Pharmaceutical

Analysis of nitrite and nitrate from microcrystalline cellulose by IC-MS

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Keywords

Nitrite, Nitrate, Microcrystalline Cellulose, IC-MS, Chromeleon

Introduction

Regulatory bodies, such as the U.S. Food and Drug Administration (FDA), have established stringent specification levels for N-nitrosamine impurities in pharmaceutical products due to their potential carcinogenicity. Nitrite, a known precursor in N-nitrosamine formation, can react with secondary or tertiary amines present during active pharmaceutical ingredient (API) synthesis or in the drug formulation process. Accurately measuring nitrite and nitrate levels is critical for drug manufacturers seeking to understand its reaction kinetics with amines and to select appropriate excipients. Similarly, excipient manufacturers must monitor nitrite content to ensure compliance with quality standards.

This study demonstrates the effectiveness of ion chromatography mass spectrometry (IC-MS) for the trace detection of nitrite and nitrate in microcrystalline cellulose, a key excipient in many drug formulations. Mass spectrometry was performed using the selected ion monitoring (SIM) mode to mitigate common interference issues associated with conductivity detection. The optimized method demonstrated strong performance, with nitrite quantitation showing excellent linearity in the concentration range of 1.0–150 ppb, with regression coefficient greater than 0.999, and nitrate linearity in the range of 1.0-70.0 ppb, where regression coefficient also exceeds 0.999. Recovery rates were 104% for nitrite and 91% for nitrate, confirming the method's accuracy and reliability.

Recommended equipment

- Thermo Scientific™ Dionex™ Reagent-Free™ Ion Chromatography (RFIC™) system
- Thermo Scientific™ Dionex™ AS-AP Autosampler
- Thermo Scientific™ Eluent Generator Cartridges (EGC) 500 KOH
- Thermo Scientific™ ISQ™ EC Single Quadrupole Mass Spectrometer
- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software

Reagents, standard and sample preparation

Reagents

- Deionized (DI) water, Type 1 reagent grade, 18 MΩ·cm resistivity or better
- Sodium nitrite, ACS Grade
- Sodium nitrate, ACS Grade

Eluent

20mM KOH was generated through EGC-KOH cartridge

Standard stocks solution

1000 ppm nitrite stock solution was prepared by dissolving 1.499 g sodium nitrite ($NaNO_2$) in 1.0 L of DI water. Likewise, 1000 ppm nitrate stock solution was prepared by dissolving 1.371 g sodium nitrate ($NaNO_3$) in 1.0 L of DI water.

Working standards

Working standards are prepared from the stock solution just before analysis. The nitrite stock solution is diluted to a concentration range of 1.0–150 ppb, while the nitrate stock solution is diluted to a concentration range of 1.0-70.0 ppb.

Sample preparation

Weigh 0.1 g of microcrystalline cellulose (MCC) and transfer it into a 10ml polypropylene (PP) volumetric flask. Fill the flask with deionized water up to the calibration mark. The solution was then vortexed for 1 minute and filtered through a 0.2 µm nylon membrane filter. The filtered solution was injected into IC-MS for analysis.

Chromatographic conditions

Columns	• Thermo Scientific™ Dionex™ IonPac™ AS11-HC IC Column, 2 mm × 250 mm (P/N 052961) • Thermo Scientific™ Dionex™ IonPac™ AS11-HC IC Guard Column, 2 mm × 50 mm (P/N 052963)
Eluent	20mM KOH, generated using an EGC-KOH cartridge
Flow Rate	0.25mL/min
Column Oven Temperature	30° C
Suppressor	Thermo Scientific™ Dionex™ DRS 600 Dynamically Regenerated Suppressor, 2mm (P/N 088667)
Suppressor Regeneration Mode	External Water Mode via AXP pump at 0.3 ml/min flow rate
Suppressor Current	13mA
Injection Volume	250µL
Run Time	30 minutes
ISQ Conditions	
Vaporization Temperature	482° C
Ion Transfer Tube Temperature	150° C
Polarity	Negative
Source Voltage	-2000 V
Sheath Gas	79.9 psig
Auxiliary Gas	7.7 psig
Sweep Gas	0.0 psig
Nitrite Monitoring	46.3 m/z
Nitrate Monitoring	62.1 m/z

Standard chromatogram

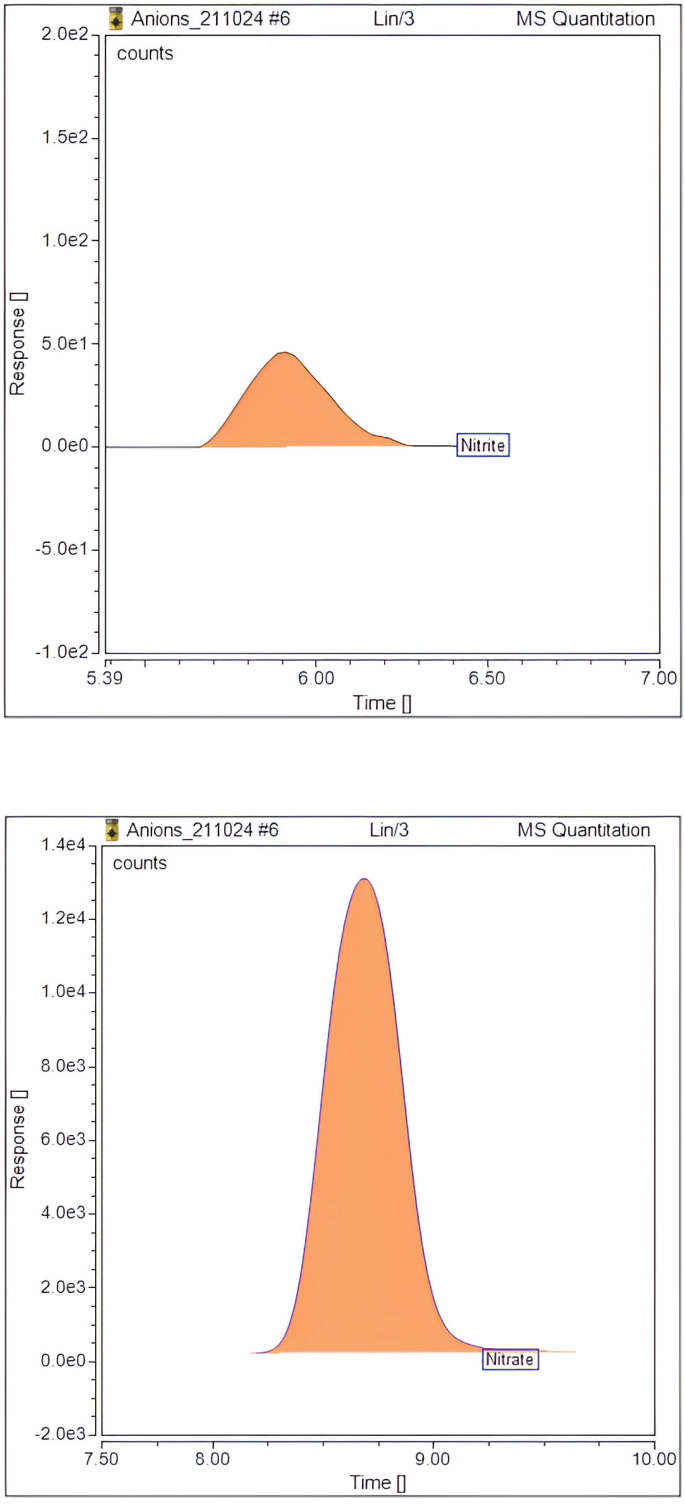

Figure 1: SIM chromatogram of a working standard solution mixture containing 10.0 ppb nitrite (left) and 10.0 ppb nitrate (right)

Sample chromatogram

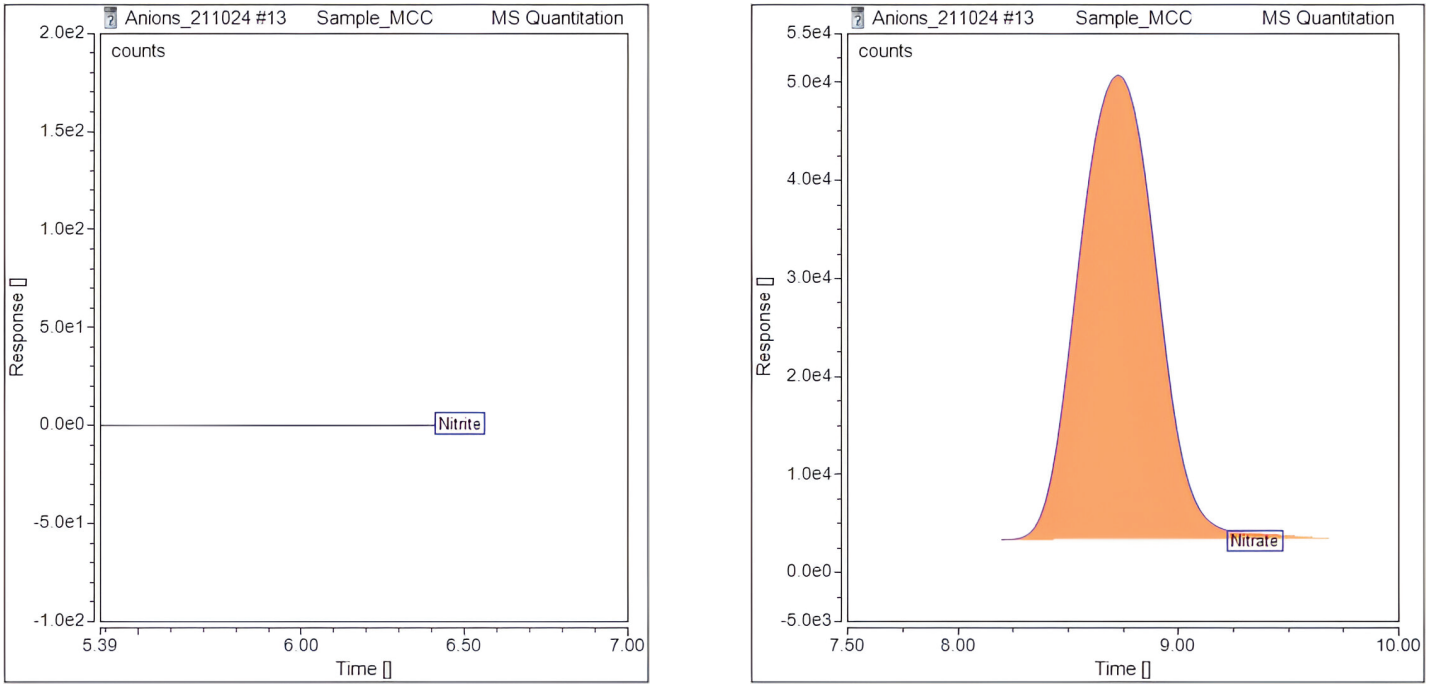

Figure 2: SIM chromatogram of nitrite (left) and nitrate (right) in microcrystalline cellulose sample

Conclusion

This method provides reliable and sensitive detection of nitrite and nitrate across a broad concentration range. The reduced detection limit enhances its effectiveness in analyzing trace levels of these compounds in microcrystalline cellulose and other pharmaceutical excipients. Strong recovery rates further validate the method's accuracy, making it a valuable tool for routine use in analytical laboratories.

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