# Beverages Applications Notebook

## Carbonated Beverages

Thermo SCIENTIFIC

# Table of Contents

# Index of Analytes

Thermo
SCIENTIFIC

# Introduction to Beverages

The global beverage industry is growing each year with the introduction of new products, such as vitamin-fortified water, energy drinks, anti-aging water, and herbal nutritional supplements. With this growth, come many more analytical challenges. These challenges are compounded by the continuing and new needs to analyze classic favorites such as sodas, fruit juices, milk drinks, alcoholic beverages, and bottled water. One such example would be the melamine contamination in milk and infant milk formula.

For all beverages, the compositional quality and safety must be monitored to help track contamination, adulteration, product consistency, and to ensure regulatory compliance from raw ingredients (water, additives, and fruits) to the final product.

Thermo Fisher Scientific is a recognized leader in providing analytical solutions for sample preparation, liquid chromatography for compositional testing, and chromatography data management for compliance and quality testing of beverages. From inorganic ions, organic acids, biogenic amines, glycols and alcohols, carbohydrates and sugar alcohols, to vitamins, additives, and sugar substitutes, we are unique in our commitment to provide fast, accurate testing and labeling information for all applications in this industry.

***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 liquid chromatography (LC), ion chromatography (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 lineup 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

# UltiMate 3000 UHPLC+ Systems

***Best-in-class HPLC systems for all your chromatography needs***

Thermo Scientific Dionex UltiMate 3000 UHPLC+ Systems provide excellent chromatographic performance while maintaining easy, reliable operation. The basic and standard analytical systems offer ultra HPLC (UHPLC) compatibility across all modules, ensuring maximum performance for all users and all laboratories. Covering flow rates from 20 nL/min to 10 mL/min with an industry-leading range of pumping, sampling, and detection modules, UltiMate™ 3000 UHPLC+ Systems provide solutions from nano to semipreparative, from conventional LC to UHPLC.

- Superior chromatographic performance
- UHPLC design philosophy throughout nano, standard analytical, and rapid separation liquid chromotography (RSLC)
- 620 bar (9,000 psi) and 100 Hz data rate set a new benchmark for basic and standard analytical systems
- RSLC systems go up to 1000 bar and data rates up to 200 Hz
- ×2 Dual System for increased productivity solutions in routine analysis
- Fully UHPLC compatible advanced chromatographic techniques
- Thermo Scientific Dionex Viper and nanoViper–the first truly universal, fingertight fitting system even at UHPLC pressures

Thermo Fisher Scientific is the only HPLC company uniquely focused on making UHPLC technology available to all users, all laboratories, and for all analytes.

*Rapid Separation LC Systems:* The extended flow-pressure footprint of the RSLC system provides the performance for ultrafast high-resolution and conventional LC applications.

*RSLCnano Systems:* The Rapid Separation nano LC System (RSLCnano) provides the power for high-resolution and fast chromatography in nano, capillary, and micro LC.

*Standard LC Systems:* Choose from a wide variety of standard LC systems for demanding LC applications at nano, capillary, micro, analytical, and semipreparative flow rates.

*Basic LC Systems:* UltiMate 3000 Basic LC Systems are UHPLC compatible and provide reliable, high-performance solutions to fit your bench space and your budget.

# IC and RFIC Systems

***A complete range of ion chromatography solutions for all customer performance and price requirements***

For ion analysis, nothing compares to a Thermo Fisher Scientific ion chromatography system. Whether you have just a few samples or a heavy workload, whether your analytical task is simple or challenging, we have a solution to match your needs and budget. And with your IC purchase, you get more than just an instrument—you get a complete solution based on modern technology and world-class support.

- Thermo Scientific Dionex ICS-5000: The world's first capillary IC system
- Dionex ICS-2100: Award-winning integrated Reagent-Free™ IC system
- Dionex ICS-1600: Standard integrated IC system
- Dionex ICS-1100: Basic integrated IC system
- Dionex ICS-900: Starter line IC system

Ranging from the Dionex ICS-900 to the ICS-5000, these IC systems cover the entire range of IC needs and budgets and come with superior support and service worldwide.

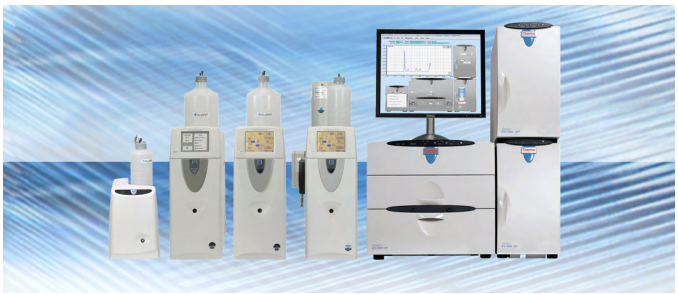
*Dionex ICS-5000:* Developed with flexibility, modularity, and ease-of-use in mind, the Dionex ICS-5000 combines the highest sensitivity with convenience

*Dionex ICS-2100:* An integrated Reagent-Free IC (RFIC™) system for electrolytically generated isocratic and gradient separations with conductivity detection, now with electrolytic sample preparation.

*Dionex ICS-1600:* The Dionex ICS-1600 combines high sensitivity with convenience. Now ready for eluent regeneration, with available dual-valve configuration for automated sample preparation.

*Dionex ICS-1100:* With dual-piston pumping and electrolytic suppression. Now ready for eluent regeneration, with available dual-valve configuration for automated sample preparation.

*Dionex ICS-900:* Can routinely analyze multiple anions and cations in 10–15 min—fully automated with Displacement Chemical Regeneration (DCR).



# MS Instruments

***Single-point control and automation for improved ease-of-use in LC/MS and IC/MS***

Thermo Fisher Scientific provides advanced integrated IC/MS and LC/MS solutions with superior ease-of-use and modest price and space requirements. UltiMate 3000 System Wellness technology and automatic MS calibration allow continuous operation with minimal maintenance. The Dionex ICS-5000 instrument and the family of RFIC systems automatically remove mobile phase ions for effort-free transition to MS detection.

- Thermo Scientific MSQ Plus mass spectrometer, the smallest and most sensitive single quadrupole on the market for LC and IC
- Self-cleaning ion source for low-maintenance operation
- Thermo Scientific Dionex Chromeleon Chromatography Data System software for single-point method setup, instrument control, and data management
- Compatible with existing IC and LC methods
- The complete system includes the MSQ Plus™ mass spectrometer, PC datasystem, electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) probe inlets, and vaccum system

You no longer need two software packages to operate your LC/MS system. Chromeleon™ LC/MS software provides single-software method setup and instrument control; powerful UV, conductivity, and MS data analysis; and fully integrated reporting.

*MS Systems and Modules:* MSQ Plus Mass Spectrometer; MSQ18LA nitrogen gas generator; Thermo Scientific Dionex AXP-MS digital auxiliary pump

Thermo SCIENTIFIC

# Chromeleon 7 Chromatography Data System Software

***The fastest way to get from samples to results***

Discover Chromeleon software version 7, the chromatography software that streamlines your path from samples to results. Get rich, intelligent functionality and outstanding usability at the same time with Chromeleon software version 7—the Simply Intelligent™ chromatography software.

- Enjoy a modern, intuitive user interface designed around the principle of operational simplicity
- Streamline laboratory processes and eliminate errors with eWorkflows, which enable anyone to perform a complete analysis perfectly with just a few clicks
- Access your instruments, data, and eWorkflows instantly in the Chromeleon Console
- Locate and collate results quickly and easily using powerful built-in database query features
- Interpret multiple chromatograms at a glance using MiniPlots
- Find everything you need to view, analyze, and report data in the Chromatography Studio
- Accelerate analyses and learn more from your data through dynamic, interactive displays
- Deliver customized reports using the built-in Excel-compatible speadsheet

Chromeleon software version 7 is a forward-looking solution to your long-term chromatography data needs. It is developed using the most modern software tools and technologies, and innovative features will continue to be added for many years to come.

The Cobra™ integration wizard uses an advanced mathematical algorithm to define peaks. This ensures that noise and shifting baselines are no longer a challenge in difficult chromatograms. When peaks are not fully resolved, the SmartPeaks™ integration assistant visually displays integration options. Once a treatment is selected, the appropriate parameters are automatically included in the processing method.

Chromeleon software version 7 ensures data integrity and reliability with a suite of compliance tools. Compliance tools provide sophisticated user management, protected database stuctures, and a detailed interactive audit trail and versioning system.

# Process Analytical Systems and Software

***Improve your process by improving your process monitoring with a Thermo Scientific Dionex on-line IC or HPLC system***

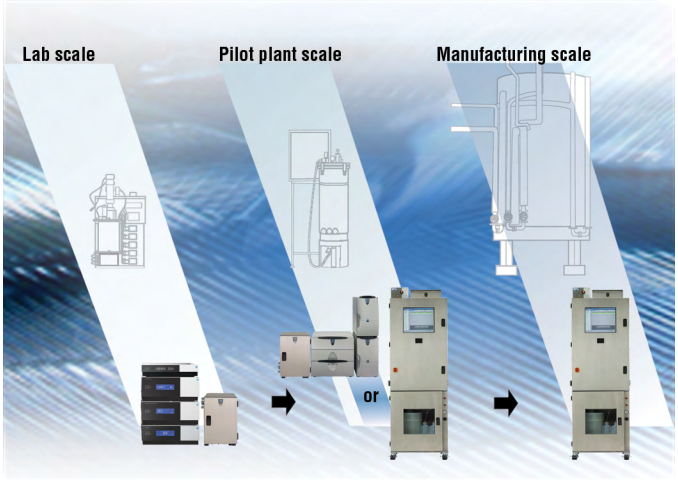
Our process analytical systems provide timely results by moving liquid chromatography-based measurements on-line. Information from the Thermo Scientific Dionex Integral process analyzer can help reduce process variability, improve efficiency, and reduce downtime. These systems provide comprehensive, precise, accurate information faster than is possible with laboratory-based results. From the lab to the factory floor, your plant's performance will benefit from the information provided by on-line LC.

- Characterize your samples completely with multicomponent analysis
- Reduce sample collection time and resources with automated multipoint sampling
- Improve your process control with more timely results
- See more analytes with unique detection capabilities
- 25 years of experience providing on-line IC and HPLC capabilities to a wide range of industries
- The Thermo Scientific Integral Migration Path approach lets you choose the systems that best meets your needs

The Integral Migration Path™ approach enables on-line IC/HPLC to generate timely, high-resolution information when monitoring a small-scale reactor in a process R&D lab, in a pilot plant, or improving current manufacturing plant processes. No matter what the application, the Integral™ process analyzer has the versatility to place a solution using on-line IC/HPLC, whenever and wherever it is needed.

*Integral:* The Integral Migration Path approach: System solutions wherever you need them: lab, pilot plant, or manufacturing

*Chromeleon Process Analytical (PA) Software:* Chromeleon PA software provides unique capabilities to support on-line IC or HPLC analysis



# Automated Sample Preparation

## ACCELERATED SOLVENT EXTRACTORS

***Two new solvent extraction systems with pH-hardened Dionium components***

We offer two solvent extraction systems. The Thermo Scientific Dionex ASE 150 Accelerated Solvent Extractor is an entry-level system with a single extraction cell, for laboratories with modest throughput. The Dionex ASE™ 350 system is a sequential extraction system capable of automated extraction of up to 24 samples. Both systems feature chemically inert Dionium components that allow the extraction of acid- or base-pretreated samples.

Thermo
SCIENTIFIC

## SOLID-PHASE EXTRACTION SYSTEMS

***Faster, more reliable solid-phase extraction while using less solvent***

The Thermo Scientific Dionex AutoTrace 280 Solid-Phase Extraction (SPE) instrument unit can process six samples simultaneously with minimal intervention. The instrument uses powerful pumps and positive pressure with constant flow-rate technology. Current analytical methods that require SPE sample preparation include gas chromatography (GC), GC-MS, LC, and LC-MS, IC and IC-MS. The Dionex AutoTrace™ 280 instrument is approved or adapted for U.S. EPA clean water methods and safe drinking water methods (600 and 500 series) and can extract the following analytes:

- PCBs (polychlorinated biphenyls)
- OPPs (organophosphorus pesticides), OCPs (organochlorine pesticides), and chlorinated herbicides
- BNAs (base, neutral, acid semivolatiles)
- Dioxins and furans
- PAHs (polyaromatic hydrocarbons)
- Oil and grease or hexane extractable material

With SPE, large volumes of liquid sample are passed through the system and the compounds of interest are trapped on SPE adsorbents (cartridge or disk format), then eluted with strong solvents to generate an extract ready for analysis. Automated SPE saves time, solvent, and labor for analytical laboratories.

*Dionex AutoTrace Systems:* The new Dionex AutoTrace 280 system provides fast and reliable automated solid phase extraction for organic pollutants from liquid samples

*Dionex AutoTrace Accessories:* High-quality parts and accessories are available for Dionex AutoTrace 280 instruments

Thermo
SCIENTIFIC

# Analysis of Carbonated Beverages

# Determination of Inorganic Ions and Organic Acids in Non-Alcoholic Carbonated Beverages

## INTRODUCTION

The determination of inorganic anions and cations and organic acids in non-alcoholic carbonated beverages is of importance from both health-related and manufacturing perspectives. Organic acids such as citrate and malate, and inorganic anions such as phosphate are monitored due to their function as acidifiers or flavor enhancers. Chloride is monitored due to restrictions imposed by different countries and many Group I and II metals are monitored for purposes of mass balance. Thus, the content of these compounds needs to be monitored by the manufacturer to maintain product quality and to investigate possible patent infringements in competitive products.

Ion chromatography (IC) is a well established technique for the determination of ions in solution. This application note describes the use of ion exchange or ion exclusion chromatography (ICE) with suppressed conductivity detection for the determination of inorganic anions, cations, and organic acids in several popular carbonated beverages.

## EQUIPMENT

A Dionex chromatographic system consisting of:
- Gradient Pump
- Chromatography Module
- Conductivity Detector
- Eluent Organizer or Eluent Degas Module
- Autosampler

Dionex PeakNet Chromatography Workstation

## REAGENTS AND STANDARDS

Deionized water (DI $H_2O$), 17.8 MΩ-cm or better

### Anion Analysis

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

Methanol (EM Science)

### Cation Analysis

Methanesulfonic acid, >99% pure (Fluka Chemika-BioChemika)

### Organic Acid Analysis

Perchloric acid (Fluka Chemika-BioChemika)

Tetrabutylammonium hydroxide (Dionex, P/N 39602)

## PREPARATION OF SOLUTIONS AND REAGENTS

### 100 mM Sodium Hydroxide

Weigh 992 g (992 mL) of 17.8 MΩ deionized water into a 1-L eluent reservoir bottle. Vacuum degas the water for approximately 10 minutes. Tare the bottle on the balance and add 8.00 g (5.25 mL) of 50% sodium hydroxide directly to the bottle. Quickly transfer the eluent bottle to the instrument and pressurize it with helium.

### 1 mM Sodium Hydroxide

Place 990 g (990 mL) of 17.8 MΩ deionized water into a 1-L eluent reservoir bottle. Vacuum degas the water for approximately 10 minutes. Add 10 mL of 100 mM sodium hydroxide directly to the bottle. Quickly transfer the eluent bottle to the instrument and pressurize it with helium.

### 100 mM Methanesulfonic Acid

Weigh out 9.61 g of methanesulfonic acid (MSA). Carefully add this amount to a 1-L volumetric flask containing about 500 mL of deionized water. Dilute to the mark and mix thoroughly.

### 0.8 mM Perfluorobutyric Acid

Perfluorobutyric acid (heptafluorobutyric acid) is supplied by Fluka in 10.0-mL bottles. Dilute the entire contents of one 10.0-mL bottle in 1 L to obtain a 0.0772 M stock solution. Dilute 10.4 g of the stock solution in 1 L to obtain the 0.8 mM working eluent.

### 5 mM Tetrabutylammonium Hydroxide

Dilute 200 mL of the Dionex 0.1 M tetrabutylammonium hydroxide regenerant solution (P/N 39602) to 4 L with water. Alternatively, dilute 10 mL of 55% tetrabutylammonium hydroxide in 4 L of water.

## *RESULTS AND DISCUSSION*

### Inorganic Anions

Inorganic anions such as chloride, nitrate, and sulfate present in carbonated beverages are usually derived from the water used in production. Some anions, however, such as phosphate may be added deliberately to impart a particular flavor or acidity. The  water can be monitored by ion chromatography to ensure purity and consistency, while the final product is monitored to maintain product quality.

Inorganic anions are separated by anion-exchange chromatography, and monitored by suppressed conductivity detection; Table 1 lists the experimental conditions. When performing gradient elution on the AS11 column, a hydroxide eluent system is used instead of a carbonate eluent, because of its lower background conductivity. An Anion Trap Column (ATC) should be installed between the gradient pump and the injection valve to minimize baseline shifts resulting from the elution of anionic contaminants in the eluent.

Figures 1–4 show the separations of inorganic and organic anions in a variety of carbonated beverages using the AS11 column. The samples were degassed and diluted 1:10 prior to injection. The sodium hydroxide concentration in eluent 1 is weak enough that not only is fluoride eluted after the void, but several weakly retained monovalent organic acids are also resolved. Thus, using the conditions described in Table 1, it is possible to separate not only the strong acid anions, but also a variety of weak organic acids. To obtain a flat baseline for these chromatograms, the baseline subtraction option in the PeakNet software was used.

Figures 1, 2, and 4 show that phosphate or citrate was used to acidify the beverages. Figure 3, which shows the separation of anions in a flavored carbonated water, naturally has no phosphate or citrate. All four beverages contain chloride and sulfate, with all but the water also containing some nitrate. A small amount of fluoride,

**Table 1 Experimental Conditions for the Separation of Inorganic Anions in Carbonated Beverages Using the IonPac AS11 Column**

| | | | | | |
|---|---|---|---|---|---|
| Column: | IonPac AS11 Analytical (4 mm)<br>IonPac AG11 Guard (4 mm)<br>ATC-1 Anion trap | | | | |
| Eluent 1: | Deionized water | | | | |
| Eluent 2: | 1 mM Sodium hydroxide | | | | |
| Eluent 3: | 100 mM Sodium hydroxide | | | | |
| Eluent 4: | Methanol | | | | |
| Gradient: | Time | E1 | E2 | E3 | E4 |
| | Initial | 80 | 20 | — | — |
| | 0.00 | 80 | 20 | — | — |
| | 5.00 | 66 | 20 | — | 14 |
| | 18.00 | 42 | — | 38 | 20 |
| Flow Rate: | 2 mL/min | | | | |
| Inj. Volume: | 25 µL | | | | |
| Detection: | Suppressed conductivity, ASRS, external water mode | | | | |

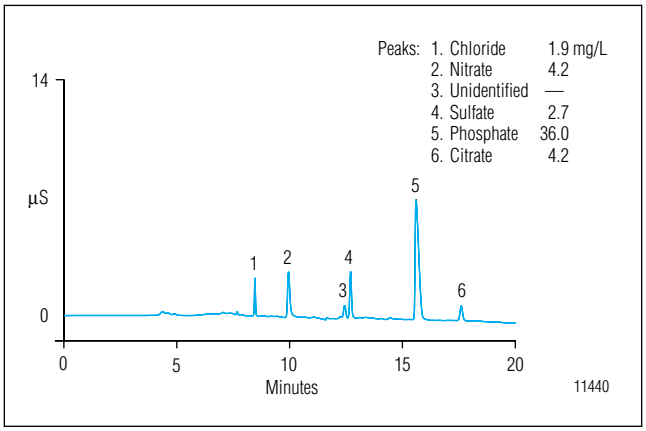



*Figure 1. Separation of inorganic anions and organic acids in a cola by anion exchange chromatography. Conditions as listed in Table 1.*

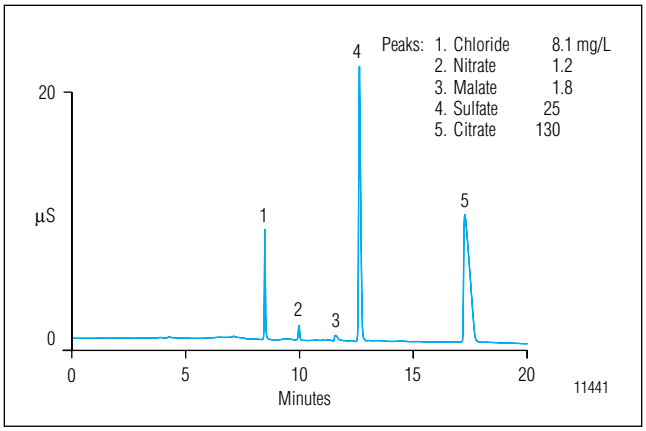



*Figure 2. Separation of inorganic anions and organic acids in a carbonated lemon drink by anion exchange chromatography. Conditions as listed in Table 1.*

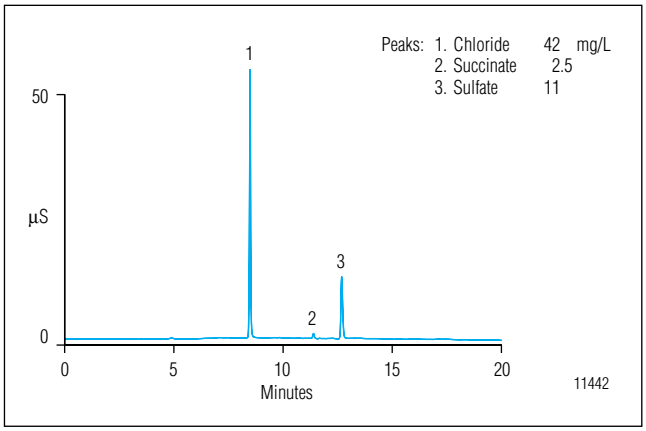



*Figure 3. Separation of inorganic anions and organic acids in a flavored carbonated water by anion exchange chromatography. Conditions as listed in Table 1.*

which is sometimes added to municipal water supplies to prevent tooth decay, is also found in the synthetic grape flavored beverage.

Reproducibility for this method is on the order of 0.5% or better for retention times and 2% or better for peak areas. Linearity is good over the range tested (1.5 orders of magnitude), with a coefficient of determination, $r^2 = 0.999$ for most of the analytes. These statistics were determined prior to baseline subtraction.

### Inorganic Cations

As is the case with the inorganic anions, many inorganic cations are introduced into carbonated beverages from the water. Others are introduced as counterions to added ingredients. The four major cations in carbonated beverages are sodium, potassium, calcium, and magnesium.

Inorganic cations can be separated by ion exchange chromatography, and monitored by suppressed conductivity detection, as described in Table 2. The step gradient allows the separation of barium and strontium in addition to the standard 5 cations, sodium, ammonium, potassium, magnesium, and calcium. A step change at 5 minutes from the weak eluent to a stronger one allows for the elution of sharp peaks for the divalent cations. If it is not necessary to monitor for barium or strontium, the conditions can be changed to allow isocratic elution of the 5 cations shown in Figure 7, in less than 10 minutes. For isocratic elution, the eluent is 20 mM methane-sulfonic acid.

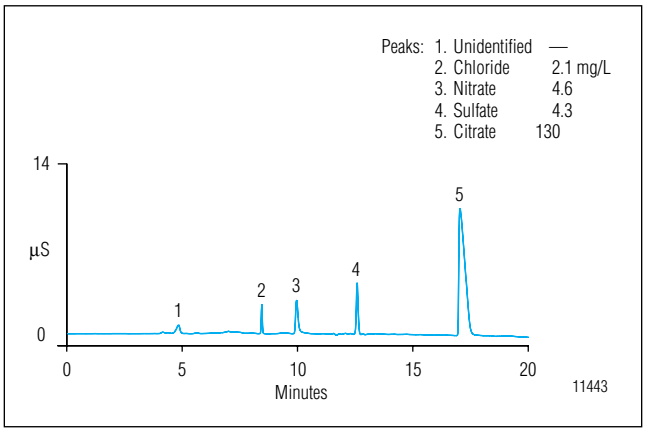



*Figure 4. Separation of inorganic anions and organic acids in a carbonated synthetic grape drink by anion exchange chro-matography. Conditions as listed in Table 1.*

**Table 2 Experimental Conditions for the Separation of Inorganic Cations in Carbonated Beverages Using the IonPac CS12 Column**

| | | | |
|---|---|---|---|
| Column: | IonPac CS12 Analytical (4 mm) | | |
| | IonPac CG12 Guard (4 mm) | | |
| | CTC-1 Cation trap | | |
| Eluent 1: | Deionized water | | |
| Eluent 2: | 100 mM Methanesulfonic acid | | |
| Gradient: | Time | E1 | E2 |
| | Initial | 84 | 16 |
| | 5.00 | 84 | 16 |
| | 5.01 | 60 | 40 |
| | 10.00 | 60 | 40 |
| Flow Rate: | 1.0 mL/min | | |
| Inj. Volume: | 25 µL | | |
| Detection: | Suppressed conductivity, CSRS, recycle mode | | |

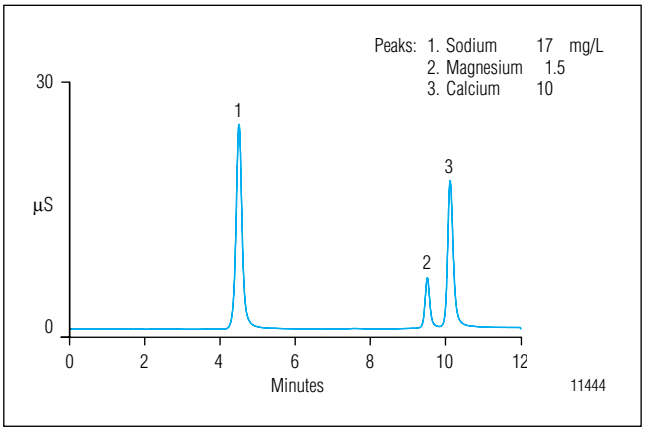



*Figure 5. Separation of inorganic cations in a carbonated lemon drink by cation exchange chromatography. Conditions as listed in Table 2.*

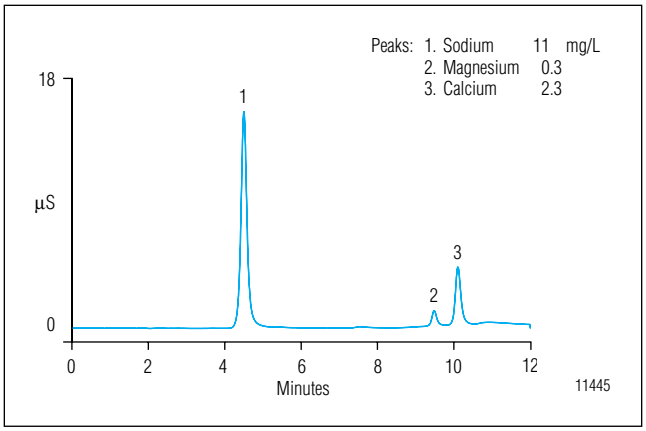



*Figure 6. Separation of inorganic cations in a carbonated, synthetic grape drink by cation exchange chromatography. Conditions as listed in Table 2.*

Figures 5–8 show the separation of cations in a series of carbonated beverages by cation exchange chromatography. The samples were degassed and diluted 1:10 prior to injection. The two synthetic beverages shown in Figures 5 and 6 contain only sodium, magnesium, and calcium, but the two carbonated juices shown in Figures 7 and 8 also contain a considerable quantity of potassium. The reproducibility of this method is on the order of 0.5% or better for retention times and 2% or better for peak areas. Linearity was good over the range tested (2 orders of magnitude) with a coefficient of determination, $r^2 = 0.999$ or better for all but ammonium.

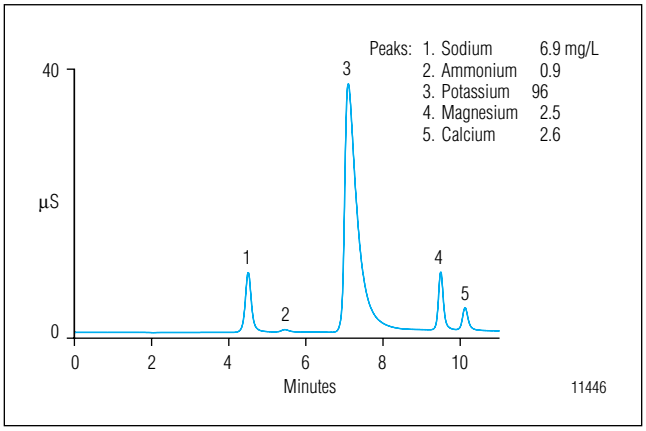



*Figure 7. Separation of inorganic cations in a carbonated apple juice by cation exchange chromatography. Conditions as listed in Table 2.*

## Organic Acid Analysis

Organic acids such as citrate or malate are often introduced into carbonated beverages in definite proportions to impart a particular flavor. For carbonated fruit juice beverages, some organic acids may be present naturally in the fruit. In addition, the presence of some organic acids can be used to reveal potential food adulteration.

One way to separate and detect organic acids is with ion exclusion chromatography using suppressed conductivity detection. The IonPac® ICE-AS6 column is an ion exclusion column designed for efficient separation of low molecular weight aliphatic organic acids including hydroxy-substituted organic acids, as well as for aliphatic alcohols and glycols. Using this separation mechanism, weakly ionized acids are separated based on differences in their $pK_a$s. Strong inorganic acid anions are not retained by the stationary phase and elute in the excluded volume of the column. The standard eluent for use with

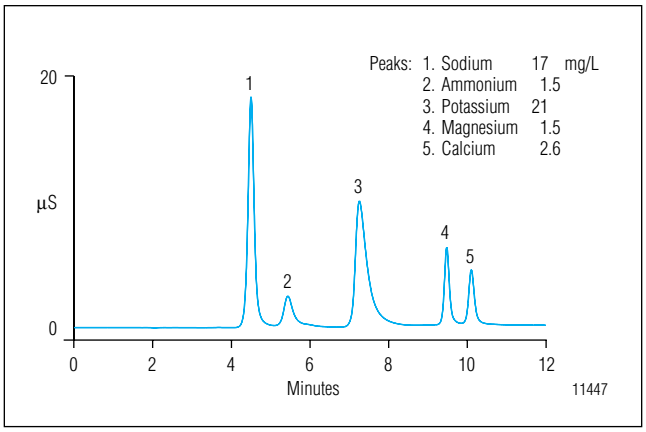



*Figure 8. Separation of inorganic cations in a carbonated grape juice by cation exchange chromatography. Conditions as listed in Table 2.*

**Table 3 Experimental Conditions for the Separation of Organic Acids in Carbonated Beverages by Ion Exclusion Chromatography Using the IonPac ICE-AS6 Column**

| | |
|---|---|
| Column: | IonPac ICE-AS6 Analytical |
| Eluent: | 0.8 mM Heptafluorobutyric acid |
| Flow Rate: | 1.0 mL/min |
| Inj. Volume: | 25 µL |
| Detection: | Suppressed conductivity, AMMS-ICE |
| Regenerant: | 5 mN Tetrabutylammonium hydroxide at 5 mL/min |

the IonPac ICE-AS6 is 0.4 mM heptafluorobutyric acid (perfluorobutyric acid). Although other monoprotic acids can be used as eluents, to do so will increase both the background conductivity and the noise. The experimental conditions are listed in Table 3.

Figures 9–12 show the separation of organic acids in a group of carbonated beverages by ion exclusion chromatography. The samples were degassed and diluted 1:10 (1:50 for the carbonated grape juice) prior to injection. Inorganic anions are eluted on either side of the water dip and do not interfere with the separation of most of the organic acids. The major exception is with oxalate, which is also eluted at the water dip. Figure 9 shows the organic acid profile in a flavored carbonated water, which contains no organic acids as expected. Figures 10 and 11 show the organic acid profiles in two synthetic carbon-ated beverages with only citrate being readily apparent. Citrate is often added to carbonated beverages to impart a certain acidity, but is also present in citric fruit; thus, explaining the higher concentration of citrate in the carbonated lemon drink compared to the cola. Figure 12 shows the organic acid profile in a carbonated apple juice. There are many different organic acids present naturally in apples, malate being particularly prevalent.

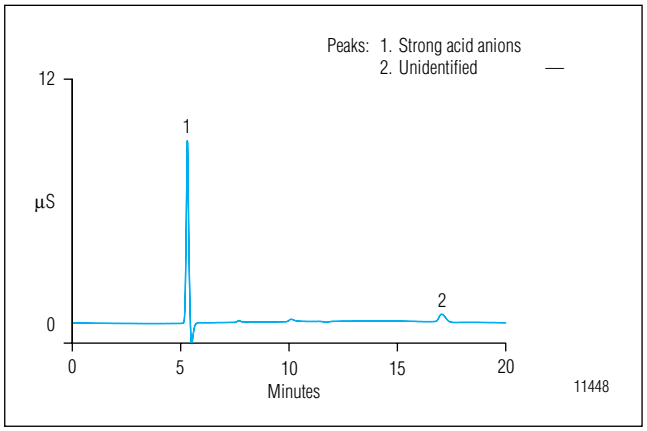



*Figure 9. Anionic profile of a flavored carbonated water by ion exclusion chromatography. Conditions as listed in Table 3.*

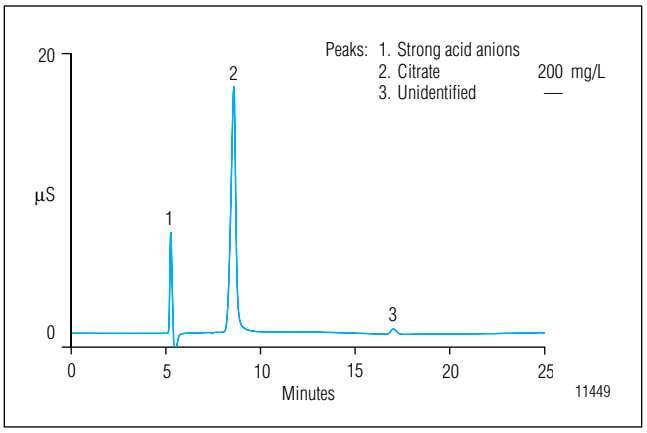



*Figure 10. Separation of organic acids in a carbonated lemon drink by ion exclusion chromatography. Conditions as listed in Table 3.*

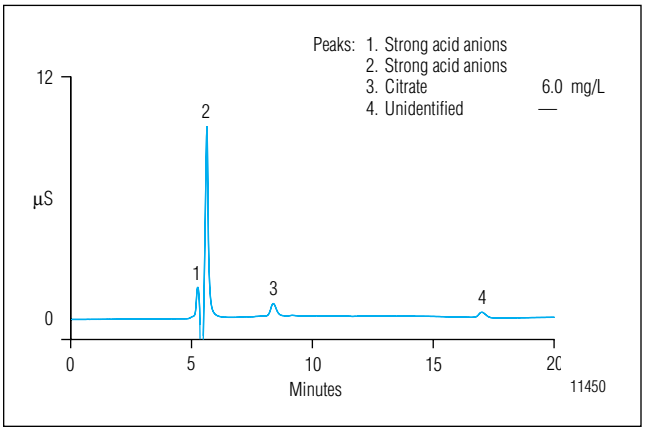



*Figure 11. Separation of organic acids in a cola by ion exclusion chromatography. Conditions as listed in Table 3.*

## CONCLUSION

IC has been applied successfully to the analysis of carbonated beverages for a variety of inorganic and organic components. Total analysis time is approximately 20 minutes for ICE analysis, 30 minutes for anion analysis, and 16 minutes for cation analysis due to the need for column reequilibration. Minimal sample pre-paration is required. The three methods described in this application note provide a rapid and convenient means to obtain complete profiles of the ionic components in carbonated beverages.

## LIST OF SUPPLIERS

EM Science, P.O. Box 70, 480 Democrat Road, Gibbstown, New Jersey, 08027, USA, 1-800-222-0342.

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

Fluka Chemika-BioChemika, Fluka Chemie AG, Industriestrasse 25, CH-9471 Buchs, Switzerland, +81 755 25 11.

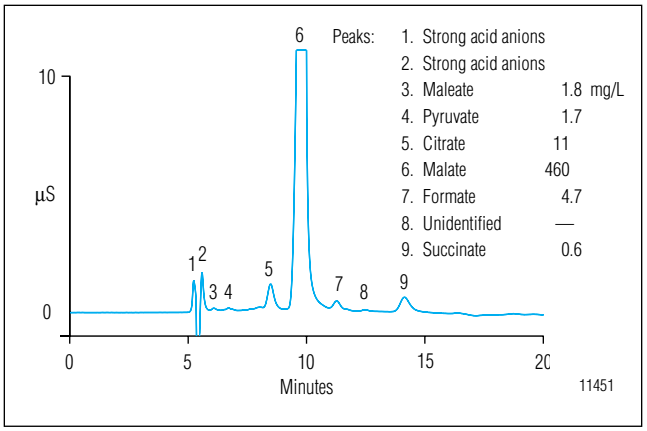



*Figure 12. Separation of organic acids in a carbonated apple juice by ion exclusion chromatography. Conditions as listed in Table 3.*

# Rapid Determination of Phosphate and Citrate in Carbonated Soft Drinks Using a Reagent-Free™ Ion Chromatography System

## INTRODUCTION

Soft drinks are complex mixtures containing a variety of substances such as coloring compounds, flavoring agents, acidifiers, sweeteners, preservatives, and caffeine. Acidulants reduce the soft drink's pH and thereby assist in beverage preservation for long-term storage. Acidulants can also be used as chelating agents, buffers, coagulators, and flavoring agents. In the latter role, the acidulant imparts a tart taste.[1] The most common acidulants used in soft drinks are phosphoric and citric acids. Phosphoric acid is more effective in lowering the pH than organic acids, while citric acid produces a stronger tartness.[1] Phosphoric acid is commonly found in colas whereas citric acid is typically added to fruit flavored beverages. However, these acids may be used alone or blended together to produce a more distinctive taste.

The National Soft Drink Association estimates that a modern bottling facility can produce as many as 2,000 cans of soft drinks per minute on each line of operation.[2] This results in the production of nearly three million cans of soft drinks per day. To maintain product consistency and quality, it is critical that an accurate amount of acidulant is used for each production line and bottling facility. This requires a rapid, accurate, and rugged analytical method to confirm that an appropriate amount of phosphoric and/or citric acid has been added to the soft drink formulation. Traditionally, analysts perform labor-intensive colorimetric assays to quantify the amount of phosphoric acid in soft drinks. These methodologies require highly skilled operators, are time-consuming, and generally poor in precision and accuracy. A separate chromatographic assay is required to determine the amount of citric acid added to the beverage. Accurate and reproducible preparations of the mobile phase are essential for this assay to produce consistent citric acid retention times.

Ion chromatography (IC) can simultaneously determine phosphoric and citric acids in soft drinks by measuring the corresponding anions, phosphate, and citrate. The introduction of Reagent-Free IC (RFIC™) systems has significantly improved the automation and ease-of-use of IC compared to other available methodologies. RFIC systems combine electrolytic generation of a potassium hydroxide eluent from deionized water, an electrolytic continuously regenerated trap column, and electrolytic suppression. The ability to accurately program the exact eluent concentration through the software has allowed analysts to streamline the development process of many analytical methods, improve the method's precision, and provide better reproducibility between laboratories and operators. Because this application requires rapid determination of the polyvalent anions phosphate and citrate, a new hydroxide-selective column—the IonPac® Fast Anion III—was developed. This application note presents a new RFIC method for the simultaneous determination of phosphate and citrate in carbonated soft drinks using the hydroxide-selective Fast Anion III column.

## EQUIPMENT

A Dionex ICS-2000 Reagent-Free Ion Chromatography (RFIC)* System was used for this work. The ICS-2000 is an integrated ion chromatograph and consists of:

Eluent Generator

Column Heater

Pump with Degasser

EluGen® EGC-KOH Cartridge (Dionex P/N 058900)

CR-ATC (Dionex P/N 060477)

AS50 Autosampler

Chromeleon® 6.6 Chromatography Workstation

## REAGENTS AND STANDARDS

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

Sodium citrate dihydrate (Sigma-Aldrich)

Phosphate standard 1000 mg/L, 100 mL (Ultra Scientific, VWR P/N ICC-005)

## CONDITIONS

| | |
|---|---|
| Column: | IonPac Fast Anion III Analytical, 3 × 250 mm (Dionex P/N 062982)** |
| Eluent: | 20 mM potassium hydroxide |
| Eluent Source: | ICS-2000 EG with CR-ATC |
| Flow Rate: | 1.0 mL/min |
| Temperature: | 30 °C |
| Inj. Volume: | 1.2 µL |
| Detection: | Suppressed conductivity, ASRS® ULTRA II, 2 mm (Dionex P/N 061562), Recycle mode, 70 mA |
| Background Conductance: | <1 µS |
| System Backpressure: | ~2300 psi |
| Run Time: | 5 min (6 min injection-to-injection) |

*This application note is also applicable to other RFIC systems.

**Note: The guard column was eliminated for this application to increase the analysis speed. Adding the guard column will increase the run time by approximately 6%.

### Recommended Chromeleon Integration Parameters

We recommend using the following integration parameters as a starting point for the proper integration of phoshate and citrate (if present) peaks.

| Time | Parameter | Parameter Value |
|---|---|---|
| 0.000 | Minimum area | 5.0 [Signal]*min |
| 2.500 | Minimum area | 0.03 [Signal]*min |
| 4.800 | Minimum area | 0.001 [Signal]*min |

## PREPARATION OF SOLUTIONS AND REAGENTS

### Citrate Stock Standard Solution

To prepare a 1000-mg/L citrate stock standard, weigh 0.156 g sodium citrate dihydrate, add to a 100-mL volumetric flask, and dilute to volume with deionized (DI) water.

### Calibration Standard Solutions

Prepare calibration standards for phosphate and citrate by using appropriate dilutions of the 1000-mg/L stock solutions. In this application note, phosphate was prepared at 250, 500, and 750 mg/L and citrate was prepared at 25, 50, 100, and 200 mg/L. These concentrations are expected to cover the concentration range found in most soft drinks.

## SAMPLE PREPARATION

Thoroughly degas all carbonated beverages in an ultrasonic bath with a vacuum pump. The beverage should be sonicated without vacuum first to release most of the dissolved carbon dioxide prior to placing the beverage under vacuum. This prevents the beverage from being pulled into the vacuum pump. The entire process takes approximately 20 min. Samples are then injected directly without further preparation.

## SYSTEM PREPARATION AND SETUP

Install the EGC-KOH cartridge in the ICS-2000 and configure it with the Chromeleon Chromatography Management Software. Condition the cartridge as directed by the EGC II Quickstart (Document No. 031909) for 30 min with 50 mM KOH at 1 mL/min. Upon completing the cartridge conditioning process, disconnect the backpressure tubing temporarily installed in place of the column set. Install a CR-ATC between the EGC II KOH cartridge and EGC degas. For more information on installing the CR-ATC, consult the EGC II Cartridge Quickstart Guide.

Install and configure the AS50 autosampler. Install a 250-µL sample syringe and set the syringe speed to 4 or 5 to make faster sample injections. Enter the correct sample syringe volume and a sample loop size of 25 µL in the AS50 Plumbing Configuration Screen. A larger sample loop size than the actual injection loop volume of 1.2 µL was used in this application to assure that the sample loop was completely filled for each injection. In the Chromeleon system configuration window, set the AS50 to operate in the sample overlap mode to reduce the time between injections and therefore increase sample throughput. Refer to the AS50 Autosampler Operator's Manual (Document No. 31169) for further details on configuring the AS50.

Install a 3 × 250 mm IonPac Fast Anion III analytical column. Make sure that the system pressure displayed by the pump is at an optimal pressure of ~2300 psi when 20 mM KOH is delivered at 1.0 mL/min to allow the degas assembly to effectively remove hydrolysis gases from the eluent. If necessary, install additional backpressure tubing supplied with the ICS-2000 ship kit to adjust the pressure to 2300 ± 200 psi. Because the system pressure can rise over time, trim the backpressure coil as necessary to maintain a system pressure between 2100–2500 psi.

Prepare the ASRS ULTRA II for use by hydrating the suppressor. Use a disposable plastic syringe and push approximately 3 mL of degassed DI water through the "Eluent Out" port and 5 mL of degassed DI water through the "Regen In" port. Allow the suppressor to sit for approximately 20 min to fully hydrate the suppressor screens and membranes. Install the ASRS ULTRA II for use in the recycle mode according to the Installation and Troubleshooting instructions for the ASRS ULTRA II (Document No. 031956).

## RESULTS AND DISCUSSION

The Association of Official Analytical Chemists (AOAC) describes a standard method for the determination of orthophosphate by reacting ammonium molybdate and potassium antimonyl tartrate in an acidic medium with a dilute solution of phosphate to form the antimonyl phosphomolybdate complex.[3] Upon reduction with ascorbic acid, this complex forms an intense blue color that absorbs at a wavelength of 660 nm. This method can also be automated in a flow-injection mode to produce one-minute analysis times.[4] The AOAC indicates that the analytical range for this method is 0.06–3 mg/L orthophosphate. The assay described by the AOAC is complex, time-consuming, often poor in precision and accuracy, and requires significant sample dilutions if applied to the analysis of soft drinks. The reported standard deviation of the method for the determination of orthophosphate in water ranged from 6–34% with a bias of –6 to –1.8%, based on 26 analysts in 16 laboratories.[3]

IC with suppressed conductivity detection has been demonstrated to be the method of choice for the determination of anions, including citrate.[5] In addition, IC is capable of simultaneously determining phosphate and citrate that are present in many carbonated beverages. Phosphate can also be calibrated at higher concentrations than the colorimetric method, thereby eliminating sample dilutions and further simplifying the analysis. The use of IC combined with electrolytic generation of a potassium hydroxide eluent significantly improves the method by avoiding the off-line preparation of eluents and therefore produces better precision and accuracy between analysts and laboratories. Previously, we developed an RFIC method for determining phosphate and citrate in pharmaceutical formulations using the IonPac AS11 column.[6] The total run time for the analysis was 10 min using an electrolytically generated 20-mM potassium hydroxide eluent at 2 mL/min. Although this method resulted in good precision and accuracy, the analysis time and eluent consumption were greater than desired for this application. Because of the large number of soft drinks produced each day, a faster analysis was required to increase sample throughput for the determination of phosphate and citrate in beverages. Therefore, we developed a new hydroxide-selective column that was optimized for this application. We evaluated this column in terms of linearity, precision, and ruggedness for the determination of phosphate and citrate in carbonated soft drinks.

The IonPac Fast Anion III column is an important advancement in Dionex polymer-bonding technology that uses an anion-exchange resin with an optimized selectivity and capacity (55 µeq/column) for the rapid determination of phosphate and citrate. The Fast Anion III stationary phase consists of a novel hyper-branched anion-exchange condensation polymer that is electrostatically attached to the surface of a wide-pore polymeric substrate. The substrate is surface-sulfonated exactly the same as the Dionex latex-coated, anion-exchange materials; however, the resin of the Fast Anion III column contains alternating

| Table 1. Concentrations of Phosphate and Citrate Found in Different Cola Samples | | | | | | |
|---|---|---|---|---|---|---|
| | Citrate | | | Phosphate | | |
| Sample | Amount Found (mg/L) | Retention Time (RSD[a]) | Peak Area (RSD) | Amount Found (mg/L) | Retention Time (RSD[a]) | Peak Area (RSD) |
| Low Carb Cola A | 444 | 0.13 | 0.11 | 41.9 | 0.10 | 0.22 |
| Diet Cola A | 502 | 0.06 | 0.18 | 214 | 0.11 | 0.19 |
| Regular Cola A | 539 | 0.12 | 0.09 | — | — | — |
| Diet Cola B | 335 | 0.13 | 0.19 | 189 | 0.10 | 0.21 |
| Low Carb Cola B | 502 | 0.05 | 0.16 | 87.4 | 0.10 | 0.17 |

[a]RSD = relative standard deviation, n = 20

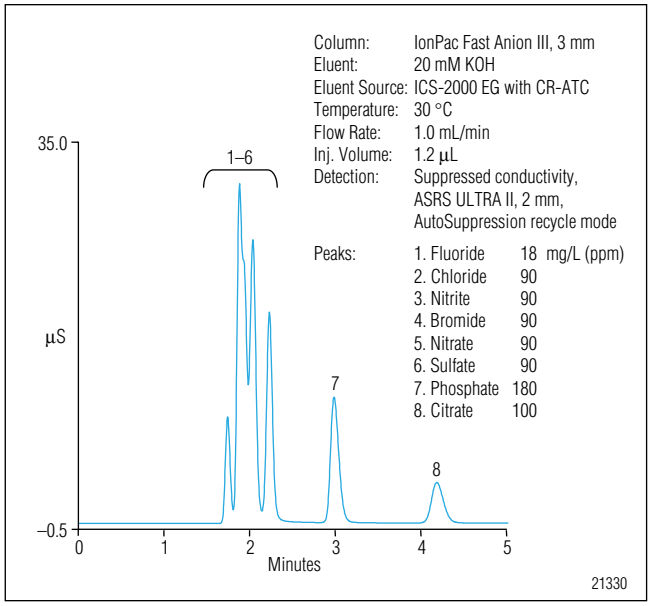



*Figure 1. Analysis of seven common anions and citrate on the IonPac Fast Anion III column.*

treatments of epoxy monomer and amines that produce a coating that grows directly off the substrate. The capacity of the resin is carefully controlled by the number of alternating coating cycles resulting in a polymer that is extremely hydrophilic and therefore has excellent selectivity for hydroxide eluents, allowing the use of lower eluent concentrations. Figure 1 shows an analysis of seven common anions and citrate on the Fast Anion III column using a 20-mM electrolytically generated potassium hydroxide eluent at 1.0 mL/min. As this figure shows, phosphate is well resolved from the other common anions and citrate in < 5 min. The low hydroxide eluent concentration and flow rate used in this application provide ideal characteristics for creating a rugged RFIC method.

Phosphoric acid in cola drinks is routinely monitored to maintain product quality and to minimize production costs. The concentration of phosphoric acid is typically measured during the manufacturing of cola syrup and during the bottling of soft drinks. In some soft drinks, citric acid may be present alone or in combination with phosphoric acid. The method described in this application note provides a convenient, reliable, precise, and rugged method for the simultaneous determination of phosphate and citrate in soft drinks. A variety of regular, diet, and flavored colas were analyzed for the presence of phosphate and citrate. A calibration curve was generated with phosphate in the range of 250–750 mg/L using three concentration levels and 25–200 mg/L for citrate using four concentration levels. Each calibration curve was linear over the specified ranges using a least-squares regression curve with correlation coefficients ($r^2$) of 0.9999 and 0.9998 for phosphate and citrate, respectively. The use of a small sample volume (1.2 µL) enables the injection of cola samples containing high concentrations of phosphate without overloading the column, thereby eliminating the need for sample dilutions.

Table 1 summarizes the results of the determination of phosphate and citrate in five carbonated soft drinks. Phosphate concentrations ranged from ~330–540 mg/L and citrate concentrations ranged from ~40–215 mg/L.

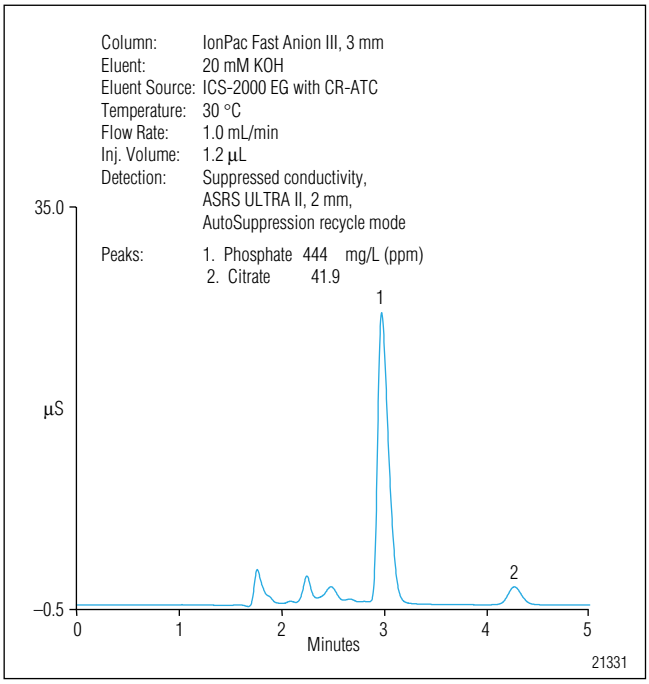



*Figure 2. Determination of phosphate and citrate in low- carbohydrate cola A on the IonPac Fast Anion III column.*

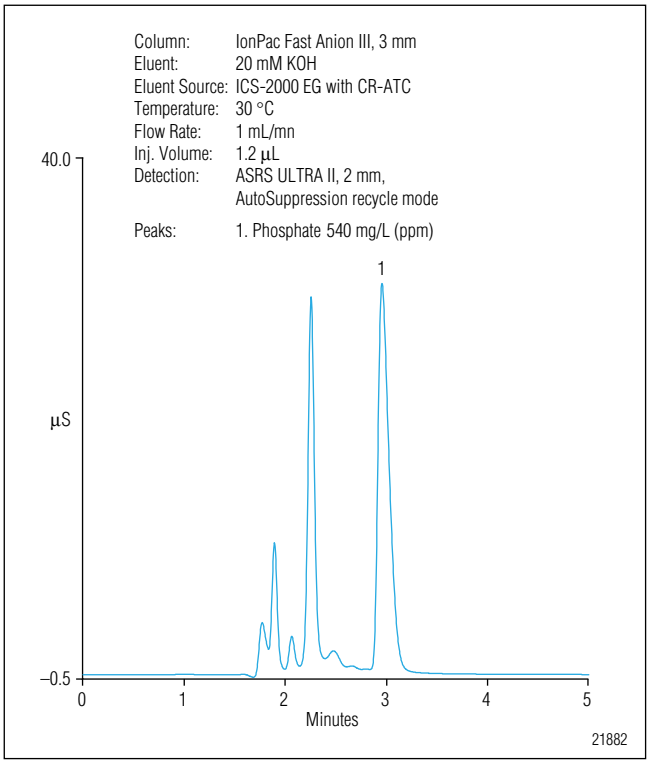



*Figure 3. Determination of phosphate in regular cola A on the IonPac Fast Anion III column.*

The precision of twenty replicate sample injections produced retention time and peak area RSDs of <0.15% and <0.25%, respectively. The excellent retention time and peak area precisions reflect results typically observed when using an RFIC system. Figure 2 demonstrates the described RFIC method for the analysis of a low-carbohydrate cola containing phosphoric and citric acids. As shown, both analytes are well resolved from other anionic components in the sample in less than 5 min. Figure 3 shows a regular cola containing only phosphoric acid with a measured phosphate concentration of approximately 540 mg/L. For the determination of only phosphate, the analysis time may be reduced to 4 min to further increase sample throughput.

Production facilities that produce large quantities of soft drinks each day not only require methods with a high sample throughput, but also require methods that are rugged and can meet the high demand of large analysis batches. Therefore, we evaluated the ruggedness of the IonPac Fast Anion III column to determine the number of cola samples that can be injected on the column without considerable loss of column capacity, which is often observed by reduced retention time. For a series of consecutive sample injections, we monitored the following criteria: 1) nitrate/phosphate resolution, 2) retention time and peak area precision of phosphate, 3) change in phosphate retention time, and 4) change in system pressure. The samples used to evaluate the column ruggedness included regular cola, diet cola, and two diet flavored colas. Although citrate was present in the diet colas, we were only interested in monitoring phosphate for the purpose of evaluating column ruggedness.

As column capacity decreases, the elution of phosphate can potentially be reduced at a faster rate than that of other common anions due to its trivalent charge. Therefore, to evaluate this effect we used nitrate, a monovalent anion, as a standard ‘marker’ to determine if a high number of cola injections would have any influence on the resolution and accurate quantification of phosphate.

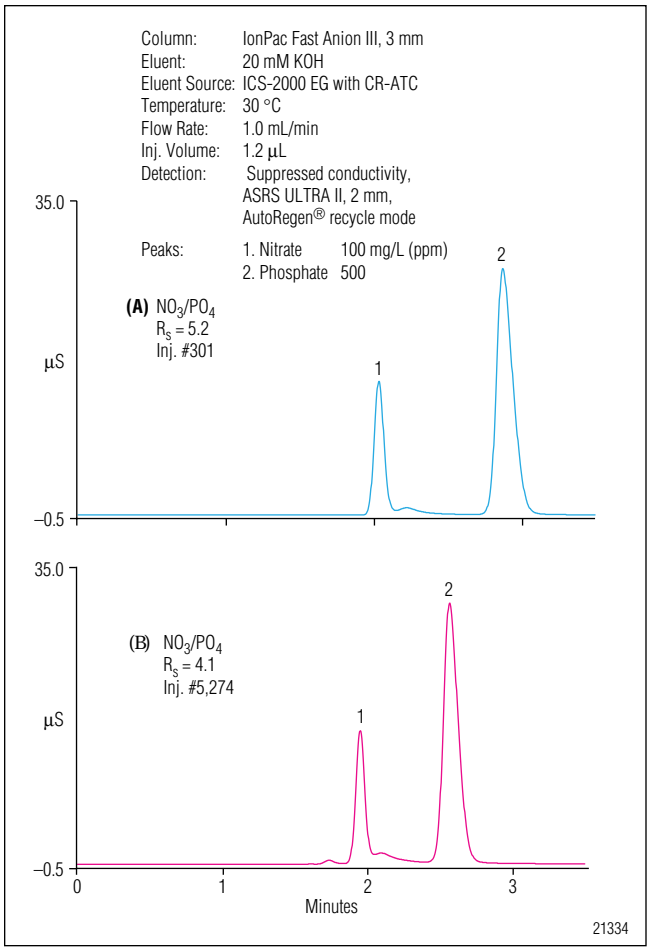



*Figure 4. Resolution of a standard separation of nitrate and phosphate for (A) injection #301 and (B) injection #5,274.*

Figure 4a shows a standard separation of nitrate and phosphate on the Fast Anion III column with a resolution ($R_s$) of 5.2 for injection #301. Figure 4b shows the same separation for injection #5,274 with an $R_s$ = 4.1. We determined no significant resolution loss between nitrate and phosphate for >5,000 total injections.

Table 2 summarizes the number of sample injections and average phosphate concentrations determined for the cola samples used to evaluate column ruggedness. Because a high number of sample injections can falsely result in high or low precision data, sets of 120 consecutive sample injections were used to evaluate the retention time and peak area precisions. Figure 5 shows a scatter plot of the calculated peak area precisions for the sample injection sets. Overall, peak area precisions ranged from 0.08–0.44% for 41 sets of injections (4,920 sample injections), while retention time precisions ranged from 0.10–0.18% (results not shown). The actual total

**Table 2. Average Phosphate Concentrations and Number of Cola Sample Injections Used to Evaluate the IonPac Fast Anion III Column Ruggedness**

| Sample | Analyte | # of Injections | Avg. Amount Found ± SD (mg/L) |
|---|---|---|---|
| Regular Cola B | Phosphate | 2,750 | 512 ± 7.0 |
| Diet Cola C | Phosphate | 2,040 | 219 ± 1.5 |
| Diet Flavored Cola D | Phosphate | 120 | 223 ± 0.6 |
| Diet Flavored Cola E | Phosphate | 120 | 218 ± 0.3 |

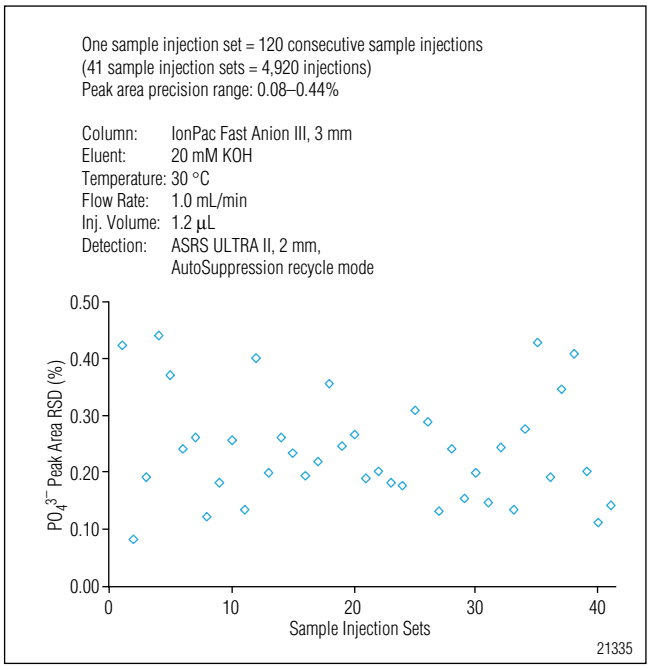



*Figure 5. Phosphate peak area precisions for multiple sets of injections (1 sample injection set = 120 sample injections).*

number of sample injections was >5,000 because sample injection sets <120 were not included with these data sets. Thirty-six injection sets (4,320 sample injections) produced phosphate peak area precisions <0.4%. These results indicate that the described RFIC method can reliably produce the same results each day. The high reproducibility of the method is primarily attributed to the use of the RFIC system because it requires no manual preparation of eluents or suppressor regenerents, and, therefore operates continuously with only an occasional replenishment of the deionized water used to supply the system.

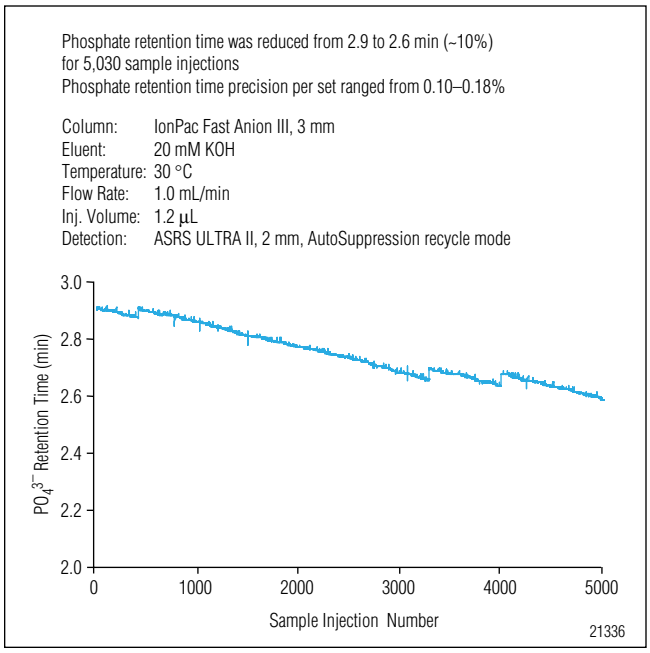



*Figure 6. Change in phosphate retention time in cola samples for 5,030 sample injections.*

Figure 6 shows the change in phosphate retention time for >5,000 injections of cola samples. The phosphate retention time was reduced by 0.3 min for 5,030 sample injections. This amounts to a decrease of only 3.6 sec per 1,000 sample injections. Considering the nature of the cola samples and the high number of samples injected on the Fast Anion III column, the total decline in phosphate retention time is insignificant. Figure 7 further demonstrates that this loss in retention had no influence on the resolution between phosphate and the earlier eluting anions. Therefore, no influence on the accurate quantification of phosphate was observed throughout this study. In addition, the system pressure was within ± 2% of the starting pressure, indicating that >5,000 sample injections had no effect on the system components (i.e., tubing, column, suppressor, etc.). The results of this study indicate the ruggedness of the IonPac Fast Anion III column for the repetitive determination of phosphate and citrate in cola samples, and its suitability for use in any soft drink production facility.

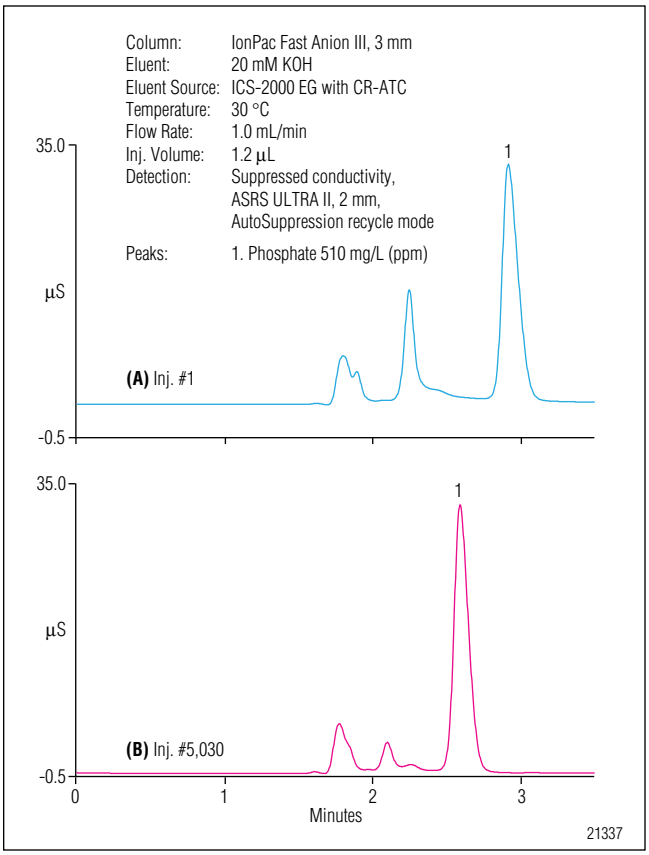



*Figure 7. Comparison of the separation of phosphate in regular cola B for (A) sample injection #1 and (B) sample injection #5,030.*

## CONCLUSION

A Reagent-Free IC (RFIC) method using a low-capacity hydroxide-selective IonPac Fast Anion III column with suppressed conductivity detection is a simple, rapid, accurate, precise, and rugged approach for the simultaneous determination of phosphate and citrate in carbonated soft drinks. The RFIC method is a significant improvement to the AOAC colorimetric assay by eliminating the use of additional reagents and unnecessary dilutions of cola samples that can result in poor precision and accuracy. The IonPac Fast Anion III produces a rapid separation (<5 min) of phosphate and citrate, and the ruggedness of the column permits the injection of >5,000 cola samples, providing the required characteristics for use in a soft drink production facility. Furthermore, an RFIC system significantly simplifies system operation and thereby improves the method's precision for use by multiple operators and laboratories.

## PRECAUTION

Some diet and diet flavored colas may contain a small interfering peak that can produce a slightly inaccurate phosphate concentration relative to the target value. Therefore, we recommend reducing the potassium hydroxide concentration from 20 to 16 mM to provide better resolution between phosphate and the unknown interferent.

## REFERENCES


1. Kuntz, L. A. Food Product Design, May 1993.
2. National Soft Drink Association, About Soft Drinks, available at http://www.ndsa.org/softdrinks/History/funfacts.html.
3. Association of Official Analytical Chemists (AOAC), Phosphorus in Water: Photometric Method, Method 973.55; Gaithersburg, MD, 1997.
4. Association of Official Analytical Chemists (AOAC), Phosphorus in Water: Automated Method, Method 973.56; Gaithersburg, MD, 1997.
5. Singh, R. P.; Smesko, S. A; Abbas, N. M. *J. Chromatogr. A* **1997**, *774,* 21–35.
6. Assay for Citrate and Phosphate in Pharmaceutical Formulations Using Ion Chromatography. Application Note 164; Dionex Corporation, Sunnyvale, CA.

# Determination of Additives in Carbonated Beverages

## INTRODUCTION

The soft drink industry is one of the largest in the world, with revenue from sales of carbonated soft drinks totaling billions of dollars annually. While the recipes for these beverages remain closely guarded trade secrets, the additives used in the formulations are critical components subject to determination for quality control. These components must be identified on the beverage's label.

Additives such as benzoate and sorbate function as preservatives. Citrate is used as both a preservative and a flavor agent. Caffeine is an integral part of the overall profile of most colas. Aspartame, acesulfame, and saccharin are three common artificial sweeteners which function as sugar substitutes.

Many methods have been developed for determining sweeteners and additives in drink formulations.[1-4] In this Application Note, we separate caffeine, sorbate, benzoate, citrate, aspartame, acesulfame and saccharin (structures are shown in Figure 1) in carbonated drinks using the Acclaim® Mixed-Mode WAX-1 column. This column features a new mixed-mode silica-based packing material that incorporates both hydrophobic and weak anion-exchange properties.[5] Unlike traditional reversed-phase substrates, the new packing features an alkyl longchain with an ionizable terminus. The column demonstrates great potential for separating a wide range of samples that contain a mixture of anionic and neutral compounds, including pharmaceutical, food, beverage, and chemical samples.

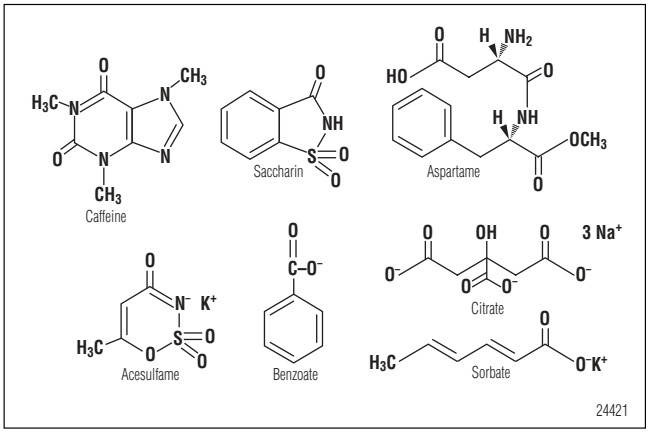



*Figure 1. Structures of the seven analytes.*

The Acclaim column's mixed-mode capability provides determination of the compounds of interest in many samples in a single injection, rather than two separate injections on two different types of columns.

| Table 1. Preparation of Mixed Stock Standard Solution | | | | |
|---|---|---|---|---|
| **Analyte** | **Concentration of Stock Standard (mg/L)** | **Volume of Added Stock Standard (mL)** | **Volume of Mixed Stock Standard (mL)** | **Concentration of Each Standard (mg/L)** |
| Caffeine | 1400 | 1.43 | 100 (diluted with water) | 20 |
| Aspartame | 500 | 12.00 | | 60 |
| Acesulfame | 1000 | 20.00 | | 200 |
| Saccharin | 1000 | 6.00 | | 60 |
| Sorbate | 1000 | 10.00 | | 100 |
| Benzoate | 1000 | 10.00 | | 100 |
| Citrate | 1500 | 40.00 | | 600 |

## *EQUIPMENT*

UltiMate®-3000 HPLC system

HPG 3400A pump with SRD 3400 Solvent Rack w/ degasser

TCC-3000 Thermostatted Column Compartment

WPS-3000TSL Autosampler

VWD-3400 UV/Vis detector

Chromeleon® 6.80 SP1 Chromatography Workstation

## *REAGENTS AND STANDARDS*

Water, Milli-Q® Gradient A10

Methanol ($CH_3OH$) and Acetonitrile ($CH_3CN$), HPLC grade, Fisher

Methanesulfonic acid (MSA), > 99.5%, Aldrich

Potassium dihydrogen phosphate ($KH_2PO_4$), analytical grade, SCRC, China

Caffeine, analytical grade, SCRC, China

Sorbate, analytical grade, SCRC, China

Benzoate, analytical grade, SCRC, China

Citrate, analytical grade, SCRC, China

Aspartame, analytical grade, Niutang Chemical Co. Ltd, China

Acesulfame, analytical grade, Supelco

Saccharin, analytical grade, Alfa Aesar

## *PREPARATION OF STANDARDS*

### Stock standard solutions

The concentrations of stock standard solutions were 500 mg/L for aspartame, 1000 mg/L for acesulfame, saccharin, sorbate and benzoate, 1400 mg/L for caffeine, and 1500 mg/L for citrate.

### Mixed stock standard solution

The mixed stock standard solution was prepared according to the procedure specified in Table 1.

### Mixed working standard solutions

The mixed stock standard solution was diluted with mobile phase to prepare the mixed working standard solutions used for calibration. The concentrations of each analyte in the mixed working standard solutions are shown in Table 2.

| Table 2. Concentrations of Mixed Working Standard Solutions | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Analyte | Concentration (mg/L) | | | | | | | | |
| | # 1 | # 2 | # 3 | # 4 | # 5 | # 6 | # 7 | # 8 | # 9* |
| Caffeine | 2 | 2.5 | 3.33 | 4 | 5 | 6.67 | 10 | 20 | 70 |
| Aspartame | 6 | 7.5 | 10 | 12 | 15 | 20 | 30 | 60 | — |
| Acesulfame | 20 | 25 | 33.3 | 40 | 50 | 66.67 | 100 | 200 | — |
| Saccharin | 6 | 7.5 | 10 | 12 | 15 | 20 | 30 | 60 | — |
| Sorbate | 10 | 12.5 | 16.7 | 20 | 25 | 33.3 | 50 | 100 | — |
| Benzoate | 10 | 12.5 | 16.7 | 20 | 25 | 33.3 | 50 | 100 | — |
| Citrate | 60 | 75 | 100 | 120 | 150 | 200 | 300 | 600 | — |

* This additional standard is prepared by adding 50 µL of the 1400 mg/L caffeine stock standard to 950 µL of mobile phase.

## *SAMPLE PREPARATION*

Six bottled carbonated drinks (named as samples A to E, respectively) were purchased from a local supermarket. Prior to injection, the samples were filtered though a 0.45-µm filter and diluted with mobile phase.

## *CHROMATOGRAPHIC CONDITIONS*

| | |
|---|---|
| Column: | Acclaim Mixed-Mode WAX-1, 5 µm, 4.6 × 150 mm (P/N = 064984) |
| Column Temp.: | 30 °C |
| Mobile Phase: | 120 mM $KH_2PO_4$-$CH_3CN$ (45% : 55%, v/v, pH 3.0, adjusted with MSA) |
| Flow rate | 1.5 mL/min |
| Inj. Volume: | 5 µL |
| Detection: | Absorbance at 210 nm |

## *RESULTS AND DISCUSSION*

### Optimized chromatographic conditions

Separations with the Acclaim Mixed-Mode WAX-1 column can be optimized by changing the following conditions of the mobile phase: 1) concentration of organic solvent, 2) ionic strength (buffer concentration), and 3) pH. The effects of changing these parameters are demonstrated by recording the retention time changes of a polar compound (4-hydroxybenozic acid) and nonpolar compound (butylbenzene) with controlled changes of the mobile phase.[6] Retention time for the nonpolar compound was almost unchanged when the buffer concentration changed from 100 to 20 mM, It altered only slightly when pH was changed from 6 to 2.6, and increased significantly when the organic solvent concentration was decreased from 50% to 45%. Retention time of the polar compound increased significantly when the buffer concentration was changed from 100 to 20 mM. Retention time decreased significantly when pH was changed from 6 to 2.6, and decreased slightly when the organic solvent concentration was decreased from 50% to 45%. Using these guidelines, the chromatographic conditions were optimized to obtain separation and baseline resolution of seven analytes in carbonated beverages (Figure 2).

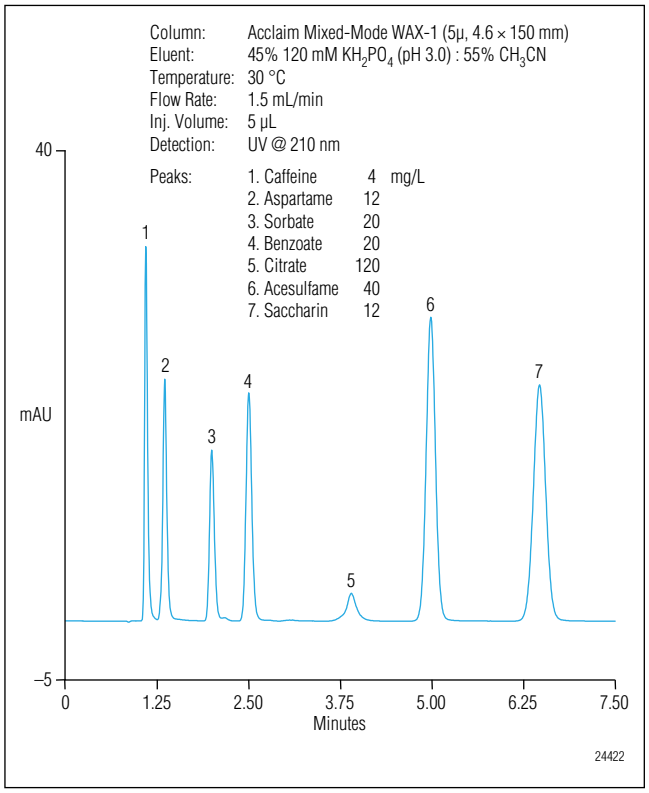



*Figure 2. Chromatogram of a mixed standard.*

### Reproducibility, linearity and detection limits

Prior to sample analysis method reproducibility was demonstrated by making seven replicate injections of a mixed standard solution. Table 3 summarizes the retention time and peak area precision data.

**Table 3. Reproducibility of Retention Times and Peak Areas**

| Analyte | RT RSD (%) | $A_{peak}$ RSD (%) |
|---|---|---|
| Caffeine | 0.000 | 0.169 |
| Aspartame | 0.000 | 0.257 |
| Sorbate | 0.000 | 0.223 |
| Benzoate | 0.130 | 0.594 |
| Citrate | 0.091 | 1.221 |
| Acesulfame | 0.065 | 0.294 |
| Saccharin | 0.098 | 0.299 |

This table shows seven injections of mixed standard #4 (Table 2)

Calibration linearity for each of the seven compounds was determined by making replicate injections of a mixed standard prepared at eight different concentrations. The external standard method was used to calculate the calibration curve and to quantify each of the compounds in the six samples tested. Table 4 shows the calibration data. The single-sided Student's *t*-distribution was used to estimate method detection limits (MDL). This data is also reported in Table 4.

### Sample analysis

Six different soft drinks were tested: One lemon-lime soda, one orange soda, two colas, and two diet colas. The diet colas contained no added sugar. Figures 3 through 8 show chromatograms of each sample and the same sample spiked with a mixed standard. Results, amounts of each additive per beverage, and recovery data are summarized in Table 5.

**Table 4. Calibration Data and MDLs**

| Analyte | Equations | r | RSD (%) | MDL (mg/L) |
|---|---|---|---|---|
| Caffeine | A = 0.3700C + 0.0181 | 0.9999 | 1.6036 | 0.6 |
| Aspartame | A = 0.1043C + 0.0735 | 0.9998 | 1.2287 | 0.2 |
| Sorbate | A = 0.0520C + 0.0417 | 0.9995 | 1.8813 | 1.1 |
| Benzoate | A = 0.0829C + 0.1198 | 0.9992 | 2.3464 | 1.2 |
| Citrate | A = 0.0028C – 0.0774 | 0.9988 | 2.6191 | 8.5 |
| Acesulfame | A = 0.0917C + 0.1352 | 0.9998 | 1.2345 | 1.2 |
| Saccharin | A = 0.3143C + 0.1499 | 0.9999 | 1.6036 | 0.4 |

Note: The single-sided Student's *t* test method (at the 99% confidence limit) was used to determine MDL, where the standard deviation (SD) of the peak area of seven injections is multiplied by 3.14 (at n = 7) to yield the MDL

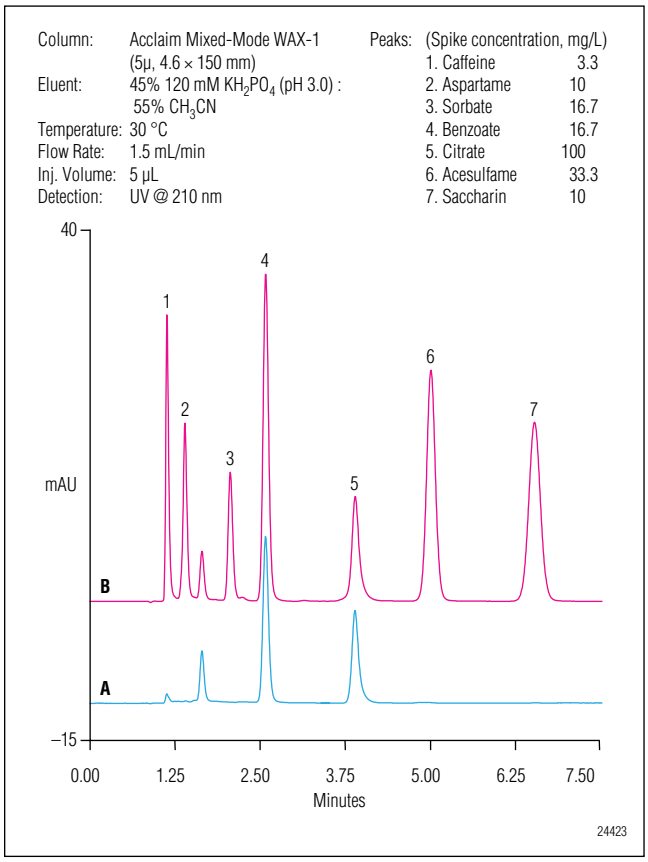



*Figure 3. Overlay of chromatograms of a lemon-lime carbonated beverage, diluted ten-fold (chromatogram A) and the same sample spiked (chromatogram B).*

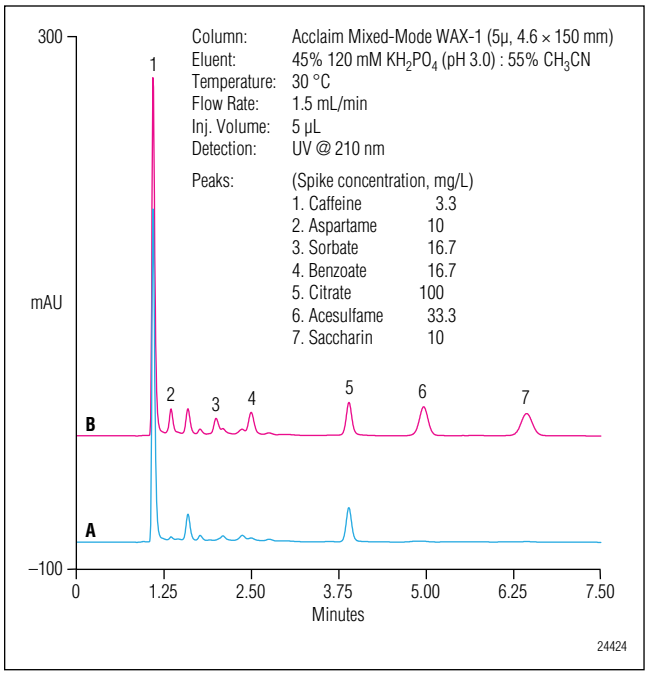



*Figure 4. Overlay of chromatograms of cola #1, diluted three-fold (chromatogram A) and the same sample spiked (chromatogram B).*

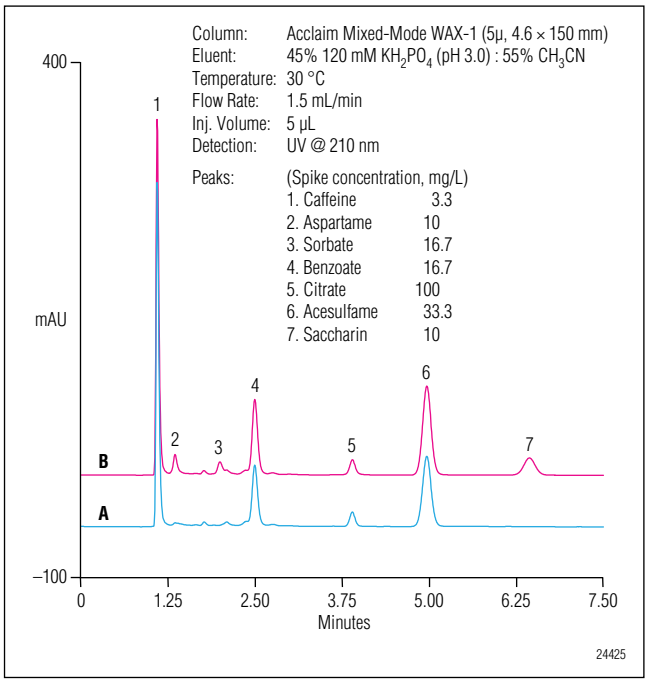



*Figure 5. Overlay of chromatograms of diet cola #1, diluted three-fold (chromatogram A) and the same sample spiked (chromatogram B).*

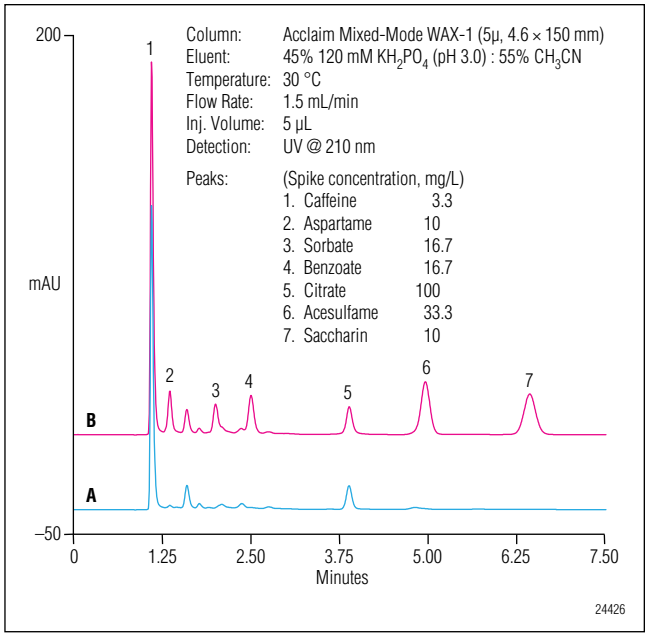



*Figure 6. Overlay of chromatograms of cola #2, diluted five-fold (chromatogram A) and the same sample spiked (chromatogram B).*

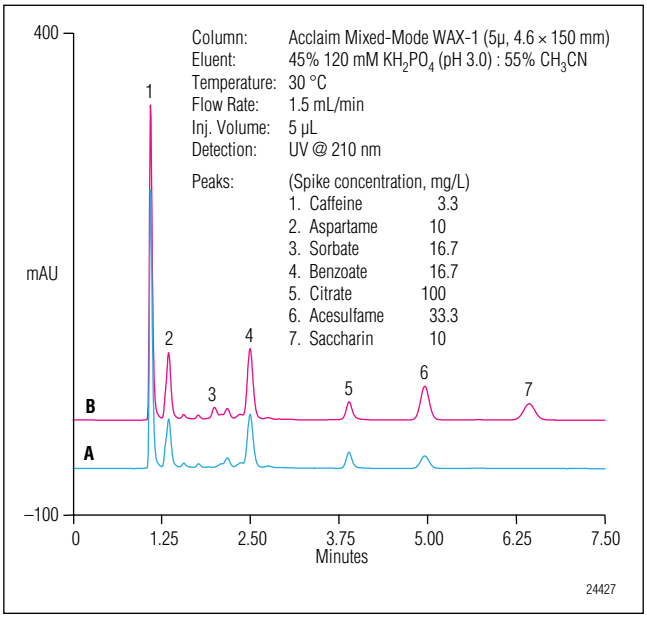



*Figure 7. Overlay of chromatograms of carbonated beverage diet cola #2, diluted three-fold (chromatogram A) and the same sample spiked (chromatogram B).*

Samples show an unidentified peak eluting at approximately 1.6 min, however, this peak was not present in either of the diet colas tested (samples 3 and 5). Tests showed the peak to correspond to fructose (Figure 9).

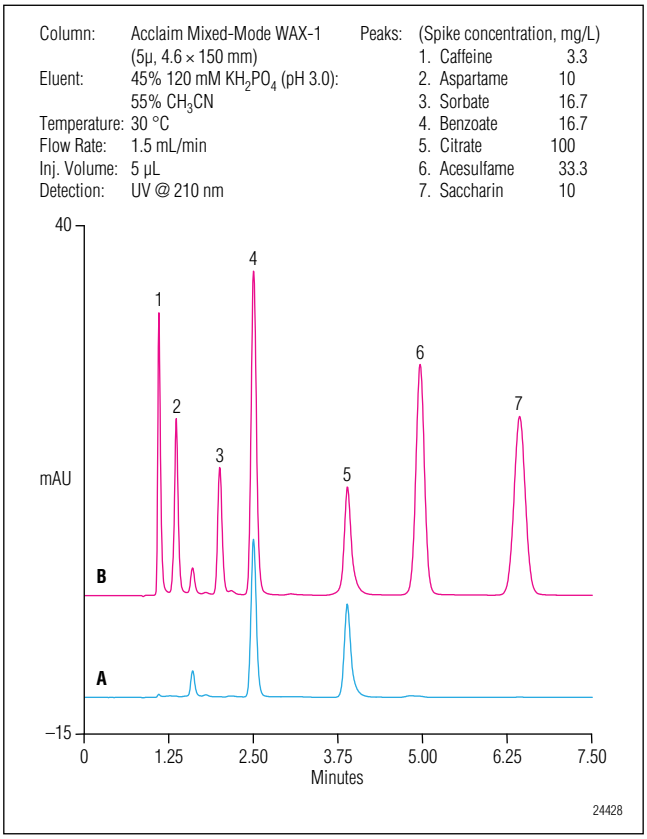



*Figure 8. Overlay of chromatograms of an orange soda sample, diluted ten-fold (chromatogram A) and the same sample spiked (chromatogram B).*

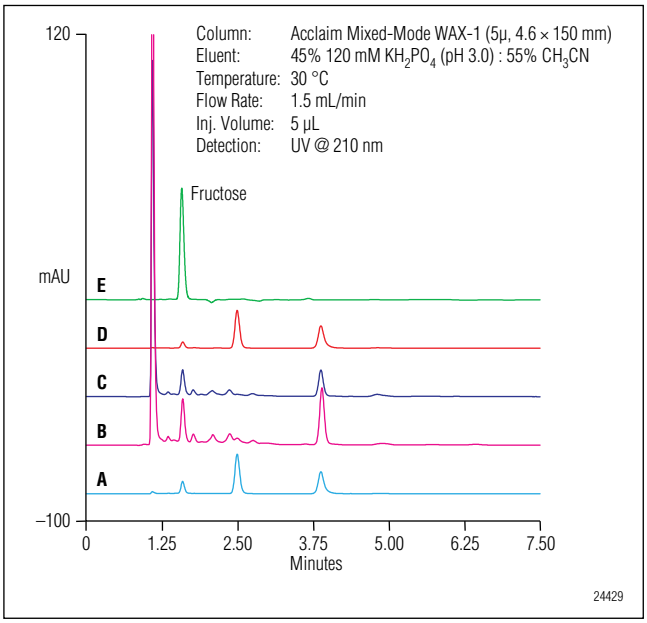



*Figure 9. Overlay of chromatograms of a lemon-lime carbonated beverage diluted ten-fold (chromatogram A), cola #1, diluted three-fold (chromatogram B), cola #2, diluted five-fold (chromatogram C), orange soda, diluted ten-fold (chromatogram D) and single standard of fructose, 5%, w/w (chromatogram E).*

| Table 5. Analysis Results for the Carbonated Drinks[1] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Analyte** | **Lemon-Lime Soda (Diluted 10-fold)** | | | | **Cola #1 (Diluted 3-fold)** | | | |
| | **Detected (mg/L)[3]** | **Added (mg/L)** | **Found (mg/L)[4]** | **Recovery (%)** | **Detected (mg/L)** | **Added (mg/L)** | **Found (mg/L)** | **Recovery (%)** |
| Caffeine | ND[2] | 3.3 | 3.4 | 103 | 94 | 3.3 | 3.6 | 109 |
| Aspartame | ND | 10 | 9.3 | 93 | ND | 10.0 | 11.3 | 113 |
| Sorbate | ND | 16.7 | 17.4 | 106 | 15.6 | 16.7 | 22.4 | 108 |
| Benzoate | 200 | 16.7 | 14.9 | 89 | ND | 16.7 | 20.7 | 103 |
| Citrate | 2767 | 100 | 89 | 89 | 2250 | 100 | 106 | 106 |
| Acesulfame | ND | 33.3 | 33.9 | 102 | ND | 33.3 | 34.2 | 103 |
| Saccharin | ND | 10.0 | 10.0 | 100 | ND | 10.0 | 10.0 | 100 |
| **Analyte** | **Diet Cola #1 (Diluted 3-fold)** | | | | **Cola #2 (Diluted 5-fold)** | | | |
| | **Detected (mg/L** | **Added (mg/L)** | **Found (mg/L)** | **Recovery (%)** | **Detected (mg/L** | **Added (mg/L)** | **Found (mg/L)** | **Recovery (%)** |
| Caffeine | 131.4 | 3.3 | 3.1 | 94.0 | 99.3 | 3.3 | 3.1 | 94 |
| Aspartame | ND | 10.0 | 10.4 | 104 | ND | 10.0 | 9.4 | 94 |
| Sorbate | 15.6 | 16.7 | 17.3 | 104 | 18.9 | 16.7 | 16.9 | 101 |
| Benzoate | 104 | 16.7 | 15.5 | 93 | ND | 16.7 | 14.3 | 86 |
| Citrate | 1410 | 100 | 92 | 92 | 1852 | 100 | 106 | 106 |
| Acesulfame | 324 | 33.3 | 29 | 87 | ND | 33.3 | 36.1 | 108 |
| Saccharin | ND | 10.0 | 9.9 | 99 | ND | 10.0 | 9.9 | 99 |
| **Analyte** | **Diet Cola #2 (Diluted 3-fold)** | | | | **Orange Soda (Diluted 10-fold)** | | | |
| | **Detected (mg/L** | **Added (mg/L)** | **Found (mg/L)** | **Recovery (%)** | **Detected (mg/L** | **Added (mg/L)** | **Found (mg/L)** | **Recovery (%)** |
| Caffeine | 113.4 | 3.3 | 3.5 | 106 | ND | 3.3 | 3.5 | 106 |
| Aspartame | 113.1 | 10.0 | 10.5 | 105 | ND | 10.0 | 9.9 | 99 |
| Sorbate | ND | 16.7 | 16.6 | 99 | ND | 16.7 | 15.2 | 91 |
| Benzoate | 183 | 16.7 | 14.8 | 89 | 180 | 16.7 | 18.4 | 110 |
| Citrate | 1770 | 100 | 111 | 111 | 3815 | 100 | 100 | 100 |
| Acesulfame | 56.4 | 33.3 | 35.3 | 106 | ND | 33.3 | 33.8 | 102 |
| Saccharin | ND | 10.0 | 10.0 | 100 | ND | 10.0 | 9.7 | 97 |

Notes: 1. One sample and one spiked sample were prepared, with 3 injections made for each.

2. ND="not detected"

3. Detected = Measured Value of sample × Diluted fold

4. Found = Measured Value of spiked sample – Measured Value of sample

## *REFERENCES*


1. N.M.M. Coelho, L.M. Coelho, E.S. de Lima, A. Pastor and M. de la Guardia, *Talanta,* 66 **2005** 818.
2. B. Saad, Md.F. Bari, M.I. Saleh, K. Ahmad and M.K.M. Talib, *J. Chromatogr. A,* 1073 **2005** 393.
3. M. S. García-Falcón and J. Simal-Gándara, *Food Control,* 16 **2005** 293.
4. T. Pérez-Ruiz, C. Martínez-Lozano, V. Tomás and J. Martín, *J. Chromatogr. A,* 1026 **2004** 57.
5. User Guide for Acclaim Mixed-Mode WAX Columns, Dionex Corporation LPN 065165.
6. X. Liu and C. Pohl, A New Multimode Stationary Phase: Characterization, Chromatographic Evaluation and Application, Dionex Corporation. LPN 1846-01 **2006**.

# Determination of Sucralose in Reduced-Carbohydrate Colas using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection

## INTRODUCTION

Sucralose (trichlorogalactosucrose or 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-deoxy-α-D-galacto-pyranoside) is a non-nutritive sweetener used to manufacture diabetic and dietetic foods and beverages. Detection of sucralose and other carbohydrates is challenging because they lack a strong chromophore and, therefore, cannot be detected at low concentrations with UV detection. Furthermore, sucralose would typically be present in foods containing compounds with strong UV chromophores. Refractive index detection can be used, but the sensitivity is poor. Most carbohydrates are not ionic at pH 7, therefore suppressed conductivity detection is not a suitable option. However, because carbohydrates, amines, glycols, alcohols, and sulfur compounds are easily oxidized at specific applied potentials they can be detected with high sensitivity by pulsed amperometric detection (PAD). Thus, carbohydrates are detected by PAD in basic solutions (pH >12) using a gold working electrode and Waveform A,[1] specifically designed for carbohydrate detection.

Basic eluents (pH >12) are required to ionize carbohydrates to form oxy-anions for separation. These anions are separated by high performance anion-exchange (HPAE) chromatography, using a CarboPac™ PA20 column. Sucralose, with three chlorine atoms, is more electronegative than sucrose and is retained longer on the CarboPac PA20 column, allowing its separation from fructose and sucrose.

This application update discusses the development of a HPAE-PAD method to determine sucralose in reduced-carbohydrate colas. HPAE-PAD has been used to determine sucralose in other sugar-free beverages, after a 50-fold dilution,[2] and foods[3-5]. Reduced-carbohydrate cola samples have high concentrations of fructose and sucrose relative to sucralose, making these samples challenging for chromatographic analysis. In this application update, we optimized the sample dilution and the eluent to separate high concentrations of fructose and sucrose from sucralose, while at the same time injecting enough sample to detect the low concentrations of sucralose in beverages.

## EXPERIMENTAL

### Equipment

Dionex ICS-2500 system consisting of:
- GP50 Gradient Pump with degas option and gradient mixer (GM-4 for microbore, P/N 049136; GM-3 for standard bore, P/N 042126)
- ED50A Electrochemical Detector with combination pH Ag/AgCl reference (P/N 046333) electrode and Carbohydrate Certified disposable Au working electrodes (Package of 6 electrodes, P/N 060139; Package of 24 electrodes, P/N 060216)
- AS50 Autosampler
- AS50TC Thermal Compartment
- Chromeleon® Chromatography Workstation with Chromeleon 6.6

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

Vacuum pump

Polypropylene sample vials, 1.5 mL with caps and slit septa (Dionex vial kit, P/N 079812) or 0.3 mL polypropylene sample vials with caps and slit septa (Dionex vial kit, P/N 055428)

Disposable polystyrene 25-mL pipettes

Micropipettor and tips for preparing samples, standards, and pipetting samples into vials

### Reagents and Standard

Deionized water, 18 MΩ-cm resistivity or better (used for all eluent and standard preparations)

Sodium hydroxide, 50% (w/w) (Fisher Scientific)

Sodium acetate, anhydrous (Fluka, Microselect)

Sucralose, micronized (McNeil Nutritionals)

Sucrose, D-Fructose, and α-D-Glucose (dextrose) reference standards, > 99% (United States Pharmacopeial Convention, Inc.)

### Electrochemical Cell

The Dionex ED50A Product Manual[6] and theDionex Disposable Electrode Installation Guide[7] describe the calibration, handling, and installation tips on the reference electrodes and Carbohydrate Certified Disposable Au working electrodes. Dionex Technical Note 21 (TN 21)[1] contains a detailed discussion of "Waveform A".

### Conditions

| | |
|---|---|
| Columns: | CarboPac PA20 Analytical (P/N 060144) |
| | CarboPac PA20 Guard (P/N 060142) |
| Flow Rate: | 0.5 mL/min |
| Eluent A: | 100 mM sodium hydroxide, 90 mM sodium acetate |
| Temperature: | 30 ºC |
| Inj. Volume: | 25 µL, PEEK sample loop (P/N 042857), full loop injection |
| Detection (ED50A): | Pulsed Amperometric Detection, carbohydrate four-potential waveform, "Waveform A" |
| Reference Electrode: | Ag mode |
| Working Electrode: | Carbohydrate Certified disposable gold working electrode |
| Background: | 11–54 nC |
| System Backpressure: | ~2500 psi |
| Noise: | 9–27 pC |
| Run Time: | 30 min |

## *PREPARATION OF SOLUTIONS AND REAGENTS*

### Eluent Preparation

Consistent eluent preparation is critical for reproducible chromatography. Eluent preparation is thoroughly discussed in the Dionex CarboPac PA20 product manual[8] and Dionex Application Note 159.[9] These documents should be reviewed prior to eluent preparation. It is essential to use high quality water (18 MΩ-cm resistivity or better) and it should contain as little dissolved carbon dioxide as possible. It is also important to minimize carbonate and microbial contamination. Carbonate is a divalent anion at pH >12; it will bind to the column and affect resolution and efficiency. Sodium hydroxide pellets are coated with sodium carbonate and should never be used for preparation of sodium hydroxide solutions. Sodium hydroxide based eluents should always be prepared from 50% (w/w) sodium hydroxide reagents. Microbial contamination is a source of carbohydrates and particles, and will cause higher background levels.

### Eluent A (100 mM sodium hydroxide/90 mM sodium acetate)

Measure ~800 mL of deionized water into a 1-L graduated cylinder. Add a magnetic stir bar and begin stirring. Slowly pour 14.77 g of anhydrous sodium acetate (82.03 g/mole) into the graduated cylinder. Stir until it is fully dissolved and then remove the magnetic stir bar. Using a rinsed plastic pipette, measure 10.5 mL of 50% (w/w) sodium hydroxide solution and pipette the solution into the graduated cylinder. Fill and dispense (several times) the sodium acetate-sodium hydroxide solution from the pipette to fully rinse the viscous sodium hydroxide into the same graduated cylinder. Add deionized water to the 1000 mL mark and briefly stir to mix the sodium hydroxide addition. In order to remove particles, vacuum filter the solution through a 0.2-µm nylon filter unit into the 2-L eluent bottle. Measure an additional 1000 mL of deionized water with the same graduated cylinder and vacuum filter through a 0.2-µm nylon filter unit into the same 2-L eluent bottle. Connect the eluent bottle to the Eluent A line from the pump and place the eluent bottle under ~4–5 psi of helium or other inert gas. Swirl the eluent bottle to thoroughly mix the eluent. Prime the pump with the new eluent.

### Column Wash (100 mM sodium hydroxide/800 mM sodium acetate)

Measure ~800 mL of deionized water into a 1 L graduated cylinder. Add a magnetic stir bar and begin stirring. Slowly pour 65.62 g of anhydrous sodium acetate (82.03 g/mole) into the graduated cylinder. Stir until it is fully dissolved and then remove the magnetic stir bar. Using a rinsed plastic pipette, measure 5.2 mL of 50% (w/w) sodium hydroxide solution and pipette it into the graduated cylinder. Fill and dispense (several times) the sodium hydroxide/sodium acetate solution from the pipette to fully rinse the viscous sodium hydroxide into the same graduated cylinder. Add deionized water to the 1000 mL mark and briefly stir to mix the sodium hydroxide addition. To remove particles, vacuum filter the solution through a 0.2-µm nylon filter unit into the eluent bottle. Connect the eluent bottle to the column wash eluent line from the pump and place the eluent bottle under ~4–5 psi of helium or other inert gas. Swirl the eluent bottle to thoroughly mix the eluent. Prime the pump with the new column wash.

## *STANDARD PREPARATION*

The standards were prepared gravimetrically and deionized water was used as the diluent. A stock solution of 100 mM sucralose (397.64 g/mol) solution was prepared from 0.795 g of reference grade crystalline sucralose (Splenda® by McNeil Nutritionals) dissolved in deionized water to a total weight of 20.00 g. The 100 mM sucralose standard was diluted appropriately with deionized water to prepare the 0.1 µM, 0.3 µM, 0.5 µM, 5 µM, and 10 µM sucralose standards.

Fructose (180.16 g/mol), sucrose (342.30 g/mol), and glucose (180.16 g/mol) stock standards were prepared in the same way as the sucralose stock standard. Individual stock solutions of 100 mM fructose, 100 mM sucrose, and 100 mM glucose were prepared from reference grade standards, 0.360 g of fructose, 6.846 g of sucrose, and 0.360 g of glucose, respectively, and dissolved in deionized water to a final weight of 20.00 g. The 100 mM standards were diluted with deionized water to prepare 10 µM standards. All of the standards were stored in the freezer at -5 ºC and thawed prior to use. These standards determined the retention times of the fructose, sucrose, and glucose.

## *SAMPLE PREPARATION*

The carbonated beverages were degassed by freezing them overnight. The samples were thawed and diluted prior to analysis. Brand C peach citrus beverage was diluted 50-fold, as prescribed for sugar-free beverages in Dionex Application Note 159. The dilution levels of the reduced-carbohydrate colas were established during the course of this work and are described in the next section of this document.

## *RESULTS AND DISCUSSION*

According to the manufacturer's label, Brand A reduced-carbohydrate cola contains: carbonated water; high fructose corn syrup and/or sucrose; caramel color; natural flavors; phosphoric acid, potassium benzoate, and potassium citrate; caffeine; and the sweeteners aspartame, potassium acesulfame-K (potassium salt of acesulfame), and sucralose. Brand B reduced-carbohydrate cola contains:  carbonated water; high fructose corn syrup and/or sucrose; carmel color; natural flavors; caffeine; sucralose; and phosphoric acid, potassium benzoate, citric acid, and potassium citrate.

The first experiments were designed to determine the optimum dilution level that would minimize the amount of fructose–sucrose on the column while maintaining a measurable amount of sucralose. We tested the following dilutions: 2-, 10-, 50-, 100-, 500-, 1000- and 5000-fold dilutions. A 100-fold dilution minimized column overload while maintaining a measurable sucralose peak for both brands of reduced-carbohydrate colas.

The goal for this project was to establish conditions that would elute the fructose and sucrose, as early as possible, and elute the sucralose a short time later, well resolved from the other sugars. We experimented with isocratic separations, using a fixed concentration of sodium hydroxide with variable sodium acetate concentrations. Using 100 mM concentration of sodium hydroxide ensures sucralose ionization and good detection sensitivity. Acetate is a stronger eluent than hydroxide, hence it was used to control retention. Low concentrations of sodium acetate in the eluent resulted in long sucralose retention times (>25 min using <20 mM sodium acetate), or poor sucralose response (<75 mM sodium acetate). High concentrations of sodium acetate (200 mM) eluted sucralose in ~3 min, but caused it to be poorly resolved

from sucrose. A 100 mM sodium hydroxide/90 mM sodium acetate eluent achieved a good separation of sucralose from fructose and sucrose in under 10 min. Figure 1 shows an overlay of individual injections of 10 µM fructose, glucose, sucrose, and sucralose standards under the stated conditions. Note that glucose, fructose, and sucrose all elute in under 2 min while sucralose elutes at about 6 min.

We applied our HPAE-PAD method to the analysis of two reduced-carbohydrate colas, Brand A and Brand B (Figure 2). While sucralose eluted at about 6 min, there were a number of unknown compounds in these colas that eluted after sucralose (not shown). To ensure that the compounds did not carry over to the next injection, the method run time was extended to 30 min for the reduced-carbohydrate colas and no late-eluting compounds were observed in subsequent injections. To assess method ruggedness, we alternately analyzed standards (2–4 injections), reduced-carbohydrate colas (5 each), and reduced-carbohydrate colas spiked with 10 µM sucralose standard (3 injections). While Figure 3 shows that the retention time of sucralose dropped slightly during the course of the analysis, presumably as a result of the other components in the two colas, the resolution was still sufficient for quantitative analysis.

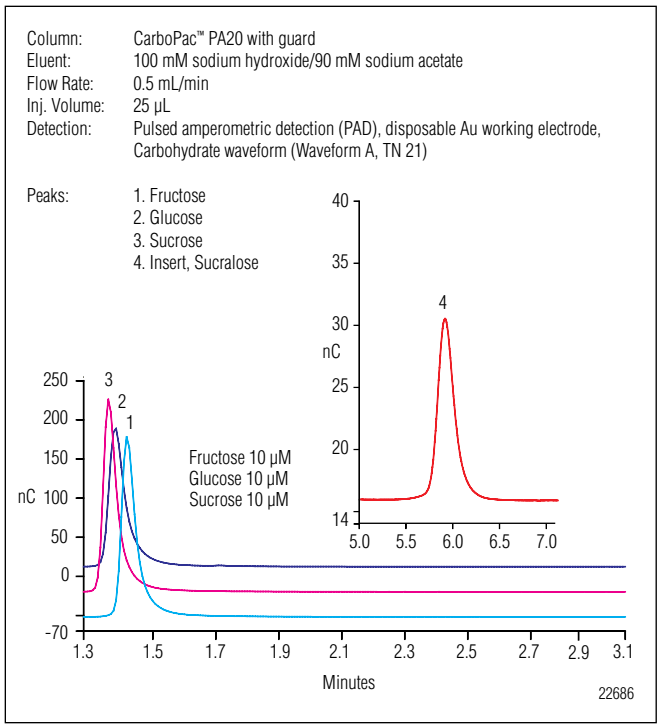



*Figure 1. HPAE-PAD analysis of 10-µM standards.*

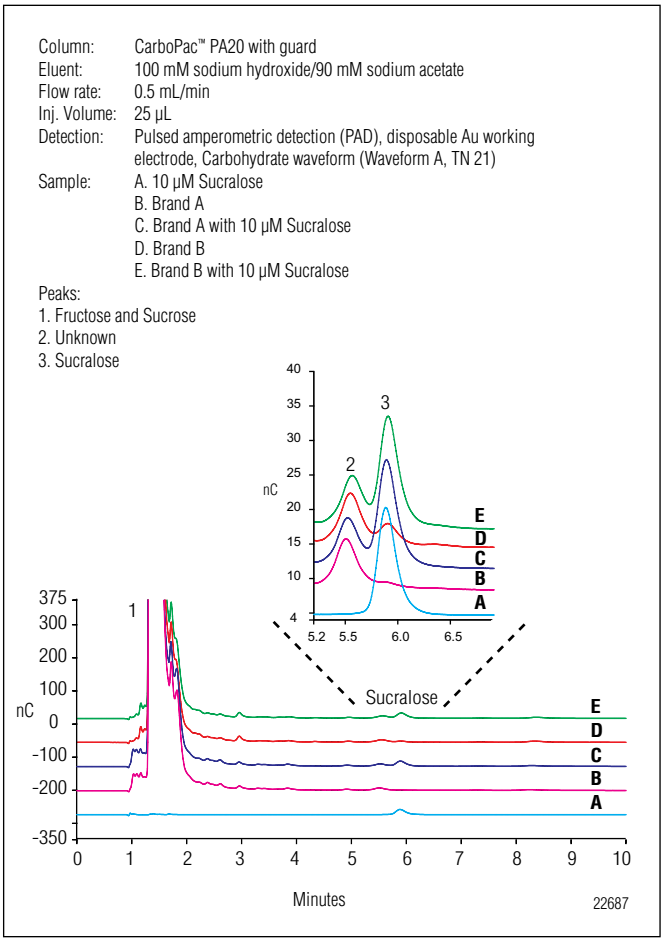



*Figure 2. HPAE-PAD analysis of sucralose in a 100-fold dilution of the reduced-carbohydrate colas.*

These other components together with excess fructose and sucrose also caused sucralose to elute earlier in Brand B reduced-carbohydrate cola compared with Brand A (Table 1). A 30-min column wash of 100 mM sodium hydroxide/800 mM sodium acetate and a 30-min equilibration were added after 24 h of analysis to restore the retention time of sucralose to its original value.

The HPAE-PAD system was calibrated with triplicate injections of sucralose standards (0.1 µM, 0.3 µM, 0.5 µM, 5 µM, and 10 µM sucralose) with 10 min runs (the additional isocratic time was not needed as the standard did not contain late-eluting compounds). This calibration was linear ($r^2 > 0.999$) and was used to determine the sucralose calibrations in the colas. From the sample analyses in Figure 3, we determined the concentration of sucralose in the two reduced-carbohydrate colas. In the 100-fold dilutions measured, Brands A and B reduced-carbohydrate colas had concentrations of

0.26 ± 0.02 µM sucralose and 1.88 ± 0.16 µM sucralose, respectively (Table 2). To ensure that we were accurately determining these concentrations, we conducted a spike recovery study. Table 2 shows that we observed good recovery for both colas. The spike recovery demonstrates that the observed variability in retention time of the reduced-carbohydrate colas (Table 1) is not interfering with sucralose quantification.

This method was optimized for sucralose and does not fully resolve fructose, sucrose, and glucose for quantification. Fructose, sucrose, and glucose can be determined separately with a 10,000-fold dilution using gradient or isocratic methods.[9] This high dilution (i.e., 10,000-fold dilution) illustrates the sucralose concentration disparity between the diet colas and the reduced-carbohydrate colas, and the difficulty of determining low concentrations of sucralose in a high fructose/sucrose matrix.

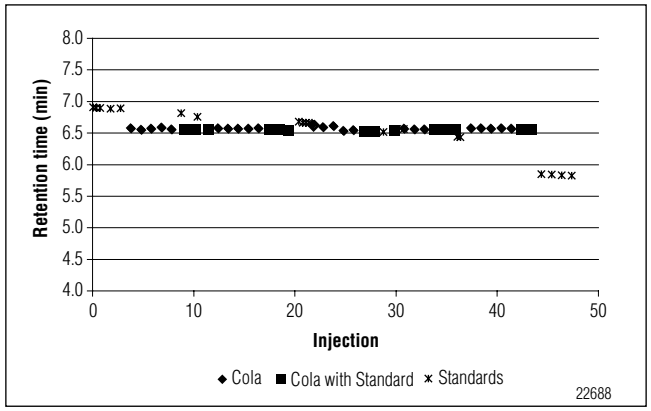



*Figure 3. Retention time stability of sucralose during a one-day analysis of reduced-carbohydrate colas.*

**Table 1. Retention Time and Area Stability of Reduced-Carbohydrate Colas and Beverages**

| Beverage | Retention Time (min) | Area (nC-min) |
|---|---|---|
| Brand A Reduced-Carbohydrate Cola | 6.54 ± 0.05<br>n = 24 | 0.08 ± 0.01<br>n = 24 |
| Brand B Reduced-Carbohydrate Cola | 6.02 ± 0.24<br>n = 21 | 0.68 ± 0.06<br>n = 21 |
| Brand C Peach Citrus* | 6.23 ± 0.03<br>n = 21 | 2.07 ± 0.02<br>n = 21 |
| 9.86 µM Sucralose Standard | 6.33 ± 0.46<br>n = 13 | 3.44 ± 0.26<br>n = 13 |

* Analysis performed on a separate day.

We also determined sucralose concentration in a low carbohydrate, peach citrus beverage. The peach citrus beverage is a store brand beverage, marketed as a caffeine free, low sodium, low carbohydrate, fruit beverage. According to the manufacturer's label, it contains mostly water with citric acid, potassium citrate, sodium benzoate, potassium sorbate, EDTA, gum acacia, along with sodium hexametaphosphate, natural flavors, the sweeteners sucralose, acesulfame-K, food dye Yellow 6, and food dye Red 40. This sample does not contain sucrose and fructose in high concentrations that can interfere

**Table 2. Determination of Sucralose in Reduced-Carbohydrate Colas and Beverages**

| Beverage | Measured Concentration of Diluted Beverage (µM) | Calculated Undiluted Concentration (µM) | Spike Recovery (%) |
|---|---|---|---|
| Brand A Reduced-Carbohydrate Cola | 0.26 ± 0.02<br>n = 24 | 25.6 ± 1.9<br>n = 24 | 101.9 ± 4.3<br>n = 20 |
| Brand B Reduced-Carbohydrate Cola | 1.88 ± 0.16<br>n = 21 | 188 ± 16<br>n = 21 | 88.1 ± 6.5<br>n = 23 |
| Brand C Peach Citrus[a] | 7.77 ± 0.08<br>n = 21 | 389 ± 4<br>n = 21 | —[b] |

[a] 50-fold dilution.
[b] not determined

with sucralose determinations. Figure 4 shows that a 50-fold dilution of the peach citrus beverage has no interference from fructose and sucrose. We also found that sucralose in citrus peach beverage did not exhibit the same retention loss with sample injection as observed for the reduced-carbohydrate colas. We analyzed this sample on two separate occasions (21 days apart) and found similar retention times of 6.35 ± 0.02 (n = 6) and 6.23 ± 0.03 min (n = 21). Using the same sucralose method as for the reduced-carbohydrate colas, we determined the sucralose concentration in the peach citrus beverage to be 391 ± 4 µM (Table 2). The peach citrus beverage has very low concentrations of fructose and sucrose (compare Figures 2 and 4) and low concentrations of late-eluting unknown compounds (not shown). For this sample, the method can be shortened to 8 min for faster sample throughput. It is recommended that the sucralose retention time be evaluated after 24 h to determine if a column wash is needed.

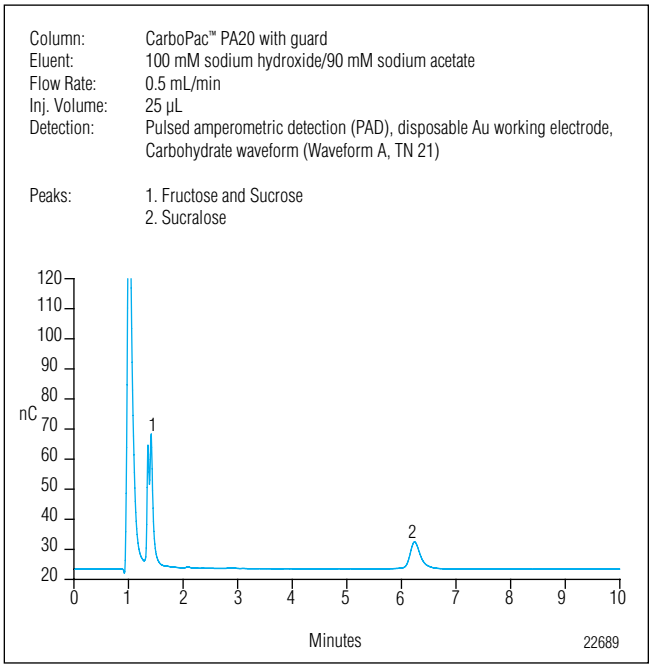



*Figure 4. Determination of sucralose in a 50-fold dilution of Brand C peach citrus low-carbohydrate beverage.*

## CONCLUSION

The HPAE-PAD method described in this application update can be used to determine low concentrations of sucralose in the difficult high fructose and sucrose matrix of reduced-carbohydrate colas. The success of this type of analysis depends on determining the appropriate sample dilution to achieve a sucralose concentration that can be measured accurately.

## PRECAUTIONS

Food and beverage samples can degrade over time, especially after they have been degassed. Reduced-carbohydrate and low carbohydrate beverages should be stored in the refrigerator until sample analysis.

## REFERENCES


1. *Optimal Settings for Pulsed Amperometric Detection of Carbohydrates Using the Dionex ED40 Electrochemical Detector*. Technical Note 21; Dionex Corporation, Sunnyvale, CA, 1998.
2. Hanko, V.; Rohrer, J. Determination of Sucralose in Splenda and a Sugar-Free Beverage Using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection. *J. Agric. Food Chem.* **2004**, *52*, 4375–4379.
3. Ichiki, H.; Semma, M.; Sekiguchi, Y.; Nakamura, M.; Ito, Y. Determination of Sucralose in Food by Anion-Exchange Chromatography with Pulsed Amperometric Detection. *Jpn. J. Food Chem. Nihon Shoku-hinkagaku Gakkaishi* **1995**, *2*, 119–121.
4. Kobayashi, C.; Nakazato, M.; Yamajima, Y.; Ohno, I.; Kawano, M.; Yasuca, K. Determination of Sucralose in Foods by HPLC. *Shokuhn-Eiseigata-Zasshi* **2001**, *42*, 139–143.
5. Kishi, H.; Kawana, K. Determination of Sucralose in Foods by Anion-Exchange Chromatography and Reversed-Phase Chromatography. *Shokuhn-Eiseigata-Zasshi* **2001**, *42*, 133–138.
6. Operator's Manual for ED50A Electrochemical Detector. LPN 031772; Dionex Corporation, Sunnyvale, CA, 2002.
7. Installation Guide for Disposable Electrodes. LPN 065040; Dionex Corporation, Sunnyvale, CA 2002.
8. Product Manual for CarboPac PA20 Guard Column and CarboPac PA20 Analytical Column. LPN 031884; Dionex Corporation, Sunnyvale, CA, 2003.
9. *Determination of Sucralose Using HPAE-PAD*. Application Note 159; LPN 1572; Dionex Corporation, Sunnyvale, CA, 2004.


## SUPPLIERS

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

McNeil Nutritionals, LLC, a Johnson and Johnson Company, Rt 2 Box 16M Industrial Rd, McIntosh, AL 36553 USA, Tel: 1-800-777-5363, www.splenda.com.

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

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

# Fast Determinations of Phosphate and Citrate in Carbonated Beverages Using On-Line Degassing with the Carbonate Removal Device (CRD) and a Reagent-Free™ Ion Chromatography System

## INTRODUCTION

Phosphoric and citric acids are critical additives to colas for flavor and preservation. Carbon dioxide is added for flavor or effervescence and also acts as a preservative. When samples are analyzed by ion chromatography (IC) with hydroxide and tetraborate eluents, carbonate in the sample can sometimes coelute and interfere with the quantification of an anion of interest. The gas bubbles from the carbonation also cause variability in the amount injected, resulting in poor peak area reproducibility. Carbonation must be removed to achieve precise and accurate phosphate and citrate determinations.

In Application Note 169[1], we showed that an ICS-2000 Reagent-Free ion chromatography (RFIC™) system outfitted with an IonPac® Fast Anion III column set was a fast and rugged solution for phosphate and citrate determinations in colas. Phosphate and citrate were accurately and precisely determined in 5 min. AN169 used off-line sample degassing for 20 min in an ultrasonic bath. To eliminate this labor, we incorporated a carbonate removal device (CRD) into the RFIC method. The CRD is typically used for removing carbonate from a sample after it is separated and has passed through a suppressor[2], but it can remove carbonate from acidic samples prior to injection. In this application update, we show that phosphate and citrate determinations of samples with carbonate removed on-line with the CRD are equivalent to determinations of samples degassed off-line.

## EXPERIMENTAL

### Equipment

Dionex ICS-2000 Reagent-Free (RFIC) fully integrated IC system

AS40 Autosampler with 5 mL PolyVial™ vials and plain caps (P/N 039532)

Chromeleon® 6.7 data management system

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

Vacuum pump

PEEK tubing

- Black (0.25-mm or 0.01-in. i.d., P/N 052306 for 5 ft) for connecting columns and ASRS ULTRA II backpressure loops.
- Orange (0.51-mm or 0.02-in. i.d., P/N 052309 for 5 ft) to make the injection line from AS40 to CRD to injection valve
- Red (0.127-mm or 0.005-in. i.d., P/N 052310 for 5 ft) to make a 1.2-µL loop
- Yellow (0.076-mm or 0.003-in. i.d., P/N 052301 for 5 ft) for system backpressure loop

Low-pressure Teflon® (E.I. du Pont de Nemours) tubing (1.6-mm or 0.063-in. i.d., P/N 014157) tubing for the CRD and degas waste lines

Micropipettor and tips for preparing samples, standards, and pipetting samples into vials

### Reagents and Standards

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

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

Sodium phosphate, dibasic anhydrous (JT Baker, BioReagent, ultrapure, P/N JT4062-1)

Trisodium citrate, dihydrate (Sigma, P/N S4641)

### Samples

Regular colas 1 and 2

Reduced sugar cola 1

Diet colas 1–4, diet colas 5–6 with flavoring

### Conditions

| | |
|---|---|
| Columns: | IonPac® Fast Anion III, 3 × 250 mm (P/N 062982) and Guard, 3 × 50 mm (P/N 062981) |
| Flow Rate: | 1.0 mL/min |
| Eluent: | EluGen KOH, 20 mM Potassium hydroxide |
| Temperature: | 30 ºC |
| Inj. Volume: | 1.2 µL |
| Sample Prep: | 4-mm CRD (P/N 062983) installed between autosampler and injection port |
| Detection: | Suppressed conductivity, in recycle mode, 70 mA |
| Background: | <1 µS |
| Backpressure: | ~2100 psi |
| Typical Noise: | <1.6 nS |
| Run Time: | 5 min |

## *PREPARATION OF SOLUTIONS AND REAGENTS*

### Eluent Preparation

It is essential to use high-quality, Type 1 water, >18.2 MΩ–cm and it should contain as little dissolved carbon dioxide as possible. Degas the deionized water before eluent preparation.

### Standard Preparation

To prepare separate stock solutions of 10,000 mg/L of phosphate and citrate, weigh the amount of reagent grade, dibasic sodium phosphate (FW 142.0 g/mol) and reagent grade, tri-sodium citrate dihydrate (FW 294.1 g/mol) respectively, into separate 125-mL polypropylene bottles (Table 1). Add degassed deionized water to a total weight of 100.0 g. Shake thoroughly to dissolve the solids.

**Table 1. Amount of Compound Used to Prepare 100.0 g of Individual 10,000 mg/L Stock Solutions**

| Anion | Compound | Mass (g) |
|---|---|---|
| Citrate | Trisodium citrate, dihydrate ($NaOOCCH_2C(OH)(COONa)(CH_2COONa{\cdot}2H_2O)$) | 1.555 |
| Phosphate | Sodium phosphate dibasic, anhydrous ($Na_2HPO_4$) | 1.495 |

To prepare combined phosphate and citrate working standards from the 10,000 mg/L stock standards (Table 2), pipette both 10,000 mg/L stock solutions into 125-mL polypropylene bottles. Dilute these working standards with degassed deionized water to 100.0 g total weight. The stock solutions are stable for more than a month when refrigerated. The working standards should be prepared weekly.

**Table 2. Amount of 10,000 mg/L Stock Solutions Used to Prepare 100.0 g of Combined Working Standards**

| Standard | Citrate Stock Solution (µL) | Phosphate Stock Solution (µL) |
|---|---|---|
| 50 mg/L citrate, 200 mg/L phosphate | 500 | 2000 |
| 100 mg/L citrate, 300 mg/L phosphate | 1000 | 3000 |
| 150 mg/L citrate, 400 mg/L phosphate | 1500 | 4000 |
| 200 mg/L citrate, 500 mg/L phosphate | 2000 | 5000 |

### Sample Preparation

Some cola samples were degassed for 5 min with ultrasonic agitation and then 15 min with ultrasonic agitation and applied vacuum. These were control samples to compare to the same colas analyzed without off-line degassing using the method in this application update.

## *SYSTEM SETUP*

To install the EluGen II potassium hydroxide cartridge, CR-ATC, columns, ASRS® ULTRA II suppressor, and backpressure loops for the suppressor and the eluent generator, refer to the Installation section of the ICS-2000 Operator's Manual.[3] Hydrate the CR-ATC and ASRS ULTRA II suppressor according to the ICS-2000 Installation and Quickstart[4] instructions. Install both the EluGen II KOH cartridge and the CR-ATC in the ICS-2000 cartridge holder and condition the EluGen II KOH cartridge. Install the columns after the injection valve and heat exchanger according to the IonPac Fast Anion III Product Manual[5] and the ICS-2000 Installation section. Install the suppressor, in recycle mode, between the columns and the conductivity cell. Install the backpressure loops after the cell and before the suppressor. After the installations are completed, check the total system pressure. The total system pressure should be >2000 psi for the eluent generator with an optimum operating pressure of 2300 psi. If the system pressure is <2000 psi, refer to the Installation section and install yellow (0.076-mm or 0.003-in. i.d.) PEEK tubing between the degas module and the injection valve to increase the system pressure to ~2300 psi. Do not allow the system pressure to exceed 3000 psi, as this could damage the degas module.

### Preparation of a 1.2-µL Sample Loop

To prepare a 1.2-µL sample loop, cut a 10-cm length of red PEEK tubing (0.127-mm or 0.005-in. i.d.). The sample loop volume must be calibrated by weight using an analytical balance because the tubing inside diameter can vary by as much as 20%. The sample loop volume is the difference between the empty loop and the loop filled with deionized water. (See Dionex Application Note 166 for an example of this calculation.)[6] The loop used for the work reported in this application update had a calibrated volume of 1.1 µL.

### CRD Installation

Typically, the CRD (for theory and operation, see the CRD manual and Technical Note 62)[2] is used to remove the carbonate peak from the sample just prior to detection, and it would be installed after the suppressor and before the detector. In this application update, the CRD is used as a sample preparation device to remove carbonate from the colas during injection; therefore it is installed between the AS40 Autosampler and the injection valve. Hydrate the 4-mm CRD according to the QuickStart Instructions.[7] Because the CRD is a membrane-based device, high pressure can irreversibly damage it. Always remove all plugs from the CRD before hydrating it or installing any tubing. Replace the PEEK injection tubing on the bleed valve of the AS40 Autosampler with a ~50-cm (18-in.) piece of orange PEEK (0.51-mm or 0.02-in. i.d.) tubing. Install the free end into the "Eluent In" port of the 4-mm CRD. Cut another ~20-cm (8-in.) piece of orange PEEK and install one end into the "Eluent Out" port of the CRD and the other end into Port 5 of the injection valve. Connect the regenerant waste line (1.6-mm or 0.063-in. i.d. Teflon) from the degas module to the "Regen In" port of the CRD. Connect one end of another length of Teflon tubing into the "Regen Out" port of the CRD and direct the other end to waste. The CRD is designed to slip over the top of the suppressor. The CRD can be positioned either outside the ICS-2000 system or secured on the ASRS ULTRA II suppressor according to Figure 1.[8]

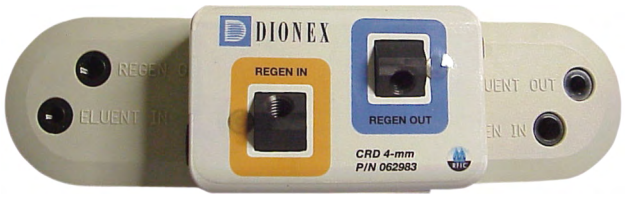



*Figure 1. Carbonate Removal Device.*

### AS40 Setup

To connect the AS40 Autosampler to the ICS-2000 system, refer to the AS40 Autosampler Operator's manual[9] and the ICS-2000 Operator's Manual.[10] After installing the AS40, configure it by selecting Concentrate, Bleed On, 3 samples per vial, and Proportional. The AS40 in Concentrate mode injects the sample at a slower flow rate, and thus applies less pressure to the CRD. The "bleed on" function bleeds off excess liquid or air to waste. Always verify after any start-up or power-up that the AS40 is in Concentrate mode.

### Configuring the AS40 in the Chromeleon Timebase and Program

To configure the timebase with only the ICS-2000 system, open the Chromeleon configure program, create a new timebase, and add the ICS-2000 system, according to the instructions in the ICS-2000 Operator's Manual. Select the TTL Input tab and select "normal edge" for TTL Input Mode and "TTL Input 1" for the Load and Inject valve. Although the AS40 Autosampler cannot be added during Server Configuration, Chromeleon 6.7 will automatically detect it and add it to the Panel.

Creating a program with the Chromeleon Program Wizard is thoroughly discussed in the ICS-2000 Operator's Manual. Enter the loading and injecting commands for the AS40 autosampler in the Injection Options tab (Table 3).

**Table 3. Entries in Chromeleon Program Wizard for the AS40 Autosampler**

| Injection Options | Entry |
|---|---|
| Trigger Load Operations | Pump_ECD_TTL_1 |
| Load Sample, Before Injection | 2.3 min |
| Inject Duration | 5 s |

It is helpful to ensure that the injection valve is in the load position at the start of the program. It is also helpful to shorten the duration commands. In the program review mode, insert on the first line of the program, "Load Position" and "Pump_ECD_TTL_1.5". Change the "Duration = 138" at -2.3 min to "Duration = 5" and delete the "Duration = 5" at 0 min.

### Verifying that the CRD is Operational

If peak reproducibility is poor, the CRD may be leaking and has failed. To test if the CRD is leaking, use the procedure described in the CRD Product Manual instructions.[11] There is also a quick pH test on the sample leaving the CRD that will identify whether the CRD is leaking. To test for a leak, remove the PEEK tubing from Injection Valve Port 2 (opposite end from Eluent Out on the CRD) and test a few drops on pH paper. If the pH is basic, then the CRD is leaking and has failed.

## *RESULTS AND DISCUSSION*

First, four colas were analyzed (two regular and two diet colas) with off-line degassing using ultrasonic agitation to establish the expected values for citrate and phosphate. Three of these colas were previously analyzed in AN169. The phosphate and citrate concentrations were consistent with those determined in AN169. The CRD was then installed and the same colas analyzed without off-line degassing. Figures 2 and 3 show that the retention times of phosphate and citrate remained the same for both sample preparation methods and both regular and diet colas. Phosphate had retention times of 2.78 to 2.79 min (Table 4). Citrate for Cola 2 and both diet colas had retention times ranging from 3.73 to 3.77 min. Cola 1 did not contain citrate. The reproducibilities for both retention time and concentration of phosphate and citrate were good, <0.3 %RSD (n = 20). The phosphate and citrate concentrations were comparable to those degassed off-line, 100.2–102.8%.

To verify the linearity of phosphate and citrate with on-line sample carbonate removal, four standards (six replicates each, from 200 to 600 mg/L and 50 mg/L to 200 mg/L, of phosphate and citrate, respectively) were analyzed. The noise over 60-min runs of five water injections was also determined by measuring the noise in 1-min intervals, from 5 to 60 min of each run. The results were comparable to the original method. The linearity for both anions was ≥0.999 and the average noise was 1.6 ± 0.5 nS (n = 5).

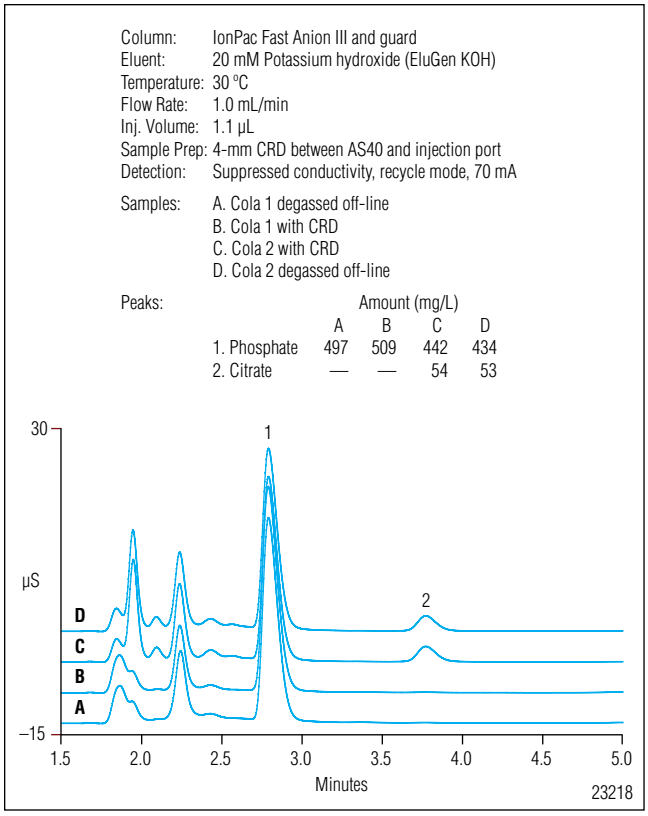



*Figure 2. Colas with carbonate removed on-line and off-line.*

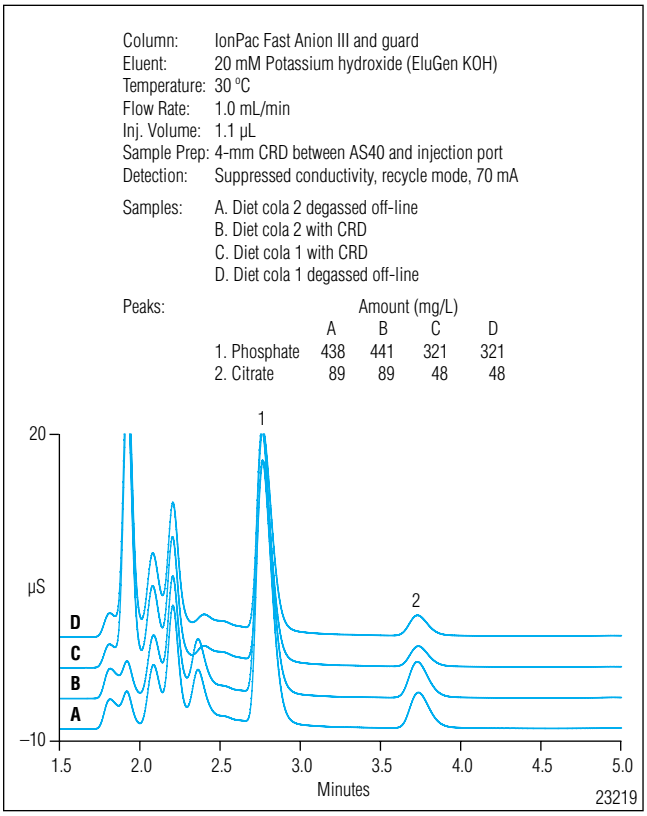



*Figure 3. Diet colas with carbonate removed on-line and off-line.*

**Table 4. Comparison of Cola Analyses Using On-Line and Off-Line Carbonate Removal**

| | | Phosphate | | | | Citrate | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Carbonate Removal** | **Retention Time (min)** | **RSD** | **Peak Area (µS-min)** | **RSD** | **Retention Time (min)** | **RSD** | **Peak Area (µS-min)** | **RSD** |
| Cola 1 | CRD | 2.79 ± 0.00 | 0.05 | 3.30 ± 0.01 | 0.22 | N.A.[b] | N.A. | N.A. | N.A. |
| Cola 1 | Off-line | 2.79 ± 0.00 | 0.00 | 3.23 ± 0.01 | 0.20 | N.A. | N.A. | N.A. | N.A. |
| Cola 2 | CRD | 2.79 ± 0.00 | 0.06 | 2.87 ± 0.00 | 0.13 | 3.77 ± 0.00 | 0.04 | 0.31 ± 0.00 | 0.08 |
| Cola 2 | Off-line | 2.79 ± 0.00 | 0.05 | 2.82 ± 0.01 | 0.20 | 3.77 ± 0.00 | 0.05 | 0.30 ± 0.00 | 0.27 |
| Diet cola 1 | CRD | 2.77 ± 0.00 | 0.06 | 2.08 ± 0.00 | 0.12 | 3.74 ± 0.00 | 0.04 | 0.28 ± 0.00 | 0.16 |
| Diet cola 1 | Off-line | 2.77 ± 0.00 | 0.04 | 2.08 ± 0.00 | 0.13 | 3.73 ± 0.00 | 0.04 | 0.28 ± 0.00 | 0.24 |
| Diet cola 2 | CRD | 2.76 ± 0.00 | 0.05 | 2.87 ± 0.00 | 0.11 | 3.73 ± 0.00 | 0.06 | 0.51 ± 0.00 | 0.22 |
| Diet cola 2 | Off-line | 2.76 ± 0.00 | 0.00 | 2.85 ± 0.01 | 0.22 | 3.73 ± 0.00 | 0.03 | 0.50 ± 0.00 | 0.25 |

[a] N.A. Not applicable.

Note: A standard deviation value of 0.00 represents a standard deviation less than the last significant figure for the experiment.

To determine whether these results were typical of on-line sample carbonate removal, we analyzed cola samples with three CRDs. Each CRD was used continuously for at least one week. During the course of these experiments, three lots of Cola 1 and two lots of Diet Cola 2 were tested. The results show excellent reproducibility and agreement between CRDs (Table 5) and cola lots. Cola 1 and Diet Cola 2 contained 509.2–513.9 mg/L and 448.7–454.7 mg/L of phosphate and 509.2–513.9 mg/L and 448.7–454.7 mg/L of citrate with an RSD of <0.15 %RSD (n = 20) for each analyte in each sample. The phosphate and citrate retention time reproducibility (2.74 ± 0.12 and 3.61 ± 0.00 min, n = 1317, respectively) and peak area reproducibility (<0.3 %RSD) for 950–2660 injections were as good as the original method.

Having established this method, it was applied to a wider range of samples, one reduced sugar cola and four diet colas (Table 6). For these five samples, the retention times for phosphate and citrate were 2.72–2.77 min and 3.56–3.70 min, respectively. The phosphate and citrate concentrations varied with product, ranging from 221–523 mg/L and 43–172 mg/L, respectively.

## CONCLUSION

Using the CRD to remove cola carbonation adds ~2.5 min to the total analysis time while retaining the precision (<0.15 %RSD for retention time), linearity ($r^2 > 0.999$), and reproducibility (<0.3 %RSD) of the original method. This eliminated the extra sample handling and the 20 min required for off-line degassing. Although only colas were analyzed, this sample preparation method can be used with other acidic carbonated samples.

**Table 5. Phosphate and Citrate Concentrations in Cola and Diet Cola Using Different CRDs**

| | Phosphate (mg/L) | | | | | | Citrate (mg/L) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CRD #1 | RSD | CRD #2 | RSD | CRD #3 | RSD | CRD #1 | RSD | CRD #2 | RSD | CRD #3 | RSD |
| Cola 1[a] | 509.2 ± 0.7 | 0.14 | 513.4 ± 0.6 | 0.12 | 513.9 ± 0.4 | 0.07 | N.D.[b] | | N.D.[b] | | N.D.[b] | |
| Diet cola 1[a] | 454.7 ± 0.7 | 0.14 | 454.3 ± 0.4 | 0.08 | 448.7 ± 0.3 | 0.06 | 86.1 ± 0.2 | 0.18 | 86.5 ± 0.2 | 0.23 | 85.6 ± 0.1 | 0.09 |

n = 20

[a] We tested three lots each of Diet cola 1 and Cola 1 during the course of the experiments. Not all three lots were tested on every CRD.

[b] N.D. None Detected

**Table 6. Product Analysis Using On-Line Carbonate Removal**

| | Phosphate | | | | Citrate | | | |
|---|---|---|---|---|---|---|---|---|
| | Retention Time (min) | RSD | Concentration (mg/L) | RSD | Retention Time (min) | RSD | Concentration (mg/L) | RSD |
| Reduced sugar cola 1 | 2.76 ± 0.00 | 0.05 | 523.3 ± 0.7 | 0.13 | 3.70 ± 0.00 | 0.05 | 90.0 ± 0.1 | 0.09 |
| Diet cola 3 | 2.77 ± 0.00 | 0.05 | 221.3 ± 0.1 | 0.07 | 3.66 ± 0.00 | 0.07 | 172.1 ± 0.2 | 0.14 |
| Diet cola 4 with flavoring | 2.72 ± 0.00 | 0.06 | 259.2 ± 0.1 | 0.04 | 3.56 ± 0.00 | 0.04 | 162.0 ± 0.2 | 0.12 |
| Diet cola 5 with flavoring | 2.72 ± 0.00 | 0.06 | 258.8 ± 0.3 | 0.10 | 3.57 ± 0.00 | 0.05 | 162.8 ± 0.2 | 0.09 |
| Diet cola 6 | 2.76 ± 0.00 | 0.06 | 397.9 ± 0.5 | 0.12 | 3.69 ± 0.00 | 0.04 | 42.9 ± 0.1 | 0.26 |

n = 10

Note: A standard deviation value of 0.00 represents a standard deviation less than the last significant figure for the experiment.

### Precautions

The AS40 injections induce pressure changes on the CRD, and over time these pressure changes will eventually cause the CRD to leak and fail. If a failure is suspected, test the CRD for leaks as discussed earlier in this document. Of the three CRDs tested for this application, only one was tested to failure. It failed after ~2660 injections. The other two CRDs were each subjected to ~950 injections.

## *REFERENCES*


1. Rapid Determination of Phosphate and Citrate in Carbonated Soft Drinks by Reagent-Free Ion Chromatography. Application Note 169, Literature Product No. 01774. Dionex Corporation, Sunnyvale, CA, 2005.
2. Reducing Carbonate Interference in Anion Determinations with the Carbonate Removal Device (CRD). Technical Note 62, Literature Product No. 01765. Dionex Corporation, Sunnyvale, CA, 2005.
3. ICS-2000 Ion Chromatography System Operator's Manual. Sections B.9 Installing and Plumbing Columns, B.13 Setting up the Eluent Generator, Literature Product No. 031857. Dionex Corporation, Sunnyvale, CA, March 2003. Pages B20–23, B30–B44.
4. Quickstart Procedure for the ASRS ULTRA II, Anion Self-Regenerating Suppressor. Literature Product No. 031951. Dionex Corporation, Sunnyvale, CA, January 2005.
5. Product Manual for IonPac Fast Anion III Guard Column and Analytical Column. Literature Product No. 065021. Dionex Corporation, Sunnyvale, CA, November 2004.
6. Application of Eluent Generation for Trace Analysis of Borated Waters. Application Note 166, p. 4, Literature Product No. 1654. Dionex Corporation, Sunnyvale, CA, October 2004.
7. Quickstart Instructions for Carbonate Removal Device (CRD). Literature Product No. 065069. Dionex Corporation, Sunnyvale, CA, June 2005.
8. Product Manual for the Carbonate Removal Device (CRD), (Figure 4, p.7, CRD Mounted on SRS ) Literature Product No. 065068. Dionex Corporation, Sunnyvale, CA, June 2005.
9. AS40 Automated Sampler Operator's Manual, Literature Product No. 034970. Dionex Corporation, Sunnyvale, CA, June 2004.
10. ICS-2000 Ion Chromatography System Operator's Manual, Sections B.19 "Connecting an AS40 Automated Autosampler (Optional)" and B.8 "Setting up Chromeleon", pp. B20–B23, B30–B44. Literature Product No. 031857. Dionex Corporation, Sunnyvale, CA, March 2003.
11. Product Manual for Carbonate Removal Device. Literature Product No. 065068-02. Dionex Corporation, Sunnyvale, CA June 2005.


## *SUPPLIERS*

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

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

Sigma-Aldrich, Inc., P.O. Box 951524, Dallas, TX 75395-1524 USA, 1-800-325-3010. www.sigmaaldrich.com

Thermo
SCIENTIFIC

# 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 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *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 | | | | | | | | | | | | | | | | | | | | | | | | |

| | | 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<br>4 × 250 mm | Carbonate | Recommended column for inorganic anions and oxyhalides. Trace bromate in drinking water. | 6 µm | 55% | - | - | 80 µeq<br>320 µeq | Alkyl quaternary ammonium | Ultralow |
| IonPac AS22 | 2 × 250 mm<br>4 × 250 mm | Carbonate | Recommended column for fast analysis of common inorganic anions. | 6.5 µm | 55% | - | - | 52.5 µeq<br>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<br>4 × 250 mm | Hydroxide | Recommended column for trace perchlorate prior to suppressed conductivity detection. | 7.5 µm | 55% | - | - | 77.5 µeq<br>310 µeq | Alkanol quaternary ammonium | Ultralow |
| IonPac AS19 | 2 × 250 mm<br>4 × 250 mm | Hydroxide | Recommended column for inorganic anions and oxyhalides. Trace bromate in drinking water. | 7.5 µm | 55% | - | - | 60 µeq<br>350 µeq | Alkanol quaternary ammonium | Low |
| IonPac AS18 | 2 × 250 mm<br>4 × 250 mm | Hydroxide | Recommended column for the analysis of common inorganic anions. | 7.5 µm | 55% | 65 nm | 8% | 75 µeq<br>285 µeq | Alkanol quaternary ammonium | Low |
| IonPac AS17-C | 2 × 250 mm<br>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<br>30 µeq | Alkanol quaternary ammonium | Low |
| IonPac AS16 | 2 × 250 mm<br>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<br>170 µeq | Alkanol quaternary ammonium | Ultralow |
| IonPac AS15 | 2 × 250 mm<br>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<br>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<br>4 × 250 mm | Carbonate | Moderate capacity for fast analysis of common inorganic anions. | 9 µm | 55% | - | - | 16 µeq<br>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<br>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<br>52 µeq | Alkyl quaternary ammonium | Medium |
| IonPac AS11-HC | 2 × 250 mm<br>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<br>290 µeq | Alkanol quaternary ammonium | Medium-Low |
| IonPac AS11 | 2 × 250 mm<br>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<br>45 µeq | Alkanol quaternary ammonium | Very Low |
| IonPac AS10 | 2 × 250 mm<br>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<br>170 µeq | Alkyl quaternary ammonium | Low |
| IonPac AS9-HC | 2 × 250 mm<br>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<br>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<br>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<br>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<br>4 × 250 mm | MSA | Recommended column for hydrophobic and polyvalent amines (biogenic amines and diamines) | 7 µm | 55% | - | - | 0.363 µeq<br>1.45 µeq | Carboxylic acid | Very Low |
| IonPac CS16 | 3 × 250 mm<br>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<br>8.4 µeq | Carboxylic acid | Medium |
| IonPac CS15 | 2 × 250 mm<br>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<br>2.8 µeq | Carboxylic acid/ phosphonic acid/ crown ether | Medium |
| IonPac CS14 | 2 × 250 mm<br>4 × 250 mm | MSA | Aliphatic amines, aromatic amines, and polyamines plus mono- and divalent cations. | 8.5 µm | 55% | - | - | 0.325 µeq<br>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<br>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<br>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<br>4 × 250 mm | Pyridine dicarboxylic acid | Recommended column for transition and lanthanide metals analysis. Aluminum analysis. | 9 µm | 55% | 140 nm<br>75 nm | 10%<br>20% | 0.02 µeq/<br>0.005 µeq<br>0.04 µeq/<br>0.01 µeq | Sulfonic acid/ alkanol quaternary ammonium | - |

## Ion-Exclusion Columns

| Column | Format | Primary Eluent | Application | Particle Diameter | Substrate Crosslinking | Latex Diameter | Latex Crosslinking | Capacity (per column) | Functional Group | Hydrophobicity |
|---|---|---|---|---|---|---|---|---|---|---|
| IonPac ICE-AS1 | 4 × 250 mm<br>9 × 250 mm | Heptafluorobutyric acid | Organic acids in high ionic strength matrices. Fast separation of organic acids. | 7.5 µm | 8% | - | - | 5.3 µeq<br>27 µeq | Sulfonic acid | Ultra Low |
| IonPac ICE-AS6 | 9 × 250 mm | Heptafluorobutyric acid | Organic acids in complex or high ionic strength matrices. | 8 µm | 8% | - | - | 27 µeq | Sulfonic and carboxylic acid | Moderate |
| IonPac ICE-Borate | 9 × 250 mm | MSA/ Mannitol | Trace concentrations of borate | 7.5 µm | 8% | - | - | 27 µeq | Sulfonic acid | Ultra Low |

## Acclaim General and Specialty Columns

| Column | Bonded Phase | USP Type | Endcapped | Substrate | Particle Shape | Particle Size | Metal Impurity (ppm) Na, Fe, AL | Average Pore Diameter | Surface Area ($m^2/g$) | Total Carbon Content |
|---|---|---|---|---|---|---|---|---|---|---|
| Mixed-Mode WAX | Proprietary alkyl amine | na | Proprietary | Ultrapure silica | Spherical | 5 µm | <10 ppm | 120 Å | 300 | na |
| Mixed-Mode HILIC | Proprietary alkyl diol | na | Proprietary | | | 5 µm | | 120 Å | 300 | na |
| Mixed-Mode WCX | Proprietary alkyl carboxyl | na | Proprietary | | | 5 µm | | 120 Å | 300 | na |
| Organic Acid (OA) | Proprietary | na | Yes | | | 5 µm | | 120 Å | 300 | 17% |
| Surfactant and Explosives E1/2 | Proprietary | na | Yes | | | 5 µm | | 120 Å | 300 | na |
| 120 C18 | C18 | L1 | Yes | | | 2, 3 and 5 µm | | 120 Å | 300 | 18% |
| 120 C8 | C8 | L7 | Yes | | | 3 and 5 µm | | 120 Å | 300 | 11% |
| 300 C18 | C18 | L1 | Yes | | | 3 µm | | 300 Å | 100 | 7% |
| Polar Advantage | Sulfamido C16 | na | Yes | | | 3 and 5 µm | | 120 Å | 300 | 17% |
| Polar Advantage II | Amide C18 | na | Yes | | | 2, 3 and 5 µm | | 120 Å | 300 | 17% |
| HILIC | Proprietary hydrophilic | | Yes | | | 3 µm | | 120 Å | 300 | |
| Phenyl-1 | Proprietary alkyl phenyl | | Yes | | | 3 µm | | 120 Å | 300 | |
| Carbamate | Proprietary alkyl group | | Yes | | | 3 and 5 µm | | 120 Å | 300 | |
| Trinity | | | Yes | | | | | 120 Å | 300 | |

# Bio Columns

## Protein

| Column | Phase | Target Applications | Base Matrix Material | Substrate Crosslinking | Capacity | Recommended Flow Rate | Solvent Compatibility | Maximum Backpressure | pH Range |
|---|---|---|---|---|---|---|---|---|---|
| MAbPac SEC-1 | | | | | | | | | |
| MAbPac SCX-10 | | | | | | | | | |
| ProPac WCX-10 | Weak Cation Exchange | High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units | 10-µm diameter nonporous substrate to which is grafted a polymer chain bearing carboxylate groups. | 55% | 6 mg/ mL lysozyme | 0.2–2 mL/min | 80% ACN, acetone. Incompatable with alcohols and MeOH | 3000 psi (21 MPa) | 2–12.0 |
| ProPac SCX-10 | Strong Cation Exchange | High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units | 10 µm diameter nonporous substrate to which is grafted a polymer chain bearing sulfonate groups. | 55% | 3 mg/ mL lysozyme | 0.2–2.0 mL/min | 80% ACN, acetone, MeOH | 3000 psi (21 MPa) | 2–12.0 |
| ProPac SCX-20 | | | | | | | | | |
| ProPac WAX-10 | Weak Anion Exchange | High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units | 10 µm diameter non-porous substrate to which is grafted a polymer chain bearing tertiary amine groups. | 55% | 5 mg/ mL BSA/ mL | 0.2–2.0 mL/min | 80% ACN, acetone, MeOH, | 3000 psi (21 MPa) | 2–12.0 |
| ProPac SAX-10 | Strong Anion Exchange | High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units | 10 µm diameter non-porous substrate with grafted polymer chain bearing quaternary ammonium groups. | 55% | 15 mg/ mL BSA | 0.2–2.0 mL/min | 80% ACN, acetone, MeOH | 3000 psi (21 MPa) | 2–12.0 |
| ProSwift RP-1S | Reversed-Phase | Fast protein separation with high capacity using Reversed Phase | Monolith; polystyrene-divinylbenzene with phenyl functional group | Monolith Standard permeability | 5.5 mg/mL Insulin | 2–4 mL/min | Most common organic solvents | 2800 psi (19.2 Mpa) | 1–14 |
| ProSwift RP-2H | Reversed-Phase | Fast protein separation with high capacity using Reversed Phase | Monolith; polystyrene-divinylbenzene with phenyl functional group | Monolith High permeability | 1.0 mg/mL Lysozyme | 1–10 mL/min | Most common organic solvents | 2800 psi (19.3 Mpa) | 1–14 |
| ProSwift RP-4H | | | | | | | | | |
| ProSwift RP-3U | Reversed-Phase | Fast protein separation with high capacity using Reversed Phase | Monolith; polystyrene-divinylbenzene with phenyl functional group | Monolith Ultrahigh permeability | 0.5 mg/mL Lysozyme | 1– 16 mL/min | Most common organic solvents | 2800 psi (19.3 Mpa) | 1–14 |
| ProSwift SAX-1S | Strong Anion Exchange | Fast protein separation with good resolution using Anion Exchange | Monolith; polymethac-rylate with quaternary amine functional group | Monolith Standard permeability | 18 mg/mL BSA | 0.5–1.5 (4.6 mm), 0.05–.25 (1.0 mm) | Most common organic solvents | 1000 psi (4.6 mm) 2000 psi (1.0 mm) | 2–12.0 |
| ProSwift SCX-1S | Strong Cation Exchange | Fast protein separation with good resolution using Cation Exchange | Monolith; polymethac-rylate with sulfonic acid fuctional group | Monolith Standard permeability | 30 mg/mL Lysozyme | 0.5–1.5 mL/min (4.6 mm) | Most common organic solvents | 1000 psi (4.6 mm) | 2–12.0 |
| ProSwift WAX-1S | Weak Anion Exchange | Fast protein separation with good resolution using Anion Exchange | Monolith; polymethacrylate with tertiary amine (DEAE) functional group | Monolith Standard permeability | 18 mg/mL BSA | 0.5–1.5 mL/min (4.6 mm), 0.05–.25 (1.0 mm) | Most common organic solvents | 1000 psi (4.6 mm) 2000 psi (1.0 mm) | 2–12.0 |
| ProSwift WCX-1S | Weak Cation Exchange | Fast protein separation with good resolution using Cation Exchange | Monolith; polymethacrylate with carboxylic acid (CM) functional group | Monolith Standard permeability | 23 mg/mL Lysozyme | 0.5–1.5 mL/min (4.6 mm), 0.05–.20 (1.0 mm) | Most common organic solvents | 1000 psi (4.6 mm) 2000 psi (1.0 mm) | 2–12.0 |
| ProPac IMAC-10 | Immobilized Metal Affinity | High resolution separation of certain metal-binding proteins and peptides | 10 µm diameter non-porous polystyrene divinylbenzene substrate with poly (IDA) grafts. | 55% | >60 mg lysozyme/ mL gel (4 x 250 mm) | 1.0 mL/min | EtOH, urea, NaCl, non- ionic detergents, glycerol, acetic acid, guanidine HCl | 3000 psi (21MPa) | 2–12 |
| ProSwift ConA-1S | | | | | | | | | |
| ProPac HIC-10 | Reversed-Phase | Protein separation using hydrophobic interaction with salt gradient elution | Spherical 5 µm, ultrapure silica, 300 A, surface area 100 $m^2$/ g, | n/a | 340 mg lysozyme per 7.8 x 75 mm column | 1.0 mL/ min | 2M Ammonium sulfate/ phosphate salts, organic solvent for cleanup | 4,000 psi | 2.5–7.5 |

## Carbohydrate

| Column | Target Applications | Base Matrix Material | Substrate Crosslinking | Latex Crosslinking | Capacity | Recommended Eluents | Recommended Flow Rate | Solvent Compatibility | Maximum Backpressure | pH Range |
|---|---|---|---|---|---|---|---|---|---|---|
| CarboPac MA1 | Reduced mono- and disaccharide analysis. | 7.5 µm diameter macroporous substrate fully functionalized with an alkyl quaternary ammonium group | 15% | No latex | 1450 µeq (4 × 250 mm) | Hydroxide | 0.4 mL/min | 0% | 2000 psi (14 MPa) | 0–14 |
| CarboPac PA1 | General purpose mono-, di-, and oligosaccharide analysis | 10 µm diameter nonporous substrate agglomerted with a 500 nm MicroBead quaternary ammonium functionalized latex | 2% | 5% | 100 µeq (4 × 250 mm) | Hydroxide, acetate/ hydroxide | 1.0 mL/min | 0–5% | 4000 psi (28 MPa) | 0–14 |
| CarboPac PA10 | Monosaccharide compositonal anaylysis | 10 µm diameter nonporous substrate agglomerated with a 460 nm MicroBead di-functionalized latex | 55% | 5% | 100 µeq (4 × 250 mm) | Hydroxide, acetate/ hydroxide | 1.0 mL/min | 0–90% | 3500 psi (24.5 MPa) | 0–14 |
| CarboPac PA20 | Fast mono-, and disaccharide analysis | 6.5 µm diameter nonporous substrate agglomerated with a 130 nm MicroBead quaternary ammonium functionalized latex | 55% | 5% | 65 µeq (3 × 150 mm) | Hydroxide, acetate/ hydroxide | 0.5 mL/min | 0–100% | 3000 psi (21 MPa) | 0–14 |
| CarboPac PA100 | Oligosaccharide mapping and analysis | 8.5 µm diameter nonporous substrate agglomerated with a 275 nm MicroBead di-functionalized latex | 55% | 6% | 90 µeq (4 × 250 mm) | Hydroxide, acetate/ hydroxide | 1.0 mL/min | 0–90% | 4000 psi (28 MPa) | 0–14 |
| CarboPac PA200 | High resolution oligosaccharide mapping and analysis | 5.5 µm diameter nonporous substrate agglomerated with a 43 nm MicroBead quaternary ammonium functionalized latex | 55% | 6% | 35 µeq (3 × 250 mm) | Hydroxide, acetate/ hydroxide | 0.5 mL/min | 0–100% | 4000 psi (28 MPa) | 0–14 |

## DNA

| Column | Target Applications | Base Matrix Material | Substrate Crosslinking | Latex Crosslinking | Capacity | Recommended Eluents | Recommended Flow Rate | Solvent Compatibility | Max. Backpressure | pH Range |
|---|---|---|---|---|---|---|---|---|---|---|
| DNAPac PA100 | Single stranded DNA or RNA oligonucleotides, restriction fragments, glycoprotein isoforms. | 13-µm diameter nonporous substrate agglomerated with a 100-nm MicroBead alkyl quaternary ammonium functionalized latex. | 55% | 5% | 40 µeq | Chloride, acetate, bromide, perchlorate: in lithium sodium or ammonium forms | 1.5 mL/min | 0–100% | 4000psi (28MPa) | 2–12.5 |
| DNAPac PA200 | High resolution single stranded DNA or RNA oligonucleotides, restriction fragments, glycoprotein isoforms. | 8-µm diameter nonporous substrate agglomerated with a 130-nm MicroBead alkyl quaternary ammonium functionalized latex. | 55% | 5% | 40 µeq | Chloride, acetate, bromide, perchlorate: in lithium sodium or ammonium forms | 1.2 mL/min | 0–100% | 4000psi (28MPa) | 2–12.5 |
| DNASwift | | | | | | | | | | |

AccuStandard is a trademark of AccuStandard Inc.
Acrodisc is a registered trademark of Pall Corporation.
Anke is a registered trademark of Anting Scientific Instrumental Factory.
Anotop is a trademark of Whatman.
BD and Bacto are trademarks of Becton, Dickinson, and Company.
Bigelow is a registered trademark of R.C. Bigelow, Incorporated.
Diet Rite is a registered trademarks of Royal Crown Company, Inc.
Enfamil is a registered trademark of Bristol-Myers Squibb Co., Mead Johnson Nutritionals.
Good Start and Alsoy are registered trademarks of Nestle USA, Inc., Nutrition Division.
IKA is a registered trademark of IKA Works.
Kudos is a registered trademark of Kudos Ultrasonic Instrumental Company.
Lipton is a registered trademark of the Unilever Group of Companies.
MICRO DIST is a trademark of Lachat Instruments, a Hach Company.
Milli-Q and Millex are registered trademarks of Millipore Corporation.
Minute Maid is a registered trademark of the Coca-Cola Company.
PEEK is a trademark of Victrex PLC.
Phenomenex is a trademark of Phenomenex, Inc.
Pyrex is a registered trademark of Corning Glass Works.
Similac and Isomil are registered trademarks of Abbott Laboratories, Ross Products Division.
Splenda is a registered trademark of McNeil-PPC, Inc.
Strata is a trademark of Shine Technology.
Sunett is a registered trademark of Nutrinova.
Supelcosil is a trademark of Sigma-Aldrich Co.
SUPELCOWAX 10 is a trademark of Supelco, Inc.
Teflon is a registered trademark of E. I. du Pont de Nemours.
TurboVap is a registered trademark of Caliper Life Sciences.
ULTREX is a registered trademark of J.T. Baker.

All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.



**Dionex Products:** 1228 Titan Way, PO Box 3603, Sunnyvale, CA 94088-3603, (408) 737-0700
**North America:** U.S./Canada (847) 295-7500
**South America:** Brazil (55) 11 3731 5140
**Europe:** Austria (43) 616 51 25, Benelux (31) 20 683 9768 (32) 3 353 4294
Denmark (45) 36 36 90 90, France (33) 1 39 30 01 10, Germany (49) 61125 991 0
Ireland (353) 644 0064, Italy (39) 02 51 62 1267, Sweden (46) 8 473 3380,
Switzerland (41) 62 205 9966, United Kingdom (44) 1276 691722
**Asia Pacific:** Australia (61) 2 9420 5233, China (852) 2428 3282, India (91) 22 2764 2735,
Japan (81) 6885 1213, Korea (82) 2 2653 2580, Singapore (65) 6289 1190,
Taiwan (886) 2 875 6655

**www.thermoscientific.com/dionex**

LPN 3085 02/12

Now sold under the
Thermo Scientific brand

Thermo
SCIENTIFIC