DIONEX

Application Note 25

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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)				
	IonPac AG11 Guard (4 mm)				
	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				

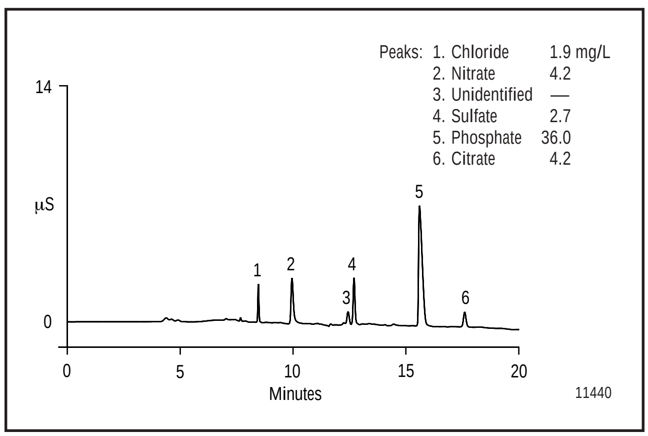

Figure 1 *Separation of inorganic anions and organic acids in a cola by anion exchange chromatography. Conditions as listed in Table 1.*

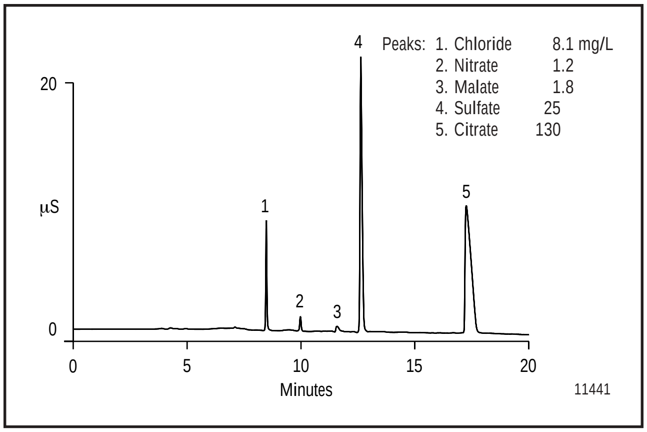

Figure 2 *Separation of inorganic anions and organic acids in a carbonated lemon drink by anion exchange chromatography. Conditions as listed in Table 1.*

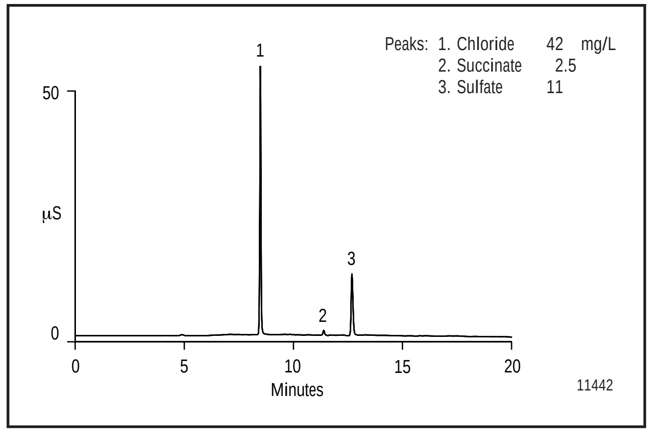

Figure 3 *Separation of inorganic anions and organic acids in a flavored carbonated water by anion exchange chromatography. Conditions as listed in Table 1.*

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 methanesulfonic acid.

Figures 5–8 show the separation of cations in a series of carbonated beverages by cation exchange chromatography. The samples were degassed and diluted

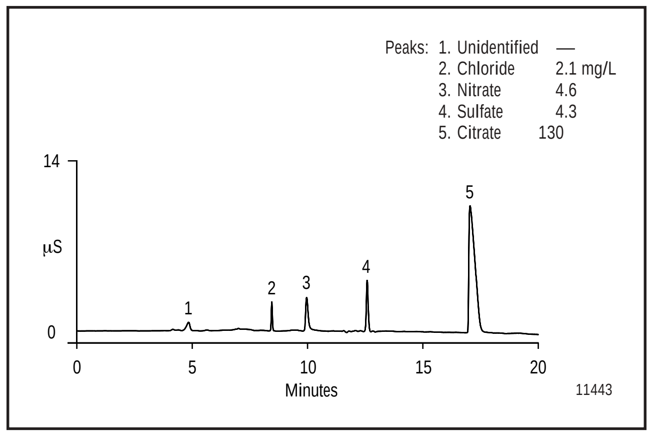

Figure 4 *Separation of inorganic anions and organic acids in a carbonated synthetic grape drink by anion exchange chromatography. Conditions as listed in Table 1.*

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		

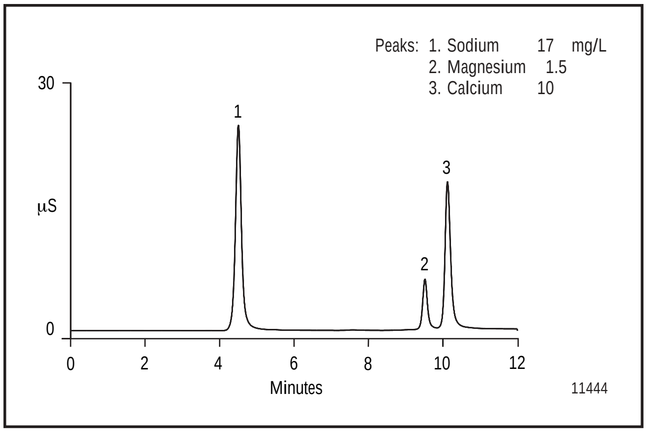

Figure 5 *Separation of inorganic cations in a carbonated lemon drink by cation exchange chromatography. Conditions as listed in Table 2.*

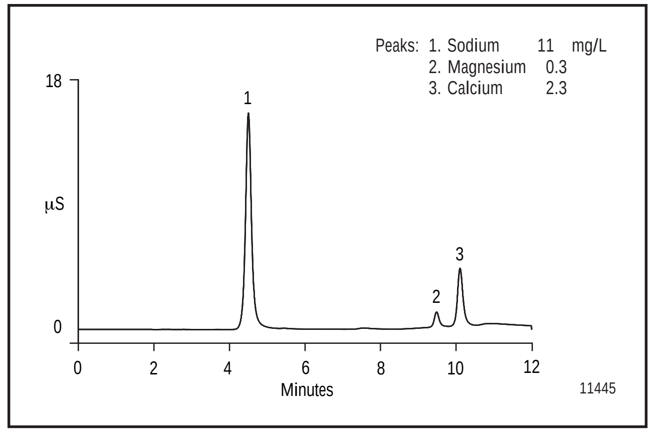

Figure 6 *Separation of inorganic cations in a carbonated, synthetic grape drink by cation exchange chromatography. Conditions as listed in Table 2.*

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.

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_as. 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 the IonPac ICE-AS6 is 0.4 mM heptafluorobutyric acid (perfluorobutyric acid). Although

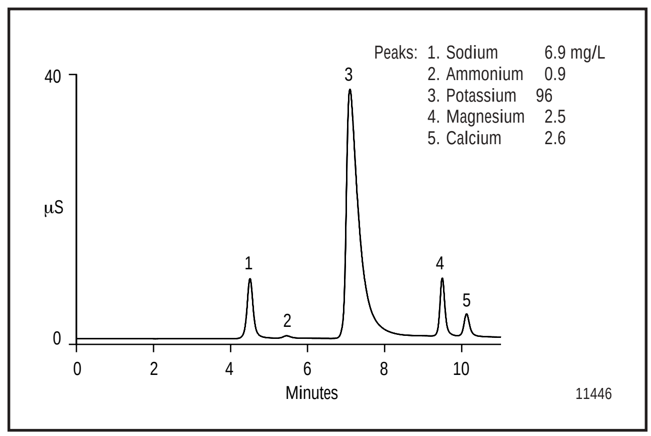

Figure 7 *Separation of inorganic cations in a carbonated apple juice by cation exchange chromatography. Conditions as listed in Table 2.*

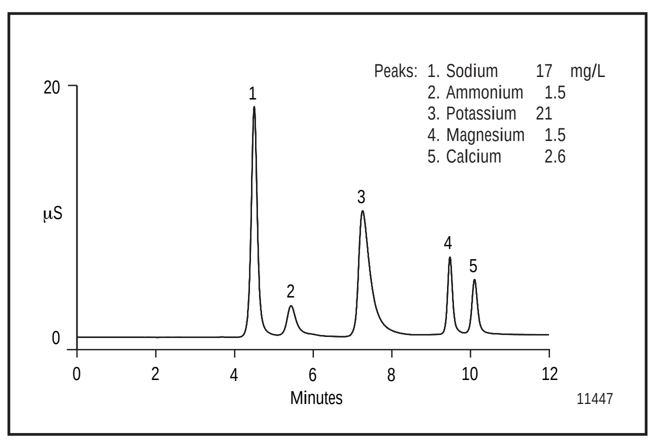

Figure 8 *Separation of inorganic cations in a carbonated grape juice by cation exchange chromatography. Conditions as listed in Table 2.*

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

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 carbonated 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.

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 preparation 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.

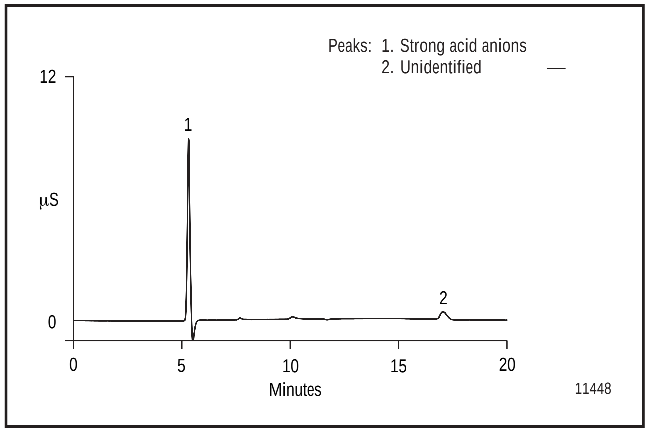

Figure 9 *Anionic profile of a flavored carbonated water by ion exclusion chromatography. Conditions as listed in Table 3.*

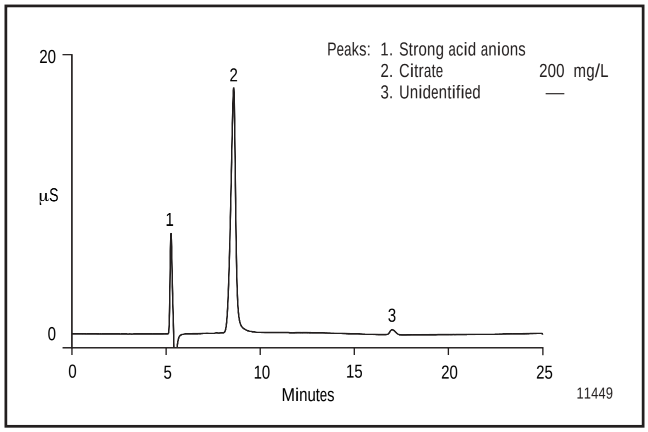

Figure 10 *Separation of organic acids in a carbonated lemon drink by ion exclusion chromatography. Conditions as listed in Table 3.*

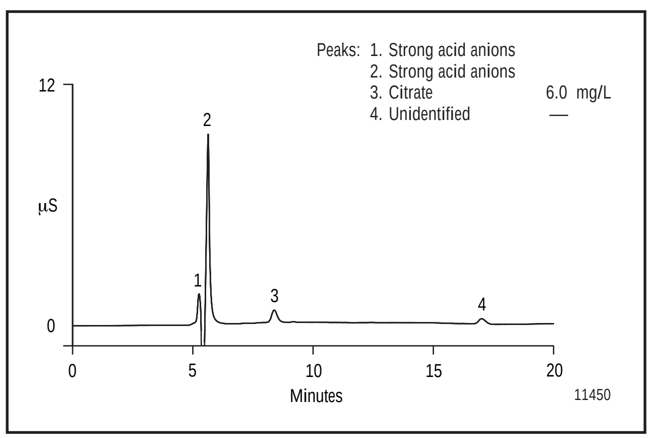

Figure 11 *Separation of organic acids in a cola by ion exclusion chromatography. Conditions as listed in Table 3.*

LIST OF SUPPLIERS

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

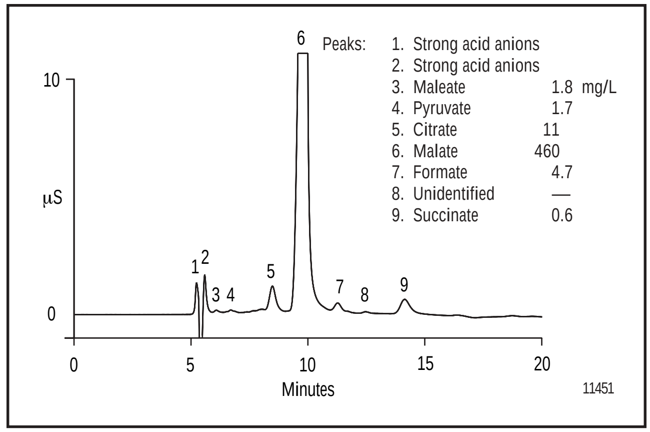

Figure 12 *Separation of organic acids in a carbonated apple juice by ion exclusion chromatography. Conditions as listed in Table 3.*

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