Application Note 1088

Determination of Thiosulfate and Pyrophosphate in Crayfish Wash Powder

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Key Words
Dionex IonPac AG/AS19 Capillary Column Set, Capillary Ion Chromatography (IC), Mass Spectrometric Detection, Food Safety

Goal
To develop a simple, efficient, and sensitive IC method for the determination of thiosulfate and pyrophosphate in crayfish wash powder

Introduction
Crayfish—freshwater crustaceans that resemble small lobsters—are a type of seafood enjoyed by many people around the globe. However, eating too much of this delicacy can cause excessive myoglobin in the blood and thereby trigger rhabdomyolysis.[1] To make crayfish look more attractive to consumers, they are often cleaned with wash powder, and it is this wash powder that is suspected of causing the problems associated with excessive ingestion of crayfish. The reported harmful ingredients of crayfish wash powder are thiosulfate and phosphate-related compounds such as tripolyphosphate, pyrophosphate, trimetaphosphate, and phosphite.[1] Therefore, it is necessary to establish an efficient method to determine these components in crayfish wash powder.

Thiosulfate and phosphate-related compounds are charged analytes that can be detected at low levels using suppressed conductivity detection. Thus, IC is an effective technique for their determination. Recently, IC combined with mass spectrometry (MS) has become a technique of choice in many applications because it fulfills key requirements for speed, sensitivity, selectivity, and peak-assignment certainty for the determination of low concentrations in complex samples.[2-7] Therefore, IC/MS was applied to the analysis of components in crayfish wash powder and an effective method was developed using a Reagent-Free™ IC (RFIC™) system, the Thermo Scientific™ Dionex™ ICS-4000 Integrated Capillary HPIC™ System, capable of supporting high-pressure IC.

Equipment
- Dionex ICS-4000 Integrated Capillary HPIC system with 0.4 µL sample loop
- Thermo Scientific Dionex AS-AP Autosampler
- Thermo Scientific Dionex EGC Potassium Hydroxide (KOH) Capillary Eluent Generator Cartridge (P/N 072076)
- Thermo Scientific™ Dionex™ ACES™ 300 Anion Capillary Electrolytic Suppressor
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software, version 6.8 SR13 or higher
- Thermo Scientific™ TSQ Vantage™ Triple Stage Quadrupole mass spectrometer
- Thermo Scientific™ Xcalibur™ software, version 1.2

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Consumables

- Thermo Scientific™ Target2™ Nylon Syringe Filters, 0.45 µm, 30 mm (P/N F2500-1)
- Thermo Scientific™ Dionex™ OnGuard™ RP Cartridge (P/N 039595)
- Dionex OnGuard II Na Cartridge, 2.5 cc (P/N 062962)
- Thermo Scientific Sun-SRi Luer-Lock Syringe (Fisher Scientific P/N 14-823-261)

Reagents and Standards

- Deionized (DI) water, 18.2 MΩ-cm resistivity
- Sodium Fluoride, Powder/Certified ACS (Fisher Scientific P/N S299-100)
- Sodium Chloride, Crystalline/Biological, Certified (Fisher Scientific P/N S671-500)
- Sodium Bromide, Granular, Certified ACS (Fisher Scientific P/N S255-500)
- Sodium Sulfate, Anhydrous, Powder/USP/FCC (Fisher Scientific P/N S429-500)
- Sodium Phosphate Tribasic Dodecahydrate, Crystalline/Certified ACS (Fisher Scientific P/N S377-500)
- Sodium Nitrite, Crystalline/Certified ACS (Fisher Scientific P/N S347-250)
- Sodium Nitrate, Crystalline/Certified ACS (Fisher Scientific P/N S343-500)
- Sodium Oxalate, Certified ACS (Fisher Scientific P/N S487-500)
- Sodium Thiosulfate, Crystalline/USP/FCC (Fisher Scientific P/N S474-500)
- Potassium Citrate Monohydrate, Granular/USP/FCC (Fisher Scientific P/N P218-500)
- Sodium Tripolyphosphate, for Analysis (Fisher Scientific P/N AC39396-0250)
- Sodium Pyrophosphate Decahydrate, Crystalline/Certified ACS (Fisher Scientific P/N S390-500)
- Sodium Trimetaphosphate, Powder (Fisher Scientific P/N AA89063A1)
- Sodium Phosphite (Fisher Scientific P/N ICN209073)
- Sodium Hydroxide (NaOH) Solution, 50% w/w/Certified (Fisher Scientific P/N SS254-500)

Chromatographic Conditions (Applicable to Figures 1 and 4)	
System:	Dionex ICS-4000 Integrated Capillary HPIC system with eluent generation
Columns:	Thermo Scientific™ Dionex™ IonPac™ AG19 Capillary Guard, 0.4 × 50 mm (P/N 072065) Dionex IonPac AS19 Capillary, 0.4 × 250 mm (P/N 072064)
Eluent Source:	Dionex EGC-KOH Capillary Cartridge
Eluent:	KOH
Gradient:	0–15 min, 6.5 mM; 30 min, 40 mM; 37–39 min, 67 mM; 40–46 min, 6.5 mM
Flow Rate:	0.012 mL/min
Injection Volume:	0.4 µL
Temperature:	30 °C
Detection:	Suppressed conductivity, Auto Suppression recycle mode, power setting 14 mA
System Backpressure:	1680 psi
Background Conductance:	0.314 µS

Mass Spectrometric Conditions (Applicable to Figure 2)*	
System:	TSQ Vantage mass spectrometer
Ionization Mode:	Negative ion mode
Spray Voltage:	3000 V
Vaporizer Temp:	350.0 °C
Sheath Gas Pressure:	40.0 bar
Ion Sweep Gas Pressure:	0 bar
Auxiliary Gas Pressure:	5.0 bar
Collision Gas Pressure:	0 mTorr

*Note: The Dionex ICS-2100 system coupled with the TSQ Vantage mass spectrometer was used to identify the sample ingredients, and the Auto Suppression external water mode was used for IC-MS. The external water was delivered pneumatically with nitrogen gas. This system used a Dionex IonPac AS19-4µm Analytical (2 × 250 mm, P/N 083223) at a flow rate of 0.25 mL/min.

Preparation of Solutions and Reagents

Working Standard Solutions for Calibration

To prepare stock standard solutions, weigh the correct amounts of the compounds that contain the 14 analytes—sodium fluoride, sodium chloride, sodium bromide, sodium sulfate, sodium phosphate tribasic dodecahydrate, sodium nitrite, sodium nitrate, sodium oxalate, sodium thiosulfate, potassium citrate monohydrate, sodium tripolyphosphate, sodium pyrophosphate, sodium trimetaphosphate, and sodium phosphite—and dilute to 100 mL in 14 separate volumetric flasks with a 5 mM NaOH solution. The concentration of each anion stock standard will be 1000 mg/L.

To prepare a mixed standard containing the 14 analytes, add 5 mL of each of the stock standard solutions (total of 70 mL) to a volumetric flask and dilute to 100 mL with a 5 mM NaOH solution. The concentration of each analyte will be 50 mg/L.

The preparation of working standards for calibration will depend on the target ions found in the crayfish wash powder samples. For example the detected harmful ingredients in the samples here were thiosulfate and pyrophosphate; therefore, a mixed stock standard containing thiosulfate and pyrophosphate was prepared by adding 5 mL of each of the stock standards of sodium thiosulfate and sodium pyrophosphate (concentration 1000 mg/L each) to a volumetric flask and diluting to 100 mL with a 5 mM NaOH solution. The concentration of each of the two analytes in the stock mixed standard was 50 mg/L.

Add the correct amounts of the mixed stock standard of thiosulfate and pyrophosphate and dilute with a 5 mM NaOH solution to make a series of working standard solutions with concentrations of 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, and 7.5 mg/L to generate a calibration curve for each.

Sample Preparation

Cartridge Activation

Use the Dionex OnGuard RP and Dionex OnGuard II Na cartridges for sample pretreatment to remove hydrophobic organics and metal ions. Both cartridges require activation before use. To activate the Dionex OnGuard RP cartridge, pass 10 mL of methanol through the cartridge followed by 15 mL of DI water, then allow it to stand for 15 min before use. To activate the Dionex OnGuard II Na cartridge, pass 10 mL of DI water through the cartridge and allow it to stand for 15 min.

Sample Pretreatment

Two crayfish wash powder samples were generously donated by a customer from Jiangsu, China.

Dissolve 100 mg of crayfish wash powder with 80 mL of DI water, then dilute to 100 mL with DI water. After filtering 10 mL of the sample solution through a 0.45 µm syringe filter, pass the filtrate through a Dionex OnGuard RP cartridge and then a Dionex OnGuard II Na cartridge. Discard the first 3 mL of solution and collect the remaining solution. Prior to injection, dilute the collected solution with a 5 mM NaOH solution if necessary and filter the solution through a 0.45 µm syringe filter.

Results and Discussion

Separation of 14 Analytes

The Dionex IonPac AS19 capillary column with hydrophilic quaternary ammonium groups provides excellent separation of a variety of anions (including inorganic anions, oxyhalides, oxyanions, and organic acids), but requires only 1/100th the eluent flow rate of a 4 mm diameter column.[8] The capillary format offers the advantage of less eluent consumption and thus reduced operating costs. Figure 1 shows a chromatogram of a mixed standard containing 14 target anions separated by a Dionex IonPac AS19 capillary column under the specified chromatographic conditions.

Identification of Sample Ingredients

The ingredients in the crayfish wash samples were identified by comparing their retention time to that of the standards using IC, then their masses were determined by MS.

Figure 2 shows the mass spectra of Samples 1 and 2 in the retention time window of thiosulfate and pyrophosphate using an electrospray ionization (ESI) source in the negative ion mode. The ion in the mass spectra of Samples 1 and 2 with the *m/z* 112.9 (Figure 2, Chromatograms A and B) was identified as thiosulfate and the ion in Sample 2 with the *m/z* 176.9 (Figure 2, Chromatogram C) was identified as pyrophosphate.

Eluent: Hydroxide gradient

Time (min)	Concn (mM)
0	6.5
15	6.5
30	40
37	67
39	67
40	6.5
46	6.5

Peaks:		Concn (mg/L)
1.	Fluoride	1
2.	Chloride	2
3.	Nitrite	4
4.	Bromide	10
5.	Nitrate	8
6.	Phosphite	5
7.	Sulfate	2
8,	Phosphate	5
9.	Thiosulfate	4
10.	Oxalate	6
11.	Citrate	6
12.	Pyrophosphate	10
13.	Trimetaphosphate	6
14.	Tripolyphosphate	6

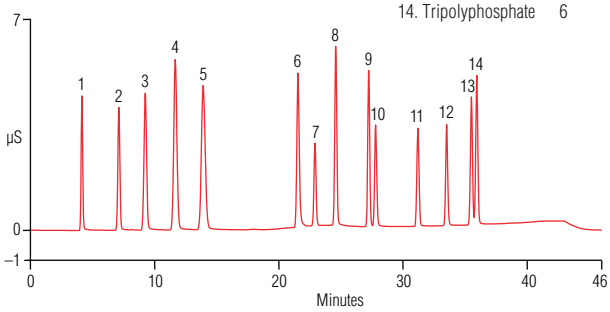

Figure 1. A standard mix of 14 anions separated on a Dionex IonPac AS19 capillary column.

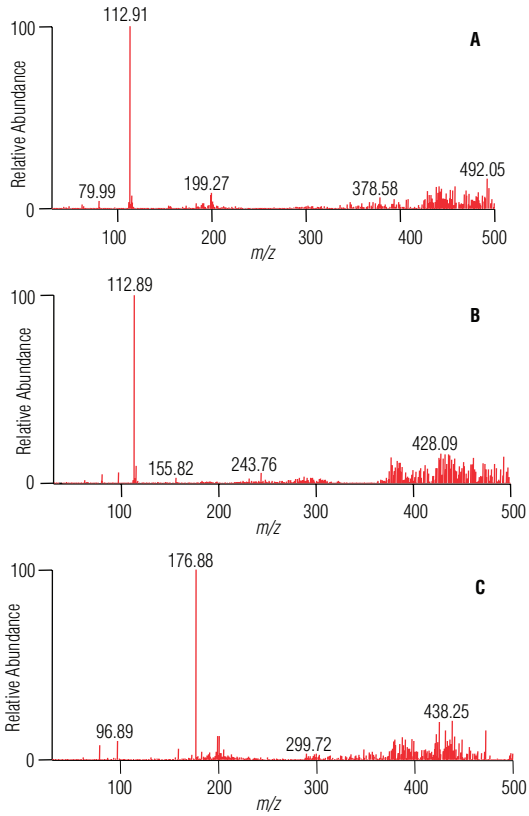

Figure 2. Mass spectra of (A) Sample 1 and (B) Sample 2 in the retention time window of thiosulfate, and (C) Sample 2 in the retention time window of pyrophosphate using an ESI source in the negative ion mode.

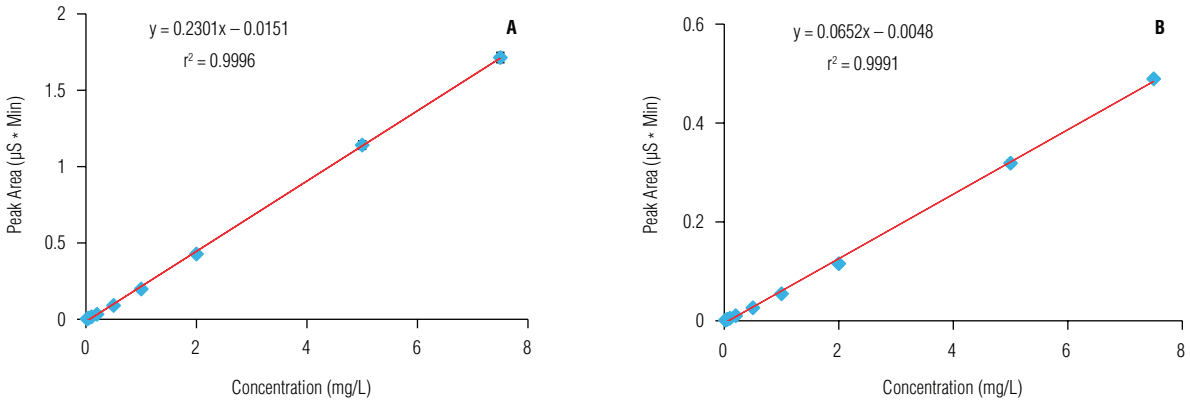

Figure 3. Calibration curves for (A) thiosulfate and (B) pyrophosphate.

Reproducibility, Calibration, and Detection Limits

Six consecutive 0.4 µL injections of a mixed standard of thiosulfate and pyrophosphate (5.0 mg/L each) under the specified chromatographic conditions showed that the RSDs of thiosulfate retention time and peak area were 0.01 and 0.57%, respectively; for pyrophosphate, the values were 0.019 and 0.54%, respectively. These results indicate that the method has good short-term reproducibility. Day-to-day reproducibility was also investigated by making three injections of the above standards each day for three consecutive days. The RSD of peak area was 0.714% for thiosulfate and 1.01% for pyrophosphate, demonstrating good longer-term reliability.

Calibration linearity was investigated by making 0.4 µL injections of mixed standards of thiosulfate and pyrophosphate at different concentrations: 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, and 7.5 mg/L (three injections for each concentration). The results are shown in Table 1.

Five replicate injections of a mixed standard with a concentration of 0.05 mg/L were used for estimating method detection limits (MDLs) using a signal-to-noise ratio of 3. The results are listed in Table 1. Figure 3 shows the calibration curves for thiosulfate and pyrophosphate.

Sample Analysis

Figure 4 shows chromatograms of the crayfish wash powder samples. Thiosulfate (Peak 1) was detected in Samples 1 and 2, whereas pyrophosphate (Peak 2) was detected only in Sample 2. Peak 3 was also found in both samples; however, comparisons of retention time in IC and the *m/z* 96.98 in MS all indicate that Peak 3 was not one of the 14 target analytes. To judge method accuracy, three injections of Sample 1 spiked with a mixed standard were made. Table 2 summarizes the analysis results.

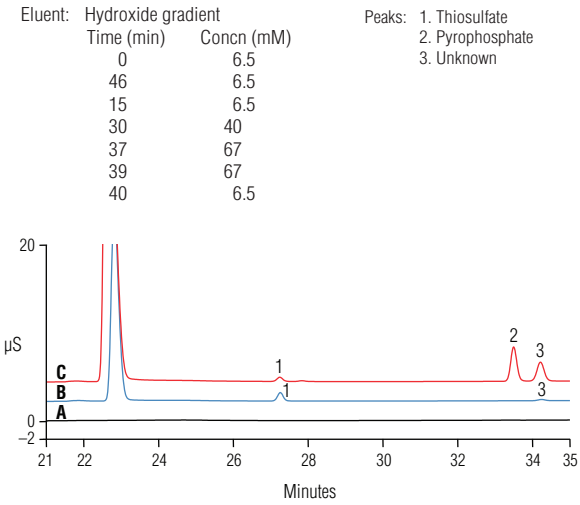

Figure 4. (A) Blank, (B) crayfish wash powder Sample 1, and (C) crayfish wash powder Sample 2.

Table 1. Calibration data and MDLs.

Analyte	Range (mg/L)	Regression Equation	r^2	MDL (µg/L)
Thiosulfate	0.02–7.5	A = 0.2301c – 0.0151	0.9993	6
Pyrophosphate	0.02–7.5	A = 0.0652c – 0.0048	0.9991	11

Table 2. Sample analysis results.

Sample Analyte	Sample 1				Sample 2
	Detected (mg/Kg)	Added (mg/Kg)	Found (mg/Kg)	Recovery (%)	Detected (mg/Kg)
Thiosulfate	27.2	20	22	110	4.6
Pyrophosphate	Not Detected	20	16	80	103.8

Conclusion

This work describes a reproducible and accurate capillary IC method for determining thiosulfate and pyrophosphate in crayfish wash powder. This application is easily executed using an RFIC system controlled by Chromeleon CDS software. The analyst simply adds water and samples to separate thiosulfate and pyrophosphate on the Dionex IonPac AS19 capillary column, thus enabling accurate determination.

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