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

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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 Thermo Scientific Application Note (AN) 169: *Rapid Determination of Phosphate and Citrate in Carbonated Soft Drinks Using a Reagent-Free™ Ion Chromatography System*[1], we showed that a Thermo Scientific Dionex ICS-2000 Reagent-Free ion chromatography (RFIC™) system outfitted with an Thermo Scientific™ Dionex™ 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 Thermo Scientific Dionex CRD Carbonate Removal Device into the RFIC method. The Dionex CRD device 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 Dionex CRD device are equivalent to determinations of samples degassed off-line.

Equipment

Dionex ICS-2000 RFIC fully integrated IC system*:

- AS40 Autosampler with 5 mL PolyVial™ vials and plain
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) Software, Workstation 6.7
- Filter unit, 0.2 µm nylon
- Vacuum pump
- PEEK tubing
  - Black (0.25 mm or 0.01 in. i.d., for 5 ft) for connecting columns and Thermo Scientific™ Dionex™ ASRS™ ULTRA II suppressor backpressure loops.
  - Orange (0.51 mm or 0.02 in. i.d., for 5 ft) to make the injection line from Thermo Scientific Dionex AS40 autosampler to Dionex CRD device to injection valve
  - Red (0.127 mm or 0.005 in. i.d., for 5 ft) to make a 1.2 µL loop
  - Yellow (0.076 mm or 0.003 in. i.d., for 5 ft) for system backpressure loop
- Low-pressure Teflon® tubing (1.6 mm or 0.063 in. i.d.) tubing for the Dionex CRD device and degas waste lines
- Micropipettor and tips for preparing samples, standards, and pipetting samples into vials

**Equivalent or improved results can be achieved using the Thermo Scientific Dionex ICS-2100 system.*

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
- Trisodium citrate, dihydrate

Samples

- Regular colas 1 and 2
- Reduced sugar cola 1
- Diet colas 1–4, diet colas 5–6 with flavoring

Conditions	
Columns:	Dionex IonPac Fast Anion III, 3 × 250 mm Dionex IonPac Guard, 3 × 50 mm
Flow Rate:	1.0 mL/min
Eluent:	Thermo Scientific Dionex EluGen KOH cartridge, 20 mM Potassium hydroxide
Temperature:	30 °C
Inj. Volume:	1.2 µL
Sample Prep:	4 mm Dionex CRD device 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. 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 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 ($NaOOCCH_2C(OH)(COONa)$ $(CH_2COONa{\bullet}2H_2O)$)	1.555
Phosphate	Sodium phosphate dibasic, anhydrous (Na_2HPO_4)	1.495

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 Dionex EluGen II potassium hydroxide cartridge, Thermo Scientific Dionex CR-ATC Continuously Regenerating Anion Trap Column, columns, Dionex ASRS ULTRA II suppressor, and backpressure loops for the suppressor and the eluent generator, refer to the Installation section of the Dionex ICS-2000 Operator's Manual.[3] Hydrate the Dionex CR-ATC column and Dionex ASRS ULTRA II suppressor according to the Dionex ICS-2000 system Installation and Quickstart[4] instructions. Install both the Dionex EluGen II KOH cartridge and the Dionex CR-ATC column in the Dionex ICS-2000 cartridge holder and condition the Dionex EluGen II KOH cartridge. Install the columns after the injection valve and heat exchanger according to the Dionex IonPac Fast Anion III Product Manual[5] and the Dionex ICS-2000 system 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 [now part of Thermo Scientific] 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.

Dionex CRD Device Installation

Typically, the Dionex CRD device (for theory and operation, see the Dionex CRD device 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 Dionex CRD device is used as a sample preparation device to remove carbonate from the colas during injection; therefore it is installed between the Dionex AS40 autosampler and the injection valve. Hydrate the 4 mm Dionex CRD device according to the QuickStart Instructions.[7] Because the Dionex CRD device is a membrane- based device, high pressure can irreversibly damage it. Always remove all plugs from the Dionex CRD device before hydrating it or installing any tubing. Replace the PEEK injection tubing on the bleed valve of the Dionex 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 Dionex CRD device. Cut another ~20 cm (8 in.) piece of orange PEEK and install one end into the “Eluent Out” port of the Dionex CRD device 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 Dionex CRD device. Connect one end of another length of Teflon tubing into the “Regen Out” port of the Dionex CRD device and direct the other end to waste. The Dionex CRD device is designed to slip over the top of the suppressor. The Dionex CRD device can be positioned either outside the Dionex ICS-2000 system or secured on the Dionex ASRS ULTRA II suppressor according to Figure 1.[8]

Figure 1. Carbonate removal device.

Dionex AS40 Autosampler Setup

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

Configuring the Dionex AS40 Autosampler in the Chromeleon Timebase and Program

To configure the timebase with only the Dionex ICS-2000 system, open the Chromeleon CDS software configure program, create a new timebase, and add the Dionex ICS-2000 system, according to the instructions in the Dionex 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 Dionex AS40 autosampler cannot be added during Server Configuration, Chromeleon CDS software, version 6.7 will automatically detect it and add it to the Panel.

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

Table 3. Entries in Chromeleon CDS Software Program Wizard for the Dionex 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 Dionex CRD Device is Operational

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

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.

Column: Dionex IonPac Fast Anion III and guard
Eluent: 20 mM Potassium hydroxide (Dionex EluGen KOH cartridge)
Temperature: 30 °C
Flow Rate: 1.0 mL/min
Inj. Volume: 1.1 µL
Sample Prep: 4 mm Dionex CRD device between Dionex AS40 autosampler and injection port
Detection: Suppressed conductivity, recycle mode, 70 mA
Samples: A. Cola 1 degassed off-line
B. Cola 1 with CRD
C. Cola 2 with CRD
D. Cola 2 degassed off-line

Peaks: Amount (mg/L)

	A	B	C	D
1. Phosphate	497	509	442	434
2. Citrate	—	—	54	53

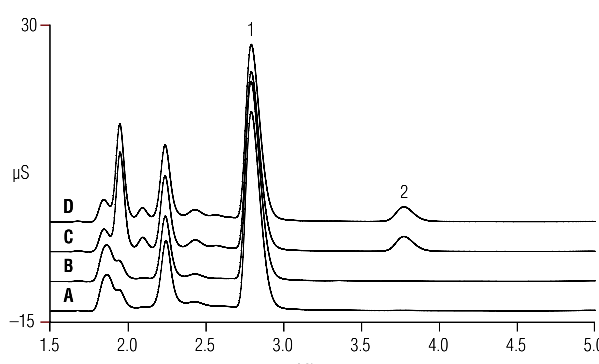

Figure 2. Colas with carbonate removed on-line and off-line.

Column: Dionex IonPac Fast Anion III and guard
Eluent: 20 mM Potassium hydroxide (Dionex EluGen KOH cartridge)
Temperature: 30 °C
Flow Rate: 1.0 mL/min
Inj. Volume: 1.1 µL
Sample Prep: 4 mm Dionex CRD device between Dionex AS40 autosampler and injection port
Detection: Suppressed conductivity, recycle mode, 70 mA
Samples: A. Diet cola 2 degassed off-line
B. Diet cola 2 with CRD
C. Diet cola 1 with CRD
D. Diet cola 1 degassed off-line

Peaks: Amount (mg/L)

	A	B	C	D
1. Phosphate	438	441	321	321
2. Citrate	89	89	48	48

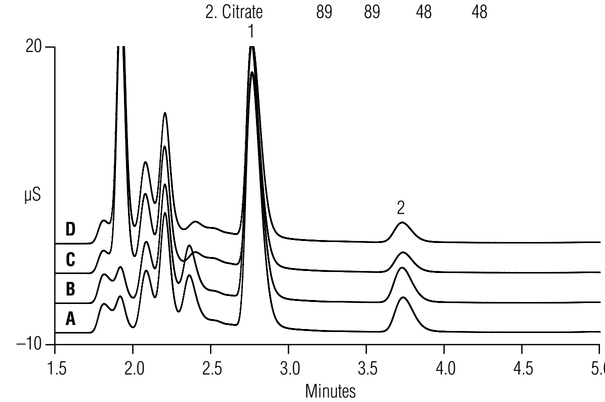

Figure 3. Diet colas with carbonate removed on-line and off-line.

Table 4. Comparison of cola analyses using on-line and off-line carbonate removal.

	Carbonate Removal	Phosphate				Citrate			
		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.[a]	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.

Table 5. Phosphate and citrate concentrations in cola and diet cola using different CRDs.

	Phosphate						Citrate					
	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	Retention Time (min)	RSD	Retention Time (min)	RSD	Retention Time (min)	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.

Conclusion

Using the Dionex CRD device 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.

Precautions

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

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