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Note d'application

UPLC-MS/MS Analysis of the N-Nitrosamine, NDMA, in Ranitidine

Hitesh Shrimal, Dr. Padmakar Wagh

Waters Corporation

Abstract

The goal of the work described herein, was to develop a sensitive and robust method for the LC-MS/MS quantification of NMDA containing the drug product, ranitidine. This application highlights accurate NDMA quantification, achieving LOQs of 0.75 ppb (11 picograms on-column), using the Waters ACQUITY UPLC H-Class PLUS System and XSelect HSS T3 Column for chromatographic separation, and the Xevo TQ-S cronos tandem MS configured with APCI probe for highly sensitive MS detection.

Benefits

- A simple and reproducible method for quantification of NDMA
- Use of XSelect HSS T3 Column for excellent reversed-phase chromatographic retentivity of NDMA, and resolution from the drug product ranitidine
- NDMA quantification from 0.75–1,200 ppb

Introduction

Genotoxic impurities (GTIs), arising from the synthesis/manufacturing from many drug products, specifically suspected carcinogenic N-nitroso compounds, have been found in several medicines. This has resulted in many drug recalls.

Through the testing thus far, FDA have found levels of NDMA in ranitidine that are similar exposure levels from eating common foods like grilled or smoked meats.[1]

Based on methods described in the 2018 ICH Guidance M7(R1) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk,1 the FDA has set the acceptable daily intake limit for NDMA at 96 ng/day (0.32 ppm) for ranitidine.

The goal of the work described herein, was to develop a sensitive and robust method for the LC-MS/MS quantification of NMDA containing the drug product, ranitidine.

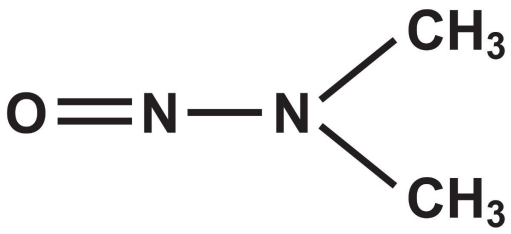

Figure 1. NDMA structure.

Experimental

Sample preparation

Active pharmaceutical ingredient (API)

- 10 mL of a 50:50 water:methanol solutionb was added to 300 mg API.
- Sample was vortexed and transferred into glass vials for subsequent analysis.

GTI NDMA standard preparation

- 2 mL of NDMA standard of 7.5 ng/mL was added to 8 mL of a 50:50 water:methanol solution.
- Standard solution was vortexed.
- Final concentration of NDMA was 1.5 ng/mL.

NMDA/API sample preparation

- 2 mL of NDMA standard of 7.5 ng/mL was added to 8 mL of 300 mg prepared API solution.
- Final concentration of NDMA and ranitidine API was, 1.5 ng/mL and 30 mg/mL, respectively.

Accuracy/recovery quality control (QC) sample preparation

Three QC levels of NDMA (1.5, 3, and 10 ng/mL) containing 30 mg/mL ranitidine API was prepared.

- Concentrated NDMA stock solutions (7.5, 15.0, and 50 ng/mL) were added to 300 mg ranitidine, to yield final QC concentrations, all containing 30 mg/mL final concentration of ranitidine.

- All the samples were processed according to the method described previously (NMDA/API Sample Preparation).

LC Conditions

LC system:	ACQUITY UPLC H-Class PLUS
Vials:	Waters Total Recovery Vials [p/n 186007197C]
Column:	XSelect HSS T3 5 µm, 4.6 × 150 mm (p/n 186004791)
Column temp.:	40 °C
Sample temp.:	10 °C
Injection volume:	15 µL
Flow rate:	0.6 mL/min
Mobile phase A:	0.1% Formic acid in water
Mobile phase B:	0.1% Formic acid in methanol
Diluent:	50% methanol in water
Needle wash:	50% methanol in water

Time (min)	Flow rate mL/min	%A	%B
Initial	0.6	99	1
1.5	0.6	99	1
7	0.6	70	30
11	0.6	30	70
11.5	0.8	5	95
16	0.8	5	95
18	0.8	99	1
19	0.6	99	1
25	0.6	99	1

Table 1. UPLC gradient conditions.

MS Conditions

MS system:	Xevo TQ-S cronos
Ionization mode:	APCI positive ion mode
Corona voltage:	4.7 kV
Desolvation gas:	1000 L/Hr
Cone gas:	20 L/Hr
Desolvation temp.:	600 °C
MS software:	MassLynx v4.2, TargetLynx XS

Compound name	Parent ion (*m/z*)	Daughter ion (*m/z*)	Dwell (s)	Cone voltage (V)	Collision energy (eV)
NDMA	75.2	57.9	0.100	20	10

Table 2. MRM conditions for NDMA, including precursor and fragment ions.

Results and Discussion

Using the Waters Xevo TQ-S cronos Tandem Quadrupole MS coupled to the ACQUITY UPLC H-Class PLUS System (Figure 2) and chromatographic separation using the Waters XSelect HSS T3 5 m, 4.6 × 150 mm Column, and MRM transitions listed in Table 2, a lower Limit of Quantification (LOQ) of NDMA 0.75 ppb (S/N 30) was observed (Figure 3). Linear dynamic range (R^2 0.9998) of this assay was 0.75–1200 ppb (Figure 3), with no carry over observed in the blank sample proceeding the highest calibration standard. Representative QC performance is highlighted in Table 3. Use of the Waters XSelect HSS T3 Column provided necessary retention of NDMA, while providing adequate resolution fromdetecon.

Figure 2. ACQUITY UPLC H-Class PLUS System and Xevo TQ-S cronos Tandem Quadrupole Mass Spectrometer.

NDMA QC levels	NDMA QC concentrations (ppb)	NDMA % recovery
QC-1	1.5	117.7
QC-2	3.0	102.7
QC-3	10.0	101.5

Table 3. Representative QC sample quantitative performance for NMDA.

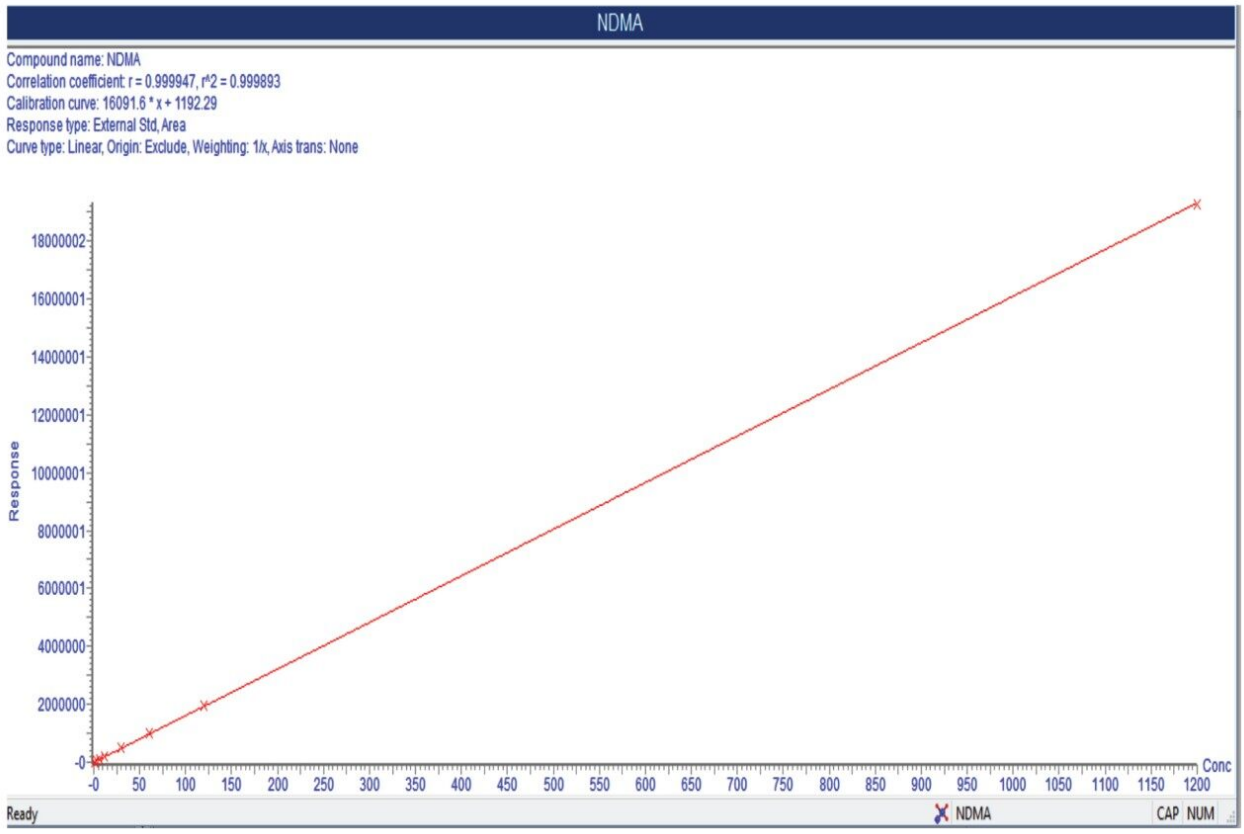

Figure 3. NMDA standard calibration curve (0.75–1200 ppb).

Conclusion

This application highlights accurate NDMA quantification, achieving LOQs of 0.75 ppb (11 picograms on-column), using the Waters ACQUITY UPLC H-Class PLUS System and XSelect HSS T3 Column for chromatographic separation, and the Xevo TQ-S cronos tandem MS configured with APCI probe for highly sensitive MS detection.

References

1. https://www.fda.gov/news-events/press-announcements/statementnew-testing-results-including-low-

levels-impurities-ranitidine-drugs (US FDA For Immediate Release: November 01, 2019.

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