

Determination of Trace Sodium in Cranberry Powder

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Key Words

Dietary Supplements, Ion Chromatography, Dionex IonPac CS12A Column, Food, Suppressed Conductivity

Introduction

The number of consumers taking dietary supplements has increased significantly within the past decade, partially due to soaring health care costs influencing Americans' decisions to try alternative remedies,¹ and to a better-educated, more health-conscience consumer as a preventative measure. In 2011, nearly 70% of U.S. adults reported taking dietary supplements, with just over 50% taking supplements on a daily basis. The most popular supplements used by consumers include vitamins and minerals; herbs and botanicals; and other ingredients including glucosamine, fish oils, and probiotics.² In 2010, dietary supplements grew about 4.5% to reach a total sales of nearly \$28 billion. More than 50% of the sales were vitamins, herbs, and botanical supplements.³ Therefore, the quality and safety of these products is critical and must be verified and maintained.

In 1995, the National Institute of Health's Office of Dietary Supplements (ODS) was created based on the signing of the 1994 Dietary Supplement Health and Education Act. The primary objectives of the ODS are to explore the potential role of dietary supplements, to promote scientific research to examine the benefits of dietary supplements, and to serve as the principal advisor to other government agencies. The ODS collaborates with the National Institute of Standards and Technology (NIST), Food and Drug Administration (FDA), and other agencies to create standard reference materials and calibration standards related to dietary supplements. In 2007, NIST initiated the Dietary Supplements Quality Assurance Program (DSQAP) to help improve the accuracy of measurements in the dietary supplements community. The program includes the measurements of nutritional elements, marker compounds, contaminants, and fat- and water-soluble vitamins in foods, botanical supplement ingredients, and finished products.⁴

Botanical and herbal supplements have become increasingly popular in the dietary supplement market and accounted for nearly 20% of sales in 2010.² In this category, superfruits have gained a growing share of the consumer market because these fruits are known to be rich in anthocyanins (a class of antioxidants) and nutrients.

In 2010, the top-selling superfruits (including açai, pomegranate, coconut, elderberry, and goji berry) grew by 7.2%. However, the five fastest growing superfruits are blueberry, elderberry, cranberry, coconut, and noni. Sales of cranberry supplements increased by about 30% in 2010 from 2009 and this trend appears to be continuing.⁵ The popularity of cranberries is primarily associated with their reported ability to combat urinary tract infection, particularly in women.⁶

Cranberry powder contains organic acids, proanthocyanidins, and polyphenols. The powders are generally recognized as safe (GRAS) and can be used in beverages, foods, cosmetics, and supplements.⁷ Dionex (now part of Thermo Scientific) Application Brief 112 describes the determination of organic acids in cranberry extracts.⁸ Organic acids in cranberries impart flavors and their specific ratios can be used to detect adulteration. Although inductively coupled plasma-optical emission spectroscopy (ICP-OES) is the most common approach for determining nutritional elements in cranberry powder, ion chromatography (IC) is a feasible alternate approach. In addition to determining sodium in cranberry powder, IC can also be used to determine other important components that cannot be determined by ICP-OES (e.g., organic acids).

Goal

To accurately determine sodium in cranberry powder using a Reagent-Free™ IC (RFIC™) system with suppressed conductivity detection.

Equipment

- Thermo Scientific Dionex ICS-5000 system* including:
 - DP Dual Pump module**
 - EG Eluent Generator module
 - DC Detector/Chromatography module
 - AS Autosampler***
- Thermo Scientific Dionex EGC III MSA Methanesulfonic Acid Eluent Generator Cartridge (P/N 074535)
- Thermo Scientific Dionex CR-CTC II Continuously-Regenerated Cation Trap Column (P/N 066262)
- Thermo Scientific Dionex Chromeleon Chromatography Data System software version 7.1
- Vial Kit, 0.3 mL Polyprop with Caps and Septa (P/N 055428)
- Thermo Scientific Dionex OnGuard II A Cartridges, 2.5 cc (pkg of 48, P/N 057092)

*This application is also compatible with other RFIC systems.

**An SP Single Pump module can also be used with this application.

***An AS-AP Autosampler can also be used with this application.

Reagents and Standards

- Deionized (DI) water, Type I reagent grade, 18 M Ω -cm resistivity or better
- Sodium chloride (NaCl) (J.T. Baker P/N 4058-05)
- Combined Six Cation Standard-II, 50 mL (P/N 046070)
- Nitric acid, 70%, ultrapure reagent (J.T. Baker P/N 6901-05)

Chromatographic Conditions

Columns:	Thermo Scientific Dionex IonPac CG12A Guard, 2 × 50 mm (P/N 046076) Dionex IonPac™ CS12A Analytical, 2 × 250 mm (P/N 046075)
Eluent:	20 mM Methanesulfonic Acid (MSA)
Eluent Source:	Dionex EGC III MSA with CR-CTC II
Flow Rate:	0.25 mL/min
Inj. Volume:	5 μ L
Temperature:	30 °C (lower compartment) 30 °C (upper compartment)
Detection:	Suppressed conductivity, Thermo Scientific Dionex CSRS 300 Cation Self-Regenerating Suppressor (2 mm), Recycle mode, 15 mA current
System	
Backpressure:	~2300 psi
Background Conductance:	~0.1 to 0.2 μ S
Noise:	~0.1 to 0.2 nS/min peak-to-peak
Run Time:	20 min

Sample

Cranberry powder (provided by the NIST)

Preparations of Solutions and Reagents

Stock Standard Solution

Prepare a 1000 mg/L sodium stock solution by dissolving 0.2542 g of sodium chloride in DI water and dilute to 100 mL. Alternatively, a certified 1000 mg/L sodium standard can be purchased from a reputable source. Store the stock solution in a high-density polyethylene or polypropylene bottle at 4 °C.

Primary Dilution Standard

Prepare 10 mg/L of sodium in a 20 mL scintillation vial by combining 200 μ L of the 1000 mg/L sodium stock standard solution with 19.8 mL of DI water.

Calibration Standards

Prepare calibration standards of sodium from the 10 mg/L primary dilution standard using appropriate dilutions for each standard. Prepare five levels of calibration standards, ranging from 100 to 1000 μ g/L. Store the calibration standards at 4 °C when not in use.

Sample Preparation and Precautions

When working with nitric acid, wear the correct protective gear and work in a fume hood.

To prepare the cranberry powder for analysis, weigh approximately 0.500 g (record exact amount to 3 digits) of the sample in a 120 mL high-density polypropylene bottle. Then add 3 mL of DI water, mix, and add 7 mL of concentrated HNO₃ in a well-ventilated fume hood while wearing protective equipment, such as powder-free nitrile gloves. Securely tighten the bottle cap, then vortex or manually shake the mixture for \geq 1 min. Place a water bath in the fume hood and secure the sample bottle in the bath using a support stand and clamp to hold the bottle upright, then loosen the cap.

Be sure the bottle is secured upright in the water bath to avoid spilling the nitric acid solution. Test this beforehand using a 120 mL high-density polypropylene bottle filled with 10 mL of water. If the bottle does not sit upright, remove water from the water bath.

Digest the sample in the water bath at 100 °C for 30 min, then carefully remove the bottle from the water and allow the solution to cool to room temperature in the fume hood with the cap still loosely fastened. When the solution has cooled to room temperature, securely tighten the cap, mix the solution, and then add approximately 40 mL of DI water. Before neutralizing the sample solution with a Dionex OnGuard™ II A cartridge, dilute the solution 1:6 with DI water.

Prepare the Dionex OnGuard II A cartridge by slowly passing ~30 mL of DI water (~1 to 2 mL/min) through the cartridge to a waste container. Then aspirate ~10 mL of sample and dispense the first 6 mL to waste before directly collecting ~0.3 mL in the polypropylene sample vial.

Sodium is a ubiquitous analyte; therefore, the potential for contamination when performing trace sodium analysis is significant. Use extreme caution throughout the entire process to eliminate (or at least minimize) the potential for contamination. Wear disposable, powder-free gloves at all times when working on the IC system, standards, samples, and blanks. Some sources of contamination may include the IC system flow path; the autosampler; the autosampler vials; the sample preparation process; and containers used for samples, standards, or blanks. Soak all autosampler vials overnight in DI water and rinse several times before use. In addition, analyze a representative sample blank that covers the same process as the cranberry sample to measure the contamination from that process.

Results and Discussion

The analysis of botanical dietary supplements is challenging due to the complexity of the sample matrix. This is further complicated by the lack of well-developed methods and no consensus in the dietary supplement community regarding the best analytical approach for uncharacterized materials. The determination of elements at low concentrations can also be problematic due to possible interferences and contamination from various sources. One of the goals of the DSQAP is to identify problems and challenges for the dietary supplement analytical community. In addition, participants are able to compare their results to other laboratories to improve their measurement capabilities.

In this study, trace concentrations of sodium were determined in cranberry powder that was provided by the NIST as part of the DSQAP. The sample provided by the NIST was assumed to be similar to SRM 3281 (cranberry fruit) based on the certificate of analysis; however, this information was not specifically provided by the NIST. Trace elements (i.e., calcium, iron, magnesium, potassium, and sodium) are typically determined by ICP-OES after a microwave digestion with concentrated HNO₃ or concentrated HNO₃/HF mixture.⁹ However, IC provides an alternate approach for this determination. In addition, IC is also suitable for other determinations, such as organic acids, that are of particular interest in botanical dietary supplements and would otherwise be unsuitable for analysis by ICP.

The method to determine sodium in cranberry powder uses a Dionex IonPac CS12A column with an electrolytically generated MSA eluent and suppressed conductivity detection. Although the focus of this study was on the determination of sodium as a nutritional element in cranberry powder, the method can also be applied to other cations, such as potassium, magnesium, and calcium. Figure 1 shows a standard separation of six common cations in <15 min on the Dionex IonPac CS12A column using 20 mM MSA. The retention time for sodium under the conditions described here is approximately 4.5 min.

Column: Dionex IonPac CG12A, CS12A, 2 mm
 Eluent: 20 mM MSA
 Eluent Source: Dionex EGC III MSA with CR-CTC II
 Flow Rate: 0.25 mL/min
 Inj. Volume: 5 µL
 Temperature: 30 °C
 Detection: Dionex CSRS™ 300, 2 mm, recycle mode, 15 mA
 Standard: 1000× dilution of Cation II standard
 Peaks:

1. Lithium 0.05 mg/L
 2. Sodium 0.20
 3. Ammonium 0.25
 4. Potassium 0.50
 5. Magnesium 0.25
 6. Calcium 0.50

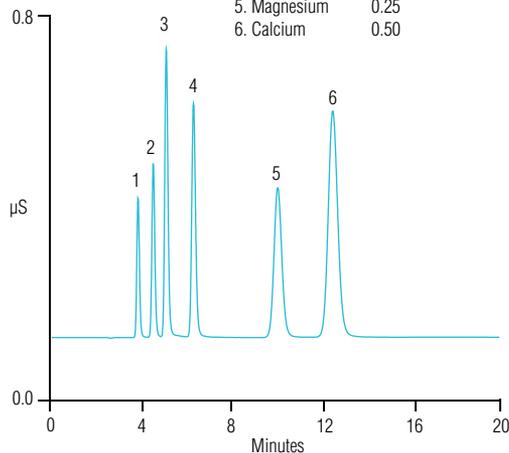


Figure 1. Separation of six common cations on the Dionex IonPac CS12A column.

Linearity, Limit of Quantitation, Limit of Detection

To determine the linearity of the method, calibration standards were injected in duplicate at five concentration levels in the range from 100 to 1000 µg/L of sodium. A plot of peak area vs concentration produced a coefficient of determination (r^2) value of 0.9980 using a least squares regression fit (Table 1). To determine the LOD and LOQ, the baseline noise was first determined by measuring the peak-to-peak noise in a representative one-minute segment of the baseline where no peaks elute. Typical baseline noise for this method using the Dionex CSRS 300 suppressor in the recycle mode is ~0.1 to 0.2 nS/min. The LOQ for sodium was determined to be ~1.2 µg/L ($S/N = 10$) and the LOD was estimated at ~0.35 µg/L ($S/N = 3$).

Table 1. Linearity, LOD, and LOQ for sodium.

Analyte	Range (µg/L)	Linearity (r^2)	LOD ^a (µg/L)	LOQ ^a (µg/L)
Sodium	100–1000	0.9980	0.35	1.2

^aLODs estimated from $3 \times S/N$

^bLOQs estimated from $10 \times S/N$

Sample Analysis

It is important to establish a blank and ensure the stability of the blank analysis over several days. The time required to establish a low blank concentration will vary from laboratory to laboratory, depending on the cleanliness of the laboratory, purity of the water source, presence of contaminants in the IC system, and other factors that can contribute to high blank levels. In addition, it is also critical to establish a sample preparation blank, particularly in cases where the preparation of the sample is a multistep process. Establishing the concentration of the target analyte in the blanks is critical to minimizing the impact of the sample analysis results. When significant contamination of a target analyte is observed, it is preferable to eliminate the sources of contamination rather than try to compensate for them. In this study, a DI water blank was analyzed at various intervals over a seven-day period, resulting in an average sodium concentration of $0.6 \pm 0.2 \mu\text{g/L}$ ($n = 8$). In addition, a sample preparation blank that followed the same procedural process as the real sample was analyzed. This produced an approximate sodium concentration of $6.6 \mu\text{g/L}$, which is ~ 10 times more than that generated by the DI water blank.

Table 2. Determination of sodium in cranberry powder.

Preparation	n	Average Calculated Concentration ($\mu\text{g/g}$)	Retention Time RSD	Peak Area RSD
1	3	210	0.07	0.59
2	3	215	0.00	0.29
3	3	215	0.04	2.50

Three independent preparations of the cranberry powder were tested to determine the concentration of sodium. For each preparation, the samples were analyzed in triplicate. Table 2 summarizes the results from these analyses. As shown, the calculated sodium concentration was consistent between preparations with overall concentrations only varying slightly from 210 to 215 $\mu\text{g/g}$. The peak area RSDs from the independent preparations ranged from 0.3 to 2.5%, based on triplicate injections. The overall average sodium concentration was 214 $\mu\text{g/g}$ with a peak area RSD of 1.6% ($n = 9$). Figure 2 shows a typical chromatogram for the separation of sodium in cranberry powder after acid digestion with HNO_3 followed by treatment with a Dionex OnGuard II A cartridge. In comparison to the results reported in this study, the NIST reported an average sodium concentration of 253 $\mu\text{g/g}$, which is approximately a 15% difference between these results. This study included a total of 23 participating laboratories that submitted results to the NIST. Overall, the consensus average concentration was $268 \pm 83 \mu\text{g/g}$ with a range of reported values varying from 140 to 901 $\mu\text{g/g}$. Although comparison of this data to the NIST and other participating laboratories is a good indication of the validity of the data, it is also recommended to spike the sample with a known concentration of sodium and then calculate the recovery. Therefore, the sample was spiked with 340 $\mu\text{g/L}$ of sodium after acid digestion but before further treatment with a Dionex OnGuard II A cartridge. The spiked concentration is approximately the same as the concentration measured in the original sample. The average recovery from the spiked sample was 90%.

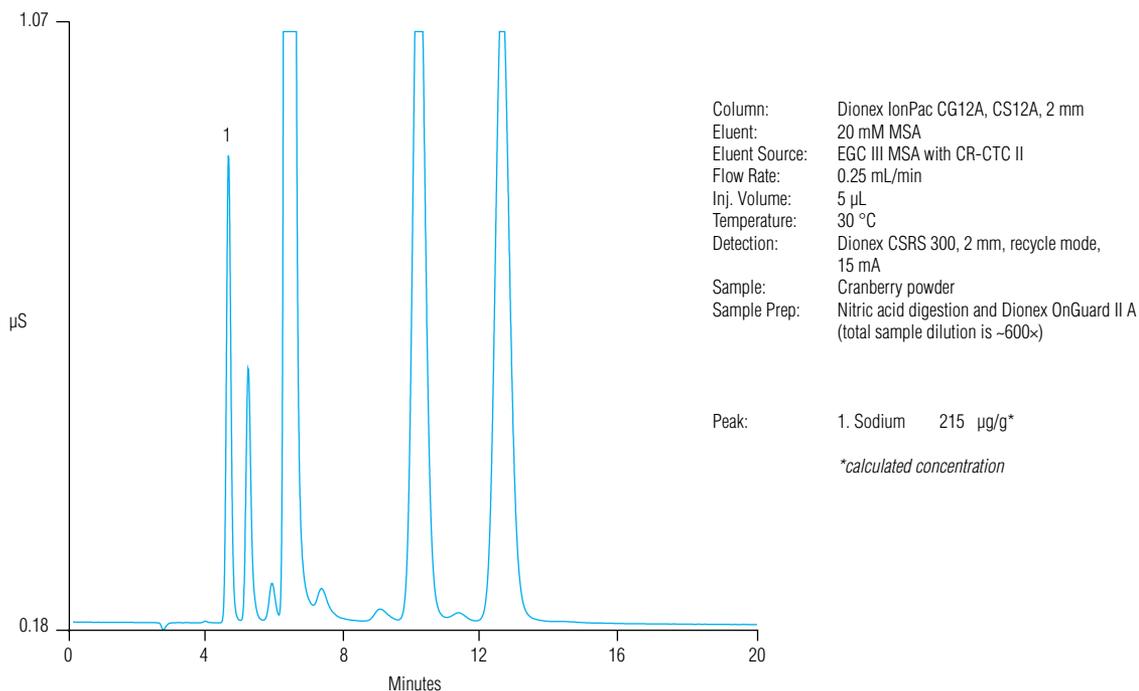


Figure 2. Determination of sodium in cranberry powder.

Conclusion

This study demonstrates the ability to determine trace concentrations of sodium in cranberry powder using an electrolytically generated MSA eluent with the Dionex IonPac CS12A column and suppressed conductivity detection. Cranberry powder is a complex matrix that requires acid digestion to extract the sodium from the sample. However, the results reported in this study are within 15% of the results reported by the NIST, indicating that IC is a viable alternative to other analytical techniques for this analysis. In addition, IC can be used to determine other important analytes in this matrix, such as organic acids, that are not possible by spectroscopic techniques. Furthermore, a Reagent-Free system enhances the level of automation and ease of use of the IC system, improving inter- and intralaboratory data reproducibility.

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