

Determination of sodium, potassium, and calcium in rice and wheat flours using ion chromatography

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Goal

To develop an ion chromatography method for the determination of sodium, potassium, and calcium in rice and wheat flours

Introduction

For the past 20 years, ion chromatography (IC) with suppressed conductivity detection has proven to be a robust and reliable technique for the determination of alkali and alkaline earth metals. The National Institute of Standards and Technology (NIST) established a Health Assessment Measurements Quality Assurance Program (HAMQAP) to support the measurement needs of the food, dietary supplement, and clinical communities.¹ 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.

Rice and wheat flours were provided by NIST to measure nutritional elements including calcium, potassium, and sodium. Here, we employed IC to make those measurements. The method uses a Thermo Scientific™ Dionex™ IonPac™ CS16 column, an electrolytically generated methanesulfonic acid (MSA) eluent, and suppressed conductivity detection on a Reagent-Free™



Ion Chromatography (RFIC™) system. The Dionex IonPac CS16 column is a high-capacity cation exchange column that has medium hydrophobicity and is solvent compatible with 100% aqueous eluents, 100% acetonitrile, or 20% tetrahydrofuran without loss of performance.² The high capacity of 3000 µeq/column is achieved by using a particle diameter of 5.5 µm and high density of grafted carboxylic acid cation exchange groups. The flour samples were extracted under acidic conditions and then separated on a Dionex IonPac CS16 column set. The cations were measured by suppressed conductivity detection. The contents of the cations in different flour samples vary greatly. Therefore, it is important to have a high capacity column to determine small amounts of one cation in the presence of large amounts of other cations, including the hydronium ions from the extraction solution. In our study, linearity, limits of detection and quantification, accuracy, and precision are demonstrated.

Experimental

Equipment

- Thermo Scientific™ Dionex™ ICS-6000 HPIC system* including:
 - Thermo Scientific™ Dionex™ ICS-6000 DP Pump module
 - Thermo Scientific™ Dionex™ ICS-6000 EG Eluent Generator module with high-pressure degasser module
 - Thermo Scientific™ Dionex™ ICS-6000 DC Detector/Chromatography module
 - CD Conductivity Detector
 - Tablet control
- Thermo Scientific Dionex AS-AP Autosampler (P/N 074921) that includes sample syringe, 250 µL (P/N 074306) and buffer line, 1.2 mL (P/N 074989)

*This method can be run on any Thermo Scientific™ Dionex™ Reagent-Free Ion Chromatograph (RFIC™) system, and an SP pump module can be used rather than the DP pump module.

Consumables

- Thermo Scientific™ Dionex™ EGC 500 MSA 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)
- Thermo Scientific™ Dionex™ IC PEEK Viper™ Fitting Kit for Dionex ICS-6000 2 mm systems with CD (Analytical) (P/N 302965)
- Thermo Scientific™ Nalgene™ Syringe Filters, PES, 0.2 µm (Fisher Scientific P/N 725-2520)
- Air-Tite™ All-Plastic Norm-Ject™ Syringes, 5 mL, Sterile (Fisher Scientific P/N 14-817-28)

Software

Thermo Scientific™ Chromeleon™ Chromatography Data System software version 7.2

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistivity or better
- Thermo Scientific™ Dionex™ Combined Six Cation Standard-II, 50 mL (P/N 046070)
- Sodium chloride, (Crystalline/Certified ACS), Fisher Chemical™ (Fisher Scientific P/N S271-500)
- Potassium chloride, (Crystalline/Certified ACS), Fisher Chemical (Fisher Scientific P/N P217-500)
- Calcium chloride dihydrate, (Certified ACS), Fisher Chemical (Fisher Scientific P/N C79-500)

Samples

- One bottle of rice flour
- One bottle of wheat flour

The samples were provided by NIST.

IC conditions	
Columns	Dionex IonPac CG16 Guard, 3 × 50 mm (P/N 079931) Dionex IonPac CS16 Analytical, 3 × 250 mm (P/N 059596)
Eluent source	Dionex EGC 500 MSA Eluent Generator Cartridge with CR-CTC 600 trap column
Eluent	30 mM MSA
Flow rate	0.36 mL/min
Column temperature	40 °C
Detector temperature	20 °C
Injection volume	25 µL, (Full Loop)
Detection	Suppressed conductivity, Dionex CDRS 600 Suppressor (2 mm), recycle mode, Use the recommended voltage at constant voltage mode or 32 mA in constant current mode.
System backpressure	~2600 psi (with backpressure coils) (100 psi = 0.6894 MPa)
Background conductance	~0.2 µS/cm
Run time	25 min

Preparation of solutions and reagents

Stock solution

To prepare 1000 mg/L stock solutions of each cation of interest, accurately weigh the amounts of reagent-grade salts in Table 1, transfer to a 100 mL plastic volumetric flask, and fill to the mark with DI water. Mix thoroughly and store at 4 °C. Stock standards are stable for at least three months. Our experiences and official ISO standards indicate that all solutions used, including the standard solutions, should be acidified. This prevents false negative bias due to the formation of calcium and magnesium carbonates.

Table 1. Amounts of compounds used to prepare 100 mL of 1000 mg/L stock solutions

Cation	Compound	Mass (mg)
Sodium	Sodium chloride	254.2
Potassium	Potassium chloride	190.7
Calcium	Calcium chloride	366.8

Working standard solutions calibration

Prepare the calibration standard solutions by diluting the 1000 mg/L stock standard with DI water. The calibration concentrations were 0.02, 0.05, 0.2, 0.4, 2, 4, 10, and 20 mg/L for sodium; 0.05, 0.125, 0.5, 1, 5, 10, 25, and 50 mg/L for potassium; and 0.05, 0.125, 0.5, 1, 5, 10, 25, and 50 mg/L for calcium. For example, to prepare the 20 mg/L sodium, 50 mg/L potassium, and 50 mg/L calcium mixed standard, add 2 mL 1000 mg/L sodium, 5 mL potassium, and 5 mL calcium into a 100 mL plastic volumetric flask and bring to volume with DI water.

Sample preparation

Weigh 1 g of the flour samples and extract them in 100 mL 3% (w/w) acetic acid solution. Shake the mixture for at least 5 min at room temperature. Centrifuge sample solutions at 5000 rpm for 30 min; then pass through a 0.22 µm Nalgene PES syringe filter. Prepare three samples from one bottle of each flour provided.

Recovery study

The samples were spiked with appropriate known amounts of the analytes, then subjected to the sample preparation procedure. Use the overall mean of the unspiked samples for the recovery calculation.

Results and discussion

Separation and detection

The separation of six common cations was performed on a Dionex IonPac CS16 column set with 30 mM MSA at a flow rate of 0.36 mL/min and a column temperature of 40 °C because the selectivity of the column for maximizing peak efficiencies is optimized at that temperature.

Figure 1 shows the chromatogram of six common cations on a Dionex IonPac CS16 column set. Sodium, potassium, and calcium were well resolved and separated from other cations that could be in the sample.

Column: Dionex IonPac CG16 Guard, 3 × 50 mm
 Dionex IonPac CS16 Analytical, 3 × 250 mm
 Eluent: 30 mM MSA
 Eluent source: Dionex EGC 500 MSA cartridge with CR-CTC 600
 Temperature: 40 °C
 Flow rate: 0.36 mL/min
 Inj. volume: 25 µL
 Detection: Dionex CDRS 600 suppressor,
 2 mm, recycle mode, 32 mA

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

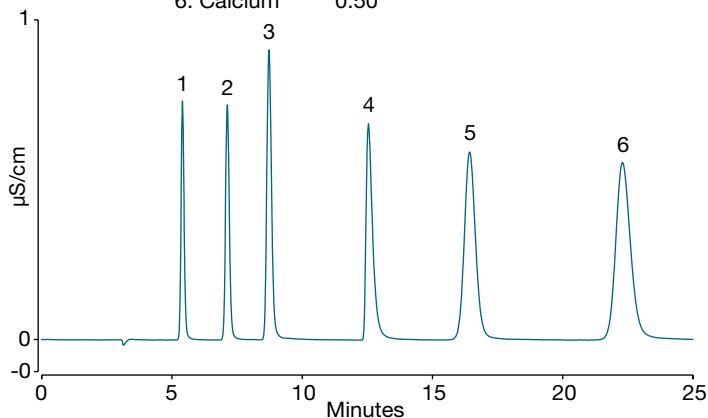


Figure 1. Separation of six common cations on a Dionex IonPac CS16 column set

Calibration, limit of detection, and limit of quantitation

Calibration curves with eight concentration levels were constructed from 0.02 mg/L to 20 mg/L for sodium, from 0.05 mg/L to 50 mg/L for potassium, and from 0.05 mg/L to 50 mg/L for calcium (Figure 2). Each of the standards was injected in triplicate. The results yielded a linear relationship of peak area to concentration with a coefficient of determination (r^2) of 1.0 for each (Table 2).

A signal-to-noise ratio 3:1 is generally considered acceptable for estimating the limit of detection (LOD), and a signal-to-noise ratio 10:1 for limit of quantification (LOQ).³ To determine the LOD and LOQ, the baseline noise is determined by measuring the peak-to-peak noise in a representative 1 min segment of the baseline where no peaks elute but close to the peak of interest. The LOD and LOQ were then calculated from the average peak height of five injections of 0.08 µg/L sodium, 0.2 µg/L potassium, and 0.2 µg/L calcium. The results of the calibration, LOD, and LOQ are summarized in Table 2.

Table 2. Method calibration, LOD, and LOQ

Compound	Range (mg/L)	Coefficient of determination (r ²)	LOD ^a (µg/L)	LOQ ^b (µg/L)
Sodium	0.02–20	1.0	0.087	0.290
Potassium	0.05–50	1.0	0.264	0.880
Calcium	0.05–50	1.0	0.139	0.462

^aLOD = 3 × S/N

^bLOQ = 10 × S/N

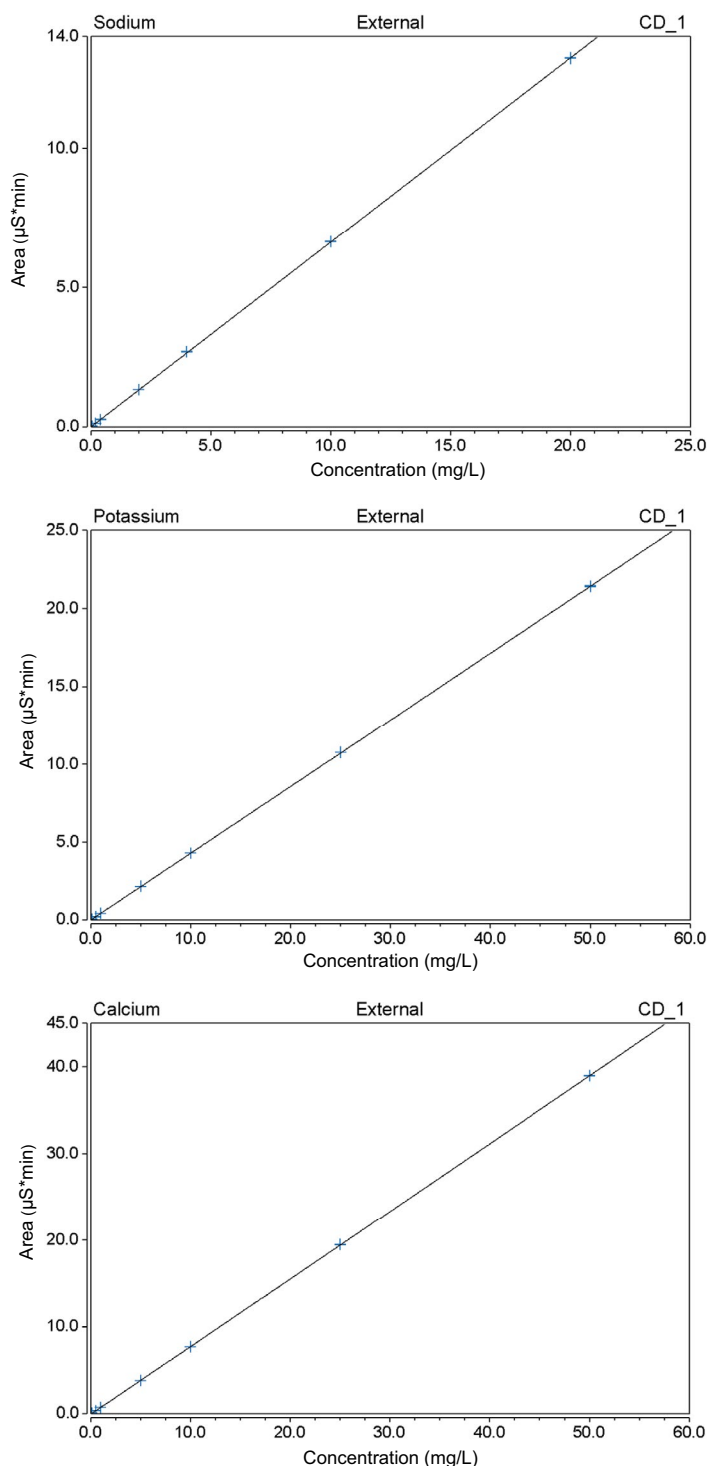


Figure 2. Calibration curves of sodium (0.02–20 mg/L), potassium (0.05–50 mg/L), and calcium (0.05–50 mg/L)

Sample analysis

To accurately determine sodium, potassium, and calcium, we assessed the performance of acetic acid concentration on the extraction of these cations from rice and wheat flours. Figures 3 and 4 show the overlaid chromatograms of rice and wheat flours treated with 0, 0.1, 0.5, 1, and 3% acetic acid solution. When the samples were extracted with acidified solution, the recoveries were improved compared to extraction with water. The addition of acetic acid was found to have more influence on rice flour than wheat flour. The lower percentages of acetic acid were effective too. To make the protocol of sample preparation universal and reproducible, the 3% acetic acid solution was standardized to extract rice and wheat flours.

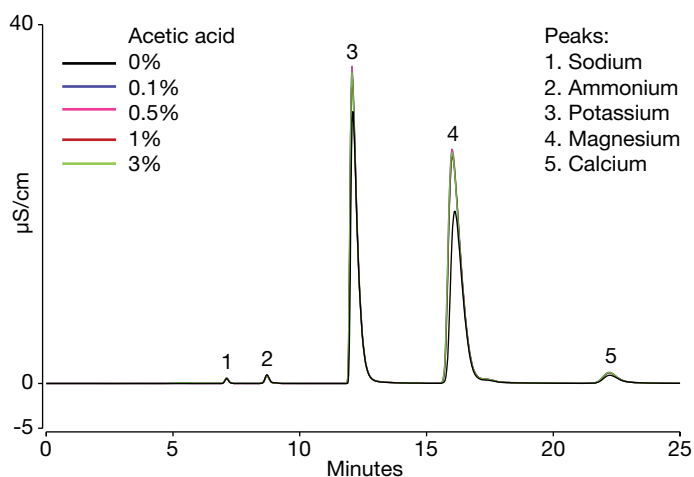


Figure 3. Overlaid chromatograms of rice flour extracted with 0, 0.1, 0.5, 1, and 3% acetic acid solution

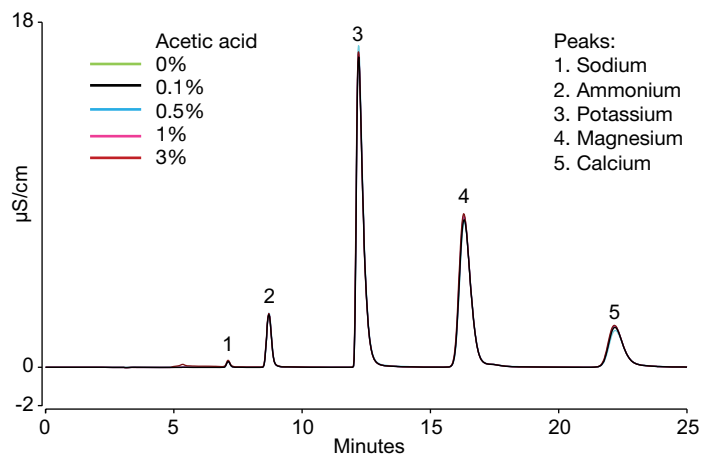


Figure 4. Overlaid chromatograms of wheat flour extracted with 0, 0.1, 0.5, 1, and 3% acetic acid solution

The high capacity of the Dionex IonPac CS16 column allows loading of samples containing 3% acetic acid without affecting resolution of the target analytes. Six samples, three from each flour, were analyzed for sodium, potassium, and calcium contents. Figure 5 shows chromatograms of the three rice flour replicate samples and three wheat flour replicate samples.

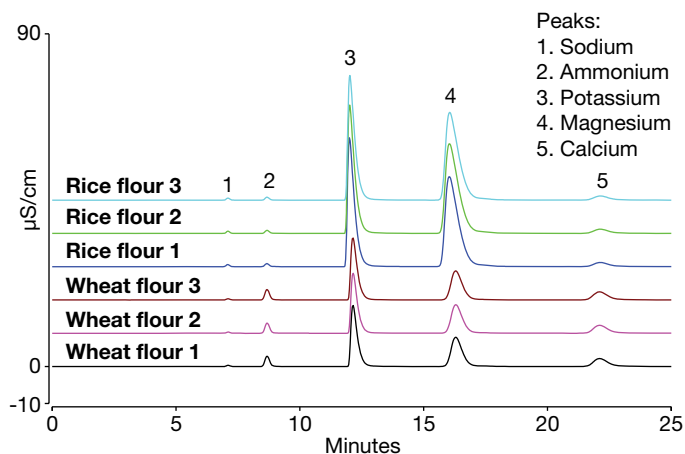


Figure 5. Chromatograms of three rice flour and three wheat flour samples

The concentrations (mg/L) of sodium, potassium, and calcium were calculated using their calibration curves. The contents (mg/kg) of sodium, potassium, and calcium in rice and wheat flours were calculated as below:

$$\text{Content (mg/kg)} = \frac{\text{Calculated concentration (mg/L)}}{0.001 \text{ kg}} \times 0.1 \text{ L}$$

As shown in Table 3, rice flour contains an average of 15.8 mg/kg sodium, 2700 mg/kg potassium, and 113 mg/kg calcium, while wheat flour contains an average of 9.18 mg/kg sodium, 1220 mg/kg potassium, and 199 mg/kg calcium.

Precision

Method precision 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 retention time RSDs were $\leq 0.1\%$ and the peak area RSDs ranged from 0.14 to 0.60% (Table 4). If using manually prepared mobile phases, the precisions—especially for retention times—likely will not be as low as when using electrolytically generated eluent.

Table 3. The contents of sodium, potassium, and calcium in rice and wheat flours

	Sodium (mg/kg)	Potassium (mg/kg)	Calcium (mg/kg)
Rice flour 1	15.9	2740	113
Rice flour 2	16.0	2730	113
Rice flour 3	15.6	2640	112
Average	15.8	2700	113
RSD (n=3)	1.33	1.91	0.704
Wheat flour 1	8.97	1220	197
Wheat flour 2	9.19	1200	196
Wheat flour 3	9.38	1240	203
Average	9.18	1220	199
RSD (n=3)	2.23	1.77	1.63

Table 4. Retention time and peak area precisions (n=9)

	Rice flour		Wheat flour	
	Retention time RSD	Peak area RSD	Retention time RSD	Peak area RSD
Sodium	0.07	0.48	0.05	0.60
Potassium	0.05	0.48	0.05	0.14
Calcium	0.10	0.59	0.08	0.34

Accuracy

Method accuracy was validated by determining the recovery of sodium, potassium, and calcium in spiked rice and wheat flours in triplicate. The recoveries in rice flour were checked with 0.2 mg/L sodium, 2 mg/L potassium, and 0.5 mg/L calcium, while they were checked in wheat flour with 0.1 mg/L sodium, 2 mg/L potassium, and 2 mg/L calcium. Table 5 shows the recovery ranged from 79 to 110%, indicating that this method can be applied to the determination of sodium, potassium, and calcium in rice and wheat flours.

Table 5. Method accuracy (n=3)

	Rice flour		Wheat flour	
	Spike level (mg/L)	Recovery (%)	Spike level (mg/L)	Recovery (%)
Sodium	0.2	93	0.1	83
Potassium	2	79	2	110
Calcium	0.5	92	2	99

Conclusion

This study describes an IC method for the determination of sodium, potassium, and calcium in rice and wheat flours. The method uses a Dionex IonPac CS16 column combined with suppressed conductivity detection on an RFIC system. The sample preparation was optimized for the extraction of sodium, potassium, and calcium in rice and wheat flours. The method showed broad linearity, excellent sensitivity, good accuracy, and high precision.

References

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