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Determination of Sulfate Counter Ion and Anionic Impurities in Aminoglycoside Drug Substances by Ion Chromatography with Suppressed Conductivity Detection

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ABSTRACT

Many drug substances are manufactured in specific salt forms to promote solubility, stability, and bioavailability. Sulfate is the most common pharmaceutical counter ion used in the development of aminoglycoside antibiotics. The accurate determination of the sulfate concentration is critical to confirm the correct formula weight of the drug, the stoichiometry, and the completeness of salt formation. During the early stages of drug development, it is also critical to determine the concentrations of unknown ionic impurites. In this poster, we compare two hydroxide-selective anion-exchange columns for the determination of sulfate counter ion and anionic impurities in eight different aminoglycosides. The sulfate percentages found in the aminoglycoside samples ranged from 13.7–30.2% and the total anionic impurities were <0.4%. We report the linearity, detection limits, precision, and recovery of sulfate and anionic impurities in aminoglycosides.

EXPERIMENTAL

Dionex ICS-3000 Reagent-Free™ ion chromatography system with Eluent Generation (RFIC-EG™ system) consisting of a DP dual gradient pump, EG Eluent Generator with an EGC II KOH cartridge and CR-ATC trap column, and a DC detector compartment with dual temperature zones.

Method

	1	**2**
Columns:	IonPac® AG18 (2 × 50 mm) IonPac AS18 (2 × 250 mm)	IonPac AG11-HC (2 × 50 mm) IonPac AS11-HC (2 × 250 mm)
Flow Rate:	0.25 mL/min	0.38 mL/min
Temperature:	30 °C	30 °C
Inj. Volume:	5 µL	5 µL
Detection:	Suppressed Conductivity (ASRS® ULTRA II, 2-mm)	Suppressed Conductivity (ASRS ULTRA II, 2-mm)

RESULTS AND DISCUSSION

Method 1

The IonPac AS18 column was used to determine sulfate and anionic impurities in various aminoglycoside sulfate compounds. Common inorganic anions can be separated on this column in <16 min. Therefore, this column is recommended for high-throughput analysis of well characterized samples. Table 1 summarizes the calibration data and estimated limits of detections (LODs) for the target anions.

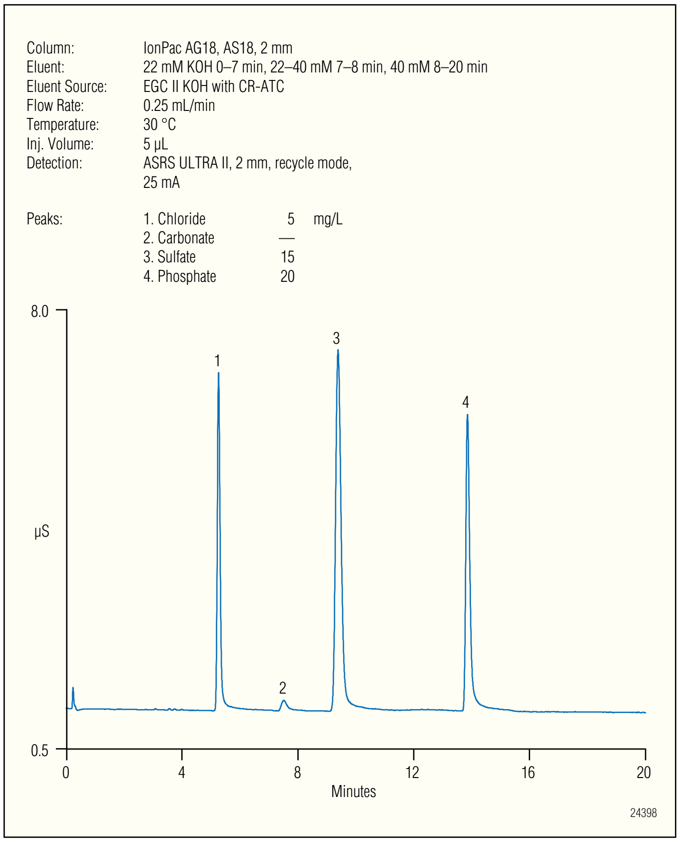
Table 1: Calibration Data and Detection Limits Using Method 1

Analyte	Range (mg/L)	Linearity r^2	LODs[a] (µg/L)
Chloride	0.025–0.15	0.9998	3.0
Sulfate	5.0–25	0.9994	7.7
Phosphate	0.020–0.15	0.9999	9.3

[a]LODs estimated from 3 × S/N

Pittcon 2008 Presentation

Figure 1 shows the separation of target anions on the IonPac AS18 column in <16 min. For samples that do not contain phosphate, the run time can be reduced to 12 min to increase sample throughput.


Column: IonPac AG18, AS18, 2 mm
Eluent: 22 mM KOH 0–7 min, 22–40 mM 7–8 min, 40 mM 8–20 min
Eluent Source: EGC II KOH with CR-ATC
Flow Rate: 0.25 mL/min
Temperature: 30 °C
Inj. Volume: 5 µL
Detection: ASRS ULTRA II, 2 mm, recycle mode, 25 mA

Peaks:
1. Chloride 5 mg/L
2. Carbonate —
3. Sulfate 15
4. Phosphate 20

Figure 1. Separation of target anions on the IonPac AS18 column.

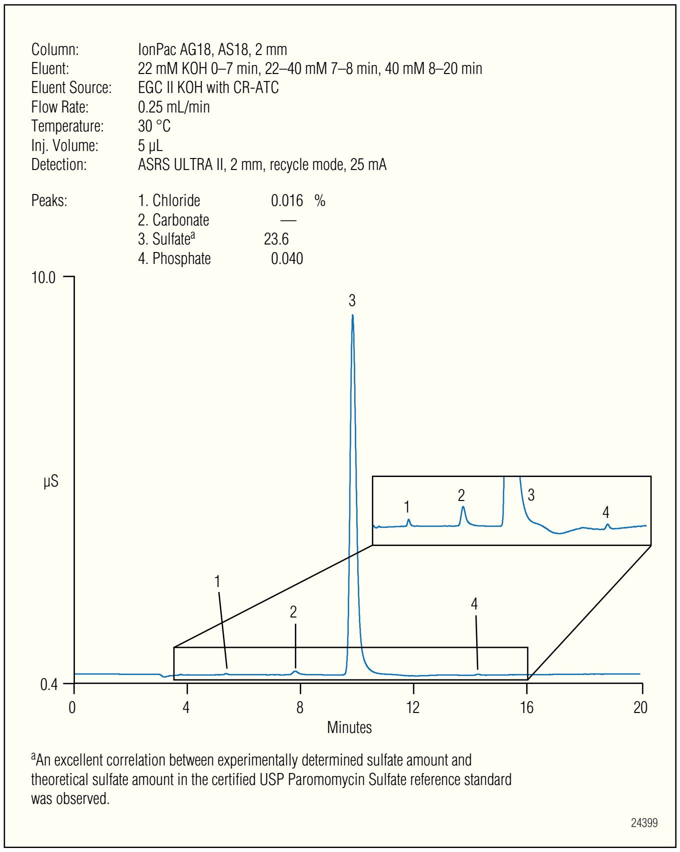
Table 2 summarizes the average percentages (n = 3) of sulfate and the anionic impurities chloride and phosphate determined in the aminoglycoside sulfate compounds.

Table 2. Percentages of Sulfate Counter Ion and Anionic Impurities Determined in Anhydrous Aminoglycoside Sulfate Compounds Using Method 1

Aminoglycoside Sample	Theoretical Sulfate (%)	Experimental Sulfate (%)	Chloride (%)	Phosphate (%)
Amikacin	24.6	22.3	0.110	—
Dihydrostreptomycin	19.7	16.8	0.052	—
Kanamycin A	16.5	13.7	0.057	—
Kanamycin B	28.2	24.8	0.065	—
Neomycin	29.1	25.0	0.090	0.042
Paromomycin, Sigma Lot 1	23.7	22.5	0.021	0.097
Paromomycin, Sigma Lot 2	23.7	24.2	0.036	0.058
Paromomycin, USP®	23.7	23.6	0.016	0.040
Sisomicin	34.6	30.2	0.056	—
Streptomycin	18.7	17.3	0.098	—

The stoichiometry between the aminoglycoside freebase to sulfate was determined to be in agreement with the data provided by Sigma Aldrich. For aminoglycoside samples where the stoichiometry was not provided, such as paromomycin and kanamycin B (bekanamycin), the aminoglycoside freebase-to-sulfate ratio was determined to be 1:2.

Figure 2 demonstrates the separation of sulfate, trace chloride, and trace phosphate in USP-grade paromomycin sulfate.


Column: IonPac AG18, AS18, 2 mm
Eluent: 22 mM KOH 0–7 min, 22–40 mM 7–8 min, 40 mM 8–20 min
Eluent Source: EGC II KOH with CR-ATC
Flow Rate: 0.25 mL/min
Temperature: 30 °C
Inj. Volume: 5 µL
Detection: ASRS ULTRA II, 2 mm, recycle mode, 25 mA

Peaks:
1. Chloride 0.016 %
2. Carbonate —
3. Sulfate[a] 23.6
4. Phosphate 0.040

[a] An excellent correlation between experimentally determined sulfate amount and theoretical sulfate amount in the certified USP Paromomycin Sulfate reference standard was observed.

Figure 2. Separation of sulfate counter ion and anionic impurities in USP-grade paromomycin sulfate.

Accuracy and Precision

For triplicate sample injections, the peak area RSDs were <3% for chloride and phosphate and <2% for sulfate.

To verify the accuracy of the method, each sample was spiked with known concentrations of the target anions. The average recoveries (n = 3) were in the range of 92–112% for chloride, 97–101% for sulfate, and 91–103% for phosphate.

Method 2

The IonPac AS11-HC column was used to determine sulfate and anionic impurities in Humatin®, a broad-spectrum antibiotic supplied as a water-soluble paromomycin sulfate capsule that contains the equivalent of 250 mg paromomycin. This sample was previously analyzed using a CarboPac® PA1 column and IPAD to determine the paromomycin freebase concentration.[1]

The IonPac AS11-HC column is recommended for the determination of a wide range of inorganic and organic anions in uncharacterized samples. Table 3 summarizes the calibration data and estimated LODs using Method 2.

Table 3. Calibration Data and Detection Limits Using Method 2

Analyte	Range (mg/L)	Linearity (r^2)	LODs[a] (µg/L)
Acetate	1.0–10	0.9998	50
Chloride	0.50–5.0	0.9998	12
Sulfate	50–150	0.9998	25
Phosphate	5.0–15	0.9996	53
Pyrophosphate	0.50–5.0	0.9999	150

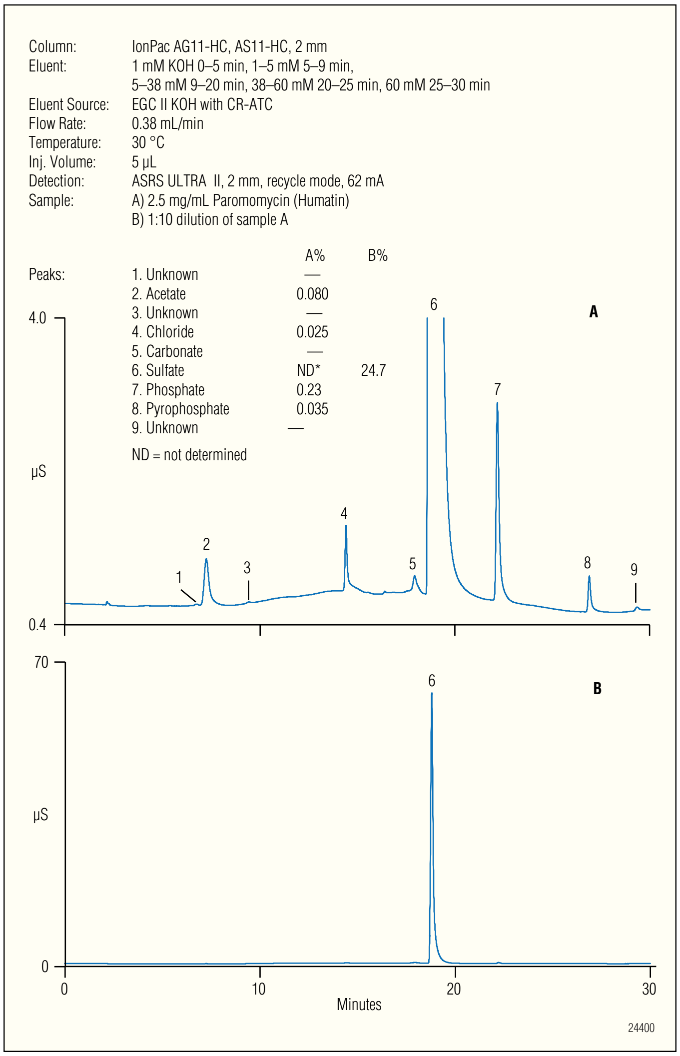
[a] LODs estimated from 3 × S/N

Table 4 summarizes the data obtained from the analysis of Humatin using the IonPac AS11-HC column. As shown, the experimental sulfate percentage found in the sample was 24.7%, which is 1.0% higher than theory. In addition, the inorganic impurities ranged from 0.025–0.23% with total impurities of 0.37%, which is nearly three times higher than the impurities detected in the Sigma-Aldrich samples.

Table 4. Percentages of Sulfate Counter Ion and Anionic Impurities Detected in Humatin Using Method 2.

Sample	Theoretical Sulfate (%)	Experimental Sulfate (%)	Acetate (%)	Chloride (%)	Phosphate (%)	Pyrophosphate (%)
Humatin	23.7	24.7	0.080	0.025	0.23	0.035

Figure 3A shows the determination of impurities in Humatin and Figure 3B shows the same sample diluted 1:10 to determine the sulfate percentage.



Column: IonPac AG11-HC, AS11-HC, 2 mm
Eluent: 1 mM KOH 0–5 min, 1–5 mM 5–9 min, 5–38 mM 9–20 min, 38–60 mM 20–25 min, 60 mM 25–30 min
Eluent Source: EGC II KOH with CR-ATC
Flow Rate: 0.38 mL/min
Temperature: 30 °C
Inj. Volume: 5 µL
Detection: ASRS ULTRA II, 2 mm, recycle mode, 62 mA
Sample: A) 2.5 mg/mL Paromomycin (Humatin)
B) 1:10 dilution of sample A

Peaks:	A%	B%
1. Unknown	—	
2. Acetate	0.080	
3. Unknown	—	
4. Chloride	0.025	
5. Carbonate	—	
6. Sulfate	ND*	24.7
7. Phosphate	0.23	
8. Pyrophosphate	0.035	
9. Unknown	—	

ND = not determined

Figure 3. Separation of sulfate counter ion and anionic impurities in Humatin using the IonPac AS11-HC.

The peak area RSDs for triplicate injections of the target anions were <3%. The average recoveries from the spiked sample were 97.5% for sulfate and 93–111% for the anionic impurities detected in the sample.

CONCLUSIONS

- The IonPac AS18 column separates sulfate counter ion and anionic impurities in well-characterized aminoglycoside sulfate compounds with good accuracy and precision in <16 min.
- The IonPac AS11-HC column can separate a wide range of anions in uncharacterized pharmaceutical formulations, enabling the analyst to obtain additional information on the sample content.
- The use of an RFIC-EG system eliminates the need to manually prepare eluents and increases automation and ease-of-use, which improves the reproducibility of analysis between analysts and laboratories.
- The excellent sensitivity of the RFIC-EG system enables the detection of anionic impurities to <0.1%.

REFERENCE

1. Dionex Corporation. Analysis of Paromomycin by HPAE-IPAD. Application Note 186, LPN 1942. Sunnyvale, CA, 2007

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