



Assay of sodium thiosulfate and ionic impurities in sodium thiosulfate using ion chromatography

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Goal

To validate the ion chromatography (IC) methods for the assay of sodium thiosulfate and its ionic impurities in the proposed United States Pharmacopeia monographs

Keywords

Dionex IonPac AS12A column,
suppressed conductivity detection,
pharmaceutical, USP Monograph,
drug substance, drug product

Introduction

Sodium thiosulfate is an active pharmaceutical ingredient (API) approved by the United States Food and Drug Administration. Dosing sequentially with sodium nitrite, Sodium Thiosulfate Injection solution is used for the treatment of acute cyanide poisoning that is judged to be life-threatening.^{1,2} Sodium thiosulfate is being tested as an extravasation antidote for cancer treatment to lessen the side effects of cisplatin (a chemotherapy agent).^{3,4}

The United States Pharmacopeia (USP) has embarked on a global initiative to modernize many of the existing monographs across all compendia. As part of the USP modernization effort, an ion chromatography (IC) method has been proposed to replace existing titration-based assays in the Sodium Thiosulfate and Sodium Thiosulfate Injection monographs. In addition, another IC method has also been proposed for determining chloride, sulfate, and sulfite impurities in Sodium Thiosulfate; and sulfate and sulfite impurities in Sodium Thiosulfate Injection.^{5,6}

This application note evaluates both methods with sodium thiosulfate following the guidelines outlined in USP General Chapter <1225>, Validation of Compendial Methods.⁷⁻⁹ A Thermo Scientific™ Dionex™ ICS-5000⁺ ion chromatography system with a Thermo Scientific™ Dionex™ IonPac™ AS12A anion-exchange column and a Thermo Scientific™ Dionex™ AERS 500 (4 mm) Anion Electrolytically Regenerated Suppressor for suppressed conductivity detection were used for both method evaluations.

Experimental Equipment

- A Thermo Scientific Dionex ICS-5000⁺ ion chromatography (RFIC) system*, which includes:
 - Pump
 - Column Heater
 - Degasser
 - Conductivity Detector
- Thermo Scientific™ Dionex™ AS-AP Autosampler, with 250 µL syringe (P/N 074306), 1.2 mL buffer line assembly (P/N 074989), 25 µL injection loop
- Thermo Scientific™ Chromeleon™ 7.2 Chromatography Workstation

*This method can be run on any system supporting an electrolytic suppressor or any Thermo Scientific Dionex ion chromatography system using a chemically regenerated suppressor. Please note that this method was not tested with a chemically regenerated suppressor.

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistance or better
- Sodium Thiosulfate anhydrous USP reference standard (USP, P/N 1615107)
- Sodium chloride (Sigma-Aldrich, 746398-500g)
- Sodium sulfite (Fisher Scientific, S430-500 98.1%)
- Sodium sulfate (EM, > 99%)
- D-mannitol (Acros Organic, 98+%)
- Sodium thiosulfate salt (J.T Baker, USP grade)

Conditions

Table 1. Chromatography conditions for the sodium thiosulfate assay.

Columns:	Dionex IonPac AS12A 4-mm Analytical, 4 × 250 mm (P/N 046034) Dionex IonPac AG12A 4-mm Guard, 4 × 50 mm (P/N 079801)
Eluent:	13.5 mM Na ₂ CO ₃ /1.5 mM NaHCO ₃
Flow Rate:	1.5 mL/min
Injection Volume	25 µL in Push-Full mode
Temperature:	30 °C
Detection:	Suppressed conductivity, Dionex AERS 500 (4 mm) Anion Electrolytically Regenerated Suppressor, recycle mode, 106 mA current
System Backpressure:	~2500 psi
Background Conductance:	~26 µS
Noise:	<5 nS/min
Run Time	10 min

Table 2. Chromatography conditions for the sodium thiosulfate ionic impurity method.

Columns:	Dionex IonPac AS12A 4-mm Analytical, 4 × 250 mm (P/N 046034) Dionex IonPac AG12A 4-mm Guard, 4 × 50 mm (P/N 079801)	
Eluent Solution A:	2.7 mM Na ₂ CO ₃ /0.3 mM NaHCO ₃	
Eluent Solution B:	13.5 mM Na ₂ CO ₃ /1.5 mM NaHCO ₃	
Gradient:		
Time (min)	Solution A (%)	Solution B (%)
-5	100	0
0	100	0
14	100	0
16	0	100
21	0	100
23	100	0
30	100	0
Flow Rate:	1.5 mL/min	
Injection Volume	25 µL in Push-Full mode	
Temperature:	30 °C	
Detection:	Suppressed conductivity, Dionex AERS 500 (4 mm) Anion Electrolytically Regenerated Suppressor, recycle mode, 106 mA current	
System		
Backpressure:	~2500 psi	
Background		
Conductance:	~13–26 µS	
Noise:	<5 nS/min	
Run Time	35 min (includes 5 min equilibrium time)	

Preparations of solutions and reagents

Note: Do not use glassware to prepare the solutions. Polymeric containers made of high-density polyethylene (HDPE) are recommended.

Stock standard solution for sodium thiosulfate assay, 1.000 mg/mL in water

Accurately weigh 100.0 mg of USP Sodium Thiosulfate into a 125 mL polypropylene bottle and dissolve in 100 mL (100.00 g) of DI water. Keep at 4 °C for up to a month.

Standard solution for sodium thiosulfate assay, 100 µg/mL in water

Mix 1.0 mL (1.0 g) of 1.0 mg/mL of sodium thiosulfate stock standard solution and 9.0 mL (9.0 g) of DI water to make the standard solution for assay. Prepare fresh for each sequence. This standard is also used as the system suitability solution for the assays.

Sodium thiosulfate calibration standards, 0.2, 20, 50, 75, 100, 125, 150, 200 µg/mL

To prepare calibration standard solutions, dilute the stock standard solution (1.0 mg/mL) to the appropriate concentrations with DI water.

Diluent: 2.0 g/L of D-mannitol in water

Accurately weigh 4.0 g of D-mannitol solid into a 2 L polypropylene bottle and dissolve in 2 L of DI water to make the diluent. The diluent is used to prepare the samples and standards in the sodium thiosulfate ionic impurity method.

Stock standard solutions for the ionic impurity method, in diluent

Accurately weigh a pure anhydrous salt (using USP reference standard if available) into a polypropylene bottle and dissolve in 100 mL (100.00 g) of diluent to make each stock standard solution. Mix 40.0 mg of sodium chloride to make 0.400 mg/mL sodium chloride stock, 100 mg of sodium sulfite to make 1.00 mg/mL sodium sulfite stock, and 200.0 mg of sodium sulfate to make 2.00 mg/mL of sodium sulfate stock. Keep stock standard solutions at 4 °C.

Mixed standard stock solution for the ionic impurity method, in diluent

Mix the stock standard solutions (1.00 mL (1.0 g) each of sodium chloride and sodium sulfate stock, 2.00 mL of sodium sulfite stock) and 96.0 mL (96.0 g) of the diluent to make the mixed standard stock solution containing 4.0 µg/mL of sodium chloride, 20.0 µg/mL of sodium sulfite, and 20.0 µg/mL sodium sulfate.

Calibration standard solutions for the ionic impurity method, in diluent

Dilute the mixed standard stock solution to the appropriate concentrations with diluent to make the calibration standards (Table 3). The system suitability solution is the level 4 calibration standard solution.

Table 3. Concentration of standard solutions for the ionic impurity method (µg/mL of the salt (e.g., sodium chloride)).

Analyte	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6
Chloride	0.04	0.08	0.2	0.4	0.8	2
Sulfite	0.2	0.4	1	2	4	10
Sulfate	0.2	0.4	1	2	4	10

Sample preparation

Sodium thiosulfate stock sample solution, 1.000 mg/mL in water

Accurately weigh 100.0 mg of JT Baker sodium thiosulfate salt into a 125 mL polypropylene bottle and dissolve in 100 mL (100.0 g) DI water.

Sodium thiosulfate sample solution for assay, 0.100 mg/mL (100 µg/mL) in water

Mix 1.00 mL (1.00 g) of 1.00 mg/mL of the sodium thiosulfate sample stock and 9.00 mL (9.00 g) of DI water to make the sample solution for the sodium thiosulfate assay.

Spiked sodium thiosulfate sample solutions

Mix 1.00 mg/mL of sodium thiosulfate sample stock, 1.000 mg/mL of sodium thiosulfate standard stock solution, and DI water to make spiked samples (Table 4).

Sodium thiosulfate sample solution for ionic impurities, 2.0 mg/mL in diluent

Accurately weigh 40.0 mg of sodium thiosulfate solid sample into a 20 mL polypropylene bottle and dissolve in 20 mL (20.0 g by weight) diluent to make the sample solution for the ionic impurities determination.

Spiked sodium thiosulfate sample solutions for the ionic impurity recovery test

Accurately weigh 200.0 mg of sodium thiosulfate into a 100 mL polypropylene bottle and dissolve in 50 mL (50.0 g by weight) of diluent to make 4.0 mg/L sample stock solution. Mix 1.00 mL of 0.40 mg/mL sodium chloride stock, 2.00 mL of 1.0 mg/L of sodium sulfite stock, 1.00 mL of 2.0 mg/mL of sodium sulfate stock, and 96.0 mL (96.0 g) of the diluent to make the mixed spike stock solution containing 4 µg/mL sodium chloride, 20 µg/mL sodium sulfite, and 20 µg/mL sodium sulfate. Mix the sample stock and appropriate amount of the mixed spike stock with diluent to make the spiked samples (Table 5) for the recovery test.

Table 4. Preparation of spiked samples for assay recovery test.

Sodium Thiosulfate Spiked (µg/mL)	10	25	50*	50	75	100
Sample Stock (mL)	1.000	1.000	0.500	1.000	1.000	1.000
Standard Stock (mL)	0.100	0.250	0.500	0.500	0.750	1.000
DI water (g)	8.90	8.75	9.00	8.50	8.25	8.00

*50 µg/mL spiked in 50 µg/mL sample. All others are spiked in 100 µg/mL sample.

Table 5. Concentration of ionic impurities spiked in sodium thiosulfate samples.

	Sample	Spiked Sample 5	Spiked Sample 4	Spiked Sample 3	Spiked Sample 2	Spiked Sample 1
Sodium thiosulfate (mg/mL)	2.000	2.000	2.000	2.000	2.000	2.000
Sodium chloride (µg/mL)		2	1	0.4	0.2	0.02
Sodium sulfite (µg/mL)		10	5	2	1	0.1
Sodium sulfate (µg/mL)		10	5	2	1	0.1

Eluent preparation

Weigh 83.7 g of sodium carbonate monohydrate (mw = 124.0) and 6.3 g of sodium bicarbonate (mw = 84.0) in a 1 L polypropylene flask. Add DI water to the mark to make 50x concentrated eluent stock.

Dilute 20.0 mL of the 50x concentrated eluent stock to 1.00 L to make the eluent of 13.5 mM Na₂CO₃/1.5 mM NaHCO₃ for the sodium thiosulfate assay. This is also the eluent solution B used for the sodium thiosulfate ionic impurity method.

Dilute 22.0 mL of the 50x concentrated eluent stock to 1.00 L to make the +10% eluent (14.85 mM Na₂CO₃/1.65 mM NaHCO₃). Dilute 18.00 mL to 1.00 L to make -10% eluent (12.15 mM Na₂CO₃/1.35 mM NaHCO₃) for the robustness test.

Mix one part of the eluent solution B with four parts DI water to make eluent solution A for the sodium thiosulfate ionic impurity method, which is 2.7 mM Na₂CO₃/0.3 mM NaHCO₃.

Robustness study

Following the guidelines in USP General Chapter <1225>, Validation of Compendial Methods,⁹ and USP General Chapter <621> Chromatography,¹⁰ the robustness of this method was evaluated by examining the retention time (RT), peak asymmetry, and assay results of a 100 mg/L sodium thiosulfate sample after imposing small variations ($\pm 10\%$) in procedural parameters (e.g., flow rate, eluent gradient concentration, column temperature). A system suitability standard containing 100 mg/L of sodium thiosulfate was injected. The same procedure was applied to two column sets from two different lots. The following variations were tested:

- Flow rate at 1.5 mL/min, 1.35 mL/min, 1.65 mL/min
- Column temperature at 30 °C, 27 °C, 33 °C
- Eluent concentrations at, 13.5 mM Na₂CO₃/1.5 mM NaHCO₃, 12.15 mM Na₂CO₃/1.35 mM NaHCO₃, 14.85 mM Na₂CO₃/1.65 mM NaHCO₃

Results and discussion

Sodium thiosulfate assay

Separation

Figure 1 shows the chromatogram of sodium thiosulfate mixed with anions including fluoride, chloride, nitrite, bromide, sulfate, nitrate, and phosphate. Using a Dionex IonPac AS12A column set under the prescribed isocratic conditions, thiosulfate is well separated from the common anions. Figure 2 shows a chromatogram of 100 µg/mL of sodium thiosulfate. The retention time of thiosulfate is in agreement with the proposed USP method, which states about 7 min. For two lots of the Dionex IonPac AS12A column, retention time was 7.20 and 7.68 min. The data from both columns passed the proposed USP method suitability requirements. The asymmetry values for thiosulfate were 1.3 and 1.47 (USP requires these values be not more than (NMT) 2 and the relative standard deviations were 0.7% and 0.03% (USP NMT 2.0%), respectively.

Columns: Dionex IonPac AG12A, 4 × 50 mm and Dionex IonPac AS12A, 4 × 200 mm
Eluent: 13.5 mM Na₂CO₃ / 1.5 mM NaHCO₃
Flow Rate: 1.5 mL/min
Inj. Volume: 25 µL
Column Temp.: 30 °C
Detection: Suppressed conductivity, Dionex AERS 500 (4mm) Suppressor, 25 °C, 106 mA, recycle mode
Samples: 50 mg/L of Sodium Thiosulfate in DI-water spiked with anions (4 to 30 mg/L of Fluoride, Chloride, Nitrite, Bromide, Nitrate, Phosphate, and Sulfate)

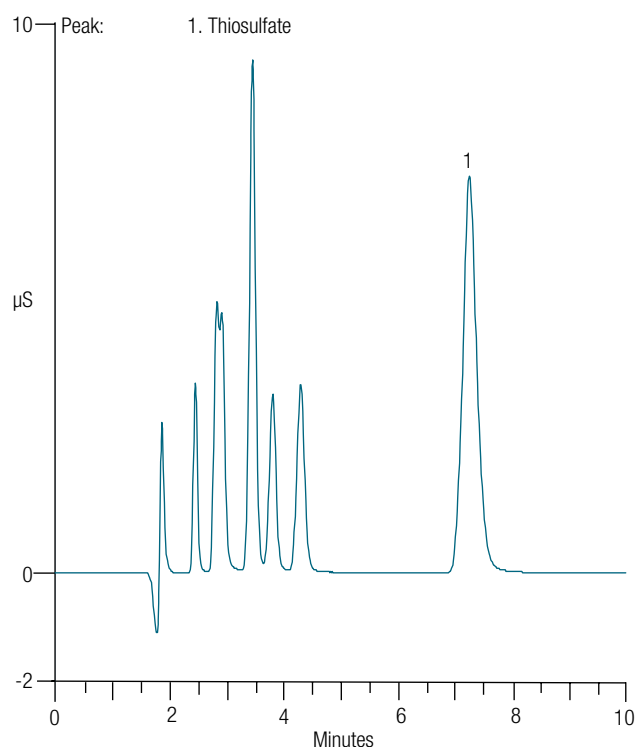


Figure 1. Separation of sodium thiosulfate from other anions.

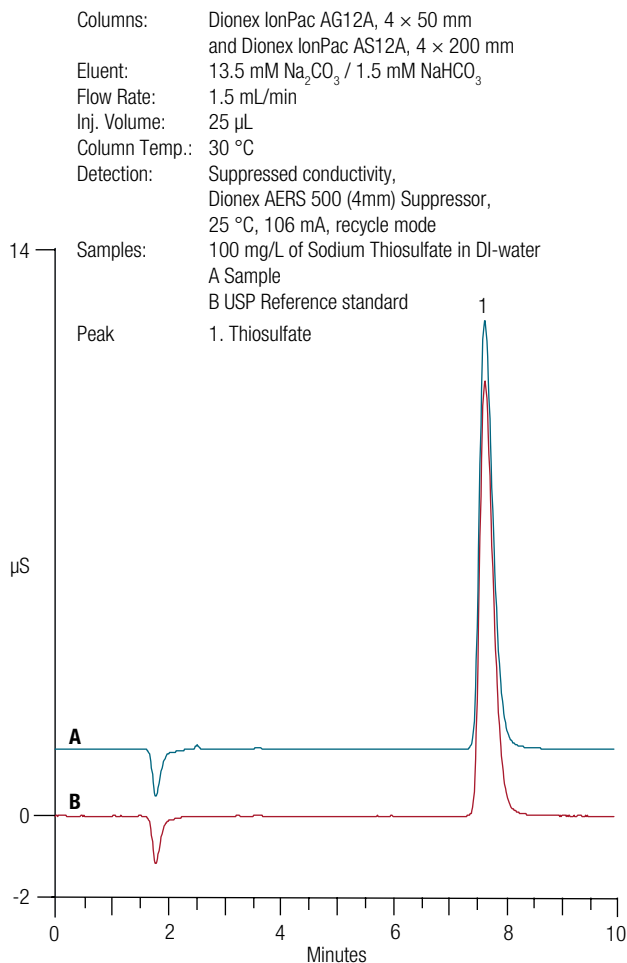


Figure 2. Chromatogram of 100 µg/mL of sodium thiosulfate.

Calibration, limit of detection (LOD), and limit of quantitation (LOQ)

The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the USP General Chapter <1225> guidelines recommend a minimum of five concentrations to establish linearity in

an assay. For a drug substance or finished product, the minimum specified range is from 80% to 120% of the test concentration.

In this study, sodium thiosulfate was calibrated at eight concentration levels ranging from 0.2 to 200 µg/mL. When the high concentration of 200 µg/mL is included, the measured coefficient of determination (r^2) was 0.998, therefore the data should be fit with using a quadratic function if including a concentration > 150 µg/mL. From 0.2 to 150 µg/mL, there was a linear relationship of peak area to concentration with a coefficient of determination (r^2) of 0.999. (Table 6 and Figure 3). As calibration is linear, the IC method for assay in the proposed USP Sodium Thiosulfate monograph using a one-point calibration at 100 µg/mL is an acceptable method for assay.

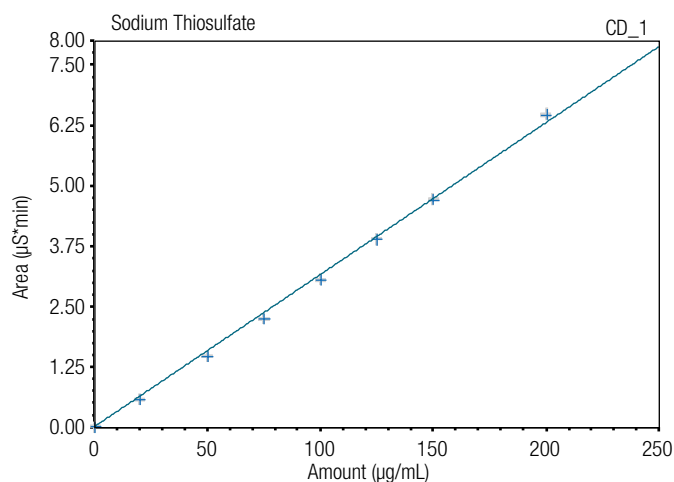


Figure 3. Calibration plot for sodium thiosulfate illustrating linearity.

Table 6. Comparison of calibration methods, LOD, and LOQ for sodium thiosulfate.

Method	Calibration Standards (µg/mL)	Calibration Type	r^2	Response Factor (µS*min/(µg/mL))	LOD (µg/mL)	LOQ (µg/mL)
A (USP method)	100	One level	n. a	0.031	0.05	0.17
B	0.2–200	Quadratic	1	n. a		
C	0.2–150	Linear, through origin	0.999	0.031		
D	0.2–200	Linear, through origin	0.998	0.032		

The LOD and LOQ were determined by seven injections of 0.20 µg/mL sodium thiosulfate. The baseline noise was determined by measuring the peak-to-peak noise in a representative 1 min segment of the baseline where no peaks elute but close to the peak of interest. The LOD and LOQ were determined for the concentration at the signal-to-noise ratio 3x and 10x (Table 6). The LOD is 0.05 µg/mL and the LOQ is 0.17 µg/mL.

Sample analysis

The proposed USP monograph requires that sodium thiosulfate contain 98.0–102.0% on the dried basis. In this study, the USP Sodium Thiosulfate Reference Standard was used to prepare the standard solutions. A purchased USP grade sodium thiosulfate salt was used to prepare the 100 µg/mL sample solution in DI water.

Two quantification methods were compared and evaluated to calculate the percentage of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) in the portion of sodium thiosulfate taken. As shown in Table 7, the sodium thiosulfate % calculated from method A (proposed monograph method) is similar to that determined using a calibration curve (method B). The assay results from both methods show that this sodium thiosulfate sample (98.8% purity) passed the acceptance criteria of 98.0–102.0% in the proposed USP monograph. The assay result agrees with USP grade stated on the sample bottle.

Table 7. Percentage of sodium thiosulfate in a sample using two quantification methods.

	Method A* (%)	Method B**(%)
Average	98.80	98.85
RSD (n=3)	0.25	0.28

*Method A: Proposed USP IC method for assaying sodium thiosulfate using one-point calibration.

**Method B: Eight-point calibration using quadratic fitting.

Sample accuracy and precision

Assay precision was evaluated by injecting 0.1 mg/mL sodium thiosulfate sample solutions, and expressed as the RSD of the results (sodium thiosulfate % in sample by method A). The method is precise with intraday precision from 0.2% to 0.6% and interday precision of 0.8% (Table 8).

Table 8. Precision of the sodium thiosulfate assay.

Analyte	Injection Precision Range (%) *	Intraday Precision Range (%) **	Interday Precision (%) ***
Sodium Thiosulfate	0.04–0.3	0.2– 0.6	0.8

* Injection precisions calculated from n=3 injections/sample for each sample.

** Intraday precision range is from independently prepared 100 µg/mL sodium thiosulfate samples, n=3 injections/sample, 2-3 samples/day for four days.

*** Interday precision is from 10 independently prepared 100 mg/L sodium thiosulfate samples, n=3 injections/sample, the samples were analyzed on four separate days.

Method accuracy was validated by spiked recovery of USP Sodium Thiosulfate Reference Standard in sodium thiosulfate samples over five concentration levels from 10 to 100 µg/mL in both 50 and 100 µg/mL samples. Table 9 summarizes the recovery results. For the calibration range of 0.2–150 µg/mL (150% of assay concentration), the method is accurate with sodium thiosulfate recovery ranging from 99 to 108%. The results from two columns are similar.

Robustness

Assay robustness was evaluated by measuring the influence of small variations ($\pm 10\%$) in procedural parameters (e.g., flow rate, eluent concentration, and column temperature) on the RT, peak asymmetry, and sodium thiosulfate purity results. These tests were carried out on two column sets from two different lots. The peak asymmetry was measured following the USP standard. Table 10 summarizes the results for sodium thiosulfate. These results indicate the method was robust to both changes in chromatography parameters and column change.

Table 9. Recovery data for sodium thiosulfate spiked in 50 µg/mL and 100 µg/mL samples.

Sodium Thiosulfate Added (µg/mL)	Column A		Column A		Column B	
	50 µg/mL Sodium Thiosulfate		100 µg/mL Sodium Thiosulfate		100 µg/mL Sodium Thiosulfate	
	Total Found (µg/mL)	Recovery %	Total Found (µg/mL)	Recovery %	Total Found (µg/mL)	Recovery %
0			98.0–100.7*		98.6–99.0	
10	59.6	101	109.3–109.4**	103		
25			125.2–126.1**	101–105	125	104
50	99.2	99	152.6–154.1**	105–108	152.5	107
75			178.8–183.1**	105–111	180.8	109
100			202.7–210.4**	102–110	210.0	111

*n=7 independently prepared 100 µg/mL sodium thiosulfate samples over four days

**n=5 independently prepared spiked sodium thiosulfate samples over four days

Table 10. Robustness of the IC-based assay for sodium thiosulfate (injected sample: 100 µg/mL sodium thiosulfate).

Parameter	Value	Column A					
		Ret.Time (min)		Amount (µg/mL)		Asym.	
		Average	% Diff	Average	% Diff	Average	% Diff
Flow Rate (mL/min)	1.65	6.49	-9.8	98.04	0.0	1.27	-1.8
	1.5	7.20		98.08		1.30	
	1.35	7.88	9.5	98.09	0.0	1.30	0.3
Column Temp. (°C)	27	6.41	-11.1	98.07	0.0	1.26	-2.6
	30	7.20		98.08		1.30	
	33	6.25	-13.2	98.09	0.0	1.26	-3.1
Eluent Conc. (mM) Na ₂ CO ₃ / NaHCO ₃	12.15/1.35	7.71	7.1	98.20	0.1	1.29	-0.8
	13.5/1.5	7.20		98.08		1.30	
	14.85/1.65	6.81	-5.5	98.23	0.2	1.27	-1.8
Parameter	Value	Column B					
		Ret.Time (min)		Amount (µg/mL)		Asym.	
		Average	% Diff	Average	% Diff	Average	% Diff
Flow Rate (mL/min)	1.65	6.95	-9.5	98.59	-0.2	1.45	-1.1
	1.5	7.68		98.83		1.47	
	1.35	8.52	10.9	98.61	-0.2	1.47	0.0
Column Temp (°C)	27	7.01	-8.8	98.58	-0.3	1.44	-1.6
	30	7.68		98.83		1.47	
	33	6.91	-10.0	98.27	-0.6	1.44	-1.8
Eluent Conc. (mM) Na ₂ CO ₃ / NaHCO ₃	12.15/1.35	8.47	10.2	98.65	-0.2	1.49	1.6
	13.5/1.5	7.68		98.83		1.47	
	14.85/1.65	7.08	-7.9	98.82	0.0	1.46	-0.7

Ionic impurities in the sodium thiosulfate method

In the proposed Sodium Thiosulfate and Sodium Thiosulfate Injection monograph revisions, an IC method was also used to determine the ionic impurities (chloride, sulfite, and sulfate). The sample solution for the ionic impurities determination is 2.0 mg/mL of sodium thiosulfate in diluent. All sample and standards for impurity determination were dissolved in the diluent (2.0 g/L of D-mannitol) to prevent oxidation of sulfite.

Separation

Figure 4 shows a chromatogram of chloride, sulfite, and sulfate spiked in sodium thiosulfate with an enlarged view of the analytes of interest. Using a Dionex IonPac AS12A column set under the gradient conditions, chloride, sulfite, and sulfate are separated and also well resolved from thiosulfate in 30 min. The gradient is modified from the proposed USP revision method (eluent A from 0 to 14 min, instead of 12 min) to allow complete separation of sulfate from the rise in the baseline due to the eluent gradient. Resolution between sulfite and sulfate is 2.6 for column A and 3 for column B, both passing the proposed USP method suitability requirement NLT 2. Relative retention times for chloride, sulfite, and sulfate are 0.31, 0.84, and 1 for column A and 0.28, 0.84, and 1 for column B (Table 11). The proposed USP method suitability requirements are 0.22, 0.84, and 1. The relative retention of chloride varies from column to column and neither matched the proposed USP method value. However, it was observed that this had no impact on the determination of ionic impurities.

Columns:	Dionex IonPac AG12A, 4 × 50 mm and Dionex IonPac AS12A, 4 × 200 mm	
Eluent:	A: 2.7 mM Na ₂ CO ₃ / 0.3 mM NaHCO ₃ B: 13.5 mM Na ₂ CO ₃ / 1.5 mM NaHCO ₃	
Time (min)	A (%)	B (%)
0	100	0
14	0	100
16	100	0
21	0	100
23	100	0
30	100	0
Flow Rate:	1.5 mL/min	
Inj. Volume:	25 µL	
Column Temp.:	30 °C	
Detection:	Suppressed conductivity, Dionex AERS 500 (4 mm) Suppressor, 25 °C, 106 mA, recycle mode	
Peaks:	1. Chloride	2.0 µg/mL
	2. Sulfite	5.0
	3. Sulfate	10.0
	4. Thiosulfate	100.0

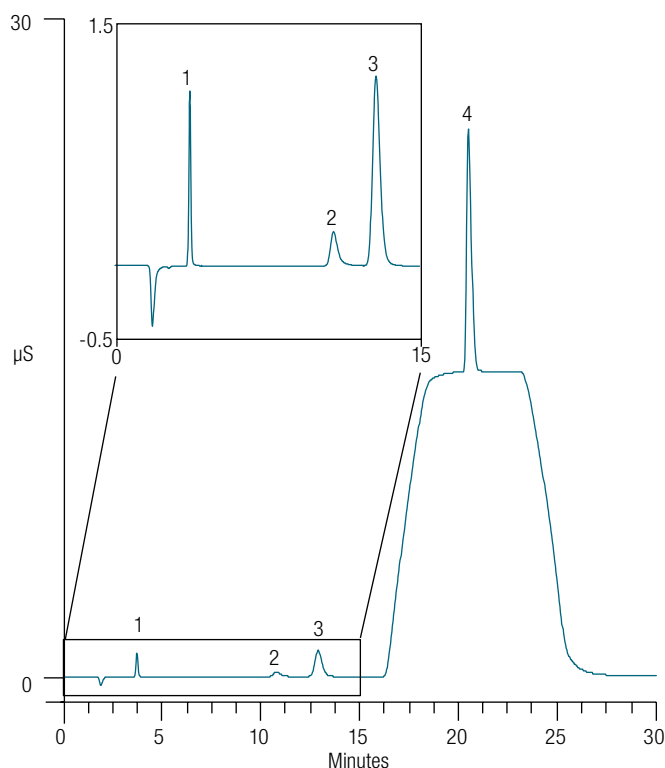


Figure 4. Chromatogram of chloride, sulfite, and sulfate in sodium thiosulfate.

Table 11. Retention time and resolution of impurity ions.

Analyte	Column A			Column B		
	Ret. Time (min)	Relative Ret. Time	Resolution	Ret. Time (min)	Relative Ret. Time	Resolution
Chloride	3.37	0.31	14	3.70	0.28	16
Sulfite	9.29	0.84	2.6	11.01	0.84	3
Sulfate	11.04	1.00		13.17	1.00	

Calibration, limit of detection (LOD), and limit of quantitation (LOQ)

According to the ICH and the USP guidelines, a minimum calibration range of 50% to 120% is required for determination of an impurity with a minimum of five concentrations to establish its calibration curve.

In this study, ionic impurities (chloride, sulfite, and sulfate) were calibrated at six concentration levels following the proposed monograph revision method. The range of chloride is from 0.04 to 2 µg/mL, the range of sulfite is from 0.2 to 10 µg/mL, and the range of sulfate is from 0.2 to 10 µg/mL. The results yield a linear relationship of peak area to concentrations for all three impurities (Table 12 and Figure 5). The coefficients of determination (r^2), were 1 for chloride, 0.9995–0.9998 for sulfite, and 1 for sulfate, and all passed the suitability requirements (NLT 0.995).

Using similar methodology, LOD and LOQ were determined with repeat injection of low levels (approximately 3 times the LOQ or approximately 10 times the LOD) of chloride, sulfite, and sulfate standards. The impurity method is sensitive with LOD of chloride at 0.004 µg/mL, sulfite at 0.09 µg/mL, and sulfate at 0.02 µg/mL, and LOQ of chloride at 0.01 µg/mL, sulfite at 0.3 µg/mL, and sulfate at 0.08 µg/mL.

Sample accuracy and precision

The ionic impurities (chloride, sulfite, and sulfate) in the sodium thiosulfate sample were determined using 2.0 mg/mL of sodium thiosulfate in the diluent. The limits of acceptance criteria are 0.02% for chloride, 0.1% for sulfite, and 0.5% for sulfate. Table 13 compares the results of chloride, sulfite, and sulfate in the sodium thiosulfate sample to the limit in the monographs revisions. This sodium thiosulfate sample did not pass the acceptance criteria limit stated in the proposed monographs revision because it exceeds the limit of 0.02% chloride. Recall that this sample was a purchased chemical and not an actual drug substance.

Table 12. Summary of calibration, limits of detection (LODs), and limits of quantitation data (LOQs) for ionic impurities.

Analyte	Calibration Standards (µg/mL)*	Coefficient of Determination Range (r^2)	LOD (µg/mL)	LOQ (µg/mL)
Chloride	0.04–2	1	0.004	0.01
Sulfite	0.2–10	0.9995–0.9998	0.09	0.3
Sulfate	0.2–10	1	0.02	0.08

*This is the concentration of its sodium salt

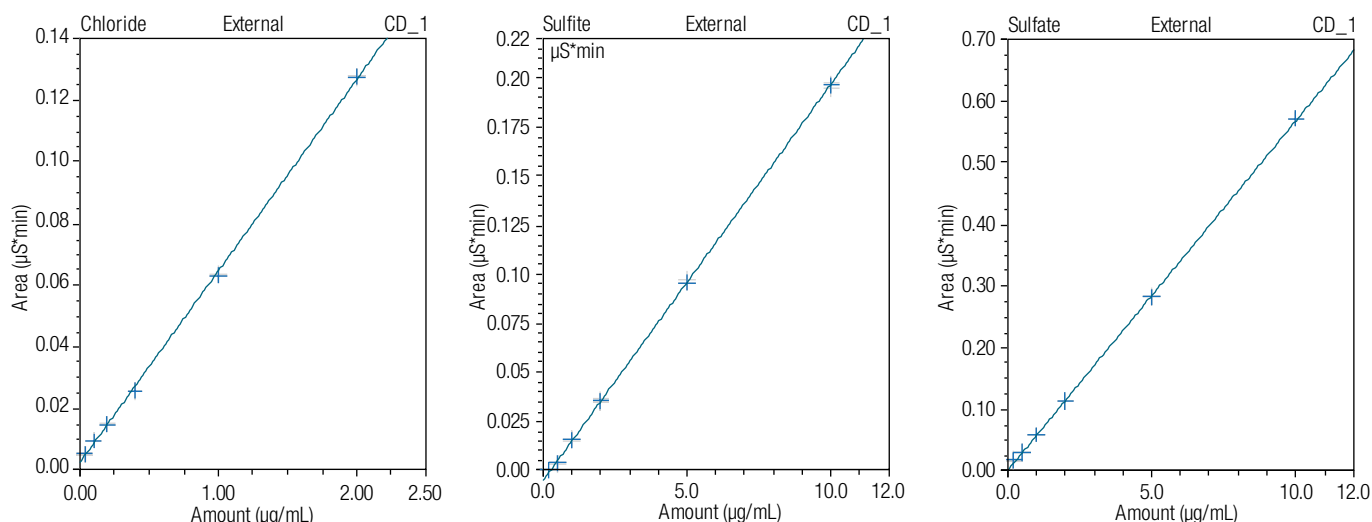


Figure 5. Calibration plots for ionic impurities illustrating linearity.

Table 13. Chloride, sulfite, and sulfate in sodium thiosulfate sample.

		Chloride	Sulfite	Sulfate
Acceptance Criteria (No more than)	In Sodium Thiosulfate (%)	0.02	0.1	0.5
	In 2.0 mg/mL Solution (µg/mL)	0.4	2	10
Sodium Thiosulfate Sample*	In Sodium Thiosulfate (%)	0.022	0.023	0.06
	In 2.0 mg/mL Solution (µg/mL)	0.44 ± 0.03	0.46 ± 0.06	1.19 ± 0.01

*Average result of n=3 each day over three days

Method accuracy was validated by spiked recovery of chloride, sulfite, and sulfate in 2.000 mg/mL of sodium thiosulfate in sample diluent solution at low concentration, with three replicates of each concentration and repeated twice on different days. Table 14 summarizes the recovery results. The method is accurate with chloride recovery ranges of 95–101%, sulfite of 86–100%, and sulfate of 107–109%.

Method precision was evaluated by injecting ($n \geq 3$ per day) the system suitability solution containing 0.4 µg/mL of chloride, 1 µg/mL of sulfite, and 2 µg/mL of sulfate. The impurity method is precise with intraday precision range of chloride at 0.2–0.8%, sulfite at 1.0–3.3%, and sulfate at 0.04–0.9%. The interday precisions are chloride at 4.1%, and sulfite and sulfate at 1.9%. These precision results surpass the suitability requirement in the proposed USP method (<15%) (Table 15).

Table 14. Recovery data for mixed chloride, sulfite, and sulfate spiked in a sodium thiosulfate sample containing 2.0 mg/mL sodium thiosulfate.

	Chloride		Sulfite		Sulfate	
	Added (µg/mL)	Recovery (%)	Added (µg/mL)	Recovery (%)	Added (µg/mL)	Recovery (%)
Spiked in 2.0 mg/mL Sodium thiosulfate	0.4	95–99	2	86–88	2	107
	1	99–100	5	90–96	5	108
	2	101	10	98–100	10	109

*n=2 independently prepared spiked sample over 2 days

Table 15. Precisions for analysis of the system suitability solution.

Analyte	Chloride	Sulfite	Sulfate
Intraday Precision range* (%)	0.2–0.8	1.0–3.3	0.04–0.9
Interday** Precision (%)	4.1	1.9	1.9

*n=3 or > 3 for each day

**n=5 days. Two days with column set A and three days with column set B

Conclusion

This study evaluated two IC methods included in the proposed Sodium Thiosulfate and Sodium Thiosulfate Injection monograph revisions. Both IC methods use a Dionex IonPac AS12A anion-exchange column and suppressed conductivity detection. Following the guidelines outlined in USP General Chapter <1225> (Validation of Compendial Methods) and the monograph instructions for each method, both methods were validated. Deliberate variations in the IC method parameters (e.g., mobile phase concentration, column temperature, etc.) were also made to test robustness.

The sodium thiosulfate assay method, a 10 min isocratic method, is linear ($r^2 = 0.999$) over the established analytical range of 0.2 to 150 µg/mL. The method is sensitive (LOQ at 0.17 mg/L), accurate (recovery 99–108%), precise (intraday precision 0.2–0.6% and interday precision of 0.8%), and specific for sodium thiosulfate determination. The method is robust as IC method parameter changes had no impact on the purity determination. The sodium thiosulfate impurity method, a 35 min gradient method, is linear over the established analytical range for impurities, precise, and accurate.

In conclusion, both IC methods meet the guidelines outlined in USP General Chapter <1225> and can be used to replace existing titration-based assays in the Sodium Thiosulfate monograph and likely the Sodium Thiosulfate Injection monograph (we were unable to test the drug product).

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