

Determination of Anions in Dried Distillers Grains with Solubles

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Key Words

Dionex IonPac AS11 Column, Minerals, Ethanol Coproducts, Nutrient Composition, Biofuel, Animal Feed

Introduction

Distillers grains are a coproduct of the biofuel production process and have been used as animal feed for nearly a century. Due to an increased interest in alternative fuels coupled with the rising cost of corn and soybean meal, the distillers grains industry has witnessed significant growth. When present in livestock rations, distillers grains are an excellent source of energy, protein, and essential minerals that provide three times the amount of nutrients available from unprocessed grain.¹ Because the nutritional composition of dried distillers grains with solubles (DDGS) can vary from batch to batch, the distillery industry is interested in determining DDGS composition to properly formulate a nutritionally balanced diet for the animal feed industry.²

Many factors contribute to the nutrient variability of DDGS, including corn hybrid and yield, fermentation characteristics, processing equipment and chemicals, and drying times and temperatures. The mineral variability of DDGS in a single study reported coefficients of variation as high as 25%.² This nutrient variability can have negative effects on the distillers grains industry by requiring the consumer to supplement the diet of feed animals to meet their nutritional needs. High concentrations and low bioavailability of some minerals can lead to serious nutritional disorders.

Two minerals that contribute to the variability of DDGS are sulfur and phosphorus. Total sulfur intake is monitored at a maximum tolerable level of 0.4% to minimize the risk of developing polioencephalomalacia (PEM), a serious disorder that can cause blindness, comas, seizures, and even death. Sulfur is an essential nonmetallic mineral that occurs naturally in DDGS at ~0.2%; however, it has been reported at levels as high as 1%.



Excess sulfur is seen in the form of inorganic sulfur or sulfate, and predominantly originates from the sulfuric acid used in the production process, in particular from the cleaning of the distillation columns.³

Phosphorus is another essential mineral for animal diets. Total phosphorus concentrations in DDGS can range from 0.5–1%. However, many forms of phosphorus exist and not all can be digested by nonruminant animals, such as poultry and swine.^{4,5} Total available phosphorus is reported at 0.04% in corn and 0.7% in DDGS.⁶ The increase of available phosphorus in DDGS is due to the enzymatic and nonenzymatic hydrolytic release of phosphate groups from phytate, as a result of increased temperatures and pH changes during fermentation and processing. Therefore, phosphate levels are indicative of the extent of phytate hydrolysis and can be used to determine phosphorus bioavailability.⁵

Goal

To develop a rapid (<10 min) method for the determination of chloride, sulfate, and phosphate in DDGS

Equipment

- Thermo Scientific™ Dionex™ ICS-2100* Integrated Reagent-Free™ Ion Chromatography (RFIC™) system, including:
 - Isocratic Pump
 - Vacuum Degasser
 - Eluent Generator
 - Column Heater Enclosure
 - High-Pressure, 6-Port Injector
 - Conductivity Cell and Detector
- Thermo Scientific Dionex AS-AP Autosampler**
- Thermo Scientific Dionex Potassium Hydroxide Eluent Generator Cartridge, EGC III KOH (P/N 074532)
- Thermo Scientific Dionex Chromeleon™ Chromatography Data System software version 7.1
- Helium or nitrogen; 4.5 grade (99.995%) or better
- Vial Kit, 1.5 mL Polypropylene with Caps and Septa (P/N 079812)
- Fisherbrand™ Easy Reader Plastic Centrifuge Tube Sterile, Polypropylene; Flat top closure, 15 mL (Fisher Scientific P/N 07-200-886)

*A Dionex ICS-5000 system, or any other Dionex ICS system capable of eluent generation that also uses 2 mm columns, can also be used for this application.

**A Dionex AS or AS-DV Autosampler can also be used with this application.

Reagents and Standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistance or better
- Sodium Chloride, Crystalline, Certified ACS (Fisher Scientific P/N S271500)
- Potassium Sulfate, Crystalline, Certified ACS (Fisher Scientific P/N P304-500)
- Potassium Phosphate Dibasic Anhydrous, Crystalline Powder, Certified ACS (Fisher Scientific P/N P288-500)
- Thermo Scientific Dionex Chloride Standard, 1000 µg/mL in water (P/N 037159)
- Thermo Scientific Dionex Sulfate Standard, 1000 µg/mL in water (P/N 037160)
- Phosphate Standard, 1000 µg/mL in water (low TOC, <50 ppb) (Ultra Scientific P/N ICC-005)

Samples

DDGS (Sample was generously donated)

Conditions

Columns:	Dionex IonPac™ AS11 Analytical, 2 × 250 mm (P/N 044077) Dionex IonPac AG11 Guard, 2 × 50 mm (P/N 044079)
Eluent:	-5–0 min 3 mM KOH, 0–5 min 3–15 mM KOH, step at 5 min to 25 mM KOH, 5–7.5 min 25 mM KOH
Eluent Source:	Dionex EGC III KOH with Thermo Scientific Dionex CR-ATC Continuously Regenerated Anion Trap Column
Flow Rate:	0.5 mL/min
Injection Volume:	5 µL
Temperature:	30 °C
Detection:	Suppressed conductivity, Thermo Scientific Dionex ASRS™ 300 Anion Self-Regenerating Suppressor™, 2 mm (P/N 064555) in the recycle mode, 31 mA
System Backpressure:	~2200 psi
Background Conductance:	<0.4 µS
Typical Noise:	<1.4 nS
Run Time:	7.5 min

Preparation of Solutions and Reagents 10,000 mg/L Stock Standard Solutions

Prepare separate 10,000 mg/L stock solutions by weighing the appropriate amounts of the required analytes in a 125 mL polypropylene bottle (Table 1). Tare the balance, add 100 mL of DI water, cap the bottle, and shake until the solid particles are fully dissolved. The solutions are stable for at least one month when stored at 4 °C.

1000 mg/L Stock Standard Solutions

Purchase certified standards from Thermo Fisher Scientific or another reputable source. Alternatively, prepare separate 1000 mg/L stock solutions by weighing the appropriate amounts of the required analytes in a 125 mL polypropylene bottle (Table 1). Tare the balance, add 100 mL of DI water, cap the bottle, and shake until the solid particles are fully dissolved. The solutions are stable for at least one month when stored at 4 °C.

Table 1. Stock standard solution preparation.

Analyte	Compound	Amount Added to Prepare a 10,000 mg/L Solution (g)	Amount Added to Prepare a 1000 mg/L Solution (g)	Amount of DI Water Added (g)
Chloride	Sodium Chloride	1.65	0.165	100
Sulfate	Potassium Sulfate	1.81	0.181	100
Phosphate	Potassium Phosphate Dibasic Anhydrous	1.83	0.183	100

Mixed Stock Calibration Standard Solution

Prepare a mixed stock calibration standard solution containing 5, 40, and 40 mg/L of chloride, sulfate, and phosphate, respectively. Place a 125 mL polypropylene bottle on a balance and tare it. Using the 1000 mg/L stock standard solutions, add 0.5, 4.0, and 4.0 mL of chloride, sulfate, and phosphate, respectively. Add DI water until the weight reaches 100 g, cap, and shake to mix.

Working Calibration Standard Solutions

Use the mixed stock solution to perform six dilutions to provide a total of seven calibration standards. For this study, calibration standards were prepared at 5, 2.5, 1.2, 0.6, 0.3, 0.16, 0.08 mg/L for chloride and 40, 20, 10, 5, 2.5, 1.2, and 0.6 mg/L for sulfate and phosphate. The mixed stock calibration standard solution was used as the highest calibration working solution.

Sample Preparation

To ensure a representative DDGS sample is analyzed, mix the solid material thoroughly and then take portions from three different areas of the DDGS. Weigh 1 g of DDGS into a 125 mL polypropylene bottle and then add DI water until the scale reads 100 g. Cap the bottle, shake to mix, and sonicate for 5 min. Transfer a 12 mL portion of the liquid to a 15 mL disposable polypropylene centrifuge tube with cap and centrifuge at 5000 rpm for 10 min. Perform a final 1:10 dilution by transferring 1 g of the supernatant to a 20 mL polypropylene vial and bring to volume by weight (10 g) with DI water.

Spiked Sample Solutions

Add the appropriate amount of the 10,000 mg/L stock standard solution of each anion directly to 1 g from a representative portion of the dry DDGS sample and continue by following the Sample Preparation procedure.

Results and Discussion

Separation and Detection

Separation of chloride, sulfate, and phosphate was achieved on a Dionex IonPac AS11 (2 × 250 mm) column in <10 min using a hydroxide eluent gradient and detected by suppressed conductivity detection. The Dionex IonPac AS11 column is designed to separate a wide range of small organic acids, which are often present in these types of samples, from common inorganic anions. Figure 1 shows the highest calibration standard containing 5, 40, and 40 mg/L of chloride, sulfate, and phosphate, respectively. This chromatogram shows excellent peak shape and asymmetry with tailing factors <1.5 for all three anions. Figure 2 shows a typical DDGS sample prepared using the conditions described in this study. Retention times for chloride, sulfate, and phosphate were 1.7, 4.5, and 6.8 min, respectively. Unidentified peaks, which are believed to be small organic acids and residuals of the fermentation process, are present at 1.1, 3.8, 4.8, and 7.5 min.

Column: Dionex IonPac AG11, AS11, 2 mm
 Eluent: -5–0 min 3 mM KOH, 0–5 min 3–15 mM KOH, step at 5 min to 25 mM KOH, 5–7.5 min 25 mM KOH
 Eluent Source: Dionex EGC III KOH with Dionex CR-ATC
 Flow Rate: 0.5 mL/min
 Inj. Volume: 5 µL
 Temp: 30 °C
 Detection: Dionex ASRS 300 (2 mm), recycle mode, 31 mA
 Sample: Calibration standard

Peaks: mg/L
 1. Chloride 5
 2. Sulfate 40
 3. Phosphate 40

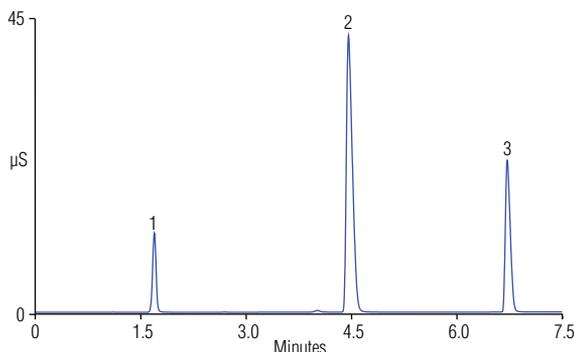


Figure 1. Separation of chloride, sulfate, and phosphate on the Dionex IonPac AS11 column.

Column: Dionex IonPac AG11, AS11, 2 mm
 Eluent: -5–0 min 3 mM KOH, 0–5 min 3–15 mM KOH, step at 5 min to 25 mM KOH, 5–7.5 min 25 mM KOH
 Eluent Source: Dionex EGC III KOH with Dionex CR-ATC
 Flow Rate: 0.5 mL/min
 Inj. Volume: 5 µL
 Temp: 30 °C
 Detection: Dionex ASRS 300 (2 mm), recycle mode, 31 mA
 Sample: DDGS sample

Peaks:		mg/L	%*
1. Chloride	1.3	0.13	
2. Sulfate	15.4	1.54	
3. Phosphate	11.2	1.12	

*Calculated amount

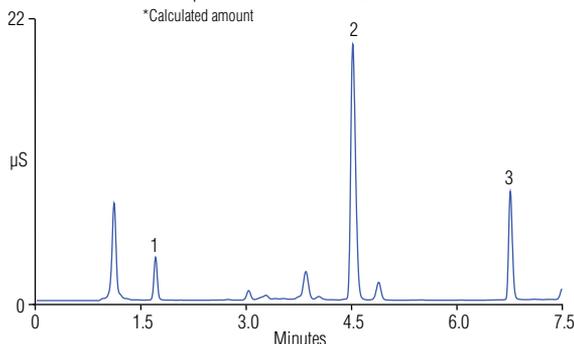


Figure 2. Determination of anions in a DDGS sample.

Linearity, Limit of Detection, and Limit of Quantification

To determine method linearity, calibration standards were injected in triplicate over seven concentration levels within the calibration ranges listed in Table 2. Calibration levels were determined based on typical amounts reported in the literature.^{2,6} All three analytes demonstrated coefficients of determination >0.999 .

The limits of detection (LODs) and limits of quantification (LOQs) were estimated for the method using a prepared DDGS sample. The LOD and LOQ are defined as three times the signal-to-noise ratio (S/N) and 10 times the S/N, respectively. By measuring the peak-to-peak noise between 5 and 6 min over seven consecutive injections of the DDGS sample, the system baseline noise for the LOD and LOQ was determined to be 1.4 nS. This method demonstrated sample LODs and LOQs in the low $\mu\text{g/L}$ for all three anions (1.7–5.7 $\mu\text{g/L}$ and 5.7–18.8 $\mu\text{g/L}$, respectively). Table 2 summarizes the calibration, LOD, and LOQ data.

Table 2. Linearity, LODs, and LOQs.

Analyte	Calibration Range (mg/L)	Linearity ¹ (r^2)	LOD ² ($\mu\text{g/L}$)	LOQ ³ ($\mu\text{g/L}$)
Chloride	0.08–5.0	0.9999	1.7	5.7
Sulfate	0.6–40.0	0.9999	3.3	10.9
Phosphate	0.6–40.0	0.9999	5.7	18.8

¹Seven calibration levels, each level injected in triplicate

²LOD calculated as $3 \times \text{S/N}$

³LOQ calculated as $10 \times \text{S/N}$

Sample Analysis

Prior to analysis, anions were extracted from a 1 g sample of DDGS by ultrasonic-assisted extraction into 100 mL of DI water, followed by centrifugation and a 10-fold dilution. The average amount of chloride, sulfate, and phosphate in three separate preparations were 1.3, 15.4, and 11.2 mg/L (0.13, 1.5, and 1.1%) with peak area RSDs of 0.55, 0.96, and 0.89%, respectively, over 21 injections (three samples injected seven times each). The measured values for chloride and phosphate agreed with values reported by the distillery and feed industries; however, sulfate levels were slightly higher than typical.^{2,7}

Accuracy and Precision

Precision of the method was evaluated by making several injections of standards and sample solutions. Peak area precision was measured by evaluating RSDs for five replicate injections of the lowest and highest concentrations in the calibration range. At the lowest end of the calibration range, peak area RSDs were <1 and at the highest end, peak area RSDs were <0.2 . Samples were also prepared at 75%, 100%, and 125% of the starting grain weight (0.75 g, 1.0 g, and 1.25 g, respectively). Over this range, the amounts of chloride, sulfate, and phosphate in the samples were determined to be 0.13%, 1.49–1.52%, and 1.08–1.11%, respectively, with peak area precisions of $<0.5\%$ for samples prepared at 75% and 100% starting grain weight and $<0.7\%$ for samples prepared at 125% starting grain weight. These data are summarized in Table 3.

Table 3. Analytes measured in different starting grain weights.

(n = 7)	Chloride		Sulfate		Phosphate	
Amount Sampled (g)	Calculated Average Amount of Chloride in the Sample (%)	Peak Area RSD	Calculated Average Amount of Sulfate in the Sample (%)	Peak Area RSD	Calculated Average Amount of Phosphate in the Sample (%)	Peak Area RSD
0.75	0.13	0.41	1.52	0.44	1.11	0.43
1.00	0.13	0.40	1.49	0.50	1.08	0.48
1.25	0.13	0.59	1.49	0.63	1.09	0.69

Table 4. Chloride, sulfate, and phosphate recovery in spiked DDGS samples.

Chloride		Sulfate		Phosphate	
Final Spiked Concentration (mg/L)	Amount Recovered (%)	Final Spiked Concentration (mg/L)	Amount Recovered (%)	Final Spiked Concentration (mg/L)	Amount Recovered (%)
0.27	107	3.13	102	2.30	104
0.55	100	5.87	99.2	4.59	97.5
1.16	100	12.4	101	9.15	99.0
1.49	97.5	15.5	98.4	11.5	96.2
1.71	97.9	18.5	98.8	13.7	96.5

Accuracy of the method was evaluated by calculating the recovery of the analytes from adding known concentrations of the target anions directly into the dry grain at ~1.2, 1.0, 0.8, 0.4, and 0.2 times the expected amount. The recoveries ranged from 97–107%, 98–102%, and 96–104% for chloride, sulfate, and phosphate, respectively (Table 4). These values suggest good method accuracy.

Conclusion

This method provides a simple and accurate approach for determining water-soluble anions (e.g., chloride, sulfate, and phosphate) that contribute to the nutritional composition of DDGS. The separation of these anions is achieved on a Dionex IonPac AS11 column in <10 min. The method demonstrates excellent peak shape, linearity ($r^2 > 0.999$), accuracy (spiked recoveries 96–107%), precision (peak area RSDs <1), and detection limits in the low $\mu\text{g/L}$ range (LODs 1.7–5.7 $\mu\text{g/L}$ and LOQs 5.7–18.8 $\mu\text{g/L}$). The simple sample preparation and the electrolytic eluent generator enhance the ease of use, reproducibility, and automation level of the method.

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