# Assay of Nitrite and Determination of Nitrate Impurity in Sodium Nitrite Using a Reagent-Free Ion Chromatography System

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Application Note 1148

**Key Words**
Dionex IonPac AS12A Column, Suppressed Conductivity Detection, Pharmaceutical, USP Monograph

## Goal

To confirm an ion chromatography (IC) method for the determination of nitrite and nitrate in sodium nitrite using a RFIC system with suppressed conductivity detection.

## Introduction

Sodium nitrite is indicated for sequential use with sodium thiosulfate for the treatment of acute cyanide poisoning that is judged to be life-threatening. Sodium nitrate is the possible anionic impurity in sodium nitrite. The U.S. Pharmacopeia (USP) monograph describes a sodium nitrite assay by titration with potassium permanganate. That assay is time-consuming and uses hazardous reagents.

The USP has embarked on a global initiative to modernize many of the existing monographs across all compendia. In response to this initiative, an alternative analytical method to assay nitrite and determine nitrate impurity in sodium nitrite was developed and subsequently published in *Pharmacopeia Forum* (PF).[1] This method uses ion chromatography with a Thermo Scientific™ Dionex™ IonPac™ AS12A anion-exchange column and suppressed conductivity detection to assay the nitrite content of sodium nitrite. The same IC method is used to determine nitrate impurity in sodium nitrite and is also described in the proposed revision to the USP sodium nitrite injection monograph.[2]

Ion chromatography (IC) offers a significant improvement to the existing assay for nitrite because it can simultaneously determine nitrite and nitrate in a single injection. In addition, using a Reagent-Free™ Ion Chromatography (RFIC™) system with electrolytically generated potassium carbonate and bicarbonate eluent significantly simplifies the method and enhances method reproducibility between laboratories.

This application note reports our evaluation of the IC method for nitrite assay and nitrate determination in the proposed revision of the USP monograph for sodium nitrite using an electrolytically generated potassium carbonate/bicarbonate eluent to execute the method rather than the manually prepared sodium carbonate/sodium bicarbonate eluent described in the PF proposal. The required eluent is generated using a Thermo Scientific Dionex EGC 500 $K_2CO_3$ Eluent Generator and EPM 500 pH modifier. The Thermo Scientific Dionex AERS 500 (4 mm) Anion Electrolytically Regenerated Suppressor produces the regenerate ion necessary for eluent suppressor and allows continuous operation with minimum maintenance. Because the RFIC system requires only deionized (DI) water as the carrier, it significantly simplifies system operation and improves analytical reproducibility. This method was validated following the guidelines outlined in USP General Chapter <1225>, Validation of Compendial Methods.[3]

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## Equipment

- A Thermo Scientific™ Dionex™ ICS-2100 Reagent-Free Ion Chromatography (RFIC) system was used in this work. The Dionex ICS-2100 RFIC is an integrated ion chromatograph that includes the following:
  - Eluent Generator
  - 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), and 25 µL injection loop
- Dionex EGC 500 $K_2CO_3$ Cartridge (P/N 088453)
- Dionex EPM 500 Electrolytic pH Modifier (P/N 088471)
- Dionex EGC Carbonate Mixer Kit, 4 mm (P/N 042126)
- Thermo Scientific Dionex AERS 500 Anion Electrolytically Regenerated Suppressor, 4 mm (P/N 082541)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software version 7.2

The column temperature of an ICS-2100 system can only be set at a minimum of 30 °C. Therefore, a Thermo Scientific™ Dionex™ ICS-5000 Reagent-Free Ion Chromatography (RFIC™) system was used in the method robustness test for column temperature (i.e. to set temperatures lower than 30 °C).

## Reagents and Standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistance or better
- Sodium nitrite USP reference standard (Sigma-Aldrich® Cat # 1614454-1G)
- Sodium nitrite, extra pure (Sigma-Aldrich Cat # 13447-1KG-R)
- Sodium nitrate USP reference standard, 99.995% METALS BASIS (Sigma-Aldrich Cat # 229938-10G)
- Sodium and potassium salts, A.C.S. reagent grade for preparing the Quality Assurance Report (QAR) standard mix

| Conditions | |
|---|---|
| Columns: | Dionex IonPac AS12A, 4 mm Analytical, 4 x 250 mm (P/N 046034)<br>Dionex IonPac AG12A, 4 mm Guard, 4 x 50 mm (P/N 079801) |
| Eluent: | 2.7 mM $K_2CO_3$ /0.3 mM $KHCO_3$ |
| Eluent Source: | Dionex EGC 500 $K_2CO_3$ cartridge with EPM 500 electrolytic pH modifier |
| Flow Rate: | 1.5 mL/min |
| Injection Volume: | 25 µL in Push-Full mode |
| Temperature: | Ambient (~24 °C) |
| Detection: | Suppressed conductivity, Dionex AERS 500 (4 mm) Suppressor, recycle mode, 22 mA current |
| System Backpressure: | ~2000–2100 psi |
| Background Conductance: | ~12.5 µs |
| Noise: | <10 nS/min |
| Run Time: | 18 min |

## Preparations of Solutions and Reagents

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

**Sodium Nitrite Stock Standard Solution, 1200 mg/L**
Accurately weigh 12.0 mg of USP sodium nitrite into a 20 mL polypropylene bottle and dissolve in 10 g of DI water.

**Sodium Nitrite Working Standard Solution, 120 mg/L**
Transfer 1.0 mL of sodium nitrite stock standard solution (1200 mg/L) into a 20 mL polypropylene bottle and mix with 9.0 g of DI water.

**Sodium Nitrite Calibration Standards, 30, 60, 90, 120, 150, 180 mg/L (Nitrite 20–120 mg/L)**
To prepare calibration standard solutions, dilute the stock standard solution (1200 mg/L) to the appropriate concentrations with DI water (Table 1).

Table 1. Preparation of sodium nitrite calibration standard solutions.

| Sodium Nitrite Concentration (mg/L) | 30 | 60 | 90 | 120 | 150 | 180 |
|---|---|---|---|---|---|---|
| Sodium Nitrite Stock Standard (1200 mg/L) (mL) | 0.25 | 0.5 | 0.75 | 1 | 1.25 | 1.5 |
| DI $H_2O$ (g) | 9.75 | 9.5 | 9.25 | 9 | 8.75 | 8.5 |

### Sodium Nitrate Stock Standard Solution, 1200 mg/L

Accurately weigh 12.0 mg of USP sodium nitrate into a 20 mL polypropylene bottle and dissolve in 10 g of DI water.

### Sodium Nitrate Working Standard Solution, 10 mg/L

Dilute the stock standard solution (1200 mg/L) to the appropriate concentration with DI water by pipetting 0.5 mL of Sodium Nitrate Stock Standard Solution to 59.5 g of DI $H_2O$.

### Sodium Nitrate Calibration Standard, 0.5, 1, 1.5, 2, 5, 10 mg/L, (Nitrate 0.365–7.30 mg/L)

To prepare sodium nitrate calibration standard solutions, dilute the working standard solution to the appropriate concentrations with DI water (Table 2).

Table 2. Preparation of sodium nitrate calibration standard solutions.

| Sodium Nitrate Concentration (mg/L) | 0.5 | 1 | 1.5 | 2 | 5 | 10 |
|---|---|---|---|---|---|---|
| Working Sodium Nitrate Standard (10 mg/L) (mL) | 0.5 | 1 | 1.5 | 2 | 5 | 10 |
| DI $H_2O$ (g) | 9.5 | 9 | 8.5 | 8 | 5 | 0 |

### Robustness Test Standard, (Nitrite 10 mg/L, Nitrate 20 mg/L)

Sodium nitrite stock standard solution (1200 mg/L) contains 800.2 mg/L of nitrite. Sodium nitrate stock standard solution (1200 mg/L) contains 875.3 mg/L of nitrate. Dilute and mix the stock standard solutions to the appropriate concentration with DI water (Tables 3 and 4).

Table 3. Stock standard solutions.

| Nitrite and Nitrate Stock Mixture | Nitrite (80 mg/L) + Nitrate (160 mg/L) |
|---|---|
| Nitrite Stock (800.2 mg/L) (mL) | 2.00 |
| Nitrate Stock (875.3 mg/L ) (mL) | 3.66 |
| DI $H_2O$ (g) | 14.34 |

Table 4. Working mixtures.

| Nitrite and Nitrate Working Mixture | Nitrite (10 mg/L) + Nitrate (20 mg/L) |
|---|---|
| Nitrite and Nitrate Stock Mixture (mL) | 12.5 |
| DI $H_2O$ (g) | 87.5 |

## Sample Preparation

### Sodium Nitrite Stock Sample Solution, 1200 mg/L, Prepared Using Sodium Nitrite, Extra Pure

Accurately weigh 12.0 mg of sodium nitrite into a 20 mL polypropylene bottle and dissolve in 10 g of DI water.

### Sodium Nitrite Working Sample Solution, 120 mg/L

Transfer 1.0 mL of sodium nitrite stock sample solution (1200 mg/L) into a 20 mL polypropylene bottle and mix with 9.0 g of DI water.

### Sodium Nitrite Recovery Test Sample Solution

To prepare 120 mg/L sodium nitrite sample solution spiked with 30, 60, 90, 120, 150, and 180 mg/L of USP grade sodium nitrite, dilute and mix sodium nitrite sample stock (1200 mg/L) with sodium nitrite USP standard stock (1200 mg/L) to the appropriate concentration with DI water (Table 5).

Table 5. Preparation of sodium nitrite sample solution.

| Sodium Nitrite Spiked (mg/L) | 30 | 60 | 90 | 120 | 150 | 180 |
|---|---|---|---|---|---|---|
| **Sodium Nitrite Stock Sample (1200 mg/L) (mL)** | 1 | 1 | 1 | 1 | 1 | 1 |
| **Sodium Nitrite Stock Standard (1200 mg/L) (mL)** | 0.25 | 0.5 | 0.75 | 1 | 1.25 | 1.5 |
| **DI $H_2O$ (g)** | 8.75 | 8.5 | 8.25 | 8 | 7.75 | 7.5 |

### Sodium Nitrate Recovery Test Sample Solution

To prepare 120 mg/L sodium nitrite sample solution spiked with 0.25, 0.5, 0.75, 1, 2.5, and 5 mg/L of sodium nitrate, dilute and mix sodium nitrite stock sample (1200 mg/L) with USP sodium nitrate working standard (10 mg/L) to the appropriate concentration with DI water (Table 6).

Table 6. Preparation of sodium nitrate sample solution.

| Sodium Nitrate Spiked (mg/L) | 0.25 | 0.5 | 0.75 | 1 | 2.5 | 5 |
|---|---|---|---|---|---|---|
| **Sodium Nitrite Stock Sample (1200 mg/L) (mL)** | 1 | 1 | 1 | 1 | 1 | 1 |
| **Sodium Nitrate Working Standard (10 mg/L) (mL)** | 0.25 | 0.5 | 0.75 | 1 | 2.5 | 5 |
| **DI $H_2O$ (g)** | 8.75 | 8.5 | 8.25 | 8 | 6.5 | 4 |

## Robustness Study

Following the guidelines of USP Physical Test, <621> Chromatography, evaluate the robustness of this method by examining the retention time (RT), peak asymmetry, and resolution of the two analytes in the robustness test standard after imposing small variations (±10%) in procedural parameters (e.g., flow rate, eluent gradient concentration, and column temperature). Apply the same procedure to two column sets from two different lots. Test the following variations:

- Flow rate at 1.5 mL/min, 1.35 mL/min, 1.65 mL/min
- Column temperature at 24 °C, 21 °C, 27 °C, and 30 °C (Using the ICS-5000 system)
- Eluent concentrations at 2.7 mM $K_2CO_3$/0.3 mM $KHCO_3$, 2.43 mM $K_2CO_3$/0.27 mM $KHCO_3$, and 2.97 mM $K_2CO_3$/0.33 mM $KHCO_3$

## Results and Discussion

### Separation

The separation of nitrite and nitrate was achieved using a Dionex IonPac AS12A column set with the specified isocratic conditions. Figure 1 shows the separation of ten anions using the proposed method. The other anions do not interfere with the determination of nitrite and nitrate.

Figure 2A shows the chromatogram of a sodium nitrite sample (120 mg/L sodium nitrite) with an enlarged view (Figure 2B) showing the separation of nitrate. The relative retention times are 1 for nitrite and 1.9 for nitrate, similar to the 1 and 1.85 reported in the proposed monograph revision. Peak resolution between nitrite and nitrate is >11, exceeding the USP requirement of 3. The asymmetry value for both nitrite and nitrate is <1.2 (USP specification is not more than (NMT) 2).

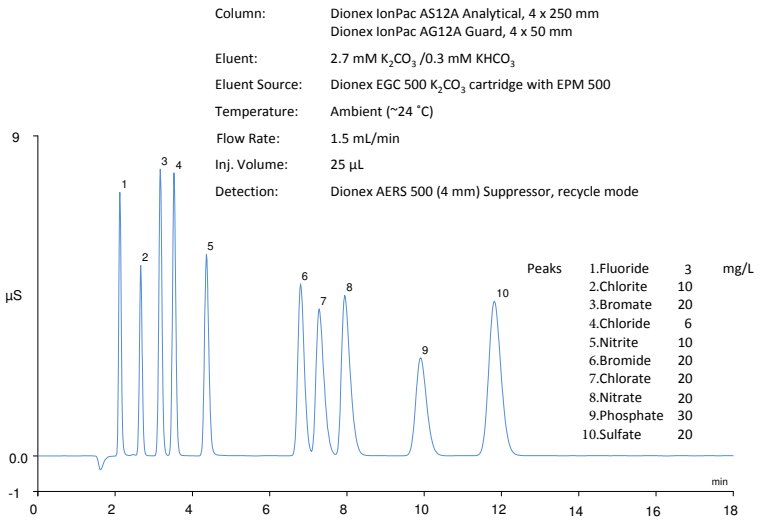


Figure 1. Separation of ten anions.

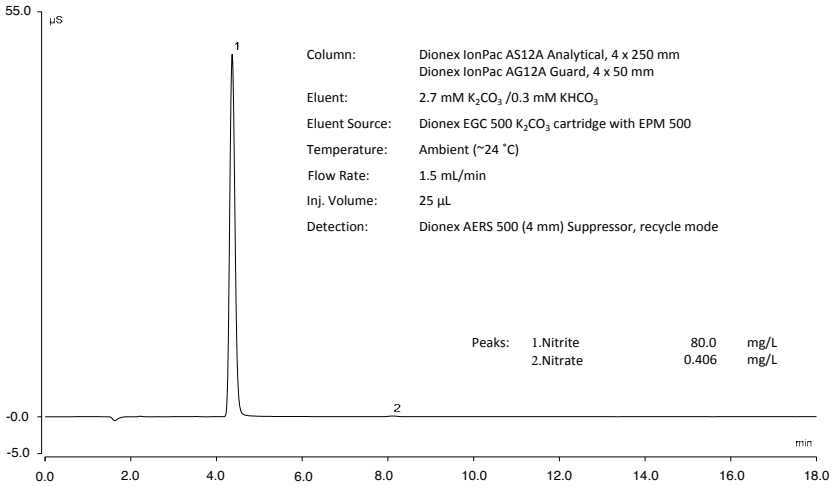


Figure 2A. Sodium nitrite sample (80 mg/L nitrite).

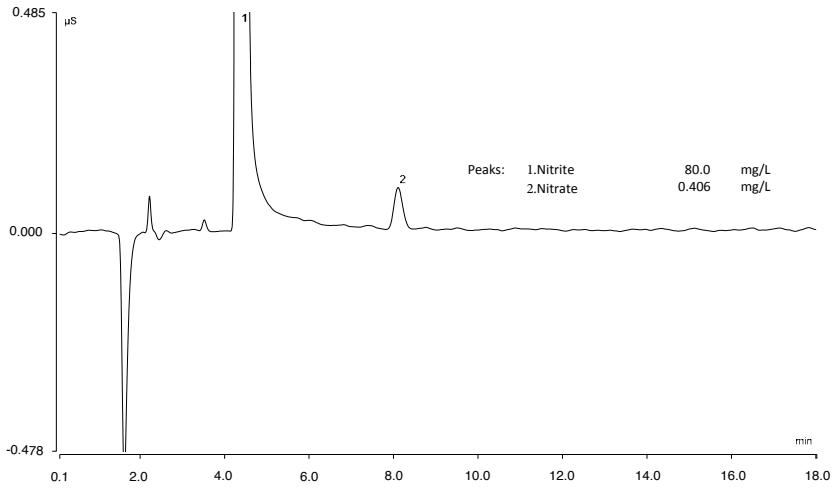


Figure 2B. Enlarged view of Chromatogram A showing the nitrate peak.

## 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.[3-5] For a drug substance or finished product, the minimum specified range is from 80% to 120% of the test concentration. A minimum range from 50% to 120% is required for determination of an impurity. In this study, nitrite was calibrated at six concentration levels ranging from 20 to 120 mg/L. The results yield a linear relationship of peak area to concentration with a coefficient of determination ($r^2$) of 0.9999. Nitrate was calibrated at six concentration levels ranging from 0.365 to 7.20 mg/L with an $r^2$ of 0.9999 (Table 7).

Table 7. Calibration, LODs, and LOQs of nitrite and nitrate.

| Anion | Calibration Range (mg/L) | $r^2$ | LOD (µg/L) | LOQ (µg/L) |
|---|---|---|---|---|
| **Nitrite** | 20–120 | 0.9999 | 43.6 | 145 |
| **Nitrate** | 0.365–7.30 | 0.9999 | 97.3 | 324 |

To determine the LODs and LOQs for nitrite and nitrate, the baseline noise was first determined by measuring the peak-to-peak noise in a representative 1-min segment of the baseline where no peaks elute but is close to the peaks of interest. The signal was determined from the average peak height of three injections of a sample of 200 µg/L nitrite and 365 µg/L nitrate. The LODs and LOQs were then determined by multiplying the signal-to-noise ratio 3x and 10x, respectively (Table 7). The LODs of nitrite and nitrate were 43.6 and 97.3 µg/L, respectively. The LOQs of those two analytes were 145 and 324 µg/L, respectively. In the sodium nitrite sample (120 mg/L), when nitrate is at the LOD level (97.3 µg/L), the sodium nitrate percentage is 0.11; when nitrate is at the LOQ level (324 µg /L), the sodium nitrate percentage is 0.37. Both are less than the USP acceptance criterion for nitrite (0.4%).

## Sample Analysis

The USP monograph requires that sodium nitrite contain 98.0%–102.0% on the dried basis. In this study, the USP sodium nitrite reference standard (Sigma-Aldrich Cat # 1614454-1G) was used to prepare the standard solution of 120 mg/L sodium nitrite. Sodium nitrite (Sigma-Aldrich Cat # 13447-1KG-R) was used to prepare the sample solution of 120 mg/L.

Two quantification methods were compared and evaluated to calculate the percentage of sodium nitrite in the sample solution (Table 8).

### Sodium Nitrite Percentage Method A: USP Sodium Nitrite Single Standard Point (120 mg/L) (Proposed monograph revision method)

Calculate the percentage of sodium nitrite ($NaNO_2$) in the portion of sodium nitrite taken:

$$\text{Result} = \left(\frac{ru}{rs}\right) \times \left(\frac{Cs}{Cu}\right) \times 100$$

ru = Peak area from the sample solution

rs = Peak area from the standard solution

Cs = Concentration of USP Sodium Nitrite RS in the standard solution (mg/L)

Cu = Concentration of sodium nitrite in the sample solution (mg/L)

### Sodium Nitrite Percentage Method B: USP Sodium Nitrite Calibration Standard Curve

- Build a calibration curve with 30–180 mg/L of USP Sodium Nitrite Reference Standard.
- Prepare sodium nitrite sample solution of 120 mg/L.
- Calculate the true concentration of sample solution using the calibration curve.
- Calculate the percentage of sodium nitrite.

$$\text{Result} = \frac{\textit{Sodium Nitrite Concentration calculated from calibration curve (mg/L)}}{120\ \textit{(mg/L)}} \times 100$$

Table 8. Sodium nitrite percentage in sample using two quantification methods.

| | Method A (%) | Method B (%) |
|---|---|---|
| **Ave** | 99.1 | 99.5 |
| **RSD (n=3)** | 0.14 | 0.14 |

The USP monograph requires that sodium nitrite contain no more than 0.4% of sodium nitrate. Three quantification methods were compared and evaluated to calculate the percentage of sodium nitrate in the portion of sodium nitrite taken (Table 9).

### Sodium Nitrate Percentage Method A: Nitrate Response Factor (Proposed monograph revision method)

Calculate the percentage of sodium nitrate ($NaNO_3$) in the portion of the sample taken:

$$\text{Result} = \left(\frac{ru}{rs}\right) \times \left(\frac{1}{F}\right) \times 100$$

ru = Peak response of nitrate from the sample solution

rs = Peak response of nitrite from the sample solution

F = Response factor for nitrate, 0.7

**Sodium Nitrate Percentage Method B:**
**USP Sodium Nitrate Single Standard Point (1 mg/L)**

Calculate the percentage of sodium nitrate ($NaNO_3$) in the portion of sodium nitrite taken:

Calculate sodium nitrate concentration using single sodium nitrate standard as follows:

$$cu = cs \times \left(\frac{ru}{rs}\right)$$

ru = peak area from the sample solution

rs = peak area from the standard solution

Cs = concentration of USP Sodium Nitrate RS in the standard solution (mg/L)

Cu = concentration of sodium nitrate in the sample solution (mg/L)

$$\text{Result} = \frac{\textit{Sodium Nitrate Concentration calculated from single standard (mg/L)}}{\textit{Sodium Nitrite Concentration (mg/L)}} \times 100$$

**Sodium Nitrate Percentage Method C:**
**USP Sodium Nitrate Calibration Standard Curve**

- Build a calibration curve with 0.5–3 mg/L of USP Sodium Nitrate Standard.
- Prepare sodium nitrite sample solution of 120 mg/L.
- Calculate true sodium nitrate concentration using calibration curve.
- Calculate % of sodium nitrate in the portion of sodium nitrite taken.

$$\text{Result} = \frac{\textit{Sodium Nitrate Concentration calculated from calibration curve (mg/L)}}{\textit{Sodium Nitrite Concentration (mg/L)}} \times 100$$

As shown in Table 8, the $NaNO_2$ percentage calculated from the method A (monograph revision method) gives a similar result to the standard curve calibration method. As shown in Table 9, the $NaNO_3$ percentage calculated from the method A (monograph revision method) also gives similar result as the calibration method.

Table 9. Sodium nitrate percentage in sample using three quantification methods.

| Sample | | Method A | Method B | Method C |
|---|---|---|---|---|
| **120 mg/L $NaNO_2$** | AVE | 0.499 | 0.503 | 0.528 |
| | RSD (n=3) | 0.589 | 0.589 | 0.542 |
| **120 mg/L $NaNO_2$ spiked with 0.25 mg/L $NaNO_3$** | AVE | 0.694 | 0.699 | 0.721 |
| | RSD (n=3) | 0.442 | 0.442 | 0.459 |
| **120 mg/L $NaNO_2$ spiked with 0.5 mg/L $NaNO_3$** | AVE | 0.891 | 0.898 | 0.915 |
| | RSD (n=3) | 0.500 | 0.500 | 0.512 |
| **120 mg/L $NaNO_2$ spiked with 0.75 mg/L $NaNO_3$** | AVE | 1.08 | 1.09 | 1.10 |
| | RSD (n=3) | 0.707 | 0.707 | 0.624 |
| **120 mg/L $NaNO_2$ spiked with 1 mg/L $NaNO_3$** | AVE | 1.28 | 1.29 | 1.30 |
| | RSD (n=3) | 0.833 | 0.833 | 0.817 |
| **120 mg/L $NaNO_2$ spiked with 2.5 mg/L $NaNO_3$** | AVE | 2.53 | 2.55 | 2.53 |
| | RSD (n=3) | 0.492 | 0.492 | 0.506 |
| **120 mg/L $NaNO_2$ spiked with 5 mg/L $NaNO_3$** | AVE | 4.62 | 4.66 | 4.59 |
| | RSD (n=3) | 0.465 | 0.465 | 0.478 |

## Sample Accuracy and Precision

Method accuracy was validated by spiked recovery of sodium nitrite and sodium nitrate in sodium nitrite sample over six concentration levels, with three replicates of each concentration. Tables 10 and 11 summarize recovery results for sodium nitrite and sodium nitrate. Sodium nitrite recovery ranges from 94 to 103% for the two quantification methods and sodium nitrate recovery ranges from 93% to 101% for all three quantification methods.

Table 10. Recovery data for sodium nitrite spiked in sodium nitrite sample containing 119 mg/L sodium nitrite.

| Sodium Nitrite Added (mg/L) | Peak Area RSD | Method A | | Method B | |
|---|---|---|---|---|---|
| | | Total Found (mg/L) | Recovery % | Total Found (mg/L) | Recovery % |
| **0** | 0.2 | 119 | - | 120 | - |
| **30** | 0.05 | 150 | 103 | 151 | 102 |
| **60** | 0.14 | 181 | 103 | 182 | 103 |
| **90** | 0.19 | 211 | 101 | 211 | 101 |
| **120** | 0.2 | 240 | 100 | 240 | 100 |
| **150** | 0.37 | 268 | 99.2 | 269 | 98.8 |
| **180** | 0.24 | 296 | 98.1 | 296 | 97.7 |

Table 11. Recovery data for sodium nitrate spiked in sodium nitrite sample containing 120 mg/L sodium nitrite.

| Sodium Nitrate Added (mg/L) | Peak Area RSD | Method A | | Method B | | Method C | |
|---|---|---|---|---|---|---|---|
| | | Total Found (mg/L) | Recovery % | Total Found (mg/L) | Recovery % | Total Found (mg/L) | Recovery % |
| **0** | 0.19 | 0.59 | - | 0.599 | - | 0.63 | - |
| **0.25** | 0.5 | 0.83 | 94.4 | 0.837 | 95.1 | 0.86 | 93.5 |
| **0.5** | 0.31 | 1.07 | 95.5 | 1.08 | 96.2 | 1.10 | 94.4 |
| **0.75** | 0.49 | 1.30 | 94.6 | 1.31 | 95.3 | 1.33 | 93.4 |
| **1** | 0.79 | 1.54 | 95.1 | 1.55 | 95.3 | 1.56 | 93.5 |
| **2.5** | 0.24 | 3.06 | 98.5 | 3.08 | 99.3 | 3.06 | 97.2 |
| **5** | 0.21 | 5.60 | 100 | 5.64 | 101 | 5.57 | 98.8 |

Assay precision was evaluated by injecting seven replicates of the test sample 120 mg/L sodium nitrite (119 mg/L sodium nitrite + 0.568 mg/L sodium nitrate) spiked with 1 mg/L sodium nitrate and expressed as the RSDs of RT and peak area (Table 12). The assay exhibited good short-term precision.

Table 12. Retention time and peak area precisions of sodium nitrite 119 mg/L spiked with USP 0.5 mg/L sodium nitrate.

| Compound | Conc (mg/L) | RT RSD (n=7) | Peak Area RSD ( n=7) |
|---|---|---|---|
| **Sodium Nitrite** | 119 | 0.050 | 0.070 |
| **Sodium Nitrate** | 1.56 | 0.060 | 1.66 |

## Robustness

Assay robustness was evaluated by measuring the influence of small variations (±10%) in procedural parameters (e.g., flow rate, eluent concentration, column temperature on the RT, peak asymmetry, and resolution of the two analytes on two column sets from two different lots). The peak asymmetry was calculated using the USP formula. The resolution was determined relative to the previous peak in a chromatogram using the USP formula. A standard mix (10 mg/L nitrite, 20 mg/L nitrate) was injected three times at each chromatographic condition. The resolution of nitrate to nitrite ranged from 11.6 to 12.0 on column 1 and from 11.2 to 12.0 on column 2. Tables 13 and 14 summarize the results for nitrite and nitrate, respectively. These results indicate the method is robust for both analytes.

Table 13. Robustness of the IC-based assay for nitrite (injected sample: 10 mg/L nitrite and 20 mg/L nitrate; average of three injections).

| Parameter | | Column 1 | | | | | | Column 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Nitrite RT (min) | Diff (%) | Asym. | Diff (%) | Resol. | Diff (%) | Nitrite RT (min) | Diff (%) | Asym. | Diff (%) | Resol. | Diff (%) |
| Eluent Conc (mM) $K_2CO_3/KHCO_3$ | 2.7/0.3 | 4.77 | - | 1.09 | - | 11.8 | - | 4.77 | - | 1.09 | - | 11.8 | - |
| | 2.43/0.27 | 4.94 | 3.72 | 1.07 | -1.53 | 11.9 | 1.19 | 4.94 | 3.72 | 1.07 | -1.53 | 11.9 | 1.19 |
| | 2.97/0.33 | 4.61 | -3.22 | 1.10 | 0.92 | 11.7 | -1.19 | 4.61 | -3.22 | 1.10 | 0.92 | 11.7 | -1.19 |
| Flow Rate (mL/min) | 1.5 | 4.77 | - | 1.09 | - | 11.8 | - | 4.77 | - | 1.09 | - | 11.8 | - |
| | 1.35 | 5.30 | 11.1 | 1.09 | 0.00 | 12.0 | 1.72 | 5.30 | 11.1 | 1.09 | 0.00 | 12.0 | 1.69 |
| | 1.65 | 4.31 | -9.54 | 1.09 | 0.00 | 11.6 | -2.06 | 4.31 | -9.54 | 1.09 | 0.00 | 11.6 | -2.09 |
| Column Temp (˚C) | 24 | 4.37 | - | 1.15 | - | 11.9 | - | 4.79 | - | 1.09 | - | 11.6 | - |
| | 21 | 4.44 | 1.58 | 1.16 | 0.87 | 12.0 | 0.93 | 4.86 | 1.52 | 1.10 | 0.92 | 11.7 | 0.60 |
| | 27 | 4.31 | -2.93 | 1.17 | 0.86 | 11.8 | -1.17 | 4.71 | -3.00 | 1.10 | 0.00 | 11.5 | -1.63 |
| | 30 | 4.25 | -2.72 | 1.16 | 0.87 | 11.7 | -1.43 | 4.65 | -2.86 | 1.09 | 0.00 | 11.2 | -3.45 |

Table 14. Robustness of the IC-based assay for nitrate (injected sample: 10 mg/L nitrite and 20 mg/L nitrate; average of three injections).

| Parameter | | Column 1 | | | | Column 2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Nitrate RT (min) | Diff (%) | Asym. | Diff (%) | Nitrate RT (min) | Diff (%) | Asym. | Diff (%) |
| Eluent Conc (mM) $K_2CO_3/KHCO_3$ | 2.7 /0.3 | 8.73 | - | 1.40 | - | 8.73 | - | 1.40 | - |
| | 2.43 /0.27 | 9.10 | 4.33 | 1.39 | -0.24 | 9.10 | 4.33 | 1.39 | -0.24 |
| | 2.97/0.33 | 8.41 | -3.67 | 1.40 | 0.48 | 8.41 | -3.67 | 1.40 | 0.48 |
| Flow Rate (mL/min) | 1.5 | 8.73 | - | 1.40 | - | 8.73 | - | 1.40 | - |
| | 1.35 | 9.71 | 11.3 | 1.41 | 0.95 | 9.71 | 11.3 | 1.41 | 0.95 |
| | 1.65 | 7.85 | -10.0 | 1.39 | -0.72 | 7.85 | -10.0 | 1.39 | -0.72 |
| Column Temp (˚C) | 24 | 7.90 | - | 1.53 | - | 8.73 | - | 1.38 | - |
| | 21 | 8.16 | 3.30 | 1.57 | 2.61 | 9.018 | 3.26 | 1.40 | 1.45 |
| | 27 | 7.67 | -6.04 | 1.5 | -4.46 | 8.47 | -6.10 | 1.36 | -2.86 |
| | 30 | 7.45 | -5.76 | 1.48 | -3.27 | 8.22 | -5.87 | 1.33 | -3.62 |

## Conclusion

This study describes and evaluates an IC-based assay for simultaneous determination of nitrite and nitrate in sodium nitrite that was proposed to modernize two USP monographs.[1-2] The two analytes were separated on an anion-exchange column and detected by suppressed conductivity within 18 min. This assay for nitrite and nitrate was validated to meet the analytical performance characteristics outlined in USP General Chapter <1225>. Compared to the time-consuming assays in the USP sodium nitrite monograph, this IC-based assay executed with an RFIC system offers a simple, accurate, and robust measurement of the two analytes without handling hazardous regents.

## References


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