APPLICATION NOTE 73450

Simultaneous determination of nitrate and nitrite in spinach and meat by ion chromatography

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Goal

To develop a method for determination of nitrite and nitrate in foods such as meat homogenates and slurried spinach using ion chromatography (IC)

Introduction

Nitrate and nitrite salts are often used as food additives in processed meats like bacon, ham, and sausages to stabilize the color of red meat. They also function as preservatives, helping to prevent the growth of microorganisms that cause food poisoning. The presence of nitrite and nitrate in processed meat is believed to increase the risk of cancer in the digestive tract. The reaction between nitrite with secondary and tertiary amines leads to the formation of carcinogenic chemicals known as N-nitroso compounds.[1] The nitrate content of food is also important, as nitrate can be reduced to nitrite by bacterial enzymes present in the posterior part of the tongue and in the stomach under acidic conditions.[2] For these reasons, use of nitrate and nitrite salts as food additives is strictly regulated worldwide. For example, the European Commission established the maximum allowable concentration for nitrate and nitrite salts in processed meat as 150 mg/kg.[3]

Nitrate and nitrite are also found naturally in vegetables and fruits. The amount of nitrate in vegetables depends on agricultural practices,[4] environmental variables (season, light, temperature, etc.), and genetic factors. Most vegetables usually have low levels of nitrate, with leafy vegetables such as lettuce, spinach, and arugula having the highest levels.[5] Conversely, only trace amounts of nitrite (<10 mg/kg) are present in vegetables. Exceptions to this are poorly stored vegetables or vegetables stored for extended periods, due to the bacterial reduction of nitrate to nitrite.

Ion chromatography (IC) is the most used and well-established technique for the analytical determination of nitrite and nitrate.[6-8] Application Note 112 describes anion exchange chromatography with UV detection for the determination of nitrate and nitrite in meat products.[9]

A Thermo Scientific™ Dionex™ IonPac™ AS11 column was used in that work. In this application note, we developed an IC method using a high capacity version of the Dionex IonPac AS11 column to separate nitrite and nitrate from other anions present in a meat homogenate and slurried spinach. The Dionex IonPac AS11-HC column is a high capacity column that allows relatively large injection volumes, thus facilitating the determination of low nitrate and nitrite concentrations.[10] After the separation, nitrate and nitrite were detected by suppressed conductivity detection. The two food samples were extracted with deionized water and subjected to a series of clean up steps before they were analyzed on the IC system.

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 KOH Eluent Generator Cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 600 Continuously Regenerated Anion Trap Column (P/N 088662)
- Thermo Scientific™ Dionex™ ADRS 600 Anion Dynamically Regenerated Suppressor (2 mm) (P/N 088667)

*This method can be executed on any Thermo Scientific Dionex IC system with eluent generation capable of creating multiple step eluent gradients.

Software

Thermo Scientific™ 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 (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™ Vials, 1.5 mL polypropylene, with caps and septa (P/N 079812)
- Thermo Scientific™ Dionex™ OnGuard™ II Ag/H Cartridges, 2.5 mL (P/N 057410)
- Thermo Scientific™ Dionex™ OnGuard™ II RP Cartridges, 2.5 mL (P/N 057084)

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistivity or better
- Sodium nitrate, Crystalline/Certified ACS (Fisher Chemical, P/N S343-500)
- Sodium nitrite, Crystalline/Certified ACS (Fisher Chemical, P/N S347-500)

Samples

- Meat homogenate
- Slurried spinach

Table 1. Chromatographic conditions

Parameter	Value
System	Dionex ICS-6000 system
Columns	Dionex IonPac AG11-HC Guard, 2 × 50 mm (P/N 052963) Dionex IonPac AS11-HC Analytical, 2 × 250 mm (P/N 052961)
Eluent	5–10 mM KOH (0–9 min); 10 mM KOH (9–13 min); 50 mM KOH (13–16 min); 5 mM KOH (16–20 min)
Eluent source	Dionex EGC 500 KOH cartridge with Dionex CR-ATC 600
Flow rate	0.38 mL/min
Injection volume	25 µL in Push-Full mode
Column temperature	30 °C
Detection	Suppressed Conductivity
Suppressor	Dionex ADRS 600 (2 mm) Suppressor, recycle mode, 48 mA current
Detection/suppressor compartment temperature	15 °C
Background conductance	<0.4 µS/cm
System backpressure	<2500 psi (100 psi = 689.5 kPa)
Noise	0.5–0.8 nS/cm
Run time	20 min

Preparation of solutions and reagents

1000 mg/L sodium nitrate stock standard

Prepare a 1000 mg/L stock standard solution by dissolving 0.1371 g of sodium nitrate in 100 mL of DI water.

1000 mg/L sodium nitrite stock standard

Prepare a 1000 mg/L stock standard solution by dissolving 0.1500 g of sodium nitrite in 100 mL of DI water.

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. For method linearity studies, the following standards of nitrite and nitrate were used: 1500, 1000, 500, 200, 100, 50, 25, 10, and 5 µg/L. Table 2 lists the volumes of nitrate and nitrite standards added to prepare calibration standards in a 250 mL volumetric flask.

Table 2. Calibration standards

Calibration standard (µg/L)	Volume of 1000 mg/L nitrite std (µL)	Volume of 1000 mg/L nitrate std (µL)	DI water up to (mL)
5	1.25	1.25	250
10	2.5	2.5	250
25	6.25	6.25	250
50	12.5	12.5	250
100	25	25	250
200	50	50	250
500	125	125	250
1000	0	250	250
1500	0	375	250

Sample preparation

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

*Both meat and spinach samples were received ground from the National Institute of Standards and Technology (NIST).

Step 2: Centrifuge an aliquot of the solution from step 1 at 4000 RPM for 20 min at 20 °C. Aspirate the supernatant and pass it through a 0.22 µm syringe filter.

Step 3: Prepare a Dionex OnGuard II RP, 2.5 mL cartridge by flushing it with 10 mL methanol, and then connect the Dionex OnGuard II Ag/H cartridge in series such that it sits on the top of the Dionex OnGuard II RP cartridge. Flush with 15 mL of DI water at a flow rate of less than 2 mL/min, then discard the effluent. Load 8 mL of sample and discard the first 6 mL into a waste container. Collect the next 2 mL for analysis.

Note: A Dionex OnGuard II RP cartridge removes hydrophobic substances such as aromatic hydrocarbons from samples. The Dionex OnGuard II Ag/H cartridge layers the resins from both the Dionex OnGuard II Ag and Dionex OnGuard II H cartridges. A Dionex OnGuard II Ag cartridge removes chloride, bromide, and iodide from samples, while in this application a Dionex OnGuard II H cartridge traps any silver that may leach from the Ag cartridge and other cations found in the sample.[11]

Results and discussion

Separation

A good separation is required for the determination of low nitrate and nitrite concentrations in complex matrices like meat homogenate and slurried spinach to avoid overestimation of the content as a result of analyte co-elution. We used a Dionex IonPac AS11-HC column to separate nitrate and nitrite from matrix anions. 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 seven-anion standard analyzed using the conditions listed in the QAR.

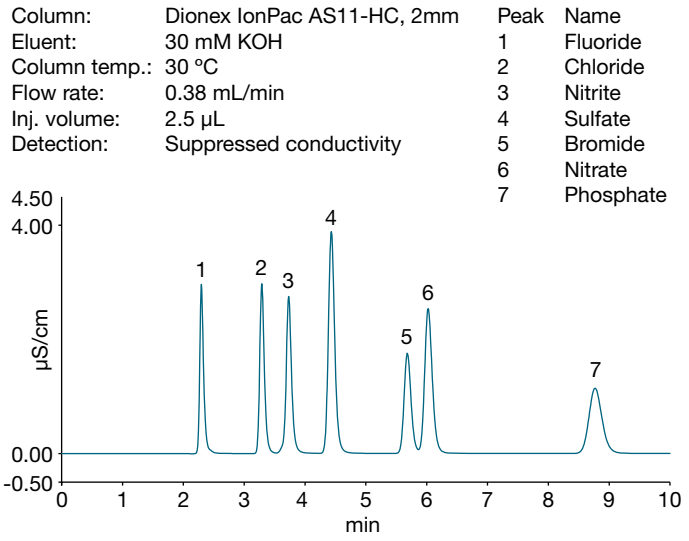

Figure 1. Chromatogram of the Dionex IonPac AS11-HC column QAR standard

Nitrate and nitrite were separated on a 2 × 250 mm Dionex IonPac AS11-HC column at a 0.38 mL/min flow rate using KOH eluent produced by the Dionex EGC 500 KOH eluent generator cartridge. The complete separation of nitrite and nitrate from matrix anions was accomplished by using the gradient elution program listed in Table 1.

Calibration and quantification

In this study, calibration curves with seven and nine concentrations ranging from 5 to 500 µg/L and 5 to 1500 µg/L were constructed for nitrite and nitrate, respectively. Calibration standards for nitrate and nitrite were prepared in DI water. The calibration plots of peak area versus concentration were fit using linear regression functions that yielded coefficients of determination (r^2) greater than 0.999. Figure 2 displays the calibration curves of nitrite and nitrate. Table 3 summarizes the calibration results.

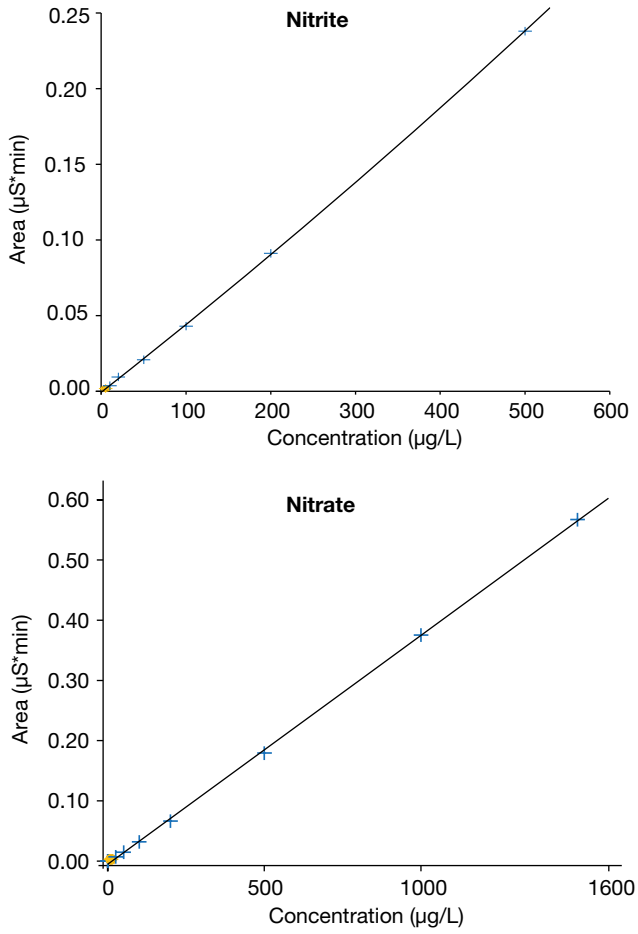

Figure 2. Nitrite and nitrate calibration curves

Table 3. Results of calibration, LOD, and LOQ on nitrite and nitrate

Analyte	Range (µg/L)	r^2	LOD[a] (µg/L)	LOQ[b] (µg/L)
Nitrite	5–500	0.9999	1.33	4.42
Nitrate	5–1500	0.9998	2.26	7.52

[a] LOD = 3 × signal-to-noise ratio (S/N)
[b] LOQ = 10 × S/N

The sensitivity of the method was assessed by estimating the limit of detection (LOD) and limit of quantitation (LOQ). To determine the LOD and LOQ, the baseline noise was determined by measuring the peak-to-peak noise in a representative one-minute segment of the baseline where no peaks elute, but close to the peak of interest. The signal was determined from the average peak height of three injections of a 1 µg/L nitrite and nitrate standard solution. The estimated LODs and LOQs are summarized in Table 3.

Sample pretreatment

The samples were treated with a series of cleanup steps described in the sample preparation section and then run on the IC system. A Dionex OnGuard II RP cartridge was used to remove lipids from the meat and spinach samples. A Dionex OnGuard II Ag/H cartridge (a layered cartridge containing OnGuard II Ag and OnGuard II H resins) was used to remove excess chloride and other halides from the extraction solution that may interfere with the detection of nitrite. These cleanup steps are essential. Not removing chloride from the sample will create a sample of higher ionic strength, which will limit the ability to increase injection volume, and for some samples, lead to column overload.

Sample analysis

Nitrite and nitrate concentrations were determined in slurried spinach and meat homogenate. Both samples were provided by NIST for Exercise 4 of the HAMQAP (Health Assessment Measurements Quality Assurance Program). Figure 3 displays the chromatograms of the meat homogenate and slurried spinach samples along with a 5 µg/L nitrite and nitrate standard. Both nitrite and nitrate are well resolved from other peaks in the meat homogenate sample. Both nitrite and nitrate content are well below the maximum allowable limit of 150 mg/kg. For the slurried spinach sample, nitrate is well resolved, but the nitrite peak is not very well resolved and is present at a level lower than LOQ of the method. As discussed earlier, only trace amounts of nitrite are present in vegetables. Table 4 lists the amount of nitrite and nitrate measured in these samples.

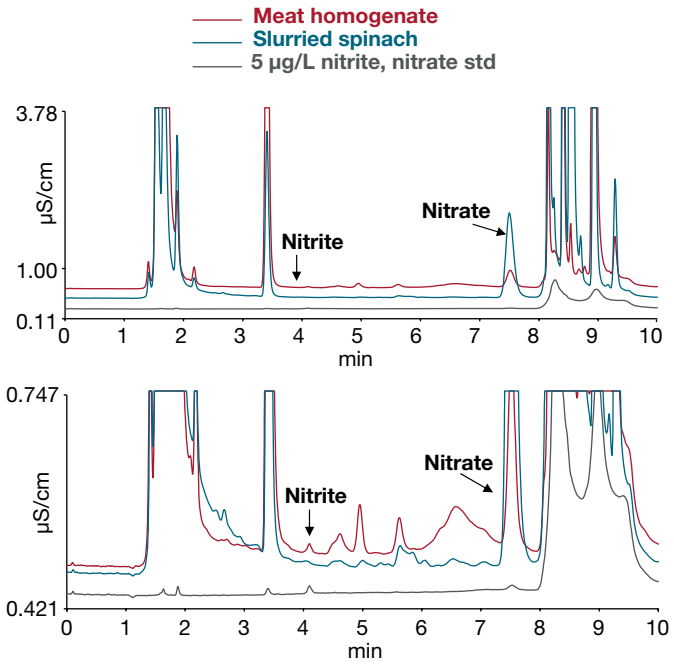

Figure 3. Chromatograms of meat homogenate and slurried spinach sample along with a 5 µg/L standard

Table 4. Amount of nitrite and nitrate in samples

Sample	Nitrite		Nitrate	
	Avg (mg/kg)	RSD (n=9)	Avg (mg/kg)	RSD (n=9)
Meat homogenate	0.597	1.44	24.2	0.38
Slurried spinach	<LOQ		130	0.12

Accuracy and precision

To evaluate the method accuracy, recovery experiments were carried out by spiking with 10 µg/L and 200 µg/L of nitrite and nitrate, respectively. The recovery percentages were calculated using the formula shown below:

$$\%\ Recovery = \frac{C\ spiked\ sample - C\ unspiked\ sample}{C\ analyte\ added} \times 100$$

Figures 4 and 5 show the chromatograms of the meat homogenate sample and the slurried spinach sample unspiked and spiked with 10 µg/L nitrite and 200 µg/L nitrate. The method showed recoveries ranging from 89 to 100% (Table 5).

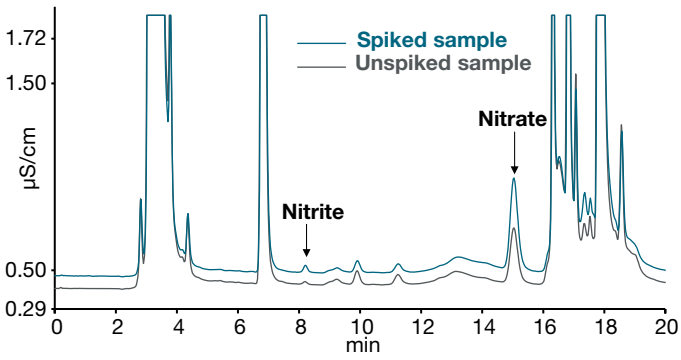

Figure 4. Chromatogram of meat homogenate sample unspiked and spiked (10 µg/L nitrite and 200 µg/L nitrate)

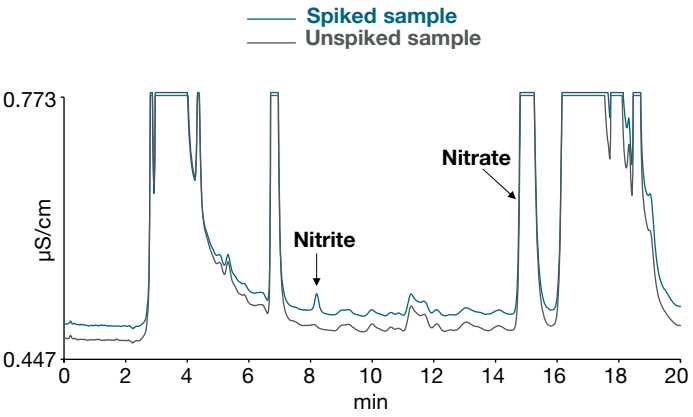

Figure 5. Chromatogram of slurried spinach sample unspiked and spiked (10 µg/L nitrite and 200 µg/L nitrate)

Table 5. Recovery of nitrite and nitrate

Sample	Nitrite				Nitrate			
	Found (µg/L)	Spiked (µg/L)	Recovered (µg/L)	Recovery (%)	Found (µg/L)	Spiked (µg/L)	Recovered (µg/L)	Recovery (%)
Meat homogenate	5.97	10.0	15.9	99.3	242	200	419	88.8
Slurried spinach	n.a				1300	200	1490	95.0

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 are listed in Table 6. The peak area RSD for nitrite in the slurried spinach sample is higher than it is for the meat, ~10%. It is present at a concentration lower than the LOQ, thus a higher variation is expected.

Table 6. Precision (RSD, n=9) of peak area and retention time of nitrite and nitrate

	Nitrite		Nitrate	
Sample	RT	Peak area	RT	Peak area
Meat homogenate	0.06	4.63	0.02	3.29
Slurried spinach	0.15	9.25	0.04	1.12

Conclusion

Using a high capacity Dionex IonPac AS11-HC column and a large volume injection, low concentrations of nitrite and nitrate were determined in meat homogenate and slurried spinach samples. The method showed good precision with RSDs <0.2% and <5% (n=9) for retention time and peak area, respectively. The recoveries from meat homogenate and slurried spinach sample ranged from 89 to 100%.

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