Application note | 004315

Ion chromatography

Determination of nitrite and nitrate from lactose by ion chromatography using the NGES-A suppressor

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Introduction

Nitrite (NO_2^-) and nitrate (NO_3^-) impurities in pharmaceutical excipients are of growing concern due to their potential role in forming carcinogenic N-nitrosamines when secondary or tertiary amines are present in the active pharmaceutical ingredients or formulation process.[1,2] Consequently, accurate quantification of these anions at trace levels is essential for ensuring drug safety and compliance with regulatory standards.[1]

Ion chromatography (IC) with suppressed conductivity has emerged as the preferred analytical technique for the simultaneous determination of nitrite and nitrate. Since its development in the 1970s, IC has become widely adopted in laboratories due to its high sensitivity, selectivity, and capacity for multianalyte separation.[3,4] Moreover, IC methods have been incorporated into official pharmacopeial monographs, underpinning their relevance for quality control in the pharmaceutical industry.[3]

In pharmaceutical matrices, analytical challenges, such as high concentrations of chloride ions that may mask nitrite peaks, have been successfully addressed.[5,6] For example, UV detection at ~210 nm, in conjunction with conductivity detection in IC,

can effectively mitigate chloride interference, enabling sensitive nitrite quantification down to sub-µg/L levels, with excellent linearity and recovery rates; however, use of UV can also be challenging because of interference from UV-absorbing organic compounds in complex matrices and because of an inconsistent baseline during gradient analysis.[5] Alternative approaches, such as gas chromatography–mass spectrometry (GC-MS), have also been investigated. One headspace GC-MS method achieved a quantification limit of 0.05 ppm for nitrite across diverse excipients, offering robustness and specificity, but sometimes requiring specialized instrumentation that can be more difficult to operate.[2] These headspace GC-MS methods often involve derivatization under acidic conditions or the use of secondary amines, which can lead to the formation of nitrosamines. These nitrosamines pose a safety concern that complicates the analysis, introducing uncertainty, variability, and unwanted complexity into the analytical workflow. Also, inconsistent derivatization efficiency can cause poor reproducibility and quantification errors.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods have also been utilized, but these methods often require derivatization for enhanced sensitivity in detecting nitrites. This frequently involves more complex sample handling and risks contamination, highlighting the balance between method simplicity and sensitivity in pharmaceutical analysis.[1]

Thus, IC stands out as a robust, reliable, and accessible tool for the simultaneous detection of nitrite and nitrate in excipients. Its adaptability across detection modes (conductivity, UV) and sensitivity to low levels make it a valuable asset for safeguarding drug quality.

IC, though widely used for nitrite analysis in pharmaceuticals, faces several limitations when applied to complex excipient matrices. The relatively low molar absorptivity of nitrite at 210 nm further challenges sensitivity, particularly in ultra-trace-level (low parts per billion or parts per trillion level) quantification, which might be required for nitrosamine risk assessment.[5] Sample preparation may be minimal, but excipients with high ionic loads can necessitate dilution or cleanup to prevent column overload and detector saturation. Additionally, suppressed conductivity detection may suffer from baseline instability in complex formulations.[5] Derivatization-based methods like Griess or DAN (2,3-diaminonapthalene) provide good sensitivity and specificity, but their derivatization reaction can be hindered by constituents present in the sample.[5] IC with post-column derivatization using Griess can be used to match the required sensitivity for nitrite.[5,7]

Here, we demonstrate the application of a novel suppressor for multiple application notes (ANs) proposed by the U.S. Pharmacopeia (USP) for the determination of nitrite and nitrate using suppressed conductivity. The USP ANs specify the Thermo Scientific™ Dionex™ IonPac™ AS19-4µm Analytical Column (4 × 250 mm) for excellent separation of anions.[8–10] The method detailed here was found to determine nitrite and nitrate from lactose monohydrate and colloidal silicon dioxide excipients successfully, and it can also be applied to other pharmaceutical excipients. One notable difference is the use of the Thermo Scientific™ Dionex™ NGES Next Generation Electrolytic Suppressor for anion analysis (NGES-A) here, which provides improved robustness over previous generations of suppressors without any sacrifice to performance. This ensures constant and routine testing of pharmaceuticals without interruption, saving the user time and intervention. The new suppressor also provides the advantage of faster baseline stabilization and decreased noise relative to older generation suppressors.

Summarily, this method helps labs ensure compliance with USP methodology while ensuring the pharma excipient products are free of harmful nitrosamine products and precursors.

Experimental

Equipment

- Thermo Scientific™ Dionex™ Integrion™ HPIC™ System (Part No. 22153-60305),* including:
  - Thermo Scientific™ Dionex™ EGC KOH 500 Eluent Generator Cartridge (Part No. 075778)
  - CD detector
  - Thermostatted column oven
  - Thermo Scientific™ Dionex™ AS-AP Autosampler (Part No. 074926) with 10 mL Thermo Scientific™ Dionex™ Vial Tray (Part No. 074938)
  - Thermo Scientific™ Dionex™ AS-AP Autosampler Vial Kit, 10 mL polystyrene with caps and septa (Part No. 055058)

*A Thermo Scientific™ Dionex™ Inuvion™ IC System with Reagent-Free IC (RFIC™) or a Thermo Scientific™ Dionex™ ICS-6000 HPIC™ System can also be used for this application.

Software

- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), software version 7.3.2.14225 MUe

Reagents, standards, and samples

- Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistance or better
- Nitrite Standard for IC, 1000 mg/L (Merck Part No. 67276)
- Nitrate Standard for IC, 1000 mg/L (Merck Part No. 74246)
- Potassium Hydroxide (KOH) solution 45 wt. % in water (Merck Part No. 417661)
- Helium or nitrogen; 4.5 grade (99.995%) or better (Praxair)
- Lactose monohydrate (Loba Chemie product code 0433000500, Lot No. SG37271202)
- Colloidal Silicon Dioxide IP (Aay Cee Cellulose Industry, B. No. ACC/C50/25/099)

Consumables

Thermo Scientific™ Dionex™ IonPac™ AS19-4µm Guard Column, 4 × 50 mm (Part No. 083221)

Dionex IonPac AS19-4µm analytical column, 4 × 250 mm (Part No. 083217)

Dionex NGES-A suppressor, 4 mm (Part No. 060001)

Dionex EGC 500 KOH eluent generator cartridge (Part No. 075778)

Thermo Scientific™ Dionex™ CR-ATC 600 Continuously Regenerated Anion Trap Column (Part No. 088662)

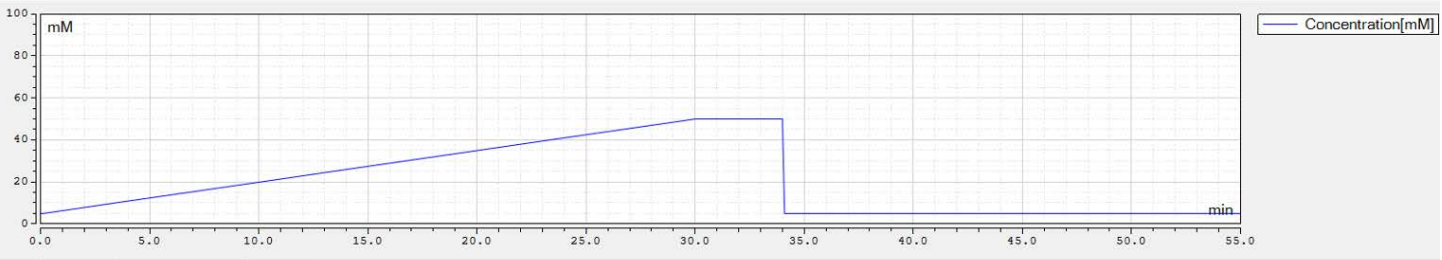
Millipore™ Millex™ Nylon Syringe Filter, 0.2 µm x 25 mm (Part No. SLGNDZ5)

Instrument method

System	Dionex Integrion RFIC
Columns	Dionex IonPac AS19-4µm, analytical, 4 x 250 mm Dionex IonPac AG19-4µm, guard, 4 x 50 mm
Eluent source	RFIC-KOH cartridge
Gradient	See “Eluent gradient program” below
Flow rate	1.0 mL/min
Injection volume	200 µL (full loop)
Temperature	40 °C (column oven) 35 °C (detector compartment)
Autosampler vial temperature	15 °C
Suppressor	NGES-A, 4 mm in Recycle mode
Suppressor current	124 mA
Detection	Conductivity
System backpressure	~3000 psi
Background conductance	<2.0 µS
Typical noise	<3 nS
Run time	55 min

Eluent gradient program: Potassium hydroxide concentration gradient

Time (min)	KOH (mM)
0.00	5.0
0.10	5.0
30.00	50.0
34.00	50.0
34.10	5.0
55.00	5.0



No	Time	Concentration [mM]	Curve
1	0.000	Run	
2	0.000	5.00	
3	0.100	5.00	
4	30.000	50.00	
5	34.000	50.00	
6	34.100	5.00	
7	55.000	5.00	
8	*New Row*		
9	55.000	Stop Run	

KOH gradient conditions

Preparation of solutions and reagents

Diluent: 5 mM KOH in DI water

Standard Stock Solution-A (100 µg/mL of nitrite and 200 µg/mL of nitrate): Transfer 1 mL of nitrite standard (1000 mg/L) and 2 mL of nitrate standard (1000 mg/L) into a 10 mL volumetric flask. Dilute to 10 mL final volume with diluent.

Standard Stock Solution-B (1000 µg/mL of nitrite and 2000 µg/mL of nitrate): A series of calibration standard solutions was prepared by diluting the Standard Stock Solution-B (1000 µg/L of nitrite and 2000 µg/L of nitrate) with diluent as outlined in Table 1.

LOQ Solution (2.5 µg/L for nitrite and 5.0 µg/L for nitrate): Transfer 0.125 mL of Standard Stock Solution-B (1000 µg/L of nitrite and 2000 µg/L of nitrate) into a 50 mL volumetric flask and dilute to a final volume of 50 mL with diluent.

Table 1: Standard calibration preparation.

Name	Volume of Standard Stock Solution-B (mL)	Diluted to volume (mL)	Concentration of nitrite after dilution (µg/L)	Concentration of nitrate after dilution (µg/L)	Concentration of nitrite with respect to sample (µg/g)*	Concentration of nitrate with respect to sample (µg/g)*
Diluent	0.0	50	0.0	0.0	0.0	0.0
Linearity Level 1 (LOQ)	0.125	50	2.5	5	0.1	0.2
Linearity Level 2	0.25	50	5	10	0.2	0.4
Linearity Level 3	0.5	50	10	20	0.4	0.8
Linearity Level 4	2.0	50	40	80	1.6	3.2
Linearity Level 5	5.0	50	100	200	4.0	8.0
Linearity Level 6	5.0	25	200	400	8.0	16.0
Linearity Level 7	10.0	20	500	1000	20.0	40.0
Linearity Level 8	20.0	20	1000	2000	40.0	80.0

*Concentration of nitrite and nitrate with respect to 25 mg/mL of sample

Sample Solution (25 mg/mL of lactose/colloidal silicon dioxide): Accurately weigh and transfer 250 mg of sample into a 10 mL volumetric flask, add about 8 mL of diluent, sonicate to dissolve, and dilute to a final volume of 10 mL with diluent. Filter using a 0.2 µm nylon membrane syringe filter.

System conditions

The IC system was configured as shown in Figure 1. To start conditioning the system, the pump was primed with eluent and system flow was set to 1 mL/min through the tubing leading to the column. Condition the guard column by flushing for at least 15 min with 50 mM KOH at 0.5 mL/min before connecting it to the Dionex IonPac AS19-4µm analytical column. Flush the guard and analytical columns together for at least 60 min with 50 mM KOH at 0.5 mL/min before connecting them to the suppressor.

Before installing the Dionex NGES-A suppressor, follow the NGES installation guide instructions to check the system, suppressor, and CD pressures and to hydrate the membranes properly.

Connect the suppressor in Recycle mode and equilibrate the system using the initial gradient conditions.

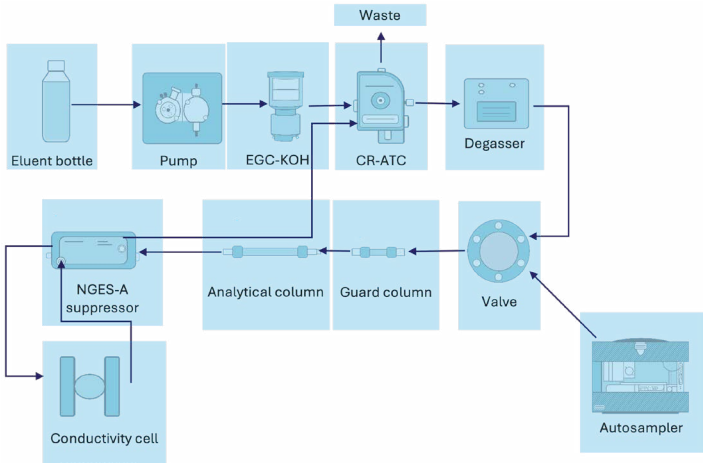


Figure 1. System configuration and flow diagram.

Analysis

Examine diluent (blank), standard solution (6 replicates), and sample solutions. Calculate the concentration, in µg/g, of nitrite and nitrate in the sample solution using the following equation:

Content of nitrite/nitrate (µg/g) = ((Ru-Rb)/Rs) x (Cs/Cu)

where:

Ru = Peak response of nitrite/nitrate from the *Sample solution*

Rb = Peak response of nitrite/nitrate from the *Sample blank solution*

Rs = Peak response of nitrite/nitrate from the *Standard solution*

Cs = Concentration of nitrite/nitrate in the *Standard solution* (µg/L)

Cu = Concentration of the *Sample solution* (mg/mL)

Results and discussion

Separation and detection

Separation of nitrite and nitrate was achieved using a Dionex IonPac AS19-4µm analytical column with a gradient of potassium hydroxide as the eluent. The Dionex IonPac AS19-4µm analytical column, with highly cross-linked (55%) particles and a small particle size of the reactive surface, allows excellent mass transfer and thus high efficiency. This is critical in this application, as the high capacity of the column is needed to effectively separate nitrite from samples with high concentrations of other anions. For example, chloride can coelute with nitrite if the analytical column efficiency is not high enough, causing problems when trying to quantitate nitrite in more complex samples. Notably, this column is designated as USP L103 and was specifically chosen for the USP ANs.[6–8]

The Dionex NGES-A suppressor maintains peak efficiency and its performance, helping to sustain accurate results with exceptional peak resolution. Faster baseline equilibration and lower noise were observed when compared to ASRS/ADRS suppressors. For example, we achieved Linearity Level 1 (LOQs) that were half the value of those reported in the USP ANs, indicating lower noise levels.

Figure 2 illustrates the chromatogram obtained from an anion mixed standard solution. Nitrite and nitrate peaks were well separated from other anions without any coelution of peaks.

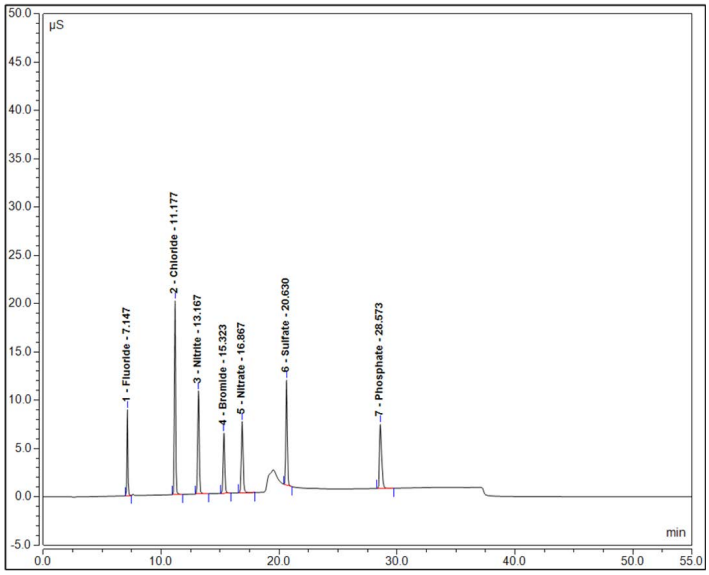


Figure 2. Chromatogram of a standard mix of anions detected using suppressed conductivity detection.

To ensure we were meeting performance criteria defined by the USP, we evaluated our system performance using the system suitability requirements outlined in the USP ANs below:

(1) Correlation coefficient (r) of nitrite and nitrate from the calibration curve generated from calibration standards should be >0.99.

(2) Signal-to-noise ratio (S/N) for nitrite and nitrate peaks in the Linearity Level 1 (LOQ) should be no less than 20.

(3) USP tailing of nitrite and nitrate peaks from the calibration standard should be no less than 0.8 and no more than 1.8.

(4) System precision %RSD of 6 replicate injections of Calibration Standard 1 (LOQ) should be no more than 20%.

An S/N of 20 was used as a basis for LOQ determination (0.1 µg/g of nitrite and 0.2 µg/g of nitrate), and S/N values for samples spiked at LOQ were greater than 20, indicating acceptable sensitivity for trace-level analysis of nitrite and nitrate in lactose monohydrate.

Linearity was evaluated within the concentration range of 0.1 µg/g to 40 µg/g for nitrite using conductivity detection with a correlation coefficient >0.99. Linearity was evaluated within the concentration range of 0.2 µg/g to 80 µg/g for nitrate using conductivity with a correlation coefficient >0.99.

Procedure accuracy was evaluated by the analysis of spiked solutions at 3 concentrations within the range (nitrite: 0.1, 4.0, and 40.0 µg/g; nitrate: 0.2, 8.0, and 80.0 µg/g). The average recovery for the nitrite and nitrate in the spiked recovery samples was within 100 ± 30.0% at all levels studied.

This study was conducted using ICH guidelines and the system suitability parameters mentioned above.

Table 2: System suitability study criteria and results.

Specificity

- Diluent, sensitivity solution, individual standard solution, sample solution, and spiked sample solution were prepared and injected
- No interference was observed for nitrite and nitrate peaks from diluent injections
- Nitrite and nitrate peaks were separated from the impurity peaks in the spiked sample solution by a resolution of >1.5

Linearity

- Calibration standard solutions from 2.5 µg/L to 1000 µg/L for nitrite and from 5.0 µg/L to 2000 µg/L for nitrate[a] were prepared and injected
- Correlation coefficients (r) for nitrite and nitrate were >0.9995

LOQ precision

- 3 replicate injections of Linearity Level 1 (LOQ) solution were done
- %RSD of the 3 replicate injections results for nitrite and nitrate were 1.71 and 0.96, respectively
- The S/N values for nitrite and nitrate were 57 and 77, respectively

Accuracy and reproducibility

- 3 spiked sample solutions for each recovery level were injected, including a recovery lower level,[b] a recovery middle level,[c] and a recovery upper level[d]
- %RSD observed for each level are tabulated below

Name	Nitrite (%RSD)	Nitrate (%RSD)
Sample solution (n=3)	1.52	0.66
Recovery at lower level (n=3)	0.04	0.04
Recovery at middle level (n=3)	0.87	0.05
Recovery at upper level (n=3)	0.11	0.11

- Average recovery observed for each level is tabulated below

Recovery	Nitrite (%)	Nitrate (%)
At lower level (n=3)	79.22	118.15
At middle level (n=3)	98.44	97.90
At upper level (n=3)	109.08	114.95

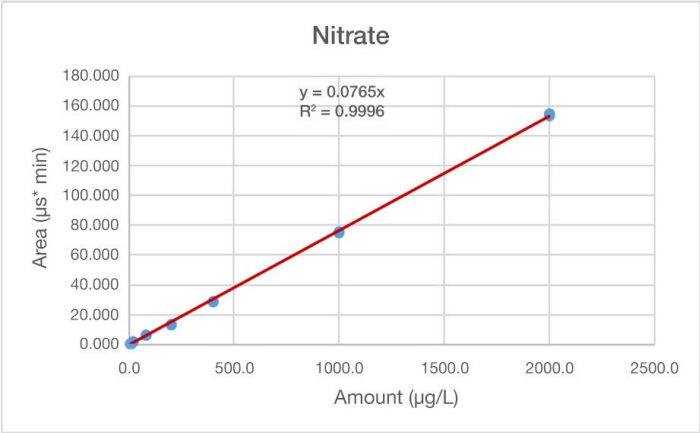
[a]0.1 µg/g–40 µg/g for nitrite and 0.2 µg/g–80 µg/g for nitrate with respect to 25 mg/mL sample concentration.

[b]Recovery lower level—0.1 µg/g of nitrite and 0.2 µg/g of nitrate.

[c]Recovery middle level—4 µg/g of nitrite and 8 µg/g of nitrate.

[d]Recovery upper level—40 µg/g of nitrite and 80 µg/g of nitrate.

Linearity studies for nitrite and nitrate were conducted from 2.5 µg/L to 1000 µg/L and from 5.0 µg/L to 2000 µg/L, respectively (Figure 3). The correlation coefficient for nitrate was 0.9996 with a slope of 0.0765 and 0 intercept. The correlation coefficient for nitrite was 0.9999 with a slope of 0.0974 and 0 intercept.



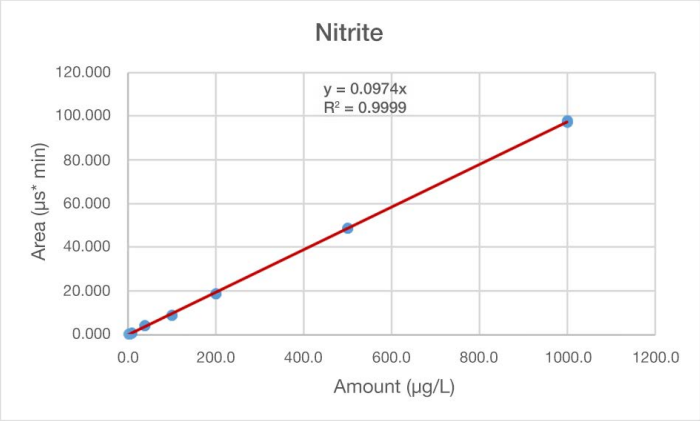


Figure 3. Calibration plots for nitrite and nitrate using suppressed conductivity detection.

Results observed in Table 2 show that the method is specific, linear in the given range, precise, accurate, and repeatable. Therefore, this method can be useful to quantitate nitrite and nitrate from lactose monohydrate samples.

Sample results

Samples of lactose monohydrate and colloidal silicon dioxide were analyzed for nitrite and nitrate content. Results are tabulated in Table 3.

Table 3. Nitrite and nitrate concentrations in lactose and colloidal silicon dioxide samples.

Sample	Batch No./ Lot No.	Nitrite concentration (µg/g)	Nitrate concentration (µg/g)
Lactose Monohydrate_ Preparation1	SG37271202	0.187	0.346
Lactose Monohydrate_ Preparation2	SG37271202	0.181	0.344
Lactose Monohydrate_ Preparation3	SG37271202	0.185	0.341
Colloidal silicon dioxide	ACC/C50/25/099	0.187	0.391

Figure 4 shows the chromatogram of a diluent injection. Notably, both nitrate and nitrite peaks are absent.

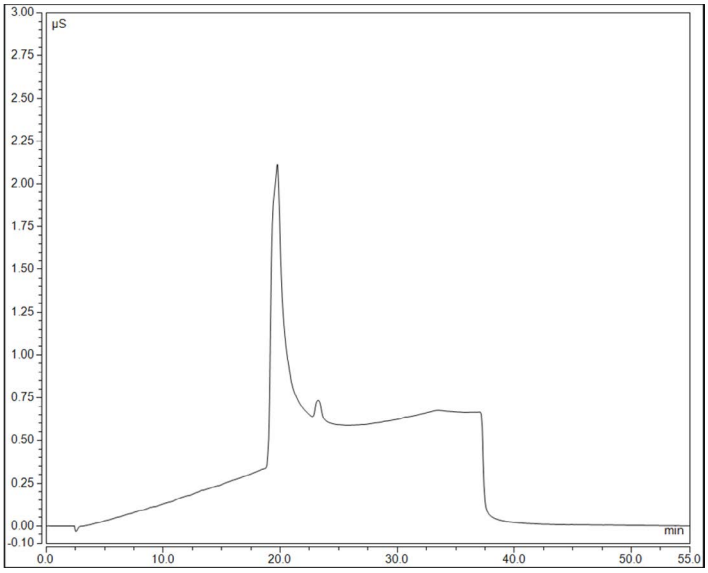


Figure 4. Chromatogram of diluent (blank) using suppressed conductivity detection.

Figure 5 shows the chromatogram of a standard injection containing a mixture of 10 µg/L nitrite and 20 µg/L nitrate. Both peaks were well separated without any notable interference from other anions.

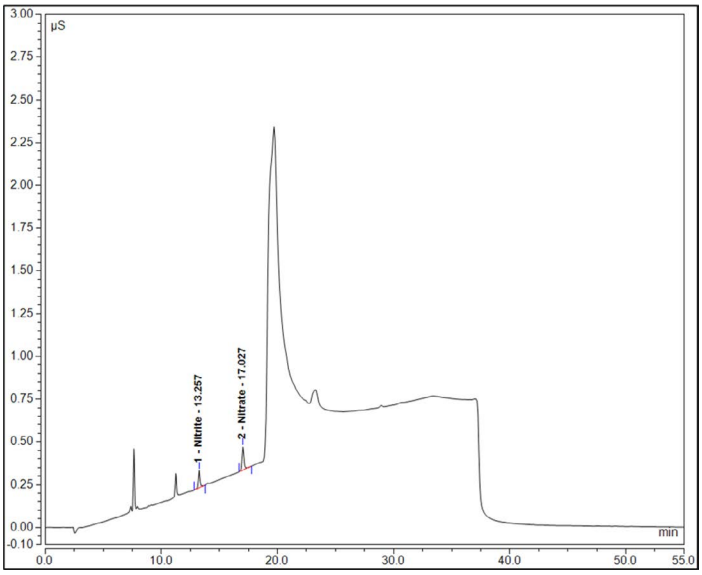


Figure 5. Chromatogram of a standard containing both nitrite and nitrate at 10 µg/L and 20 µg/L concentrations, respectively.

Figure 6 shows the chromatogram of a 25 mg/mL lactose monohydrate sample. Once again, both nitrite and nitrate peaks were very well separated from one another as well as from other anions in the sample.

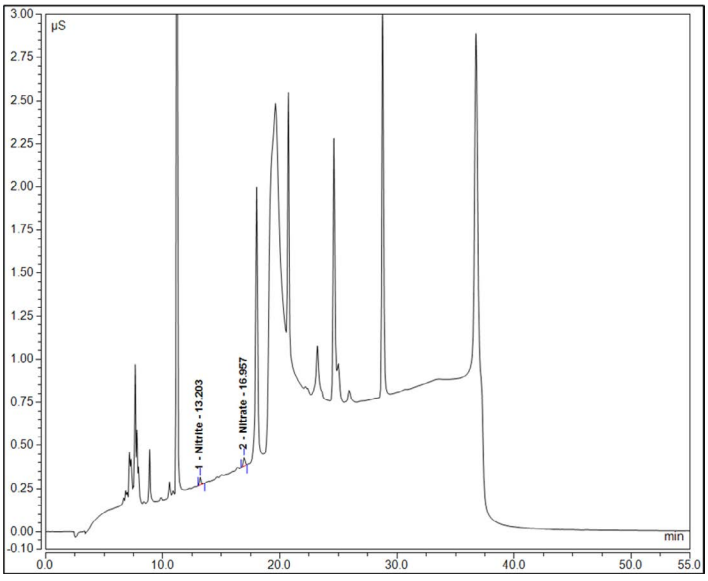


Figure 6. Chromatogram of a sample of lactose monohydrate prepared at a concentration of 25 mg/mL.

Figure 7 shows the chromatogram of a 25 mg/mL silicon dioxide sample. Nitrite and nitrate are separated effectively from other anions of the sample matrix here as well.

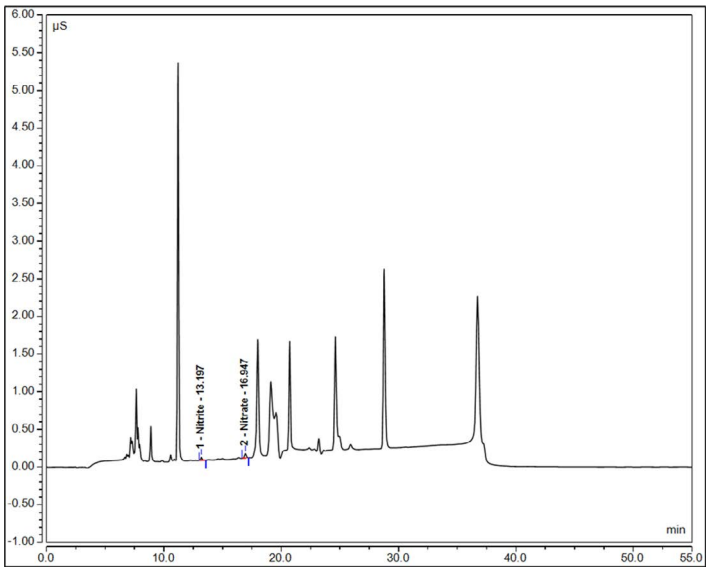


Figure 7. Chromatogram of a sample of colloidal silicon dioxide prepared at a concentration of 25 mg/mL.

Conclusion

This application note presents an IC method that enables precise and selective quantification of nitrite and nitrate in lactose monohydrate and colloidal silicon dioxide using a Dionex NGES-A suppressor. The technique demonstrates reliable detection at trace levels—specifically 0.1 µg/g nitrite and 0.2 µg/g nitrate—based on a 25 mg/mL sample concentration. Its sensitivity makes it well-suited for low-level nitrite and nitrate impurity analysis in lactose and colloidal silicon dioxide. Use of the Dionex NGES-A suppressor for this application afforded decreased noise, which improved detection limits of nitrite and nitrate and enabled faster equilibration.

This analytical approach can assist pharmaceutical manufacturers in conducting comprehensive risk assessments and implementing control measures. These may include choosing suitable excipients, verifying supplier quality, determining optimal excipient levels, and refining the production process.

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