

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

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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, coagulants, 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 2000 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 Thermo Scientific™ Dionex™ 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 Thermo Scientific™ Dionex™ IonPac™ Fast Anion III column—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 Dionex IonPac Fast Anion III column.

Equipment

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

- Eluent Generator
- Column Heater
- Pump with Degasser
- Thermo Scientific Dionex EluGen EGC-KOH Cartridge
- Thermo Scientific Dionex CR-ATC Continuously Regenerating Anion Trap Column
- Thermo Scientific Dionex AS50 Autosampler
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) Software, Workstation 6.6

Reagents and Standards

- Deionized water, Type I reagent grade, 18 MΩ-cm resistivity or better
- Sodium citrate dihydrate
- Phosphate standard 1000 mg/L, 100 mL

Conditions

Columns:	Dionex IonPac Fast Anion III Analytical, 3 × 250 mm**
Eluent:	20 mM potassium hydroxide
Eluent Source:	Dionex ICS-2000 EG with Dionex CR-ATC column
Flow Rate:	1.0 mL/min
Temperature:	30 °C
Inj. Volume:	1.2 µL
Detection:	Suppressed conductivity, Thermo Scientific™ Dionex™ ASRS™ ULTRA II Anion Self-Regenerating Suppressor, 2 mm, 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. Equivalent or improved results can be achieved using a Thermo Scientific Dionex ICS-2100 system.

** 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 phosphate 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 Dionex EGC-KOH cartridge in the Dionex ICS-2000 system and configure it with Chromeleon CDS software. Condition the cartridge as directed by the Thermo Scientific Dionex 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 Dionex CR-ATC column between the Dionex EGC II KOH cartridge and Dionex EGC degas. For more information on installing the Dionex CR-ATC column, consult the Dionex EGC II Cartridge Quickstart Guide.

Install and configure the Dionex 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 Dionex AS50 autosampler 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 CDS software configuration window, set the Dionex AS50 autosampler to operate in the sample overlap mode to reduce the time between injections and therefore increase sample throughput. Refer to the Dionex AS50 Autosampler Operator's Manual (Document No. 31169) for further details on configuring the Dionex AS50 autosampler.

Install a 3 × 250 mm Dionex 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 Dionex ICS-2000 system 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 Dionex ASRS ULTRA II suppressor 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 Dionex ASRS ULTRA II suppressor for use in the recycle mode according to the Installation and Troubleshooting instructions for the Dionex ASRS ULTRA II suppressor (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 Dionex 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 Dionex IonPac Fast Anion III column is an important advancement in Thermo Scientific Dionex polymer-bonding technology that uses an anion-exchange resin with an optimized selectivity and capacity (55 $\mu\text{eq}/\text{column}$) for the rapid determination of phosphate and citrate. The Dionex IonPac Fast Anion III column 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 Dionex IonPac Fast Anion III column contains alternating 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.

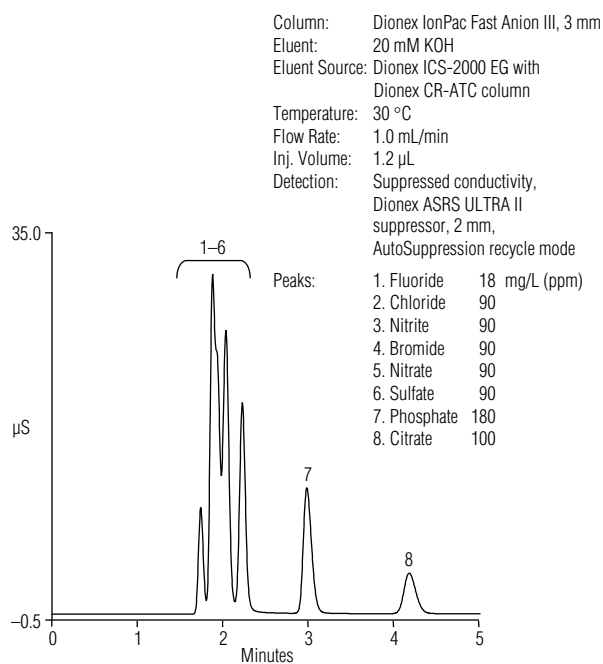


Figure 1. Analysis of seven common anions and citrate on the Dionex IonPac Fast Anion III column.

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 μ L) 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. 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 precision 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.

Table 1. Concentrations of phosphate and citrate found in different carbonated colas using the IonPac Fast Anion III column.

Sample	Phosphate			Citrate		
	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

^aRSD = relative standard deviation, $n = 20$

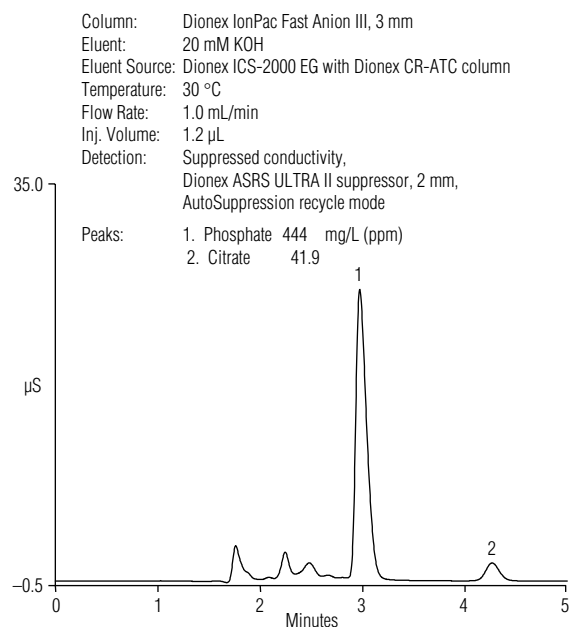


Figure 2. Determination of phosphate and citrate in low carbohydrate Cola A on the Dionex IonPac Fast Anion III column.

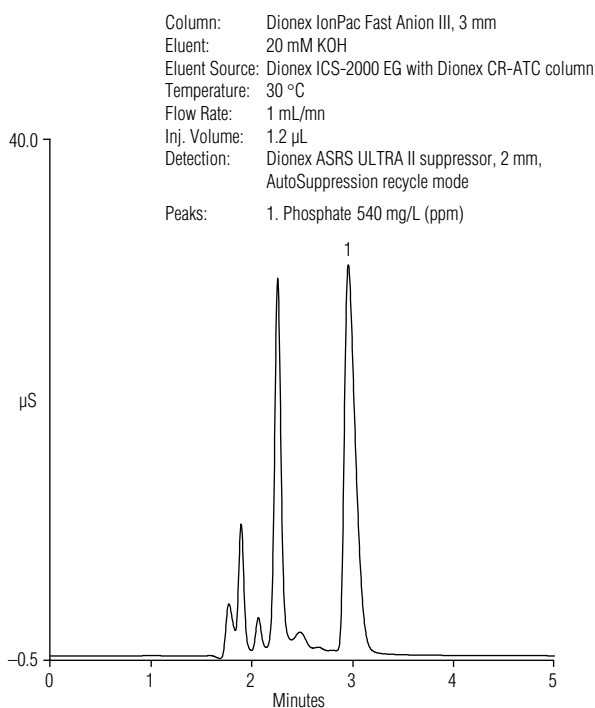


Figure 3. Determination of phosphate in regular Cola A on the Dionex IonPac Fast Anion III column.

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 Dionex 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 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 #5274 with an $R_s = 4.1$. We determined no significant resolution loss between nitrate and phosphate for >5000 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 (4920 sample injections), while retention time precisions ranged from 0.10–0.18% (results not shown). The actual total number of sample injections was >5,000 because sample injection sets <120 were not included with these data sets. Thirty-six injection sets (4320 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.

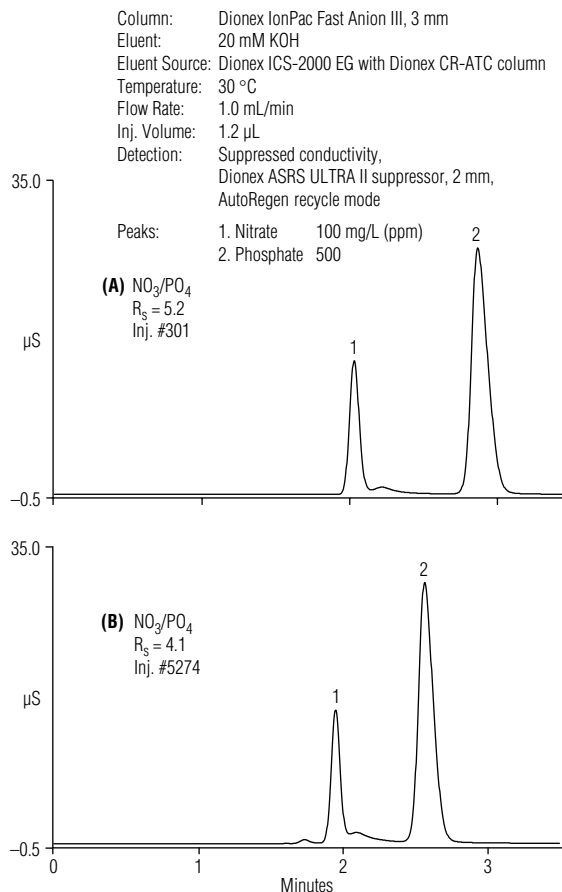


Figure 4. Resolution of a standard separation of nitrate and phosphate for (A) injection #301 and (B) injection #5274.

Table 2. Average phosphate concentrations and number of cola sample injections used to evaluate the Dionex IonPac Fast Anion III column ruggedness.

Sample	Analyte	# of Injections	Avg. Amount Found \pm SD (mg/L)
Regular Cola B	Phosphate	2750	512 \pm 7.0
Diet Cola C	Phosphate	2040	219 \pm 1.5
Diet Flavored Cola D	Phosphate	120	223 \pm 0.6
Diet Flavored Cola E	Phosphate	120	218 \pm 0.3

One sample injection set = 120 consecutive sample injections
 (41 sample injection sets = 4920 injections)
 Peak area precision range: 0.08–0.44%

Column: Dionex IonPac Fast Anion III, 3 mm
 Eluent: 20 mM KOH
 Temperature: 30 °C
 Flow Rate: 1.0 mL/min
 Inj. Volume: 1.2 µL
 Detection: Dionex ASRS ULTRA II suppressor, 2 mm,
 AutoSuppression recycle mode

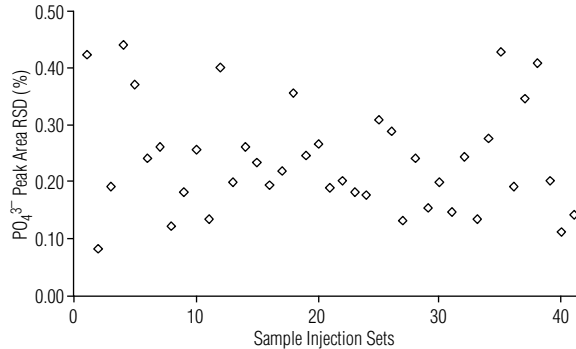


Figure 5. Phosphate peak area precisions for multiple sets of injections (1 sample injection set = 120 sample injections).

Phosphate retention time was reduced from 2.9 to 2.6 min (~10%)
 for 5,030 sample injections
 Phosphate retention time precision per set ranged from 0.10–0.18%

Column: Dionex IonPac Fast Anion III, 3 mm
 Eluent: 20 mM KOH
 Temperature: 30 °C
 Flow Rate: 1.0 mL/min
 Inj. Volume: 1.2 µL
 Detection: Dionex ASRS ULTRA II suppressor, 2 mm,
 AutoSuppression recycle mode

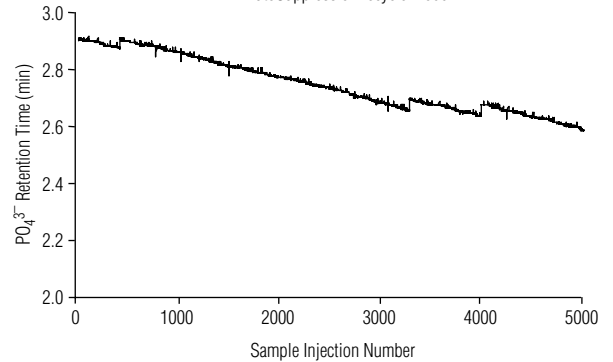


Figure 6. Change in phosphate retention time in cola samples for 5030 sample injections.

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 shows the change in phosphate retention time for >5000 injections of cola samples. The phosphate retention time was reduced by 0.3 min for 5030 sample injections. This amounts to a decrease of only 3.6 sec per 1000 sample injections. Considering the nature of the cola samples and the high number of samples injected on the Dionex IonPac 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 $\pm 2\%$ of the starting pressure, indicating that >5000 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 Dionex 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.

Column: Dionex IonPac Fast Anion III, 3 mm
 Eluent: 20 mM KOH
 Eluent Source: Dionex ICS-2000 EG with Dionex CR-ATC column
 Temperature: 30 °C
 Flow Rate: 1.0 mL/min
 Inj. Volume: 1.2 µL
 Detection: Suppressed conductivity,
 Dionex ASRS ULTRA II suppressor, 2 mm,
 AutoSuppression recycle mode

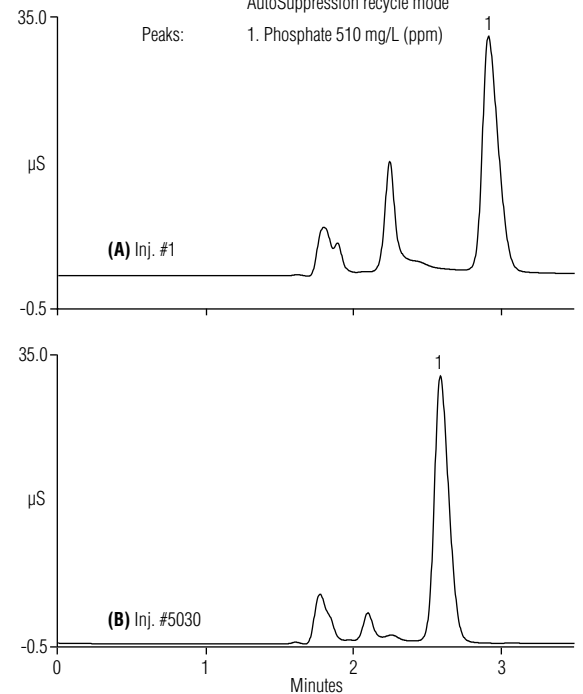


Figure 7. Comparison of the separation of phosphate in regular Cola B for (A) sample injection #1 and (B) sample injection #5030.

Conclusion

An RFIC method using a low capacity hydroxide-selective Dionex 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 Dionex IonPac Fast Anion III produces a rapid separation (<5 min) of phosphate and citrate, and the ruggedness of the column permits the injection of >5000 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.

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