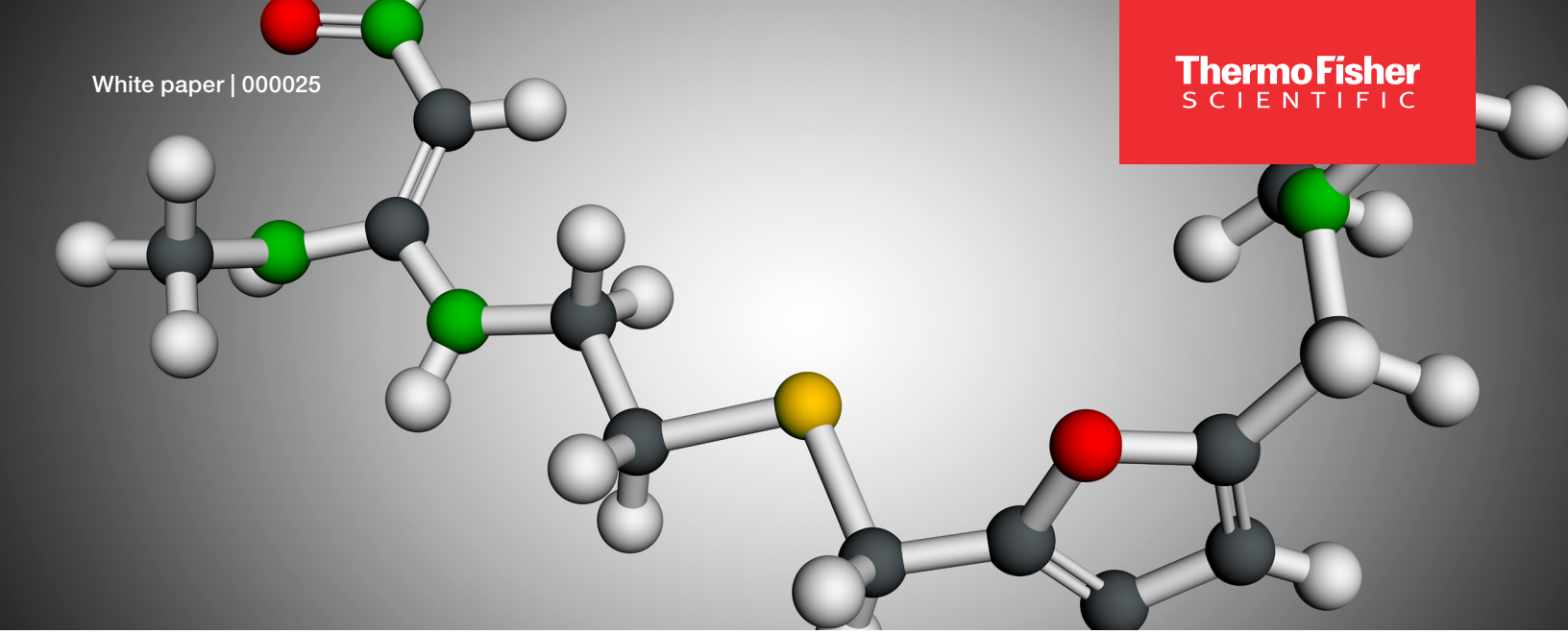

White paper | 000025

# Ion chromatography determination of nitrate and nitrite in ranitidine drug substance and drug products

Authors: Dr. Chetan Chavan,
Dr. Chanakya Thaker, and
Mr. Chetan Chaudhari;
Thermo Fisher Scientific,
Powai Mumbai, India

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## Introduction

Ranitidine HCl is a medicine used to reduce stomach acid production, which helps to treat and prevent stomach acid related disorders such as heartburn and acid reflux diseases. It is a commonly used drug with manufacturing processes that can potentially produce nitrite and, or nitrate. In 2019, Ranitidine was recalled because it may contain unacceptable levels of N-nitrosodimethylamine (NDMA). NDMA is one of the simplest members of a large class of N-nitrosamines, which are a known carcinogens. Following the advice from the FDA, pharmaceutical companies had to recall their products and test for NDMA to ensure it is less than the acceptable daily intake limit.

Nitrate is an impurity that can be found in many starting materials and intermediates in a drug substance process, and can be easily reduced to nitrite. Nitrite is a potential impurity in chemical reactions that can form nitroso-compounds. This can potentially be a nitrosamine impurity which will need to be controlled. Therefore, an ion chromatography method was developed and validated to measure both nitrite and nitrate levels in ranitidine drug substance and drug products.

## Summary

The aim of this white paper is to provide analytical conditions and data for determination of nitrate and nitrite in ranitidine API and tablets by ion chromatography (IC) using suppressed conductivity detection.

This method provides good resolution among other anions like fluoride, bromide, sulfate and phosphate, which might present in raw materials and the API. This method also provides the best resolution between a high concentration of chloride and a low concentration of nitrite in ranitidine HCl.

This paper provides a hardware setup, reagents, standards, eluent preparations, precautions, and chromatographic conditions required to perform the determination of nitrite and nitrate in ranitidine API and tablets by IC.

## Hardware setup

### Ion chromatography

A Thermo Scientific™ Dionex™ ICS-6000 HPIC system consisting of a gradient pump, thermostatted autosampler, suppressor, column, and conductivity detector was used.

## Requirements

### Reagents

All reagents used shall be HPLC grade purity or better. The aqueous solution should be prepared with deionized water (DI water, conductivity = 0.05 µS and TOC <10 ppb which is ASTM Type I reagent grade water)

- Sodium nitrate (Sigma Aldrich PN S5022)
- Sodium nitrite (Sigma Aldrich PN 237213)
- 50% NaOH (19.1 Molar) (Sigma Aldrich PN 415413)

### Preparation of Eluent (must be freshly prepared prior to analysis)

- **Port A of Pump:** Prepare 1 L of 100 mM NaOH by diluting 5.23 mL 50% NaOH solution with DI water
- **Port B of Pump:** DI water
- Diluent is DI water

### Test procedure

### Sample preparation

For API, 0.3 g of sample were dissolved in 2.0 mL of diluent in a 15 mL centrifuge tube. It was then vortexed to dissolve. Pass the dissolved sample through a 0.2 µm nylon membrane filter, and then through a Thermo Scientific™ Dionex™ OnGuard™ II RP (1cc) cartridge, PN 082760. The sample is ready to inject.

For tablets, the average weight of 15 tablets was taken, they were crushed to a fine powder with a mortar & pestle. From this, 0.3838 g was taken and 2.0 mL of diluent were added. This sample was then vortexed for five minutes and then centrifuged for 15 minutes at 6000 rpm. Supernatant was taken, filtered through 0.2 µm nylon membrane filter, and used for injection.

## Precautions

1. Condition column before analysis:
   - Pass 60 mM NaOH through the column at 1.0 mL/min for 20 min
   - Pass 5 mM NaOH through the column at 1.0 ml/min for 20 min
2. Column storage after analysis:
   - Pass 60 mM NaOH through the column at 1.0 mL/min for 30 min

## Preparation of standards

Separate 1000 mg/L (ppm) of nitrate and nitrite standards were prepared from their respective salts. For linearity, mixtures of 0.9, 10.0, 30.0, 45.0, 75.0 and 100.0 mg/L (ppm) each of nitrate and nitrite were prepared. For LOD and LOQ, mixtures of 0.3 mg/L (ppm) each and 0.9 mg/L each nitrate and nitrite were prepared. For Precision, a mixture of 45.0 mg/L each of nitrate and nitrite was prepared.

## Chromatographic conditions

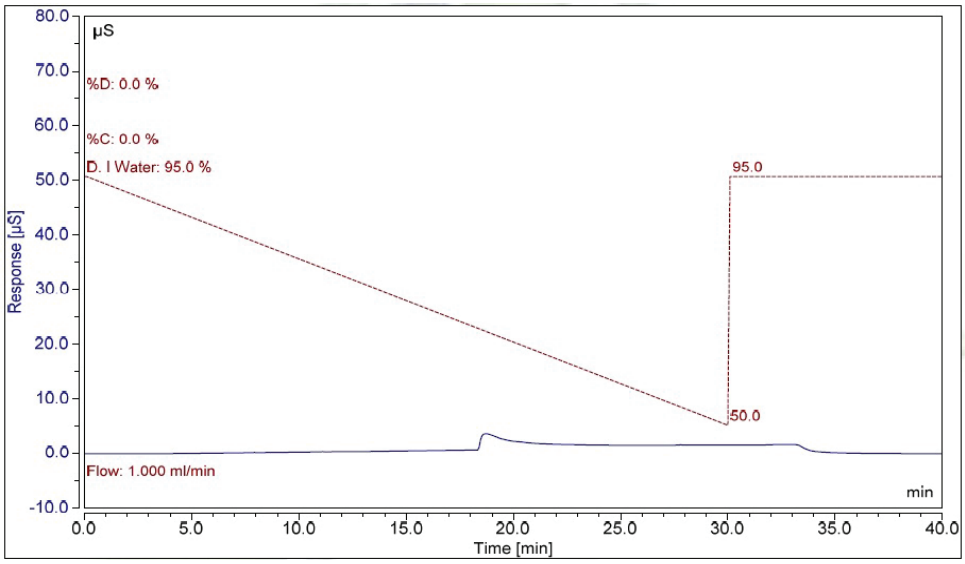
Column: Thermo Scientific™ Dionex™ IonPac™ AS19 analytical column, 4x250 mm, 4 µm, (PN 083217) and its guard, Thermo Scientific™ Dionex™ IonPac™ AG19 guard column (PN 083221)
Injection Volume: 10 µL
Run Time 40 min
Eluent Flow Rate: 1.0 mL/min
Column Oven Temperature: 40 °C
Autosampler Temperature: 10 °C
Suppressor: Thermo Scientific™ Dionex™ ADRS600 suppressor, 4 mm (PN 088666) (Recycle mode)
Detection: Conductivity Detector
*Note: The NaOH gradient can also be delivered using RFIC-NaOH/KOH Cartridge*

Gradient Conditions:

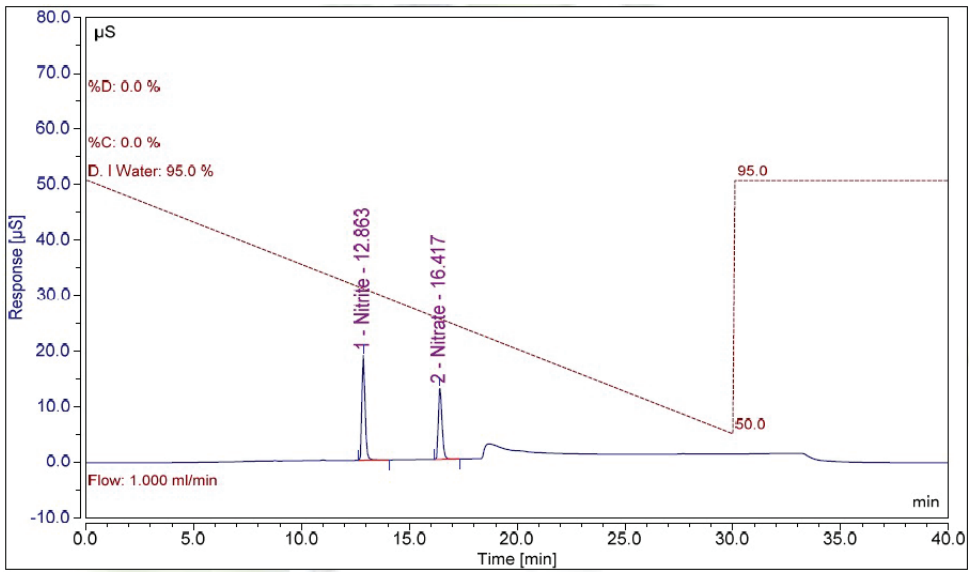
| Time, min | % A = 100 mM NaOH | %B = D. I. Water |
|---|---|---|
| 0.0 | 5.0 | 95.0 |
| 0.1 | 5.0 | 95.0 |
| 30.0 | 50.0 | 50.0 |
| 30.1 | 5.0 | 95.0 |
| 40.0 | 5.0 | 95.0 |

## Chromatograms and data

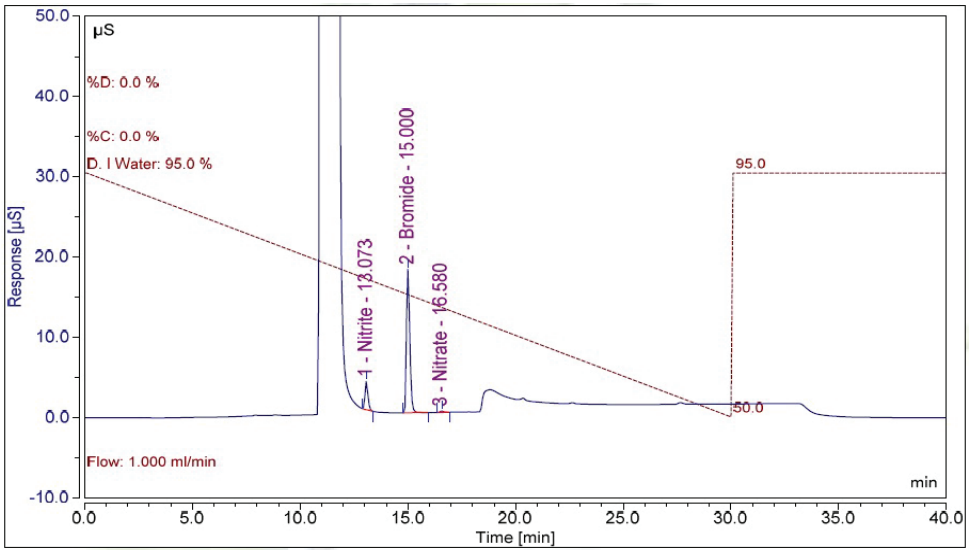
1. Diluent chromatogram:



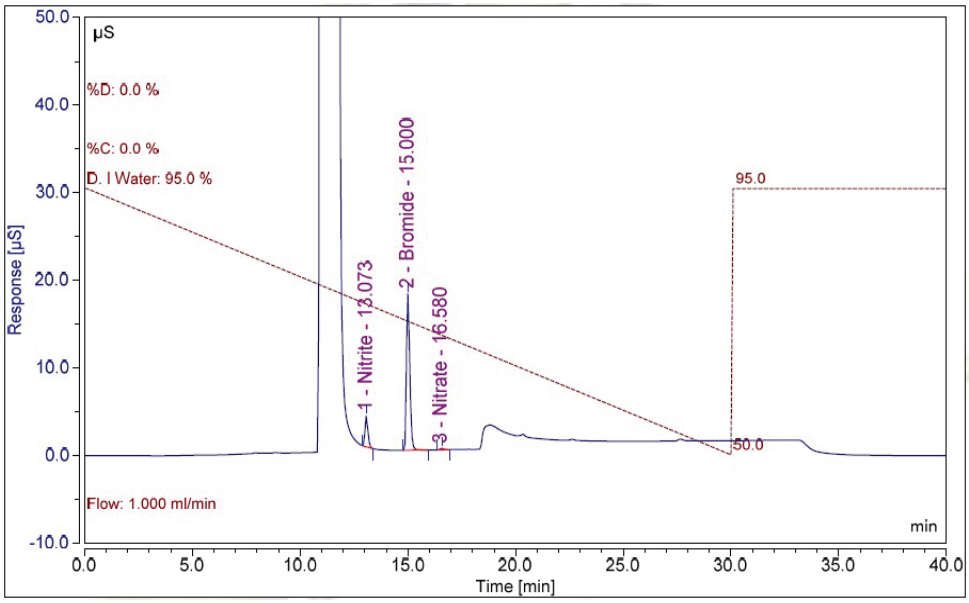

2. Standard chromatogram:



3. Sample chromatogram:
   API: B. No. xxx

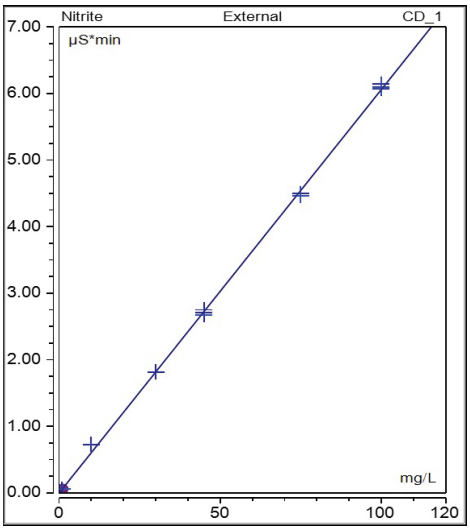


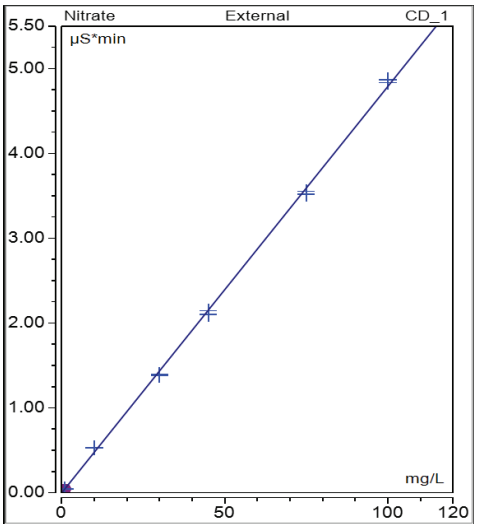

Tablet: B. No. xxx



Results:

1. Calibration curves:




| Peak № | Ret. Time (min) | Peak name | Cal. Type | Points | Corr. Coeff. | Offset | Slope | Curve |
|---|---|---|---|---|---|---|---|---|
| 1 | 12.67 | Nitrite | Lin | 18 | 0.999 | 0.0000 | 0.0606 | 0.0000 |
| 3 | 16.28 | Nitrate | Lin | 18 | 0.999 | 0.0000 | 0.0479 | 0.0000 |

2. Standard precision:

| Precision study | 45 mg/L of nitrite and nitrate standard each used for precision study | | |
|---|---|---|---|
| Nitrite | Area µS*sec | Nitrate | Area µS*sec |
| Standard_Injection1 | 198.64 | Standard_Injection1 | 154.03 |
| Standard_Injection2 | 196.16 | Standard_Injection2 | 153.83 |
| Standard_Injection3 | 197.64 | Standard_Injection3 | 152.88 |
| Standard_Injection4 | 198.68 | Standard_Injection4 | 154.06 |
| Standard_Injection5 | 199.17 | Standard_Injection5 | 154.05 |
| Standard_Injection6 | 199.61 | Standard_Injection6 | 155.72 |
| Avg. | 198.31 | Avg. | 154.09 |
| Std. Dev. | 1.245 | Std. Dev. | 0.918 |
| RSD | 0.628 | RSD | 0.596 |

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3. Sample results: API

| Sample Name | Nitrite, mg/Kg | Nitrate, mg/Kg |
|---|---|---|
| Sample_1 (n=6) | 49.9 | 4.03 |
| Sample_2 (n=2) | 45.9 | 3.50 |

Tablet

| Sample Name | Nitrite, mg/Kg | Nitrate, mg/Kg |
|---|---|---|
| Sample_1 (n=6) | 7.31 | 3.78 |
| Sample_2 (n=2) | 7.59 | 3.88 |

4. Avg. Recoveries from LOQ, 50%, 100%, and 150% levels for nitrite and nitrate were observed in the range of 90% to 115%.

## Conclusions

This ion chromatography method provides simultaneous determination of nitrite and nitrate in single run. In the same injection, it can also provide the determination of other anions such as fluoride, bromide, phosphate and sulfate from ranitidine HCl raw materials and API or similar products.

### References


1. 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 July 7, 2021).
2. AN73987: Determination of Nitrite in Pharmaceuticals (accessed March 23, 2021).
3. TN74093: Designing Ion Chromatography Methods for Determining Amines in Pharmaceuticals.


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