Application Note 1075

Ion Chromatography Assay for Chloride and Sulfate in Adenosine

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High-Pressure Ion Chromatography, Suppressed Conductivity, Fast Separation, Pharmaceuticals, Drug Products, Dionex IonPac AS18-4 µm Column

Introduction

Adenosine (Figure 1) is a nucleoside composed of adenine attached to ribose in the furanose conformation. The major therapeutic uses of adenosine are for treating surgical and nerve pain, pulmonary hypertension, and irregular heartbeat; for controlling blood pressure during anesthesia/surgery; and for cardiac stress tests. For these indications, adenosine is administered either as a bolus intravenous injection or an intravenous infusion.

Adenosine is produced by the biochemical condensation of ribose and adenine or by fermentation. Anions such as chloride and sulfate are possible impurities in adenosine preparations and the U.S. Pharmacopeia (USP) monograph for adenosine has assays to determine the amounts of both anions in adenosine. For measuring chloride, the sample and standard solutions are treated with nitric acid and silver nitrate and their turbidities are compared. The acceptance criterion is "sample solution is not more turbid than the standard solution" (i.e., not more than [NMT] 0.007% chloride).

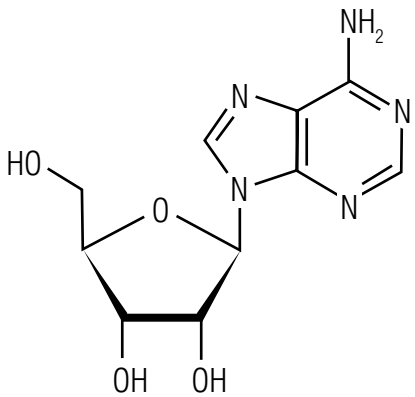

Figure 1. Chemical structure of adenosine.

Similarly for sulfate, the sample solution and a standard solution are both treated with barium chloride and hydrochloric acid, and then their turbidities compared. The acceptance criteria for sulfate is also a visual comparison to confirm that "sample solution is not more turbid than the standard solution."[1] This correlates to NMT 0.02% sulfate in an adenosine test solution. However, both of these assays are subjective, use toxic chemicals, and generate hazardous waste.

Goal

To develop a fast and robust ion chromatography (IC)-based method for the determination of chloride and sulfate in adenosine—a method that can replace the USP monograph's existing turbidity-based assays of these compounds

Thermo SCIENTIFIC

Equipment

- Thermo Scientific™ Dionex™ ICS-5000+ HPIC™ IC system, capable of supporting high-pressure IC, including:
  - SP Single Pump or DP Dual Pump
  - EG Eluent Generator
  - DC Detector/Chromatography Compartment
  - CD Conductivity Detector
- Thermo Scientific Dionex AS-AP Autosampler
- Thermo Scientific Dionex EGC 500 Potassium Hydroxide (KOH) Cartridge (P/N 075778)
- Thermo Scientific Dionex CR-CTC 500 Continuously Regenerated Cation Trap Column (P/N 075551)
- Injection Loop, 10 µL
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System Software

Conditions

Columns:	Thermo Scientific™ Dionex™ IonPac™ AG18-4 µm Guard, 4 × 30 mm (P/N 076035) Dionex IonPac AS18-4 µm Analytical, 4 × 150 mm (P/N 082314)
Eluent:	28 or 42 mM KOH for high-pressure IC method
Flow Rate:	0.8 mL/min or 1.5 mL/min for high-pressure IC method
Inj. Volume:	10 µL
Column Temp:	30 °C
Detector Temp:	20 °C
Detection:	Suppressed conductivity with the Thermo Scientific™ Dionex™ ERS™ 500 Electrolytically Regenerated Suppressor, recycle mode, power setting 56 mA for standard method or 110 mA for high-pressure IC method
Backpressure:	2800 or 4500 psi for high-pressure IC method
Background Conductance:	<0.50 µS
Noise:	~0.1–0.2 nS

Reagents and Standards

- Deionized (DI) water, 18 M -cm resistance or better
- Adenosine, 99+% (Fisher Scientific P/N AC16404-0050)
- Chloride Standard (Fisher Scientific P/N 037159)
- Sulfate Standard (Fisher Scientific P/N 037160)

Sample Preparation

Ammonia Standards

Prepare standards gravimetrically by making appropriate dilutions of a commercial 1000 mg/L standard with DI water. Store standard solutions at 4 °C when not in use.

Adenosine Sample Solution

In the current USP monograph for chloride assay in adenosine, sample preparation steps are: suspend 0.2 g in 10 mL of water, stir for 30 s, pass through a coarse filter, and use filtrate for the assay. The sample preparation for the sulfate assay in the same monograph is the same as for chloride, except 0.75 g is suspended in 15 mL of water.[1]

For the IC-based methods presented here, use a single preparation of adenosine (prepared as per the steps for the chloride assay in the USP monograph) for assaying both chloride and sulfate. The acceptance criterion for chloride is 0.007% (i.e., 1.4 ppm) and for sulfate is 0.02% (i.e., 4 ppm).

Note: Use sample within 24 h.

General Design of the Robustness Study

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small variations in the procedural parameters. The robustness of the method was studied by determining the response of a standard solution containing 1.4 and 5 ppm chloride and sulfate, respectively, under typical variations of analytical conditions. Variations in the following were tested:

- Mobile phase concentration (±2 mM KOH*)
- Flow rate (±10%)
- Temperature of column (±2 °C)
- Column-to-column (column sets from two different production batches)

*Not typical for eluent generation but rather for manually prepared eluents

Results

Separation

The anions in adenosine were separated on a Dionex IonPac AS18-4 µm column set, a high efficiency anion-exchanger designed for fast, isocratic separation of common anions. Figure 2 shows separation of chloride and sulfate standards (Chromatogram A) and a 20 mg/mL adenosine sample (Chromatogram B) using a Dionex IonPac AS18-4 µm column set on a Dionex ICS-5000⁺ HPIC system. Chloride elutes at 3.6 min and sulfate elutes at 5.9 min. Carbonate elutes at 4.8 min between the chloride and sulfate peaks. Chloride and sulfate both have a peak asymmetry of 1.2. The total run time is 8 min using a flow rate of 0.8 mL/min (backpressure ~2800 psi) and an eluent concentration of 28 mM KOH. After increasing the eluent concentration to 40 mM KOH, chloride and sulfate elute at 3.09 and 4.76 min, respectively, with a total run time of 5 min (data not shown). The amounts of chloride and sulfate in the adenosine sample (Figure 2, Chromatogram B) are 0.06 and 0.1 mg/L, both well under their respective limits of 1.4 and 4 mg/L in the USP monograph.

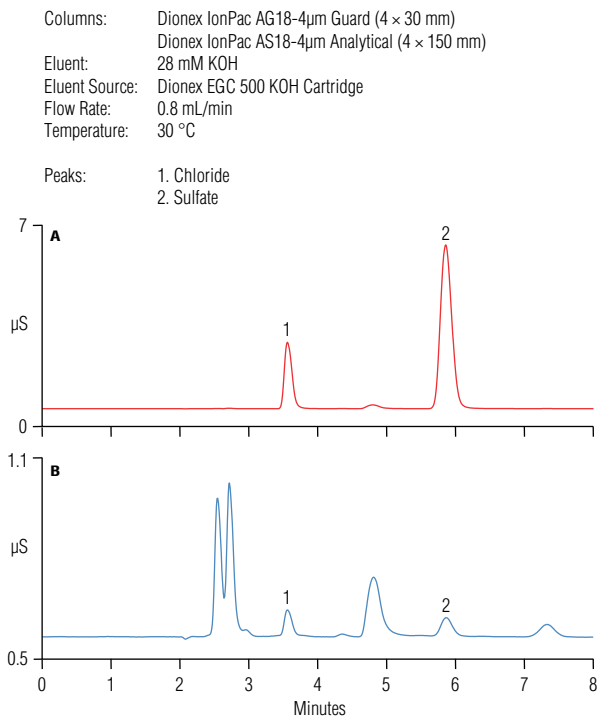

Figure 2. Separation of (A) chloride and sulfate and (B) an adenosine sample (20 mg/mL) on a Dionex IonPac AS18-4 µm column set.

The smaller resin particles used in the Dionex IonPac AS18-4 µm column produce increased backpressure compared to the traditional Dionex IonPac AS18 column. Because the Dionex ICS-5000⁺ HPIC system is capable of handling high backpressures, an even faster separation can be achieved by increasing the flow rate to 1.5 mL/min. Use an eluent concentration of 40 mM KOH to further reduce run time. This yields a separation of chloride and sulfate with a total run time of 3.5 min (Figure 3) and a backpressure of 4500 psi. Chloride elutes at 1.69 min, sulfate at 2.09 min, and both have a peak asymmetry of 1.2. Compared to the same eluent concentration (i.e., 40 mM) at 0.8 mL/min, the high-pressure IC system enables a 45% reduction in run time for this analysis.

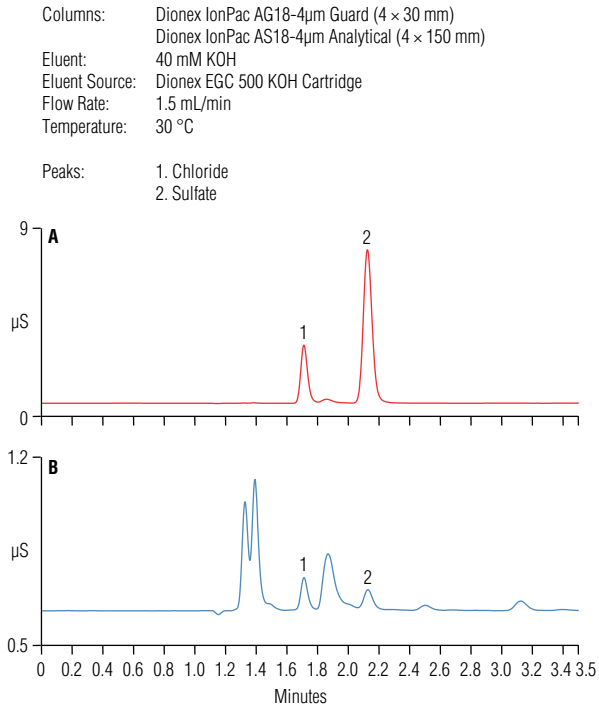

Figure 3. High-pressure IC separation of (A) chloride and sulfate and (B) an adenosine sample (20 mg/mL) on a Dionex IonPac AS18-4 µm column set.

Table 1. Recovery of chloride and sulfate in adenosine.

Day	Chloride			Sulfate		
	Amount Present (mg/L)	Measured (mg/L)	Recovery (%)	Amount Present (mg/L)	Measured (mg/L)	Recovery (%)
1	0.0568	0.5513	99	0.0928	0.5895	99
	0.0568	0.5562	100	0.0928	0.589	99
	0.0568	0.5502	99	0.0928	0.5814	98
2	0.0568	0.5513	99	0.0928	0.5895	99
	0.0568	0.5562	100	0.0928	0.589	99
	0.0568	0.5502	99	0.0928	0.5814	98
3	0.0527	0.5607	102	0.0956	0.5769	96
	0.0527	0.553	100	0.0956	0.5904	99
	0.0527	0.5722	104	0.0956	0.6103	103

Accuracy

Method accuracy was verified by determining recoveries of ammonium in spiked adenosine samples over three consecutive days (Table 1). The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the USP General Chapter <1225> guidelines recommend that accuracy be determined using a minimum of nine measurements (i.e., three concentration levels and three replicates of each concentration).[2]

The amount of chloride and sulfate in the adenosine sample were 0.06 and 0.1 mg/L, respectively. The samples were spiked with 0.5 mg/L chloride and sulfate. Recoveries were calculated from the difference in response between the spiked and unspiked samples. The average recovery of chloride and sulfate ranged from 96 to 104%.

Precision

The precision of an analytical procedure is typically expressed as the RSD of a series of measurements. It is determined by assaying a sufficient number of aliquots of a sample that have undergone the complete analytical procedure from sample preparation to final test. Precision for the two anions is summarized in Table 2. The retention time (RT) RSD was <0.2% and the peak area RSD ranged from 0.25 to 1.3%.

Detection Limit

The limits for chloride and sulfate in adenosine in the current USP monograph are NMT 0.007% and 0.02% ppm, respectively. In the test solution used here, this correlates to 1.4 mg/L chloride and 4 mg/L sulfate.

For this IC method, the limit of detection (LOD) and limit of quantitation (LOQ) for chloride in adenosine (i.e., concentrations that resulted in peaks that were 3× and 10× the noise) were 0.005 and 0.02 mg/L, respectively. For sulfate, the LOD and LOQ (determined in a manner similar to that of chloride) were 0.013 and 0.04 mg/L, respectively. The low LOD and LOQ levels indicate that this IC method is sensitive for both chloride and sulfate and can easily assay the two anions at the limits in the USP adenosine monograph.

Table 2. Precision.

	Concn (mg/L)	RT RSD	Peak Area RSD
Chloride	0.2	0.11	0.25
	0.6	0.05	0.67
Sulfate	0.1	0.12	1.26
	1	0.04	0.71

Linearity

The ICH/USP recommendations for establishing linearity of an impurity in a drug substance or a finished product are a minimum of five concentrations ranging from 50 to 120% of the acceptance criteria.

For chloride, linearity was investigated in the range of 0.2–5 mg/L (0.02, 0.6, 1.0, 1.4, 2.0, and 5.0 mg/L). The highest concentration investigated was 3.6× the acceptance criterion (NMT 1.4 mg/L) for chloride in adenosine. The coefficient of determination for the linear regression analysis of the relationship of chloride peak area to concentration was 0.9992.

For sulfate, linearity was investigated in the range of 0.1–20 mg/L (0.1, 1.0, 2.0, 5.0, 10.0, and 20.0 mg/L) and the highest concentration was 5× that of the acceptance criterion of NMT 4 mg/L. The coefficient of determination for linear regression analysis of relationship of sulfate peak area to concentration was 0.9999.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small variations in the procedural parameters. A mix of standards (i.e., chloride at 1.4 mg/L and sulfate at 5 mg/L) and an adenosine sample were used to measure the robustness of this method. The data are summarized in Tables 3 and 4. The ranges of peak asymmetry for chloride (1.2) and sulfate (1.0–1.2) and the resolution of chloride and sulfate (3.6–6.5) in adenosine solution under the different conditions indicate that both IC assays are robust.

Table 3. Robustness of the standard method.

Sample: Mix of Chloride and Sulfate Standards (0.8 mL/min)										
Conditions	Chloride						Sulfate			
	Column 1			Column 2			Column 1		Column 2	
	RT (min)	*Asym*	Resolution	RT (min)	*Asym*	Resolution	RT (min)	*Asym*	RT (min)	*Asym*
0.8 mL/min, 28 mM KOH, Column Temp 30 °C	3.55	1.17	5.72	3.45	1.16	5.34	5.83	1.08	5.61	1.09
0.8 mL/min, 28 mM KOH, Column Temp 32 °C	3.56	1.16	6.05	3.47	1.16	5.14	6.01	1.09	5.76	1.09
0.8 mL/min, 28 mM KOH, Column Temp 28 °C	3.54	1.17	5.04	3.44	1.15	4.77	5.65	1.07	5.43	1.08
0.8 mL/min, 26 mM KOH, Column Temp 30 °C	3.67	1.16	6.78	3.57	1.16	6.49	6.47	1.08	6.21	1.09
0.8 mL/min, 30 mM KOH, Column Temp 30 °C	3.45	1.18	4.50	3.36	1.17	4.15	5.31	1.08	5.11	1.09
0.75 mL/min, 28 mM KOH, Column Temp 30 °C	3.94	1.17	5.67	3.82	1.17	5.32	6.44	1.08	6.20	1.10
0.85 mL/min, 28 mM KOH, Column Temp 30 °C	3.35	1.17	5.67	3.25	1.16	5.30	5.50	1.08	5.29	1.09

Table 4. Robustness of the high-pressure IC method.

Conditions	Sample	Chloride			Sulfate	
		RT (min)	*Asym*	Resolution	RT (min)	*Asym*
1.5 mL/min, 40 mM KOH, Column Temp 30 °C	Mix of Chloride and Sulfate Standards	1.70	1.3	4.06	2.09	1.2
	Adenosine	1.69	1.2	4.08	2.09	1.2
1.5 mL/min, 40 mM KOH, Column Temp 28 °C	Mix of Chloride and Sulfate Standards	1.70	1.2	3.75	1.89	1.2
	Adenosine	1.70	1.1	3.87	2.07	1.2
1.5 mL/min, 38 mM KOH, Column Temp 30 °C	Mix of Chloride and Sulfate Standards	1.72	1.3	4.67	2.20	1.2
	Adenosine	1.72	1.3	4.60	2.20	1.2
1.5 mL/min, 42 mM KOH, Column Temp 30 °C	Mix of Chloride and Sulfate Standards	1.67	1.2	3.45	2.00	1.2
	Adenosine	1.67	1.1	3.63	2.00	1.0
1.35 mL/min, 40 mM KOH, Column Temp 30 °C	Mix of Chloride and Sulfate Standards	1.88	1.3	4.11	2.31	1.2
	Adenosine	1.88	1.1	4.10	2.31	1.2
1.5 mL/min, 40 mM KOH, Column Temp 32 °C	Mix of Chloride and Sulfate Standards	1.71	1.3	4.41	2.14	1.2
	Adenosine	1.70	1.3	4.40	2.13	1.1
1.65 mL/min, 40 mM KOH, Column Temp 30 °C	Mix of Chloride and Sulfate Standards	1.56	1.3	3.98	1.94	1.2
	Adenosine	1.55	1.3	4.01	1.93	1.2

Conclusion

The Dionex ICS-5000⁺ HPIC system combined with the Dionex IonPac AS18-4 µm column set enables a significantly faster determination of chloride and sulfate in adenosine compared to a traditional IC system. A run time of 3.5 min supports high sample throughput. The electrolytically generated eluent and the self-regenerating suppressor eliminate the labor of eluent and regenerant preparation, thereby increasing productivity and contributing to the robustness and sensitivity of the method. Only water is used for eluent generation; no hazardous chemicals are required.

The precision (RT RSD <0.2% and peak area RSD <2%), accuracy (average recovery 96–103%), linearity, detection and quantitation limits, and robustness of this IC assay for chloride and sulfate meet the analytical performance characteristics recommended in USP General Chapter <1225>. Thus, this new approach provides an alternative to the turbidity-based methods described in the USP monograph for adenosine.

References

1. Adenosine 2079. *U.S. Pharmacopeial Convention (USP)*; Rockville, MD, USP35-NF30.
2. Validation of Compendial Procedures, General Chapter <1225>. *U.S. Pharmacopeia/National Formulary*; Rockville, MD, 2012; pp 877–881.

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