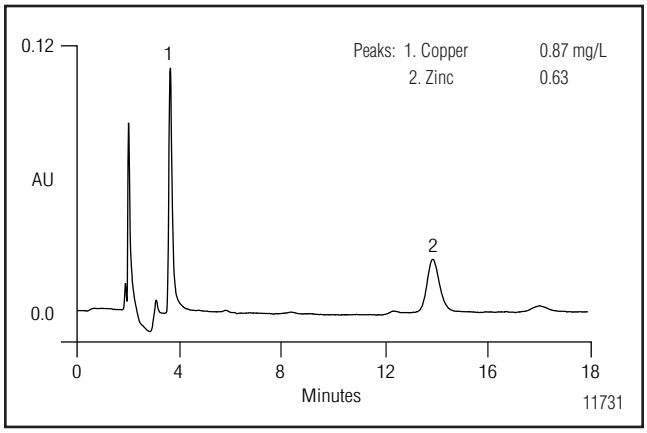

Figure 3. Determination of copper and zinc in serum (IonPac CS5, Oxalic Acid Eluent).

also be purged from the analytical column by pumping 0.1 M sodium sulfite (12.6 g/L Na_2SO_3) through the column for 2 hours.

Table 2 Precision and Recovery Data for Transition Metals in Whole Blood[a]

Analyte	Amount Found (mg/L)	Amount Spiked (mg/L)	Mean Recovery (%)	RSD (%)
Iron(III)[b]	420	—	—	2.7
Zinc	1.2	3.0	95	1.3

[a]n = 7 replicates, 50 µL injected.
[b]Iron(III) was not spiked.

Table 3 Precision and Accuracy Data for Transition Metals in Serum[a]

Analyte	Amount Found (mg/L)	Amount Spiked (mg/L)	Mean Recovery (%)	RSD (%)	MDL[b] µg/L
Copper	0.57	1.0	98	3.8	45
Zinc	0.85	2.0	95	2.4	70

[a]n = 7 replicates, 50 µL injected.
[b]MDL = SD x $(t_s)_{99\%}$.

An alternative eluent system uses an oxalic acid-based eluent, which is a moderate strength complexing agent that separates the metals by a mixed mode mechanism. The oxalate eluent separates lead, copper, cobalt, zinc, and nickel (see Figure 3). Cadmium and manganese coelute with this eluent.

The separated metals from the analytical column enter a postcolumn reaction system where they are derivatized with 4-(2-pyridylazo)resorcinol and then detected at 520–530 nm using a UV/visible absorbance detector. This method is ideal for complex matrices such as physiological fluids. This method is highly sensitive, specific, and precise. Analyte recoveries for various physiological fluid matrices are listed in Tables 2 and 3.

PRECAUTIONS

The analytical flow path must have no metal components. This includes tubing end fittings, stainless steel washers, omni-fittings, etc., as well as columns and valves that contain stainless steel. Use caution in preparing and transferring reagents to minimize contamination. The prepared PAR is easily oxidized. If at anytime the PAR takes on a red color, it has been contaminated and should be discarded. Prepared reagents should be stored under an inert gas, such as nitrogen or helium, and used within two weeks of preparation. Be sure that the eluent is being pumped through the columns when the postcolumn pneumatic controller is turned on. Failure to do so may cause the PAR reagent to back up through the analytical column.

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SUPPLIERS

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Fisher Scientific, 711 Forbes Avenue
Pittsburgh, Pennsylvania 15219-4785, USA
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Fluka Chemika-BioChemika, Fluka Chemie AG
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Tel.: (800) 325-3010

Elimination of Iron and Aluminum as Matrix Interferences for Determination of Transition Metals Using Chelation Ion Chromatography

INTRODUCTION

The naturally occurring high concentrations of certain metals, such as iron, aluminum, alkali metals and alkaline earth elements, usually interfere with the determination of the trace transition metals. For atomic spectroscopy techniques, several common methods are used in order to minimize the sample matrix interferences. These include spectral background corrections, standard additions, sample dilution, and matrix matching. For chromatographic separation with postcolumn derivitization, the high concentrations of iron and aluminum not only interfere with the separation, but also the detection of other elements of interest. Sample dilution and standard addition are commonly used to reduce matrix effects. These methods help to minimize matrix interferences; however, these methods often result in degraded detection limits and accuracy.

The chelation concentration technique offers the analyst a solution to the detection limit and interference problems commonly experienced when analyzing complex matrices. Selective ion exchange materials such as chelating resins can concentrate transition metals while eliminating alkali and alkaline earth elements. This selective elimination process has been extended to the elements iron and aluminum. By using a matrix selective complexing agent, 95% to 99% of the iron and aluminum are removed, while the other transition metals are quantitatively retained.

In this application note, the determination of trace transition metals in samples that contain high levels of iron and aluminum will be discussed.

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INSTRUMENT REQUIREMENTS

The chelation IC system is comprised of the components listed below. For complete details, please refer to the system configuration for Chelation IC System 2 in Dionex Technical Note 25.

Gradient Pump Module (GPM-2, P/N 37098) or Advanced Gradient Pump (AGP, P/N 43116)

Sample Concentration Module (SCM, P/N 42134)

Reagent Delivery Module (RDM, P/N 37030)

Variable Wavelength Detector Module (VDM-2, P/N 39646, internal or remote cell)

Eluent Degas Module (EDM-2, P/N 39550)

Eluent Container Set, Glass (P/N 38752)

Valve, 4-Way Slider Double Stack, 2000 psi/13.7 MPa (P/N 35914), three (3) required

IonPac® Membrane Reactor (P/N 35354, optional)

Knitted Reaction Coil (P/N 39349)
MetPac™ CC-1 Column (P/N 42156)
TMC-1 Column (P/N 42155)
IonPac CG2 (2, P/N 35370)
IonPac CG5 (P/N 37029)
AI-450 or other data acquisition system

SOLUTIONS AND REAGENTS

Ultrapure 2.0 M ammonium acetate, pH 5.5
(1 L, P/N 33440; 6 L, P/N 33441)
Ultrapure 2.0 M nitric acid (1 L, P/N 33442; 6 L, P/N 33443)
Ultrapure 0.1 M ammonium nitrate (1L, P/N 33445)
20 mM Pyrophosphoric acid / 2.0 M ammonium acetate
20% Ultrapure ammonium hydroxide
Ultrapure glacial acetic acid
Chelex-100™, 50–100 mesh (Bio-Rad Laboratories)

The first three reagents used for chelation concentration are available from Dionex in a ready-to-use form. If you wish to prepare your own reagent solutions, please refer to "Preparation of Solutions and Reagents". The other ultrapure reagents are manufactured by Seastar Chemical and Ultrex Reagents. Seastar reagents are available internationally through Fisher Scientific; in North America, Fisher Scientific sells these reagents under the OPTIMA® label. Ultrex reagents are available internationally through J.T. Baker. Chelex-100 is used for eluent purification.

CONDITIONS

Chelation Concentration

Columns:	MetPac CC-1, TMC-1
Eluent 1:	20mM Pyrophosphoric acid/ 2.0M ammonium acetate, pH 5.5
Eluent 2:	2.0 M ammonium acetate, pH 5.5±0.1
Eluent 3:	1.0 M Nitric acid
Eluent 4:	0.1 M Ammonium nitrate, pH 3.4±0.3

Analytical Chromatography

Column:	IonPac CS5
Eluent:	0.0060 M Pyridine-2,6-dicarboxylic acid, 0.040 M sodium hydroxide, 0.090 M acetic acid
Flow Rate:	1.0 mL/min
Postcolumn Derivitization Reagent:	0.5 mM 4-(2-Pyridylazo)resorcinol 1.0 M 2-Dimethylaminoethanol 0.5 M Ammonium Hydroxide 0.3 M Sodium Bicarbonate
Reagent Addition:	Membrane reactor or mixing tee
Reagent Flow Rate:	0.5 mL/min
Reactor:	Packed or knitted reaction coil
Detector:	VDM-2 or UDM, 520 or 530 nm

PREPARATION OF SOLUTIONS AND REAGENTS

Three concentrated reagents are required for eluents in chelation concentration: nitric acid, acetic acid, and ammonium hydroxide. For ultratrace level determinations (sub-ppb), the reagents must be ultrapure grade. For determinations above 5 ppb, high quality trace-metal grade reagents can be used. Any metal impurity in these reagents will be concentrated with your sample, constituting a system blank.

Preparation of Chelex-100 for Eluent Purification

Suspend approximately 30 g of Chelex-100 (50-100 mesh) resin in 300 mL of 2.0 M nitric acid (trace-metal grade) in a 1-L polyethylene bottle. Using a stir bar, stir the solution for approximately 10 min. Decant the nitric acid and the fine resin particles. Repeat the acid cleaning step twice before rinsing the resin with 500 mL of deionized water. Store the cleaned resin in 200 mL of 2.0 M ammonium acetate, pH 5.5, until use.

20 mM Pyrophosphoric Acid / 2.0 M Ammonium Acetate, pH 5.5 (Eluent 1)

Dissolve 3.7 g of pyrophosphoric acid (97%, Aldrich Chemical Co., Inc.) in 1 L of 2.0 M ammonium acetate, pH 5.5. Since pyrophosphoric acid is not available in ultrapure grade reagent, the trace transition metal contaminants in this solution can be removed by using Chelex-100 resin. Place the cleaned Chelex-100 resin into the pyrophosphoric acid / ammonium acetate solution and stir using a stir bar. After stirring the solution for 1 hr, decant the pyrophosphoric acid / ammonium acetate solution into the glass eluent container.

20 mM Pyrophosphoric Acid / 2.0 M Ammonium Acetate, pH 8.5 (Eluent 1 for Manganese Determination)

Dissolve 3.7 g of pyrophosphoric acid in 200 mL of 2.0 M ammonium acetate, pH 5.5. Add 500 mL of ammonium acetate to this solution. Then, add 60 g (65 mL) of 20% ultrapure ammonium hydroxide. Calibrate a pH meter to pH 7. Pour about 10 mL of the buffer into a small container (e.g., scintillation vial, 10-mL disposable beaker), and measure the pH. If the pH

is below 8.5, add about 5 mL of ammonium hydroxide to the buffer solution. If the pH is above 8.5, add 5 g of acetic acid. Adjust the pH of the ammonium acetate to 8.5 ± 0.1 using ammonium hydroxide. Once the pH is 8.5 ± 0.1, bring to a volume of 1 L. Place the cleaned Chelex-100 resin into the pyrophosphoric acid / ammonium acetate solution and stir using a stir bar. After stirring the solution for 1 hr, decant the pyrophosphoric acid / ammonium acetate solution into the glass eluent container.

2.0 M Ammonium Acetate pH 5.5 ± 0.1 (Eluent 2)

Place 600 mL of deionized or high purity water into a clean 1-L glass eluent container. Tare the bottle. Add 121 g (115 mL) of ultrapure glacial acetic acid and mix thoroughly. In a fume hood, slowly add 120 g (130 mL) of 20% ultrapure ammonium hydroxide and mix thoroughly. Agitate the bottle to thoroughly mix the solution. Calibrate a pH meter to pH 7. Pour about 10 mL of the buffer into a small container (e.g., scintillation vial, 10-mL disposable beaker, etc.), and measure the pH. If the pH is below 5.4, add about 5 mL of ammonium hydroxide to the buffer solution. If the pH is above 5.6, add 5 g of acetic acid. Adjust the pH of the ammonium acetate to 5.5 ± 0.1 using acetic acid if the pH is greater than 5.5, or ammonium hydroxide if the pH is less than 5.5. Once the pH is 5.5 ± 0.1, bring to a volume of 1 L.

1.0 M Nitric Acid (Eluent 3)

Place 200 mL of deionized or high purity water in a clean 1-L glass eluent container. Add 89.5 g (63 mL) of ultrapure nitric acid. Add deionized water to bring the final volume to 1 L and mix thoroughly.

0.10 M Ammonium Nitrate, pH 3.4 ± 0.3 (Eluent 4)

Place 200 mL of deionized water into a clean 1-L glass eluent container. Add 8.9 g (6.3 mL) of ultrapure nitric acid. Next, add 7.6 g (8.5 mL) of ultrapure 20% ammonium hydroxide. Add sufficient water deionized water to give a final volume of 1 L and mix thoroughly. Calibrate pH meter to pH 4.0. Take a 10-mL aliquot of the solution and measure the pH. Add either 0.10 M ammonium hydroxide or 0.10 M nitric acid in 3 to 5-mL aliquots to the bulk solution to adjust the pH. Continue taking aliquots and adjusting the pH to 3.4 ± 0.3.

PDCA Stock Solution

0.060 M PDCA
0.40 M Sodium Hydroxide
Place 200 mL of deionized water into a clean 1-L polyethylene bottle. Add 32 g (21 mL) of 50% sodium hydroxide and stir with a stir bar. While stirring, add 10.0 g of pyridine-2,6-dicarboxylic acid. Continue to stir for about 10 min or until all the PDCA has dissolved. Dilute to 1 L and stir thoroughly. Label the solution "0.060 M PDCA, 0.40 M NaOH".

Acetic Acid Stock Solution

0.90 M Acetic Acid
Place 200 mL of deionized water into a clean 1-L polyethylene bottle. Add 54 g (52 mL) of trace-metal grade acetic acid and dilute to 1 L. Label the solution "0.90 M Acetic Acid".

PDCA Eluent

0.0060 M PDCA
0.040 M Sodium Hydroxide
0.090 M Acetic Acid
Add 100 g (100 mL) of the PDCA and acetic acid solutions to a 1-L glass eluent container. Dilute to 1 L with deionized water. Label the container "0.0060 M PDCA, 0.040 M NaOH, 0.090 M Acetic Acid". The eluent should have a final pH of 4.6.

PAR Postcolumn Reagent

0.5 mM 4-(2-Pyridylazo)resorcinol
1.0 M 2-Dimethylaminoethanol
0.5 M Ammonium Hydroxide
0.3 M Sodium Bicarbonate
Prepare PAR directly in 1-L plastic reagent reservoir container (P/N 37054). To 200 g (200 mL) of deionized water, add 31 g (35 mL) of trace-metal grade ammonium hydroxide. Next, add 0.12 g of 4-(2-pyridylazo)resorcinol, monosodium, monohydrate, and ultrasonicate for 5 min. Stir the solution for several minutes with a stir bar to ensure that PAR has completely dissolved. Add 500 g (500 mL) of deionized water and then 89 g of 2-dimethylaminoethanol (DMAEOH). The solution should turn from red to orange yellow. Add 25.4 g of sodium bicarbonate and stir thoroughly until dissolved. Fill the reagent container with deionized water up to the threads on the neck, and stir. The color of the final solution should be yellow to yellow orange. Place the reagent container in the reagent reservoir.

PREPARATION OF STANDARDS

Standards should be prepared daily. Certain metals, especially iron (III), are not stable at pH 5.5 for more than a day. The standards described below are intended for the determination of metals in the low-ppb (ng/mL) range. If quantification at high levels is required, standards can be prepared at concentrations five times greater than those listed. The standards listed below are intended for use with the PDCA eluent.

Transition Metal Stock Solution

Using a variable volume micropipet, add the following volumes of 1000-ppm atomic absorption standards to a 100-mL volumetric flask.

Metal Ion	Volume (µL)	Final Concentration (µg/mL)
Fe^{3+}	200	2.00
Cu^{2+}	200	2.00
Ni^{2+}	400	4.00
Zn^{2+}	400	4.00
Co^{2+}	400	4.00
Mn^{2+}	400	4.00

Next, add about 1 mL of concentrated nitric acid (ultrapure) and dilute to volume. This stock solution will be used to prepare the calibration standards. The calibration standards can be prepared in 100-mL volumetric flasks or LDPE bottles. Be sure that the flasks or bottles have been thoroughly cleaned.

Single Level Calibration Standard

If you are using a 100-mL volumetric flask to prepare the standard, add 200 µL of stock solution and 15 mL of the 2 M ammonium acetate buffer and bring to volume.

If you are using a polyethylene or Teflon bottle, tare the empty bottle (without the cap) on a top loading balance.

Using a micropipet, add 200 µL of stock solution and 15 g of the 2 M ammonium acetate buffer. Next, add water to give a total mass of 100 g (±0.1 g). This single level calibration standard will have the following concentrations.

Metal Ion	Concentration (ng/mL)
Fe^{3+}	4.0
Cu^{2+}	4.0
Ni^{2+}	8.0
Zn^{2+}	8.0
Co^{2+}	8.0
Mn^{2+}	8.0

For calibration, concentrate at least 5 mL of this solution.

Multilevel Calibration Standards

The multilevel calibration method recommended uses standards at four concentrations. Using the procedure given in "Single Level Calibration Standard," prepare standards as given below.

Level	Volume, Stock Solution (µL)
L1	100
L2	200
L3	500
L4	1000

The multilevel calibration standards will have the following concentrations of metal ions.

Metal Ion	L1	L2	L3	L4
	Concentration (mg/mL)			
Fe^{3+}	2.0	4.0	10.0	20.0
Cu^{2+}	2.0	4.0	10.0	20.0
Ni^{2+}	4.0	8.0	20.0	40.0
Zn^{2+}	4.0	8.0	20.0	40.0
Co^{2+}	4.0	8.0	20.0	40.0
Mn^{2+}	4.0	8.0	20.0	40.0

Depending on the concentration of metal ions in the sample, the volume of the standards to be concentrated can be varied.

SAMPLE PREPARATION

To avoid hydrolysis of transition metals during long storage, it is recommended that the pH of the sample be maintained at 1–2. If the sample is digested with concentrated acid, it must be neutralized with ammonium hydroxide to pH 1–2 prior to analysis. Avoid using pipets and glassware, which usually contaminate the samples.

If the sample contains more than 400-ppm iron and more than 600-ppm aluminum in the final dilution, the sample should be further diluted. Note that the mass ratio of iron to aluminum and transition metals should not exceed 20,000 to 1. For example, if the sample contains 2% iron and 1-ppm copper, the sample must be diluted at least 50-fold to give less than 400-ppm iron and 2.5-ppb copper.

Caution: Samples should not contain high (%) levels of silica. Silicate may be precipitated in the column at pH 5.5. Digestion of such sample with concentrated hydrofluoric acid prior to chromatography is strongly recommended.

DISCUSSION OF THE METHOD

The method described in this application note was developed for determining trace transition metals in complex matrices containing high levels of alkali metals, alkaline earth elements, iron, and aluminum. The removal of iron and aluminum is based upon the electroselectivity difference between iron and aluminum with pyrophosphate eluate and iron and aluminum with iminodiacetate functionality on the stationary phase (MetPac CC-1). The other transition metals are not eluted by pyrophosphate and are quantitatively retained by the MetPac CC-1 chelating resin. The alkali and alklaline earth metals in the MetPac CC-1 column are removed with ammonium acetate eluent.

The selective elimination of iron and aluminum using chelation sample pretreatment is possible only with an on-line buffering system. Off-line buffering of the samples that contain the high levels of iron and aluminum (<100 ppm) would result in precipitation of iron and aluminum. The SCM is equipped with a high pressure valve system that allows the acidified sample to be neutralized on-line with a known quantity of buffer solution. The neutralization by on-line buffering is instantaneous and the sample preconcentration processes occur in a few seconds. As a result, hydrolysis of aquated metal ions and adsorption of metal–hydroxide complexes on the surface of inert polymeric tubing is prevented.

The chelation concentration with selective elimination of iron and aluminum can be described as shown in Figure 1. A complexing agent is used which will selectively bind iron and aluminum, thus preventing uptake by the chelating resin during concentration. This approach not only prevents the precipitation of iron and aluminum at high concentration, it also allows an effective removal of iron and aluminum from the MetPac CC-1 column. The relatively stable metal–pyrophosphate (PP) complexes formed during on-line neutralization step (in-situ) do not interact with iminodiacetate and are not retained in the column. Figure 1 shows the scheme of the selective concentration using MetPac CC-1 with complexing agents.

The high levels of iron, aluminum, and manganese that are normally found in rock, sediment, and soil samples can be separated from trace transition metals by using 20 mM pyrophosphate, pH 5.5. Moreover, the rare earth, lanthanide, and transuranium elements, which normally are present in rock samples and may interfere with the analysis, are eliminated from the MetPac CC-1. The analytes of interest, such as copper, cadmium, cobalt, nickel, and zinc, are quantitatively retained by the MetPac CC-1 chelating resin. The IC/ICAP data indicated in Figure 2 shows recovery of metals using the MetPac CC-1 at various pyrophosphate concentrations. If manganese is one of the analytes of interest, it can be determined by using 10 mM pyrophosphate at pH 8.5 as noted in Table 1.

The recommended eluent for this application is pyridine-2,6-dicarboxylic acid (PDCA), which is a strong complexing agent that separates metal ion complexes by anion exchange. The 0.006M PDCA eluent is best suited for iron, copper, nickel, zinc, cobalt, cadmium, and manganese (see Technical Note 25 for complete details). Lead, on the other hand, can be determined using the oxalic acid eluent (again, see Technical Note 25 for more details).

The elimination of iron and aluminum by chelation ion chromatography has been applied to the determination of transition metals in geological materials. The matrix components that potentially interfere with the chromatographic separation and the postcolumn derivatization are eliminated during chelation concentration. An example of matrix components eliminated by chelation concentration is shown in Table 2. This table summarizes the interfering species present in the USGS Basalt rock sample. The results of analysis and the chromatogram are shown in Figure 2.

Example - Iron, Aluminum with Pyrophosphate (M^{3+} – Al^{3+}, Fe^{3+})

In-Situ -

$$M^{3+} + PP^{4-} \overset{K_{MPP}}{\rightleftharpoons} MPP^{-}$$

$$M^{3+} + 2PP^{4-} \overset{K_{MPP_2}}{\rightleftharpoons} M(PP)_2^{5-}$$

$$2M^{3+} + PP^{4-} \overset{K_{M_2PP}}{\rightleftharpoons} M_2PP^{2+}$$

Resin -

$$M^{3+} + R - N(CH_2COO^-)_2 \overset{K_R}{\rightleftharpoons} R - N(CH_2COO)M^{+}$$

$$M^{3+} + 2R - N(CH_2COO^-)_2 \overset{K_{R_2}}{\rightleftharpoons} 2R - N(CH_2COO)M^{+}$$

For Selective Concentration: $K_{MPP} >> K_R$ and $K_{MPP_2} >> K_{R_2}$

Figure 1 Chelation concentration using MetPac CC-1 with complexing agents

Table 1 Metal recovery from high calcium–iron–aluminum matrix using MetPac CC-1 with pyrophosphate for complexation

Element	Amount Added (ng/g)	Found (pH 5.5) ng/g (%RSD)*	Found (pH 8.5) ng/g (%RSD)*
Ca	50,000	15	15
Al	50,000	5	5
Fe	100,000	10	10
Pb	100	98.4 (3.08%)	99.3 (0.93%)
Cd	100	98.1 (1.20%)	99.5 (0.61%)
Cu	100	104 (0.80%)	135 (0.31%)
Co	100	100 (0.96%)	132 (0.25%)
Mn	100	70.2 (2.46%)	135 (0.49%)
Ni	100	98.3 (1.09%)	132 (1.07%)
Zn	100	98.2 (1.69%)	136 (1.17%)

*n = 4

Table 2 Matrix composition of USGS Basalt (BHVO-1) rock sample	
Element	**Concentration**
SiO_2	49.94%*
Al_2O_3	13.80%
Fe_2O_3	12.23%
MnO	0.168%
MgO	7.23%
CaO	11.40%
TiO_2	2.71%

* If sample contains high concentration of SiO_2, it must be treated with hydrofluoric acid prior to chelation concentration.

SYSTEM PREPARATION, SET-UP, AND TEST

For complete details in system preparation and setup, operation and automation; refer to Technical Note 25.

1. A schematic diagram of the SCM configured for operation in Chelation IC System 2 is provided in Figure 4 in Technical Note 25. Confirm that the system is configured and plumbed as shown.

2. Ensure that there are no metal components in the flow path, including tubing end fittings (stainless steel washers, omni-fittings), columns, and valves, which contain stainless steel. Replace all omni-grippers with Thermo-Flare™ washers.

3. Follow the step-by-step "System Preparation" instructions given in Technical Note 25.

4. Four eluents are required for this application:

   E1: 20 mM pyrophosphoric acid / 2.0 M ammonium acetate
   E2: 2.0 M ammonium acetate
   E3: 1.0 M nitric acid
   E4: 0.1 M ammonium nitrate

5. If a 5-mL sample loop is used, enter the program provided in Table 3 into the gradient pump. For other sample loops, refer to the Appendix for instructions on how to create an appropriate gradient pump program.

6. Follow the step-by-step "System Test" instructions given in Technical Note 25.

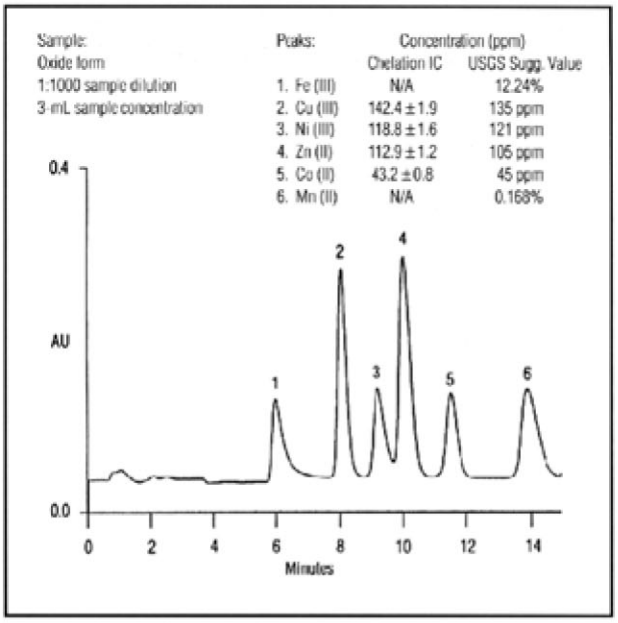

Figure 2 Determination of transition metals in USGS Basalt (BHVO-1) by chelation IC

SYSTEM OPERATION

The sequencing and operation of the system components are described below. This system configuration has been designed for use in the fully automated mode, except for the sample introduction step. If the required sample volume is not more than 3 mL, the Dionex ASM autosampler may be used. The sample must be introduced using a syringe or peristaltic pump unless the autosampler used is capable of delivering more than 5 mL of sample.

1. Confirm that the system is configured as given in Technical Note 25. Check to see that the system has a 80–120 psi (550–830 kPa) inert gas supply.

2. Turn the absorbance detector on. If the VDM-2 is being used, set the wavelength to 530 nm. If a filter based detector is being used, be sure the filter is 520 or 530 nm. Turn on the visible lamp and set the sensitivity to 0.2 AUFS. Be sure that the detector output is connected to a data collection system (integrator or ACI/AI-450).

3. Enter the program listed in Table 3. Check the program carefully by listing each step of the program.

4. Reset the gradient program at time 0.0 min. Turn on the carrier pump (Pump 1, 1.0 mL/min) and the RDM. Confirm that the PAR reagent is flowing through the detector by measuring the flow rate out of the waste line.

Table 3 Gradient program for chelation concentration

E1: 20 mM Pyrophosphoric Acid / 2.0 M Ammonium Acetate
E2: 2.0 M Ammonium Acetate
E3: 1.0 M Nitric Acid
E4: 0.1 M Ammonium Nitrate

t	%E1	%E2	%E3	%E4	V5	V6	Flow Rate (mL/min)
0.0	100	0	0	0	1	0	3.0
2.0	100	0	0	0	0	1	2.0
6.0	100	0	0	0	1	0	3.0
6.1	0	100	0	0	1	0	3.0
7.0	0	100	0	0	1	0	1.2
7.1	0	0	100	0	1	1	1.2
12.0	0	0	100	0	1	1	1.2
12.1	0	0	0	100	1	1	2.0
13.0	0	0	0	100	0	0	3.0
15.0	0	0	0	100	0	0	3.0
15.1*	0	0	100	0	1	0	4.0
16.0	0	0	100	0	1	0	4.0
17.0	0	0	100	0	1	0	4.0
18.0	0	100	0	0	1	0	0.0

* Begin sample analysis

5. Turn on the integrator or monitor and begin to monitor the baseline. At 0.2 AUFS, an essentially noise-free and drift-free baseline should be observed.

6. Step 1: Once the baseline is stable, start the gradient pump and press RUN. Confirm that valve 5 is ON and valve 6 is OFF. The sample or the standard can be loaded via the autosampler. If the autosampler is not used, use a syringe to load the sample by drawing the sample through the sample inlet. The sample pH should be 1–2. While the sample introduction step is in progress, the gradient pump is pumping 20 mM pyrophosphate to regenerate / equilibrate the MetPac CC-1 column. Note that the next step occurs at 2.0 min. If the sample introduction takes more than 2.0 min, adjust the gradient pump program accordingly (see the Appendix).

7. Step 2: Valve 5 is OFF and valve 6 is ON. The sample pump delivers the deionized water through the sample loop that was previously loaded with the sample. The sample stream is now mixing with the 20 mM pyrophosphate from the gradient pump, and the buffered sample passes through the MetPac CC-1 column.

8. Step 3: Valve 5 is ON and valve 6 is ON. Valve E now is switched to the LOAD position in which the TMC-1 column is placed in-line with the gradient pump. The gradient pump delivers 1.0 M nitric acid to the MetPac CC-1 column. The concentrated metal ions are eluted off the column, and the 1.0 M acid stream is diluted on-line to approximately 0.37 M with deionized water from the sample pump before it passes through the TMC-1 column. This step maximizes the removal of concentrated metal ions from the MetPac CC-1 and places them on the TMC-1 column in a tight band.

9. Step 4: Valve 5 is OFF and valve 6 is OFF. The gradient pump delivers 0.1 M ammonium nitrate to the TMC-1. This step is required to convert the TMC-1 from the hydronium form to the ammonium form.

10. Step 5: Valve 5 is ON and valve 6 is OFF. Valve E is now switched to the INJECT position in which the TMC-1 column is placed in-line with the IonPac CS5 column. The gradient pump delivers 1.0 M nitric acid to the MetPac CC-1 for 2 min (4.0 mL/min), followed by 2.0 M ammonium acetate for 1.0 min (4.0 mL/min) before the end of the chelation concentration process.

If you wish to start the chelation concentration on the next sample, you may start at this time. Remember that the analysis of the previous sample must be completed before proceeding to step 3 in which the TMC-1 column is switched in-line with the MetPac CC-1. The time to complete the analysis of transition metals is normally within 15 min. The gradient pump may be adjusted so that step 3 can proceed at 15.0 min.

11. Reset the gradient pump and data collection device. Start the overall cycle *without* injecting the sample. This run will represent the system blank. Repeat this cycle at least three times or until the blank is reproducible.

12. At this point, the system is ready for calibration and sample analysis. Refer to the appropriate section of Technical Note 25 for details on calibration and quantification.

APPENDIX: SAMPLE LOOP

The 1-mL and 5-mL sample loops are available and supplied with the SCM. If you wish to prepare a sample loop larger than 5.0 mL, use an appropriate length of 1/8-in. I.D. Tefzel® tubing.

To create a proper gradient program for a new sample loop, use the worksheet shown in Table 3 and follow the steps below:

1. Determine the loop loading time (*L*) starting from the sample source to the sample loop. If an autosampler is used, determine how much time the autosampler completes the loading step. If a syringe is used, 2.0 min is appropriate. Be sure that the sample loop is completely filled. A minimum of 2.0 min is required for the first step.

2. Determine the sample loading time (*C*) from sample loop to the MetPac CC-1 column. This value can be obtained by divided the sample loop size (mL) by the sample pump flow rate (mL/min). For proper sample loading, an additional 1.0 min is normally included.

   Example: Sample loop size = 7 mL
   Sample pump flow rate = 2.0 mL/min
   Sample loading time = C =
   (7 mL/2.0 mL/min) + 1.0 min = 4.5 min

3. Enter the *L* and *C* values in the work sheet shown in Table 3. Calculate and enter the new time. Enter the new program into the gradient pump.

Table 3 Gradient Program Work Sheet

t (min)	Enter New Time	E1	E2	E3	E4	V5	V6	Flow (mL/min)
0.0		100	0	0	0	1	0	3.0
L		100	0	0	0	0	1	2.0
L + C		100	0	0	0	1	0	3.0
L + C + 1.1		0	100	0	0	1	0	3.0
L + C + 2.0		0	100	0	0	1	0	1.2
L + C + 2.1		0	0	100	0	1	1	1.2
L + C + 7.0		0	0	100	0	1	1	1.2
L + C + 7.1		0	0	0	100	1	1	2.0
L + C + 8.0		0	0	0	100	0	0	3.0
L + C + 10		0	0	0	100	0	0	3.0
L + C + 10.1*		0	0	100	0	1	0	4.0
L + C + 11		0	0	100	0	1	0	4.0
L + C + 12		0	0	100	0	1	0	4.0
L + C + 13		0	100	0	0	1	0	0.0

*Begin sample analysis

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Determination of Trace Transition Metals in Reagent Grade Acids, Bases, Salts, and Organic Solvents Using Chelation Ion Chromatography

INTRODUCTION

The SEMI (Semiconductor Equipment and Materials International) specifications for maximum permitted levels of transition metals in concentrated acids used in fabrication of semiconductor devices is in the range of 0.1 to 1.0 part per billion (ppb). Labor-intensive manual preconcentration methods are usually required prior to analytical measurements. Typical preconcentration procedures involve evaporation of a specific volume of sample for 2.5 to 4 hours on a hot plate before transfer to a volumetric flask and analysis. Also, a class-100 clean room environment is normally required during sample pretreatment.

The chelation ion chromatographic technique (chelation IC) provides the sample preconcentration step and direct determination of trace transition metals by ion chromatographic separation and postcolumn derivatization prior to detection. Preconcentration is performed in minutes rather than hours. Sampling, preconcentration, and analyte transfer to the IC system is on-line and automated. The sample is never exposed to the ambient atmosphere during preconcentration. Typical recoveries are 95 to 100% in the 2 to 5-ppb concentration range in concentrated acids. Detection limits are in the 0.5 to 5-ppb range for most metals using 5 mL of sample.

Methodology for the determination of transition metals in concentrated acids using chelation ion chromatography is applicable to semiconductor grade bases and solvents. Currently, the chelation IC is applicable to iron, copper, nickel, zinc, cobalt, cadmium, and manganese. Typical detection limits for semiconductor solvents and bases using chelation IC are one to two orders of magnitude better than by using direct flame atomic absorption spectroscopy and plasma emission spectroscopy.

This application note describes a method for the determination of trace transition metals in trace metal grade reagents by chelation IC. The detection limits for most elements present in concentrated trace-metal grade reagents are in the sub part-per-billion range.

INSTRUMENT REQUIREMENTS

Two chelation IC system configurations can be used for this application. Both systems include a gradient pump, a Sample Concentration Module (SCM), a Reagent Delivery Module (RDM), and a Variable Wavelength Detector Module (VDM-2). System 1 is configured for manual operation, while system 2 is for automated operation. Refer to Dionex Technical Note 25 for complete details on configuring these two chelation chromatography systems.

Chelation IC System 1

Gradient Pump Module (GPM-2, P/N 37098) or
Advanced Gradient Pump (AGP, P/N 43116)
Sample Concentration Module (SCM, P/N 42134)
Reagent Delivery Module (RDM, P/N 37030)
Variable Wavelength Detector Module
(VDM-2, P/N 39646, internal or remote cell)
Eluent Degas Module (EDM-2, P/N 39550)
Eluent Container Set, Glass (P/N 38752)
IonPac® Membrane Reactor (P/N 35354, optional)
Knitted Reaction Coil (P/N 39349)
MetPac™ CC-1 Column (P/N 42156)
TMC-1 Column (P/N 42155)
IonPac CG5 (P/N 37029)
AI-450 or other data acquisition system

Chelation IC System 2

Gradient Pump Module (GPM-2, P/N 37098) or
Advanced Gradient Pump (AGP, P/N 43116)
Sample Concentration Module (SCM, P/N 42134)
Reagent Delivery Module (RDM, P/N 37030)
Variable Wavelength Detector Module
(VDM-2, P/N 39646, internal or remote cell)
Eluent Degas Module (EDM-2, P/N 39550)
Eluent Container Set, Glass (P/N 38752)
Valve, 4-Way Slider Double Stack (3),
2000 psi (P/N 35914)
IonPac Membrane Reactor (P/N 35354, optional)
Knitted Reaction Coil (P/N 39349)
MetPac CC-1 Column (P/N 42156)
TMC-1 Column (P/N 42155)
IonPac CG2 (2, P/N 35370)
IonPac CG5 (P/N 37029)
AI-450 or other data acquisition system

SOLUTIONS AND REAGENTS

Ultrapure 2.0 M ammonium acetate, pH 5.4 ± 0.1
(1 L, P/N 33440; 6 L, P/N 33441)
Ultrapure 2.0 M nitric acid (1 L, P/N 33442; 6 L,
P/N 33443)
Ultrapure 0.1 M ammonium nitrate, pH 3.5 ± 0.3
(1L, P/N 33445)
20% Ultrapure ammonium hydroxide
Ultrapure glacial acetic acid
0.006 M Pyridine-2,6-dicarboxylic acid (PDCA)
0.040 M sodium hydroxide
0.090 M acetic acid
0.5 mM 4-(2-pyridylazo) resorcinol (PAR)
1.0 M 2-dimethylaminoethanol
0.5 M ammonium hydroxide
0.3 M sodium bicarbonate

The first three reagents used for chelation concentration are available from Dionex in a ready-to-use form. If you wish to prepare your own reagent solutions, please refer to "Preparation of Solutions and Reagents". The remaining ultrapure reagents are manufactured by Seastar Chemical and Ultrex Reagents. Seastar reagents are available internationally through Fisher Scientific; in North America, Fisher Scientific sells these reagents under the OPTIMA® label. Ultrex reagents are available international through J.T.Baker.

CONDITIONS

The conditions for chelation concentration and analytical chromatography are presented in Table 1.

PREPARATION OF SOLUTIONS AND REAGENTS

Three concentrated reagents are required for eluents in chelation concentration: Nitric acid, acetic acid, and ammonium hydroxide. For ultratrace level determinations (sub-ppb), the reagents must be ultrapure grade. For determination above 1 ppb, high quality trace-metal grade reagents can be used. Any metal impurity in these reagents will be concentrated with your sample, constituting a system blank.

2.0 M Ammonium Acetate pH 5.4 ± 0.1 (Eluent 2)

Place 600 mL of deionized or high purity water into a clean 1-L glass eluent container. Tare the bottle. Add 121 g (115 mL) of ultrapure glacial acetic acid and mix thoroughly. In a fume hood, slowly add 120 g (130 mL) of 20% ultrapure ammonium hydroxide and mix thoroughly. Agitate the bottle to thoroughly mix the solution. Calibrate a pH meter to pH 7. Pour about 10 mL of the buffer into a small container (e.g., scintillation vial, 10-mL disposable beaker, etc.) and measure the pH. If the pH is below 5.4, add about 5 mL of ammonium hydroxide to the buffer solution. If the pH is above 5.5, add 5 g of acetic acid. Adjust the pH of the ammonium acetate to 5.4 ± 0.1 using acetic acid if the pH is greater than 5.5, or ammonium hydroxide if the pH is less than 5.3. Once the pH is 5.5 ± 0.1, bring to a volume of 1 L.

2.0 M Nitric Acid (Eluent 3)

Place 200 mL of deionized or high purity water into a clean 1-L glass eluent container. Add 179 g (126 mL) of ultrapure nitric acid. Add deionized water to bring the final volume to 1 L and mix thoroughly.

0.10 M Ammonium Nitrate, pH 3.5 ± 0.3 (Eluent 4)

Place 200 mL of deionized water into a clean 1-L glass eluent container. Add 8.9 g (6.3 mL) of ultrapure nitric acid. Next, add 7.6 g (8.5 mL) of ultrapure 20% ammonium hydroxide. Add sufficient water deionized water to give a final volume of 1 L and mix thoroughly. Calibrate pH meter to pH 4.0. Take a 10-mL aliquot of the solution and measure the pH. Add either 0.10 M ammonium hydroxide or 0.10 M nitric acid in 3 to 5-mL aliquots to the bulk solution to adjust the pH. Continue taking aliquots and adjusting the pH to 3.4 ± 0.3.

Table 1
Chelation Concentration Operation Conditions

Chelation Concentration–Transition Metals

Columns:	MetPac CC-1, TMC-1
Eluents:	E1: H_20
	E2: 2.0 M ammonium acetate, pH 5.4 ± 0.1
	E3: 2.0 M nitric acid
	E4: 0.10 M ammonium nitrate, pH 3.5 ± 0.3

Gradient Program–System 1

t (min	%E1	%E2	%E3	%E4	V5	V6	Flow (mL/min)
0.0	0	100	0	0	1	0	3.0
0.1	0	100	0	0	1	1	3.0
2.5	0	100	0	0	1	1	3.0
2.6	72	0	28	0	0	1	3.0
5.0	72	0	28	0	0	1	1.0
5.1	0	0	0	100	0	0	3.0
6.6	0	0	0	100	1	0	1.0
6.7	0	0	100	0	1	1	3.0
7.7	0	0	100	0	1	1	3.0
7.8	0	100	0	0	1	1	3.0
9.3	0	100	0	0	1	1	3.0
9.4	100	0	0	0	1	1	0.0

Gradient Program–System 2

t (min	%E1	%E2	%E3	%E4	V5	V6	Flow (mL/min)
0.0	0	100	0	0	1	0	3.0
2.0	0	100	0	0	0	1	2.0
5.0	0	100	0	0	1	0	3.0
7.0	0	100	0	0	1	0	1.2
7.1	50	0	50	0	1	1	1.2
12.0	50	0	50	0	1	1	1.2
12.1	0	0	0	100	1	1	2.0
13.0	0	0	0	100	0	0	3.0
15.0	0	0	0	100	0	0	3.0
15.1*	0	0	100	0	1	0	4.0
16.0	0	0	100	0	1	0	4.0
17.0	0	0	100	0	1	0	4.0
18.0	0	100	0	0	1	0	0.0

*begin sample analysis

Analytical Chromatography–Transition Metals

Column:	IonPac CS5
Eluent:	0.0060 M Pyridine-2,6-dicarboxylic acid, 0.090 M acetic acid, 0.040 M sodium hydroxide or 0.050 M oxalic acid, 0.095 M lithium hydroxide
Eluent Flow Rate:	1.0 mL/min

Postcolumn Derivatization

Reagent:	4×10^{-4} M 4-(2-pyridylazo) resorcinol 1.0 M 2-dimethylaminoethanol 0.50 M ammonium hydroxide 0.30 M sodium bicarbonate
Reagent Addition:	Membrane reactor or mixing tee
Reagent Flow Rate:	0.5mL/min
Reactor:	Packed or knitted reaction coil

Detection

Detector:	Visible absorbance, VDM or UDM
Wavelength:	520 or 530 nm
Time Constant:	1 s

PDCA Stock Solution

0.060 M PDCA

0.40 M Sodium Hydroxide

Place 200 mL of deionized water into a clean 1-L polyethylene bottle. Add 32 g (21 mL) of 50% sodium hydroxide and stir with a stir bar. While stirring, add 10.0 g of pyridine-2,6-dicarboxylic acid. Continue to stir for about 10 min or until all the PDCA has dissolved. Dilute to 1 L and stir thoroughly. Label the solution "0.060 M PDCA, 0.40 M NaOH".

Acetic Acid Stock Solution

0.90 M Acetic Acid

Place 200 mL of deionized water into a clean 1-L polyethylene bottle. Add 54 g (52 mL) of trace metal grade acetic acid and dilute to 1 L. Label the solution "0.90 M Acetic Acid".

PDCA Eluent

0.0060 M PDCA

0.040 M Sodium Hydroxide

0.090 M Acetic Acid

Add 100 g (100 mL) of the PDCA and acetic acid solutions to a 1-L glass eluent container. Dilute to 1 L with deionized water. Label the container "0.0060 M PDCA, 0.040 M NaOH, 0.090 M Acetic Acid". The eluent should have a final pH of 4.6.

PAR Postcolumn Reagent

0.5 mM 4-(2-Pyridylazo)resorcinol

1.0 M 2-Dimethylaminoethanol

0.5 M Ammonium Hydroxide

0.3 M Sodium Bicarbonate

Prepare PAR directly in 1-L plastic reagent reservoir container (P/N 37054). To 200 g (200mL) of deionized water, add 31 g (35 mL) trace-metal grade ammonium hydroxide. Next, add 0.12 g of 4-(2-pyridylazo) resorcinol, monosodium, monohydrate, and ultrasonicate for 5 min. Stir solution for several minutes with a stir bar to ensure that PAR has completely dissolved. Add 500 g (500 mL) of deionized water and then 89 g of 2-dimethylaminoethanol (DMAEOH). The solution should turn from red to orange yellow. Add 25.4 g of sodium bicarbonate and stir thoroughly until dissolved. Fill the reagent container with deionized water up to the threads on the neck, and stir. The color of the final solution should be yellow to yellow orange. Place the reagent container in the reagent reservoir.

STANDARD PREPARATION

Standards should be prepared daily. Maintain the sample pH between pH 1–2. The standards listed below are intended for the determination of metals in the low ppb (ng/mL) range. If quantitation at higher levels is required, standards can be prepared at concentrations five times greater than those listed. If Chelation IC System 1 is used, the pH of the standard solution must be adjusted to 5.5 with ammonium acetate prior to sample introduction.

SAMPLE PREPARATION

Since the trace-metal grade samples contain a very low level of metal contaminants, dilution of the concentrated sample must be minimized. One important step in sample preparation is to adjust the concentrated acid samples to pH 1–2, which requires a great deal of precaution. Several guidelines will be discussed that are applicable to most of the concentrated acid samples.

The sample should be prepared in clean poly–ethylene containers. Avoid using pipets and glassware, which can contaminate the samples. For the concentrated acid sample, it is advisable to keep the acid sample in a cooling bath during neutralization with ammonium hydroxide and ammonium acetate.

For inorganic salts (e.g., ammonium chloride, ammonium nitrate) and organic solvents, the sample solution must be acidified to pH 1–2 with ultrapure nitric acid. For concentrated acid, the sample must be neutralized and buffered with ultrapure concentrated ammonium hydroxide/ammonium acetate solution. For example, calculate 0.5 mole equivalent of concentrated acid (g/L) and weigh it in a clean 100 mL volumetric polyethylene container (e.g., 44.5 g of HNO_3 or 49 g of HC1). Place the sample container in the ice bath and slowly add 10 g of ultrapure 6.0 M ammonium acetate buffer (pH 5.5) to the sample. In a separate container, weigh 30 g of saturated ammonium hydroxide (20%). Slowly add ammonium hydroxide (drop-wise) to the sample with constant swirling. WARNING: The sample will become very HOT. Allow the sample to cool to room temperature and dilute sample to volume with deionized water. Check that the final pH is 1–2. Note the amount of 6.0 M ammonium acetate added to the sample. Prepare the blank solution by using the exact amount of saturated ammonium hydroxide and 6.0 M ammonium acetate. For concentrated bases, neutralize the sample with ultrapure concentrated nitric acid and ammonium acetate. For organic solvents, it is strongly recommended that the samples be acidified to stabilize the trace metals. If Chelation IC System 1 is used, the sample pH must be adjusted to 5.5 with ammonium acetate prior to sample introduction.

For complete details on system preparation and setup, operation, and automation, refer to Dionex Technical Note 25.

DISCUSSION OF THE METHOD

The method described in this application note was developed for a high ionic strength matrix of acid, base, and salt samples. In general the high ionic strength matrices usually interfere with the chromatographic separations and degrade the detection limits for many transition metals. Chelation IC not only offers a sample concentration capability to enhance the detection limits, it also standardizes or matches the sample matrix to the standard matrix without an off-line sample pretreatment step. For discussion of the chelation IC method, refer to Technical Note 25 for complete details.

The chelation IC method has been applied to the analysis of trace transition metals in magnesium chloride, sodium hydroxide, hydrochloric acid, and acetonitrile matrices as shown in Figures 1 through 4. The detection limits for most metals are in the sub-part-per-billion to low-part-per-billion range. Spike recoveries in these matrices are listed in Table 2.

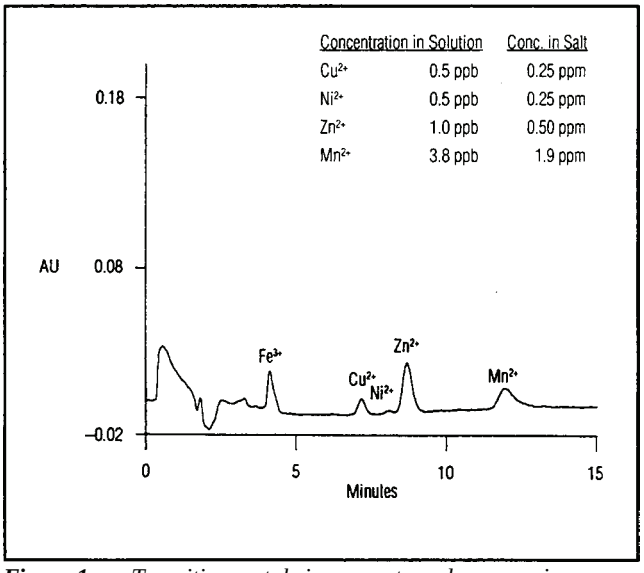

Figure 1 *Transition metals in reagent grade magnesium chloride*

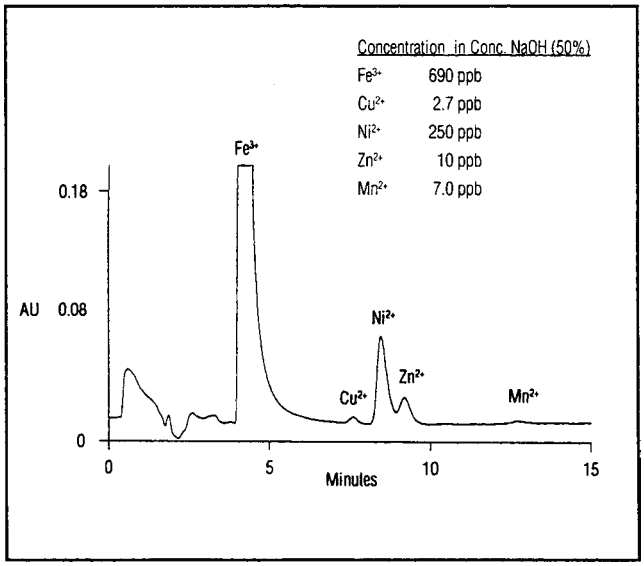

Figure 2 *Transition metals in sodium hydroxide*

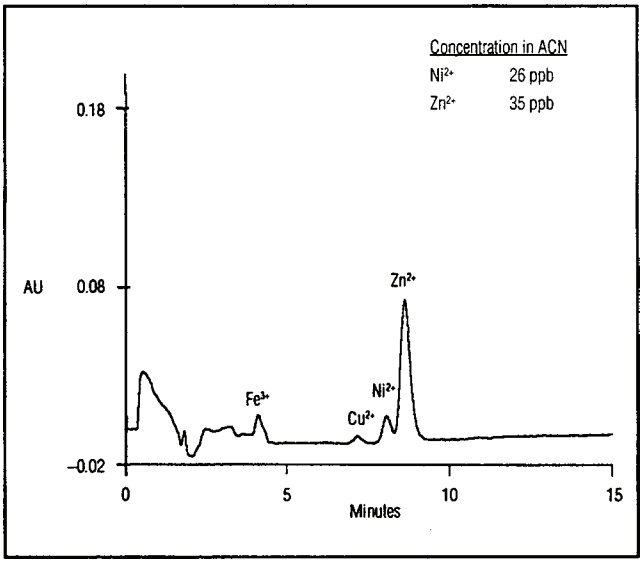

Figure 4 *Trace metals acetonitrile*

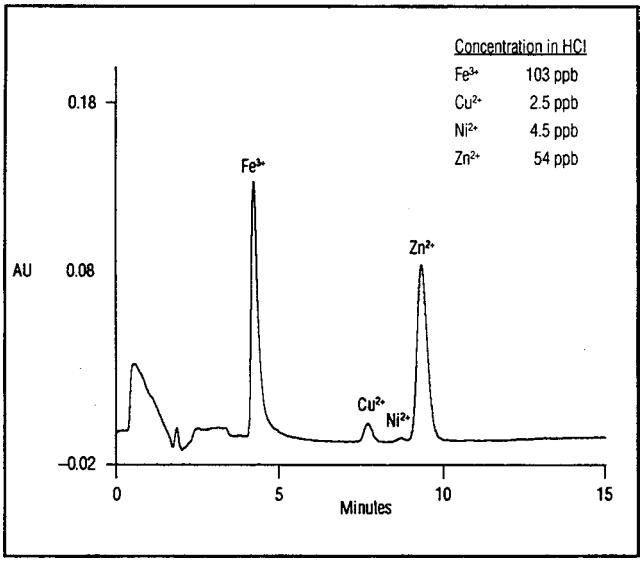

Figure 3 *Trace metals in hydrochloric acid*

Table 2 Spike Recoveries in Various Matrices

Element	Spike Level (ppb) in Each Sample	Concentration in Matric (ppb)			Percent Recovery		
		NaOH	HCl	ACN	NaOH	HCl	ACN
Fe^{3+}	20	690	103	–	27	92	40
CU^{2+}	20	2.7	2.5	–	105	103	97
Ni^{2+}	40	250	4.5	–	106	106	100
Zn^{2+}	40	10	54	1.8	96	98	102
Co^{2+}	40	–	–	–	98	103	103
Mn^{2+}	60	7	–	–	91	75	96

Determination of Trace Transition Metals in Reagent Grade Acids, Bases, and Salts Using Ion Chromatography/Inductively Coupled Argon Plasma Spectroscopy (IC/ICAP)

INTRODUCTION

The SEMI (Semiconductor Equipment and Materials International) specifications for maximum permitted levels of transition metals in concentrated acids used in fabrication of semiconductor devices is in the range of 0.1 to 1.0 part per billion (ppb). Labor intensive manual preconcentration methods are usually required before analytical measurement by flame atomic absorption spectroscopy. Typically, evaporation of a specific volume of sample for 2.5 to 4 hours on a hot plate is required before analysis. Also, a Class 100 clean-room environment is normally required during sample pretreatment.

Automated chelation concentration ion chromatography automates the sample preconcentration step before determination by plasma emission spectroscopy. Preconcentration is performed in minutes rather than hours. Sampling, preconcentration, and delivery to the plasma are automated. The sample is never exposed to the ambient atmosphere during preconcentration.* Typical recoveries are 95% to 100% in the 1 ppb concentration range in concentrated acids. Detection limits are in the 0.2 to 2 ppb range for most metals using 5 mL of sample.

Methodology for the determination of transition metals in concentrated acids using coupled IC/ICAP is applicable to semiconductor grade bases and solvents. Typical detection limits for semiconductor solvents and bases using IC/ICAP are one to two orders of magnitude better than by using flame atomic absorption spectroscopy or plasma emission spectroscopy directly.

**Note:* The chelation concentration methods discussed in this application note can also be used for "off-line" preconcentration in conjunction with atomic absorption spectroscopy.

This application note describes a method for the determination of trace transition metals in trace metal grade reagents by IC/ICAP. The detection limits for most elements present in concentrated trace metal grade reagents are below 1 ppb.

EQUIPMENT

Dionex Advanced Gradient Pump (AGP)

Dionex Sample Concentration Module (SCM)

Thermo Jarrell Ash simultaneous ICAP spectrometer

IBM® or 100% compatible computer with TJA ThermoSpec™software

REAGENTS

Ultrapure 2.0 M ammonium acetate, pH 5.5 (1 L, P/N 33440; 6 L, P/N 33441)

Ultrapure 2.0 M nitric acid (1 L, P/N 33442; 6 L, P/N 33443)

20% Ultrapure ammonium hydroxide

Ultrapure glacial acetic acid

Ultrapure concentrated nitric acid

The first two reagents used for chelation concentration are available from Dionex in a ready-to-use form. If you wish to prepare your own reagent solutions, information for ordering ultrapure acids and ammonium hydroxide are given below in *Preparation of Solutions and Reagents*.

SAMPLE PREPARATION

Since trace metal grade samples contain very low concentrations of metal contaminants, dilution of the concentrated sample must be minimized. One important step in sample preparation is to adjust the concentrated acid samples to pH 1–2, which requires a great deal of precaution. Several guidelines will be discussed that apply to most of the concentrated acid samples.

The sample should be prepared in clean polyethylene containers. Avoid using pipets and glassware that may contaminate the samples. For the concentrated acid sample, it is advisable to keep the sample in a cooling bath during neutralization with ammonium hydroxide and ammonium acetate.

For inorganic salts (e.g., ammonium chloride, ammonium nitrate), the sample solution must be acidified to pH 1–2 with ultrapure nitric acid. For concentrated acid, the sample must be neutralized and buffered with ultrapure concentrated ammonium hydroxide/ammonium acetate solution. For example, weigh 0.5 mole equivalent of concentrated acid in a clean 100-mL volumetric polyethylene container (e.g., 44.5 g HNO_3 or 49 g HCl). Place the sample container in an ice bath and slowly add 10 g 6.0 M ammonium acetate buffer (pH 5.5) to the sample. In a separate container, weigh 30 g concentrated ammonium hydroxide. Slowly add ammonium hydroxide (dropwise) to the sample with constant swirling. WARNING: The sample will become very HOT. Allow the sample to cool and dilute the sample to volume (100 mL) with deionized water. Check that the final pH is 1–2. Note the amount of6.0 M ammonium acetate added to the sample. Prepare the blank solution using the same amount of saturated ammonium hydroxide and 6.0 M ammonium acetate. For concentrated bases, neutralize the sample with ultrapure concentrated nitric acid and ammonium acetate.

PREPARATION OF SOLUTIONS AND REAGENTS

Three concentrated reagents are required for eluents in chelation concentration: nitric acid, acetic acid, and ammonium hydroxide. For ultratrace level determinations (sub ppb), the reagents must be ultrapure grade. For determination above 1 ppb, high quality trace metal grade reagents can be used. Any metal impurity in these reagents will be concentrated with the sample, constituting a system blank.

2.0 M Ammonium Acetate pH 5.5 ± 0.1

If Dionex ultrapure 2.0 M ammonium acetate is used, no further preparation is required. Otherwise, place 600 mL of deionized or high purity (18 MΩ) water into a clean 1-L glass eluent container. Add 121 g (115 mL) of ultrapure glacial acetic acid and mix thoroughly. In a fume hood, slowly add 120 g (130 mL) of 20% ultrapure ammonium hydroxide and mix thoroughly. Agitate the bottle to thoroughly mix the solution. Adjust the pH of the ammonium acetate to 5.5 ± 0.1 using acetic acid if the pH is greater than 5.5, or ammonium hydroxide if the pH is less than 5.5. Once the pH is 5.5 ± 0.1, bring to a volume of 1 L.

2.0 M Nitric Acid

If Dionex ultrapure 2.0 M nitric acid is used, no further preparation is required. Otherwise, place 200 mL of deionized or high purity (18 MΩ) water into a clean 1-L glass eluent container. Add 179 g (126 mL) of ultrapure nitric acid. Add deionized water to bring the final volume to 1 L and mix thoroughly.

0.1 M Nitric Acid—Carrier Solution

Place about 1000 mL of deionized water into the 4-L plastic eluent container. Add 89 g (63 mL) of concentrated nitric acid; then, add deionized water to bring the final volume to 4 L and mix thoroughly.

The ultrapure reagents are manufactured by Seastar Chemical and Ultrex Reagents. Seastar reagents are available internationally from Fisher Scientific, who sells these reagents under the OPTIMA® label. Ultrex reagents are available internationally through J.T. Baker.

STANDARD PREPARATION

Since the analytes of interest must be concentrated, the concentration of the “high” standard should not exceed 1 ppm. Typically, a working “high” standard in the range of 5 to 100 ppb is used for ultratrace analysis work. It is convenient to prepare a 10X concentrate of stock solution of the standard and prepare the high standard by dilution of the stock solution.

SYSTEM OPERATION

In Dionex Technical Note 28, "IC/ICAP: A New Technique for Trace Metal Determinations", the gradient program described for the standard configuration may be used for the method described below. To eliminate alkaline earth elements in the sample, the amount of time to wash the column with the ammonium acetate buffer may be shortened if these elements are not at high concentrations. For complete details in system preparation and setup, operation, and automation, refer to Technical Note 28.

DISCUSSION OF THE METHOD

The method described in this application note was developed for a high ionic strength matrix of acid, base, and salt samples. In many instances, a high salt matrix (>6.0 M) precipitates in the sample flow path and in the nebulizer, causing mechanical problems in ICAP instruments. On the other hand, IC not only offers a sample concentration capability to enhance the ICAP detection limits, it also standardizes or matches the sample matrix to the standard matrix, without the need for an off-line matrix matching step.

The MetPac™ CC-1 chelating resin has very high affinity for transition metals compared to the alkali and alkaline earth metals. The resin does not concentrate anions such as the halides, nitrate, sulfate, phosphate, or organic anions. Thus, transition metals can be quantitatively concentrated from high concentrations of anions, alkali, and alkaline earth elements. The chelation concentration process consists of four steps. A known volume of the sample is buffered to pH 1–2 with ammonium hydroxide and ammonium acetate and concentrated on the MetPac CC-1. Most polyvalent cations are quantitatively concentrated, while anions pass through the column. Weakly bound alkaline earth metal ions such as magnesium and calcium are selectively eliminated using a 2.0 M ammonium acetate eluent (pH 5.5). Next, the concentrated transition metals are eluted in a 100 to 200-µL volume to the ICAP. Finally, the MetPac CC-1 is converted to the ammonium form with 2.0 M ammonium acetate eluent. Figure 1 shows a typical time scan of trace metal analysis of cadmium by IC/ICAP.

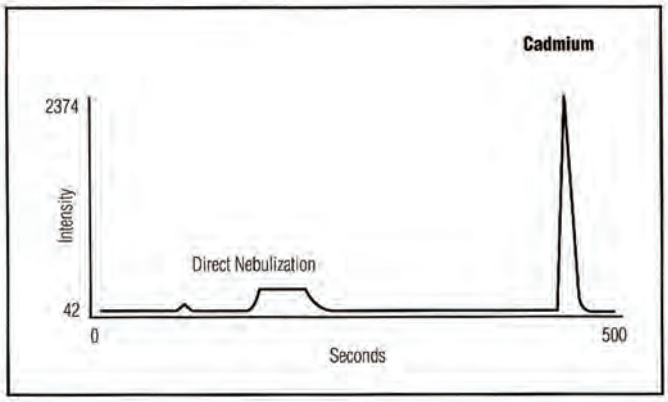

Figure 1 A typical scan of trace metal analysis by IC/ICAP

Tables 1 and 2 show the results of typical trace metals and concentrations found in trace metal grade concentrated nitric acid and hydrochloric acid. These samples were buffered with saturated ammonium hydroxide/6.0 M ammonium acetate. The results of the analysis of buffered blank solutions were used to correct the amount of trace metals in acid samples.

Spike/recovery of trace metals in the ppb range in acid matrix was evaluated. Tables 3 and 4 list the percent recoveries of trace metals in concentrated nitric acid and phosphoric acid.

Table 5 gives the trace metal detection limits attained by IC/ICAP with 5-mL sample concentrations.

Table 1 Typical trace transition metals and concentrations found in trace metal grade nitric acid

Element	Buffer Blank (ppm)	Buffered HNO_3 (ppm)	Blank Corrected (ppm)
Lead	0.0013 ± 0.001	0.0056 ± 0.0016	0.0043
Cadmium	0.0028 ± 0.0007	0.0034 ± 0.0002	0.0006
Copper	0.0001 ± 0.0003	0.0032 ± 0.0002	0.0031
Cobalt	–0.0001 ± 0.0000	0.0004 ± 0.0002	0.0004
Zinc	0.0001 ± 0.0000	0.0012 ± 0.0002	0.0011
Nickel	0.0006 ± 0.0002	0.0024 ± 0.0002	0.0018
Manganese	–0.0009 ± 0.0001	0.0006 ± 0.0004	0.0006
Iron	–0.0065 ± 0.0006	–0.0026 ± 0.0010	ND
Aluminum	–0.0167 ±0.0005	0.0082 ± 0.0010	0.0082

Table 2 Typical trace transition metals and concentrations found in trace metal grade hydrochloric acid

Element	Buffer Blank (ppm)	Buffered HCl (ppm)	Blank Corrected (ppm)
Lead	0.0013 ±0.0001	0.0075 ±0.0005	0.0062
Cadmium	0.0028 ±0.0007	0.0047 ±0.0002	0.0019
Copper	0.0001 ±0.0003	0.0024 ±0.0008	0.0023
Cobalt	–0.0001 ±0.0000	0.0008 ±0.0002	0.0008
Zinc	0.0001 ±0.0000	0.0133 ±0.0006	0.0132
Nickel	0.0006 ±0.0002	0.0127 ±0.0004	0.0121
Manganese	–0.0009 ±0.0001	0.0039 ±0.0006	0.0039
Iron	–0.0065 ±0.0006	0.0562 ±0.0091	0.0562
Aluminum	–0.0167 ±0.0005	0.0543 ±0.0096	0.0543

Table 3 % Recovery of trace transition metals in 30% nitric acid

Element	Conc. (ppm)	Spike	Found (ppm)	% Recovery
Lead	0.0010 ±0.0010	0.0500	0.0510 ±0.0018	100
Cadmium	0.0009 ±0.0002	0.0200	0.0194 ±0.0004	93
Copper	0.0009 ±0.0002	0.0200	0.0212 ±0.0002	101
Cobalt	0.0006 ±0.0002	0.0200	0.0207 ±0.0002	100
Zinc	0.0013 ±0.0002	0.0200	0.0214 ±0.0003	101
Nickel	0.0000 ±0.0001	0.0200	0.0208 ±0.0001	104
Manganese	0.0012 ±0.0002	0.0200	0.0206 ±0.0003	97
Iron	0.0035 ±0.0006	0.0500	0.0473 ±0.0034	88
Aluminum	0.0074 ±0.0011	0.0500	0.0579 ±0.0022	101

Table 4 % Recovery of transition metals in 30% phosphoric acid

Element	Conc. (ppm)	Spike	Found (ppm)	% Recovery
Lead	0.0043 ±0.0020	0.0500	0.0534 ±0.0017	98
Cadmium	0.0214 ±0.0002	0.0200	0.0412 ±0.0005	100
Copper	0.0011 ±0.0001	0.0200	0.0210 ±0.0001	100
Cobalt	0.0007 ±0.0002	0.0200	0.0207 ±0.0002	100
Zinc	0.0961 ±0.0006	0.0200	0.1146 ±0.0006	99
Nickel	0.0130 ±0.0006	0.0200	0.0327 ±0.0006	99
Manganese	0.0023 ±0.0004	0.0200	0.0215 ±0.0003	96
Iron	0.0810 ±0.0006	0.0500	0.0193 ±0.0005*	15*
Aluminum	ND*	0.0500	ND*	ND*

*Fe and Al are poorly retained on the MetPac CC-1 in samples containing a high concentration of phosphate.

Table 5 Detection limits by IC/ICAP with 5-mL sample concentration

Element	Conc. (ppb)*
Lead	2.0
Cadmium	0.2
Copper	0.2
Cobalt	0.2
Zinc	0.2
Nickel	0.2
Manganese	0.4
Iron	0.8
Aluminum	0.8

*Estimate based on 2 x standard deviation.

Determination of Trace Metals in Water Miscible Organic Solvents by Ion Chromatography/Inductively Coupled Argon Plasma Spectroscopy (IC/ICAP)

INTRODUCTION

The direct analysis of organic solvents such as low molecular weight alcohols and acetonitrile by inductively coupled argon plasma spectroscopy (ICAP) requires significant changes in the operating parameters of ICAP. In addition, calibration standards made in the same base solvent are required. Due to the high solvent loading of the plasma, high power levels are often required to maintain a stable plasma. Analytical results tend to drift over time because of the varying vapor pressure as a function of ambient temperature. Cooled spray chambers are often employed to minimize this effect.

A method for determining trace metals in water miscible organic solvents has been developed. The method uses a sol-vent compatible MetPac™ CC-1 chelating column to extract the metals of interest from the organic solvent. The MetPac CC-1 selectively retains the transition metals and allows the solvent matrix to go to waste. The concentrated metals are eluted with a nitric acid eluent and delivered to the plasma. As a result, this technique normalizes the matrix from the organic solvent to that of normal dilute acid solutions in water. Normally, cali-bration standards are made up in the solvent (matrix matching) before analytical measurements of the unknown samples. The IC system eliminates the solvent matrix and the trace metals are extracted into an aqueous environment. As a result, IC eliminates the need to change the operating parameters of the ICAP when changing from aqueous to organic samples.

Table 1 SEMI Standard for Water Miscible Organic Solvents

Element	Methanol	Acetone	Propanol
Copper	0.1 ppm max.	0.1 ppm max.	0.1 ppm max.
Iron	0.1	0.1	0.1
Lead	0.1	0.1	0.1
Manganese	0.1	0.1	0.1
Nickel	0.1	0.1	0.1
Zinc	0.1	0.1	0.1

Source: SEMI BOSS 1990

This method can also be applied to determining selected transition elements in organic solvents used in semiconductor fabrication. The SEMI (Semiconductor Equipment and Materials International) guidelines for maximum allowable concentrations of selected transition metals in several water miscible solvents are listed in Table 1.

EQUIPMENT

Dionex Advanced Gradient Pump (AGP)
Dionex Sample Concentration Module (SCM)
Thermo Jarrell Ash simultaneous ICAP spectrometer
IBM® or 100% compatible computer with TJA ThermoSpec™ software

REAGENTS

Ultrapure 2.0 M ammonium acetate, pH 5.5 (1 L, P/N 33440; 6 L, P/N 33441)

Ultrapure 2.0 M nitric acid (1 L, P/N 33442; 6 L, P/N 33443)

20% Ultrapure ammonium hydroxide

Ultrapure glacial acetic acid

Ultrapure concentrated nitric acid

The first two reagents used for chelation concentration are available from Dionex in a ready-to-use form. If you wish to prepare your own reagent solutions, information for ordering ultrapure acids and ammonium hydroxide are given below in *Preparation of Solutions and Reagents*.

SAMPLE PREPARATION

Since trace metal grade organic solvents contain very low concentrations of metal contaminants, dilution of the sample must be minimized. Undiluted samples can be introduced directly into the IC without sample modification. If sample modification (e.g., dilution, buffering) is required, the sample must be prepared in clean polyethylene containers. Avoid using pipets and glassware that may contaminate the samples. It is strongly recommended to acidify the organic solvent sample to stabilize the trace metals.

PREPARATION OF SOLUTIONS AND REAGENTS

Three concentrated reagents are required for eluents in chelation concentration: nitric acid, acetic acid, and ammonium hydroxide. For ultratrace level determinations (sub ppb), the reagents must be ultrapure grade. For determination above 1 ppb, high quality trace metal grade reagents can be used. Any metal impurity in these reagents will be concentrated with the sample, constituting a system blank.

2.0 M Ammonium Acetate pH 5.5 ± 0.1

If Dionex ultrapure 2.0 M ammonium acetate is used, no further preparation is required. Otherwise, place 600 mL of deionized or high purity (18 MΩ) water into a clean 1-L glass eluent container. Add 121 g (115 mL) of ultrapure glacial acetic acid and mix thoroughly. In a fume hood, slowly add 120 g (130 mL) of 20% ultrapure ammonium hydroxide and mix thoroughly. Agitate the bottle to thoroughly mix the solution. Adjust the pH of the ammonium acetate to 5.5 ± 0.1 using acetic acid if the pH is greater than 5.5, or ammonium hydroxide if the pH is less than 5.5. Once the pH is 5.5 ± 0.1, bring to a volume of 1 L.

2.0 M Nitric Acid

If Dionex ultrapure 2.0 M nitric acid is used, no further preparation is required. Otherwise, place 200 mL of deionized or high purity (18 MΩ) water into a clean 1-L glass eluent container. Add 179 g (126 mL) of ultrapure nitric acid. Add deionized water to bring the final volume to 1 L and mix thoroughly.

0.1 M Nitric Acid—Carrier Solution

Place about 1000 mL of deionized water into the 4-L plastic eluent container. Add 89 g (63 mL) of concentrated nitric acid; then, add deionized water to bring the final volume to 4 L and mix thoroughly.

The ultrapure reagents are manufactured by Seastar Chemical and Ultrex Reagents. Seastar reagents are available internationally from Fisher Scientific, who sells these reagents under the OPTIMA® label. Ultrex reagents are available internationally through J.T. Baker.

STANDARD PREPARATION

Since the analytes of interest must be concentrated, the concentration of the "high" standard should not exceed 1 ppm. Typically, a working "high" standard in the range of 5 to 100 ppb is used for ultratrace analysis work. It is convenient to prepare a 10X concentrate or stock solution of the standard and prepare the high standard by dilution of the stock solution.

SYSTEM PREPARATION

Modification of the standard SCM (P/N 42134) is required for this application. The 1-mL sample loop that normally provides a portion of the raw or unprocessed sample to the nebulizer is replaced with a 10-µL loop (P/N 42949).

For complete details in system preparation and setup, operation, and automation, refer to Dionex Technical Note No. 28: "IC/ICAP: A New Technique for Trace Metal Determinations".

DISCUSSION OF THE METHOD

The method described here has been applied to 100% denatured ethanol, 100-proof distilled spirits, methanol, and acetonitrile. The organic solvent samples containing 1%

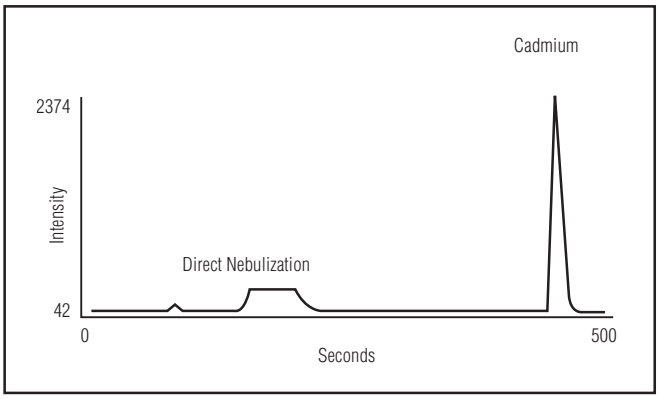

Figure 1 A typical time scan of trace metal analysis of cadmium by IC/ICAP

nitric acid to stabilize the trace metals are automatically buffered with 2.0 M ammonium acetate prior to the loading onto the column. The trace metals are desorbed from the column by 1.5 M nitric acid. The output of the SCM is connected directly to the nebulizer.

The gradient program described in Technical Note 28 for the standard configuration may be used for this application. If desired, the amount of time allocated for loading the sample may be shortened since the 1-mL loop for the direct nebulization run is not required. Also, the amount of time used to wash the column with the ammonium acetate buffer to eliminate alkaline earth elements may be shortened if these elements are not at high concentrations in the solvent.

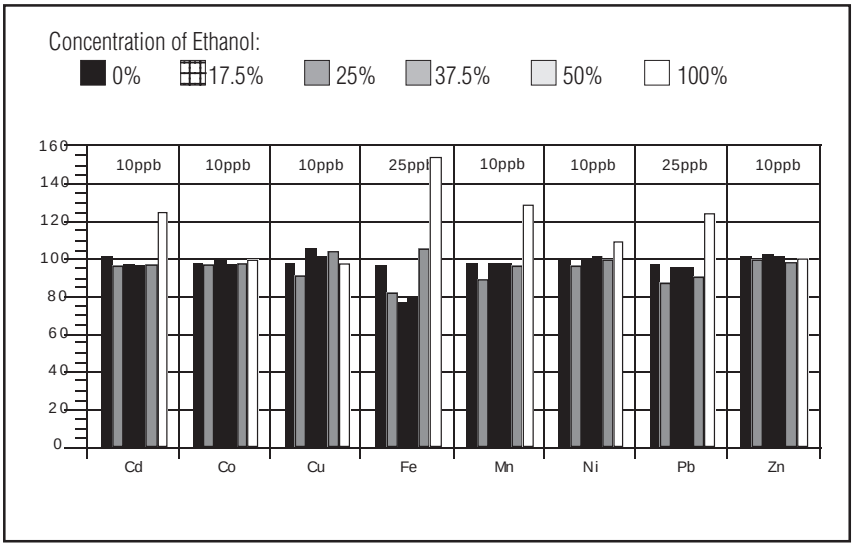

Figure 2 Graphic plot of recovery versus varying concentration of ethanol

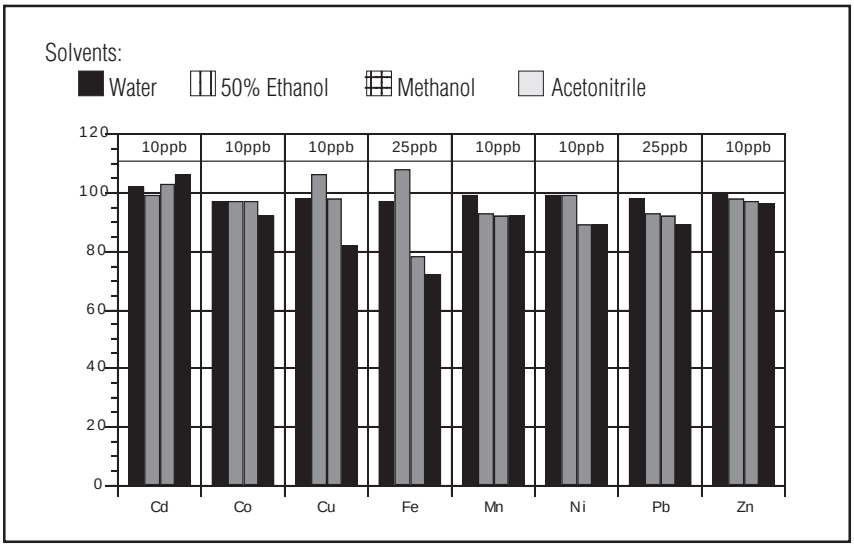

Figure 3 Sample spiking levels as in Figure 2 for different solvents

Chelation Concentration

A MetPac CC-1 chelating resin has very high affinity for transition metals compared to the alkali and alkaline earth metals. The resin does not concentrate anions such as the halides, nitrate, sulfate, phosphate, or organic anions. Thus, transition metals can be quantitatively concentrated from high concentrations of anions, alkali and alkaline earth elements. The chelation concentration process consists of four steps. A known volume of the sample is concentrated on the MetPac CC-1. Most polyvalent cations are quantitatively concentrated while anions pass through the column. Weakly bound alkaline earth metal ions such as magnesium and calcium are selectively eliminated using a 2.0 M ammonium acetate eluent (pH 5.5). Next, the concentrated transition metals are eluted in a 100 to 200-µL volume to the ICAP. Finally, the MetPac CC-1 is converted to the ammonium form with 2.0 M ammonium acetate eluent. Figure 1 shows a typical time scan of trace metal analysis of cadmium by IC/ICAP.

All analytical results presented in this application note are based upon aqueous calibration standards. All data relating to recovery reflect this fact. Figure 2 shows a graphic plot of recovery versus varying concentrations of ethanol. The concentrations of ethanol varied from 0% on the left to 100% on the right side of the block for each ele-

Table 2 Analysis of 100-proof ethanol		
Element	Average	Std. Dev.
Cadmium	0.0002	0.0001
Cobalt	0.0003	0.0002
Copper	0.0017	0.0002
Manganese	0.0005	0.0005
Nickel	0.0002	0.0003
Lead	0.0009	0.0008
Zinc	0.0005	0.0005

All units in ppm/4 replicates

Table 3 Analysis of methanol solvent		
Element	Average	Std. Dev.
Cadmium	0.0002	0.0002
Cobalt	0.0003	0.0001
Copper	0.0014	0.0002
Manganese	0.0005	0.0004
Nickel	0.0002	0.0003
Lead	0.0007	0.0006
Zinc	0.0004	0.0001

All units in ppm/4 replicates

ment. All compositions below 100% were prepared from the distilled spirits. The 100% composition was obtained from denatured ethanol reagent. The result shows that the 100% ethanol peaks are higher than the others, indicating potential contamination effects from the ethanol reagent. Also, since the reagent contained a denaturing agent that is not totally water soluble, residual organic may be present in the portion being eluted to the plasma. Spike recoveries for all the solutions prepared from the ethanol spirits were close to 100%. This indicates that the MetPac CC-1 chelating column was efficient in retaining the transition metals. The only element that appeared to exhibit poor recovery was iron. The spike concentration was 0.010 ppm for all elements except lead and iron, which were spiked at levels of 0.025 ppm.

Figure 3 shows the sample spiking levels as in Figure 2 for different solvents. The results indicate that the chelation concentration is effective in extracting the transition metals from these common matrices. Iron, however, exhibits erratic behavior as in Figure 2.

Note that the spiking levels of these elements are very close to the detection limits obtained from normal sample introduction with a pneumatic nebulizer. The spiking level for lead at 0.025 ppm was at the detection limit. The use of the chelation concentration sample pretreatment not only eliminates the sample matrix but retains all the benefits of preconcentration. To estimate the limits of detection that may be obtained, Tables 2–4 show data for the analyses of the original solvents without any spikes. The standard deviations obtained may be scaled to a specific definition of detection limit.

Table 4 Analysis of acetonitrile		
Element	Average	Std. Dev.
Cadmium	0.0003	0.0002
Cobalt	0.0001	0.0001
Copper	0.0010	0.0004
Manganese	0.0008	0.0004
Nickel	0.0001	0.0002
Lead	0.0004	0.0009
Zinc	0.0002	0.0001

All units in ppm/4 replicates

Table 5 Low level spike recovery in 50% ethanol		
Element	Average	Std. Dev.
Cadmium	0.0011	0.0001
Cobalt	0.0003	0.0002
Copper	0.0014	0.0006
Iron	0.0026	0.0008
Manganese	0.0005	0.0006
Nickel	0.0002	0.0003
Lead	0.0007	0.0011
Zinc	0.0004	0.0001
Vanadium	0.0008	0.0001

All units in ppm/4 replicates
All elements at 0.001 except Fe and Pb which are 0.0025

Determination of Transition Metals in Complex Matrices Using Chelation Ion Chromatography

INTRODUCTION

Chelation ion chromatography facilitates the determination of low concentrations (µg/L and lower) of transition metals in samples including seawater, brines, estuarine waters, and a variety of biological samples. These types of samples are characterized by high concentrations of alkali and alkaline earth metals that can interfere with many spectroscopic techniques for metal determinations. Chelation ion chromatography removes alkali and alkaline earth metals while concentrating the sample, then determining the analytes of interest. The principle of chelation concentration and matrix elimination is described in Dionex Technical Note 25; Determination of Transition Metals in Complex Matrices by Chelation Ion Chromatography in the summary of the Method and Discussion of the Method sections. TN 25 Table I reports that metals that can be concentrated using chelation concentration, and Table II shows a step-by-step summary of chelation ion chromatography chemistry. Here, the authors simplify the system configuration described in TN 25, using an ICS-3000 system. The separation has also been updated to include the IonPac® CS5A/CG5A column set. The CS5A column demostrates improved selectivity and peak efficiency for seapartion of transition metals compared to the CS5.

In Application Note 131, a 2 mm CS5A is used for transition metal determinations at sub-µg/L levels. The IonPac CS5A is available in 2 and 4 mm column formats; this application update supports both. The successful analysis of transition metals in a seawater sample confirms the updated chelation ion chromatography configuration reported here.

EQUIPMENT

ICS-3000 system consisting of the following modules and accessories:

- DP Dual pump (Gradient/Isocratic) (P/N 061710)
- TC Thermal Compartment; one 6-port valve, one 10-port valve (P/N 064651)
- VWD Variable wavelength absorbance detector (P/N 064377)
- Absorbance cell, 11 µL, PEEK® (P/N 066346)
- EO eluent organizer with four 2 L bottles (P/N 062628)
- AS (P/N 063120) or AS-HV (P/N 064051) Autosampler
- PC10 postcolumn pneumatic delivery system 2 mm (P/N 053591), or 4 mm (P/N 050601)
- AXP pump (P/N 064507)
- 4 L plastic container (P/N 063292)
- Chromeleon® 6.8 Data Management System software

CONSUMABLES

MetPac™ CC-1 concentrator column, pkg. of 2 (P/N 042156)

TMC-1 concentrator column (P/N 049000)

IonPac CG5A (4 mm) (P/N 046104) or 2 mm (P/N 052836)

IonPac CS5A (4 mm) (P/N 046100) or 2 mm (P/N 052576)

$^{1}/_{16}$" o.d. × 0.010 i.d. PEEK (black) tubing (P/N 052306 for 5 feet) 10 feet (300 cm)

$^{1}/_{16}$" o.d. × 0.030 i.d. PEEK (green) tubing (P/N 44777–per inch) 40 feet (1.2 m). Approximately 225 cm green tubing is required for every mL of sample loop.

Fitting, 10-32 bolt, (P/N 062980)

Fitting, ferrule, double cone, (P/N 043276)

Mixing tee, 3-way (10-32), 2, (P/N048227)

REAGENTS AND STANDARDS

Deionized water (DI), Type I reagent grade, 18.2 MΩ-cm resistivity or better.

Atomic absorption spectroscopy metal standards: iron (III), copper, nickel, zinc, cobalt, cadmium, and manganese, traceable to NIST reference materials (e.g. Sigma-Aldrich Iron Atomic Absorption Standard Solution, P/N 305952).

Nitric acid, 2.0 M, 6 × 1L (P/N 033443) or ultrapure grade equivalent

Ammonium acetate, 2.0 M, 6 × 1 L (P/N 033441) or ultrapure grade equivalent

Ammonium nitrate, 0.1 M, pH 3.5, 6 × 1 L (P/N 033445)

MetPac PAR postcolumn diluents, 1 L (P/N 046094) or prepared as described in TN 25

Pryridine-2,6-dicarboxylic acid (PDCA), 20 g (P/N 039671)

(4-pyridylazo) resorcinol (PAR), monosodium monohydrate, 5 g, (P/N 039672)

Formic acid (ACS grade)

Potassium hydroxide (ACS grade)

Potassium chloride (ACS grade)

CONDITIONS

Guard Column:	IonPac CG5A, 4 mm (P/N 046104) or 2 mm (P/N 052836)
Column:	IonPac CS5A, 4 mm (P/N 046100) or 2 mm (P/N 052576)
Trap Column:	MetPac CC-1 concentrator column (2) (P/N 042156, pkg of 2) TMC-1 concentrator column (P/N 049000)
Eluent:	6 mM PDCA, 96 mM KOH, 94 mM formic acid and 10 mM KCl
Flow Rate:	1.2 mL/min for 4 mm or 0.30 mL/min for 2 mm
Sample Volume:	5 mL for 4 mm or 1.0 mL for 2 mm
Column Oven:	30 ºC for 2 mm or 40 ºC for 4 mm
Pressure:	1100–1300 psi
Detection:	Absorbance: 520 nm, after postcolumn derivatization with PAR
Postcolumn Flow:	0.5 mL/min (4 mm) or 0.12 mL/min (2 mm)

PREPARATION OF SOLUTIONS AND REAGENTS

See Appendices A and B of Technical Note 25 for additional information on preparation of reagents and standards.

All prepared standards must be acidified to pH 1–2 to avoid formation of metal oxides. The authors strongly recommend that all reagents used be obtained from Dionex; alternately, obtain ultrapure grade reagents from other sources. Contamination can originate from the container used to store the prepared standard and this contamination can increase with storage. Common contaminants such as Fe (III) and Zn should be maintained at sub-µg/L concentrations. The MetPac CC-1 is used to maintain an adequate blank from the ammonium acetate. This column must be regenerated periodically using 20 mL of 2.0 M nitric acid.

PREPARATION OF PDCA ELUENT

Add 5.4 g of KOH to 200 mL DI water in a 1 L volumetric flask. Add 1.0 g PDCA while stirring until completely dissolved. Add 5.25 mL formic acid (>99%) and 500 mL of DI water while stirring. Add 0.75 g of KCl and measure pH. Adjust the eluent pH with KOH or formic acid to 4.2. Bring to volume with DI water.

SYSTEM SETUP

Use DP pump 2 (gradient) for delivery of the chelation reagents, and the DP pump 1 (isocratic) for the analytical separation. The inline degasser is not recommended for use with this application and should be bypassed. To accomplish this, connect the eluent lines directly to the DP proportioning valve or to DP pump inlet. The AXP pump delivers ammonium acetate for matrix elimination and buffering.

1. Install columns and postcolumn reagent as described in the ICS-3000 manual.
2. Connect DP pump 2 to the 10-port valve port #10 with an appropriate length of 0.010" i.d. PEEK (black) tubing.
3. Connect port #8 and #9 with an appropriate length of 0.010" i.d. PEEK (black) tubing.
4. Install a sample loop between port #4 and #7 (5 mL for 4 mm or 1 mL for 2 mm).
5. Connect port #5 and #6 to the autosampler inlet and outlet lines respectively.
6. Connect the MetPac CC-1 column on the AXP pump outlet line. This column is used for ammonium acetate purification.
7. Connect the MetPac CC-1 column outlet to the tee with an appropriate length of 0.010" i.d. PEEK (black) tubing.
8. Connect port #3 to one of the two remaining ports of the tee with an appropriate length of 0.010" i.d. PEEK (black) tubing.
9. Connect the remaining port of the tee to the second MetPac CC-1 column inlet with an appropriate length of 0.030" i.d. PEEK (green) tubing. Place the column inside the TC compartment.
   Using the second tee, connect one port to 10-port valve port #1, one to the second MetPac CC-1 column outlet, and the third outlet to 6-port valve port #5 with an appropriate length of 0.030" i.d. (PEEK) (green) tubing.
10. Plug the 10-port valve port # 2 with a 10-32 end fitting. This is represented in Figure 1 by the magenta circle marked with an X.
11. Install a TCC-1 concentrator between ports #1 and #4 of the 6-port valve.

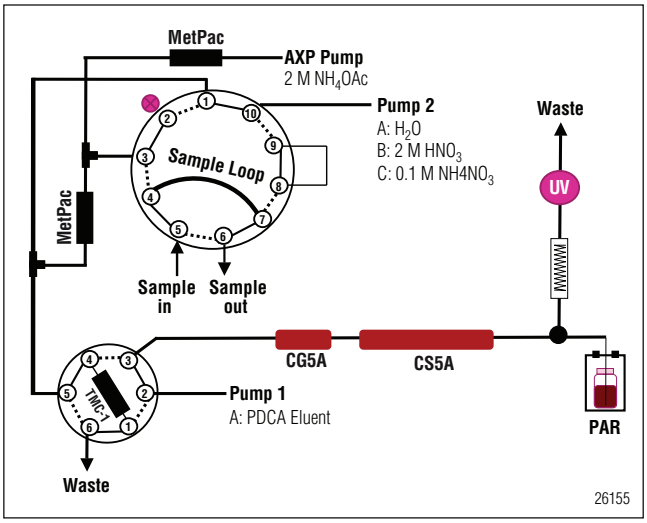

Figure 1. Chelation IC system schematic.

CHELATION IC STEPS

A total of three pumps and two valves are required to deliver the necessary reagents and change flow directions in the Chelation IC process (Figure 1). Refer to Tables 1 and 2 for the Chromeleon program steps for 5 mL (4 mm column set) and 1 mL (2 mm column set) sample loading.

1. ***Sample Loading and MetPac Conditioning.*** The AXP delivers ammonium acetate directly to the MetPac CC-1 at 2 mL/min. While the 10-port valve is in the A position and the 6-port is in the Inject position, the DP pump 2 flushes ports #10 and #1 with DI water at 2.0 mL/min. DP pump 1 delivers PDCA to the analytical system through the TMC-1.
2. ***Chelation Concentration and Matrix Elimination.*** The 10-port valve switches to B position. The DP pump 2 flushes sample from the loop with DI water. The sample stream combines with ammonium acetate and the solution flows into the MetPac CC-1 and out to waste through the 6-port valve. The AXP pump stops at the end of this step.
3. ***Water Rinse.*** Without valve switching, the DP pump 2 continues to flush the MetPac CC-1 with DI water to remove excess ammonium acetate.
4. ***Metal Elution (Acid Elution).*** The 6-port valve switches to Load position, the DP pump 2 pumps a solution of 75% water and 25% nitric acid to the MetPac CC-1 and out to the TMC-1 concentrator. Metals are removed from the MetPac CC-1 and trapped on TMC-1 concentrator.

Table 1. Valve Control Program for a 5 mL Injection Loop

Time	DP2*	10-port	6-port	AXP*	Comments
Load	Motor on	A	Inject	OFF	Autosampler loading time
–23.0	water	A	Inject	ON	Equilibration
–22.0	water	B	—	—	Start Chelation step
–14.0	water	—	—	OFF	Start water rinse
–13.0	water	—	—	—	Stop water rinse
–12.9	25% HNO_3	—	Load	—	Start acid elution
–5.00	25% HNO_3	—	—	—	Stop acid elution
–4.90	NH_4NO_3	A	—	—	Start TMC-1 conversion
0.00	NH_4NO_3	—	Inject	—	Begin analysis
0.10	50% HNO_3	B	—	—	MetPac CC-1 cleanup
5.00	50% HNO_3	—	—	—	MetPac CC-1 cleanup
5.10	water	A	—	—	Water rinse
10.0	Motor off	—	—	—	End chelation steps
15.0	End program				

*DP2 and AXP flow rates: 2 mL/min.

Table 2. Valve Control Program for a 1 mL Injection Loop

Time	DP2*	10-port	6-port	AXP*	Comments
Load	Motor on	A	Inject	OFF	Autosampler loading time
–16.0	water	A	Inject	ON	Equilibration
–15.0	water	B	—	—	Start chelation step
–11.0	water	—	—	OFF	Start water rinse
–10.0	water	—	—	—	Stop water rinse
–9.90	25% HNO_3	—	Load	—	Start acid elution
–5.00	25% HNO_3	—	—	—	Stop acid elution
–4.90	NH_4NO_3	A	—	—	Start TMC-1 conversion
0.00	NH_4NO_3	—	Inject	—	Begin analysis
0.10	50% HNO_3	B	—	—	MetPac CC-1 cleanup
5.00	50% HNO_3	—	—	—	MetPac CC-1 cleanup
5.10	water	A	—	—	Water rinse
10.0	Motor off	—	—	—	End chelation steps
15.0	End program				

*DP2 and AXP flow rates: 2 mL/min.

5. ***TMC-1 Conversion.*** The 10-port valve switches to A position and the DP pump 2 switches to ammonium nitrate. The TMC-1 in acid form converts to the ammonium form during this time. The 6-port valve switches to the Inject position at the end of this step to begin separation of metals.

RESULTS AND DISCUSSION

Chelation Concentration and Matrix Elimination

The chelation concentration and matrix elimination conditions in this application update (Tables 1 and 2) have been optimized for either 5 mL (4 mm column) or 1 mL (2 mm column) of seawater or brine. Larger volumes or samples with high concentrations of alkali and alkaline earth metals will require a larger ammonium acetate rinse volume. However, be aware that increasing the ammonium acetate rinse may affect the analytical blank. A typical blank run using the 4 mm column set is shown in Figure 2. The concentrations of Fe(III) and Zn(II) are both <1 µg/L. All data shown in this application update are with the 4 mm column set. The major benefits of using the 2 mm column set are reduction in eluent consumption and improved mass sensitivity. The latter can be significant if sample sizes are limited, which can be true for biological samples, but not typically for seawater and brine samples.

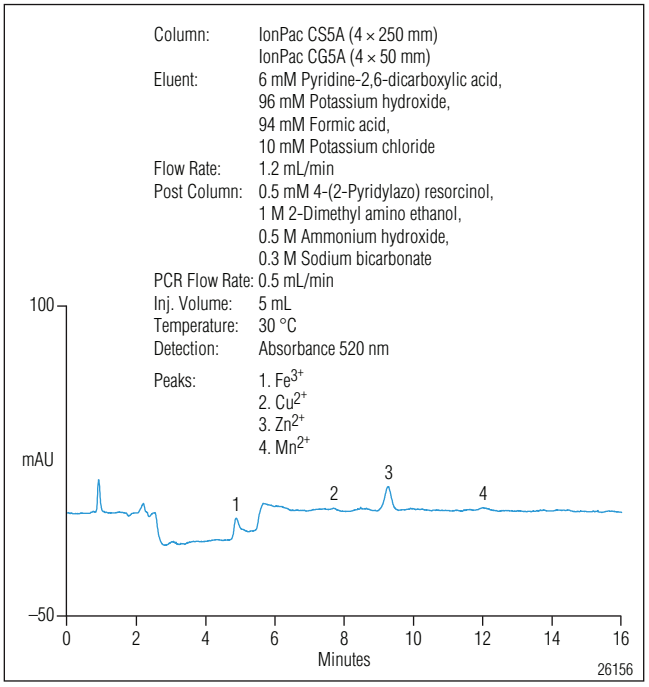

Figure 2. Typical blank chromatogram obtained from a 5 mL injection (4 mm IonPac CS5A column set).

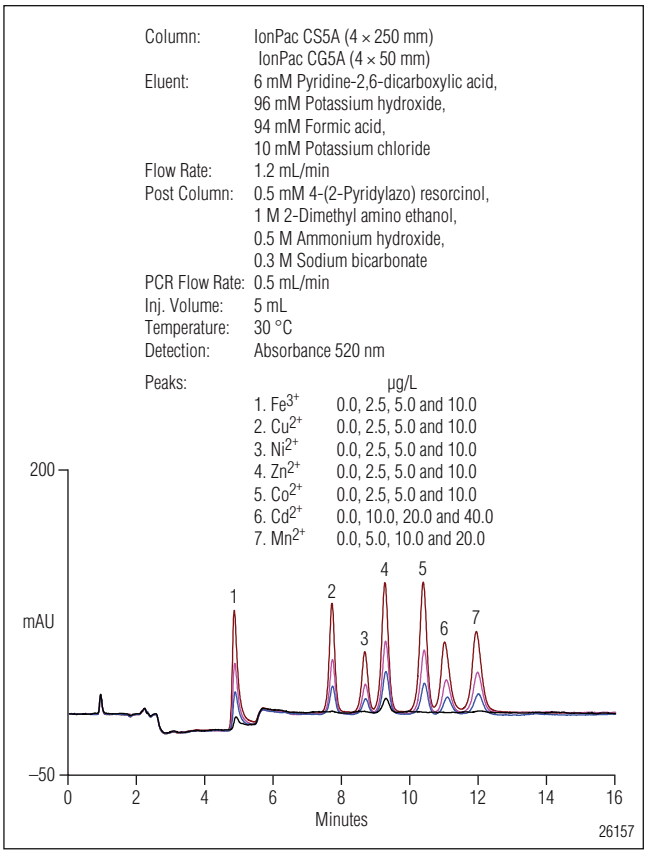

Figure 3. Overlay of chromatograms of the blanks and three standard concentrations (4 mm column set). Concentrations are shown in Table 3A.

Table 3A. Standard Concentrations

Peak Name	Concentration (µg/L) Level 1 (blank)	Level 2	Level 3	Level 4
Fe^{3+}	0.0	2.5	5.0	10.0
Cu^{2+}	0.0	2.5	5.0	10.0
Ni^{2+}	0.0	2.5	5.0	10.0
Zn^{2+}	0.0	2.5	5.0	10.0
Co^{2+}	0.0	2.5	5.0	10.0
Cd^{2+}	0.0	10.0	20.0	40.0
Mn^{2+}	0.0	5.0	10.0	20.0

Table 3B. Calibration Report from Chromeleon

Peak Name	Cal. Type	Points	R-square * 100	Offset	Slope
Fe^{3+}	LOff	4	99.8428	1.9046	1.6481
Cu^{2+}	LOff	4	99.9846	0.1225	1.6689
Ni^{2+}	LOff	4	99.8911	–0.0036	0.9831
Zn^{2+}	LOff	4	99.9977	11.6026	9.2632
Co^{2+}	LOff	4	99.9983	–0.3297	2.5727
Cd^{2+}	LOff	4	99.7806	–0.1697	0.3811
Mn^{2+}	LOff	4	99.9933	0.1281	0.9987

Metal Separation

After being trapped on the MetPac CC-1 the transition metals are eluted onto the TMC-1 with nitric acid. The TMC-1 is then converted from acid to salt form. The metals elute from the TMC-1 and separated on the IonPac CS5A column set using PDCA eluent. The composition of the PDCA used in this application update differs from the standard PDCA eluent used for transition metal analysis by direct injection or after concentrating large volumes of ultrapure water as in AN 131. The PDCA eluent composition has been adjusted for higher buffering capacity to compensate for the ammonium and residual hydronium from the TMC-1. Because the authors used the same size TMC-1 with the 2 mm column set that was used with the the 4 mm column set, it is also necessary to adjust the PDCA eluent composition due to the lower capacity of the 2 mm CS5A compared to the 4 mm. This adjustment is unnecessary if the column temperature is increased from 30 °C to 40 °C.

Calibration

Figure 3 shows an overlay of the chromatograms of the blank and three standards (Table 3A). Fe(III), Cu(II), Ni(II), Zn(II), Co(II), Cd(II), and Mn(II) are baseline resolved in under 15 min. The four-point calibration from 0 to 10 µg/L (blank included) shows that the analyte response is linear in this range (Table 3B). Note the offsets for Fe(III) and Zn(II) due to the presence of these metals in the blank. The sensitivity of this method ultimately depends on establishing a low blank. AN 131 demonstrated that this separation and detection method can achieve double-digit ng/L sensitivity, however, in that example, complex sample matrices such as seawater or brine were not analyzed. The reagents used in chelation IC—ammonium acetate, nitric acid, and ammonium nitrate —are additional sources of possible metal contamination.

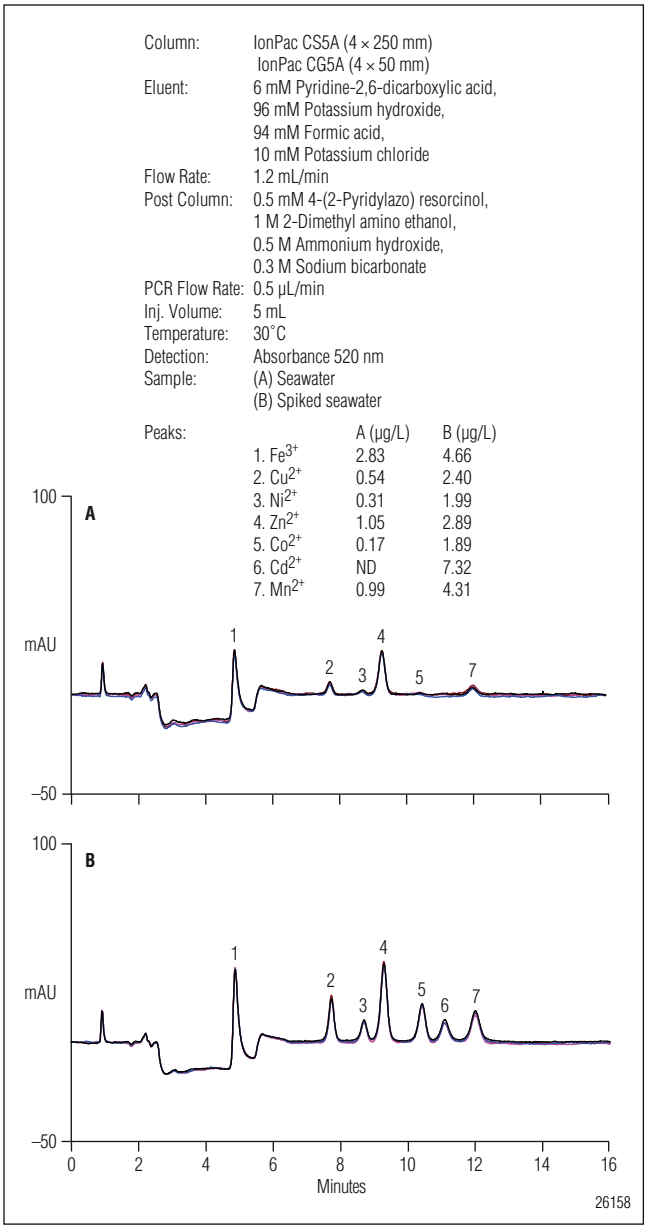

Figure 4. Analysis of seawater (A), and spiked seawater (B) using chelation ion chromatography with 5 mL loop and IonPac CS5A 4 mm column set (Overlay of 4 injections).

Table 4A. Quantification and Spike Recovery of Transition Metals in Seawater Sample

Sample Name	Sample (µg/L)	Spike (µg/L)	Sample + Spike (µg/L)		Calculated % Recovery
			Found	Expected	
Fe^{3+}	2.83	2	4.66	4.83	96.50
Cu^{2+}	0.54	2	2.40	2.54	94.63
Ni^{2+}	0.31	2	1.99	2.31	85.84
Zn^{2+}	1.05	2	2.89	3.05	94.75
Co^{2+}	0.17	2	1.89	2.17	87.29
Cd^{2+}	ND	8	7.32	8.00	91.48
Mn^{2+}	0.99	4	4.31	4.99	86.45

Table 4B. Method Precision Using 5 mL Spiked Seawater Injections and 4 mm IonPac CS5A Column

	Amount (µg/L)						
Sample #	Fe^{3+}	Cu^{2+}	Ni^{2+}	Zn^{2+}	Co^{2+}	Cd^{2+}	Mn^{2+}
1	4.55	2.34	1.98	2.85	1.89	7.95	4.57
2	4.68	2.27	1.89	2.82	1.89	6.76	4.37
3	4.70	2.40	2.15	2.88	1.87	7.60	3.77
4	4.73	2.60	1.92	3.01	1.90	6.97	4.53
Average	*4.66*	*2.40*	*1.99*	*2.89*	*1.89*	*7.32*	*4.31*
RSD	*1.71*	*5.87*	*5.95*	*2.97*	*0.60*	*7.49*	*8.54*

Sample Analysis

After calibration, the chelation IC system was used to analyze a seawater sample collected from the Gulf of Thailand for transition metals (the sample was adjusted to pH 2.0 with nitric acid upon collection.) Figure 4A shows an overlay of the chromatograms of four consecutive 5 mL seawater injections. Six of the seven metals in the standard were detected in the samples at concentrations < 3 µg/L. The concentrations detected are shown in the second column of Table 4A. To evaluate the accuracy of the method the authors spiked the samples with 2 µg/L of each of five metals, 8 µg/L Cd(II), and 4 µg/L Mn(II). Chromatography of four consecutive injections of the spiked samples is shown in Figure 4B and Table 4A. Table 4B shows good method precision, with all metals recovered at >85%.

SUMMARY

This application update describes the setup of Chelation IC on the ICS-3000 system and provides methods for determination of transition metals analysis in seawater and brine samples. By effectively removing the high background interference, chelation IC facilitates the determination of low concentrations of transition metals in high-ionic strength samples.

RERERENCES

1. Dionex Corporation, *Determination of Transition Metals in Complex Matrices by Chelation Ion Chromatography*, Technical Note 25, LPN 034365, Sunnyvale CA, 1990.
2. Dionex Corporation, *Determination of Transition Metals in High-Purity Water and SC2 (D-clean) Baths*, Application Note 131, LPN 1058, Sunnyvale CA, 1998.

Automated Sample Preconcentration of Metals in Drinking Water for Inductively Coupled Argon Plasma (ICAP) Spectroscopy

INTRODUCTION

Ion exchange is a technique that has long been used for concentration and separation of trace metals. The documentation of ion exchange is extensive and its application for sample pretreatment prior to spectroscopic analysis is well known. Ion exchange offers a solution to detection limits commonly experienced when analyzing ultratrace concentration of metals in high purity water or drinking water by inductively coupled argon plasma (ICAP) spectroscopy. Using a simple form of mixed bed ion exchange resin, the analytes of interest, both anions and cations, can be concentrated from a relatively clean matrix. In this technical note, we describe a technique based on direct coupling of a sample preconcentration system (an ion chromatograph) to a simultaneous ICAP for trace metals in drinking water. This sample preconcentration method lowers the ICAP detection limits at least 50-fold for most metals.

EQUIPMENT

A sample preconcentration system comprising:

Advanced Gradient Pump (AGP, P/N 42144/115V; P/N 42145/220V)

Sample Concentration Module (SCM, P/N 42134/115V, P/N 42135/220V)

IC/ICAP Installation Kit (P/N 43169; contains eluent containers, air regulator, tubing, power cords and fittings for installation)

IonPac® CG5 (P/N 37029), 2 required

IonPac CG2 (P/N 35370), 3 required

Any Thermo Jarrell Ash (TJA) simultaneous ICAP instrument (Model 61, 61E, 1100, 9000) can be interfaced to the Dionex IC as long as the simultaneous spectrometer is used with an IBM or IBM-compatible computer with TJA ThermoSpec software. For system automation, a ThermoSpec-supported autosampler is required. A TJA type 22 or TJA 300 autosampler can be used. The autosampler should use the large sample racks (type 24) to ensure sufficient sample volume.

Questions concerning the compatibility of interfacing a particular TJA simultaneous ICAP instrument to a Dionex IC should be directed to your TJA sales or service representative.

REAGENTS

2.0 M Ultrapure Nitric Acid (1L, P/N 33442; 6 L, P/N 33443)

Atomic Absorption Standard (1000 ppm) for each metal of interest

ELUENTS AND STANDARD PREPARATION

Before preparing the eluent and standard, thoroughly clean the eluent containers as directed in "System Preparation", later in this technical note. Be sure that the eluent bottle caps have a white TFE seal, NOT a black rubber seal. The 2.0 M nitric acid is available in a ready-to-use form. If you wish to prepare your own solution, Optima grade reagents (Fisher Scientific), SeaStar Ultrapure Reagents (SeaStar Chemical), and Ultrex reagents (Van Waters and Rogers Scientific) can be used. For ultratrace level determination (sub-ppb), it is necessary to use ultrapure grade. Any metal impurity in the reagents will be concentrated with your sample constituting a system blank.

Eluent 1: Ultrapure Water

Eluent 2: 2.0 M Nitric Acid

If Dionex ultrapure reagent is used, no further preparation is required. Otherwise, place 200 mL of ultrapure water into a clean 1-L glass eluent container. Add 179 g (126 mL) of ultrapure nitric acid. Add water to bring the final volume to 1.0 L and mix thoroughly.

Carrier Solution: Ultrapure Water

Working standards can be prepared from 1000-ppm atomic absorption standard solutions. Since the analytes of interest are concentrated, the concentration of the high standard used should not exceed 1 ppm. It is convenient to first prepare a 10X concentrate or stock solution of the standard, and then prepare the high standard by dilution of the stock solution.

DISCUSSION OF THE METHOD

The method described in this technical note was developed to improve the ICAP detection limit of common metals present in relatively clean matrix samples (e.g. ultrapure water, drinking water). For complex matrices such as high ionic strength or high salt matrices, the chelation concentration sample pretreatment is strongly recommended (see Dionex Technical Note 28).

The column used for sample preconcentration, the IonPac CG5, which has both anion and cation exchange sites, contains a 13-µm latex agglomerated surface sulfonated polystyrene/divinylbenzene copolymer. The anion exchange latex is low crosslinked, aminated with a hydrophillic amine and has a diameter of 0.2 µm. The cation and anion exchange capacities are 30 microequivalents and 14 microequivalents per column, respectively. The resin can be used with acid or base up to 6 M without degradation.

The sample preconcentration process consists of two steps. First, the sample is passed through the column. Metal ions are concentrated in a tight band. Then, the concentrated metals are eluted off the column with 1.5 M nitric acid and the column is ready for the next run.

The sample pretreatment system allows two measurements per run. A typical ICAP time scan is shown in Figure 1. Using 10 mL sample concentration, the ICAP detection limit is lowered at least 50 fold for most metals. It is important to use reagents and water which have very low metal contamination. Any trace metals in the reagents will be concentrated as a "blank" and subsequently eluted with the sample. Care must be taken to minimize reagent and sample contamination during preparation and handling. Reagent purity will usually dictate the detection limits.

SYSTEM CONFIGURATION AND SET-UP

Figure 2 shows a detailed pneumatic and hydraulic schematic of the sample preconcentration system. The SCM is factory-configured for chelation concentration sample pretreatment. The following set-up procedure is required for this application.

Pneumatic Connections

Locate the four colored air tubings at the rear panel of the AGP and SCM. Using the small barbed couplers (P/N 42241), couple the air tubing together by matching the color (orange-orange, yellow-yellow, green-green and blue-blue). Next, connect about 2 ft (60 cm) of air tubing (P/N 30091) to the small barbed fitting on the back of the AGP. Insert a barbed tee (P/N 30538) into the end of this fitting. One arm of the tee will go to the nitrogen or argon source (regulator) and the other arm will go to the inlet of the eluent bottle regulator (P/N 38201). Using the required length of tubing, connect the tee to the gas source and to the eluent pressure regulator. Use the 1/4-in.-to-10/32 brass reducer (P/N 30087) and the 10/32 x 1/16-in. barbed fitting (P/N 30071) to connect the air tubing to the source regulator.

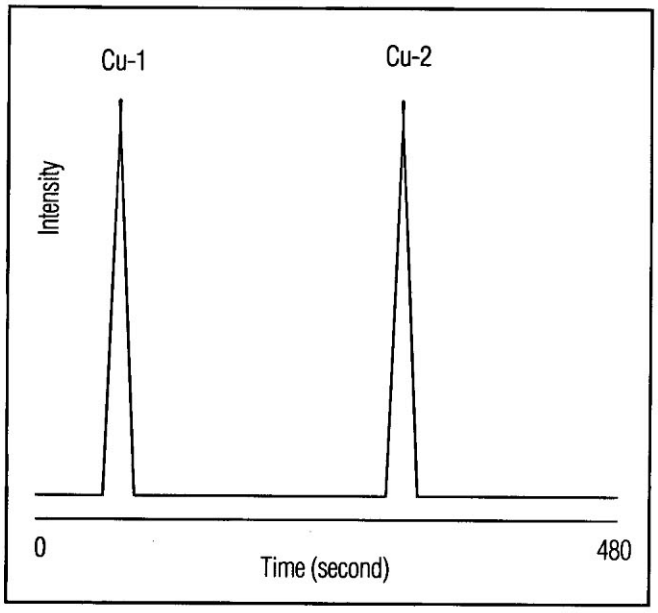

Figure 1 *A typical ICAP time scan.*

Next, connect the air tubing to the eluent container caps (P/N 41004). Start by cutting one of the two 1/8-in. Teflon® lines flush with the bottom of the cap. Repeat this for the other eluent container cap. Next, cut the same tubing about 2 in. (5 cm) above the eluent container cap. This line will be used to connect the argon or nitrogen for pressurizing the eluent bottles. Insert a barbed coupler (P/N 42241) into the trimmed Teflon line of cap E1. Insert a barbed tee (P/N 30538) into the trimmed Teflon line of cap E2. Connect the eluent caps using the air tubing (P/N 30091 or equivalent).

This completes the pneumatic set-up.

Hydraulic Connections

Refer to the *AGP* and *SCM Operator's Manuals* for details pertaining to the installation and operation of the respective modules. Begin the hydraulic connections by connecting the two eluent lines from the three eluent container caps to the front panel eluent ports of the AGP. Ensure that the eluent lines are connected to the appropriate eluent port of the AGP.

Locate the four valves in the SCM. Remove all the tubings connected to the four valves. The first valve on the far left will not be used for this configuration. Designate each valve by starting from the second valve to your right as A, B, and C. Confirm that valves A and B are controlled by E5 and valve C is controlled by E6 of the AGP. Plumb the system as indicated in Figure 2. The 0.020-in. I.D. tubings (blue tubings) are used for all connections except for the sample loop. The two 10-mL sample loops can be made from 1/8- in. I.D. tubing.

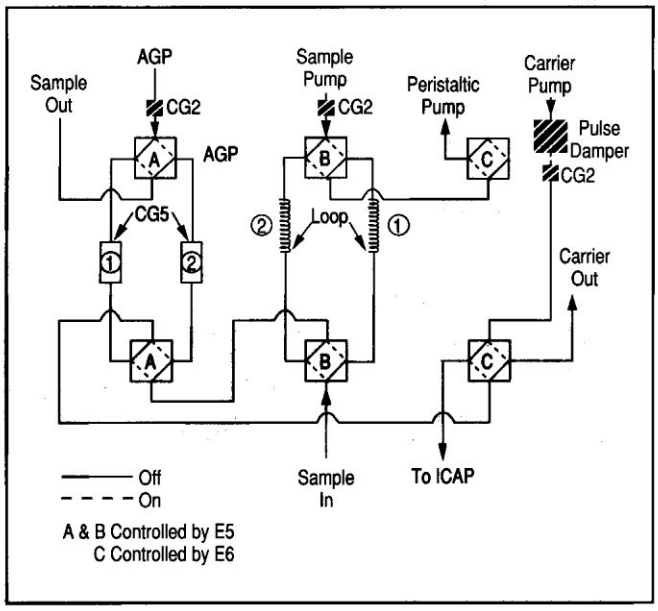

Figure 2 Pneumatic and hydraulic schematic of the sample preconcentration system

Next, connect the eluent line from the 4-liter plastic eluent container (P/N 39164) to the CARRIER IN port of the SCM rear panel. Next, connect the three blue waste lines (P/N 39441) to the ports of the SCM rear panel labeled CARRIER OUT, AGP OUT, and SAMPLE OUT and place them in a waste container. For the sample inlet line, connect the 0.037-in. I.D. x 36-in. (92 cm) length of pink tubing to valve B, port 7, in the SCM. Locate the SAMPLE IN port of the rear panel and use the 1/8-in. I.D. tubing to connect this port to the peristaltic pump inlet. Finally, remove the end fitting from the 0.020-in. I.D. x 36-in. (92 cm) tubing connected to valve C, port 8, of the SCM. Using a pair of pliers, stretch the end of this tubing to taper the tubing to about two-thirds of its original outside diameter. Using about 3/8-in. (1 cm) of 0.03-in. I.D. Tygon tubing as a coupler, connect the tapered tubing to 6-in. (15 cm) of the nebulizer tubing. This is the actual liquid interface between the IC and the ICAP. Place the tubing in a waste container for the system test.

This completes the hydraulic connections.

Electrical Connections

Verify that the front PUMP 1 and PUMP 2 Power switches of the SCM are off. Using the power cords provided (P/N 96078), connect the ac receptacles on the rear panels of the SCM and AGP to the white outlets of the power strip located on the rear upper section of the system enclosure. Next, connect the ac receptacle of the power strip enclosure to an ac (110V) power outlet.

Next, install the interface cable (P/N 43044). One end of the interface cable connects to the rear panel of the AGP and the other end connects to a connector in the ICAP main power board. Start by turning off the line voltage to the ICAP intelligent controller. The circuit breaker to be turned off is located on the rear power panel of the ICAP instrument. Next, loosen (or remove) the screws on the rear power panel and carefully open the power panel to reveal the electronics. On the left side of the electronics panel is a printed circuit board containing three 12-pin Molex nylon connects in a row. Carefully remove the center Molex connector (J3-N) by gripping the sides of the connector and pulling straight up. Note the positions of the wires in the connector. Using the Molex pin extractor tool, remove the wire(s) at the 1 and 10 or 4 and 7 positions

from the Molex connector attached to the ICAP and insert them into the same positions in the Molex connector which is attached to the interface cable. Finally, plug the interface cable Molex connector into the circuit board where the original Molex connector was located. Carefully close the power panel, being careful not to pinch or sever the interface cable.

To the right of the Molex connectors are nine relay sockets. Check to see that there is a relay (labeled SOURCE or K1) installed in the relay socket. This is the relay which will be used to control the IC.

Finally, connect the interface cable to the relay connector on the rear panel of the AGP. Check to see that the AGP relay "dip" switches located on the right top cover of the AGP are in the "off" (forward) position. When the ICAP initiates an "exposure," the AGP will be reset and will begin to execute the AGP program.

This completes the electrical connections.

SYSTEM PREPARATION

1. Confirm that the SCM is configured as shown in Figure 2. Be sure that the three IonPac CG2 columns are installed between: 1) the sample pump and valve B; 2) the carrier pump and valve C; and 3) the AGP and valve A (see Figure 2). Install the two IonPac CG5 columns in valve A.

2. Prepare 1 L of 0.2 M oxalic acid by dissolving 25.2 g of reagent grade oxalic acid dihydrate in 1 L of deionized water. This eluent will be used to clean the AGP eluent flow path.

3. Connect the 0.2 M oxalic acid to port 1 (E1) of the AGP. Pump the oxalic acid through the AGP and to waste at 2.0 mL/min for 10 minutes. Repeat this procedure for port 2. This helps to remove any trace metals from the AGP flow path.

4. Place the sample inlet tubing into the 0.20 M oxalic acid solution. Fill each of the two 1-L eluent glass bottles and the 4-L carrier bottle with 500 mL of 0.2 M oxalic acid. Enter the program listed in Table 1 on the front panel of the AGP. Refer to the *AGP Operator's Manual* for instructions on programming the AGP.

5. Before turning the pump switch on, confirm that the sample pump and carrier pump are primed. Adjust the sample pump and carrier pump flow rates at 5.0 mL/min and 2.0 mL/min, respectively. Turn the peristaltic pump switch on and adjust its flow rate to at least 5.0 mL/min. Start the AGP and run the program two or three times.

6. Replace the 0.2 M oxalic acid in the 4-L carrier bottle with water. Rinse several times. Be sure that the cap has an O-ring for proper sealing.

7. Clean two 1-L glass eluent bottles by filling them with 0.2 M oxalic acid. Allow the acid to remain in the eluent bottles for at least 4 hours. Prepare eluents as indicated in Eluent Preparation. Use caution in preparing and transferring these reagents in order to minimize contamination. Connect the filled eluent bottles to the appropriate eluent cap connected to the AGP. Be sure that the eluents are plumbed to the proper ports of the AGP (E1: water and E2: 2.0 M nitric acid). Adjust the eluent bottle regulator to 4 to 6 psi (30 to 40 kPa) and check for gas leaks.

8. Prime the AGP with each eluent as indicated in the *AGP Operator's Manual*. Be sure to tighten the needle valve upon completing the priming.

This completes the system preparation.

Table 1 Gradient Program

Time	E1	E2	E5	E6	Flow (mL/min)
0.0	25	75	0	1	1.7
4.0	25	75	1	1	1.7
9.0	25	75	0	0	1.7

E1: water
E2: 2.0 M nitric acid

System Test

The purpose of this system test is to ensure that all chromatographic and chemical components of the system are operating properly. Refer to Figure 2 for the system schematic. Be sure to check all fittings for leaks during the system test.

1. The system test begins with a test of the hydraulic system. If the system fails the hydraulic test at any point, determine the source of the plumbing error. Begin by using the program listed in Table 1 with the AGP in the stop-hold position. Press RESET toset the program to time 0.0.

2. Press start on the AGP, and the nitric acid eluent should begin to flow to the IonPac CG5-column 1 and out to the nebulizer. Check the interface tubing on valve C, port 8, of the SCM to confirm that eluent is flowing to the ICAP.

3. Next, prime the carrier pump by loosening the tubing fitting screwed into the outlet check valve. Because the carrier reservoir is pressurized (5 psi/35 kPa), the carrier solution should begin to flow out of the check valve. As the carrier solution begins to flow, turn on the carrier pump by pressing the PUMP 1 power switch located on the SCM front panel. After 5 seconds, tighten the outlet check valve tubing fitting. It is generally only necessary to finger-tighten the fitting. If the fitting leaks, tighten it another 1/8 turn using a 5/16-in. open-end wrench.

4. Set the carrier pump flow rate to about 8.00. (Refer to the *SCM Operator's Manual* for details on adjusting the flow rate.) Confirm that the carrier is flowing out of the CARRIER OUT tubing. Calibrate the carrier pump flow rate by mass or volume to 1.8 to 2.0 mL/min. Turn off the carrier pump (PUMP 1).

5. Repeat step 3 on the sample pump. Press the pump 2 power switch on the SCM front panel. Confirm that the carrier is flowing out of the SAMPLE OUT tubing. This may take a few minutes if the 10-mL sample loop is not filled. Calibrate the sample pump flow rate by mass or volume to 3.0 mL/min. Turn off the sample pump.

6. Place the sample inlet tube in a container of deionized water and start the peristaltic pump. If the sample loop is not filled, it may take about 2 minutes before DI water begins to exit the peristaltic pump tubing. Adjust the peristaltic pump flow rate to 5.0 mL/min.

7. List the AGP program to 4.0 minutes and press RUN. This will forward the program to 4.0 minutes. Check to see that the nitric acid eluent is flowing out of the ICAP interface tubing. Start the sample pump (PUMP 2) and confirm that the carrier is flowing out of the SAMPLE OUT tubing. Turn off the AGP and the sample pump.

8. List the program to 8.0 minutes and press run. Press the pump 2 power switch. Check to confirm that the carrier is flowing to the ICAP interface tubing.

   This completes the system test.

SEQUENCING OF THE IC AND ICAP FOR AUTOMATION

This section describes the sequencing and operation of the system components. The IC/ICAP system has been designed to be used either manually or in the fully automated mode. Because the IC is controlled by the ICAP computer, the discussion below places the IC functions relative to the ICAP.

The system is designed to run in a fully automated mode when analyzing a series of samples. The first IC cycle performs the sample concentration process. The analytical data are not be obtained until the next cycle is initiated. In other words, the result of the analysis is produced at run (n + 1), where n is the number of cycles or sample number.

The following describes the various operations of the IC during the gradient program:

In the first cycle (n = 1), the autosampler position is at sample number 1 and the result of this sample will be obtained in run (n + 1) or run # 2.

1.1. Time 0.0

Valves A and B switch OFF and valve C switch is ON. The peristaltic pump begins pulling sample #1 from the autosampler through loop 1 and out to waste. The AGP pumps nitric acid to the IonPac CG5-column 1 and out to the ICAP. The sample pump begins to flush the sample from loop 2 to the IonPac CG5-column 2.

2.1. Time 4.0

Valves A, B, and C switch ON. The sample pump begins to flush the sample from loop 1 to the IonPac CG5-column 1. The AGP pumps nitric acid to the IonPac CG5-column 2 and out to the ICAP. The peristaltic pump begins pulling sample #1 from the autosampler through loop 2 and out to waste.

The second cycle is initiated at time 8.0 minutes.

In the second cycle, the autosampler position is at sample number 2. The first sample has been concentrated in the IonPac CG5 column. The result of the second sample will be obtained in run #3.

2.1. Time 0.0

Valves A and B switch OFF and valve C switch is ON. The peristaltic pump begins pulling sample #2 from the autosampler through loop 1 and out to waste. The concentrated metals of sample #1 from the IonPac CG5-column 1 are eluted to the ICAP with nitric acid delivered by the AGP. The sample pump begins to flush sample #1 from loop 2 to the IonPac CG5-column 2.

2.2. Time 4.0

Valves A, B, and C switch ON. The sample pump begins to flush the sample #2 from loop 1 to the IonPac CG5-column 1. The concentrated metals of sample #1 from the IonPac CG5-column 2 are eluted to the ICAP with nitric acid delivered by the AGP. The peristaltic pump begins pulling sample #2 from the autosampler through loop 2 and out to waste.

Remember to include the blank sample (water) at the end of the analysis since the last sample requires an additional cycle to complete the analysis. In the automated mode, the gradient program is reset by the ICAP control at 8.0 minutes and never reaches the last step, time 9.0 minutes, of the gradient program. However, in the last run when the gradient is not reset, the AGP continues to run to 9.0 minutes where the IC is put in "standby" mode. If the system operates in manual mode, make sure that the new exposure starts at time 8.0 minutes.

The IC is controlled by a relay from the ICAP system controller. When an ICAP run is initiated, the computer sends a signal to the intelligent controller. The controller either begins in a flush mode or in an exposure mode. At the initiation of an exposure, a signal is sent to the AGP. If the AGP is in the "start" mode, the signal from the ICAP controller to the AGP will reset the AGP program to time 0.0 and the program will begin to run.

IC/ICAP OPERATION

This section describes the integrated operation of the IC/ICAP system. For details on ThermoSpec software, refer to the appropriate TJA manual.

Methods Development

1. Begin by writing a ThermoSpec method titled "IC/ICAP". Under Methods Development, Set-up, select the elements of interest (F1) and also select the duplicate (F3) function for each element. Select the elements and the duplicate of each element in the order in which you want them printed in the report. Add an additional element, such as Na, which can be duplicated using the F3 function key. This element (assigned as Na-3) will be used to extend the gradient program to 8.0 minutes in the automated mode (see step 3 below for more details). Press F9 to save the element selection.

   Note: The order in which the elements are printed in the analysis report can be selected under Options, F8, from the Main Menu under Methods Development.

2. Next, press F3, Output. Change the number of repeats to 1 and the concentration to ppm or ppb. Select print limits as desired. Press F9 to save.

3. For F5, Element Info, the element heading will be changed and the standard(s) concentrations entered. Start by changing the element heading. For the elements in timing group 1 (first measurement), enter the element symbol followed by "-1." For example, "Cu-1," "Fe-1," "Na-1", etc. For the elements in the timing group 2 (second measurement), enter the element symbol followed by "-2." Assign Na-3 in timing group 3.

Enter the appropriate concentration for each element. Generally, a two-point calibration routine is used, a blank "BLANK" and a high standard "HIGH STD." Be sure that the correct concentration is entered for each element and that only a high standard and a blank appear in the method. Press F9 to save. Be sure to save the entire method.

4. At this point, it is necessary to run a time scan. Press F6 for Scan and F7 for Time Scan. Remember that the time scan will be obtained in the second cycle after the sample is introduced into the IC. The Time Scan feature will be used to determine the retention times of the first and second bands. Place the sample inlet tube into the high standard and turn on the peristaltic pump when initiating the first run cycle.

5. In the Time Scan mode, the spectrophotometer monitors the plasma for a user-specified time (much like a chromatographic detector). Enter an integration "slice" of 4.8 and press ENTER. Enter 100 for the number of time slice. This will result in a time scan of 480 s. After the first cycle, press F1, run at time 8.0 minutes. The controller will go to an exposure mode (The "EXP" LED will light), and a signal sent to the AGP resets the program to time 0 and starts the AGP program. The exposure will end in 480 seconds.

7. The results of the time scan displayed on the screen should look like Figure 1. For concentrated elements, two discrete regions are present in the time scan. The first region is the first concentration band. This represents the concentrated sample from the IonPac CG5-column 1. The second region represents the second band and is subsequently eluted off the IonPac CG5-column 2. Press F1, Expand, and move the cursor to determine the time at which the peak begins to elute. Note this time for comparison to the next run. Determine the width of the peak at the base using the cursor. Typical base width is 20 to 30 seconds.

8. Press F5, Overlay, to overlay several time scans. Notice that all the concentrated metals elute with nearly the same retention times.

   The time scan can be stored if required.

9. Repeat the time scan. The retention time of the peak should not vary by more than 3 seconds. Continue repeating the time scan until the retention time is ± 3 seconds. Return to the Main Menu for methods development.

10. Press F2, Internal Standards, to enter the timing groups. For timing group 1, enter a preintegration time (in seconds), determined from the first concentration band of the time scan. This preintegration time is the time from the beginning of the exposure to when a peak begins to elute. To allow for minor shifts in retention times, it is advisable to enter a preintegration time about 1 to 2 seconds less than the measured times. For the integration time, add about 5 seconds to the measured base width and enter this value (typically 30 to 40 seconds).

For timing group 2, enter a preintegration time and an integration time for the second concentration region. Calculate the values the same as you did for the first concentration region.

Since an 8-minute cycle is required for each run, a "dummy" timing group 3 is used to keep the program running until 8.0 minutes. Otherwise the exposure will end at the second preintegration time (timing group 2) and the next cycle will be initiated. For timing group 3, enter a preintegration time of 475 seconds for 5 seconds.

Press F9, Save, to complete the methods development. Be sure to save the entire method.

The system is ready for standardization. Standardization of the ICAP system with the IC is performed in the same manner as normal ICAP standardization. Standardization can be performed manually or with the autosampler.

Ion Chromatography/Inductively Coupled Argon Plasma (IC/ICAP): A New Technique for Trace Metal Determinations

INTRODUCTION

As the world becomes more environmentally conscious, the need for determining trace elements in diverse and complex matrices increases. Since many elements are regulated at the parts-per-billion (ppb) level, more sensitive and selective analytical methods are required. During the past decade, inductively coupled argon plasma atomic emission spectroscopy (ICAP-AES) has become the predominant technique for trace metal analysis. ICAP instruments offer detection limits in the low to mid ppb range, a range of elements encompassing more than half of the periodic table, rugged instrumentation and rapid analysis times (up to sixty elements in a minute). As with any analytical technique, ICAP does suffer from interferences. In many instances these interferences significantly compromise the detection limits and the accuracy of the determination. Common interferences in ICAP include high concentrations of alkali metals (sodium, potassium), alkaline earth metals (magnesium, calcium), and spectral interferences from elements such as iron and aluminum. As a result, ICAP detection limits are compromised in complex matrices such as brines, seawater, waste water, soils, sludges, and biological fluids and tissues.

Several common methods are used in order to minimize sample matrix effects in ICAP. These include spectral background corrections, inter-element spectral correction, standard additions and matrix matching. While all of these methods help to minimize sample matrix effects, none of these methods completely eliminates the matrix effects.

Ion exchange is a technique which has long been used for the concentration and separation of trace metals. The literature of ion exchange is extensive and the application of ion exchange for sample pretreatment prior to spectroscopic analysis is well known. Ion exchange offers the analyst a solution to the detection limit and interference problems commonly experienced when analyzing complex matrices by ICAP. Ion exchange can concentrate the analytes of interest while at the same time reduce or eliminate common interferences. Using a unique form of ion exchange sample pretreatment called chelation concentration, the analytes of interest are concentrated, while common interferences such as the alkali and alkaline earth metals are eliminated. In this Technical Note we describe a technique based upon the direct coupling of an ion chromatograph (IC) to a simultaneous ICAP for the determination of trace metals in complex matrices. Using chelation concentration, detection limits are lowered 10 to 100 times in complex matrices while eliminating common interferences.

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SUMMARY OF THE TECHNIQUE

A Dionex ion chromatograph comprising the Advanced Gradient Pump (AGP) and Sample Concentration Module (SCM) is interfaced directly to a Thermo Jarrell Ash (TJA) simultaneous ICAP (ICAP 61, or any IBM®-upgraded TJA simultaneous ICAP). The IC performs automated sample pretreatment using chelation concentration with the concentrated sample being pumped directly to the nebulizer. Most cationic transition and lanthanide metals are concentrated while alkali, alkaline earth and anionic species are eliminated.

An acidified sample, containing up to 3% (0.5 M) acid, can be loaded directly to the SCM. The SCM performs on-line buffering on the acidified sample just before the sample effluent enters the concentrator column. In the standard IC/ICAP configuration, a small volume (0.5 to 1.0 mL) of the untreated sample is pumped directly to the nebulizer while the remainder of the acidified sample is buffered with ammonium acetate and then pumped to the chelating concentrator column (MetPac™ CC-1). This configuration allows for "direct nebulization" and "concentration" in a single analysis. The direct nebulization allows for the determination of elements that are not concentrated. When concentrating 5 mL of sample, analysis time is about 8 minutes per sample.

Concentrating 5 mL of sample will lower the standard ICAP detection limits by a factor of 10 to 15. Since chelation concentration also eliminates interferences, the detection limit enhancement is generally much greater in "real world" samples. For example, using the IC to concentrate 5 mL of seawater will improve detection limits 50-fold compared to direct nebulization of seawater.

The IC/ICAP system is controlled, operated and integrated within standard TJA ThermoSpec software. The system can be fully automated with a ThermoSpec supported autosampler.

For more information about the ICAP system and ThermoSpec software, please contact your local Thermo Jarrell Ash representative. In the U.S., please contact the Thermo Jarrell Ash Corporation, 8E Forge Parkway, Franklin, MA 02038; Telephone (508) 520-1880.

INSTRUMENT REQUIREMENTS

The IC/ICAP system consists of a Dionex Chelation Concentration IC and a Thermo Jarrell Ash simultaneous ICAP spectrometer. Figure 1 shows a block schematic of the IC/ICAP system.

You will require the following instrumentation and accessories:

Chelation Concentration System (P/N 42134) comprising:

Advanced Gradient Pump (AGP, P/N 42144/115 V; P/N 42145/220 V)

Sample Concentration Module (SCM, P/N 42134/115V; P/N 42135/230V)

IC/ICAP Installation Kit (P/N 43169; contains eluent containers, air regulator, tubing, power cords and fittings for installation)

MetPac CC-1 Concentrator Column (2 pack; P/N 42156)

In addition to the items listed above, you will also need:

Electrical power

Compressed nitrogen or argon (80–120 psi/550–825 kPa)

Labcart or table for the IC

Standard analytical laboratory equipment such as a balance, pH meter, etc.

If you already own a Dionex Advanced Gradient Pump (AGP), you can perform IC/ICAP with the addition of the Sample Concentration Module (SCM).

Note: The Gradient Pump Module (GPM) may be substituted for the AGP, but only if the GPM firmware is version 3.18 or later (P/N 38546), which permits the GPM to be controlled by TJA ThermoSpec Software. Only the AGP will be referenced throughout the remainder of this technical note.

The SCM contains two single piston-type Dionex QIC Pumps (DQP), four 2000-psi (14-MPa) inert double-stack four-way pneumatically controlled slider valves, and a pulse damper. In addition, a peristaltic pump is used for loading the sample from the autosampler or sample container into the SCM. Use the peristaltic pump supplied with the ICAP. One of the DQPs is used to pump the acidified sample to the mixing tee and the other pump is used for delivering a "carrier" or eluent to the nebulizer. The MetPac CC-1 column used for concentration is also installed in the SCM. All of these components are housed in a single enclosure. The rear panel of the SCM contains bulkhead fittings for connecting wastelines, eluent lines and a sample inlet line. Refer to the *SCM Manual* (P/N 34206) for details pertaining to the operation and maintenance of the individual components of the SCM.

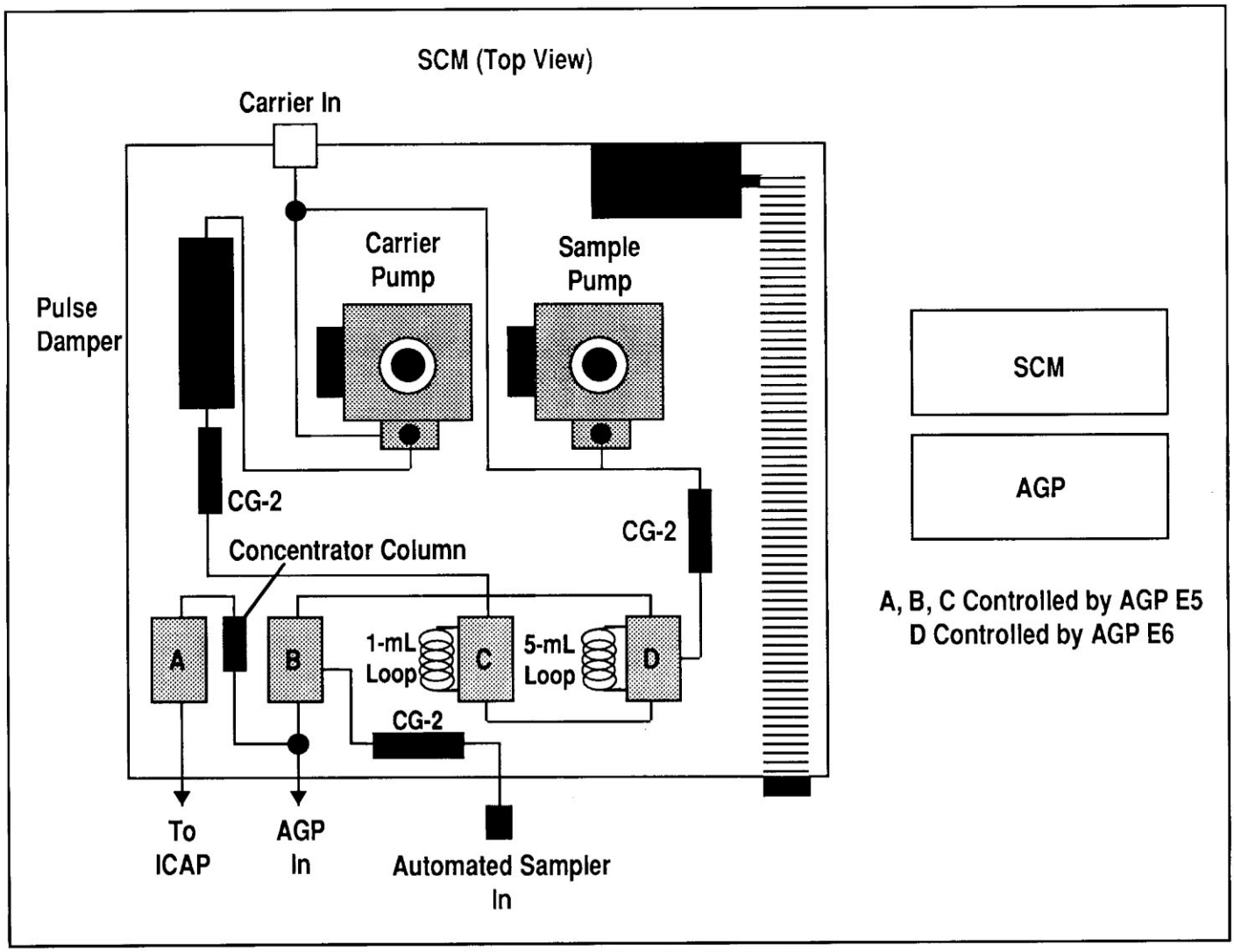


Figure 1 *Top view of the Sample Concentration Module (SCM) configured for ICAP analysis*

The AGP is a microprocessor controlled, high performance quaternary gradient IC pump. The AGP has a metal-free flow path and permits the time dependent selection of up to four eluents, flow rate, and the control of two pairs of air solenoids for external valve control. Controls 5 and 6 (E5 and E6) of the AGP are used to control the four valves present in the SCM. The AGP is programmable from the front panel and can store up to 10 different programs.

The AGP is controlled via an interface cable from the ICAP system controller. Any TJA simultaneous ICAP spectrometer (Models 61, 61E, 1100, 9000) can be interfaced to the Dionex IC as long as the simultaneous spectrometer is used with an IBM® or IBM-compatible computer with TJA ThermoSpec software. For system automation, a ThermoSpec-supported autosampler is required. A TJA type 22 or a TJA 300 autosampler can be used. The autosampler should use the larger sample racks (type 24) to ensure sufficient sample volume.

Questions concerning the compatibility of interfacing a particular TJA simultaneous ICAP instrument to a Dionex IC should be directed to your TJA sales or service representative.

CHEMICALS, REAGENTS AND STANDARDS

A complete list of reagents, preparation, and sources can be found in *Appendix A*. The two reagents used for chelation concentration, 2 M ammonium acetate, pH 5.5 (2 L; P/N 33440), and 2 M nitric acid (1 L; P/N 33442), are available ultrapure and ready to use from Dionex. See *Appendix A* for additional ordering information.

DISCUSSION OF THE METHOD

Concentration Chemistries

The method described in this report was developed to address some of the common analytical problems associated with ICAP. For many elements, the instrument detection limits obtained in ICAP would be sufficient for most analytical needs.

Unfortunately, interferences compromise detection limits. Common interferences in ICAP are the alkali and alkaline earth metals. Specifically, magnesium is a common spectral background interferant for many metals. By using selective ion exchange materials such as chelating resins, analytes may be concentrated while interferences such as the alkali and alkaline earth metals and anions are reduced or eliminated. This form of sample pretreatment is called chelation concentration. Unlike conventional ion exchange concentration methods which are typically not selective for ions of the same valency, chelation concentration is a selective concentration method.

Other common interferences of ICAP include iron and aluminum. These produce spectral interferences that are concentrated with the analytes of interest. A separate concentration method has been developed to selectively eliminate iron and aluminum from the analytes of interest (see Dionex Application Note No. 75). If iron and aluminum are not "chromatographically" removed, interfering element corrections (IECs) should be used for samples which are high in iron or aluminum.

The types of samples for which chelation concentration is applicable include seawater, brines, natural waters, waste waters, sediments, acid digested samples, fusions (KOH or $LiBO_2$), extracts and leaches, concentrated acids or bases as well as biological, botanical and geological materials. Chelation concentration is not intended for use when attempting to determine trace transition metals in the presence of large quantities of other transition metals (e.g., plating baths).

Most samples should be acid digested to ensure that the metals are free in solution and not bound by organic materials such as fulvic or humic acids. Complexing agents in the sample can interfere with concentration efficiency and recoveries. Any digestion, extraction, or fusion can be analyzed by this technique.

The column used for chelation concentration, the MetPac CC-1, contains a macroporous iminodiacetate chelating resin. The column has a capacity of 0.45 milliequivalents. The resin can be used with acid or base concentrations up to 6 M without degradation (*n.b.* do not store the resin in the acid form). The relative selectivity of the resin is:

$$\text{Lanthanides} > Hg >> Cu >> UO_2 > Ni > Pb > Zn > Co > Cd > Fe > Mn > Ba > Ca > Sr > Mg >> Na,K,Li$$

Properties of the MetPac CC-1 chelating resin are shown in Figure 2. The resin has very high affinity for transition and lanthanide metals compared to the alkali and alkaline earth metals. The selectivity of this chelating resin makes it ideal for use with a broad spectrum of sample matrices since most matrices will have high concentrations of alkali and alkaline earth metals relative to the transition metals.

The resin does not concentrate anions such as the halides, nitrate, sulfate, phosphate or organic anions. Unfortunately, anionic species such as arsenic (as arsenate or arsenite) and selenium (as selenate or selenite) are also not concentrated. Other species that are not efficiently concentrated by the MetPac CC-1 column include thallium (Tl^+) and some precious metals. Chromium as chromic ion (Cr^{3+}) is concentrated but not efficiently eluted, while chromate (CrO_4^{2-}) is not concentrated. Table 1 shows elements that are quantitatively concentrated using the MetPac CC-1.

In general, the higher the valency of the metal ion, the more strongly bound the metal ion is to the resin. Since the functional group of the resin is a weak acid (COOH) and a weak base (NH), hydronium ion (H_3O^+) competes strongly with metal ions for the chelating sites. As a result, nitric acid at 0.5 to 2.0 M is an effective eluent. Below a pH of 2.5, the MetPac CC-1 column will not concentrate transition metals. In the pH range of 5 to 6, the resin selectivity is optimized for transition and lanthanide metals relative to alkali and alkaline earth metals. By using an ammonium acetate eluent in this pH range, alkaline earth metals can be eluted while the transition and lanthanide metals remain strongly bound to the resin.

Up to 300 mL of seawater can be concentrated on the MetPac CC-1 column before breakthrough of the transition metals. Brine concentrations of 22% sodium chloride have been concentrated with quantitative recoveries. With a 1.0 M calcium solution, up to 40 mL can be concentrated with quantitative recovery of the transition elements. Flow rates up to 4.0 mL/min can be used with the MetPac CC-1 with quantitative recovery of the transition metals.

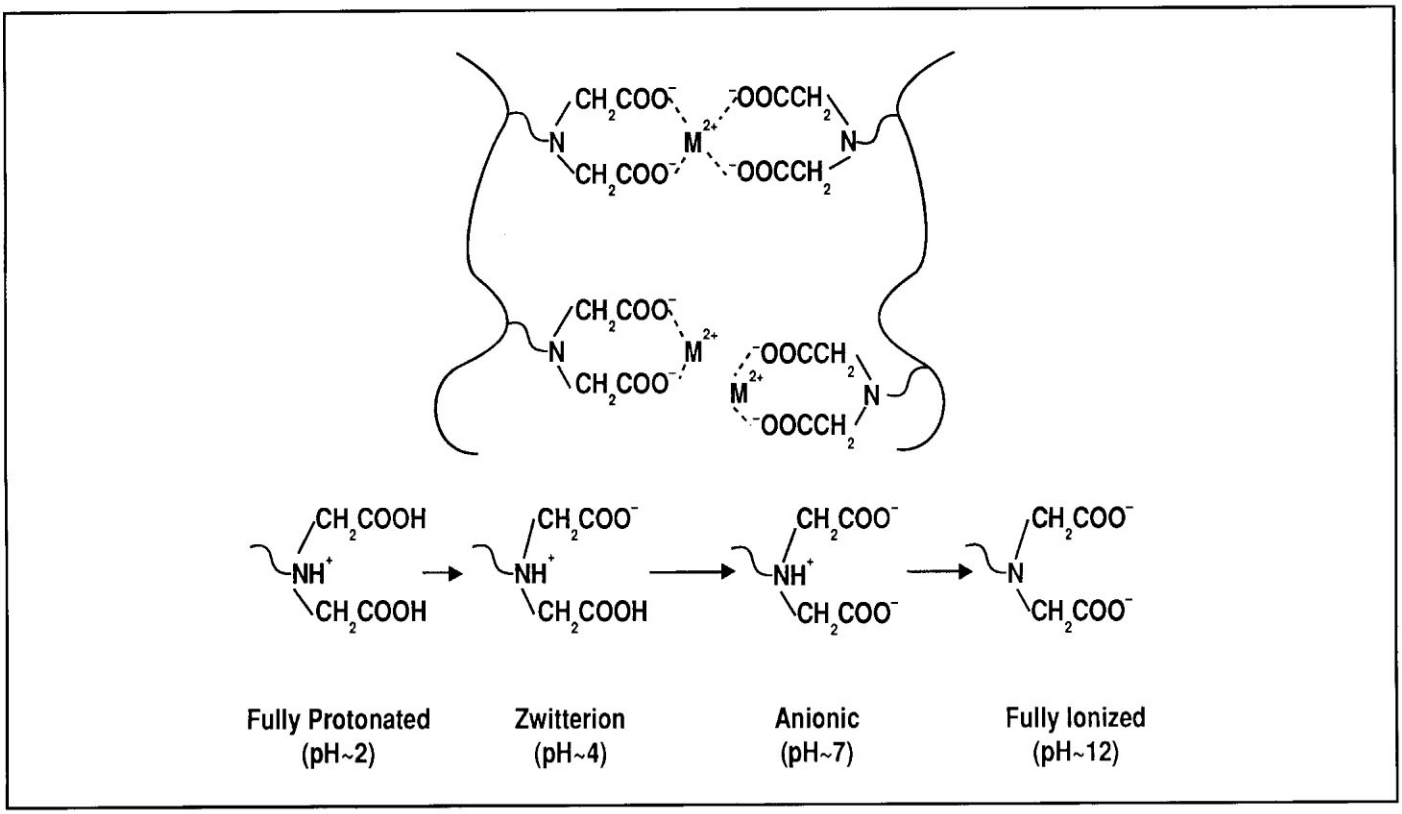


Figure 2 *Properties of MetPac CC-1 Chelating Resin*

Table 1 Retention Characteristics of the MetPac CC-1 Column

Metal Ion	Quantitative	Metal Ion	Quantitative
Ti(IV)	Yes	Cd(II)	Yes
V(IV,V)	Yes	In(III)	Yes
Cr(III)	No	Y(III)	Yes
Mn(II)	Yes	Lanthanides	Yes
Fe(II, III)	Yes	Hg(II)	Yes
Co(II)	Yes	Pb(II)	Yes
Ni(II)	Yes	Al(III)	Yes
Cu(II)	Yes	Tl(I, II)	No
Zn(II)	Yes	As(III< IV)	No
Ag(I)	No	Se(IV, VI)	No

Chelation concentration consists of four major processes.

1. A known volume of the sample is buffered on-line and passed through the MetPac CC-1 column. Most polyvalent cations are quantitatively concentrated from the sample while anions pass through the column. Alkali metals are weakly retained. Metals which are quantitatively concentrated are listed in Table 1.

2. Weakly bound alkaline earth metal ions such as magnesium and calcium are selectively eluted with a 2 M ammonium acetate eluent (pH 5.5, 9 to 12 mL), which is pumped by the AGP. During this elution process, at least 98% of the magnesium and 95% of the calcium on the column will be eliminated. Some manganese (10 to 15%) will be eluted. This does not preclude quantitation of manganese since the percentage of manganese eluted during the ammonium acetate wash is constant.

3. Next, the concentrated transition and lanthanide metals are eluted in a 100 to 200 µL volume using 1 to 2 M nitric or hydrochloric acid (8 to 10 mL) delivered from the AGP.

4. Finally, the MetPac CC-1 is converted back to the ammonium form using 2 M ammonium acetate (5 to 6 mL). This regeneration step prepares the concentrator column for the next sample.

It is important to use reagents and water which have very low metal contamination (less than 1 ppb). Any trace metals in the reagents will be concentrated as a "blank" and subsequently eluted with the sample. The system blank results from contamination in the chelation concentration reagents and the system. Generally, iron and zinc are the most common transition metal contaminants, but a small amount of copper may be observed as well. Care must be taken to minimize reagent and sample contamination during preparation and handling. Reagent purity will usually dictate the detection limits. A description of the necessary reagents are listed in Appendix A.

SEQUENCING OF THE IC AND ICAP FOR AUTOMATION

This section will describe the sequencing and operation of the system components. The IC/ICAP system has been designed for use either manually or in the fully automated mode. Table 2 presents the standard AGP program for the MetPac CC-1 column. Since the IC is controlled by the ICAP computer, the discussion below places the IC functions relative to the ICAP. The following describes the various operations of the IC during the gradient program.

1. **Time 0.0**
   Valves A, B, and C switch ON. The peristaltic pump begins pulling sample from the autosampler through the loops and out to waste. The AGP is pumping ammonium acetate through the column for regeneration.

2. **Time 2.0**
   Valves A, B, and C are OFF and valve D is ON. The carrier pump is pumping through the 1-mL loop and bringing the previously loaded sample to the nebulizer. At the same time, the sample pump has begun pumping the carrier solution (0.1 M nitric acid) at a flow rate of about 2.0 mL/min through the 5-mL loop, which was previously loaded with the sample. The acidified sample from the sample loop is mixed with 2.0 M ammonium acetate buffer from the AGP, and the buffered sample passes through the concentrator column and out to waste.

AGP Program for Chelation Concentration IC/ICAP Program 1 (for 5 mL sample loop)

E1: 2 M Ammonium acetate, pH 5.5 E2: 2 M Nitric acid E3: Deionized water

t (min)	%E1	%E2	%E3	V5	V6	Flow Rate (mL/min)	Event
0.0	100	0	0	1	0	2.0	buffer column, load loop
2.0	100	0	0	0	1	2.0	dir. neb., load column
5.0	100	0	0	0	0	4.0	selective elution
6.2	100	0	0	0	0	4.0	
6.3	0	75	25	0	0	4.0	start elution of metals
6.7	0	75	25	0	0	2.0	
6.9	0	75	25	1	1	2.0	conc. metals to neb.
8.3	0	75	25	1	1	2.0	
8.4	100	0	0	0	0	0.0	buffer column, stop EXP.

3. **Time 5.0**
   Valves A, B, C, and D are OFF. The contents of the 1-mL loop have been pumped to the nebulizer and the carrier pump is still delivering carrier to the nebulizer. The 5-mL sample has been loaded on the concentrator column and the selective elution of calcium and magnesium from the column is in progress.

4. **Time 6.3**
   Valves A, B, C, and D are OFF. The carrier pump is still delivering carrier to the nebulizer. The AGP now switches to nitric acid and begins eluting the concentrated metals. The sample pump is still on and is pumping carrier solution to waste.

5. **Time 6.9**
   Valves A, B, C, and D are ON. The AGP flow rate drops to 2.0 mL/min as the concentrated band is being eluted of the column. The 5-mL sample loop is being rinsed with the carrier (0.1 M nitric acid).

6. **Time 8.4**
   Valves A, B, C, and D are OFF. The carrier pump is now switched in line with the nebulizer. The exposure will be completed by this time. In the automated mode, a new sample cycle will begin with the autosampler proceeding to the rinse station and then to the next sample.

The IC is controlled by a relay from the ICAP system controller. When an ICAP run is initiated, the computer sends a signal to the intelligent controller. The controller either begins in a flush mode or in an exposure mode. At the initiation of an exposure, a signal is sent to the AGP. If the AGP is in the "start" mode, the signal from the ICAP controller to the AGP will reset the AGP program to time 0.0 and the program will begin to run. If the ICAP method is written with a "flush" time, the system controller begins the flush cycle but no signal is sent to the AGP. As a result, the AGP continues its program uninterrupted.

Sample loading starts at the beginning of the AGP program (t = 0.0). The flush mode is not required at the beginning of the ICAP sequence. Two steps occur at the beginning of the AGP program. At 0.0 minute, the AGP starts conditioning the chelating concentrator column by pumping the buffer solution through the column and to the nebulizer. The IC also starts loading sample to the 5-mL loop and then to the 1-mL loop, and out to waste. Typically, a minimum of 6 mL of sample is required. However, in order to prevent carryover and to completely flush the sample loops, it is recommended to pump at least 9 mL of sample. Since the peristaltic pump is operating at approximately 5 mL/min, the sample loading time of 1.8 to 2.0 min is required. The subsequent steps following the sample loading and column conditioning include on-line buffering of the acidified sample, selective elution of interfering species and acid wash.

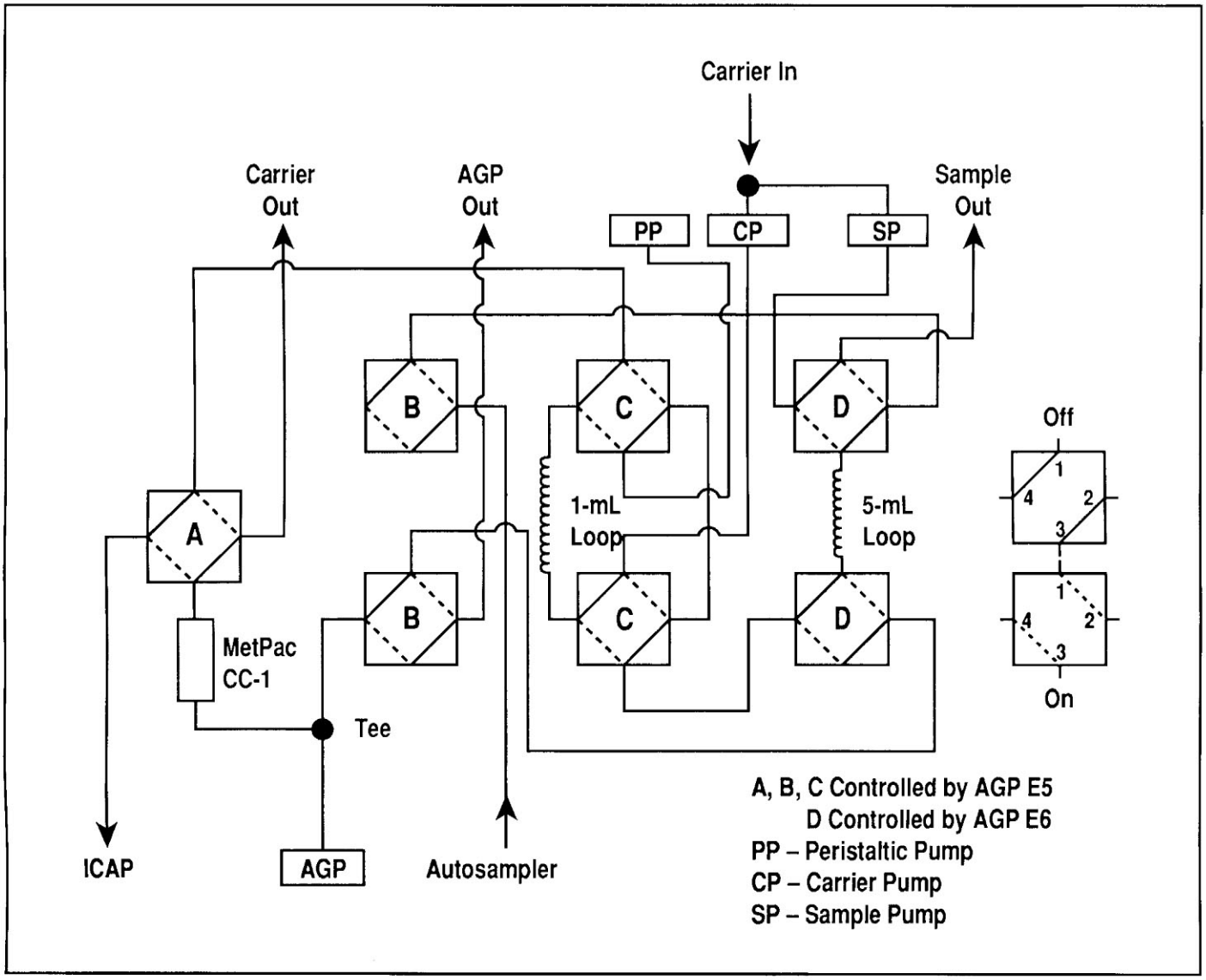


Figure 3 *Schematic for Sample Concentration Module (SCM) automated on-line buffering*

SYSTEM CONFIGURATION AND SET-UP

Figure 3 shows a detailed pneumatic and hydraulic schematic of the IC system. The SCM is factory-configured with a 1-mL sample loop for direct nebulization and a 5 mL loop for concentration. The following set-up procedure is designed for the standard (factory) SCM configuration.

Pneumatic Connections

Locate the four colored air tubings at the rear panel of the AGP and SCM. Using the small barbed couplers (P/N 42241), couple the air tubing together by matching the colors (pink-pink, yellow-yellow, green-green and blue-blue). Next, connect about 2 ft (60 cm) of air tubing (P/N 30091) to the small barbed fitting on the back of the AGP. Insert a barbed tee (P/N 30538) into the end of this tubing. One arm of the tee will go to the nitrogen or argon source (regulator) and the other arm will go to the inlet of the eluent bottle regulator (P/N 38201). Using the required lengths of tubing, connect the tee to the gas source and to the eluent pressure regulator. Use the 1/4-in. to 10/32 brass reducer (P/N 30087) and the 10/32 x 1/16-in. barbed fitting (P/N 30071) to connect the air tubing to the source regulator.

Next, connect the air tubing to the eluent container caps (P/N 41004). Start by cutting one of the two 1/8-in. Teflon® lines flush with the bottom of the cap. Repeat this for all three eluent container caps. Next, cut the same tubing about 2 in. above the eluent container cap. This line will be used to connect the argon or nitrogen for pressurizing the eluent bottles. (The eluent bottle caps should contain a white TFE O-ring (P/N 41078) and not a black rubber O-ring. If black O-rings are present, replace with the TFE O-ring.) Insert a barbed coupler (P/N 42241) into the trimmed Teflon line of cap E1. Insert a barbed tee (P/N 30538) into the trimmed Teflon lines of caps E2 and E3. Connect the eluent caps using the air tubing (P/N 30091 or equivalent). This completes the pneumatic set-up.

Hydraulic Connections

Refer to the *AGP* and *SCM Operator's Manuals* for details pertaining to the installation and operation of the respective modules. Begin the hydraulic connections by connecting the three eluent lines from the three eluent container caps to the front panel eluent ports of the AGP. Notice that the eluent lines are labeled 1, 2 and 3. Be sure that the eluent lines are connected to the appropriate eluent port of the AGP.

Connect the eluent line from the four liter plastic eluent container (P/N 39164) to the CARRIER IN port of the SCM rear panel. Next, connect the three blue waste lines (P/N 39341) to the ports of the SCM rear panel labeled CARRIER OUT, AGP OUT, and SAMPLE OUT and place them in a waste container. For the sample inlet line, connect the 0.037-in. I.D. x 36-in. (92-cm) length of pink tubing to valve B, port 2, in the SCM. Locate THE SAMPLE IN port of the rear panel, and use the 1/8-in. I.D. tubing (white tubing) to connect this port to the peristaltic pump inlet. Finally, remove the end fitting from the 0.020-in. I.D. x 36-in. (92-cm) tubing connected to valve A, port 4 of the SCM. Using a pair of pliers, stretch the end of this tubing as to taper the tubing to about two-thirds of its original outside diameter. Using about 3/8 in. (1 cm) of 0.030-in. I.D. Tygon® tubing as a coupler, connect the tapered tubing to 6 in. (15 cm) of the nebulizer tubing. This is the actual liquid interface between the IC and the ICAP. Place the tubing in a waste container for the system test. This completes the hydraulic connections.

Electrical Connections

Verify that the front PUMP 1 and PUMP 2 power switches of the SCM are off. Using the power cords provided (P/N 96078), connect the AC receptacles on the rear panels of the SCM and AGP to the white outlets of the power strip located at the upper rear section of the system enclosure. Next, connect the AC receptacle of the power strip enclosure to an AC power outlet.

Next, install the interface cable (P/N 43044). One end of the interface cable connects to the rear panel of the AGP (via the telephone plug) and the other end connects to a connector in the ICAP main power board. **Start by turning off the line voltage to the ICAP intelligent controller. The circuit breaker to be turned off is located on the rear power panel of the ICAP instrument.** Next, loosen (or remove) the screws on the rear power panel and carefully open the hinged power panel to reveal the electronics. On the left side of the electronics panel is a printed circuit board containing three 12-pin Molex nylon connectors in a row. Carefully remove the center Molex connector (J3-N) by gripping the sides of the connector and pulling straight up. Note the positions of the wires in the connector. Using the Molex pin extractor tool, remove the wire(s) at 1 and 10 or 4 and 7 position in the Molex connector attached to the interface cable (see Fig. 4) and insert the wires of the interface cable into the same position. Finally, plug the interface cable Molex connector into the circuit board where

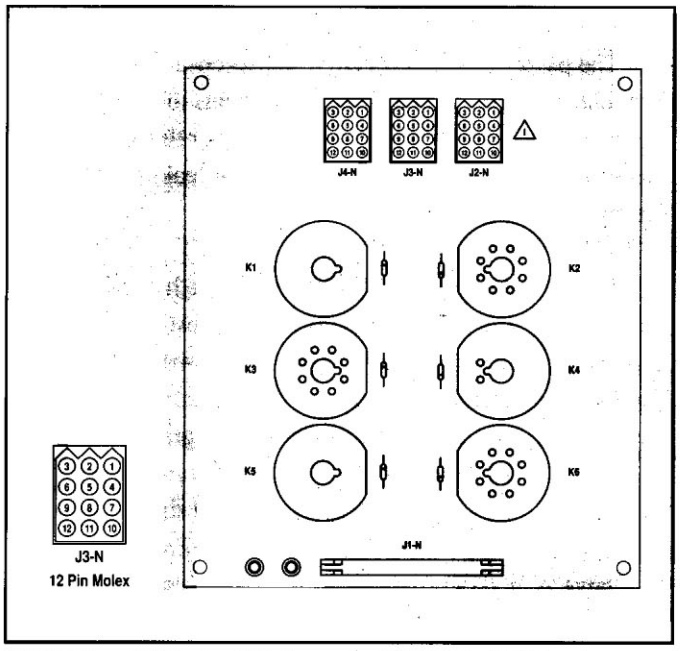


Figure 4 *Diagram of Molex connectors on the ICAP main power circuit board*

the original Molex connector was located. Carefully close the power panel being careful not to pinch or sever the interface cable.

To the right of the Molex connectors are six relay sockets. Check to see that there is a relay (labeled SOURCE or K1) installed in the relay socket labeled K1 as shown in Figure 4. This relay will be used to control the IC.

Finally, connect the interface cable to the relay connector on the rear panel of the AGP. Check to see that the AGP relay dip switches located on the right top cover of the AGP are in the OFF (forward) position. When the ICAP initiates an exposure, the AGP will be reset and will begin to execute the AGP program. This completes the electrical connections.

SYSTEM PREPARATION

1. Confirm that the SCM is configured as shown in Figure 3. Be sure that an IonPac® CG2 column is installed between the AGP and valve B (before the mixing tee) of the SCM. Confirm that the other two CG-2 columns are also installed on the two DQP outlet check valves (see Fig. 1). Install a MetPac CC-1 column between the coupled lines connecting valve A and the mixing tee.

2. Remove the mixer (GM-2 or GM-3) from the low pressure side of the AGP. This mixer is located between the valve manifold and the priming block of the AGP. Connect the two lines using a coupler (P/N 39056). Do not install a mixer on the high pressure side of the AGP (i.e., between the AGP and the SCM). Refer to the *AGP Operator's Manual* for details of the AGP.

3. Prepare 1 L of 0.20 M oxalic acid by dissolving 25.2 g of reagent grade oxalic acid dihydrate in 1 L of deionized water. This eluent will be used to clean the AGP eluent flow path.

4. Connect the 0.20 M oxalic acid to Port 1 (E1) of the AGP. Pump the oxalic acid through the AGP and to waste at 2.0 mL/min for 10 minutes. Repeat this procedure for ports 2, 3, and 4. This helps to remove any trace metals from the AGP flow path.

5. Place the sample inlet tube into the 0.20 M oxalic acid solution. Fill each of the three 1-L eluent glass bottles and the 4-L carrier bottle with 500 mL of 0.20 M oxalic acid. Enter the following program for system preparation. This program is entered from the front panel of the AGP. Refer to the *AGP Operator's Manual* for details on programming the AGP.

Time	E1	E2	E3	V5	V6	Flow
0.0	100	0	0	1	0	2.0
5.0	0	100	0	1	1	2.0
10.0	0	0	100	0	1	2.0
15.0	100	0	0	0	0	2.0
20.0	100	0	0	0	0	0.0

Before turning the pump switches on, confirm that the sample pump and the carrier pump are primed and their flow rates are set at 2.0 mL/min. Turn the peristaltic pump switch on and adjust its flow rate to at least 5.0 mL/min. Start the AGP and run the above program for 2 to 3 times.

6. Replace the 0.20 M oxalic acid in the 4-L carrier bottle with 0.1 M nitric acid. Be sure the cap has an O-ring for proper sealing. Refer to Appendix A for details on preparing the carrier solution.

7. Clean three 1-L glass eluent bottles by filling them with 0.20 M oxalic acid. Allow the acid to remain in the eluent bottle for at least 4 hours. Prepare eluents as described in Appendix A. Use caution in preparing and transferring these reagents in order to minimize contamination. Connect the filled eluent bottles to the appropriate eluent cap connected to the AGP. Be sure that the eluents are plumbed to the proper ports of the AGP (E1: 2 M ammonium acetate; E2: 2 M nitric acid; E3: deionized water). Adjust the eluent bottle regulator to 4 to 6 psi and check for any gas leaks.

8. Prime the AGP with each eluent using the following procedure. Enter Program 2 into the AGP. Set the flow rate to 3.0 mL/min and enter 100% of E1. Start the AGP and loosen the needle valve located on the pressure transducer housing. This will flush any air out of the eluent lines and the pump components and which will prime the pump. Repeat this procedure for E2 and E3. Be sure to tighten the needle valve upon completing the priming.

9. Enter the following AGP program for chelation concentration. This program is entered from the front panel of the AGP. Refer to the *AGP Operator's Manual* for details on programming the AGP. Check the program carefully for accuracy by listing the program. (This program is also shown in Table 1.)

Time	E1	E2	E3	V5	V6	Flow
0.0	100	0	0	1	0	2.0
2.0	100	0	0	0	1	2.0
5.0	100	0	0	0	0	4.0
6.2	100	0	0	0	0	4.0
6.3	0	75	25	0	0	4.0
6.7	0	75	25	0	0	1.5
6.9	0	75	25	1	1	1.5
8.3	0	75	25	1	1	1.5
8.4	100	0	0	0	0	0.0

SYSTEM TEST

The purpose of this system test is to ensure that all chromatographic and chemical components of the system are operating properly. Refer to Figure 3 for the system schematic. Be sure to check all fittings for leaks during the system test.

1. The system test begins with a test of the hydraulic system. If the system fails the hydraulics test at any point, determine the source of the plumbing error. Begin by using Program 1. With the AGP in the stop-hold position, press RESET. This will set the program to time 0.0.

2. Press START on the AGP. Eluent (E1) should begin to flow to the mixing tee, through the MetPac CC-1 and valve A, and out to the nebulizer. Check the interface tubing on valve A, port 4, of the SCM to confirm that eluent is flowing to the ICAP.

3. Next, prime the carrier pump by loosening the tubing fitting which is screwed into the outlet check valve. Since the carrier reservoir is pressurized (5 psi or 35 kPa), the 0.1 M nitric acid solution should begin to flow out of the check valve. As the carrier solution begins to flow, turn on the carrier pump by pressing the PUMP 1 power switch on the SCM front panel. After about 5 seconds, replace the outlet check valve tubing fitting. It is generally only necessary to finger tighten the fittings. If the fitting leaks, tighten it another 1/8 of a turn using a 5/16-in. open-end wrench.

4. Set the stroke dial of the carrier pump to about 8.00. (Refer to the *SCM Operator's Manual* for details on adjusting the flow rate.) After about 90 seconds, check that the carrier is flowing out of the CARRIER OUT tubing.

5. Calibrate the carrier pump flow rate by mass or volume to 1.8 to 2.0 mL/min. Be sure that the carrier reservoir is pressurized to 4 to 6 psi. Turn off the carrier pump (PUMP 1).

6. Repeat step 3 on the sample pump. Press PUMP 2 power switch on the SCM front panel. Check that the carrier is flowing out of the SAMPLE OUT tubing. Calibrate the sample pump flow rate by mass or volume to 1.8 to 2.0 mL/min. Turn off the sample pump (PUMP 2).

7. Place the sample inlet tube in a container of deionized water and start the peristaltic pump. If the sample loop is not filled, it may take about 120 seconds before DI water begins to exit the waste line of the peristaltic pump. Adjust the peristaltic pump flow rate to at least 5 mL/min.

8. List the AGP program to 2.0 minutes and press run. This will forward the program to 2.0 minutes. Check to see that the eluent is flowing out of the CARRIER OUT tubing. Stop the AGP and confirm that the eluent flow stops from the CARRIER OUT tubing. Turn on the sample pump (Pump 2). Check that the carrier is flowing out of the CARRIER OUT tubing. Turn on the carrier pump (PUMP 1) and confirm that the carrier flows out of the interface tubing.

9. List the AGP program to 5.0 minutes and press start and run. Stop and start the pump to confirm that the carrier from sample pump is flowing out of the SAMPLE OUT tubing.

   If the sample pump looses prime, prime the sample pump by loosening the tubing fitting on the outlet check valve. If there is no liquid in the check valve, use a squirt bottle and squirt some deionized water into the check valve. This will aid in priming the pump. Replace the outlet check valve fitting. If you are going to use an autosampler, connect the autosampler probe to the sample inlet line.

10. With the AGP in the start-run mode, start an ICAP exposure. As soon as the ICAP controller starts the exposure (red EXP light is on), the AGP will reset to time 0.0 and the program will be executed. If the run is aborted, the AGP will continue to run. Allow the AGP to run to the end of the program. Reset and run the program one more time to be sure that the MetPac CC-1 is thoroughly regenerated.

11. Connect the interface tubing to the nebulizer. Turn on the carrier pump and the argon to the nebulizer and torch. Confirm nebulization of the carrier by looking for mist in the spray chamber. At this point, the ICAP torch can be ignited. This completes the system test.

IC/ICAP OPERATION

This section describes the integrated operation of the IC/ICAP system. For details on ThermoSpec software, refer to the appropriate TJA manual.

Methods Development

1. Begin by writing a ThermoSpec method entitled IC/ICAP. Table 1 lists the elements which can be concentrated using the MetPac CC-1 column. Under METHODS DEVELOPMENT/SET-UP, select the elements of interest (F1) and also select the duplicate (F3) function for each element. Select the elements and the duplicate of each element in the order in which you want them printed in the report. In addition, select sodium, potassium, magnesium, and calcium, if available. Do not duplicate these elements. Press F9 to save the element selection.

   Note: The order in which the elements are printed in the analysis report can be selected by the user under F8, OPTIONS from the main menu under METHODS DEVELOPMENT.

2. Next, press F3 for output. Change the NUMBER OF REPEATS to 1 and the CONCENTRATION to ppm or ppb. Select print limits as desired. Press F9 to save.

3. For F5, ELEMENT INFO, the element heading will be changed and the standard(s) concentrations entered. Start by changing the element heading. For the elements in timing group 1 (direct nebulization), enter the element symbol followed by "-DIR". For example, Cu-DIR, Fe-DIR, Na-DIR, etc. For the elements in the timing group 2 (concentrated), enter the element symbol followed by "-CON". For example, Cu-CON, Fe-CON, etc. Next, check to see that elements labeled "-DIR" are in timing group 1 and those elements labeled "-CON" are in timing group 2.

   Enter the appropriate concentration for each element. Refer to Appendix B for recommended standard concentrations. Generally a two-point calibration routine is used, a blank and a high standard, HIGH STD. Be sure that the correct concentration is entered for each element and that only a high standard and a blank appear in the method. Press F9 to save. Be sure to save the entire method.

4. At this point it is necessary to run a time scan. Press F6 for SCAN and F7 for TIME SCAN. The TIME SCAN feature is used to determine the retention times of the direct nebulization and the concentrated bands. Place the sample inlet tube into the high standard and turn on the peristaltic pump.

5. In the TIME SCAN mode, the spectrophotometer monitors the plasma for a user-specified period of time (much like a chromatographic detector). Enter an integration "slice" of 5.0 and press ENTER. Enter 100 for the number of time slice. This will result in a time scan of 500 seconds. If the eluents are prepared properly, the concentrated sample band should elute between 425 and 475 seconds.

6. Press F1, RUN. The controller goes to an exposure mode (the EXP LED will light), a signal is sent to the AGP that resets the program to time 0.0, and the AGP program begins running. The exposure will end in 500 seconds.

7. The results of the time scan displayed on the screen should look like Figure 5. For the concentrated elements, two discrete regions are present in the time scan. The first region between 140 seconds and 160 seconds is the result of direct nebulization. This represents the 1-mL loop of raw sample that was delivered to the nebulizer by the carrier pump. The second region is typically between 425 and 475 seconds and represents the 5-mL loop of sample that was concentrated and subsequently eluted off the MetPac CC-1 column. Press F1, EXPAND, and move the cursor to determine the time at which the peak begins to elute. Note this time for comparison to the next run. Using the cursor, determine the width of the peak at the base. Typical base width is 25 to 35 seconds.

   Use the cursor to determine the time at which a steady state of (PURE SAMPLE) sample is present in the direct nebulization. Steady state occurs at the elevated, flat portion of the direct nebulization region. This usually occurs between 140 and 160 seconds. Note the time of the steady state.

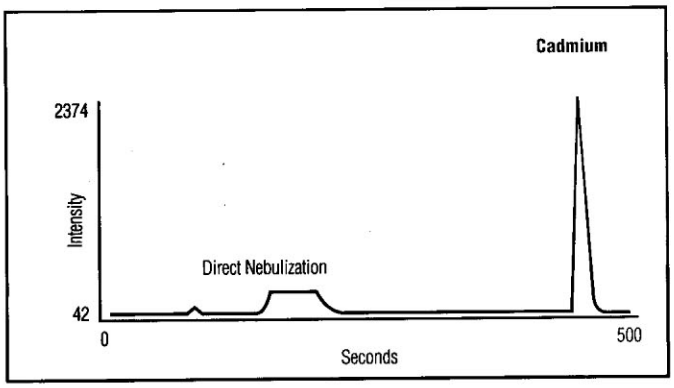


Figure 5 *Time study using chelation concentration for cadmium*

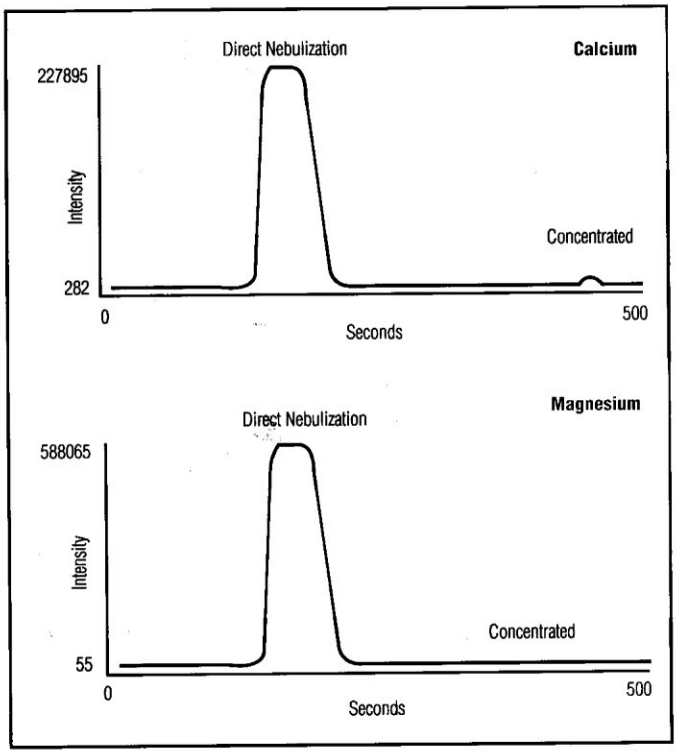


Figure 6 *Time studies using chelation concentration for calcium and magnesium*

Elements that are not present (or greatly reduced) in the concentrated band include sodium, potassium, magnesium, and calcium. Figure 6 shows the time scans for calcium and magnesium. For magnesium, notice a large signal in the direct nebulization, but virtually no signal in the concentrated band. This time scan shows the benefit of the matrix elimination capability of the method.

8. Use the F5, OVERLAY function to overlay several of the time scans. Notice that all of the concentrated metals elute with nearly the same retention times. The time scan can be stored if required.

9. Repeat the time scan. The retention time of the peak should not vary by more than 3 seconds. Continue to repeat the time scan until the retention time variation is within 3 seconds. Return to the main menu for methods development.

10. Use the F2, INTERNAL STANDARDS function to enter the timing groups. For timing group 1, enter a preintegration time (in seconds) determined from the direct nebulization region of the time scan. This preintegration time is the time from the beginning of the exposure to steady state. This is typically about 140 seconds. For the integration time, enter 10 to 20 seconds, depending on the duration of the steady state signal.

    For timing group 2, enter a preintegration time determined from the concentration region of the time scan. The preintegration time is the time from the beginning of the exposure to when the peak(s) begin to elute. It is advisable to enter a preintegration time about 3 to 5 seconds less than the measured times to allow for minor shifts in retention times. For the integration time, add about 5 seconds to the measured base width and enter this value (typically 35 to 45 seconds).

11. Press F9, SAVE, to complete the methods development. Be sure to save the entire method.

Standardization

Standardization of the ICAP system with the IC is performed in the same manner as normal ICAP standardization. Standardization can be performed manually or with the autosampler.

1. In the ANALYSIS section of ThermoSpec, press F3 for STANDARDIZATION. For the IC/ICAP method, two entries should appear: BLANK and HIGH STD. Prepare a high standard as directed in Appendix B. The blank contains 1% nitric acid (trace metal grade).

2. Run the blank. After standardization with the blank, compare the intensity values obtained for the direct ("DIR") and concentrated ("CON") timing groups for the appropriate elements. The intensity values should be approximately the same and should correspond to blank intensities obtained without the IC. The CON values for elements such as zinc, iron, and copper may be significantly higher (2 to 4 times) than the DIR values. This results from trace metals in the ammonium acetate or nitric acid solutions being concentrated on the column and subsequently eluted. In general, if the reagent solutions are relatively pure, the CON values for iron and zinc should not be greater than three times the DIR value. No appreciable copper blank should be present. If the blank values appear within range, press F9, SAVE, and continue.

3. Next, run the high standard. Compare the intensity values obtained for the direct and concentrated values. For all elements concentrated, the CON value should be greater than the DIR value. If the values are in range press, F9, SAVE, and complete the standardization (press F9 again). If the CON values are not at least two times greater than the DIR values, then either an insufficient amount of the high standard was loaded or the standard was not prepared properly.

4. Finally, run the high standard as a sample. The reported values should be within 1 to 3% of the calibrated values. If the values are out of range, run the standard again. If the result is still out of range, run another time scan and repeat the standardization procedure. This completes standardization. The system is now ready for sample analysis.

Sample Preparation

It is beyond the scope of this technical note to describe in detail all of the techniques of ultratrace analysis in terms of sample collection, storage, and handling. However, several points are discussed below that are applicable to sample preparation before analyzing samples by the method described in this technical note.

Samples should be collected in **clean** polyethylene containers. In order to stabilize the sample for storage, the sample should be acidified to a pH 1.5 to 2. Be sure to use ultrapure nitric acid to adjust the pH.

To ensure complete recovery of metals using chelation concentration, metal ions should not be bound by any strong complexing agents or be present as hydroxy complexes. Acid digestion is a general technique used to destroy complexing agents or to minimize their complexation ability. If you are analyzing samples that may contain large amounts of organic materials (e.g., humic acids), it is advisable to digest the sample. If there is solid material in the sample, it should be digested. In general, if you have used digestion for sample pretreatment prior to metal analysis, those same digestion procedures can be used in this method. **Never attempt to concentrate a sample that contains solids or suspended materials.**

Because sample preparation plays a critical role in the accuracy and the precision of analytical measurements, any contamination from sample handling or addition of reagents to the sample must be avoided or minimized. Since the pH of any digested or undigested sample is normally maintained in the acidic range, a direct introduction of such acid samples into an analytical system would minimize the sample contamination. The acidified sample can be neutralized by on-line addition of a buffer solution to the sample stream before it enters the chelator column. Because the sample pH is maintained in the acidic range, the adsorption, hydrolysis, and precipitation of metal ions that can occur during sample preparation will be avoided.

An acidified sample, containing up to 0.5 M acid, can be loaded directly. The SCM performs on-line buffering on the acidified sample just before the sample effluent enters the MetPac CC-1 column. If the sample contains more than 0.5 M acid (e.g. digested samples), it is recommended that 4 to 6 M ammonium acetate be used to neutralize the acidified sample. Use port 4 of the AGP for a 4.0 M ammonium acetate (pH 5.5) solution, and correct the percent eluent on the AGP to E4 at time 2.0 min and 5.0 min.

Sample Analysis

Samples can be run either manually or automated using the IC/ICAP method. To run samples manually, place the sample probe (inlet) in the sample solution before starting the ICAP sequence.

In the automated mode, set up the autosampler with type 24 racks (14 samples per rack). Each sample vial must contain at least 12 mL of buffered sample. Make the first sample in the first rack a blank; this will allow for proper sequencing of the IC, ICAP and autosampler. Be sure to enter the appropriate dilution factor for each sample in the autosampler table.

APPENDIX A: PREPARATION OF REAGENTS

The following reagents are required. The two eluents used for chelation concentration are available from Dionex in a ready-to-use form. If you wish to prepare your own reagent solutions, information for ordering ultrapure acids and ammonium hydroxide is also provided. Three concentrated reagents are required for eluents in this method: Nitric acid, acetic acid, and ammonium hydroxide. For ultratrace level determinations (sub-ppb) the reagents must be ultrapure grade. For determinations above 1 ppb, high quality trace metal grade reagents can be used. Any metal impurity in these reagents will be concentrated with your sample constituting a system blank.

Chelation Concentration Reagents:
Dionex Ultrapure Eluents

2 M Ammonium acetate, pH 5.5 (1 L, P/N 33440; 6 L, P/N 33441)

2 M Nitric acid (1 L, P/N 33442; 6 L, P/N 33443) or acetic acid, ultrapure (Seastar or Ultrex, 1 L)

Ammonium hydroxide, ultrapure (Seastar or Ultrex, 1 L)

Nitric acid, ultrapure (Seastar or Ultrex, 1 L)

Oxalic acid dihydrate (100 g), trace metal grade

Metal standards, 1000 or 10,000 ppm, 100 mL each

In North America, Seastar Ultrapure Reagents can be obtained through Fisher Scientific under the Optima label. Ultrex reagents can be obtained through Van Waters and Rogers (VWR) Scientific. If you cannot obtain these reagents through these sources, please contact:

Seastar Chemical
318 Second Ave. South
Seattle, WA 98104
U.S.A.
Tel: (206) 623-2855

Ultrex Reagents
J.T. Baker
222 Red School Lane
Phillipsburg, NJ 08865
U.S.A.
Tel: (201) 859-9357

Eluents and Standard Preparation

Before preparing eluents and standards, thoroughly clean the eluent containers as directed in "System Preparation", earlier in this Technical Note. Be sure that the eluent bottle caps have a white TFE seal and **not** a black rubber seal. Prepare all eluents directly in the 1-L glass eluent containers. Transfer reagents directly from their container. Avoid using pipets or graduated cylinders unless they have been thoroughly cleaned.

Chelation Concentration Eluents: Transition Metals

Use only ultrapure chemicals and deionized water (less than 0.5 ppb for each metal) for preparation of these reagents. Caution must be used in preparing this reagent in order to minimize metal contamination. Do not place anything in the eluent container (including stir bars). When adjusting the pH of the ammonium acetate and ammonium nitrate, do not place the pH electrode in the bulk solution. Instead, take aliquots of the solutions to check the pH.

Eluent 1: 2 M Ammonium Acetate, pH 5.4 ±0.1

Place 600 mL of deionized water into a clean 1-L glass eluent container. Add 121 g (115 mL) of ultrapure glacial acetic acid and mix thoroughly. In a fume hood, **slowly** add 120 g (130 mL) of 20% ultrapure ammonium hydroxide and mix thoroughly. Agitate the bottle to thoroughly mix the solution. Calibrate a pH meter to pH 7. Pour about 10 mL of the buffer into a small container (e.g., scintillation vial or 10 mL disposable beaker) and measure the pH. If the pH is below 5.3, add about 5 mL of ammonium hydroxide to the buffer solution. If above pH 5.5, add 5 g of acetic acid. Adjust the pH of the ammonium acetate to 5.4 ±0.1 using acetic acid if the pH is greater than 5.5, or ammonium hydroxide if the pH is less than 5.5. Once the pH is 5.4 ±0.1, bring up to a volume of 1 L.

Eluent 2: 2.0 M Nitric Acid

Place 200 mL of deionized water into a clean 1-L glass eluent container. Add 179 g (126 mL) of ultrapure nitric acid. Add deionized water to bring the final volume to 1 L and mix thoroughly.

Eluent 3: Deionized Water—HPLC Grade

Carrier Solution: 0.1 M Nitric Acid

Place about 1 L of deionized water into the 4 L plastic eluent container. Add 89 g (63 mL) of concentrated nitric acid and an additional 3 L of deionized water.

Concentrated Sample Buffer: 6 M Ammonium Acetate, pH 5.5

Place 200 mL of deionized water into a clean 1-L glass eluent container. Add 363 g (345 mL) of ultrapure glacial acetic acid and mix thoroughly. In a fume hood, **slowly** add 360 g (390 mL) of 20% ultrapure ammonium hydroxide and mix thoroughly. Agitate the bottle to thoroughly mix the solution. Calibrate a pH meter to pH 7. Pour about 10 mL of the buffer into a small container (e.g., scintillation vial or 10 mL disposable beaker) and measure the pH. If the pH is

below 5.3, add about 15 mL of ammonium hydroxide to the buffer solution. If above pH 5.5, add 15 g of acetic acid. Adjust the pH of the ammonium acetate to 5.4 ±0.1 using acetic acid if the pH is greater than 5.5, or ammonium hydroxide if the pH is less than 5.5. Once the pH is 5.4 ±0.1, bring up to a volume of 1 L.

APPENDIX B: PREPARATION OF STANDARDS

Listed below is a typical standard Dionex uses for a variety of sample matrices. Since the analytes of interest are concentrated, the concentration of the high standard used should not exceed 1 ppm. It is convenient to prepare a 10X concentrate or stock solution of the standard and prepare the high standard by dilution of the stock solution. Prepare the stock solution given below.

Metals	Concentration (ppm)
Alkali	200
Alkaline earth	100
Transition metals	1.0
Al, Fe, Pb	5.0

To prepare 100 mL of the standard listed below, place 10 mL of the stock solution in a 100 mL volumetric flask. Add 1 mL of concentrated nitric acid, trace metal grade. Bring the solution to a final volume of 100 mL.

Metals	Concentration (ppm)
Alkali	20
Alkaline earth	10
Transition metals	0.1
Al, Fe, Pb	0.5

Determination of Lanthanide Metals in Digested Rock Samples by Chelation Ion Chromatography

INTRODUCTION

The naturally occurring high concentrations of certain metals such as iron, aluminum, transition metals, alkali metals, and alkaline earth elements in geological materials usually interfere with the determination of the trace lanthanide metals in these samples. The predominant analytical techniques that have been used to determine lanthanide metals in the geological materials include neutron activation (NAA), isotope dilution mass spectrometry (IDMS), spark-source mass spectrometry (SSMS), inductively coupled plasma-mass spectrometry (ICP-MS), X-ray fluorescence (XRF), inductively coupled plasma-atomic emission spectroscopy (ICP-AES), and DC-plasma emission spectroscopy. In addition to the high cost of instrumentation and the need for highly skilled operators, these analytical techniques suffer from matrix interference problems. All these techniques require that the sample be taken into solution and the matrix removed prior to the analytical measurement. The removal of sample matrix by opened-column chromatography is a complex process that can take several hours per sample. If the matrix removal method is not employed, several common methods are used to minimize the sample matrix interferences. These include spectral background corrections, standard additions, sample dilution, and matrix matching. These methods help to minimize matrix interferences; however, these methods often result in degraded detection limits and accuracy.

A new technique called *chelation ion chromatography* (chelation IC) has been developed for the determination of transition and lanthanide metals in complex matrices. The sample pretreatment system (chelation concentration) coupled directly to the ion chromatograph (IC) offers the analyst a solution to the detection limit and interference problems commonly experienced when analyzing complex matrices. Chelation IC combines a selective concentration and matrix elimination with analytical separations and specific detection for lanthanide metals. Selective ion exchange materials such as chelating resins can concentrate transition and lanthanide metals while eliminating alkali and alkaline earth elements. Then the selective elimination process removes most of the iron, aluminum, and transition metals from the sample matrix. By using a matrix selective complexing agent, 90 to 99% of the iron, aluminum, and transition metals are removed while all of the lanthanides are quantitatively retained. Finally, the concentrated lanthanide metals are separated by high performance ion chromatography with specific detection. The overall analysis time after sample dissolution is approximately one hour.

INSTRUMENT REQUIREMENTS

This Technical Note gives detailed descriptions of the system components, installations, and operation of the chelation ion chromatographic system. Consult this information carefully before operating the chelation IC

system. It is important to understand each step of the sample pretreatment process to maintain the best performance of the chelation IC system. This chelation IC technique was designed to operate on a Dionex Series 4000i, 4500i, or DX-300 IC system. The recommended system configuration is shown in Figure 1.

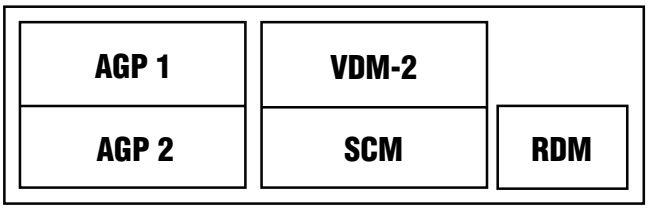


Figure 1. Chelation ion chromatography system.

A gradient pump, designated AGP1, performs the steps of chelation concentration and controls valves. Another gradient pump, designated AGP2, is used as the analytical pump for analytical separation. A DQP pump (labeled Sample Pump) in the Sample Concentration Module (SCM) is used for loading the sample from the sample loop onto the MetPac™ CC-1 chelating column. Another DQP pump (eluent pump, labeled Carrier Pump) with a pulse damper is used as an accessory pump if required. Five 4-way slider valves are located in the front section of the SCM.

To improve the detection limits of the chelation IC system, a membrane reactor is recommended in place of the mixing tee. The membrane reactor lowers detection limits for metals fivefold compared to adding reagent using the mixing tee. The membrane reactor should be used if lanthanide metals are being determined below 0.5 ppb. A beaded reaction coil (P/N 036036) or a knitted reaction coil (P/N 039349) should always be used with the membrane reactor.

The chelation ion chromatography system comprises a Dionex chromatographic system with the following components:

Advanced Gradient Pump (AGP; two required)
Sample Concentration Module
Reagent Delivery Module (RDM)
Variable Wavelength Detector Module (VDM-2)
Eluent Degas Module (EDM-2)
Eluent Container Set, glass (P/N 038752)
High Pressure 4-Way Valve (BF-2), 1/4-28 fittings (P/N 038598)
IonPac® Membrane Reactor (P/N 035354, optional)
Knitted reaction coil (P/N 039349)
MetPac CC-1 Column (P/N 042156)
TMC-1 Concentrator Column (P/N 042155)
IonPac NG1 Guard Column (P/N 039567)
HPIC CG2 Guard Column (P/N 035370)
IonPac CG5 Guard Column (P/N 037029)
ACI/AI-450 data acquisition and handling system

SYSTEM CONFIGURATION

Sample Concentration Module

The block schematic of the chelation IC system is shown in Figure 1. The SCM contains two single-piston Dionex QIC Pumps (DQP), 2000 psi (13.8 MPa); inert double stack 4-way pneumatically controlled slider valves; and a pulse damper. One of the DQPs is used to pump sample into the MetPac CC-1 column; the other pump is used as an accessory pump. All of these components are housed in a single enclosure. The rear panel of the SCM contains bulkhead fittings for connecting waste lines and eluent lines.

Figure 2 shows a detailed pneumatic and hydraulic schematic of the SCM system. From the factory, the SCM is configured to be used for sample pretreatment with an external detector (ICP). For chelation IC applications, configure and plumb the SCM as shown in Figure 2.

Advanced Gradient Pump

The AGP is a microprocessor-controlled, high performance quaternary gradient IC pump. It is chemically inert and has a metal-free flow path. The AGP permits the time-dependent selection of up to four different eluents, flow rate, and the control of two sets of air solenoids for external valve control. Controls 5 and 6 of the pump (referred to as V5 and V6, respectively) are used to control the five valves in the SCM. Valve control is programmable from the front panel and can store up to 10 different programs. Refer to the *AGP Operator's Manual* for information on operation and maintenance.

The AGP can be controlled by an integrator or the AI-450 Chromatography Workstation. For automation of the system, the Dionex Automated Sampler Module (ASM) for 1- to 3-mL sample preconcentration can also be used and controlled by the AI-450.

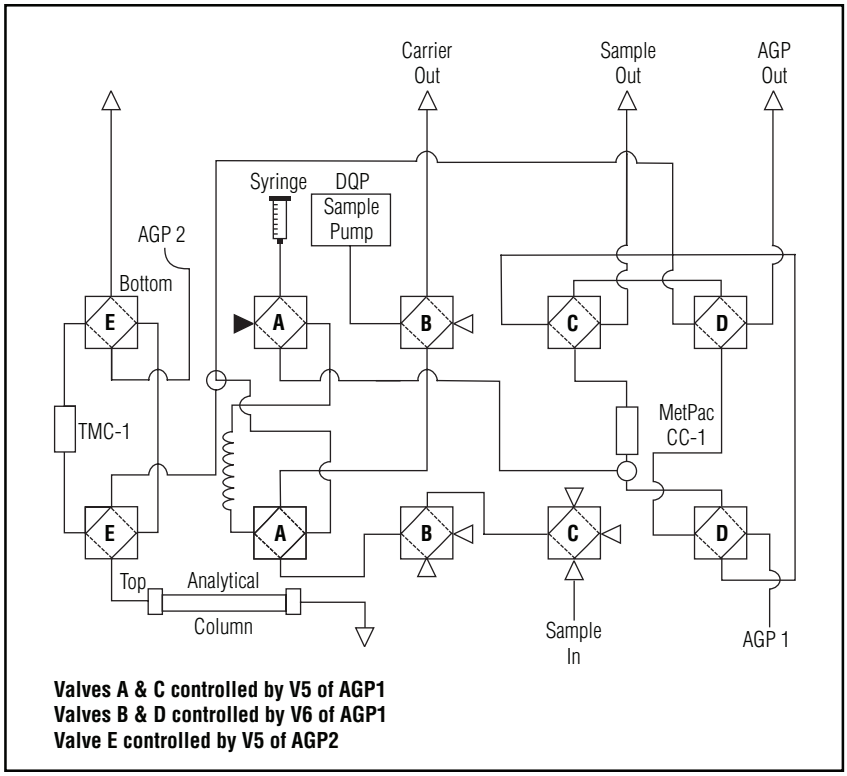


Figure 2. Schematic of chelation ion chromatography system.

Pneumatic Connections

Disconnect all the air tubings in the SCM and reconnect as indicated in Figure 2. This system configuration contains two AGPs. The first AGP, designated AGP1, performs the chelation concentration steps. The second AGP, designated AGP2, is the analytical pump used for the chromatography. Be sure that valves A and C are controlled by V5 and that valves B and D are controlled by V6 of AGP1. Valve E is controlled by V5 of AGP2. Connect the air tubing between the two AGPs and the SCM. Confirm that the air tubings are connected to the proper valves (A, B, C, D, and E) as shown in Figure 2. Also confirm that the orange tubing is connected to the tops of valves A and C and the green tubing to the tops of valves B and D. Connect the yellow tubing to the bottom of valves A and C and the blue tubing to the bottom of valves B and C. From AGP2, connect the orange tubing to the top of valve E and the yellow tubing to the bottom of the same valve. Next, connect about 2 ft (65 cm) of air tubing (P/N 030091) to the small barbed fitting on the back of the two AGPs. Insert a barbed tee (P/N 030538) into the end of this tubing. One arm of the tee will go to the nitrogen or argon source (regulator) and the other arm to the eluent regulator or EDM (optional).

Next, using a barged tee, connect the air tubing from the nitrogen or argon source (eluent regulator, or EDM) to the inlet of the eluent bottle regulator (P/N 038201). Using the required lengths of the tubing, connect the tee to the gas source and to the eluent pressure regulator. Using the 1/4-in. x 10/32 brass reducer (P/N 030087) and the 1/16-in. x 10/32 barbed fitting (P/N 030017), connect the air tubing to the gas source regulator. This completes the pneumatic set-up.

Hydraulic Connections

Refer to the *AGP Operator's Manual* and the *SCM Operator's Manual* for details on the installation and operation of the different modules. Begin the hydraulic connections by connecting the four eluent lines from the four eluent container caps to the front panel eluent port of the pump. Notice that the eluent lines are labeled 1, 2, 3, and 4. Be sure that the eluent lines are connected to the appropriate eluent port of the pump.

Connect the eluent line from the 4-L plastic eluent container (P/N 039164) to the SAMPLE IN port of the SCM rear panel. This port is connected to the check-valve inlet of the sample pump located on the right side of the SCM. Next, connect the three blue waste lines (P/N 039341) to the ports of the SCM rear panel labeled CARRIER OUT, AGP OUT, AND SAMPLE OUT, and place them in a waste container. Also, another waste line from valve E can be placed in the waste container. This completes the hydraulic connections.

Electrical Connections

Verify that the front PUMP 1 and PUMP 2 power switches of the SCM are off. Using the power cords provided (P/N 096078), connect the AC receptacles on the rear panel of the SCM to the SWITCHED AC on the rear panel of the ACI. Connect the AC receptacles on the rear panels of the AGP and VDM-2 to the white outlets of the power strip located on the rear upper section of the system enclosure. Next, connect the AC receptacles of the power strip enclosure and of the ACI to AC (110 V) power outlets.

Connect the ACI cables (ribbon cables) to the appropriate components as configured in system configuration in AI-450. Also assign AC1 in the system configuration as SP (sample pump), relay 1 as AGP2, and relay 2 as ASM (autosampler module).

Connect two relay TTL cables (P/N 042599) from the rear panels of the ACI to AGP2 and the autosampler. Consult the appropriate manuals for proper installation.

Chemicals, Reagents, and Standards

Appendix A shows a complete list of reagents and instructions for reagent preparation. The reagents used for chelation concentration — 2.0 M ammonium acetate, pH 5.5, 2.0 M nitric acid, and 0.1 M ammonium nitrate, pH 3.5 — are available from Dionex. In addition, high purity water containing less than 500 ppt of common transition metals (iron, zinc, copper, etc.) is required.

Other Supplies

In addition to the items listed above you will also need:

Electrical power

Compressed nitrogen (80-120 psi; 55-83 kPa)

Standard analytical laboratory equipment such as a balance, pH meter, etc.

DISCUSSION OF THE METHOD

The method described in this Note was developed for determining trace lanthanide metals in complex matrices containing high levels of alkali metals, alkaline earth elements, iron, aluminum, and transition metals. The elimination of alkali and alkaline earth metals is based on the nonselective property of the MetPac CC-1 chelating resin for these elements. The weakly retained alkali and alkaline earth metal ions are separated from other elements using ammonium acetate. The selective removal of iron, aluminum, and transition metals is based upon the metal chloride formation induced by a water-miscible organic eluent, for example, a hydrochloric acid/ethanol mixture. This eluent not only promotes the formation of metal chloride complexes, it also decreases the distribution coefficient of the metal complexes on the cation exchange resin. Thus, the relatively stable metal chloride complexes of iron, aluminum, and most transition metals are selectively removed from the TMC-1 cation exchange column using hydrochloric acid/ethanol eluent. On the other hand, the lanthanide metals form less stable metal chloride complexes and are retained quantitatively on the TMC-1 column. Therefore, by using the optimal concentration of hydrochloric acid/ethanol eluent, lanthanide metals are selectively concentrated on the cation exchange column, while the majority of iron, aluminum, and transition metals is eliminated. Then, the TMC-1 column is converted from hydrogen to ammonium form with ammonium nitrate. Finally, the concentrated lanthanide metals are eluted with the PDCA eluent to the IonPac CS5 column, where they are resolved chromatographically.

Note: Metal chloride complexes are formed by the hydration reduction of the cationic species by the organic solvents. Generally, the effects of increasing amounts of the organic solvent are a reduction of the water molecules around the metals, a decrease in the forces binding the coordinated hydration shell, and a decrease in the size of the outer hydration cloud. Consequently, the metal chloride complexes formed in the organic solvent are relatively stable.

Chelation Concentration and Matrix Elimination

The chelating concentrator column, the MetPac CC-1, is used to selectively concentrate lanthanide metals from the aqueous sample. Alkali and alkaline earth metals are selectively eluted to waste using 2.0 M ammonium acetate, pH 5.5. Transition metals, which are concentrated along with the lanthanide metals, are removed in a tight band to the high capacity cation exchange column (TMC-1), where further separation takes place. Next, the transition metals are eluted to waste using 1.5 M hydrochloric acid/75% ethanol, while the lanthanide metals are retained in the TMC-1 column. The experimental results suggest that at least 17 mL of the hydrochloric acid/ethanol eluent can be used without eluting lanthanide metals from the TMC-1 column.

Summary of Method

In summary, chelation concentration comprises five major processes:

1. The digested sample is loaded into the sample loop. Then, the valve diverts the sample loop in-line with the MetPac CC-1. The sample loop is flushed by water from the sample pump, and the sample stream is buffered with 2.0 M ammonium acetate (pH 5.5) and passes through the MetPac CC-1 column and out to waste. Most polyvalent cations

are quantitatively concentrated from the sample while anions and alkali metals pass through the column essentially unretained.

2. Weakly bound alkaline earth metals such as calcium and magnesium are selectively eluted. This is accomplished by using 2.0 M ammonium acetate eluent (pH 5.5).

3. Concentrated transition and lanthanide metals are eluted in a 100 to 200-µL volume using 1.0 M nitric acid. The acid effluent from the MetPac CC-1 column is diluted on-line with deionized water from the sample pump before entering the TMC-1 column. The purpose of on-line dilution is to maximize the metal retention on the TMC-1 column.

4. Next, the transition metals are selectively eliminated from the TMC-1 column using a mixture of 1.5 M hydrochloric acid/75% ethanol solution. The lanthanide metals are quantitatively retained on the TMC-1, while the transition metals are eluted to waste.

5. The MetPac CC-1 is converted back to the ammonium form using 2.0 M ammonium acetate.

Before the TMC-1 can be injected into the analytical stream, it must be converted from the acid (H^+) form to ammonium (NH_4^+) form by using 0.1 M ammonium nitrate (pH 3.5). Converting the column from the acid form to the ammonium form prevents a pH disturbance of the weak acid eluents, which causes analytical problems.

The separation of lanthanide metals is accomplished by anion exchange of lanthanide-chelator complexes. By using PDCA as an eluent chelator, the concentrated transition and lanthanide metals are eluted from the TMC-1, as metal-PDCA complexes, to the IonPac CS5 column. Transition metals form stable monovalent or divalent anionic complexes with PDCA, while lanthanide metals form stable trivalent anionic complexes with PDCA. The resulting ionic charge differences between the lanthanide and transition metals permit separation of the transition metals while the lanthanides are retained in the CS5 column. After the transition metals are separated and completely eluted from the analytical column, the lanthanide metals are separated and eluted using the oxalate and diglycolate eluent.

Separations of lanthanide metals in digested rock samples are shown in Figures 3 through 5 on page 7. This method has been applied to the United States Geological Survey (USGS) Basalt (BHVO-1), Andesite (AGV-1), and Periotite (PCC-1) Geochemical Standard Reference Materials. Table 1 shows the matrix compositions of those samples. The results of the spike/recovery experiment and the USGS Geochemical Standard Reference Materials are shown in Tables 2 and 3.

Table 1 Sample Matrix Composition of USGS Geological Samples

Element	Basalt (BHVO-1)	Andesite (AGV-1)	Periotite (PCC-1)
SiO_2*	49.94%	58.79%	41.67%
Al_2O_3	13.80%	17.14%	0.67%
Fe_2O_3	12.23%	6.76%	8.25%
MnO	0.168%	0.092%	0.119%
MgO	7.23%	1.53%	43.43%
CaO	11.40%	4.94%	0.52%
Cu	136 ppm	60 ppm	10 ppm
Ni	121 ppm	16 ppm	2360 ppm
Zn	105 ppm	88 ppm	42 ppm

*SiO_2 must be removed from the sample matrix during sample digestion with concentrated hydrofluoric acid

Table 2 Low Spike/Recovery of Trace Lanthanide in PCC-1 Matrix

Element	Spike (ppm)	Found (ppm)
La	0.07	0.088 ± 0.002
Ce	0.133	0.127 ± 0.087
Pr	0.033	0.035 ± 0.005
Nd	0.067	0.079 ± 0.003
Sm	0.017	0.018 ± 0.000
Eu	0.0067	0.007 ± 0.000
Gd	0.0167	0.017 ± 0.000
Tb	0.0033	0.004 ± 0.000
Dy	0.0167	0.020 ± 0.000
Ho	0.0033	0.004 ± 0.000
Er	0.0067	0.008 ± 0.000
Tm	0.0033	0.004 ± 0.000
Yb	0.0067	0.009 ± 0.000

Sample dilution is 1:150 and 3 mL is concentrated. All values are blank corrected.

Table 3 Analysis of Lanthanide Metals in the USGS Geochemical Standard Reference Materials Using Chelation Ion Chromatography

Element	AGV-1 (ppm) Sugg. Value	AGV-1 (ppm) Chelation IC	BHVO-1 (ppm) Sugg. Value	BHVO 1 (ppm) Chelation IC
La	38	43 ± 1	15.8	12.3 ± 0.3
Ce	67	75.83 ± 0.05	39	33.4 ± 0.4
Pr	7.6	9.2 ± 0.2	5.7	4.66 ± 0.02
Nd	33	33.26 ± 0.02	25.2	23.8 ± 0.6
Sm	5.9	5.99 ± 0.07	6.2	6.3 ± 0.1
Eu	1.64	1.5 ± 0.1	2.06	2.40 ± 0.046
Gd	5	4.58 ± 0.09	6.4	6.69 ± 0.07
Tb	0.7	0.66 ± 0.02	0.96	1.14 ± 0.01
Dy	3.6	3.556 ± 0.001	5.2	5.45 ± 0.02
Ho	0.67	NA	0.99	NA
Er	1.7	1.75 ± 0.01	2.4	2.42 ± 0.06
Tm	0.34	0.246 ± 0.003	0.33	0.485 ± 0.004
Yb	1.72	1.727 ± 0.006	2.02	1.98 ± 0.004
Lu	0.27	NA	0.291	NA

*Sample dilution is 1:150 and 3.0 mL of diluted sample is concentrated. The lanthanide concentrations are averaged from three runs.

SYSTEM PREPARATION, SET-UP, AND TEST

1. Confirm that the SCM is configured as shown in Figure 2. Be sure that an NG1 column is installed between AGP1 and valve D of the SCM. Also, confirm that the HPIC-CG2 column is installed between the sample pump and valve B. Install a MetPac CC-1, TMC-1, and IonPac CG5 and CS5 columns as indicated in Figure 2.

2. Remove the GM-2 mixer from the low pressure side of AGP1. This mixer is located between the valve manifold and the eluent priming block of the AGP. Connect the two lines using a coupler (P/N 039056). Do not install a mixer on the high pressure side of the pump (i.e., between the pump and valve D). Refer to the *AGP Operator's Manual* for details.

3. Prepare 1 L of 0.2 M oxalic acid by dissolving 25.2 g of reagent grade oxalic acid dihydrate in 1 L of deionized water. This solution will be used to clean the eluent flow path.

4. Connect the 0.2 M oxalic acid to E1 of the AGP. Pump the oxalic acid through the pump and to waste at 2.0 mL/min for 10 min. Repeat this procedure for ports 2, 3, and 4 to remove all trace metals from the flow path.

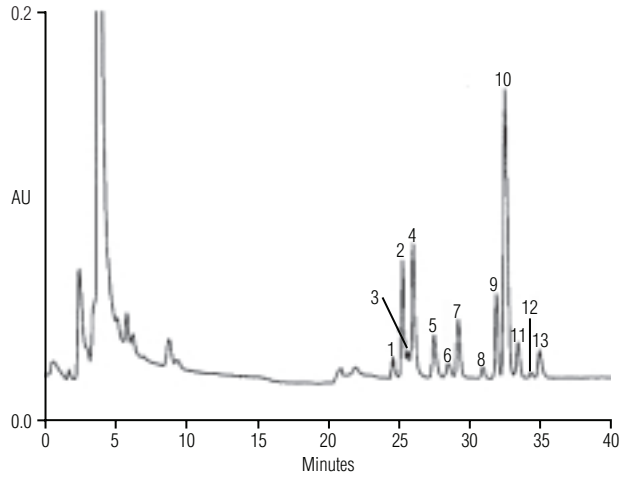
5. Place the sample inlet tube into the 0.2 M oxalic acid solution. Fill each of the four 1-L eluent bottles and the 4-L plastic eluent bottle with 500 mL of 0.2 M oxalic acid. Enter the following program for system preparation from the front panel of the AGP. Refer to the *AGP Operator's Manual* for details on programming.

Time	E1	E2	E3	E4	V5	V6	Flow Rate
0.0	100	-	-	-	1	0	2.0
5.0	-	100	-	-	1	1	2.0
10.0	-	-	100	-	0	1	2.0
15.0	-	-	-	100	0	0	2.0
20.0	100	-	-	-	0	0	0.0

6. Replace the 0.2 M oxalic acid in the 4-L plastic bottle with deionized water. Be sure that the cap has an o-ring for proper sealing.

7. Clean five 1-L glass eluent bottles by filling them with 0.2 M oxalic acid. Allow the acid to remain in the eluent bottle for at least 4 hours. Prepare eluents as described in Appendix A. Use caution in preparing and transferring these reagents to minimize contamination. Connect the filled eluent bottles to the appropriate eluent caps connected to the pump. Be sure that the eluents are plumbed to the proper ports. Adjust the eluent bottle regulator to 4 to 6 psi and check for gas leaks.

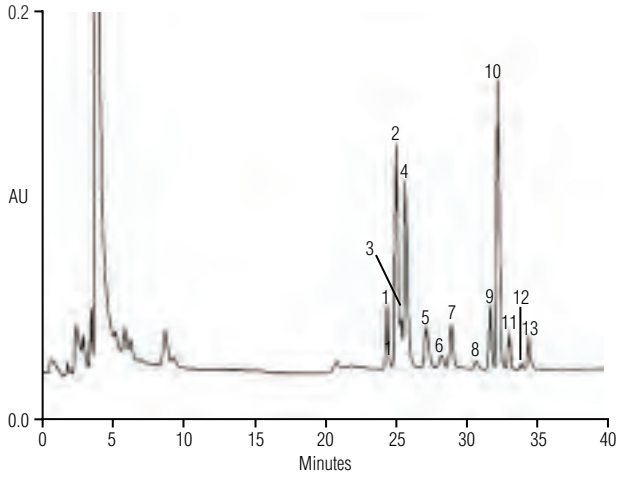
8. Prime the AGP with each eluent using the following procedure: Enter the previous program into the AGP. Set the flow rate to 3.0 mL/min and select 100% of E1. Start the AGP, hold the program, and loosen the needle valve located on the pressure transducer housing. This will flush any air out of the eluent lines and the pump compartments, thus priming the pump. Repeat this procedure for E2, E3, and E4 by selecting the next sequence of the gradient program. Be sure to hand-tighten the needle valve when priming is complete.



Peaks	Sugg. Value (ppm)	Chelation IC (ppm)
1. La	15.8	12.3 ± 0.3
2. Ce	39.0	33.4 ± 0.4
3. Pr	5.7	4.66 ± 0.02
4. Nd	25.2	23.8 ± 0.6
5. Sm	6.2	6.3 ± 0.7
6. Eu	2.06	2.40 ± 0.046
7. Gd	6.4	6.69 ± 0.07
8. Tb	0.96	1.14 ± 0.01
9. Dy	5.20	5.45 ± 0.02
10. Ho, Y	0.99, 27.6	NA
11. Er	2.4	2.42 ± 0.06
12. Tm	0.33	0.485 ± 0.004
13. Yb, Lu	2.02, 0.291	NA

1:150 Sample Dilution
3.0 mL Sample Concentration
(Estimated Sample Injected = 20 mg)

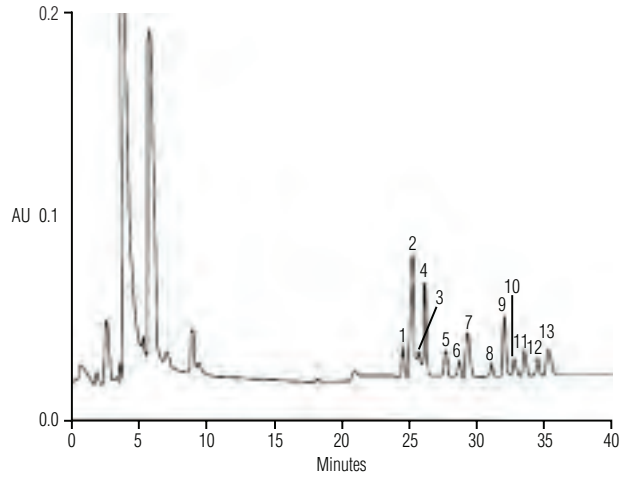
Figure 3. Determination of lanthanide metals in USGS basalt (BHVO-1).



Peaks	Sugg. Value (ppm)	Chelation IC (ppm)
1. La	38.0	43 ± 1
2. Ce	67.0	75.83 ± 0.05
3. Pr	7.6	9.2 ± 0.2
4. Nd	33.0	33.26 ± 0.026
5. Sm	5.9	5.99 ± 0.07
6. Eu	1.64	1.5 ± 0.7
7. Gd	5.0	4.58 ± 0.09
8. Tb	0.7	0.66 ± 0.02
9. Dy	3.6	3.556 ± 0.001
10. Ho, Y	0.67, 20	NA
11. Er	1.7	1.75 ± 0.01
12. Tm	0.34	0.246 ± 0.003
13. Yb, Lu	1.72, 0.27	NA

1:150 Sample Dilution
3.0 mL Sample Concentration
(Estimated Sample Injected = 20 mg)

Figure 4. Determination of lanthanide metals in USGS andesite (AGV-1).



Peaks	Spike (ppm)	Found (ppm)
1. La	0.07	0.088 ± 0.002
2. Ce	0.133	0.127 ± 0.087
3. Pr	0.033	0.035 ± 0.005
4. Nd	0.067	0.079 ± 0.003
5. Sm	0.017	0.018 ± 0.000
6. Eu	0.0067	0.007 ± 0.000
7. Gd	0.0167	0.017 ± 0.000
8. Tb	0.0033	0.004 ± 0.000
9. Dy	0.0167	0.020 ± 0.000
10. Ho, Y	0.0033	0.004 ± 0.000
11. Er	0.0067	0.008 ± 0.000
12. Tm	0.0033	0.004 ± 0.000
13. Yb, Lu	0.0067	0.009 ± 0.000

1:150 Sample Dilution
3.0 mL Sample concentration
(Estimated Sample Injected=20 mg)

Figure 5. Spike/recovery of lanthanide metals in USGS periotite (PCC-1) matrix.

9. Prepare PAR reagent as directed in Appendix A. Place PAR reagent into the RDM reagent reservoir and close the reservoir after the eluent is flowing. Turn the RDM reagent 1 switch on and immediately adjust the regulator to 60 psi. Turn the RDM reagent 1 switch off.

   Caution: Be sure that PDCA is being pumped through the columns when the RDM is switched on; failure to do so may cause the PAR reagent to back up through the IonPac CS5 column.

10. Enter the AGP1 program for chelation concentration as shown in Table 4 (page 10). This program can be entered from the front panel of the AGP or from the AI-450's Method Editor.

In fully automated system operation, a relay TTL cable (P/N 042599) is used to reset/start the AGP2 pump after the chelation concentration step is completed. Consult the *AI-450 Operator's Manual* or the *Integrator Operator's Manual* for details on installation and operation.

System Test

The purpose of this system test is to ensure that all chromatographic and chemical components of the system are operating properly. The system schematic is shown in Figure 3. Be sure to check all fittings for leaks during the system test.

1. Enter the program for chelation concentration (Table 4) from the front panel of the AGP. Refer to the *AGP Operator's Manual* for details on programming. Check the program carefully for accuracy by listing the program.

   The system test will begin with a test of the hydraulic system. If the system fails the hydraulic test at any point, determine the source of the plumbing error. Begin by using the program given above. With the AGP in the STOP-HOLD position, press RESET. This will set the program to time 0.0.

2. Press START. Eluent (E1) should begin to flow to valve D and out to the AGP OUT at the SCM rear panel. Check AGP OUT to confirm that the eluent is flowing to the SCM.

3. Next, prime the sample pump by loosening the tubing fitting that is screwed into the outlet check valve. Since the eluent reservoir is pressurized (5 psi), the deionized water should begin to flow out of the check valve. As the eluent begins to flow, turn on the carrier pump by pressing the PUMP 2 POWER switch on the SCM front panel. After about 5 seconds, replace the outlet check valve tubing fitting. Generally, this fitting requires only finger-tightening; if the fitting leaks, tighten it another one-eighth of a turn using a 5/16-in. open-end wrench.

4. Set the stroke dial of the carrier pump to about 5.00 (refer to the *SCM Operator's Manual* for details on adjusting the flow rate). Calibrate the sample pump flow rate by mass or volume to 2.0 mL/min. Be sure that the eluent reservoir is pressurized to 5 psi. Turn off the sample pump (PUMP 2).

5. Place the sample inlet tube in a container of deionized water and draw the deionized water through the sample loop using a syringe. Confirm that the solution is flowing through the sample loop.

6. List the AGP program to the next sequence (2.0 min) and press RUN and HOLD. This will forward the program to 2.0 min. Check to see that the eluent is flowing out of the SAMPLE OUT tubing at the SCM rear panel. Stop the pump and confirm that the eluent flow stops. Turn on the sample pump (PUMP 2). Check to confirm that the deionized water is flowing out of the same port at the SCM rear panel. Start the AGP, wait 30 seconds, and then measure the flow rate. The combined flow rate from the sample pump and the AGP must be 4 mL/min. Adjust the sample pump flow rate if necessary. Turn off the sample pump (PUMP 2) and the AGP.

   If the sample pump loses prime, prime the pump by loosening the tubing fitting on the outlet check-valve. If there is no liquid in the check valve, use a squirt bottle to squirt some deionized water into the check-valve. This will aid in priming the pump. Replace the check valve tube fitting. This completes the hydraulic test.

7. Reset the AGP1 to time 0.0 min. Start the AGP2. Forward the AGP2 program (see Table 4) to time 0.1 min. This should place the TMC-1 column in-line with the AGP2 eluent flow. Disconnect the TMC-1 column and confirm that eluent is flowing through the column. Stop the AGP2.

8. Reset the AGP2 program. Connect the postcolumn system to the CS5 separator column. Start the AGP2 pump. Immediately turn on the RDM reagent 1 valve to pressurize the postcolumn reagent reservoir and start the reagent flow. After 1 min, check to see that the PAR postcolumn reagent is flowing to the VDM-2. Measure the flow rate from the waste line. The flow rate should be 1.4 to 1.6 mL/min. Adjust the RDM regulator to achieve the recommended flow rate. This completes the system test.

SYSTEM OPERATION

The sequencing and operation of the system components are described below. The operating conditions are summarized in Table 4. The chelation ion chromatography system configuration has been designed for use in the fully automated mode, except for the sample introduction step. If the required sample volume is less than 3 mL, the Dionex ASM autosampler is applicable. Unless an autosampler capable of delivering more than 5 mL of sample is used, sample introduction must be performed using a syringe for larger samples.

1. Confirm that the system is configured as given in Figure 2. Check to see that the system has an 80 to 120 psi inert gas supply.
2. Turn on the absorbance detector. If a variable wavelength detector is used, set the wavelength to 530 nm. If a filter-based detector is used, be sure the filter is 520 or 530 nm. Turn on the visible lamp and set the sensitivity to 0.2 AUFS. Be sure that the detector output is connected to a data collection system (integrator or ACI/AI-450).
3. Enter the program listed in Table 4 (page 10). Check the program carefully by listing each step of the program.
4. Reset the AGP2 program at time 0.0 min. Start the AGP2 and the RDM. Confirm that the PAR reagent is flowing through the detector.
5. Turn on the integrator or monitor and begin to monitor the baseline. At 0.1 AUFS, an essentially noise-free and drift-free baseline should be observed.
6. Once the baseline is stable, start the AGP1 and press RUN.
7. Step 1 of program: Confirm that valve 5 is ON and valve 6 is OFF. The sample or the standard can be loaded via the autosampler. If the autosampler is not used, load the sample by drawing the sample through the sample inlet with a syringe. The sample pH should be 1–2. While the sample introduction step is in progress, the AGP1 is pumping 2.0 M ammonium acetate to regenerate/equilibrate the MetPac CC-1 column. Note that the next step (flushing the sample loop to the MetPac CC-1 and on-line buffering) occurs at 2.0 min. If the sample introduction takes more than 2.0 min, adjust the AGP1 program accordingly.
8. Step 2: Valve 5 is OFF and valve 6 is ON. The sample pump is pumping deionized water through the sample loop that was previously loaded with sample. The sample stream is now mixing with the 2.0 M ammonium acetate from the AGP1 and the buffered sample passes through the MetPac CC-1 column.
9. Step 3: Valve 5 is ON and valve 6 is OFF. The AGP1 is pumping 2.0 M ammonium acetate to remove the alkali and alkaline earth metals from the MetPac CC-1 column to waste.
10. Step 4: Valve 5 is ON and valve 6 is ON. The AGP1 is pumping 1.0 M nitric acid to the MetPac CC-1 column. The concentrated metal ions are eluted from the column and the 1.0 M acid stream is diluted on-line to approximately 0.37 M with deionized water from the sample pump and passes through the TMC-1 column. This step maximizes the removal of concentrated metal ions from the MetPac CC-1 and places them on the TMC-1 column in a tight band.
11. Step 5: Valve 5 is OFF and valve 6 is OFF. The AGP1 is pumping 1.5 M hydrochloric acid/75% ethanol to the TMC-1. The step performs the selective elimination of transition metals from the TMC-1 to waste. Note that the backpressure of the TMC-1 increases due to the organic eluent. The backpressure at 1.5 mL/min should not exceed 2500 psi (17 MPa).
12. Step 6: Valve 5 is OFF and valve 6 is OFF. The AGP1 is pumping 0.1 M ammonium nitrate to the TMC-1. This step is required to convert the TMC-1 from acid form to ammonium form.

Table 4 Chelation Concentration Operating Conditions

AGP1 Chelation Concentration System

Columns: MetPac CC-1, TMC-1

Eluents: E1: 1.5 M Hydrochloric acid, 75% ethanol
E2: 2.0 M Ammonium acetate, pH 5.5 ±0.1
E3: 1.0 M Nitric acid
E4: 0.1 M Ammonium nitrate, pH 3.5 ±0.3

AGP1 program for >3 mL loop (AGP1) — downloaded by the ACI

t (min)	E1	E2	E3	E4	V5	V6	Flow (mL/min)
0.0	0	100	0	0	1	0	3.0
2.5	0	100	0	0	0	1	2.0
5.0	0	100	0	0	1	0	3.0
7.0	0	100	0	0	1	0	1.2
7.1	0	0	100	0	1	1	1.2
12.0	0	0	100	0	1	1	1.2
12.1	100	0	0	0	1	0	4.0
12.5	100	0	0	0	0	0	1.5
16.0	100	0	0	0	0	0	1.5
16.1	0	0	0	100	0	0	1.5
18.0	0	0	0	100	0	0	2.0
19.0	0	0	0	100	0	0	3.0
20.0*	0	0	0	100	0	0	1.0
20.1	0	0	100	0	1	0	3.0
22.0	0	0	100	0	1	0	3.0
22.1	0	100	0	0	1	0	3.0
24.0	0	100	0	0	1	0	0.0

*Begin sample analysis (start/run AGP2)

AGP2 Chromatography System

Eluents: E1: Deionized water
E2: 0.006 M Pyridine-2,6-dicarboxylic acid, 0.040 M lithium hydroxide, 0.19 M acetic acid
E3: 0.1M Oxalic acid, 0.19 M lithium hydroxide
E4: 0.1 M Diglycolic acid, 0.19 M lithium hydroxide

AGP2 program entered at AGP2 front panel.

(Reset/start control via ACI's relay TTL cable)

t (min)	E1	E2	E3	E4	V5	V6	Flow (mL/min)
0.0	0	100	0	0	0	0	1.0
0.1	0	100	0	0	1	0	1.0
12.0	0	100	0	0	1	0	1.0
12.1	100	0	0	0	1	0	1.0
17.0	100	0	0	0	1	0	1.0
17.1	20	0	80	0	1	0	1.0
20.0	20	0	80	0	1	0	1.0
20.1	20	0	75	5	1	0	1.0
30.0	50	0	25	25	1	0	1.0
40.0	50	0	25	25	1	0	1.0
40.4	0	100	0	0	0	0	1.0

Timed Events Program

t (min)	ACI SP[1]	ACI ASM[2]	ACI AGP2[3]	ACI Begin Sampling	AGP Start	AGP Run	VDM Offset
Init	off	off	off	off	on	off	off
0.0	on	on	off	off	on	on	off
13.0	off	on	off	off	on	on	off
20.0	off	on	on	on	on	on	on

[1]AC
[2]relay 1
[3]relay 2

13. Step 7: START/RUN the AGP2 pump program.

    This step can also be initialized by the integrator or the ACI/AI-450. At time 0.1 min, the AGP2 valve 5 is ON. Valve E in the SCM is now switched to the INJECT position where the TMC-1 column is placed in-line with the CS5 column. The AGP1 is pumping 1.0 M nitric acid to the MetPac CC-1 for 2 min (3.0 mL/min), followed by 2.0 M ammonium acetate for 2.0 min (3.0 mL/min) before the end of the chelation concentration process.

    At this point, the system is ready for calibration and sample analysis.

Analytical Chromatography

Column:	IonPac CS5
Eluents:	E1: Deionized water E2: 0.0060 M Pyridine-2,6-dicarboxylic acid, 0.090 M acetic acid, 0.040 M lithium hydroxide E3: 0.1 M Oxalic acid, 0.19 M lithium hydroxide E4: 0.1 M Diglycolic acid, 0.19 M lithium hydroxide
Eluent Flow Rate:	1.0 mL/min
Postcolumn Derivatization Reagent:	0.0004 M 4-(2-Pyridylazo)-resorcinol, 3.0 M ammonium hydroxide, 1.0 M acetic acid
Reagent Addition:	Membrane reactor or mixing tee
Reagent Flow Rate:	0.5 mL/min
Reactor:	Packed or knitted reaction coil
Detection:	VDM-2 or UDM, 520 or 530 nm

Sample Preparation

It is beyond the scope of this text to describe in detail the techniques of sample preparation in terms of sample collection, storage, and handling. Several points will be discussed that are applicable to sample preparation before analyzing samples by the method described in this Technical Note.

The geological materials must be carefully digested with proper acids to dissolve the lanthanide metal ions in the solution. If the sample contains a large quantity of SiO_2, it must be digested with concentrated hydrofluoric acid. Most reagents are available free of lanthanide contamination. If you suspect that those reagents may contain any lanthanide contaminants, it is advisable to prepare and analyze a reagent blank with the samples.

The digested sample should be stored in clean polyethylene containers. Avoid sample dilution if possible. If the sample contains more than 1000 ppm of iron and aluminum and more than 800 ppm of transition metals in the final volume, a further dilution of the sample is strongly recommended. The sample should not contain more than 4% nitric or hydrochloric acid. Samples with more than 4% acid must be neutralized to pH 1–2 prior to the sample loading step.

APPENDIX A

Sample Loop

The 1-mL and 5-mL sample loops are available and supplied with the SCM.

Solutions and Reagents

Ultrapure 2.0 M ammonium acetate, pH 5.5
(1 L, P/N 033440; 6 L, P/N 033441)
Ultrapure 2.0 M nitric acid
(1 L, P/N 033442; 6 L, P/N 033443)
Ultrapure 0.1 M ammonium nitrate
(1 L, P/N 033445)
20% Ultrapure ammonium hydroxide
Ultrapure glacial acetic acid

The first two reagents used for chelation concentration are available from Dionex in a ready-to-use form. If you wish to prepare your own reagent solutions, please refer to the "Preparation of Solutions and Reagents" section below for information on ordering ultrapure acids and ammonium hydroxide.

PREPARATION OF SOLUTIONS AND REAGENTS

Three concentrated reagents are rquired for eluents in chelation concentration: Nitric acid, acetic acid and ammonium hydroxide. For ultratrace level determination (sub-ppb), the reagents must be ultrapure grade. For determination above 1 ppb, high quality trace-metal grade reagents can be used. Any metal impurity in these reagents will be concentrated with your sample, constituting a system blank.

2.0 M Ammonium Acetate pH 5.5 ± 0.1

Place 600 mL of deionized or high purity water into a clean 1-L glass eluent container. Tare the bottle. Add 121 g (115 mL) of ultrapure glacial acetic acid and mix thoroughly. In a fume hood, slowly add 120 g (130 mL) of 20% ultrapure ammonium hydroxide and mix thoroughly. Agitate the bottle to thoroughly mix the solution. Calibrate a pH meter to pH 7. Pour about 10 mL of the buffer into a small container (e.g., scintillation vial, 10-mL disposable beaker, etc.) and measure the pH. If the pH is below 5.4, add about 5 mL of ammonium hydroxide to the buffer solution. If the pH is above 5.6, add 5 g of acetic acid. Adjust the pH of the ammonium acetate to 5.5 ± 0.1 using acetic acid if the pH is less than 5.5. Once the pH is 5.5 ± 0.1, bring to a volume of 1.0 L with 18-MΩ deionized water.

2.0 M Nitric Acid

Place 200 mL of deionized or highly purity water into a clean 1-L glass eluent container. Add 179 g (126) of ultrapure nitric acid. Dilute to 1.0 L with 18-MΩ deionized water and mix thoroughly.

0.1 M Ammonium Nitrate, pH 3.4 ± 0.3

Place 200 mL of deionized water into a clean 1-L glass eluent container. Add 8.9 g (6.3 mL) of ultrapure nitric acid. Next, add 7.6 g (8.5 mL) of ultrapure 20% ammonium hydroxide. Add sufficient deionized water to give a final volume of 1 L and mix thoroughly. Calibrate the pH meter to pH 4.0. Take a 10-mL aliquot of the solution and measure the pH. Add either 0.1 M ammonium hydroxide or 0.10 M nitric acid in 3- to 5-mL increments to the bulk solution to adjust the pH. Continue taking aliquots and adjusting the pH to 3.4 ± 0.3.

The ultrapure ragents are manufacutred by Seastar Chemical and Ultrex Reagents. Seastar reagents are available internationally through Fisher Scientific; in North America, Fisher Scientific sell these reagents under the OPTIMA® label. Ultrex reagents are available internationally through J. T. Baker.

0.0060 M Pyridine-2,6-dicarboxylic Acid (PDCA) Eluent

Prepare by dissolving the following reagents, in the order listed, in 18-MΩ deionized water:

0.040 M (1.7 g) Lithium hydroxide, monohydrate
0.0060 M (1.0 g) PDCA
0.090 M (5.42 g) Glacial acetic acid

Dilute to 1.0 L with 18-MΩ deionized water.

0.10 M Oxalic Acid, 0.19 M Lithium Hydroxide

Prepare by dissolving the following reagents in 18-MΩ deionized water:

0.10 M (13 g) Oxalic acid, dihydrate
0.19 M (8.0 g) Lithium hydroxide, monohydrate

Dilute to 1.0 L with 18-MΩ deionized water.

0.10 M Diglycolic Acid, 0.19 M Lithium Hydroxide

Prepare by dissolving the following reagents in 18-MΩ deionized water:

0.10 M (13 g) Diglycolic acid
0.19 M (8.0 g) Lithium hydroxide, monohydrate

Dilute to 1.0 L with 18-MΩ deionized water.

1.5 M Hydrochloric Acid/75% Ethanol

Prepare by slowly adding 120 mL (150 g) of concentrated HCl (trace metal grade) to 750 mL of 95.5% ethanol (Baker Analyzed, J.T. Baker). Dilute to 1.0 L with 18-MΩ deionized water.

PAR Postcolumn Reagent

Place 400 mL of 18-MW deionized water in a 1-L container. Add 200 mL of trace metal grade 30% ammonium hydroxide solution. Add and dissolve 0.10 g of 4-(2-pyridylazo)resorcinol (PAR). Add 57 mL of trace metal grade glacial acetic acid. Dilute to 1.0 L with 18-MΩ deionized water.

Determination of Transition Metals in Complex Matrices by Chelation Ion Chromatography*

INTRODUCTION

The determination of trace elements in complex matrices remains one of the most challenging areas of analytical chemistry. Complex matrices include seawater, brines, estuarine waters, as well as biological, botanical, and geological materials. In general, these matrices have high levels of alkali and alkaline earth metals with trace levels (less than 1 ppm) of the transition elements. High levels of alkali and alkaline earth metals cause significant interferences and/or sensitivity losses for most analytical techniques used for trace metal determinations. This technical note describes chelation concentration, a selective concentration method, coupled directly to an ion chromatograph for the determination of transition metals in complex matrices. This new method—*Chelation Ion Chromatography*—combines analyte concentration and matrix elimination with analytical separations and selective detection for transition and lanthanide metals. The result is a chromatographic technique that permits trace and ultratrace determinations of metals in a variety of sample matrices, especially those high in alkali and alkaline earth metals.

See Dionex Technical Note 27 for a method describing the determination of lanthanides using chelation IC.

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SUMMARY OF THE METHOD

A chelating concentrator column, the MetPac™ CC-1, is used to selectively concentrate polyvalent metal ions from an aqueous sample. Typical sample volumes are 5 to 50 mL. Alkali metals and anions are not retained by the chelating column. Alkaline earth metals are selectively eluted to waste. This form of selective sample pretreatment is called *chelation concentration*. The concentrated transition metals and lanthanides are then eluted to a second concentrator column, the TMC-1. The TMC-1 is then converted to a salt form and switched in line with the analytical column, the IonPac® CS5. The concentrated metals are then separated.

*Chelation Ion Chromatography is patent pending.

Detection is accomplished by visible absorbance after postcolumn derivatization with a metallochromic indicator. The analytical chromatography for transition metals requires about 15 minutes. Detection limits range from 0.1 to 0.5 ng. Detection limits of 0.2 to 1 ng/mL (ppb) can be obtained with a 20-mL sample volume. This method is applicable to a wide variety of sample matrices.

The MetPac CC-1 will concentrate most cationic transition and lanthanide elements. Currently, the analytical chromatography is applicable to iron, copper, nickel, zinc, cobalt, manganese, cadmium, lead, and the lanthanide metals.

INSTRUMENT REQUIREMENTS

This technical note gives a detailed description of the system components, installation, and operation of the chelation ion chromatographic system. Please consult the procedures within this technical note carefully before operating the chelation IC system. It is important to understand each step of the sample pretreatment process to maintain the best performance of the chelation IC system. The two recommended system configurations are given; both configurations include the Advanced Gradient Pump (AGP), a Sample Concentration Module (SCM), a Reagent Delivery Module (RDM), and a Variable Wavelength Detector Module (VDM-2). The AGP performs the steps of chelation concentration and controls the valves. A DQP (sample pump) in the SCM is used for loading the sample onto the MetPac CC-1 chelating column. Another DQP (carrier pump) with a pulse damper is used as the eluent pump. Five 4-way valves are located in the front section of the SCM.

System 1 is configured for manual operation, while system 2 is for automated operation. System 1 requires adjustment of the sample pH to 5.5 prior to sample introduction. However, an acid sample of pH 1-2 can be loaded directly into system 2. The sample introduction mode of the two systems also differs; system 1 uses a DQP pump to deliver the sample directly to the MetPac CC-1 concentrator, and system 2 uses a fixed sample loop.

To improve the detection limits of the chelation IC system, a Membrane Reactor is recommended in place of the mixing tee. The Membrane Reactor lowers detection limits for metals 5-fold compared to reagent addition using the mixing tee. The Membrane Reactor should be used if you are determining transition metals below 0.5 ppb. A knitted or beaded reaction coil should always be used with the Membrane Reactor.

Chelation Ion Chromatograph—System 1

A schematic of system 1 is shown in Figure 1.

Advanced Gradient Pump (AGP, P/N 43116)
Sample Concentration Module (SCM, P/N 42134)
Reagent Delivery Module (RDM, P/N 37030)
Variable Wavelength Detector Module (VDM-2, P/N 39646, internal or remote cell)
Eluent Degas Module (EDM-2, P/N 39550)
Eluent Container Set, Glass (P/N 38752)
IonPac Membrane Reactor (P/N 35354—optional)
Knitted Reaction Coil (P/N 39349) or Beaded Reaction Coil (P/N 36036)
MetPac CC-1 Column (P/N 42156)
TMC-1 Column (P/N 42155)
Ion Pac CG5 (P/N 37029)
IonPac CS5 (P/N 37028)
Backpressure Regulator (P/N 39760)
Data acquisition system or AI-450 Chromatography Workstation with ACI interface

Chelation Ion Chromatography—System 2

A schematic of system 2 is shown in Figure 2.

Advanced Gradient Pump (AGP, P/N 43116)
Sample Concentration Module (SCM, P/N 42134)
Reagent Delivery Module (RDM, P/N 37030)
Variable Wavelength Detector Module (VDM-2, P/N 39646, internal or remote cell)
Air Pressure Regulator (P/N 38207)
Eluent Container Set, Glass (P/N 38752)
Slider double stack, 4-way valve, 2000 psi/13.7 MPa (P/N 35914)
Slider Single Stack, 4-way valve, 2000 psi/13.7 MPa (P/N 38754) or slider double stack, 4-way valve, 2000 psi/ 13.7 MPa (P/N 35914), two (2) required
IonPac Membrane Reactor (P/N 35354 - optional)
Knitted Reaction Coil (P/N 39349) or Beaded Reaction Coil (P/N 36036)
MetPac CC-1 Column (P/N 42156)
TMC-1 Column (P/N 42155)
IonPac CG2 (P/N 35370)
IonPac CG5 (P/N 37029), two (2) required
Data acquisition system or AI-450 Chromatography Workstation with ACI interface

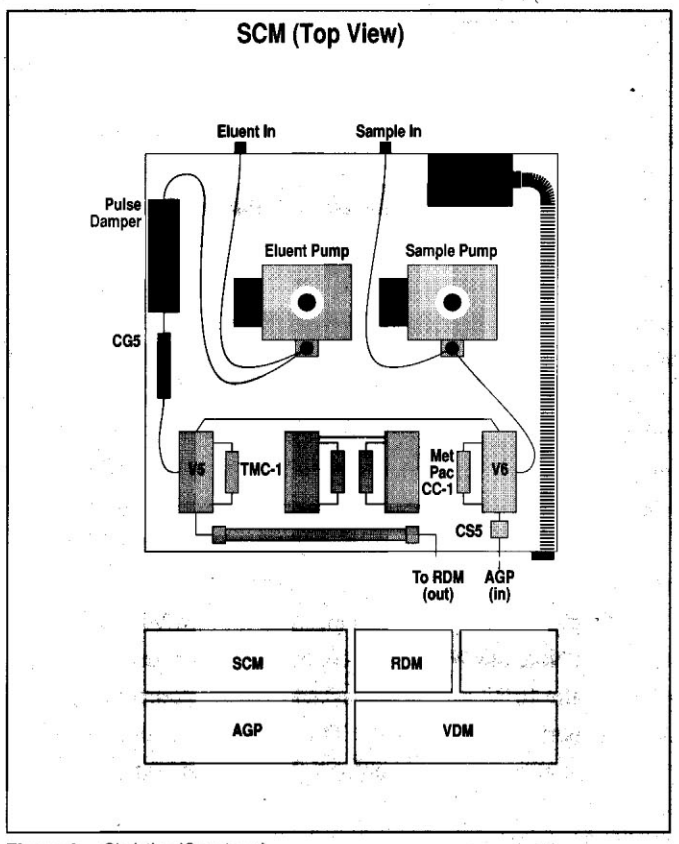

Figure 1 Chelation IC system 1

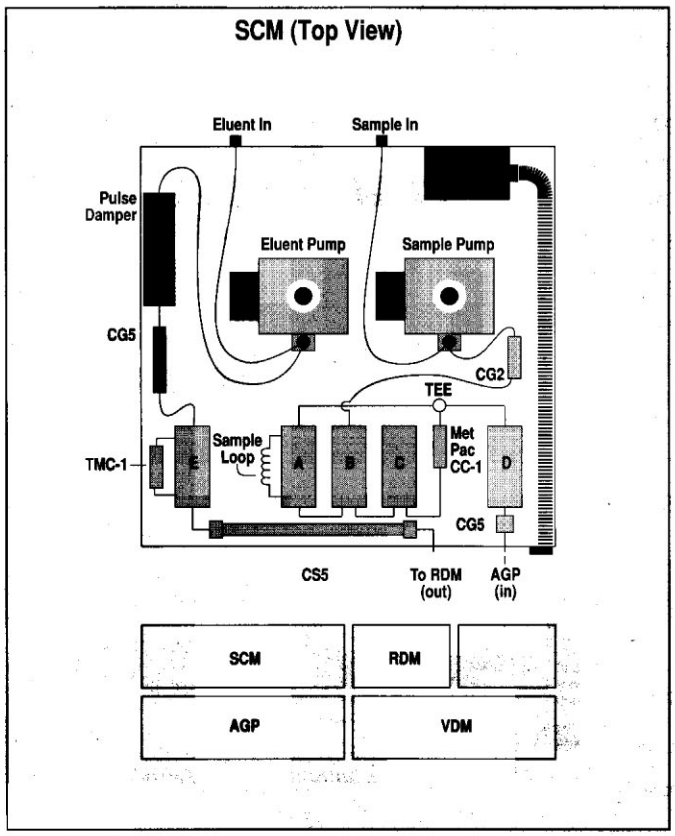

Figure 2 Chelation IC system 2

System Configuration

The two recommended systems contain the Sample Concentration Module (SCM) and the Advanced Gradient Pump (AGP).

Sample Concentration Module (SCM)

The block schematics of the SCMs used for systems 1 and 2 are shown in Figures 1 and 2. The SCM contains two single piston Dionex DQP pumps (maximum pressure1900 psi/13.1 MPa), inert double stack four-way pneumatically controlled slider valves, and a pulse damper. One DQP is used to pump sample into the system; the other DQP is used to deliver eluent to the IonPac CS5 analytical column. All of these components are housed in the SCM's single enclosure. The SCM rear panel contains bulkhead fittings for connecting waste lines and eluent lines.

Advanced Gradient Pump (AGP)

The AGP is a microprocessor controlled, high performance quaternary gradient IC pump with a metal-free flow path. It permits the time dependent selection of up to four different eluents, flow rate, and the control of two sets of air solenoids for external valve control. Controls 5 and 6 of the AGP (referred to as E5 and E6) are used to control the five SCM valves. The AGP is programmable and can store up to 10 programs. Refer to the *AGP Operator's Manual* for complete information on operation and maintenance.

Using appropriate interface hardware, the AGP can be controlled by a Dionex integrator or the AI-450 Chromatography Workstation. To automate the system for sample concentration of 1 to 3-mL samples, the Dionex Automated Sampler Module (ASM) can also be used and controlled by the integrator or the AI-450.

System 1

Figure 3 shows a pneumatic and hydraulic schematic of the chelation IC system. Configure the instrumentation as shown in Figure 1 and plumb the system as shown in Figure 3.

Connect the four air lines from the rear panel of the SCM. Color match the air lines. Valves 1, 2, and 3 of the SCM are controlled by E5 of the AGP and are connected by the orange (top) and yellow (bottom) air lines. Valve 4 of the SCM is controlled by E6 of the AGP and is connected by the blue (top) and green (bottom) air lines.

System 2

Figure 2 shows a detailed pneumatic and hydraulic schematic of the SCM system 2. The SCM is factory-configured for sample pretreatment use with an external detector (e.g., ICP). For chelation IC applications, configure and plumb the SCM as shown in Figure 4.

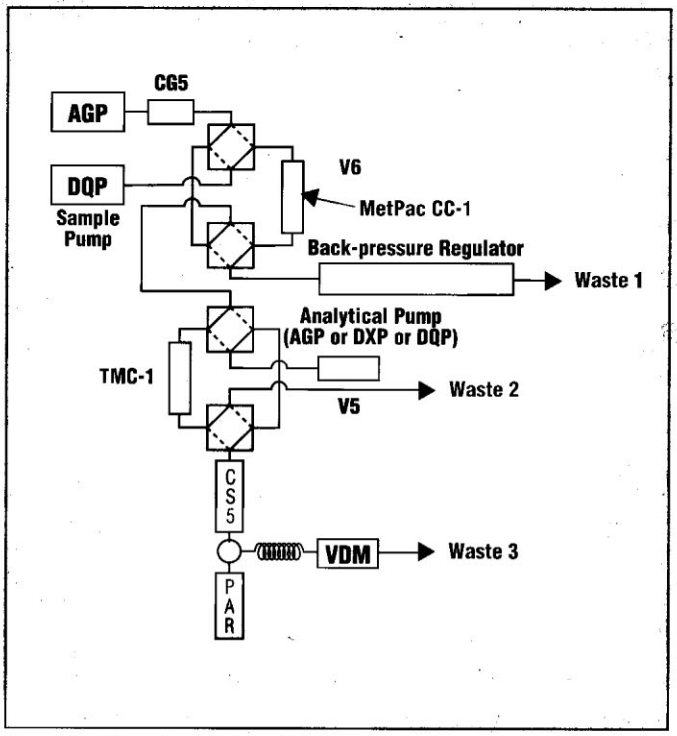

Figure 3 Schematic of Chelation IC System 1

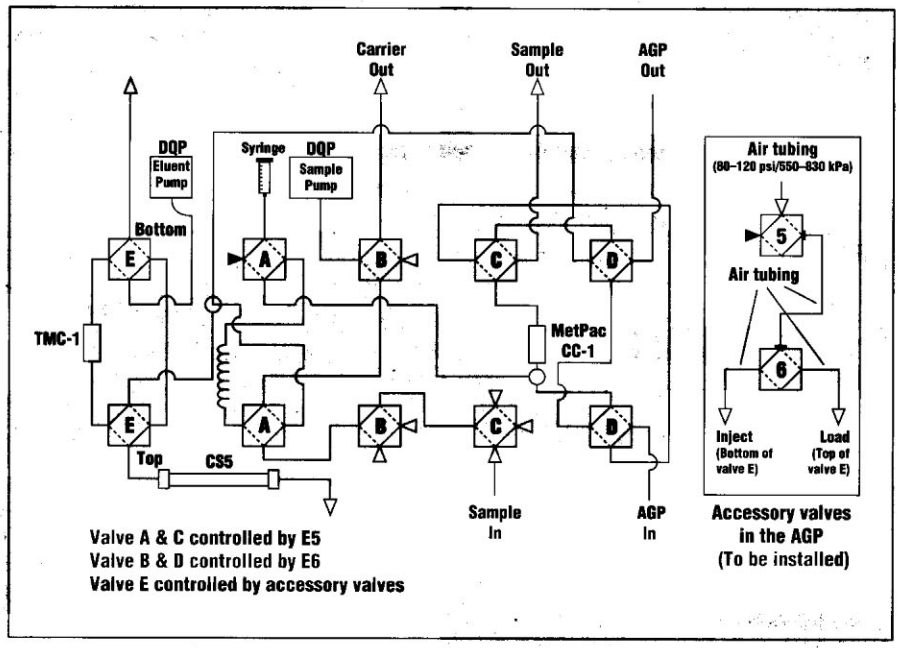

Figure 4 Schematic of Chelation IC System 2

Pneumatic Connections

Disconnect all the air tubings in the SCM and reconnect as indicated in Figure 4. Be sure that valves A and C are controlled by E5 and that valves B and D are controlled by E6. Valve E is controlled by the accessory valves that will be installed inside the AGP. To install the AGP accessory valves in the AGP and the air tubing between the AGP and the SCM, locate the four colored air tubings at the rear panel of the AGP and the SCM. Using four small barbed tees (P/N 30538), connect the air tubing by matching the colors (orange-orange, yellow-yellow, green-green and blue-blue) to the two arms of the barbed tee. Connect 12 in. (30 cm) each of the orange and green air tubing from each tee to the top of the two slider-single-stack four-way valves (P/N 38754). The valve with orange tubing on the top is designated as V1, the other as V2. Repeat this step by connecting the yellow and the blue tubings to the bottom of V1 and V2, respectively. Place V1 and V2 in the AGP. Note that V1 is now controlled by E5, and V2 by E6. Connect the 10/32 x 1/16-in. barbed fittings to port 1 and 2 of V1, and port 1, 2, and 4 of V2. Then, connect about 6 in. (15 cm) of air tubing between port 2 of V1 and port 1 of V2, as shown in Figure 3. V1 and V2 will be used to switch valve E located in the SCM. Plug port 4 of V1 with 1/8-in. fitting.

Next, connect about 24 in. (60 cm) of air tubing (P/N 30091) to the small barbed fitting on the back of the AGP. Insert a barbed tee (P/N 30538) into the end of this tubing. One arm of the tee will go to the nitrogen source (regulator) and the other arm to port 1 of V1. Connect about 36 in. (90 cm) of air tubing between port 4 of V2 and the top of valve E located in the SCM. Repeat this step with port 2 of V2 and the bottom of valve E.

Next, connect the air tubing from the nitrogen or argon source by using a barbed tee (P/N 30538) and connect the air tubing to the inlet of the eluent bottle regulator (P/N 38201). Using the required lengths of the tubing, connect the tee to the gas source and to the eluent pressure regulator. Using the 1/4-in. x 10/32 brass reducer (P/N 30087) and the 10/32 x 1/16-in. barbed fitting (P/N 30017), connect the air tubing to the gas source regulator.

Next, connect the air tubing to the eluent container caps (P/N 41004) and to the 4-L plastic eluent container cap (P/N 39164). Start by cutting one of the two 1/8-in. Teflon

lines flush with the bottom of the cap. Repeat this for all five eluent container caps. Next, cut the same tubing about 2 in. (5 cm) above the eluent container cap. This line will be used to connect the argon or nitrogen for pressurizing the eluent bottles. Confirm that the eluent bottle caps contain a white TFE O-ring (P/N 41078). Insert a barbed coupler (P/N 42241) into the trimmed Teflon line of cap E1. Insert a barbed tee (P/N 30538) into the trimmed Teflon lines of caps E2, E3, E4, and E5. Connect the eluent caps using the air tubing (P/N 30091 or equivalent). This completes the pneumatic setup.

Hydraulic Connections

Refer to the *AGP* and *SCM Operator Manuals* for complete information on the installation and operation of the respective modules. Begin the hydraulic connections by cutting the four eluent lines from the four eluent container caps to the front panel eluent port of the AGP. Notice that the eluent lines are labeled 1, 2, 3, and 4. Be sure the eluent lines are connected to the appropriate eluent port.

Connect the eluent line from the 4-L plastic eluent container (P/N 39164) to the "sample in" port of the SCM rear panel. This port is connected to the check-valve inlet of the sample pump located on the right side of the SCM. Next, connect the three blue waste lines (P/N 39341) to the ports of the SCM rear panel labeled CARRIER OUT, AGP OUT, and SAMPLE OUT, and place them in a waste container. Also, place another waste line from valve E in the waste container. This completes the hydraulic connections.

Electrical Connections (for System 1 & 2)

Verify that the front PUMP 1 and PUMP 2 power switches of the SCM are off. Using the power cords provided (P/N 96078), connect the AC receptacles on the rear panels of the SCM and AGP to the white outlets of the power strip located on the rear upper section of the system enclosure. Next, connect the AC receptacle of the power strip enclosure to an AC power outlet.

If you wish to control the system via an interface cable to a Dionex integrator, consult your Dionex service representative for further details.

CHEMICALS, REAGENTS, AND STANDARDS

A complete list of reagents and instructions on their preparation can be found in Appendix A. The reagents used for chelation concentration, 2 M ammonium acetate pH 5.5, 2 M nitric acid, and 0.1 M ammonium nitrate pH 3.5 are available from Dionex. In addition, high purity water containing less than 500 parts-per-trillion of common transition elements (iron, zinc, copper, manganese, etc.) is also required.

OTHER SUPPLIES

In addition to the items listed above, you will also need

Electrical power

Compressed nitrogen (80-120 psi/550–830 kPa)

Standard analytical laboratory equipment such as a balance, pH meter, etc.

DISCUSSION OF THE METHOD

The method described in this manual is intended for the determination of transition metals in complex matrices. The term *complex matrix* refers to any matrix containing constituents that commonly interfere with the analytical measurement. The detection limits for analytes may be severely compromised using conventional analytical techniques for complex matrices. For example, large quantities of alkali and alkaline earth metals can interfere with the determination of transition metals by IC or atomic spectroscopy. With chelation IC, it is possible to eliminate or reduce these interferences before the analytical determination, while at the same time concentrating the analytes of interest. By using selective ion exchange materials such as chelating resins, analytes can be concentrated, while common interferences such as the alkali and alkaline earth metals and anions are reduced or eliminated. This form of sample pretreatment is called *chelation concentration*. Unlike conventional ion exchange concentration methods, which are typically not selective for ions of the same valency, chelation concentration is a *selective* concentration method.

Chelation concentration is applicable to samples such as seawater, brines, natural waters, waste waters, acid digested samples, concentrated acids, bases, and biological, botanical, and geological materials. *Chelation concentration is not intended for trace transition metal determination in the presence of large quantities of other transition metals (e.g., plating baths).*

Most samples should be acid digested to ensure that the metals are free in solution and not bound by organic materials such as fulvic and humic acids. Metal ions bound by complexing agents in the sample can interfere with the concentration efficiency and recoveries.

The column used for chelation concentration, the MetPac CC-1, contains a macroporous iminodiacetate chelating resin. The column has a capacity of 0.45 milliequivalent. The relative selectivity of the resin is

Lanthanides > Hg >> Cu >> UO_2 > Ni > Pb> Zn > Co > Cd > Fe > Mn > Ba > Ca > Sr > Mg >> Na

In general, the higher the cationic charge of the metal ion, the more strongly bound the metal ion is to the resin. Anionic forms of metals, such as Cr (VI) as chromate (CrO_4^{2-}), are not retained. Since the functional group of the resin is a weak acid (COOH) and a weak base (NH), as shown in Figure 5, hydronium ion (H_3O^+) competes strongly with metal ions for the chelating sites. As a result, mineral acids such as hydrochloric or nitric acid at 0.5 to 2.0 M are effective eluents. Below pH 2.5, the MetPac CC-1 column will not concentrate transition metals. In the pH range 5–6, the resin selectivity is optimized for transition and lanthanide metals relative to alkali and alkaline earth metals. By using an ammonium acetate eluent in this pH range, alkaline earth metals can be eluted, while the transition and lanthanide metals remain strongly bound to the resin.

Chelation concentration consists of four major processes.

1. The buffered (pH 5.5) sample is passed through the chelating concentrator, the MetPac CC-1. Most polyvalent cations are quantitatively concentrated from the sample, while anions and alkali metals pass through the column essentially unretained. Metals that are quantitatively concentrated are listed in Table 1.

2. Weakly bound alkaline earth metal ions such as magnesium and calcium are selectively eluted using a 2 M ammonium acetate eluent (pH 5.5) pumped by the AGP. During this elution process, at least 95% of the magnesium and 90% of the calcium on the column will be eliminated. Some manganese (10 to 15%) will be eluted, but this is not a problem since the percentage of manganese eluted during the ammonium acetate wash is constant.

3. The concentrated transition and lanthanide metals are eluted in a 100 to 200-µL volume using nitric or hydrochloric acid delivered from the AGP.

4. After a final acid rinse (1 to 2 M) to completely remove residual metals, the MetPac CC-1 is converted back to the ammonium form using 2 M ammonium acetate.

While mineral acids efficiently elute the concentrated metal ions from the MetPac CC-1, acids are not compatible with the eluent system of the analytical column (IonPac CS5). A high capacity cation exchange concentrator must be used to retain the metal ions as they are eluted from the MetPac CC-1. The TMC-1 contains a fully sulfonated cation exchange resin with sufficient capacity (0.3 meq) to retain the metal ions under elution conditions from the MetPac CC-1. The TMC-1 interfaces the high capacity chelating column with the low capacity analytical column. Before the TMC-1 can be switched in line with the analytical stream, it must be converted from the acid (H^+) form to the ammonium (NH_4^+) form. This is accomplished using 0.1 M ammonium nitrate, pH 3.5, which is also pumped from the AGP. Converting the column from the acid form to

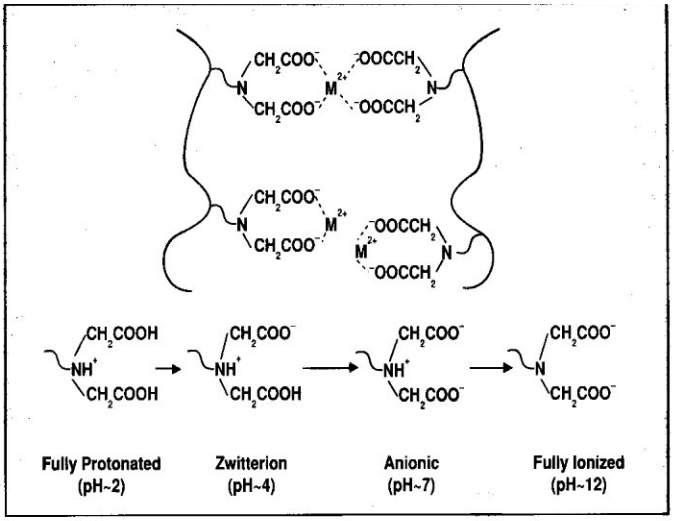

Figure 5 Properties of MetPac CC-1 chelating resin

Table 1
Retention Characteristics of the MetPac CC-1 Concentrator

Metal Ion	Quantitative	Metal Ion	Quantitative
Ti (IV)	Yes	Cd (II)	Yes
V (IV, V)	Yes	In (III)	Yes
Cr (III)	No	Y (III)	Yes
Mn (II)	Yes	Lanthanides	Yes
Fe (II, III)	Yes	Hg (II)	Yes
Co (II)	Yes	Pb (II)	Yes
Ni (II)	Yes	Al (III)	Yes
Cu (II)	Yes	Tl (I, III)	No
Zn (II)	Yes	As (III, IV)	No
Ag (I)	Yes	Se (IV, VI)	No

Table 2
Chemistry of Chelation Ion Chromatography

1. Sample pH adjusted to 5.2 to 5.6 and concentrated either on-line or off-line using the MetPac CC-1: Alkali metals and anions unretained, polyvalent metal ions concentrated.
2. Selective elution of alkaline earth metals using ammonium acetate.
3. Concentrated metals eluted to TMC-1 concentrator using nitric acid.
4. TMC-1 converted from hydronium to ammonium form: concentrated metals remain on TMC-1.
5. TMC-1 switched into analytical stream where concentrated metals are eluted to the analytical separator (CS5).

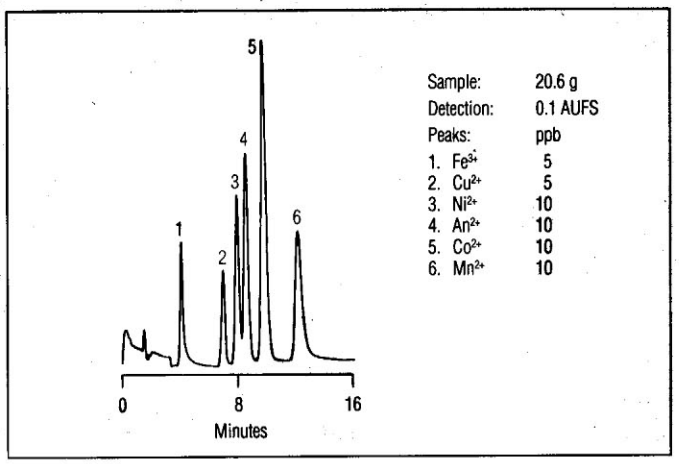

Figure 6 PDCA separation of transition metals using Chelation Ion Chromatography

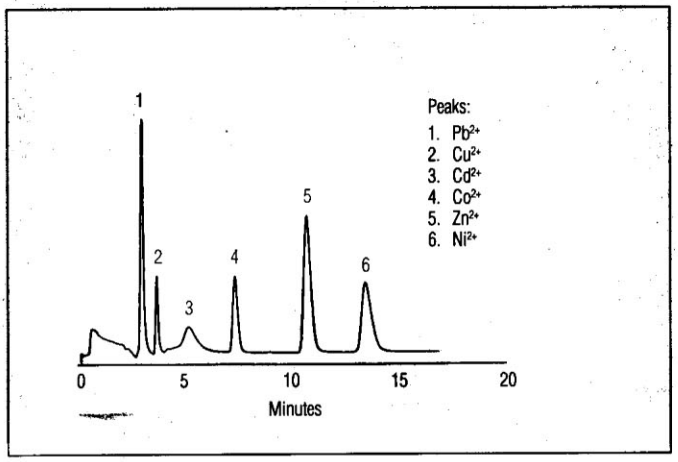

Figure 7 Oxalate separation of transition metals using Chelation Ion Chromatography

the ammonium form prevents a pH disturbance of the weak acid eluents. If the pH of the analytical eluents is disrupted, the analytical chromatography is adversely affected. If only transition metals are to be determined, the TMC-1 is switched in-line with the analytical eluent. The concentrated metals are eluted directly to the Ion Pac CS5 for the analytical separation. Table 2 summarizes the chemistries of chelation IC for transition metals.

The IonPac CS5 separations are based on one of two different eluent systems. The first is a pyridine-2,6-dicarboxylic acid (PDCA) eluent, which is a strong complexing agent that separates the metal ion complexes by anion exchange. PDCA is best suited for iron (II) and (III), copper, nickel, zinc, cobalt, and manganese. As shown in Figure 6, while iron (II) and (III) can be separated on the CS5, the nitric acid used for elution from the MetPac CC-1 causes most of the iron (II) to be oxidized to iron (III). Lead and cadmium elute under these conditions, but are so strongly bound to the PDCA that they are not sensitively detected by the postcolumn reagent.

A second, alternative eluent system uses an oxalic acid-based eluent, which is a moderate strength complexing agent that separates the metals by a mixed mode mechanism. The oxalate eluent separates lead, copper, cobalt, zinc, and nickel. This separation is shown in Figure 7. Cadmium and manganese coelute with this eluent. The isocratic analytical separations of the transition metals requires about 15 minutes.

Separated metals from the analytical column enter a postcolumn reaction system where they are derivatized with 4-(2-pyridylazo)resorcinol and then detected at 520-530 nm using a UV/visible absorbance detector.

It is important to use reagents and water with very low metal contamination (<1 ppb). Any trace metals in the reagents will be concentrated as a blank and subsequently eluted with the sample. The system blank results from contamination in the chelation concentration reagents and the system. Generally, iron and zinc are the most common transition metal contaminants, while a small amount of copper may also be observed. Care must be taken to minimize reagent and sample contamination during preparation and handling. Reagent purity will typically dictate the detection limits. The necessary reagents are described in Appendix A.

If system 1 is used, standards and samples must be buffered before concentration. To ensure complete recoveries for trace metals, the sample should be digested prior to concentration. Also, the solution to be concentrated should have an ammonium acetate concentration of 0.25 M or greater. Buffer the samples and standards to the same ammonium acetate concentration. Ensure that the samples and standards to be concentrated are pH 5.5 by adding 2 M ammonium acetate (pH 5.5).

SYSTEM PREPARATION AND SETUP

System 1

Details for operation of individual system components can be found in the appropriate Operator's Manual.

Refer to Figures 1 and 3 for set-up and plumbing of the chelation IC system. This set-up procedure uses the PDCA transition metal separation.

1. Begin the system setup by making sure there are no metal components in the flow path. This includes tubing end fittings (stainless steel washers, omni-fittings, etc.), columns, and valves that contain stainless steel. Replace all OmniFit grippers with Dionex ThermoFlare™ washers.

Table 3
Chelation Concentration Operating Conditions

Chelation Concentration—Transition Metals

Columns: MetPac CC-1, TMC-1

Eluents:
E1: H_2O
E2: 2.0 M ammonium acetate, pH 5.4 ± 0.1
E3: 2.0 M nitric acid
E4: 0.10 M ammonium nitrate, pH 3.5 ± 0.3

Gradient Program—System 1

t (min)	%E1	%E2	%E3	%E4	V5	V6	Flow (mL/min)
0.0	0	100	0	0	1	0	3.0
0.1	0	100	0	0	1	1	3.0
2.5	0	100	0	0	1	1	3.0
2.6	72	0	28	0	0	1	3.0
5.0	72	0	28	0	0	1	1.0
5.1	0	0	0	100	0	0	3.0
6.6	0	0	0	100	1	0	1.0
6.7	0	0	100	0	1	1	3.0
7.7	0	0	100	0	1	1	3.0
7.8	0	100	0	0	1	1	3.0
9.3	0	100	0	0	1	1	3.0
9.4	100	0	0	0	1	1	0.0

Gradient Program—System 2

t (min)	%E1	%E2	%E3	%E4	V5	V6	Flow (mL/min)
0.0	0	100	0	0	1	0	3.0
2.0	0	100	0	0	0	1	2.0
5.0	0	100	0	0	1	0	3.0
7.0	0	100	0	0	1	0	1.2
7.1	50	0	50	0	1	1	1.2
12.0	50	0	50	0	1	1	1.2
12.1	0	0	0	100	1	1	2.0
13.0	0	0	0	100	0	0	3.0
15.0	0	0	0	100	0	0	3.0
15.1*	0	0	100	0	1	0	4.0
16.0	0	0	100	0	1	0	4.0
17.0	0	0	100	0	1	0	4.0
18.0	0	100	0	0	1	0	0.0

*begin sample analysis

Analytical Chromatography—Transition Metals

Column: IonPac CS5

Eluent: *As shown in Figure 6:* 0.0060 M pyridine-2,6-dicarboxylic acid, 0.090 M acetic acid, 0.040 M sodium hydroxide
Or, as shown in Figure 7: 0.050 M oxalic acid, 0.095 M lithium hydroxide

Eluent Flow Rate: 1.0 mL/min

Postcolumn Derivatization

Reagent: 4×10^{-4} M 4-(2-pyridylazo) resorcinol
1.0 M 2-dimethylamino ethanol
0.50 M ammonium hydroxide
0.30 M sodium bicarbonate

Reagent Addition: Membrane reactor or mixing tee

Reagent Flow Rate: 0.5mL/min

Reactor: Packed or knitted reaction coil

Detection

Detector: Visible absorbance, VDM or UDM

Wavelength: 520 or 530 nm

Time Constant: 1 s

2. Before connecting the glass eluent bottles to the EDM, remove the endline filters. Since it is not necessary to purge the eluents, shorten the gas lines on the eluent caps so that they are not submersed into the eluents. To do so, remove the gas line, flare the end of the 1/8-in. line with the standard flaring tool, and reinstall the fitting into the eluent bottle cap. Repeat this procedure for all six eluent lines of the EDM. Each eluent bottle cap should contain a white TFE O-ring (P/N 41078), not a black rubber O-ring. Replace black O-rings with TFE O-rings as needed.

3. Remove the GM-2 mixers of the AGP. One of the mixers is located between the valve manifold and the priming block of the AGP. Connect the two lines using a union (P/N 39056). Do not install a mixer on the high pressure side of the AGP (i.e., between the AGP and valve 6).

4. Prepare 1 L of 0.2 M oxalic acid by dissolving 25.2 g of reagent grade oxalic acid dihydrate in 1 L of deionized water. This eluent will be used to clean the AGP eluent flow path.

5. Connect the 0.2 M oxalic acid to E1 of the AGP. Pump the oxalic acid through the AGP and to waste at 2.0 mL/min for 10 min. Repeat this procedure for ports 2, 3, and 4. This helps remove any trace metals from the AGP flow path.

6. Pump the 0.2 M oxalic acid through the DQP sample pump and to waste for 15 min at 2 to 3 mL/min. Finally, pump deionized water through the sample pump to remove the oxalic acid.

7. Clean four 1-L glass eluent bottles by filling them with 0.2 M oxalic acid. Allow the acid to remain in the eluent bottles for at least 4 hours. Prepare eluents as described in Appendix A. Connect the eluent bottles to the EDM and connect to the AGP. Be sure that the eluents are plumbed to the proper ports of the AGP. Pump each eluent for 5 min at 3.0 mL/min directly to waste.

8. Prepare the PAR reagent as directed in Appendix A. Place the PAR reagent in the RDM reagent reservoir and close the reservoir. Turn the RDM reagent 1 switch on and immediately adjust the regulator to 60 psi (410 kPa). Turn the RDM reagent 1 switch OFF. Be sure that PDCA is being pumped through the columns when the RDM is switched on. Failure to do so may result in PAR backing up through the IonPac CS5 column.

9. Enter the gradient program in Table 3 for chelation concentration system 1. This program can be entered from the front panel of the AGP, or if the AI-450 is being used, the program can be entered from the Method Editor.

System 2

1. Confirm that the SCM is configured as given in Figure 2. Be sure that an IonPac CG2 (P/N 35370) column is installed between the AGP and valve D of the SCM. Also, confirm that a CG2 column is present between the sample pump and valve B. Install a MetPac CC-1, TMC-1, and IonPac CG5 and CS5 columns as indicated in Figure 2.

2. Remove the GM-2 mixer from the low pressure side of the AGP. Connect the two lines using a coupler (P/N 39056). Do not install a mixer on the high pressure side of the AGP.

3. Prepare 1 L of 0.2 M oxalic acid by dissolving 25.2 g of reagent grade oxalic acid dihydrate in 1 L of the deionized water. This eluent will be used to clean the eluent flow path.

4. Connect the 0.2 M oxalic acid to E1 of the AGP. Pump the oxalic acid through the AGP and to waste at 2.0 mL/min for 10 min. Repeat this procedure for port 2, 3, and 4. This helps to remove any trace metals from the pump flow path.

5. Place the sample inlet tube into the 0.2 M oxalic acid solution. Fill each of the four 1-L eluent bottles and the 4-L plastic eluent bottle with 500 mL of 0.2 M oxalic acid. Enter the following program for system preparation. This program is entered from the front panel of the AGP. Refer to the *AGP Operator's Manual* for details on programming the AGP.

Time	E1	E2	E3	E4	V5	V6	Flow
0.0	100	-	-	-	1	0	2.0
5.0	-	100	-	-	1	1	2.0
10.0	-	-	100	-	0	1	2.0
15.0	-	-	-	100	0	0	2.0
20.0	100	-	-	-	0	0	0.0

6. Replace the 0.2 M oxalic acid in the 4-L plastic bottle with deionized water. Be sure that the cap has an O-ring for proper sealing.

7. Clean five 1-L glass eluent bottles by filling them with 0.2 M oxalic acid. Allow the acid to remain in the eluent bottle for at least 4 hours. Prepare eluents as described in Appendix A. Use caution in preparing and transferring these reagents to minimize contamination. Be sure that the eluents are plumbed to the proper ports. E5 is connected to the CARRIER IN port of the SCM rear panel. (E1: 2.0 M ammonium acetate, E2: 2.0 M nitric acid, E3: water, E4: 0.1 M ammonium nitrate, E5: PDCA or oxalic acid eluent). Adjust the eluent bottle regulator to 4–6 psi (20–41 kPa) and check for gas leaks.

8. Prime the AGP with each eluent.

9. Prepare PAR reagent as directed in Appendix A. Place PAR reagent into the RDM reagent reservoir and close the reservior. Turn the RDM reagent 1 switch on and immediately adjust the regulator to 60 psi (410 kPa). Turn the RDM reagent 1 switch off. Be sure that PDCA is being pumped through the columns when the RDM is switched on. Failure to do so may cause the PAR reagent to back up through the IonPac CS5 column.

10. Enter the gradient program for chelation concentration. This program can be entered from the front panel of the pump, or if using the AI-450, the program can be entered from the Method Editor.

SYSTEM TEST

System 1

The purpose of this system test is to ensure that all chromatographic and chemical components of the system are operating properly. Refer to Figure 3 for the schematic of system 1.

1. The system test will begin with a test of the hydraulic system. If the system fails the hydraulics test at any point, determine the source of the plumbing error. Begin by programming the AGP as follows:

   Eluent 1: 100%
   Flow: 2.0 mL/min
   Valve 5: on (1)
   Valve 6: off (0)

   Turn on the AGP. Eluent should begin to flow through valve 6 bypass and through valve 5 to waste line W2. The pressure readout of the AGP should not exceed 50 psi (340 kPa). Next, turn valve 6 on (1). Eluent 1 should begin to flow through MetPac CC-1. The pressure display of the AGP should increase. Disconnect one of the fittings connecting the MetPac CC-1 to valve 6 and confirm that eluent is flowing through the column. Reconnect the fitting when flow has been confirmed. Eluent should be flowing through line W2 to waste.

2. Turn valve 5 off (0). This should now place the TMC-1 in line with the AGP eluent flow. The AGP pressure display should increase. Disconnect one of the fittings connecting the TMC-1 to valve 5 and check for flow through the column. Reconnect the fitting and check for leaks. Turn the AGP off.

3. Using deionized water, prime the DQP sample pump. Set the flow rate to 3 mL/min. Turn valve 6 off (0) and turn on the sample pump. Deionized water should flow through the MetPac CC-1 and to waste line W1. No liquid should be exiting W2.

4. Disconnect the postcolumn system from the analytical column. Set the eluent pump (DQP or AGP) flow rate to 1.0 mL/min. Turn on the eluent pump and set valve 5 to the on (1) position. The deionized water should be flowing through the TMC-1 and to the IonPac CS5 column. Check to see that there is flow from the IonPac CS5.

5. Reconnect the postcolumn system to the IonPac CS5 column. Set the eluent pump flow rate to 1.0 mL/min and turn on the pump. Immediately, turn on the RDM reagent 1 valve to pressurize the postcolumn reagent reservoir and start reagent flow. After 1 min, check to see that the effluent from waste line W3 is yellow. This indicates that PAR is flowing. Measure the flow rate from the waste line. The flow rate should be 1.4–1.6 mL/min. Adjust the RDM regulator to achieve the recommended flow rate. If reagent is not flowing from the detector waste line, begin working backwards from the waste line to determine the source of blockage or high pressure. **Always check to see that reagent is flowing from the detector waste line when starting the system.** This completes the system test.

System 2

The purpose of this system test is to ensure that all chromatographic and chemical components of the system are operating properly. Refer to Figure 4 for a schematic of system 2. Be sure to check all fittings for leaks during the system test.

1. Enter the following gradient program for chelation concentration from the front panel of the AGP. Refer to the *AGP Operator's Manual* for details on programming the AGP. Check the program carefully for accuracy by listing the program.

Eluent 1:	100%
Flow:	2.0 mL/min
Valve 5:	on (1)
Valve 6:	off (0)

   The system test will begin with a test of the hydraulic system. If the system fails the hydraulic test at any point, determine the source of the plumbing error. Begin by using the program as given above. With the AGP in the stop-hold position, press RESET. This will set the program to time 0.0.

2. Press START on the AGP. Eluent (E1) should begin to flow to the valve D and out to the AGP OUT at the SCM rear panel. Check AGP OUT to confirm that the eluent is flowing to the SCM.

3. Next, prime the carrier pump by loosening the tubing fitting screwed into the outlet check valve. Since the eluent reservoir is pressurized (5 psi/35 kPa), the eluent (PDCA or oxalic acid) should begin to flow out of the check valve. As the eluent begins to flow, turn on the carrier pump by pressing the PUMP 1 POWER switch on the SCM front panel. After about 5 seconds, replace the outlet check valve tubing fitting. It is generally only necessary to finger-tighten these fittings. If the fitting should leak, tighten it another 1/8 of a turn using a 5/16-in. open-end wrench.

4. Set the stroke dial of the carrier pump to about 5.00 (refer to the *SCM Operator's Manual* for details on adjusting the flow rate). Disconnect the tubing between the pulse damper and the IonPac CG5 column, and confirm that the eluent is flowing out of the pulse damper. After about 90 seconds, connect the IonPac CG5 column to the pulse damper and confirm that the eluent is flowing through IonPac CG5, TMC-1, and IonPac CS5 columns. Prime each connection if necessary.

5. Calibrate the carrier pump flow rate by mass or volume to 1.0 mL/min. Be sure that the eluent reservoir is pressurized to 5 psi (35 kPa). Turn off the carrier pump (PUMP 1).

6. Repeat step 3 on the sample pump. Press the PUMP 2 POWER switch on the SCM front panel. Check to confirm that the deionized water is flowing out of the CARRIER OUT tubing at the SCM rear panel. Calibrate the sample pump flow rate by mass or volume to 2.0 mL/min. Turn off the sample pump (PUMP 2).

7. Place the sample inlet tube in a container of deionized water and draw the deionized water through the sample loop using a syringe. Confirm that the solution is flowing through the sample loop.

8. List the gradient program to the next sequence (2.0 min) and press run and hold. This will forward the program to 2.0 min. Check to see that the eluent is flowing out of the SAMPLE OUT tubing at the SCM rear panel. Stop the pump and confirm that the eluent flow stops. Turn on the sample pump (PUMP 2). Check to confirm that the deionized water is flowing out of the same port at the SCM rear panel. Start the AGP, wait 30 seconds and measure the flow rate. The combined flow rate from the sample pump and the AGP must be 4 mL/min. Adjust the sample pump flow rate as needed. Turn off the sample pump (PUMP 2) and the AGP.

   In the event that the carrier pump or sample pump loses prime, prime the pump by loosening the tubing fitting on the outlet check valve. If no liquid is in the check valve, use a squirt bottle to squirt some deionized water into the check valve. This will aid in priming the pump. Replace the check valve fitting. This completes the hydraulic test.

9. Reset the AGP to time 0.0 min. Start the carrier pump (PUMP 1). This should now place the TMC-1 column in-line with the carrier pump eluent flow. Disconnect the TMC-1 column and confirm that eluent is flowing through the column by turning off the carrier pump (PUMP 1).

10. Forward the gradient program to 8.1 min and start the pump (PUMP 2). This should now place the TMC-1 column in-line. Confirm that the eluent is flowing through the column. Reconnect the TMC-1 column and check for leaks. Turn off the AGP (PUMP 2).

11. Reset the gradient program. Connect the postcolumn system to the IonPac CS5 column. Start the carrier pump (PUMP 1). Immediately turn on the RDM reagent 1 valve to pressurize the postcolumn reagent reservoir and start the reagent flow. After 1 min, check to see that the PAR postcolumn reagent is flowing to the VDM-2. Measure the flow rate from the waste line. The flow rate should be 1.4 to 1.6 mL/min. Adjust the RDM regulator to achieve the recommended flow rate. This completes the system test.

SYSTEM OPERATION

System 1

A step-by-step operating procedure for manual operation of the system (system 1) is described below. Table 3 lists the operating parameters for the determination of transition metals.

1. Check that the system is configured as shown in Figure 3. Confirm that the system has a 80-120 psi (550–830 kPa) inert gas supply.

2. Turn on the absorbance detector. If a variable wavelength detector is being used, set the wavelength to 530 nm. If a filter-based detector is being used, ensure that the filter is 520 or 530 nm. Turn on the visible lamp and set the sensitivity to 0.2 AUFS. Be sure that the detector output is connected to an integrator or data system (AI-450).

3. Enter the program listed in Table 1 in Program 1 of the AGP used for chelation concentration. This is the complete program for chelation concentration. **Check each step of the program carefully.**

4. Turn on the analytical pump (1.0 mL/min) and the RDM. Check to see that PAR reagent is flowing from line W3. If an AGP is being used, check to see that the pressure readout of the pump does not exceed 900 psi (6.2 MPa). Check slider valve V5 for leaks.

5. Turn on the integrator or monitor and begin to monitor the baseline. At 0.2 AUFS, an essentially noise-free and drift-free baseline should be observed. If the AGP is not at the beginning of the program, reset the program. The AGP should be in the stop-hold position.

6. Once a stable baseline has been obtained, run a complete manual system test. Prepare a Level 2 standard as described in Appendix A. Prime the sample pump with the standard, and concentrate 10-20 mL of sample at a flow rate not exceeding 3.0 mL/min. Collect the sample effluent from line W1 and determine the volume or mass of the sample. Be sure to turn off the sample pump.

7. Next, start the AGP and press RUN to begin program 1. Immediately start the data system. The concentrated sample will be injected into the analytical stream at 6.6 min. Using the PDCA eluent, the first analyte from the CS5, iron(III), should elute between 9 and 11 min. For the chromatogram shown in Figure 6, the data reduction system was initiated at 6.6 min. The last analyte, manganese(II), should elute between 19 and 22 min. If the peaks are much larger than those shown in Figure 6, trace metals from the reagents and system have been eluted with the standard. The blank will decrease with subsequent runs and is not a cause for concern.

8. Reset the AGP and data collection device. Start the AGP, press RUN, and start collecting the data. This time the chromatogram will represent the system blank. Repeat this procedure at least three times or until the blank is reproducible and equivalent in magnitude to the chromatogram given in Figure 8. After 10 consecutive blank runs, if the blank is not reproducible or is still elevated, proceed to the next step.

9. Take about 100 mL of deionized water and add 15 mL of 2 M ammonium acetate. Concentrate about 50 mL of the water and analyze. If the resulting chromatograms show significantly higher concentrations of metals (greater than 15% of the blank), the deionized water has unacceptable concentrations of trace metals. The purity of the water must be improved before continuing.

10. At this point, the system is ready for calibration and sample analysis. Refer to the Calibration and Quantification/Calibration procedures later in this technical note.

System 2

This section describes the sequencing and operation of the system components. This system configuration has been designed for use in the fully automated mode, except for the sample introduction step. If the required sample volume is not more than 3 mL, the Dionex ASM autosampler can be used. Samples greater than 5 mL must be introduced using a syringe or a peristaltic pump.

1. Confirm that the system is configured as shown in Figures 2 and 4. Check to see that the system has a 80–120 psi (550–830 kPa) inert gas supply.

2. Turn the absorbance detector on. If a variable wavelength detector is being used, set the wavelength to 530 nm. If a filter-based detector is being used, check that the filter is for either 520 or 530 nm. Turn on the visible lamp and set the sensitivity to 0.2 AUFS. Be sure that the detector output is connected to a data collection system (integrator or ACI/AI450).

3. Enter the program listed in Table 1. Check the program carefully by listing each step of the program.

4. Reset the gradient program at time 0.0 min. Turn on the carrier pump (Pump 1, 1.0 mL/min) and the RDM. Confirm that the PAR reagent is flowing through the detector.

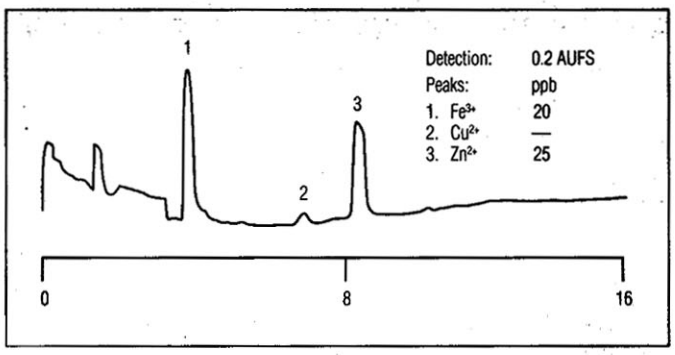

Figure 8 Typical blank obtained using Chelation Ion Chromatography

5. Turn on the integrator or monitor and begin to monitor the baseline. At 0.2 AUFS, an essentially noise-free and drift-free baseline should be observed.

6. Step 1: Once the baseline is stable, press RUN. Confirm that valve 5 is on and valve 6 is off. The sample or the standard can be loaded via autosampler. If the autosampler is not used, load the sample using a syringe to draw the sample through the sample inlet. The sample pH should be 1–2. While the sample is introduced, the AGP is pumping 2.0 M ammonium acetate to regenerate/equilibrate the MetPac CC-1 column. Note that the next step—flushing the sample loop to the MetPac CC-1 and on-line buffering—occurs at 2.0 min. If the sample introduction takes more than 2.0 min, adjust the gradient program accordingly.

7. Step 2: Valve 5 is OFF and valve 6 is ON. The sample pump is pumping deionized water through the sample loop that was previously loaded with sample. The sample stream is now mixing with the 2.0 M ammonium acetate and the buffered sample passes through the MetPac CC-1 column.

8. Step 3: Valve 5 is ON and valve 6 is ON. Valve E now is switched to the load position, where the TMC-1 column is placed in-line with the AGP. Now, the pump is pumping 1.0 M nitric acid to the MetPac CC-1 column. The concentrated metal ions are eluted from the column, and the 1.0 M acid stream is diluted on-line to approximately 0.37 M with deionized water from the sample pump and passes through the TMC-1 column. This step maximizes the removal of concentrated metal ions from the MetPac CC-1 and places them on the TMC-1 column in a tight band.

9. Step 4: Valve 5 is OFF and valve 6 is OFF. 0.1 M ammonium nitrate is pumped to the TMC-1. This step is required to convert the TMC-1 from acid form to ammonium form.

10. Step 5: Valve 5 is ON and valve 6 is OFF. Valve E is now switched to the inject position, where the TMC-1 column is placed in-line with the IonPac CS5 column. The AGP is pumping 2.0 M nitric acid to the MetPac CC-1 for 2 min (3.0 mL/min) and followed by 2.0 M ammonium acetate for 2.0 min (3.0 mL/min) before the end of the chelation concentration process.

    You can start chelation concentration at this time for the next sample. Remember that the analysis of the previous sample must be completed before proceeding to step 3 where the TMC-1 column is switched in-line with the MetPac CC-1. The analysis time for transition metals is normally within 15 min. The gradient program may be adjusted so that step 3 can proceed at 15.0 min.

11. Reset the AGP and data collection device. Start the overall cycle without injecting the sample. This run will represent the system blank. Repeat this cycle at least three times or until the blank is reproducible.

12. At this point, the system is ready for calibration and sample analysis. Refer to the appropriate section of this manual for details on calibration and quantification.

CALIBRATION

External calibration is recommended for this method and can be performed using either single level or multilevel calibration. Single level calibration generally yields good precision if the analyte concentration is within a factor of 5 to 10 of the standard concentration. For a greater working (concentration) range, a multilevel calibration is recommended.

Measurement of the standard and sample peak areas or heights and the subsequent calculation of the sample concentration is performed by either AI-450 or the integrator. Refer to the appropriate operator's manual for details of the calibration and quantification procedures.

Single Level Calibration

Single level calibration is performed by determining peak area(s) or height(s) for a standard containing a known concentration of the analyte(s) of interest. Then, the sample is run and the analyte(s) concentration is determined from the ratio of the sample peak area(s) to the standard peak area(s). Single level calibration is simple and rapid and is generally most useful for providing a relatively accurate estimation of analyte concentration. As long as the mass of sample analyte(s) is approximately equal (within a factor of three to five) to the mass of standard analyte, single level calibration will result in acceptable quantification.

Multilevel Calibration

Multilevel calibration is performed by determining peak height(s) or area(s) of standards at several concentrations. A calibration curve is established by plotting peak area or height for each analyte on the Y axis and the analyte concentrations on the X axis. A curve is fit mathematically to the points to establish a calibration curve for each analyte. The sample analyte concentration is determined by finding the point on the calibration curve that corresponds to the measured peak area or height. Multilevel calibration should be performed with two to four standards per decade of concentration. Multilevel calibrations generally give more precise quantification as well as increasing the working concentration range for quantification.

QUANTIFICATION/CALIBRATION

In the concentration mode, the quantity (mass or volume) of standards and samples concentrated must be determined with good precision. This can be accomplished by measuring time to determine volume (flow rate x time), direct volume, or mass. Measuring direct volume or mass is more precise and is recommended. A mass measurement can be readily obtained by taring a beaker on a top loading balance, collecting the sample as it passes through the concentrator (MetPac CC-1), and then weighing the beaker.

When samples are analyzed by concentration techniques, calibration is performed using the external standard method. In external calibration, a plot is made for peak area or height (Y axis) versus the mass of analytes (X axis) concentrated for a standard (single level) or a series of standards (multilevel). A best-fit curve is drawn through the points. Quantification of unknowns is accomplished by finding the point on the curve (analyte mass) that corresponds to the measured peak area. Analyte concentration is then calculated by dividing analyte mass by the volume concentrated.

For the standards, the mass of analyte is calculated from the following equation:

$$M_x = Q_s / C_x$$

where M_x is the mass of the analyte x, Q_s is the quantity (mass or volume) of the standard (or sample) concentrated, and C_x is the concentration of analyte x.

For the samples, the concentration of analyte(s) is calculated from M_x/Q_s, where M_x is obtained from the calibration plot. Refer to the *AI-450 Operator's Manual* for details on integration and calibration. Refer to Appendix B for the recommended concentrations of standards.

SYSTEM LINEARITY

The linear range of this method is approximately 0.2 to 150 ng, depending on the metal ion. The linear range is expressed in mass because we are concentrating and can readily vary the volume of standard or sample loaded. Above 150 ng of analyte, column overload occurs, distorting peak symmetry, which affects resolution and quantification. For most analytes, concentration levels of 0.2 ng approach the detection limit and thus diminish the ability to accurately measure the signal.

SAMPLE PREPARATION

It is beyond the scope of this text to describe in detail the techniques of ultratrace analysis in terms of sample collection, storage, and handling. Several points will be discussed, however, that apply to sample preparation before analyzing samples by the method described in this technical note.

Samples should be collected in *clean* polyethylene containers. In order to stabilize the sample for storage, the sample should be acidified to a pH 1.5 to 2.0. Be sure to use ultrapure nitric acid to adjust the pH.

To ensure complete recovery of metals using chelation concentration, metal ions should not be bound by any strong complexing agents or be present as hydroxy complexes. Acid digestion is a general technique used to destroy complexing agents or to minimize their complexation ability. If the samples being analyzed contain solid material and/or large amounts of organic materials (e.g., humic acids), the samples should be digested prior to analysis. In general, if you have used digestion for sample pretreatment prior to metal analysis, those same digestion procedures can be used in this method.

If system 2 is used, the sample pH must be adjusted to pH 1–2 with ultrapure ammonium hydroxide.

If system 1 is used, the sample pH must be adjusted to 5.3 to 5.6 using ultrapure 2–6 M ammonium acetate (pH 5.5). For optimum concentration, the final ammonium acetate concentration in the sample should be 0.25–1.0 M. Since the addition of any reagent to the sample adds trace metals, use the minimum amount of ammonium acetate needed for sample pH adjustment. Use care in transferring the ammonium acetate to the sample to avoid contaminating the sample with metals. Do not use plastic pipet tips because they are contaminated with zinc. Use a glass pipet for small volumes and graduated cylinders for larger volumes. Alternatively, add the ammonium acetate by mass directly from the polyethylene container. For approximately 100 mL of sample at pH 2, add 15.45 g (15.0 mL) of the 2 M ammonium acetate. If the sample has been digested and the final acid concentration is 0.5 M, a 100-mL digested sample will require about 52 g (50 mL) of the 2 M ammonium acetate. If the levels of metals in the digested sample are above 100 ppb, the sample can be diluted and the corresponding amount of ammonium acetate added to adjust the pH. Be sure to prepare and analyze a blank. The blank contains everything (nitric acid, ammonium acetate) except for sample.

If the sample contains percent levels of aluminum, iron, or silica, adding buffer solution will destabilize the solution and result in the formation of a precipitate or gel. The precipitate or gel is a hydroxide and/or oxide of the aluminum and iron resulting from hydrolysis of the aluminum and iron, which occurs at the higher pH. Since the hydrolysis reactions are quite slow (minutes) at pH 5.5, it is possible to add the buffer solution and immediately begin chelation concentration before precipitation begins. **Never attempt to concentrate a sample containing solids or suspended (collodial) materials.**

SAMPLE ANALYSIS

System 1

Before concentration, all standards and samples must be buffered to pH 5.2–5.6. This is done most readily by adding an aliquot of the 2 M ammonium acetate, pH 5.5, to the standard or sample just before concentration. The ammonium acetate concentration in the sample of standard should be at least 0.25 M.

Begin sample analysis after the instrument has been calibrated. Sample loading should be done with the AGP reset (t = 0) and in the hold position. A quality control standard should be run at least every 10 samples.

Be sure to turn the sample pump on for at least 1 minute before loading the sample on the MetPac CC-1. With valve 6 in the off (1) position, which occurs at the end of the chelation con-

centration program, sample can be pumped to waste. This minimizes sample carry-over from the pump and sample line.

In a seawater matrix, the MetPac CC-1 column can concentrate up to 300 mL before breakthrough of the trace metals occurs. In a brine matrix, which contains low levels of calcium and magnesium (less than 500 ppm), larger sample volumes can be concentrated (up to 1 L). Brine matrices containing 20% sodium chloride have been analyzed with quantitative recovery of the transition and lanthanide metals.

For concentrated acids and bases, it is best to dilute to 3 M or less and buffer. Large sample volumes (1 L) can be concentrated as long as the pH is about 5.5.

System 2

All standards and samples to be introduced in this system must not contain greater than 3% acid. If the acid content exceeds 3%, the samples or standards must be neutralized with ultrapure ammonium hydroxide. The recommended adjusted sample pH is between 1–2.

Begin sample analysis after the instrument has been calibrated. Unlike the system 1 method of operation, in which the sample introduction step is independent of the AGP sequence, the overall process (i.e., sample loading, on-line buffering, etc.) performed by system 2 is executed by the gradient program. The sample can be introduced into the system directly after the pump is in RUN mode. A quality control standard should be run at least every 10 samples.

SAMPLE VOLUME

The volume of sample to be concentrated will depend on several factors. First, to obtain good precision and to work significantly above the noise level, a minimum of 25 ng of each analyte should be loaded on the MetPac CC-1 column. Second, the mass of sample should fall within the range of the calibration standards. Finally, the mass of sample (and standard) analytes concentrated should be at least three times greater than the blank. This last point is important for iron and zinc, since these two elements exhibit the greatest contamination level. If the levels of metals in the sample are below 10 ppb, at least 10 mL of sample should be concentrated. If the approximate concentrations of metals in the samples are not known, start by concentrating between 10 and 20 mL. After determining the approximate concentrations, the appropriate volume to concentrate can be determined.

Unlike the system 1 method of operation, system 2 uses a sample loop to introduce a constant amount of sample into the system. The SCM comes factory-equipped with two sample loops—1.0 mL and 5.0 mL. The selection of the sample loop size depends on the concentration of analytes of interest. Because the sample loading step is automated by the program, consult Appendix C for more details about sample loop preparation and appropiate programs.

APPLICATIONS OF CHELATION ION CHROMATOGRAPHY

Seawater

In open ocean seawater, the concentrations of transition metals range from 0.01 to 1 ppb. In bay waters, the levels of transition elements can range from 10 ppb to sub-ppb levels. The concentrations of sodium, potassium, magnesium, and calcium in seawater and bay waters are typically 10, 0.5, 1.5, and 0.5 g/L, respectively. Figure 9 shows the determination of transition metals in bay water obtained at Monterey, California. The sample was prepared by adding 10 mL of 2 M ammonium acetate, pH 5.5, to 90 mL of the bay water. Up to 300 mL of seawater can be concentrated on the MetPac CC-1 without significant loss of the transition metals or lanthanides.

The precision of chelation IC at the low-ppb level in seawater is given in Table 4. Iron typically shows the highest relative standard deviation because of its higher blank and because iron is very strongly bound to the resin.

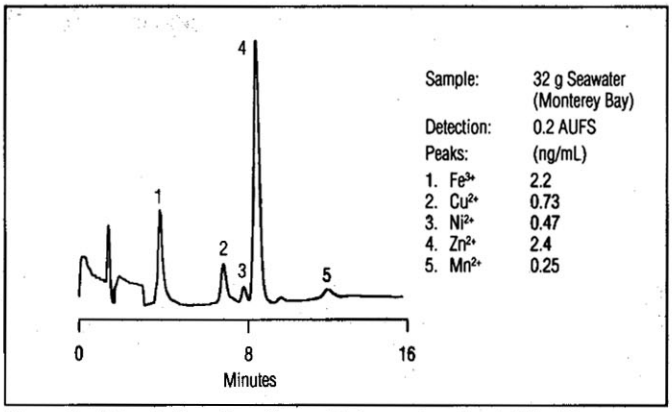

Figure 9 Determination of transition metals in seawater using chelation IC

Table 4 %RSDs for Transition Metals using Chelation Concentration

	Peak Area					
Sample	**Fe^{3+}**	**Cu^{2+}**	**Ni^{2+}**	**Zn^{2+}**	**Co^{2+}**	**Mn^{2+}**
Standard[1]	6.5	3.1	2.4	2.3	0.66	4.3
Seawater[2]	13.2	6.9	7.8	7.9	—	6.3

[1] 20 g of 5-ppb Fe^{3+}, 5-ppb Cu^{2+}, 10-ppb Ni^{2+}, 10-ppb Zn^{2+}, 10-ppb Co^{2+}, 10-ppb Mn^{2+} (n = 11).
[2] 40 g of Monterey Bay seawater.

Sodium Hydroxide

Transition metals have been determined in concentrated (50%) sodium hydroxide using chelation IC. The sample was diluted 1:10 in deionized water, and then acidified with nitric acid (0.2 mL concentrated nitric per mL of diluted sodium hydroxide). The acidified solution was then diluted 1:1 in 2 M ammonium acetate, pH 5.5. The results are shown in Figure 10.

Magnesium Chloride

Since chelation concentration effectively reduces magnesium while concentrating transition metals, the determination of trace metals in reagent grade magnesium chloride is a straightforward application of chelation IC. In this experiment, a 0.086 M solution of magnesium chloride, 0.25 M ammonium acetate (pH 5.5) solution was prepared and analyzed. See Figure 11.

Urine

Biological matrices contain large concentrations of alkali and alkaline earth metals compared to transition metals. For the determination of trace metals in biological fluids or tissues, the sample must first be acid digested. In this application, 73.4 g of urine were acid digested with 26.6 mL of concentrated nitric acid. A 10-mL aliquot of the digested urine was added to 20 mL of 2 M ammonium acetate, pH 5.5 and the sample analyzed. The results are given in Figure 12.

Oyster Tissue (SRM 1566)

Using microwave digestion for sample dissolution, a United States National Institute of Standards and Technology (NIST) standard reference material (SRM) of oyster tissue was analyzed by chelation concentration. Excellent agreement between the certified and IC values was obtained. See Figure 13 (courtesy of NIST).

Waste Water

Waste waters may contain high levels of alkali and alkaline earth metals. Figure 14 shows the determination of transition metals in an industrial waste water without interferences from the alkali and alkaline earth metals.

CASS-2 Nearshore Water

Chelation IC was used for the analysis of a Canadian seawater reference material. The results are given in Figure 15.

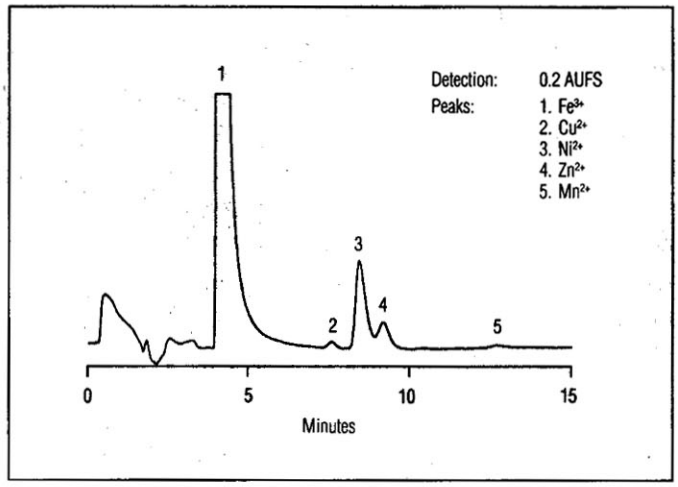

Figure 10 Determination of transition metals in sodium hydroxide using Chelation IC

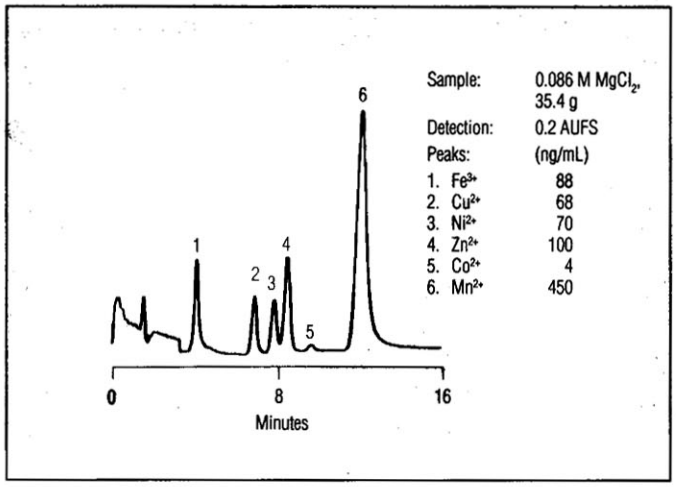

Figure 11 Determination of transition metals in reagent grade magnesium chloride using Chelation IC

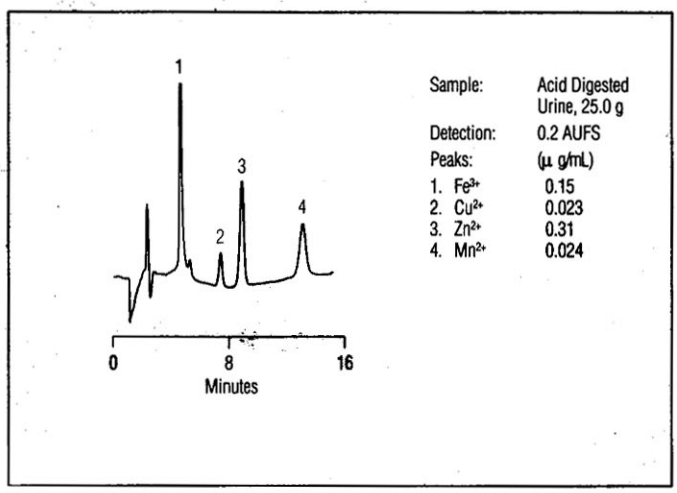

Figure 12 Determination of transition metals in urine using Chelation IC

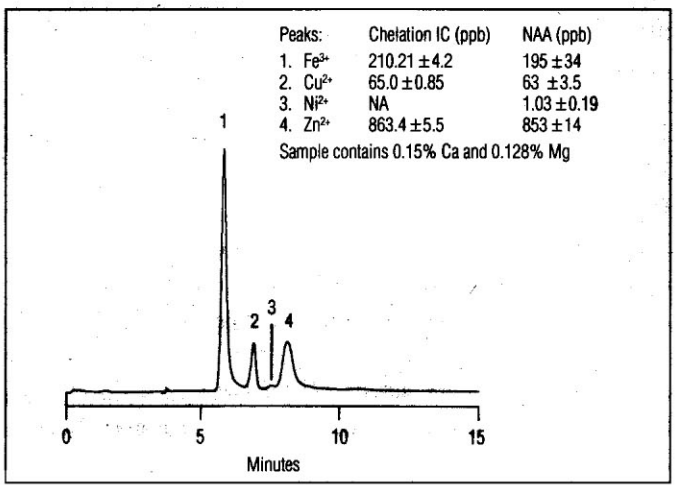

Figure 13 Determination of transition metals in tissue (SRM 1566) using Chelation IC

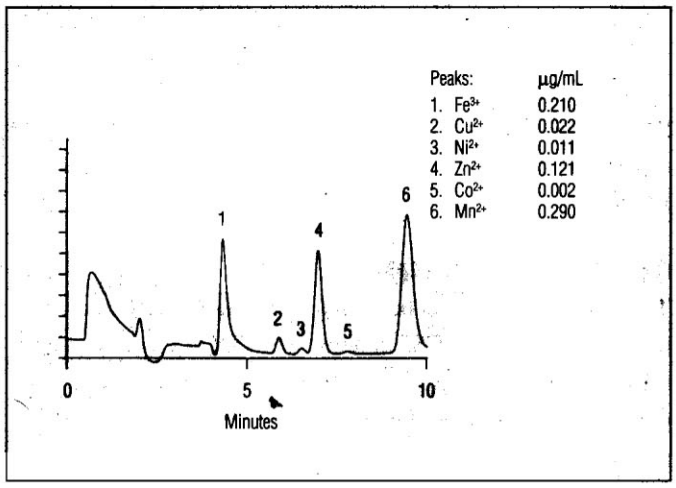

Figure 14 Determination of transition metals in acid-digested waste water by Chelation IC

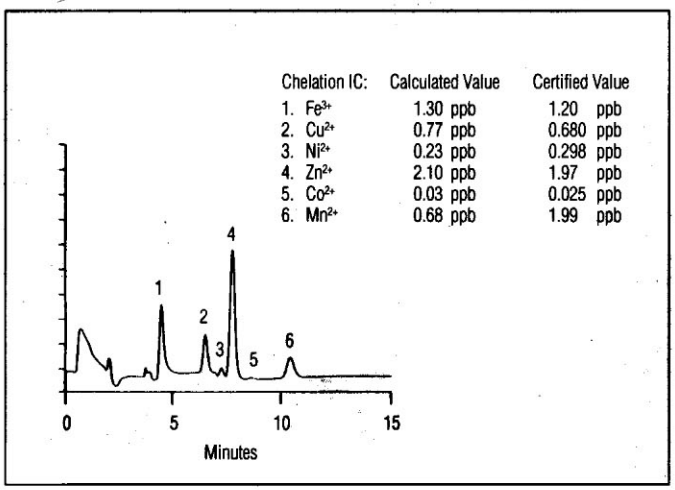

Figure 15 Determination of transition metals in nearshore seawater (CASS-2) by Chelation IC

APPENDIX A: REQUIRED REAGENTS AND STANDARDS

The following reagents are required. Minimum quantities are given in parentheses. Information for ordering ultrapure acids and ammonium hydroxide are given below. All chemicals are reagent grade unless otherwise specified.

Chelation Concentration Eluents

Prepared—Dionex Ultrapure

2 M Ammonium acetate pH 5.5 (1 L, P/N 33440; 6 L, P/N 33441)

2 M Nitric acid (1 L, P/N 33442; 6 L, P/N 33443)

0.1M Ammonium nitrate, pH 3.5 (1 L, P/N 33444; 6 L, P/N 33445)

Chelation Concentration Reagents

Acetic acid, ultrapure

Ammonium hydroxide, ultrapure

Nitric acid, ultrapure

The ultrapure reagents are manufactured by Seastar Chemical and Ultrex Reagents. Seastar reagents are available internationally from Fisher Scientific, who sells these reagents under the OPTIMA® label. Ultrex reagents are available internationally through J.T. Baker and Van Waters and Rogers (VWR).

Chromatographic Reagents

Pyridine-2,6-dicarboxylic acid (PDCA), purified (P/N 39671, 20 g)

Sodium hydroxide, 50% (w/w 0.5 L)

Acetic acid, trace metal grade (0.5 L)

Oxalic acid dihydrate (100 g)

Lithium hydroxide monohydrate, (100 g)

4-(2-pyridylazo) resorcinol, monosodium, monohydrate (P/N 39672, 5 g)

Ammonium hydroxide, trace metal grade (0.5 L)

Sodium bicarbonate (500 g)

2-Dimethylaminoethanol (1 L, Fluka Chemika–Biochemika)

Nitric acid, trace metal grade

Transition metal standards, 1000 ppm, 100 mL each: iron, copper, nickel, zinc, cobalt, manganese, and lead

Eluent and Standards Preparation

Before preparing eluents and standards, thoroughly clean the eluent containers as directed in System Preparation. Be sure that the eluent bottle caps have a white TFE seal and not a black rubber seal. Prepare all eluents directly in the one liter glass eluent containers. Transfer reagents directly from their container. Avoid using pipets or graduated cylinders unless these have been thoroughly cleaned. Do not use stir bars in chelation concentration reagents.

The 0.1 M solutions of nitric acid and ammonium hydroxide are required only if you are going to prepare the chelation concentration eluents.To make these solutions, fill two 500-mL polyethylene containers with 0.2M oxalic acid and let stand for at least four hours. Rinse the containers with deionized water. In one container, add 200 g (200 mL) of deionized water and 4.5 g (3.1 mL) of ultrapure concentrated nitric acid. Bring the final volume to 500 mL with deionized water. Label this solution 0.10 M nitric acid. In the second container, add 200 g (200 mL) of deionized water and 4.4 g (4.7 mL) of ultrapure 20% ammonium hydroxide. Bring the final volume to 500 mL. Label this solution 0.10M ammonium hydroxide. This solution will be used to adjust the pH of E4.

Chelation Concentration Eluents—Transition Metals

Use only ultrapure chemicals and deionized water (<0.5 ppb of each metal) for preparation of these reagents. Caution must be used in preparing this reagent to minimize metal contamination. Do not place anything in the eluent container (including stir bars). When adjusting the pH of the ammonium acetate and ammonium nitrate, do not place the pH electrode in the bulk solution. Instead, take aliquots of the solutions to check the pH.

E1: Deionized water

E2: 2 M Ammonium acetate, pH 5.4 ± 0.1

Place 600 mL of deionized water into a clean 1-L glass eluent container. Tare the bottle and add 121g (115 mL) of ultrapure glacial acetic acid and mix thoroughly. In a fume hood, slowly add 133 g (148 mL) of 20% ultrapure ammonium hydroxide and mix thoroughly. Agitate the bottle to thoroughly mix the solution. Calibrate a pH meter to pH 7. Pour about 10 mL of the buffer into a small container (scintillation vial, 10 mL disposable beaker) and measure the pH. If the pH is below 5.3, add about 5 mL of ammonium hydroxide to the buffer solution. If above pH 5.5, add 5 g of acetic acid. Adjust the pH of the ammonium acetate to 5.4 ± 0.1 using acetic acid if the pH is greater than 5.5, or ammonium hydroxide if the pH is less than 5.3. Once the pH is 5.4 ± 0.1, bring to a volume of 1 L. Connect the eluent container to line 2 of the EDM.

E3: 2.0 M Nitric acid

Place 200 mL of deionized water into a clean 1-L glass eluent container. Add 176 g (124 mL) of ultrapure nitric acid. Add deionized water to bring the final volume to 1 L and mix thoroughly. Connect the eluent container to line 3 of the EDM.

E4: 0.10 M Ammonium nitrate, pH 3.4 ± 0.3

Place 200 mL of deionized water into a clean 1-L glass eluent container. Add 8.9 g (6.3 mL) of concentrated nitric acid. Next, add 7.6 g (8.5 mL) of ammonium hydroxide. Add sufficient deionized water to give a final volume of 1 L and mix thoroughly. Calibrate a pH meter to pH 4.0. Take a 10-mL aliquot of the solution and measure the pH. Add either 0.10 M ammonium hydroxide or 0.10 M nitric acid in a 3–5 mL aliquot to the bulk solution to adjust the pH. Continue taking aliquots and adjusting the pH to 3.4 ± 0.3. Connect the eluent container to E4 of the EDM.

Analytical Chromatography

Two eluent systems can be used for transition metal separations with the IonPac CS5 column. The PDCA eluent is used for iron, copper, nickel, zinc, cobalt, and manganese. The oxalic acid eluent is used for lead, copper, cobalt, zinc, and nickel. Cadmium and manganese coelute using the oxalic acid eluent.

PDCA Stock Solution

0.060 M PDCA
0.400 M Sodium hydroxide

Place 200 mL of deionized water into a clean 1-L polyethylene bottle. Add 31.8 g (20.6 mL) of 50% sodium hydroxide. While stirring with a stir bar, add 10.0 g of pyridine-2,6 dicarboxylic acid. Continue to stir for about 10 minutes or until all the PDCA has dissolved. Dilute to 1 L and stir thoroughly. Label 0.060 M PDCA, 0.40 M NaOH.

Acetic Acid Stock Solution

0.90 M Acetic acid

Place 200 mL of deionized water into a clean 1-L polyethylene bottle. Add 54.2 g (51.6 mL) of trace metal grade acetic acid and dilute to 1 L. Label 0.90 M acetic acid.

PDCA Eluent

0.006 M PDCA
0.040 M Sodium hydroxide
0.090 M Acetic acid

To a 1-L glass eluent container, add 100 g (100 mL) of the PDCA and acetic acid stock solutions. Dilute to 1 L with deionized water. Label the container 0.0060 M PDCA, 0.040 M NaOH, 0.090M HOAc. The eluent should have a final pH of 4.6. Connect the eluent to line 5 of the EDM.

Oxalate Stock Solution

0.20 M Oxalic acid
0.38 M Lithium hydroxide

To a 1-L polyethylene bottle, add 500 g (500 mL) of deionized water. Next add 25.2 g of oxalic acid dihydrate and mix thoroughly to dissolve. Add 16.0 g of lithium hydroxide monohydrate and dilute to 1 L. Mix thoroughly.

Oxalate Eluent (Transition Metals)

0.050 M oxalic acid
0.095 M lithium hydroxide

To a 1-L glass eluent container, add 250 mL of the oxalate stock solution and dilute to 1 L with deionized water.

PAR Postcolumn Reagent

0.5 mM 4-(2-Pyridylazo)resorcinol
1.0 M 2-Dimethylaminoethanol
0.5 M Ammonium hydroxide
0.3 M Sodium bicarbonate

Prepare the PAR directly in the 1-L plastic reagent reservoir container (P/N 37054). To 200 g (200 mL) of deionized water add 31 g (35 mL) of trace metal grade ammonium hydroxide. Next, add 0.12 g of 4-(2-pyridylazo)resorcinol, monosodium, monohydrate and ultrasonicate for five minutes. Add a stir bar and stir for several minutes to ensure that the PAR has completely dissolved. Add 500 g (500 mL) of deionized water and then 89 g of 2-dimethylaminoethanol (DMAE). The solution should turn from red to orange-yellow. If the solution turns bright red upon addition of the DMAE, the DMAE contains metals and should be discarded. Add 25.4 g of sodium bicarbonate and stir thoroughly until dissolved. Fill the reagent container with deionized water up to the threads on the neck and stir. The color of the final solution should be yellow to yellow-orange. Place the reagent container in the reagent reservoir.

APPENDIX B: PREPARATION OF STANDARDS

Standards should be prepared daily. Certain metals, especially iron (III), are not stable at pH 5.5 for more than a day. The standards described below are intended for the determination of metals in the low-ppb (ng/mL) range. If quantification at high levels is required, standards can be prepared at concentrations five times greater than those listed. The standards listed below are intended for use with the PDCA eluent.

Transition Metal Stock Solution

Using a variable volume micropipet, add the following volumes of 1000-ppm atomic absorption standards to a 100-mL volumetric flask.

Metal Ion	Volume (µL)	Final Concentration (µg/mL)
Fe^{3+}	200	2.00
Cu^{2+}	200	2.00
Ni^{2+}	400	4.00
Zn^{2+}	400	4.00
Co^{2+}	400	4.00
Mn^{2+}	400	4.00

Next, add about 1 mL of concentrated nitric acid (ultrapure) and dilute to volume. This stock solution will be used to prepare the calibration standards. The calibration standards can be prepared in 100-mL volumetric flasks or LDPE bottles. Be sure that the flasks or bottles have been thoroughly cleaned.

Single Level Calibration Standard

If you are using a 100 mL volumetric flask to prepare the standard, add 200 µL of stock solution and 15 mL of the 2 M ammonium acetate buffer and bring to volume.

If you are using a polyethylene or Teflon bottle, tare the empty bottle (without the cap) on a top loading balance.

Using a micropipet, add 200 µL of stock solution and 15 g of the 2 M ammonium acetate buffer. Next, add water to give a total mass of 100 g (±0.1 g). This single level calibration standard will have the following concentrations.

Metal Ion	Concentration (ng/mL)
Fe^{3+}	4.0
Cu^{2+}	4.0
Ni^{2+}	8.0
Zn^{2+}	8.0
Co^{2+}	8.0
Mn^{2+}	8.0

For calibration, concentrate at least 5 mL of this solution.

MultiLevel Calibration Standards

The multilevel calibration method recommended uses standards at four concentrations. Using the procedure given in "Single Level Calibration Standard," prepare standards as given below.

Level	Volume, Stock Solution (µL)
L1	100
L2	200
L3	500
L4	1000

The multilevel calibration standards will have the following concentrations of metal ions.

	Concentration (mg/mL)			
Metal Ion	**L1**	**L2**	**L3**	**L4**
Fe^{3+}	2.0	4.0	10.0	20.0
Cu^{2+}	2.0	4.0	10.0	20.0
Ni^{2+}	4.0	8.0	20.0	40.0
Zn^{2+}	4.0	8.0	20.0	40.0
Co^{2+}	4.0	8.0	20.0	40.0
Mn^{2+}	4.0	8.0	20.0	40.0

Depending on the concentration of metal ions in the sample, the volume of the standards to be concentrated can be varied.

APPENDIX C: SAMPLE LOOP

The 1-mL and 5-mL sample loops are available and supplied with the SCM. If you wish to prepare the sample loop greater than 5.0 mL, use an appropriate length of 1/8-in. I.D. tubing.

To edit the program for a new sample loop, follow these steps:

1. Determine the loop loading time (L) starting from the sample source to the sample loop. If the autosampler is used, determine how much time the autosampler needs to complete the loading step. If the syringe is used, 2.0 minutes is appropiate. Be sure that the sample loop is completely filled. A minimum of 2.0 minutes is required for the first step ($L \geq 2.0$).

2. Determine the sample loading time (C) from sample loop to the MetPac CC-1 column. This value can be obtained by dividing the sample loop size (mL) by the sample pump flow rate (mL/min). For proper sample loading, an additional 1.0 minute is normally included.

   Example:
   Sample loop size = 7 mL
   Sample pump flow rate = 2.0 mL/min
   Sample loading time(C) =
   (7 mL/2.0 mL/min) + 1.0 min = 4.5 min

3. Enter the L and C values in the work sheet below. Calculate and enter the new time. Enter the new program into the AGP.

Table C1 Gradient Program Work Sheet

t(min) Enter new time	%E1	%E2	%E3	%E4	V5	V6	Flow Rate (mL/min)
0.0	0	100	0	0	1	0	3.0
L	0	100	0	0	0	1	2.0
L+C	0	100	0	0	1	0	3.0
L+C+2.0	0	100	0	0	1	0	1.2
L+C+2.1	50	0	50	0	1	1	1.2
L+C+7.0	50	0	50	0	1	1	1.2
L+C+7.1	0	0	0	100	1	1	2.0
L+C+8.0	0	0	0	100	0	0	3.0
L+C+10	0	0	0	100	0	0	3.0
L+C+10.1	0	0	100	0	1	0	4.0
L+C+11	0	0	100	0	1	0	4.0
L+C+12	0	0	100	0	1	0	4.0
L+C+13	0	100	0	0	1	0	0.0

*begin sample analysis

Column Selection Guide

Environmental Water Applications Notebook

Column Selection Guide

Silica Columns

			Reversed-Phase (RP)						Mixed-Mode			HILIC		Application-Specific						Example Applications
			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	
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.

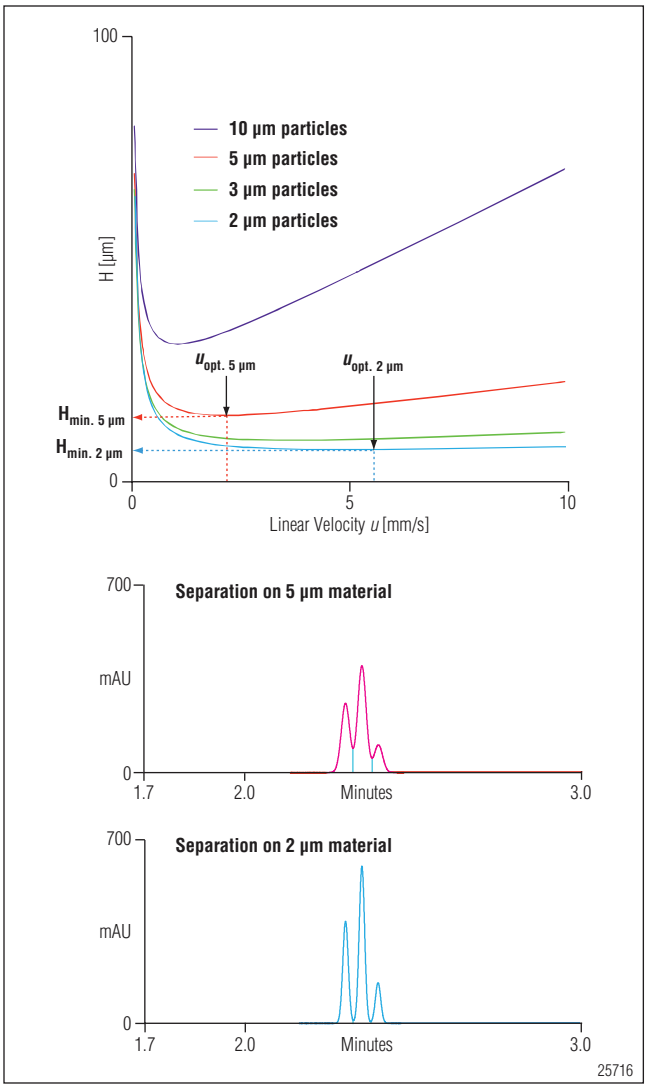

Figure 1. Smaller particles provide more theoretical plates and more resolution, demonstrated by the improved separation of three peaks (bottom) and smaller minimum plate heights H *in the Van Deemter plot (top). At linear velocities higher than uopt,* H *increases more slowly when using smaller particles, 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.

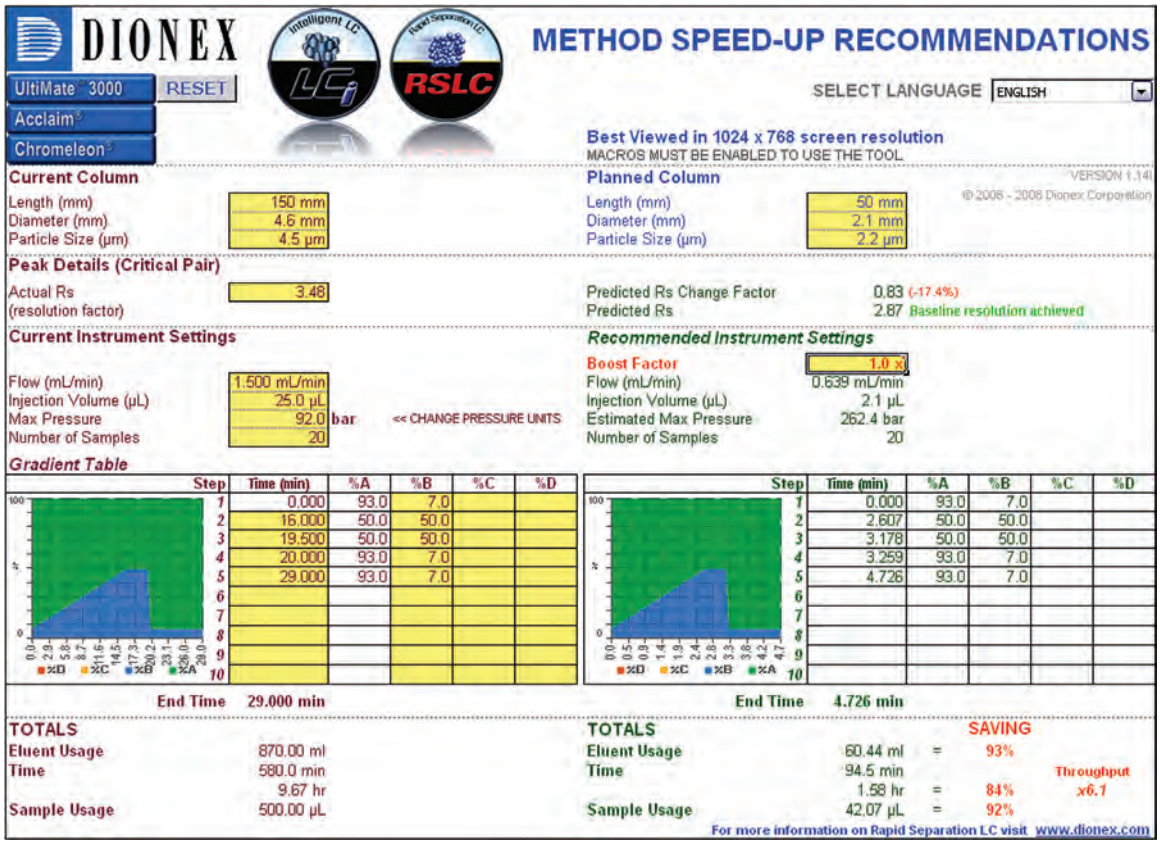

Figure 2. The Dionex Method Speed-Up Calculator transfers a conventional (current) HPLC method to a new (planned) RSLC method.

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.

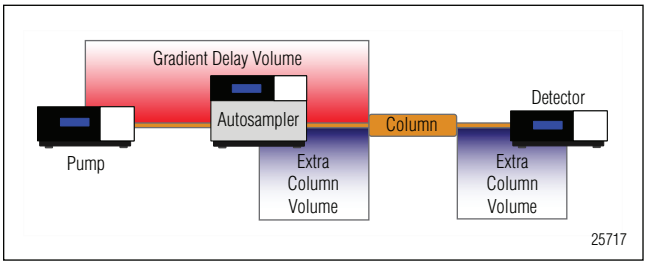

Figure 3. Gradient delay volume and extra column volume of an HPLC system. Both play an important role in method speed-up.

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

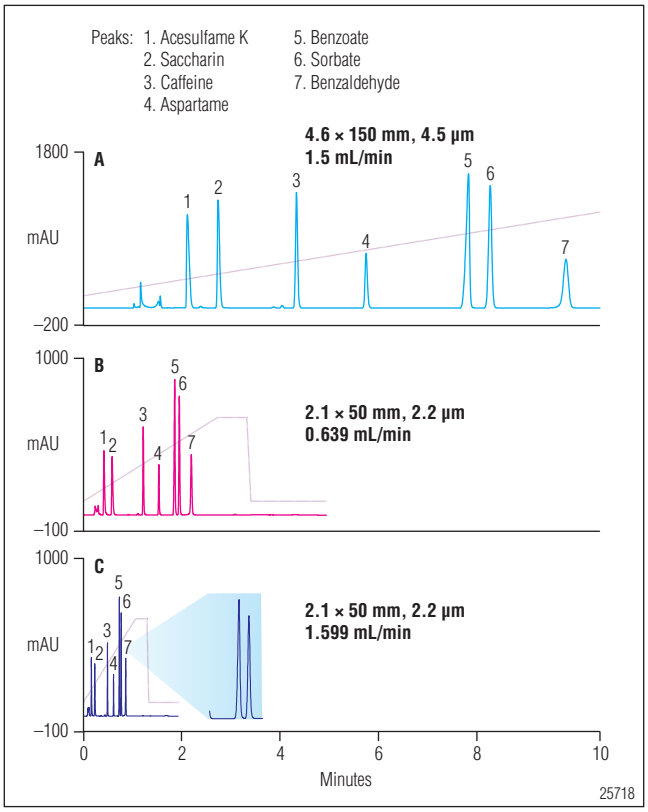

Figure 4. Method transfer with the Method Speed-Up Calculator from A) a conventional LC separation on an Acclaim 5 µm particle column, to B) and C) RSLC separations on an Acclaim 2.2 µm particle column.

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).

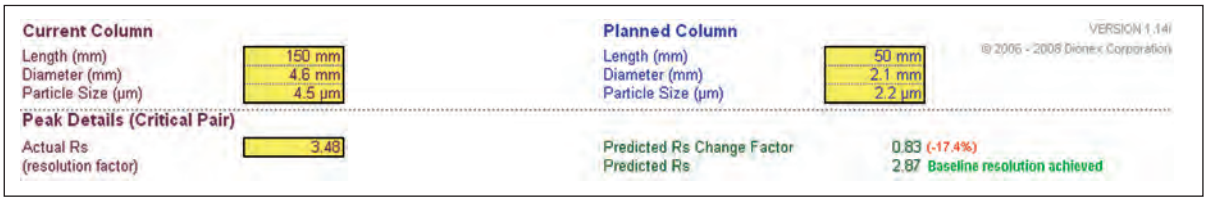

Figure 5. Column selection considering the resolution of the critical pair.

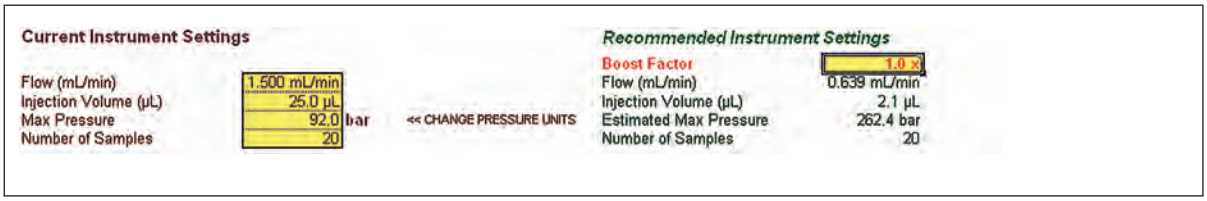

Figure 6. The flow rate, injection volume and backpressure of the current method are scaled to the new column dimension.

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).

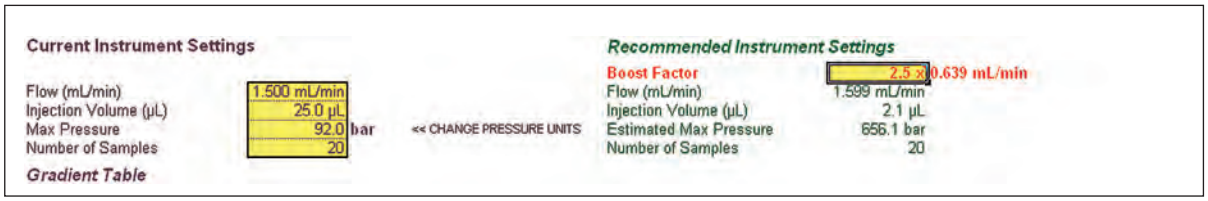

Figure 7. The new flow rate is further accelerated by applying the Boost Factor of 2.5.

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.

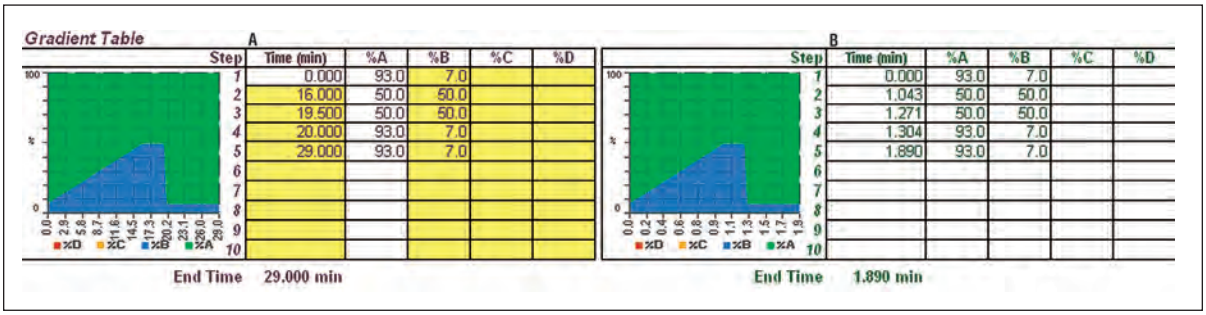

Figure 8. The gradient table of the current method (A) is adapted to the boosted method (B) according to the gradient-volume principle.

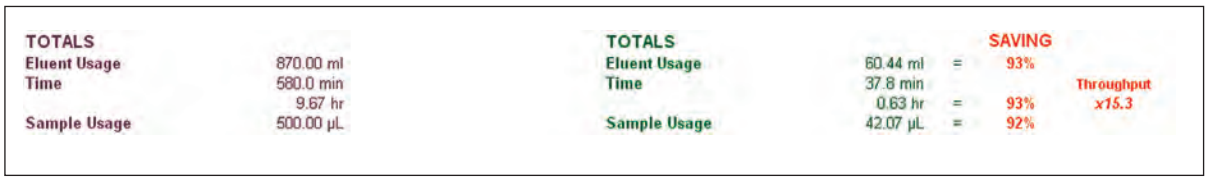

Figure 9. The absolute values for analysis time, eluent usage, and sample usage of the current (purple) and planned (green) method are calculated by the Method Speed-Up Calculator. The savings of eluent, sample, and time due to the method transfer are highlighted.

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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