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. 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. 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. 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)

**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

**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.

<table>
<thead>
<tr>
<th>Time</th>
<th>Parameter</th>
<th>Parameter Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>Minimum area</td>
<td>5.0 [Signal]*min</td>
</tr>
<tr>
<td>2.500</td>
<td>Minimum area</td>
<td>0.03 [Signal]*min</td>
</tr>
<tr>
<td>4.800</td>
<td>Minimum area</td>
<td>0.001 [Signal]*min</td>
</tr>
</tbody>
</table>

**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 molybdobdate and potassium antimonyl tartrate in an acidic medium with a dilute solution of phosphate to form the antimonyl phosphomolybdate complex. 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. 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.

IC with suppressed conductivity detection has been demonstrated to be the method of choice for the determination of anions, including citrate. 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. 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 electro-
statically attached to the surface of a wide-pore poly-
meric 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 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 hydrox-
ide 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 IonPac Fast Anion III column.](image)

Column: IonPac Fast Anion III, 3 mm
Eluent: 20 mM KOH
Eluent Source: ICS-2000 EG with CR-ATC
Temperature: 30 °C
Flow Rate: 1.0 mL/min
Inj. Volume: 1.2 µL
Detection: Suppressed conductivity, ASRS ULTRA II, 2 mm, AutoSuppression recycle mode
Peaks: 1. Fluoride 18 mg/L (ppm)
2. Chloride 90
3. Nitrite 90
4. Bromide 90
5. Nitrate 90
6. Sulfate 90
7. Phosphate 180
8. Citrate 100

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 rugged-
ness 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
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 de-
scribed in this application note provides a convenient,
reliable, precise, and rugged method for the simulta-
neous 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 (r²) 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 phos-
phate without overloading the column, thereby eliminat-
ing 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 precisions reflect results typically ob-
served when using an RFIC system. Figure 2 demon-
strates 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 concentra-
tion 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.

Phosphoric acid in cola drinks is routinely moni-
tored 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 de-
scribed in this application note provides a convenient,
reliable, precise, and rugged method for the simulta-
neous determination of phosphate and citrate in soft
drinks. A variety of regular, diet, and flavored colas
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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 rugged-
ness 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.

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### Table 1. Concentrations of Phosphate and Citrate Found in Different Carbonated Colas Using the IonPac Fast Anion III Column

<table>
<thead>
<tr>
<th>Sample</th>
<th>Phosphate</th>
<th>Citrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount Found (mg/L)</td>
<td>Retention Time (RSD)</td>
</tr>
<tr>
<td>Low carb cola A</td>
<td>444</td>
<td>0.13</td>
</tr>
<tr>
<td>Diet cola A</td>
<td>502</td>
<td>0.06</td>
</tr>
<tr>
<td>Regular cola A</td>
<td>539</td>
<td>0.12</td>
</tr>
<tr>
<td>Diet cola B</td>
<td>335</td>
<td>0.13</td>
</tr>
<tr>
<td>Low carb cola B</td>
<td>502</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* \(^{\text{RSD}} = \text{relative standard deviation, } n = 20\)

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

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 #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 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.

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Analyte</th>
<th># of Injections</th>
<th>Avg. Amount Found ± SD (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular cola B</td>
<td>Phosphate</td>
<td>2,750</td>
<td>512 ± 7.0</td>
</tr>
<tr>
<td>Diet cola C</td>
<td>Phosphate</td>
<td>2,040</td>
<td>219 ± 1.5</td>
</tr>
<tr>
<td>Diet flavored cola D</td>
<td>Phosphate</td>
<td>120</td>
<td>223 ± 0.6</td>
</tr>
<tr>
<td>Diet flavored cola E</td>
<td>Phosphate</td>
<td>120</td>
<td>218 ± 0.3</td>
</tr>
</tbody>
</table>

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

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