

# Determination of nitrite impurity in dalteparin sodium using ion chromatography

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## Goal

To demonstrate that the USP Dalteparin Sodium monograph for nitrite impurity method can be successfully executed with a Thermo Scientific™ Dionex™ IonPac™ AS15 column using an ion chromatography system

## Introduction

Dalteparin sodium is the sodium salt of a low molecular weight heparin obtained by nitrous acid depolymerization of heparin from porcine intestine or intestinal mucosa (Figure 1). Dalteparin sodium is used for prevention or treatment of deep vein thrombosis and pulmonary embolism. This drug substance is produced by a validated manufacturing and purification procedure under conditions shown to minimize the presence of species containing the N-NO group. It is important to characterize and quantify the active pharmaceutical ingredient (API) and impurities in the drug substance to ensure its quality and safety.



Ion chromatography (IC) is widely used to separate nitrite. Typically, the detection is carried out by suppressed conductivity or UV-absorbance at 215 nm. Here the anion-exchange separation is paired with electrochemical detection (ED). ED, a detection technique with a broad linear range and low detection limits, is ideally suited for detecting this impurity. One advantage is an oxidation potential can be selected for specific types of analytes while other compounds are undetected, making ED more selective than bulk property detectors (e.g., conductivity). Derivatization is not required, which simplifies the analysis. The determination of nitrite in dalteparin sodium is described in the United States and European Pharmacopoeias (USP and EP).<sup>1,2</sup> This determination uses an IC-ED method. The amperometric detection of nitrite is achieved using a glassy carbon working electrode.

In this application note, the nitrite determination method described in the USP monograph was evaluated with a Dionex IonPac AS15 column using an IC system. The method and conditions were as described in the USP Dalteparin Sodium monograph. Key performance parameters were evaluated for separation, linearity, limit of detection, accuracy, and robustness. One dalteparin sodium sample was analyzed. The nitrite result was compared with the USP acceptance criterium.

## Experimental

### Equipment

- Thermo Scientific™ Dionex™ ICS-5000+ HPIC system including\*:
  - Dionex ICS-5000+ DP Pump module
  - Dionex ICS-5000+ DC Detector/Chromatography module with ED Electrochemical Detector
  - Dionex AS-AP Autosampler with sample tray cooling, 250 µL sample syringe (P/N 074306), 1200 µL buffer line (P/N 074989), and 1.5 mL vial trays (P/N 074936)
- Dionex ICS-5000+ ED Electrochemical Detector Cell (P/N 072044)
- ED glassy carbon electrode with gasket and polishing kit (P/N 079854)
- Reference electrode Ag/AgCl (P/N 061879)
- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software, version 7.2.9

\*This method can be run on a Thermo Scientific™ Dionex™ ICS-6000 or Integrion system.

The procedure for system preparation and setup can be found in specific product manuals.<sup>3,4</sup>

### Consumables

- Thermo Scientific™ Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units with Nylon Membrane, 1000 mL, 0.2 µm pore size (Fisher Scientific P/N 09-740-46)
- Dionex AS-AP Autosampler vials 10 mL (P/N 074228)

### Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistivity or better
- Sodium acetate salt, electrochemical grade (Dionex P/N 059326)
- Orthophosphoric acid 85% (HPLC) (Fisher Scientific P/N A260-500)
- Sodium nitrite, extra pure (Sigma-Aldrich P/N 13447-1KG-R)

### Sample

A dalteparin sodium sample was purchased from Sigma-Aldrich (P/N 1162487-300MG).

### Chromatographic conditions

Column	Dionex IonPac AS15 Analytical column, 3 x 150 mm (P/N 057594)
Eluent	Sodium acetate (8.2 g/L), adjust to pH 4.3 with orthophosphoric acid
Flow rate	0.5 mL/min
Column temperature	30 °C
Injection volume	25 µL (Full loop)
Autosampler temperature	5 °C
Reference electrode	Ag/AgCl
Working electrode	Glassy carbon with a 1 mil gasket (1 mil = 25.4 µm)
DC amperometry detection potential	1 V
Detection compartment temperature	30 °C
Run time	10 min
Background	~9.5 nA
Backpressure	1850 psi

### Preparation of solutions and reagents

#### Eluent

Dissolve 8.2 g of sodium acetate in 900 mL of DI water in a 1 L volumetric flask. Adjust to pH 4.3 with orthophosphoric acid and bring to volume with DI water. Vacuum filter this solution through a 0.2 µm Nalgene, 1 L nylon filter to remove particles from the sodium acetate that can damage parts of the pump.

## Standard solutions

### Nitrite stock standard solution (50 mg/L)

Vacuum degas 2 L of DI water for 10 min. Dissolve 75 mg of sodium nitrite in a 1 L volumetric flask with degassed DI water.

### Nitrite standard solution (500 µg/L)

Dilute 1 mL of nitrite stock standard solution (50 mg/L) in a 100 mL volumetric flask with degassed DI water.

### Nitrite calibration standard solution (2.5, 5, 15, 25 µg/L)

Prepare 100 mL of calibration standard for each concentration in the calibration standard series by diluting the nitrite standard solution (500 µg/L) with degassed DI water (Table 1). For example, prepare 25 µg/L by adding 5 mL of nitrite standard to a 100 mL volumetric flask then dilute to volume with degassed DI water.

Table 1. Calibration standard preparation (100 mL)

Calibration level	Nitrite conc. (µg/L)	Volume (mL) of nitrite standard solution (500 µg/L)
1	2.5	0.5
2	5	1
3	15	3
4	25	5

Table 2. System suitability

Requirement	Description	USP criteria	Measured
Column efficiency	Theoretical plates for the nitrite peak for all calibration standard and sample solutions	≥4000	≥5278
Tailing factor	All calibration and sample solutions injections	0.8–1.2	0.8–0.95
Relative standard deviation	Peak area of six injections of calibration standard 25 ng/mL	<2%	1.7%

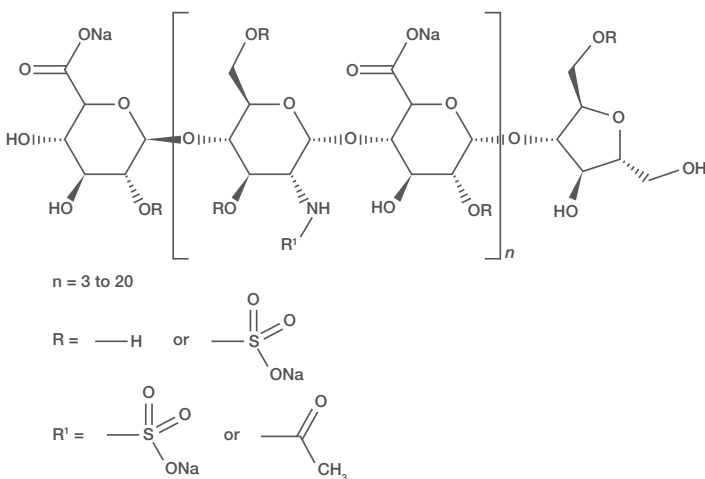


Figure 1. Structure of dalteparin sodium

## Sample preparation

### Sample solution (4 mg/mL)

Weigh 80.0 mg of dalteparin sodium into a 20 mL volumetric flask, then dilute to volume with degassed DI water.

## Results and discussion

### System suitability

In the USP's Dalteparin Sodium monograph, three system suitability requirements are specified. These requirements are that the number of theoretical plates for the nitrite peak for all calibration and sample solutions is not less than (NLT) 4000, that the nitrite peak tailing factor for all calibration and sample solutions is between 0.8 and 1.2, and that the maximum relative standard deviation (RSD) is 2% for the nitrite peak area for six injections of the calibration standard solution (25 µg/L).

Table 2 shows that the USP requirements for column efficiency, tailing factor, and repeatability are met. Figure 2 shows a separation of the system suitability solution (25 µg/L) using a Dionex IonPac AS15 column. This column is designed for high-efficiency fast analysis of inorganic anions and monovalent organic acid anions.<sup>5</sup>

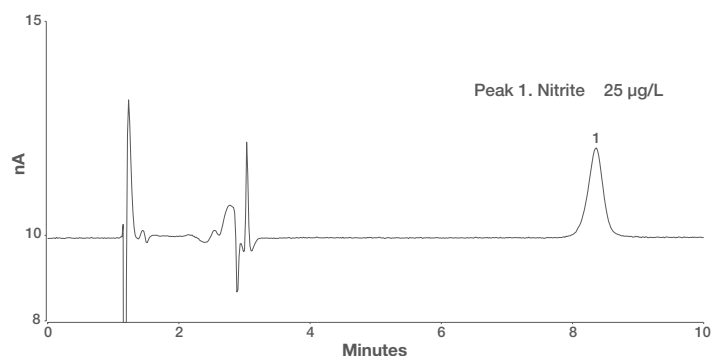


Figure 2. Separation of a system suitability standard (nitrite 25 µg/L) using a Dionex IonPac AS15 column

## Linearity

The linearity of nitrite electrochemical response to concentration was investigated in the concentration range of 2.5 to 25 µg/L (Table 1). The calibration standards were prepared by diluting the 500 µg/L standard solution with degassed DI water. Figure 3 shows the calibration curve; the coefficient of determination ( $r^2$ ) is 0.9998 using linear fitting, which passes the monograph requirement of NLT 0.995.

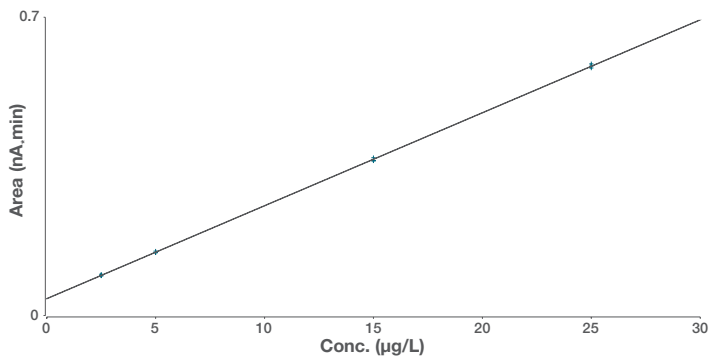


Figure 3. Calibration of nitrite 2.5–25 µg/mL (linear fitting)

## Limits of detection and quantification

The United States Pharmacopeia General Chapter <1225> on method validation specifies a signal-to-noise ratio (S/N) of 3 for the determination of the limit of detection (LOD) and a S/N of 10 for the determination of the limit of quantitation (LOQ).<sup>6</sup>

To determine the LOD and LOQ, 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 nitrite peak. The LOD and LOQ were calculated from the average peak height of three injections of nitrite (1 µg/L). Table 3 summarizes the LOD and LOQ of nitrite in the sample solution and in dalteparin sodium powder. Figure 4 shows the chromatogram of 1 µg/L nitrite.

Table 3. LOD and LOQ

Analyte	LOD (µg/L) in sample solution	LOQ (µg/L) in sample solution	LOD in dalteparin sodium powder (ng/g)	LOQ in dalteparin sodium powder (ng/g)
Nitrite	0.292	0.972	72.9	243

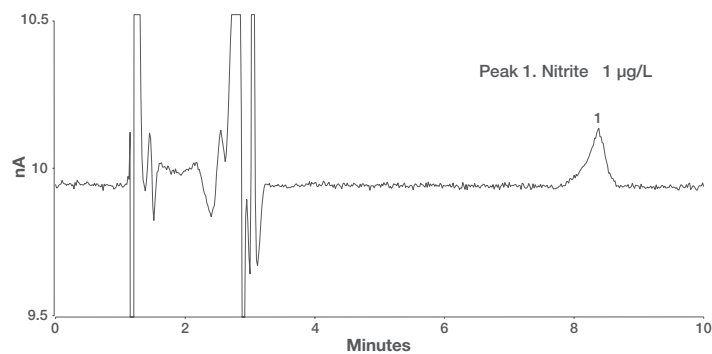


Figure 4. Nitrite standard (1 µg/L)

## Sample analysis

The dalteparin sodium sample solution (4 mg/mL) was used for nitrite impurity analysis. Figure 5 shows a chromatogram of the sample solution.

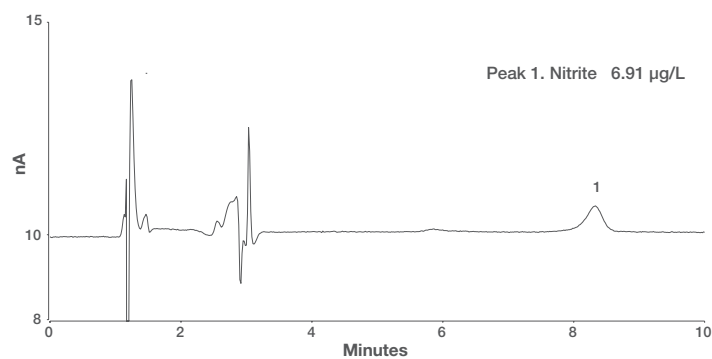


Figure 5. Dalteparin sodium sample (4 mg/mL)

The USP monograph lists acceptance criterion for nitrite impurity levels in commercial samples. For that purpose, the nitrite impurity was calculated. The sample contains 1.76 ppm (µg/g) of nitrite impurity, and it passes the USP acceptance criterion of 5 ppm.

## Method accuracy

Method accuracy was validated by standard addition experiments of nitrite in dalteparin sodium sample over four concentration levels, with three replicates of each concentration. Table 4 summarizes recovery results for nitrite. Nitrite recovery ranges from 96.2% to 105%.

Table 4. Spike recovery

Nitrite added ( $\mu\text{g/L}$ )	Sample ( $\mu\text{g/L}$ )	Spiked sample ( $\mu\text{g/L}$ )	Recovery (%)
2.5	7.04	9.61	103
5	7.04	12.3	105
10	7.04	16.7	96.2
20	7.04	27.0	100

## Robustness

Method robustness was evaluated by measuring the influence of small variations ( $\pm 10\%$ ) in procedural parameters (flow rate, eluent concentration, column temperature on the RT, peak asymmetry, column from a different lot). The peak asymmetry was calculated using the USP formula. Nitrite (20  $\mu\text{g/L}$ ) was injected three times at each chromatographic condition. Table 5 summarizes the results. These results indicate the method is robust with respect to peak asymmetry for all conditions tested.

Table 5. Robustness

Parameter		Nitrite RT (min)	Diff (%)	Asym.	Diff (%)
Eluent conc. (Sodium acetate g/L)	8.2	8.23	0	0.89	0
	7.38	8.93	8.50	0.87	-2.25
	9.02	7.57	-8.09	0.92	3.37
Flow rate (mL/min)	0.5	8.23	0	0.89	0
	0.45	9.18	11.5	0.88	-1.12
	0.55	7.45	-9.51	0.94	5.62
Column temp ( $^{\circ}\text{C}$ )	30	8.23	0	0.89	0
	27	8.47	2.84	0.89	0
	33	7.98	-3.04	0.9	1.12
Column	#1	8.23	0	0.89	0
	#2	8.30	0.81	0.91	2.25

## Precautions

It is essential to prepare sodium acetate eluents using sodium acetate that is free of impurities, like the electrochemical grade sodium acetate listed in this application note. Appropriate column performance may not be achieved when an alternate supplier of sodium acetate or lower purity water is used.

Rinse the cell body, working electrode, and gasket thoroughly with DI water and dry with a lab wipe. Do not touch the working electrode surface with any paper products as this can contaminate the working electrode. A layer of contamination may occasionally build up on the working electrode of the amperometry cell. When this occurs, the electrode must be cleaned by removing the built up layer through polishing to restore performance. The peak area may require 1 day to stabilize after electrode polishing. Indications that the working electrode needs to be polished are visible electrode discoloration or a decrease in peak area response of a known standard. The procedure for polishing the working electrode can be found in the IC system operators manual.<sup>8</sup> For best results, replace the reference electrode after six months of use. When installing a new reference electrode, calibrate it with pH 4 and 7 solutions.

An operational vacuum degasser pump (part of the analytical pump) is important to the success of this and other electrochemical detection methods. This pump can be accidentally turned off during a system restart or instrument configuration. If the vacuum degasser pump is not running, poor baseline performance and loss of column compacity can be observed. Always ensure that the vacuum degas pump is on before running a sequence. Press F8 or click the “gear” command button on the main Chromeleon instrument panel and locate the pump and then the degasser status. Make sure the degasser is “On” and “DegasserVaccum” is “OK”. If it is “OFF” and “NOT OK”, turn on the degasser by selecting “On” from the drop-down menu and wait until “DegasserVacuum” turns to “OK”.

## Conclusion

This application note demonstrates that the USP Dalteparin Sodium monograph for the nitrite impurity determination can be successfully executed with a Dionex IonPac AS15 column using a Thermo Scientific Dionex ion chromatography system. The separation, linearity, reproducibility, and sensitivity were found to meet or exceed the current USP performance requirements. This impurity analysis for nitrite was validated to meet the analytical performance characteristics outlined in USP General Chapter <1225>. This method is reliable and can be used for the routine monitoring of nitrite in dalteparin sodium.

## References

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