APPLICATION NOTE 73987

Determination of nitrite in pharmaceuticals

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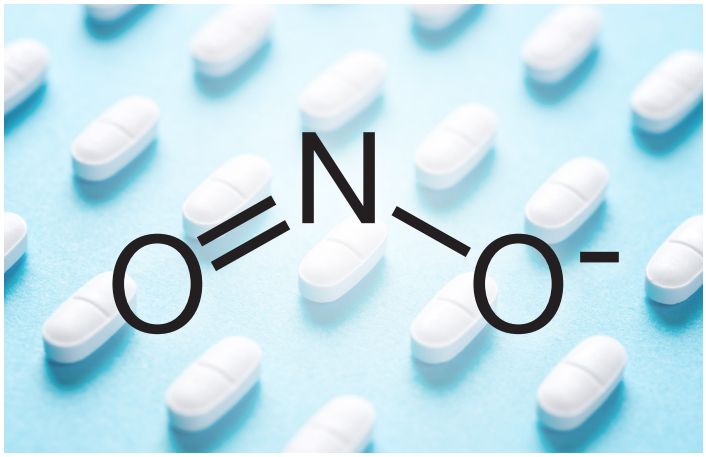
Keywords: Dionex ICS 6000 HPIC system, Dionex IonPac AS19-4µm column, losartan, metformin, ranitidine, diphenhydramine, ADRS 600 suppressor, ion chromatography, n-nitrosamine, NDMA



Goal

To develop a method to determine nitrite in pharmaceuticals to assess the likelihood of nitrosamine formation

Introduction

N-nitrosodimethylamine (NDMA) is an organic compound with the formula $(CH_3)_2NNO$. NDMA is a known carcinogen that is present in some fermented foods.[1-2] Since July 2018, a few drug products including losartan, ranitidine, and metformin have been recalled by the United States Food and Drug Administration (FDA) due to the presence of NDMA. Therefore, the FDA has provided industry guidance for controlling NDMA in human drugs.[3-8] This has increased interest in understanding the potential of NDMA formation during pharmaceutical manufacturing. The use of nitrite in a manufacturing process can represent a risk for NDMA formation if a secondary or tertiary amine is present (Figure 1).[9-10] Therefore, it is important to limit the nitrite and amine levels in drug substances and drug products.

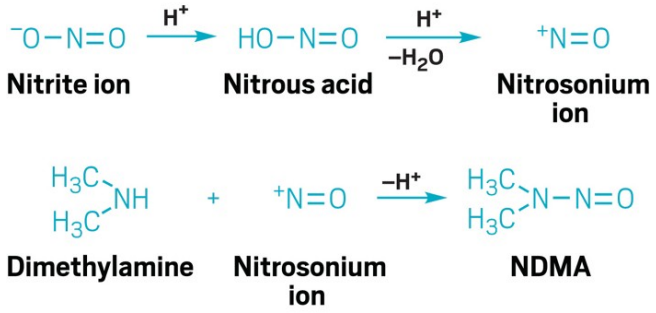


Figure 1. NDMA formation

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While there are wet chemical methods for determining nitrite, ion chromatography (IC) is the typical method. IC involves separation with an ion-exchange column and conductivity, electrochemical, UV absorption, or mass spectrometry detection. IC is especially valuable to the pharmaceutical industry for ionic analytes in products containing non-ionic components. IC-based methods are included in several USP monographs and have been applied to all aspects of pharmaceutical product manufacturing, including the determination of active ingredients, degradation products, and impurities. For most pharmaceutical samples, little or no sample preparation is required, and analyte derivatization is unnecessary. The determination of amines in pharmaceuticals by IC was discussed in TN74093.[11] For nitrite, the separation is achieved by anion-exchange chromatography and detection by UV absorbance at 210 nm. We used a high capacity Thermo Scientific™ Dionex™ IonPac™ AS19-4µm column, and due to the nature of many pharmaceutical samples, UV absorbance at 210 nm detection rather than suppressed conductivity detection. The high column capacity allows the separation of nitrite even when the sample contains high amounts of a counter ion such as chloride. The UV absorbance detection allows the sensitive and selective detection of nitrite without interference from high amounts of chloride. The method uses a Reagent-Free™ (RFIC™) ion chromatography system with electrolytically generated KOH eluent. This system requires only deionized water to produce the eluent. The method was validated and successfully applied to seven pharmaceutical samples, including metformin, losartan, ranitidine, and diphenhydramine.

Experimental

Equipment

- Thermo Scientific™ Dionex™ ICS-6000 HPIC system including:
  - Thermo Scientific™ Dionex™ ICS-6000 DP Pump module
  - Thermo Scientific™ Dionex™ ICS-6000 DC Detector/ Chromatography module with Conductivity Detector
  - Thermo Scientific™ Dionex™ AS-AP Autosampler with sample tray cooling, 250 µL sample syringe (P/N 074306), 1,200 µL buffer line (P/N 074989), and 10 mL vial trays
- Diode array detector (Thermo Scientific)
- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software, version 7.2.10

Consumables

- Thermo Scientific™ Dionex™ EGC 500 KOH Cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 600 Continuously Regenerated Anion Trap Column (P/N 088662)
- Thermo Scientific™ Dionex™ ADRS 600 Anion Dynamically Regenerated Suppressor, 2 mm (P/N 088667)
- Thermo Scientific™ Dionex™ AS-AP Autosampler Vials 10 mL (P/N 074228)
- Fisherbrand™ Narrow-Mouth field sample bottles, high density polyethylene (HDPE), 125 mL, 250 mL sizes for storage of standards and samples (Fisher Scientific P/N 02-895A, B)
- Thermo Scientific™ Nalgene™ Syringe Filter 0.2 µm PES (Fisher Scientific P/N 09-740-113)

Reagents and standards

- Deionized (DI) water, Type 1 reagent grade, 18 MΩ·cm resistivity or better
- Sodium and potassium salts, A.C.S. reagent grade or better, for preparing anions standards
- Sodium nitrite, 99% (Sigma-Aldrich P/N 13447-1KG-R)
- Losartan potassium (Sigma-Aldrich P/N PHR1602-1G)
- Metformin hydrochloride (Sigma-Aldrich P/N PHR1084-500MG)

Samples

Pharmaceutical samples used in this study are listed in Table 1.

Table 1. Pharmaceutical samples

Sample (S#)	Drug product name	API	Type	API/pill (mg)	Source	Indication
1	NA	Losartan potassium	Substance	NA	Sigma	NA
2	NA	Metformin hydrochloride	Substance	NA	Sigma	NA
3	Nytol™ QuickCaps™	Diphenhydramine HCl	Product	25	OTC	Sleep aid
4	Benadryl™	Diphenhydramine HCl	Product	25	OTC	Allergy
5	Metformin	Metformin hydrochloride	Product	500	Rx	Diabetes
6	Losartan	Losartan potassium	Product	50	Rx	High blood pressure
7	Rantidine	Ranitidine hydrochloride	Product	300	Rx	Diabetes

OTC = Over-the-counter
Rx = Prescription

Chromatographic conditions

Columns	Dionex IonPac AS19-4µm Guard Column, 2 × 50 mm (P/N 083225) Dionex IonPac AS19-4µm Analytical Column, 2 × 250 mm (P/N 083223)
Eluent	20 mM KOH from 0 to 8 min, 20–60 mM KOH from 8 to 10 min, 60 mM from 10 to 15 min, 20 mM from 15.1 to 30 min
Eluent source	Dionex EGC 500 KOH cartridge with Dionex CR-ATC 600
Flow rate	0.25 mL/min
Injection volume	25 µL in Push-Full mode
Column temperature	30 °C
Detection 1	Suppressed conductivity
Suppressor	Dionex ADRS 600 (2 mm) Suppressor, recycle mode, 38 mA
Detection/suppressor compartment	20 °C
Cell temperature	35 °C
Background conductance	<0.5 µS/cm
System backpressure	~3000 psi (100 psi = 689.5 kPa)
Noise	<1 nS/cm
Run time	30 min
Detection 2	UV 210 nm

System preparation and setup

Figure 2 shows the flow diagram of the IC system with sequential suppressed conductivity and UV detection. The Dionex ICS 6000 HPIC system is configured as a RFIC system using eluent generation following the Dionex ICS 6000 HPIC Installation and Operator's Manual.[12] The suppressor is installed in the recycle-mode.

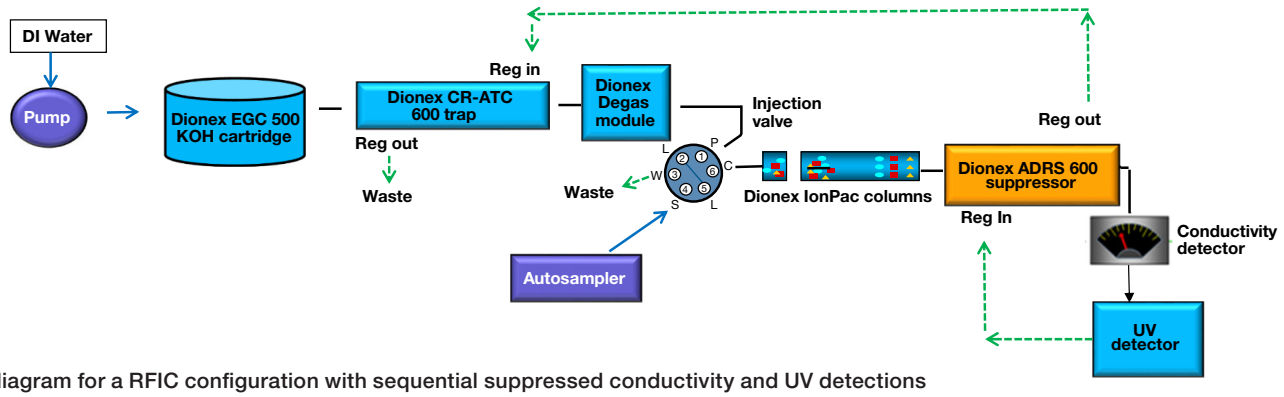

Figure 2. Flow diagram for a RFIC configuration with sequential suppressed conductivity and UV detections

Preparation of solutions and reagents

Common anions stock standard solutions

Stock standard solutions (1,000 mg/L) can be prepared by dissolving the appropriate amounts of the required analytes in 100 mL of DI water (Table 2).

Table 2. Masses of compounds used to prepare 100 mL of 1000 mg/L ion standards

Analyte	Compound	Amount (mg)
Fluoride	Sodium fluoride (NaF)	221.0
Chloride	Sodium chloride (NaCl)	164.9
Nitrite	Sodium nitrite ($NaNO_2$)	150.0
Bromide	Sodium bromide (NaBr)	128.8
Nitrate	Sodium nitrate ($NaNO_3$)	137.1
Sulfate	Sodium sulfate (Na_2SO_4)	147.9
Phosphate	Potassium phosphate, monobasic (KH_2PO_4)	143.3

Nitrite stock standard solution

Nitrite stock standard solution (1,000 mg/L) can be prepared by dissolving 150 mg of sodium nitrite in 100 mL of DI water.

Preparation of pharmaceutical samples

Two drug substances and five drug products (Table 1) were used as samples in this study to demonstrate the nitrite separation in pharmaceuticals. Prepare drug substance samples at 1 mg/mL by dissolving 50 mg of powder in 50 mL of DI water. Prepare drug products samples by dissolving the whole pill into 50 mL or 10 mL of DI water to yield a 2.5–10 mg/mL solution based on the active pharmaceutical ingredient (API) weight. Sonicate the pill with water until dissolved and centrifuge the sample extract at 8,000 × g for 15 min. Dilute the sample solution to 1 mg/mL with DI water and filter the solution with a 0.2 µm PES filter. Nitrite is fully soluble in water. The filtrate particles are likely to be insoluble matters and excipients.

Separation

The determination of common anions, including nitrite and nitrate, is usually achieved by coupling an anion-exchange separation with suppressed conductivity detection (CD). In hydrochloride drug products, chloride is the counter ion and present at a very high concentration. The integration of a small nitrite peak in the presence of a large chloride peak can be challenging when the detection is suppressed conductivity. In this study, we chose UV absorbance detection to achieve sensitive and selective detection for nitrite as chloride is not detected. We chose the Dionex IonPac AS19-4µm column for this analysis as it is a high capacity and high-resolution column, which are critical factors for determining low µg/L concentrations of nitrite in samples containing high concentrations of other anions. Figure 3 shows that nitrite was resolved from six other common anions within 30 min using this column. The top chromatogram displays the separation of the seven anions with suppressed conductivity detection. The bottom chromatogram shows that only the three analytes that have UV absorbance at 210 nm are detected. A delay time of 0.25 min was applied to the two chromatograms to match the detection time because the conductivity and absorbance detectors are connected in series.

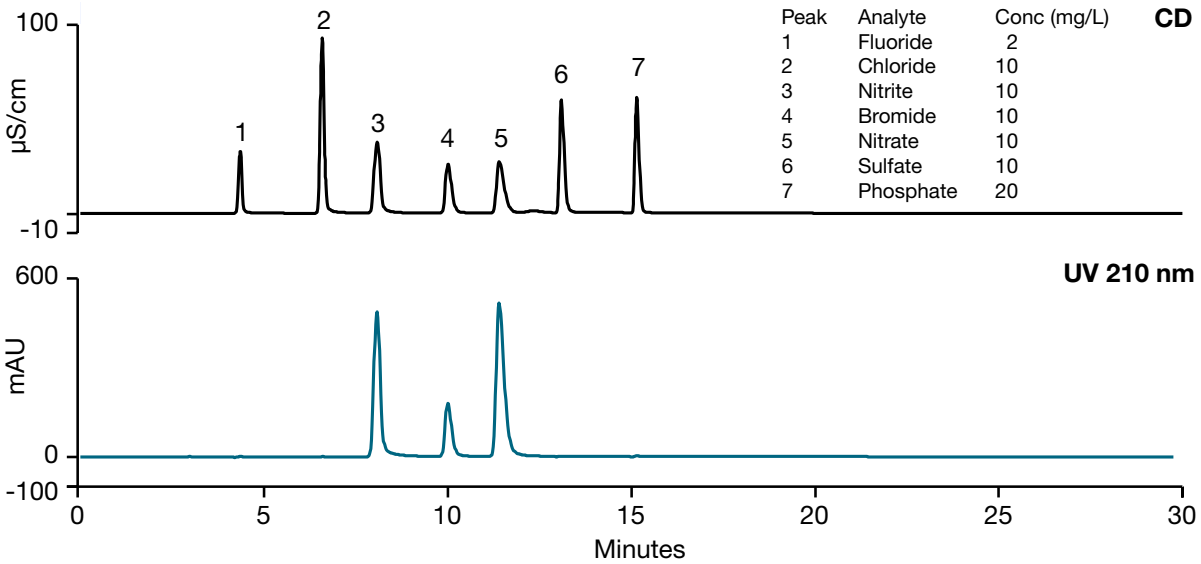

Figure 3. Separation of seven common anions using a Dionex IonPac AS19-4µm column

Limit of detection (LOD)

The determination of LOD was based on the signal-to-noise (S/N) ratio. Determination of the S/N ratio is performed by comparing measured signal from a standard with a low concentration of analyte with those of blank and establishing the minimum concentration at which the analyte can be reliably detected. A S/N = 3 is used for estimating LOD and a S/N = 10 is used for estimating the quantification limit (LOQ).[13] In this study, 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 close to the peak of interest. The signal was determined from the average height of three injections of nitrite standard (1 µg/L). The calculated LOD of nitrite in sample solution was 0.918 µg/L, which corresponds to 0.918 µg/g (ppm) of the API as the pharmaceutical samples were prepared at 1 mg/mL based on the active pharmaceutical ingredient (API) weight.

Calibration

The linearity of nitrite UV response to concentration was investigated in the concentration range of 5 to 500 µg/L. Figure 4 shows the calibration curve; the coefficient of determination (r^2) is 0.9999 using linear fitting.

Sample analysis

The amount of nitrite in the three recalled prescription drug products (losartan, metformin, and ranitidine) can provide some information for scientists investigating the formation of nitrosamines. Therefore, the three drug products and two of the corresponding drug substances (losartan potassium, metformin hydrochloride) were included as samples in this study. Two common over-the-counter (OTC) drug products (Benadryl and Nytol) were also included as samples to evaluate if this method can be applied to other amine drugs. These seven samples were previously evaluated for their dimethylamine content.[11]

The amounts of nitrite in the seven pharmaceutical samples are summarized in Table 3. Nitrite was detected in all samples except sample 1 (losartan potassium drug substance). The highest nitrite amount, 95.8 ppm (µg/g), was detected in sample 7 (ranitidine). Nitrite is detected in samples 2–6 in a range of 4.47 to 27.4 ppm. Figure 5 shows the determination of nitrite in metformin drug product (S5). The top chromatogram displays the CD detection, and the bottom chromatogram displays UV detection. The high chloride concentration in the hydrochloride drug product strongly affects nitrite detection by suppressed conductivity. However, with the UV detection, chloride does not interfere with nitrite quantification. We found this method was applicable to the other six pharmaceutical samples (Figures 6–11) and believe it should be applicable to additional pharmaceutical samples. This analysis, together with an analysis for dimethylamine or other amines, allows the analyst to determine the possibility of nitrosamine formation in the drug substance or drug product. This nitrite determination method could also be used for other components of a pharmaceutical formulation (e.g., an excipient).

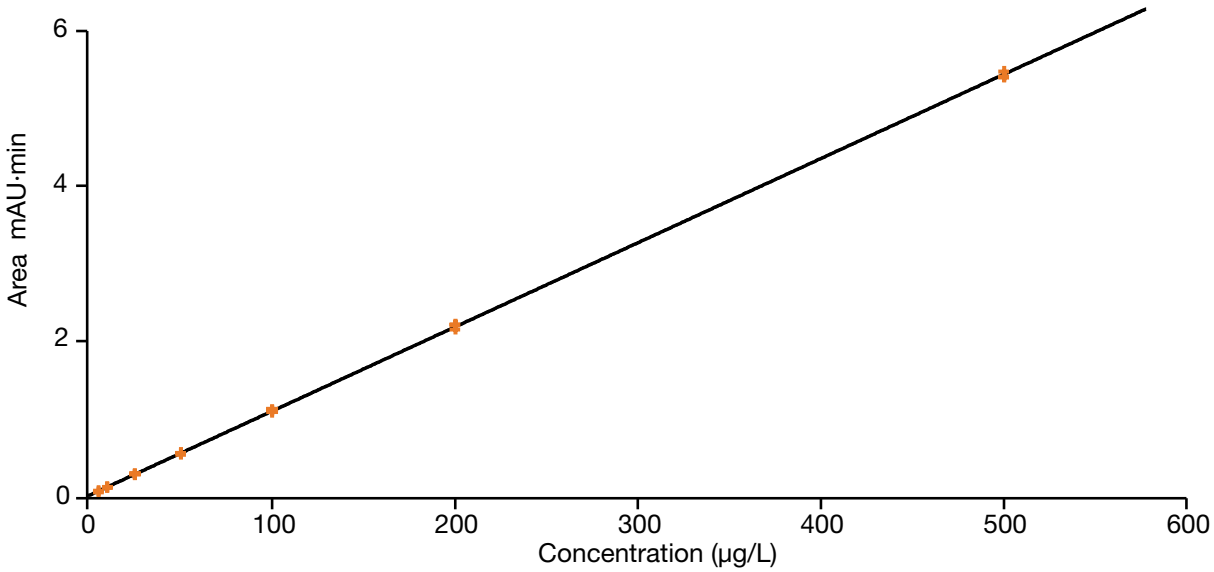

Figure 4. Nitrite calibration

Table 3. Amount of nitrite in pharmaceutical samples, ppm (µg/g API)

Sample (S#)	Nitrite	RSD (n=6)
1	<LOD	NA
2	27.0	2.3
3	17.6	2.0
4	16.2	2.3
5	6.86	2.8
6	4.45	2.9
7	95.5	1.4

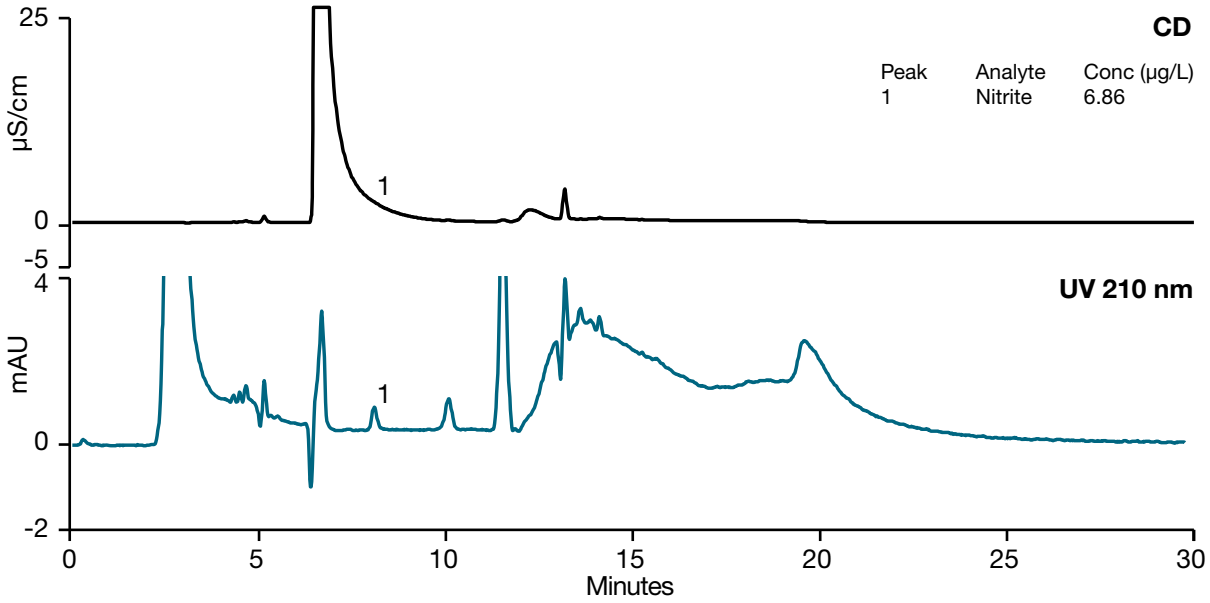

Figure 5. Nitrite in metformin drug product (S5) using a Dionex IonPac AS19-4µm column

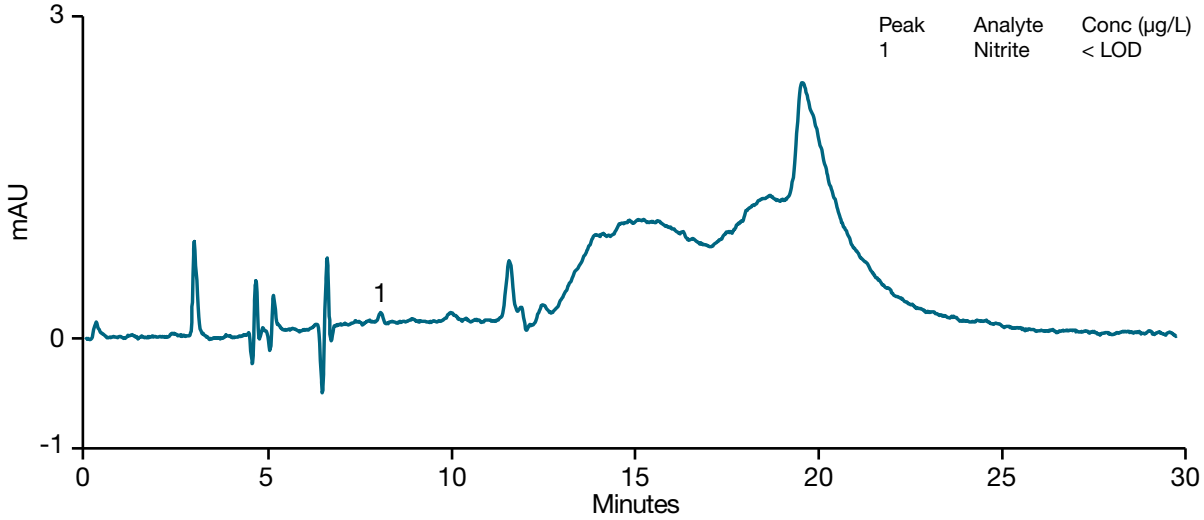

Figure 6. Nitrite in losartan potassium (S1) using a Dionex IonPac AS19-4µm column

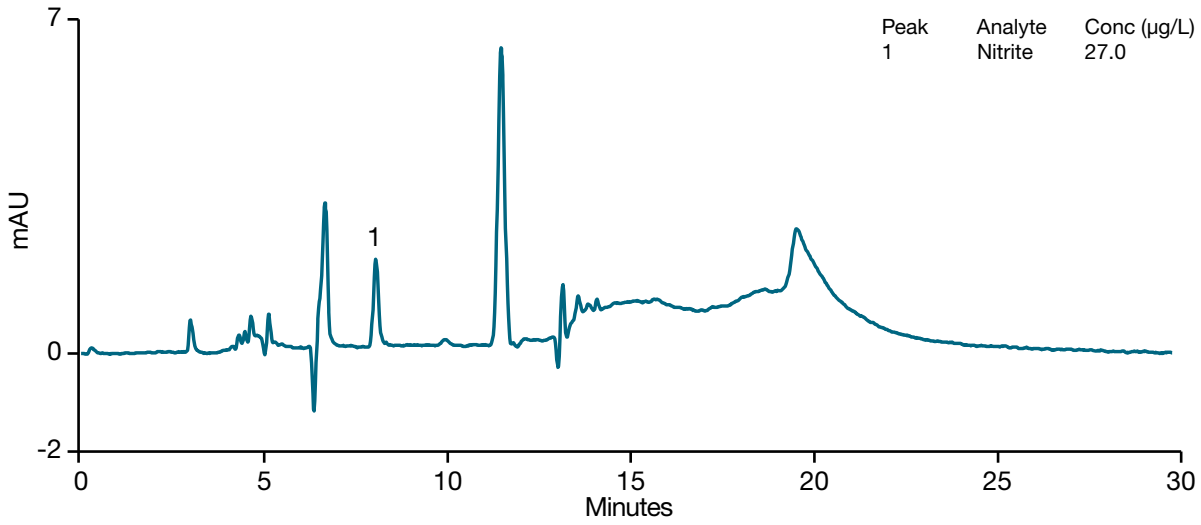

Figure 7. Nitrite in metformin hydrochloride (S2) using a Dionex IonPac AS19-4µm column

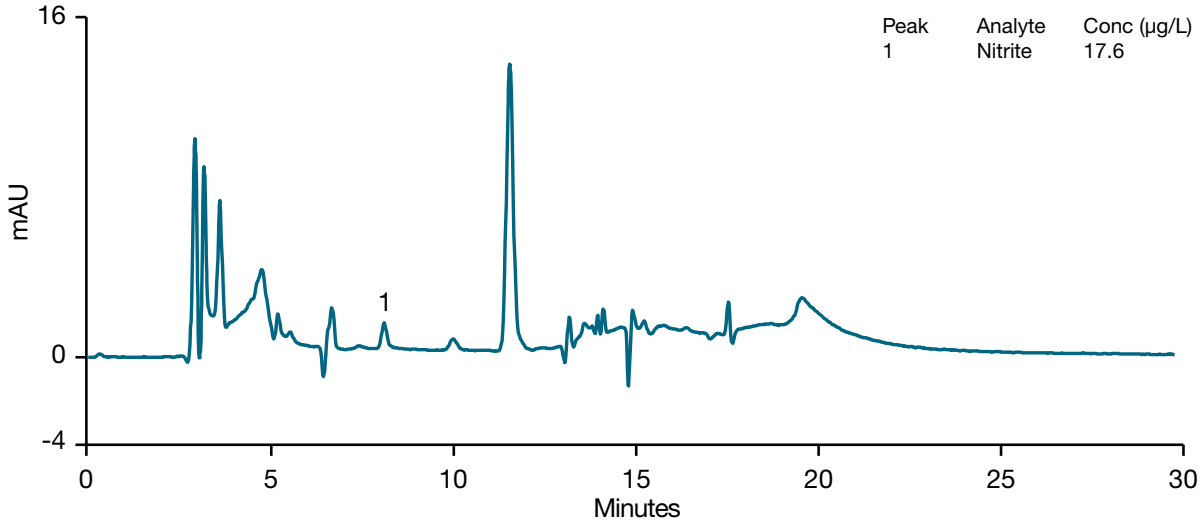

Figure 8. Nitrite in Nytol QuickCaps drug product (S3) using a Dionex IonPac AS19-4µm column

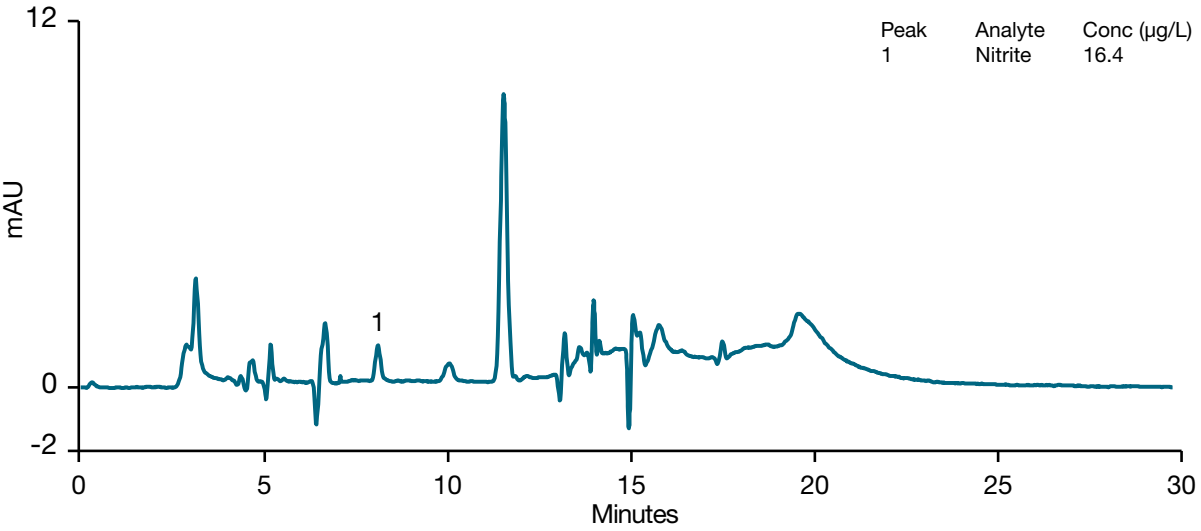

Figure 9. Nitrite in Benadryl (S4) using a Dionex IonPac AS19-4µm column

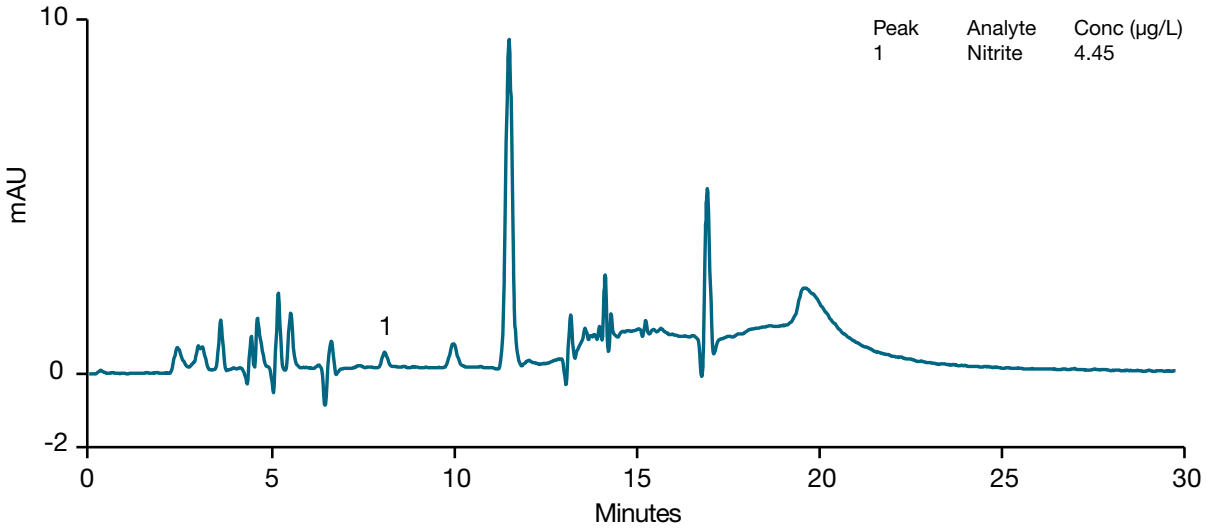

Figure 10. Nitrite in losartan drug product (S6) using a Dionex IonPac AS19-4µm column

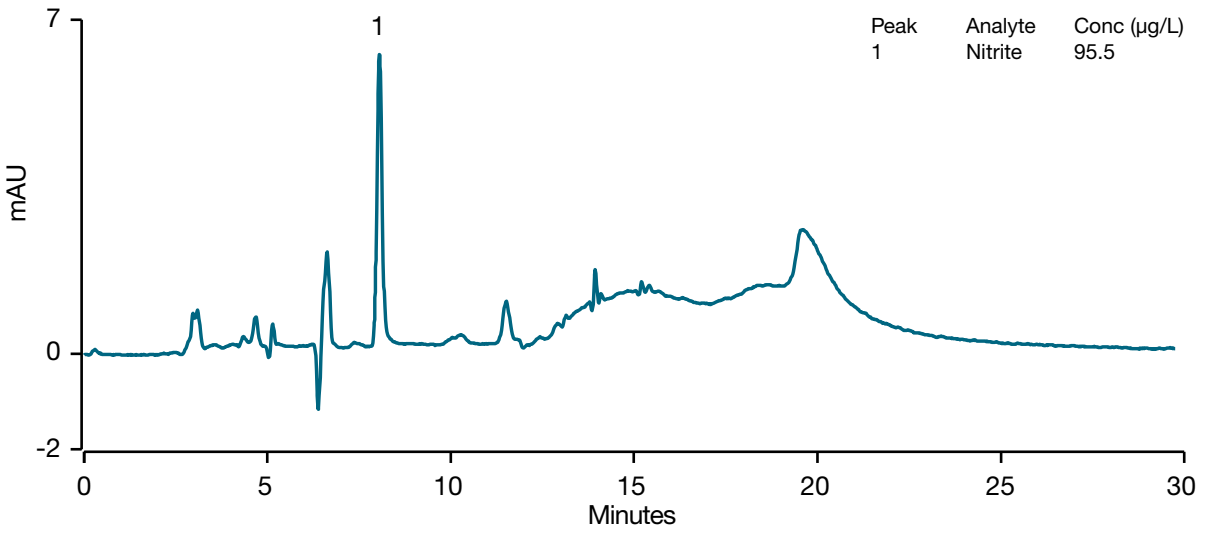

Figure 11. Nitrite in ranitidine drug product (S7) using a Dionex IonPac AS19-4µm column

Method accuracy and precision

Method accuracy was evaluated through spike recovery studies. Nitrite was spiked into each sample solution at 10 µg/L. The recovery for nitrite in the seven samples ranged from 96.3 to 101% (Table 4). Method precision was determined by injections of the 50 µg/L nitrite calibration standard on three separate days. The peak area precision was 0.56%, and retention time precision was 0.10%.

Table 4. Spike recovery of nitrite in pharmaceutical samples

Sample (S#)	Recovery (%)	RSD (n=6)
1	96.6	1.2
2	96.9	2.1
3	101	1.1
4	100	2.7
5	100	2.6
6	98.1	2.6
7	100	2.1

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Conclusion

A method was developed for the determination of nitrite in pharmaceuticals by coupling IC with UV absorbance detection. The LOD of nitrite in a pharmaceutical sample is 0.918 ppm (µg/g API). The method is accurate and precise due to the high reproducibility of the Reagent-Free ion chromatography system. This method should be applicable to the determination of nitrite throughout the manufacturing process of a drug product to assess the likelihood of nitrosamine formation.

References

1. Concise International Chemical Assessment Document, N-NITROSODIMETHYLAMINE. https://www.who.int/ipcs/publications/cicad/en/cicad38.pdf?ua=1
2. U.S. Environmental Protection Agency, Integrated Risk Information System (IRIS). https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0045_summary.pdf (accessed 10/2/20)
3. FDA announces voluntary recall of several medicines containing valsartan following detection of an impurity. https://www.fda.gov/news-events/press-announcements/fda-announces-voluntary-recall-several-medicines-containing-valsartan-following-detection-impurity (accessed 10/2/20)
4. FDA Updates and Press Announcements on NDMA in Zantac (ranitidine). https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-ndma-zantac-ranitidine (accessed 10/2/20)
5. Mylan Initiates Voluntary Nationwide Recall of Three Lots of Nizatidine Capsules, USP, Due to the Detection of Trace Amounts of NDMA (N-Nitrosodimethylamine) Impurity Found in the Active Pharmaceutical Ingredient Manufactured by Solara Active Pharma Sciences Limited. https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/mylan-initiates-voluntary-nationwide-recall-three-lots-nizatidine-capsules-usp-due-detection-trace (accessed 10/2/20).
6. FDA Updates and Press Announcements on NDMA in Metformin. https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-ndma-metformin (accessed 10/2/20)
7. Guidance Document Control of Nitrosamine Impurities in Human Drugs Guidance for Industry. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/control-nitrosamine-impurities-human-drugs (accessed 10/2/20)
8. USP General Chapter Prospectus: <1469> Nitrosamine Impurities. https://www.uspnf.com/notices/nitrosamine-impurities-gc-prospectus-20200424 (accessed 10/23/20)
9. Mirvish, S.S. Formation of N-nitroso compounds: Chemistry, kinetics and in vivo occurrence. *Toxicol. Appl. Pharmacol.* **1975**, *31*, 325–351.
10. Ashworth, I. W.; Dirat, O.; Teasdale, A.; Whiting, M. Potential for the Formation of N-Nitrosamines During the Manufacture of Active Pharmaceutical Ingredients: An Assessment of the Risk Posed by Trace Nitrite in Water. *Org. Process Res. Dev.* **2020**, DOI: 10.1021/acs.oprd.0c00224
11. Thermo Scientific Technical Note 74093: Designing ion chromatography methods for determining amines in pharmaceuticals.
12. Thermo Scientific Dionex ICS-6000+ Ion Chromatography System Operator's Manual December 2018. https://assets.thermofisher.com/TFS-Assets/CMD/manuals/man-22181-97002-ics-6000-man2218197002-en.pdf (Accessed December 17, 2020)
13. ICH Guideline Q2B, Validation of Analytical Procedures, Methodology (CPMP/ICH/281/95), Geneva, Switzerland, November 1996.

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