White paper | 000031

Ion chromatography determination of nitrate and nitrite in metformin API and tablets

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

Keywords: Impurity analysis, Genotoxic impurity, GTI, Dionex ICS-6000, metformin, n-nitrosamine

Introduction

Metformin HCl is a very effective medicine that is used to control high blood sugar. It is used in patients with type 2 diabetes. Controlling high blood sugar helps prevent kidney damage, blindness, nerve problems, and loss of limbs. Proper control of diabetes may also lessen risk of a heart attack or stroke. Metformin works by helping to restore the body's proper response to the insulin which is naturally produced. It also decreases the amount of sugar that the body's liver makes and the stomach/intestines absorb.

Nitrosamine compound formation and contamination is currently a global concern. These probable human carcinogens may form when manufacturers reuse solvents that are neutralized with nitrite containing compounds in later steps of drug or medication processing. One common example is the use of dimethyl formamide quenched with sodium nitrite.

Therefore, an ion chromatography method was developed and validated to measure the levels of nitrite and nitrate in metformin API and tablets.

Summary

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

This method provides good resolution among other anions like fluoride, bromide, sulfate, and phosphate, which might be present in raw materials and the API. Also, it provides the best resolution between a high concentration of chloride and a low concentration of nitrite in metformin 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 metformin API and tablets by ion chromatography (IC).

Hardware setup

Ion Chromatography

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

Requirements

Reagents

All reagents used should be HPLC grade purity or better. The aqueous solution should be prepared with deionized water (DI water, conductivity = 0.05µS and TOC < 10ppb 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 1L of 100 mM NaOH by diluting 5.23 mL of 50% NaOH solution with DI water.
- **Port B of pump:** DI water
- Diluent is DI water

Test procedure

Sample preparation

For the API, 0.15 g of sample was weighed in a 10 mL volumetric flask. 8 mL of diluent was added and sonicated to dissolve. Sample was passed through 0.2 µm nylon membrane filter and Thermo Scientific™ Dionex™ OnGuard™ II RP cartridge PN 082760 (1 cc) and then used for injection.

For tablets, 180 mg of sample (crushed tablets) were weighed in a 10 mL vol flask; 8 mL of diluent were added to it, and sonicated for 10 min. Volume was made up with diluent and sample was filtered through a 0.2 µm nylon filter, and passed through an OnGuard II RP cartridge (1 cc), and injected on the IC system. Some samples form an emulsion even after filtration, so they are diluted 1:1 with diluent and then used for injection.

For excipients, about 150 mg of sample were weighed in a 10 mL volumentric flask; 8 mL of diluent were added to it, and sonicated for 10 min. Volume was made up with diluent and sample was filtered through a 0.2 µm nylon filter and injected into the IC system.

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) nitrate and nitrite standards were prepared from their respective salts. For linearity, mixtures of 0.1, 1.0, 5.0, 10.0 and 25.0 mg/L (ppm) each of nitrate and nitrite were prepared. For LOD and LOQ, mixtures of 0.025 mg/L (ppm) and 0.1 mg/L each nitrate and nitrite was prepared. For precision, a mixture of 5.0 mg/L each of nitrate and nitrite was prepared.

Chromatographic conditions

Column: Thermo Scientific™ Dionex™ IonPac™AS19 column, 4 x 250 mm, 4µm (PN 083217), Thermo Scientific™ Dionex™ IonPac™ AG19 guard (PN 083221)
Injection volume: 10 µL

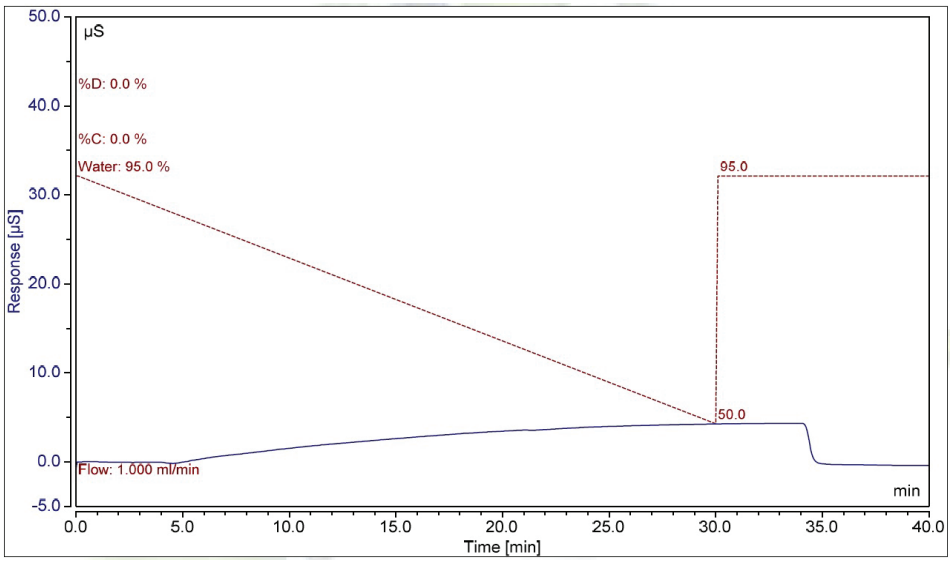
Run time: 40 min
Eluent flow rate: 1.0 mL/min
Column oven temperature: 40 °C
Autosampler temperature: 40 °C
Suppressor: Thermo Scientific™ Dionex™ ADRS 600 dynamically regenerated suppressor, 4mm (PN 088666) (recycle mode) @ 124mA
Detection: Conductivity detector
Note: The NaOH gradient can also be delivered using RFIC-NaOH/KOH Cartridge

Gradient conditions:

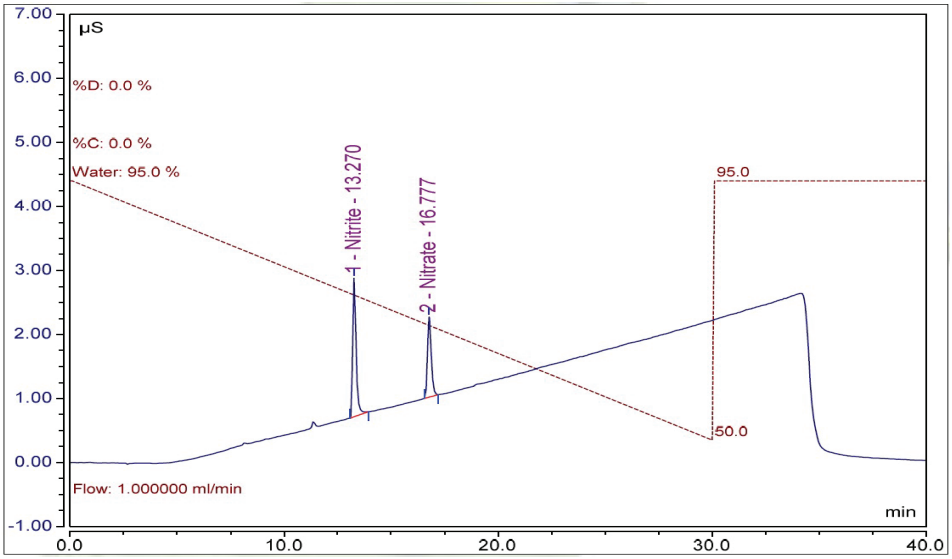
Time, min	% A = 100 mM NaOH	%B = D. I. Water
0.0	5.0	95.0
0.1	5.0	95.0
30.0	50.0	50.0
30.1	5.0	95.0
40.0	5.0	95.0

Chromatograms and data

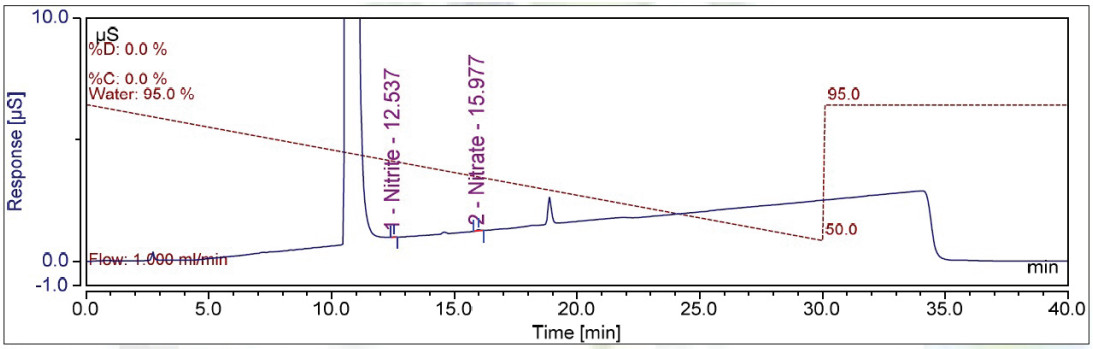
1. Diluent chromatogram:



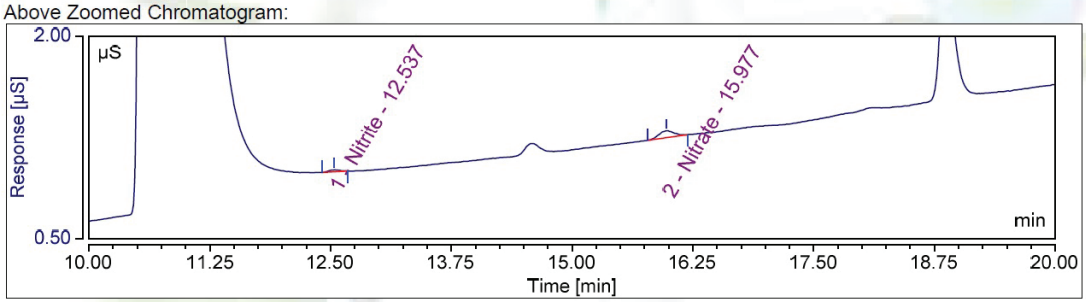
2. Standard chromatogram:



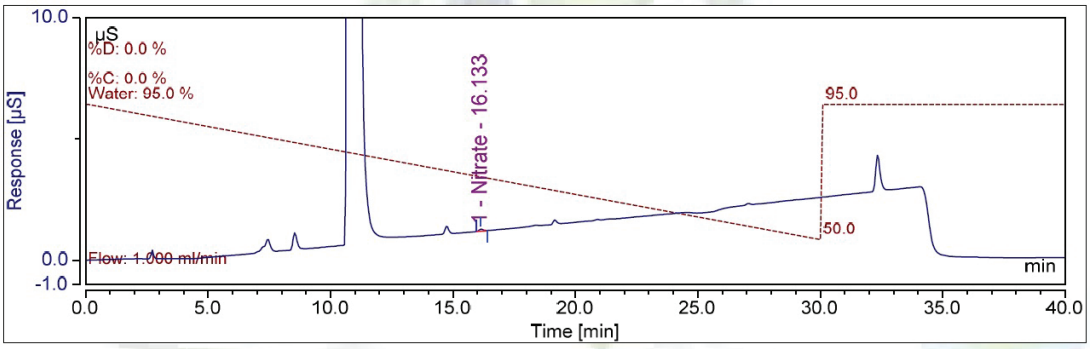
3. Sample chromatogram:
   API: B. No. xxx



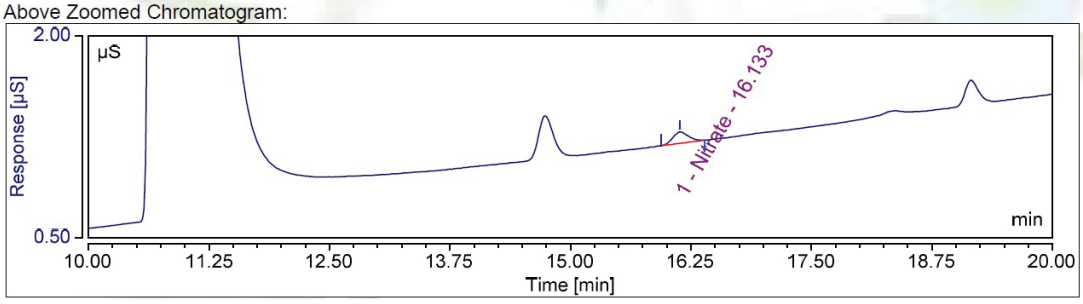
Above Zoomed Chromatogram:



Tablet: B. No. xxx

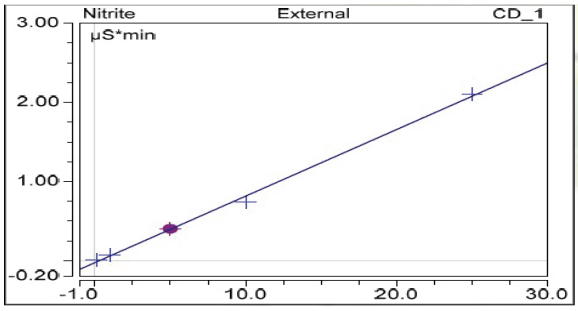


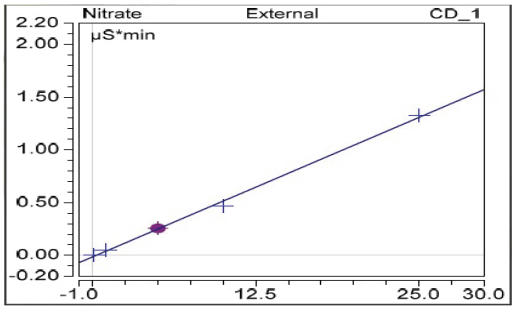
Above Zoomed Chromatogram:



Results:

1. Linearity plot:





№	Ret. Time (min)	Peak name	Cal. Type	Points	Corr. Coeff.	Offset	Slope	Curve
1	13.27	Nitrite	Lin, withOffset	5	0.999	-0.0266	0.0842	0.0000
2	16.78	Nitrate	Lin, withOffset	5	0.999	-0.0145	0.0529	0.0000

2. Sample results:
   API

Sample Name	Nitrite, mg/Kg	Nitrate, mg/Kg
Sample_1 (n=6)	Not detected	23.5
Sample_2 (n=2)	2.30	14.7

Tablet

Sample Name	Nitrite, mg/Kg	Nitrate, mg/Kg
Sample_1 (n=6)	Not detected	20.2
Sample_2 (n=2)	Not detected	25.5

Excipients

Sample Name	Nitrite, mg/Kg	Nitrate, mg/Kg
Sample_1	2.22	55.2
Sample_2	Not detected	117
Sample_3	15.6	135

3. Average recoveries from LOQ, 50%, 100% and 150% levels for nitrite and nitrate were observed in the range of 90% to 115%.

Conclusions

This method provides simultaneous determination of nitrite and nitrate in a single run for metformin API, tablets and related excipients. It also provides an analytical tool to estimate other anions like fluoride, bromide, phosphate, and sulfate from metformin HCl raw materials and API.

Reference

1. 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 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 (accessed May 19, 2021).

Learn more at **thermofisher.com/ic**