APPLICATION NOTE 73389

Determination of sodium, potassium, and calcium in dietary vitamins and sauerkraut

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Goal

To develop an IC method for the determination of sodium, potassium, and calcium in vitamin supplements and sauerkraut

Introduction

Ion chromatography (IC) is a well-established technology for the routine determination of anionic and cationic analytes in a wide variety of samples in many industries, including the pharmaceutical, biotechnology, environmental, agricultural, and food industries. Here we employed IC to analyze dietary vitamin and sauerkraut samples for nutritional elements such as sodium, potassium, and calcium. Both dietary vitamin and sauerkraut samples were provided by The National Institute of Standards and Technology (NIST) for Exercise 4 of the HAMQAP (Health Assessment Measurements Quality Assurance Program). NIST has established the HAMQAP, in part as a collaboration with the National Institutes of Health (NIH) Office of Dietary Supplements (ODS).[1] In this program, participants measure concentrations of nutritional and toxic elements, fat- and water-soluble vitamins, fatty acids, active and/or marker compounds, and contaminants in samples distributed by NIST.

In this application note, we demonstrate the determination of nutritional elements (sodium, potassium, and calcium) in dietary vitamins and sauerkraut samples using an IC method. We use a Thermo Scientific™ Dionex™ IonPac™ CS16 column for the separation of the sodium, potassium, and calcium cations. The Dionex IonPac CS16 high-capacity cation exchange column has 100% solvent compatibility and medium hydrophobicity.[2] The high capacity of 3000 µeq/column is achieved by using a smaller particle diameter (5.5 µm), a higher density of grafted carboxylic acid cation exchange groups, and a larger column format. The high capacity of the column allows loading of high concentrations of sodium, potassium, and calcium without affecting/losing resolution. After separation, the cations are measured by suppressed conductivity detection.

Experimental

Equipment

- Thermo Scientific™ Dionex™ ICS-6000 system*, including:
  - DP Dual Pump (P/N 22181-60007)
  - EG Eluent Generator (P/N 22181-60019)
  - DC Detector/Chromatography Compartment (P/N 22181-60049)
  - Conductivity Detector (P/N 079829)
- Thermo Scientific™ Dionex™ AS-AP Autosampler with tray temperature control option (P/N 074926)
- Thermo Scientific™ Dionex™ EGC 500 MSA Methanesulfonic Acid Eluent Generator Cartridge (P/N 075779)
- Thermo Scientific™ Dionex™ CR-CTC 600 Continuously Regenerated Cation Trap Column (P/N 088663)
- Thermo Scientific™ Dionex™ CDRS 600 Cation Dynamically Regenerated Suppressor (2 mm) (P/N 088670)

*This method can be executed on any Thermo Scientific Dionex IC system with column temperature control.

Software

Thermo Scientific™ Dionex™ Chromeleon™ 7.2 Chromatography Data System (CDS) software was used for all data acquisition and processing.

Consumables

- Thermo Scientific™ Nalgene™ Syringe Filters, PES, 0.2 µm (Fisher Scientific, P/N 725-2520)
- AirTite™ All-Plastic Norm-Ject™ Syringes, 5 mL, sterile (Fisher Scientific, P/N 14-817-28)
- Thermo Scientific™ Dionex™ Vial Kit, 10 mL Polystyrene with caps and blue septa; P/N 074228

  or

  Thermo Scientific™ Dionex™ Vial Kit, 1.5 mL Polypropylene with caps and septa; P/N 079812

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistivity or better
- Thermo Scientific™ Dionex™ Combined Six Cation Standard-I (P/N 040187)
- Sodium chloride, Crystal, Ultrapure Bioreagent, J. T. Baker (Product Code.15508234)
- Potassium chloride, ACS reagent, 99.0-100.5%, Sigma-Aldrich (P/N P3911)
- Calcium chloride dihydrate (Certified ACS), Fisher Chemical (Catalog No. C79-500)

Table 1. Chromatographic conditions

IC conditions	
System	Dionex ICS-6000 system
Columns	Dionex IonPac CG16 Guard, 3 × 50 mm (P/N 079931) Dionex IonPac CS16 Analytical, 3 × 250 mm (P/N 059596)
Eluent	30 mM MSA
Eluent source	Dionex EGC 500 MSA cartridge with CR-CTC 600
Eluent flow rate	0.36 mL/min
Column temperature	40 °C
Injection volume	2.5 µL
Detection	Suppressed conductivity
Suppressor	Dionex CDRS 600 (2 mm) Suppressor, 32 mA current
Detection/suppressor compartment	20 °C
Background conductance	0.2–0.3 µS/cm
System backpressure	~2250 psi (100 psi = 0.689 MPa)
Noise	0.5–0.8 nS/cm
Run time	30 min

Instrument setup and installation

System setup

The Dionex ICS-6000 HPIC system is configured with a CD Conductivity Detector with the intention to use eluent generation and temperature control of the column oven and detector/suppressor compartment.

Install and configure the Dionex AS-AP Autosampler in Push Full mode. Follow the instructions in the Dionex AS-AP Autosampler Operator's Manual (Document No. 065361)[3] to calibrate the sample transfer line volume, thereby ensuring accurate and precise sample injections. Install the Dionex EGC 500 MSA cartridge and Dionex CR-CTC 600 trap column. Condition the devices according to instructions in the product manuals[4,5] and the Dionex ICS-6000 system Operator's Manual.[6] Install the Dionex IonPac CG16 and CS16 columns. Verify that the system pressure displayed by the pump is between 2000 and 2300 psi when pumping eluent under the method conditions; this will enable the degas assembly to effectively remove electrolysis gases from the eluent. If additional pressure is needed to achieve system pressures >2000 psi, install yellow PEEK backpressure tubing (yellow PEEK, 0.076 mm i.d., 0.003 in i.d.) between the Dionex HP Degas device and the injection port (Pump port position). Because the system pressure can rise over time, if using an older Dionex IC system or IC that cannot electrolytically generate eluents over 3000 psi, trim the backpressure coil as necessary to maintain system pressure between 2000 and 2300 psi.

Prepare the Dionex CDRS 600 suppressor for use by hydrating the internal membrane. Refer to the product manual for step by step instructions on suppressor hydration.[7] Allow the suppressor to sit for 20 min to ensure complete hydration before installing it in the system.

To start the system, turn on the pump and immediately turn on the Dionex EGC 500 eluent generator cartridge. Turn on the Dionex CR-CTC 600 trap and Dionex CDRS 600 suppressor when liquid is flowing through the devices. Set the eluent concentration, column oven, compartment oven, and cell temperatures, as shown in the Conditions section in the application. Allow the system to equilibrate for 30 min and run a system blank. The system should display the background conductance and noise listed under Conditions.

Preparation of solutions and reagents

Stock standard solution

Prepare 1000 mg/L stock standard solutions of each cation of interest by accurately weighing the amounts of reagent-grade salts in Table 2. Dissolve in DI water in a 100 mL plastic volumetric flask. Dilute to volume with DI water. Store in plastic containers at 4 °C. Stock standards are stable for at least three months.

Table 2. Mass of compound required to prepare 100 mL of a 1 g/L cation solution

Analyte	Compound	Mass (g)
Sodium (Na^+)	Sodium chloride (NaCl)	0.2542
Potassium (K^+)	Potassium chloride (KCl)	0.1907
Calcium (Ca^{++})	Calcium chloride ($CaCl_2$)	0.3668

Working standards and standards for method calibration

To prepare working standards, use a calibrated pipette to deliver the appropriate volume of 1000 mg/L stock standard into a volumetric flask and dilute to volume with DI water. The calibration standard concentrations used for sodium were 1.25, 2.5, 5, 10, 20, 30, 40, and 75 mg/L; for potassium 2.5, 5, 10, 25, 50, 100, 250, and 500 mg/L; for calcium 1.25, 2.5, 5, 10, 20, 30, 50, 100, and 200 mg/L. Table 3 lists the volumes of sodium, potassium, and calcium standards added to prepare mixed calibration standards in 250 mL volumetric flasks. Prepare working standards daily.

Table 3. Mixed calibration standards (250 mL)

Level	Volume of 1000 mg/L Sodium std (mL)	Volume of 1000 mg/L Potassium std (mL)	Volume of 1000 mg/L Calcium std (mL)
Cal level 1	0.313	0.625	0.313
Cal level 2	0.625	1.25	0.625
Cal level 3	1.25	2.50	1.25
Cal level 4	2.50	6.25	2.5
Cal level 5	5.00	12.5	5
Cal level 6	7.50	25.0	7.5
Cal level 7	10.0	62.5	12.5
Cal level 8	18.75	125	25
Cal level 9	-	-	50

Sample preparation

Multivitamin tablets

Crush 30 tablets in a mixer/blender and place the powder in a sample container as the stock sample. Mix the powder thoroughly to ensure homogeneity and store it at -20 °C for further use.

Step 1: Weigh 0.4 g of the powdered sample and transfer it to a 100 mL volumetric flask, add 30 mL of 5 M acetic acid and add DI water to the mark. Shake the mixture for 5 min at room temperature.

Step 2: Centrifuge an aliquot of the solution from step 1 at 3000 RPM for 20 min. Aspirate the supernatant and filter through a 0.22 µm PES syringe filter before analyzing the sample. Dilute the sample threefold with DI water before injecting it into IC.

Sauerkraut

Mix the sauerkraut sample in a blender/mixer to ensure sample homogeneity.

Step 1: Weigh 0.52 g of the ground sample and transfer it to a 100 mL volumetric flask, add 1 mL of 1 M acetic acid and add DI water to the mark. Shake the mixture for 5 min at room temperature.

Step 2: Centrifuge an aliquot of the solution from step 1 at 3000 RPM for 20 min. Aspirate the supernatant and filter through a 0.22 µm PES syringe filter before analyzing the sample. Note: No dilution is required for the sauerkraut sample.

Results and discussion

Before running samples, check the performance of the column by reproducing the quality assurance report (QAR) chromatogram shipped with the column. Figure 1 displays a chromatogram of the six-cation standard analyzed using the conditions listed in the QAR.

Column:	Dionex IonPac CS16, 3 mm + guard	Peaks:
Eluent:	30 mM MSA	1. Lithium
Column temp.:	40 °C	2. Sodium
Flow rate:	0.36 mL/min	3. Ammonium
Inj.volume:	25 µL	4. Potassium
Detection:	Suppressed conductivity	5. Magnesium
Detection temp.:	20 °C	6. Calcium

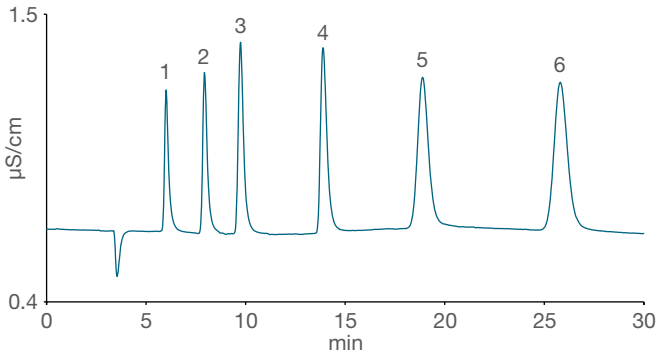

Figure 1. Chromatogram of the Dionex IonPac CS16 column QAR standard

Sample analysis

Figure 2 displays the chromatograms of the multivitamin and sauerkraut samples, run using the same conditions as the column QAR listed in Table 1. The injection volume used for samples is 2.5 µL. Except for the sodium content of the sauerkraut sample, all three elements were found in lower concentration compared to the multivitamin samples. The concentrations of Na, K, and Ca were calculated and submitted to NIST. We were informed by the NIST HAMQAP Exercise 4 study committee that the reported calcium contents in the multivitamin samples were lower than the expected values. We looked for the possible causes of lower calcium content in multivitamin samples. We first tried dissolving the samples in 10 mM acetic acid instead of DI water to check if acidic conditions improved calcium dissolution.

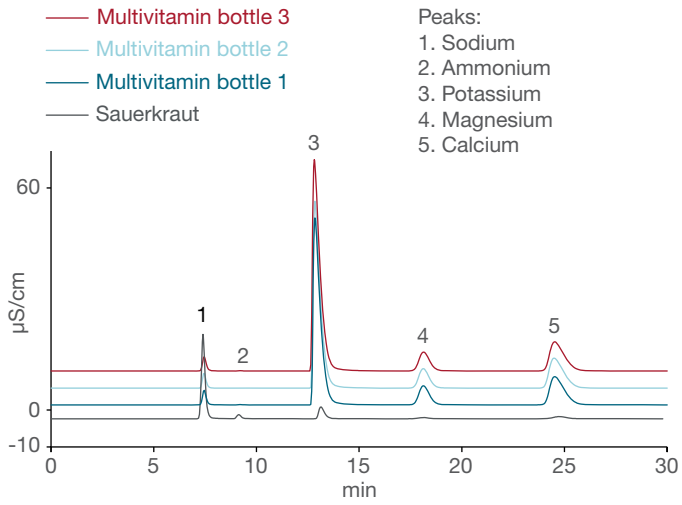

Figure 2. Chromatograms of multivitamin and sauerkraut samples

Figure 3 displays the chromatograms of a multivitamin sample prepared in DI water and 10 mM acetic acid. The concentrations of sodium and potassium were unchanged, but calcium and magnesium increased almost four times when dissolved in 10 mM acetic acid. Similarly, we also prepared the sauerkraut sample in 10 mM acetic acid. Figure 4 shows that there was no change in the concentration of the cations when the sauerkraut sample was dissolved in 10 mM acetic acid.

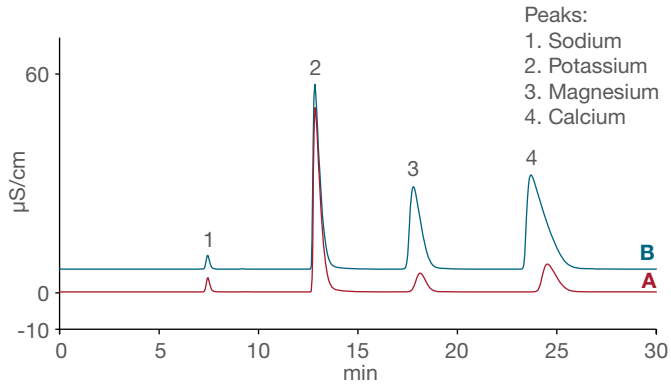

Figure 3. Chromatograms of a multivitamin sample prepared in DI water (A) and 10 mM acetic acid (B)

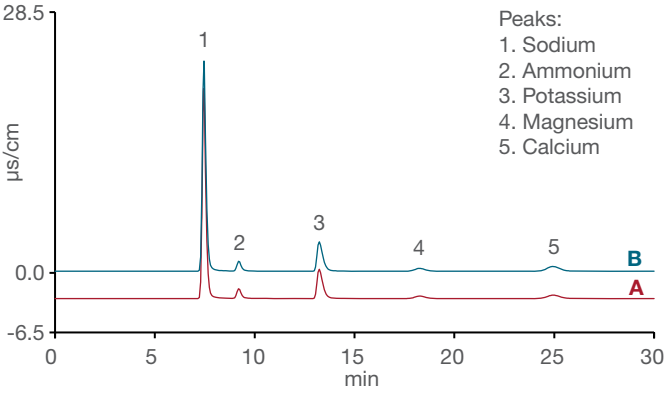

Figure 4. Chromatograms of a sauerkraut sample prepared in DI water (A) and 10 mM acetic acid (B)

To estimate the acetic acid concentration required to achieve the complete dissolution of calcium in the multivitamin sample, we prepared the multivitamin sample in a range of acetic acid concentrations: 10 mM, 100 mM, 500 mM, 1 M, 2 M, and 5 M. After dissolving the multivitamin samples in acetic acid, the amount of calcium increased. To avoid overloading the column, the samples were diluted 3× before injecting them. Figure 5 displays the chromatograms of the multivitamin sample prepared in increasing acetic acid concentration. The curve was then plotted between the acetic acid concentration versus the amount of calcium in the multivitamin. As shown in Figure 6, the curve looks almost flat between 1 M and 2 M acetic acid, such that there was little difference in the concentration of calcium when the multivitamin sample was dissolved in 1 M or 2 M acetic acid. We chose 1.5 M acetic acid (between 1 M and 2 M) to prepare the multivitamin samples.

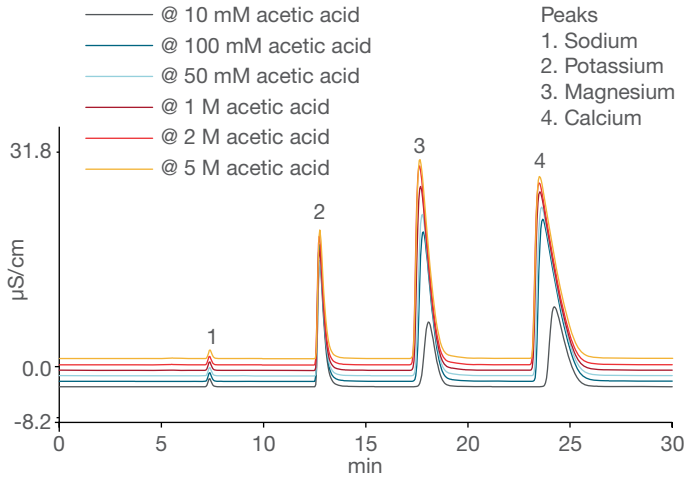

Figure 5. Chromatograms of a multivitamin sample prepared in increasing acetic acid concentrations

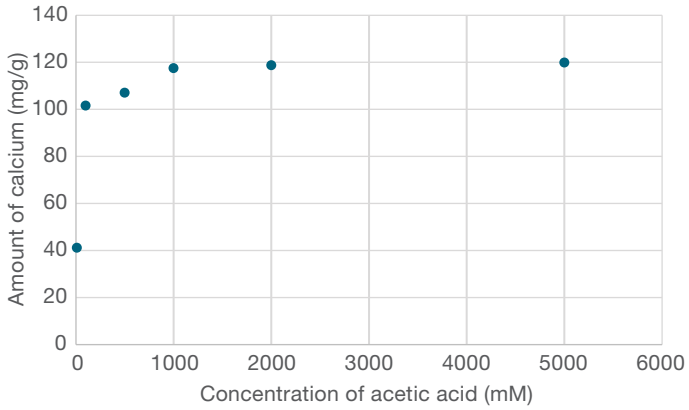

Figure 6. Plot of acetic acid concentration vs. amount of calcium in the multivitamin sample

Calibration and quantification

Calibration standards were prepared in DI water. Eight calibration standards were used for sodium and potassium and nine calibration standards for calcium. Figure 7 displays the calibration curves of sodium, potassium, and calcium. Table 4 summarizes the calibration results.

Table 5 lists the concentration of three cations calculated in multivitamin and sauerkraut samples.

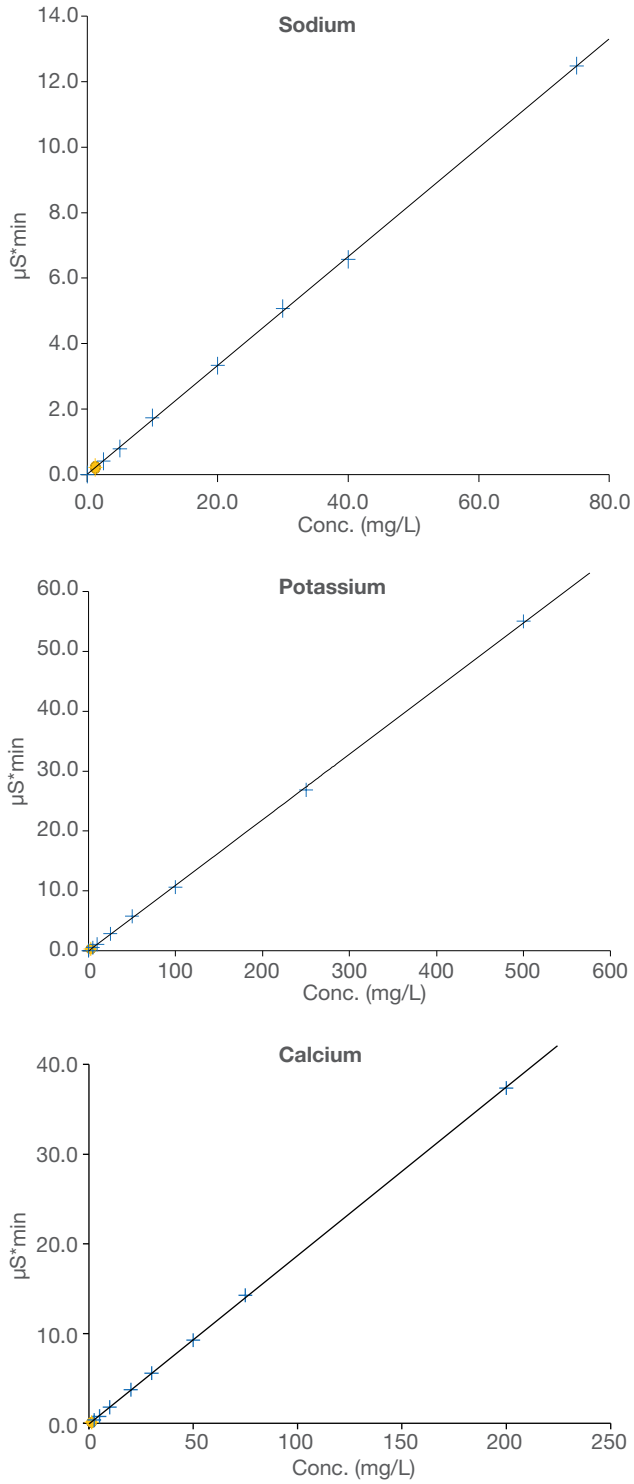

Figure 7. Calibration curves of sodium, potassium, and calcium

Table 4. Calibration data for sodium, potassium, and calcium

Analyte	Calibration range (mg/L)	Levels	Coefficient of determination
Sodium	1.25–75	8	0.9998
Potassium	2.5–500	8	0.9998
Calcium	1.25–200	9	0.9999

Table 5. Amount of sodium, potassium, and calcium in samples

Amount (mg/g)	Sauerkraut	Multi-vitamin 1	Multi-vitamin 2	Multi-vitamin 3
Sodium	5.29	1.17	1.18	1.20
Potassium	1.97	48.3	48.8	50.3
Calcium	0.39	125	117	122

Precision

The precision of the method was evaluated by triplicate injections of the samples prepared and run on three separate days. The calculation of the relative standard deviation (RSD) was performed using all nine injections. The peak area RSDs and retention time RSDs for the three cations are listed in Table 6.

Conclusion

This application used the Dionex IonPac CS16 column with 30 mM MSA eluent at 40 °C and suppressed conductivity detection to determine inorganic cations in multivitamin and sauerkraut samples at concentrations ranging from 1.98 to 200 mg/L. The method showed good precision with RSDs <0.1% and <5% (n=9) for retention time and peak area, respectively.

Table 6. Precisions (RSD, n=9) of peak area and retention time of sodium, potassium, and calcium

	Sauerkraut		Multivitamin 1		Multivitamin 2		Multivitamin 3	
	RT	Peak area	RT	Peak area	RT	Peak area	RT	Peak area
Sodium	0.06	0.74	0.05	0.92	0.05	2.61	0.05	4.08
Potassium	0.03	1.17	0.05	0.56	0.04	0.62	0.07	2.63
Calcium	0.04	3.44	0.09	2.81	0.06	1.04	0.10	3.59

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