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Rapid determination of phosphate and citrate in carbonated soft drinks using ion chromatography

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Keywords
Dionex ICS 5000+ HPIC system, Dionex IonPac Fast Anion IIIA column, conductivity detection, cola, soda pop, beverage test, beverage analysis

Goal

To develop a fast and rugged method to measure phosphate and citrate in carbonated soft drinks

Introduction

Phosphoric acid is used as a stabilizer and preservative in cola drinks. It also influences taste, making cola drinks tart. The phosphoric acid concentration in cola drinks is monitored to maintain product quality and minimize production costs. The phosphoric acid concentration is measured during the manufacturing of the cola syrup and during the bottling of the cola drink. Citric acid is used for the same purpose and both of these acids may be used in combination.

Traditionally, analysts perform a labor-intensive colorimetric assay to quantify the amount of phosphoric acid in soft drinks. This methodology requires a highly skilled operator, is time consuming, and has poor accuracy and precision. A separate assay is required to determine the amount of citric acid added to the beverage.

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Ion chromatography (IC) can simultaneously determine phosphoric and citric acids in soft drinks by measuring the corresponding anions, phosphate and citrate. Thermo Scientific Application Note 169 reported a method that rapidly determines the phosphoric and citric acid concentrations in cola drinks with an injection volume of 1.2 µL.[1] These acids were determined in approximately 5 min using an isocratic potassium hydroxide eluent and a Thermo Scientific™ Dionex™ IonPac™ Fast Anion III column. The Dionex IonPac Fast Anion IIIA analytical column is a newer column designed for the analysis of samples containing inorganic anions and citrate in less than 8 min with better separation between phosphate and the nearby peaks found in cola samples. This application note presents a new method for the simultaneous determination of phosphate and citrate in carbonated soft drinks using the Dionex IonPac Fast Anion IIIA column. In addition, the sample injection was reduced from 1.2 to 0.4 µL to increase column life.

Experimental

Equipment

- Thermo Scientific™ Dionex™ ICS-5000$^+$ HPIC™ system* including:
  - Dionex ICS-5000$^+$ DP (or SP) Pump module
  - Dionex ICS-5000$^+$ EG Eluent Generator module with high-pressure degasser module
  - Dionex ICS-5000$^+$ DC Detector/Chromatography module with Conductivity Detector and 0.4 µL Internal Injection Loop (P/N 074525)
  - Dionex AS-AP Autosampler with sample syringe, 250 µL (P/N 074306) and buffer line 1,200 µL (P/N 074989)

* This application can also be run on a Thermo Scientific™ Dionex™ Integrion™ HPIC™ system with RFIC capability.

Software

- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2 SR4

Consumables

- Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 500 Electrolytic trap column (P/N 075550)
- Thermo Scientific™ Dionex™ AERS 500 suppressor, 2 mm (P/N 082541)

Reagents and standards

- Deionized water, Type I reagent grade, 18 MΩ·cm resistivity or better
- Sodium phosphate, dibasic anhydrous (J.T.Baker, Bioreagent, ultrapure, P/N JT4062-01)
- Trisodium citrate (Sigma P/N S-4641)
- Sodium and potassium salts, A.C.S. reagent grade or better, for preparing anion standards

Samples

Regular cola A and B, diet cola, cherry cola, zero-calorie cola, and lemon-lime soft drink were obtained from a local grocery store.

IC Conditions	
Columns:	Dionex IonPac Fast Anion IIIA, Guard Column, 3 x 50 mm (P/N 062966) Dionex IonPac Fast Anion IIIA, Analytical Column, 3 x 250 mm (P/N 062964)
Eluent:	22 mM KOH
Eluent Source:	Dionex EGC 500 KOH Eluent Generator Cartridge with Dionex CR-ATC 500 Continuously Regenerated Anion Trap Column
Flow Rate:	1 mL/min
Column Temperature:	30 °C
Detector Compartment Temperature:	20 °C
Detector Temperature:	35 °C
Injection Volume:	0.4 µL (Full loop)
Detection:	Suppressed conductivity, Dionex AERS 500 Anion Electrolytically Regenerated Suppressor, 2 mm, recycle water mode, 55 mA
Run Time:	8 min
Background Conductance:	< 0.3 µS
System Backpressure:	~ 2,400 psi

Preparation of standards

Specifics of the standards used are described in Tables 1–3.

Stock solutions

Single phosphate and citrate stocks

Table 1. Masses of compounds used to prepare 100 mL of 1,000 mg/L anion standards.

Analyte	Compound	Amount (mg)
Phosphate	Sodium phosphate, dibasic	149.5
Citrate	Trisodium citrate	155.5

Single anion stocks

Table 2. Masses of compounds used to prepare 100 mL of 1,000 mg/L anion standards.

Analyte	Compound	Amount (mg)
Fluoride	Sodium fluoride (NaF)	221.0
Chloride	Sodium chloride (NaCl)	164.9
Nitrite	Sodium nitrite ($NaNO_2$)	150.0
Bromide	Sodium bromide (NaBr)	128.8
Nitrate	Sodium nitrate ($NaNO_3$)	137.1
Sulfate	Sodium sulfate (Na_2SO_4)	147.9

Working standard solutions

Table 3. Calibration standards.

Calibration Standard Level	Phosphate (ppm)	Citrate (ppm)
1	200	25
2	350	50
3	500	100
4	650	250

Sample preparation

Thoroughly degas all carbonated beverages in an ultrasonic bath with a vacuum pump. The beverages should be sonicated without vacuum first to release most of the dissolved carbon dioxide prior to placing the beverage under vacuum. This prevents the beverages from being pulled into the vacuum pump. The entire process takes approximately 20 min. Cola samples are then injected directly into the IC system. The lemon-lime soft drink sample was diluted 10-fold with DI water and then injected into the IC system.

System preparation and setup

Install the Dionex EGC KOH 500 eluent generator cartridge in the Dionex ICS 5000+ HPIC system and condition the cartridge as directed in the Dionex EGC 500 eluent generator cartridge quick start instructions. Install a Dionex CR-ATC continuously regenerated anion trap column between the Dionex EGC 500 eluent generator and Dionex EGC degas assembly. Install and configure the Dionex AS-AP autosampler. Enter a sample loop size of 5 µL in the Dionex AS-AP autosampler configuration and enter loop overfill 10 in the AS-AP method wizard. A larger sample loop size value than the actual injection loop volume 0.4 µL was entered into the method wizard in this application to ensure that the sample loop was completely filled for each injection. Set the Dionex AS-AP autosampler to operate in the sample overlap mode to reduce the time between injections and therefore increase sample throughput. Install a Dionex IonPac Fast Anion IIIA column and make sure the system pressure is over 2,000 psi to allow the degas assembly to effectively remove hydrolysis gases from the eluent. If necessary, install additional backpressure tubing (P/N 053765) to adjust the pressure to 2,300 ± 200 psi. Prepare the Dionex AERS 500 anion electrolytically regenerated suppressor by installing the suppressor and plumbing in the recycle mode. Pump ≤10 mM eluent at the application flow rate into the suppressor for 5 min from the eluent IN port. The power to the suppressor must be off during this step. Turn off the pump and allow the suppressor to sit for approximately 20 min to fully hydrate the suppressor resin and membranes.

Results and discussion

Separation

Previously, a method using an RFIC system was developed for determining phosphate and citrate in carbonated soft drinks using the Dionex IonPac Fast Anion III column. The total run time for the analysis was 5 min using an electrolytically generated 20 mM potassium hydroxide eluent at 1 mL/min with an injection volume of 1.2 µL. After extensive application of this method, a peak in cola samples was found to coelute with phosphate, resulting in higher apparent phosphate concentrations than the actual concentration. Therefore, a new column, the Dionex IonPac Fast Anion IIIA column, was developed to improve the separation of phosphate from cola sample components. This column was optimized for a 30 °C operating temperature to ensure reproducible retention times. This new column was evaluated in this application in terms of linearity, precision, and ruggedness for the determination of phosphate and citrate in carbonated soft drinks. In order to increase the column life, a sample injection of 0.4 µL was used in this application. Figure 1 shows a separation of seven common anions and citrate on the Dionex IonPac Fast Anion IIIA column using a 22 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 <8 min.

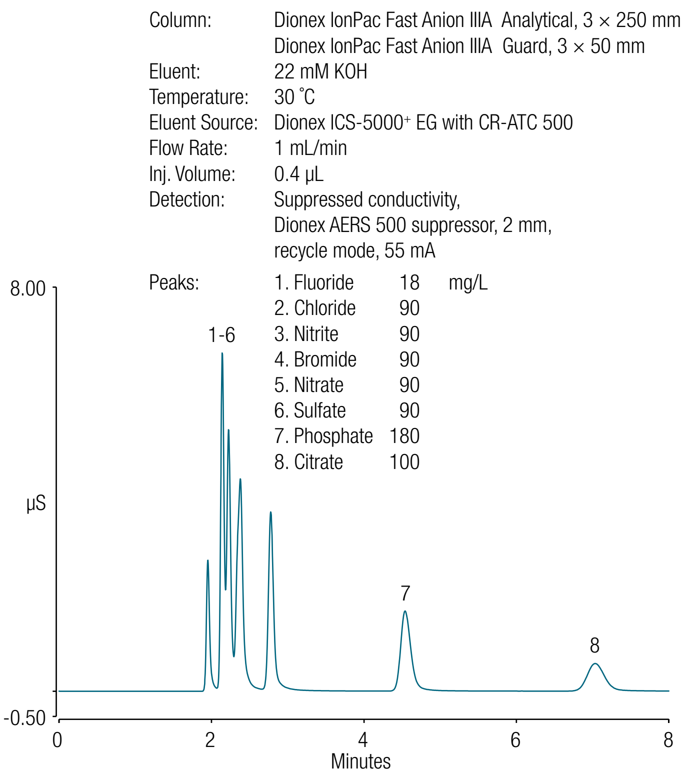

Figure 1. Separation of seven common anions and citrate using a Dionex IonPac Fast Anion IIIA column.

Method linear calibration ranges

Four calibration levels of phosphate and citrate were analyzed to cover the expected concentration range found in typical soft drinks. Table 4 summarizes the calibration experiments for phosphate and citrate. Due to the incomplete dissociation of citric acid at high concentrations, its calibration curve shows deviation from linearity in the selected calibration range. Therefore, the calibration plot of peak area versus concentration was fit using a quadratic regression function. Figures 2 and 3 show examples of the calibration curves of phosphate and citrate, respectively.

Table 4. Calibration of phosphate and citrate.

Analyte	Range (mg/L)	Coefficient of Determination (r^2)	Fitting
Phosphate	200–650	1.0000	Linear
Citrate	25–250	1.0000	Quadratic

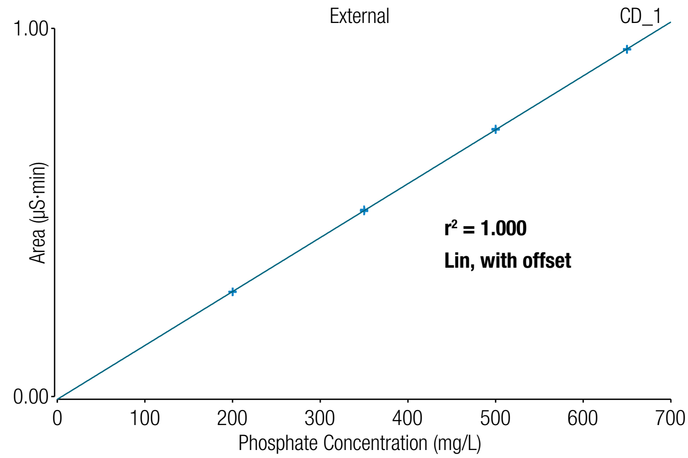

Figure 2. Phosphate calibration curve.

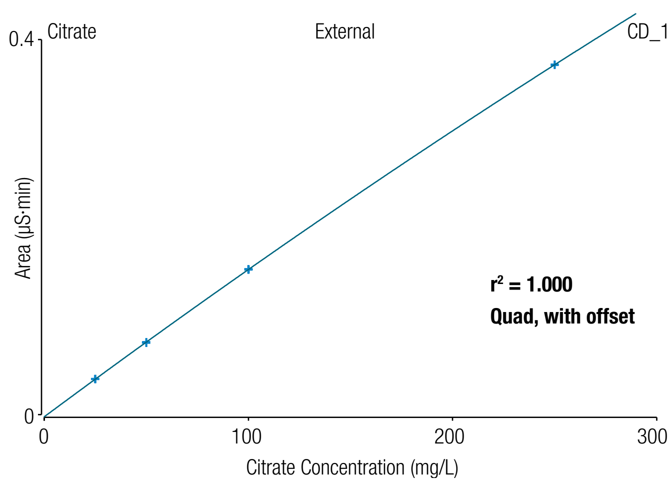

Figure 3. Citrate calibration curve.

Analysis of cola samples

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, and lemon-lime soft drink were analyzed for the presence of phosphate and citrate. Table 5 summarizes the results of the determination of phosphate and citrate in six carbonated soft drinks. The precision of ten replicate sample injections produced retention time and peak area RSDs of <0.1% and <0.5%, respectively. The excellent retention time and peak area precisions reflect results typically observed when using an RFIC system. Figure 4 shows a chromatogram of a regular cola containing only phosphoric acid. Figure 5 shows a chromatogram of a diet cola containing phosphoric and citric acids and Figure 6 shows the chromatogram of a lemon-lime soft drink containing only citric acid. As shown, both analytes are well resolved from other anionic components in their respective samples in less than 8 min. For the determination of only phosphate, the analysis time can be reduced to 5 min to further increase sample throughput (Figure 7).

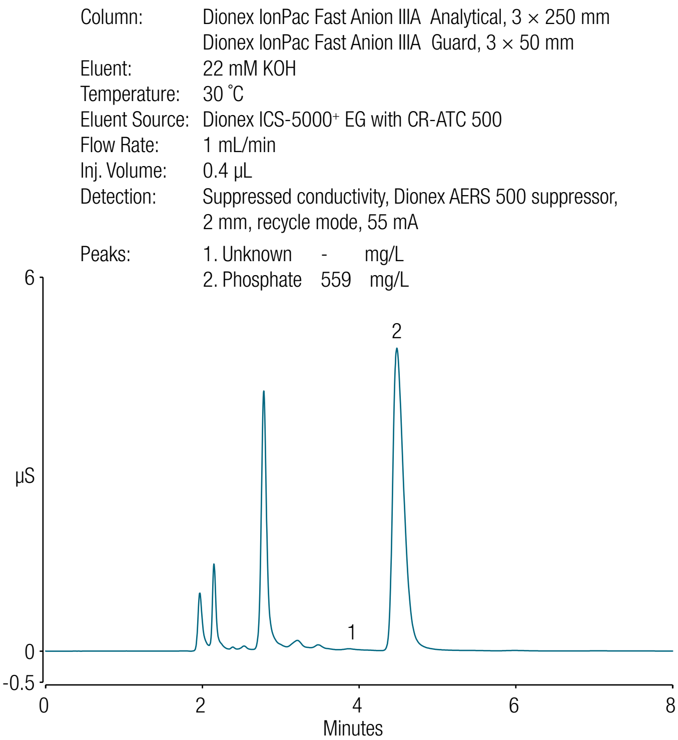

Figure 4. Determination of phosphate in regular cola A using a Dionex IonPac Fast Anion IIIA column.

Table 5. Concentrations of phosphate and citrate in soft drinks (n=10).

Sample	Phosphate			Citrate		
	Concentration Found (mg/L)	Retention Time (RSD)	Peak Area (RSD)	Concentration Found (mg/L)	Retention Time (RSD)	Peak Area (RSD)
Regular Cola A	559	0.07	0.25	-	-	-
Regular Cola B	572	0.06	0.30	-	-	-
Diet Cola	268	0.03	0.20	180	0.02	0.20
Cherry Cola	521	0.04	0.28	-	-	-
Zero Calorie Cola	508	0.06	0.26	89.0	0.03	0.31
Lemon-Lime Soft Drink	-	-	-	1,320	0.02	0.49

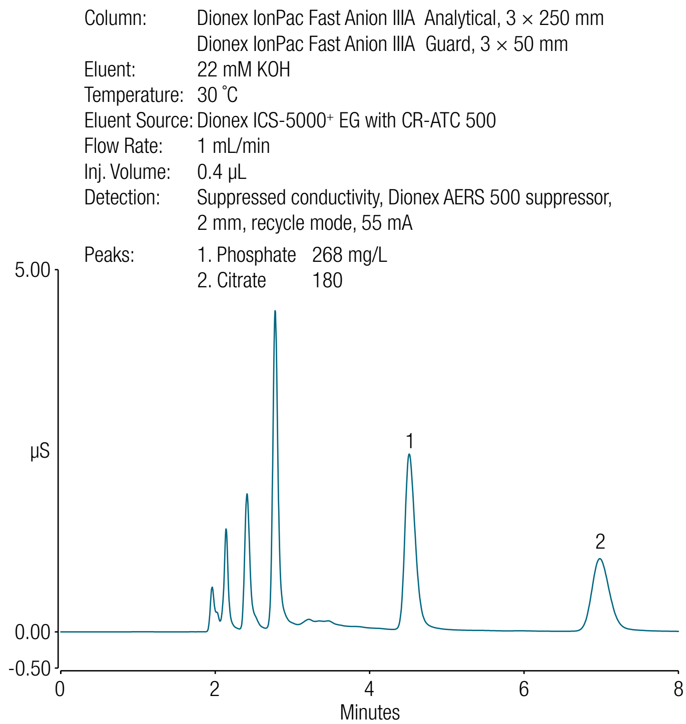

Figure 5. Determination of phosphate and citrate in diet cola using a Dionex IonPac Fast Anion IIIA column.

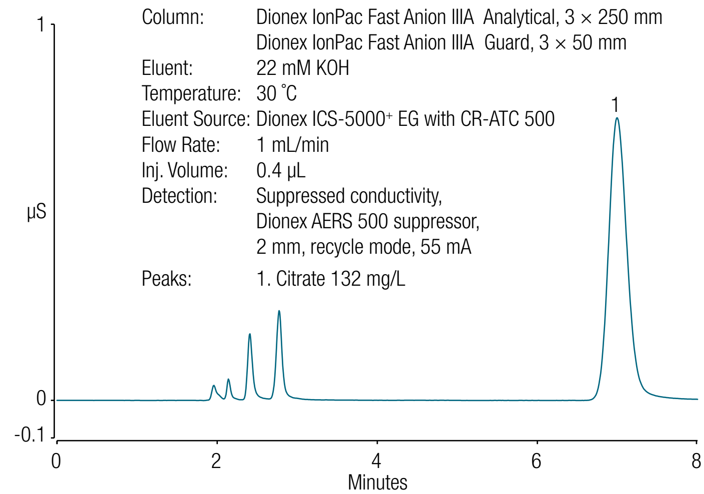

Figure 6. Determination of citrate in lemon-lime soft drink (10× dilution) using a Dionex IonPac Fast Anion IIIA column.

Method accuracy

Method accuracy was evaluated by determining recoveries of phosphate and citrate in spiked soft drinks (Table 6). Recoveries were calculated from the difference in response between the spiked and unspiked samples. The recovery for phosphate and citrate ranged from 100% to 105%, indicating that this method can accurately determine phosphate and citrate in soft drinks.

Method precision

Method precision was evaluated by calculating the average phosphate concentration of 5,000 injections of regular cola A. In this experiment, the run time was reduced from 8 to 5 min. Table 7 shows that the average phosphate of the 5,000 injections is 566 mg/L with a standard deviation of 7 mg/L.

Table 7. Average phosphate concentrations and number of cola sample injections used to evaluate the Dionex IonPac Fast Anion IIIA column ruggedness.

Sample	Analyte	Number of Injections	Average Found ± SD (mg/L)
Regular Cola A	Phosphate	5,000	566 ± 7

Table 6. Recoveries of phosphate and citrate spiked in soft drinks.

Sample	Phosphate			Citrate		
	Found (mg/L)	Added (mg/L)	Recovery (%)	Found (mg/L)	Added (mg/L)	Recovery (%)
Regular Cola A	559	100	102	-	-	-
Regular Cola B	572	100	105	-	-	-
Diet Cola	268	50	101	180	40	103
Cherry Cola	521	100	102			
Zero Calorie Cola	508	100	101	89	20	102
Lemon-Lime Soft Drink (10× diluted)	-	-	-	132	25	101

Method robustness

Production facilities that produce large quantities of soft drinks each day not only require methods with high sample throughput, but also require methods that are rugged and can meet the high demand of large analysis batches. Therefore, the ruggedness of the method was evaluated by determining the number of cola samples that can be injected on the column without significant loss of column capacity, which is observed as reduced retention time. For a series of consecutive sample injections, the following are monitored 1) Retention time and peak area precision of phosphate, 2) Change in phosphate retention time, and 3) Unknown peak/phosphate resolution.

The sample used to evaluate column ruggedness was Regular Cola A. Figure 7A shows Regular Cola A separation #100 with an unknown peak/phosphate resolution of 2.4. Figure 7B shows the same separation for injection #5000 with a resolution of 2.4 despite some loss of capacity (retention time change from 4.41 min to 4.18 min). There was no significant resolution loss between the unknown peak and phosphate for >5000 total injections despite the retention time loss. An injection volume of 0.4 µL not only increases the column life, but also improves the peak shape of phosphate compared to a 1.2 µL injection volume. Peak symmetry of phosphate was 3.37 with an injection volume of 1.2 µl and is reduced to 1.52 with an injection volume of 0.4 µL.

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 regenerants, and therefore operates continuously with only the occasional addition of DI water to supply the system.

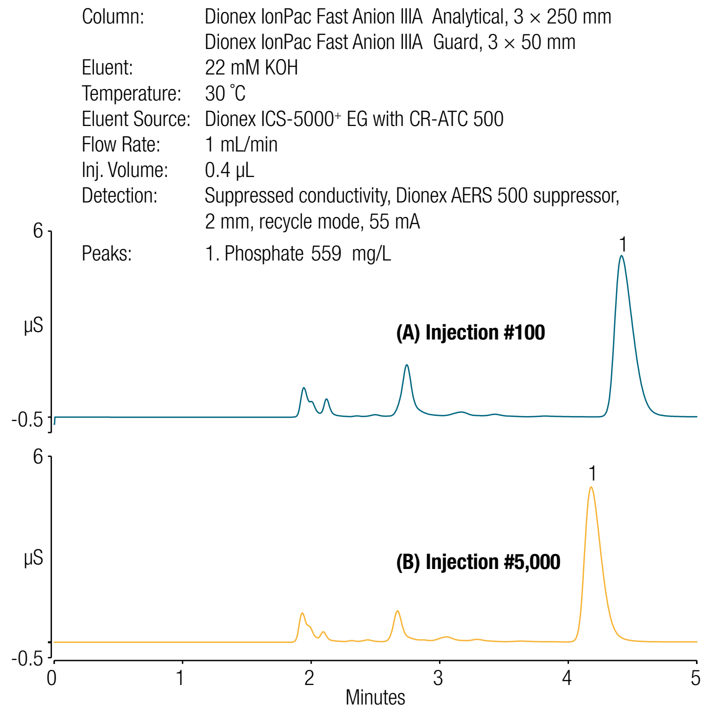

Figure 7. Comparison of the separation of phosphate in regular cola A for (A) sample injection #100, and (B) sample injection #5000.

Conclusion

A method that uses an RFIC system with a Dionex IonPac Fast Anion IIIA column with suppressed conductivity detection is simple, rapid, accurate, precise, and rugged for the simultaneous determination of phosphate and citrate in carbonated soft drinks. The column can separate phosphate from other soft drink sample components better than the Dionex IonPac Fast Anion III column and therefore improves method accuracy. The method also improves the column life with an injection volume of only 0.4 µL. Furthermore, an RFIC system significantly simplifies system operation and thereby improves method precision.

References

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