

Quick Analysis of N-nitrosodimethylamine (NDMA) in Ranitidine Drug Substance and Drug Product by HPLC/UV

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The ACQUITY Arc System with PDA Detector, integrated with an ACQUITY QDa Mass Detector, enables accurate analysis of NDMA in ranitidine drug substance and drug product.

GOAL

To demonstrate quick and accurate analysis of NDMA in ranitidine drug substance and drug product by UV detection, with the added benefit of mass confirmation by mass spectral data using an ACQUITY™ QDa™ Mass Detector.

BACKGROUND

N-nitrosodimethylamine (NDMA) is classified as a probable human carcinogen, a compound that can cause DNA mutations, potentially leading to cancer.¹ The ICH M7 recommends that known mutagenic carcinogens, such as nitrosamines, should be controlled at or below the acceptable cancer risk level to ensure safety of the pharmaceutical products.

Since July 2018, several prescription and over-the-counter medications containing ranitidine were found to contain NDMA.² Since then, the industry has been working to develop suitable analytical methods to accurately measure levels of this impurity in pharmaceutical products at all stages of the development and manufacturing process, as well as in final API and drug products.

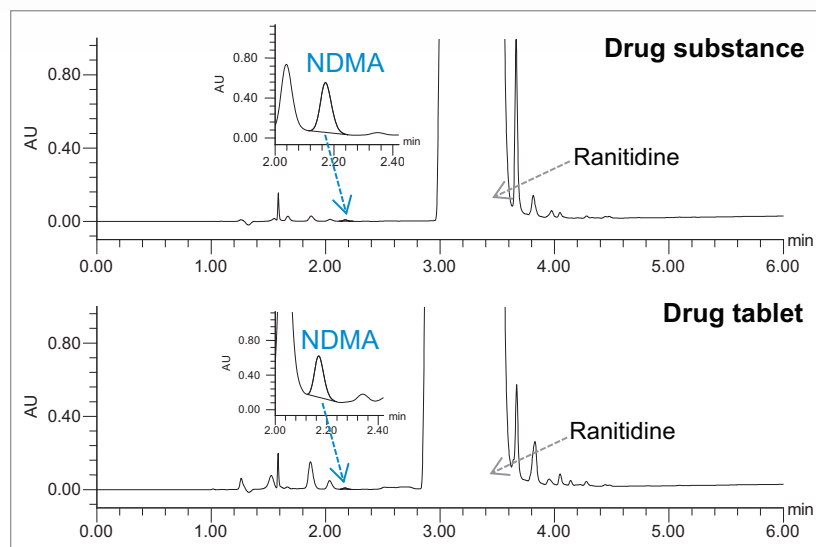


Figure 1. Chromatographic separation of NDMA (300 ng/mL) spiked into the drug substance and drug tablet formulation sample solutions containing 30 mg/mL of ranitidine. UV at 245 nm.

In this work, we present an HPLC method with UV detection for quick and accurate analysis of NDMA in ranitidine drug substance and drug tablet formulation using an ACQUITY Arc™ System with PDA Detector coupled to an ACQUITY QDa Mass Detector. In combination, the UV data is used for the determination of NDMA in test sample solutions, while the mass spectral data from the ACQUITY QDa Mass Detector enables quick and accurate peak identity confirmation.

THE SOLUTION

Ranitidine drug substance test samples were prepared in water at 30 mg/mL. Drug product tablet formulations were prepared by dissolving crushed tablets in water to contain 30 mg/mL of ranitidine. After extraction, sample test solutions were centrifuged for 30 minutes at 4000 rpm and filtered using 0.2 um PVDF syringe filters. NDMA commercially available stock (Sigma P/N CRM40059) was diluted in 20% MeOH to 250 µg/mL. This solution was subsequently diluted with water to make NDMA calibration standards ranging from 10 to 300 ng/mL.

The HPLC separation was performed using an XSelect™ HSS T3 Column with the conditions described in Table 1. The method provided excellent retention of NDMA and separation from the ranitidine peak (Figure 1).

The analysis of drug substance and drug tablet test sample solutions prepared at 30 mg/mL of ranitidine showed presence of NDMA impurity (Figure 2). The age, expiration and storage conditions of the ranitidine drug substance and drug product was unknown. Both the drug substance and drug product test samples were spiked with the NDMA standard to evaluate accuracy in the matrix.

| Parameter | Description | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|------------------|--|------------|------------|------|---------|-------|---|---------|------|-----|---------|---|------|------|-----|---|---|------|-----|------|---|---|------|-----|------|---|---|------|------|-----|---|---|------|------|-----|---|
| LC systems | ACQUITY Arc System with 2998 PDA and ACQUITY QDa detectors, passive pre-heater, and flow path 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Column | XSelect HSS T3 4.6 x 100 mm, 3.5 µm | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Column temp. | 40 °C | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Flow rate | 1.0 mL/min | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Injection volume | 25.0 µL | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mobile phase | A: water with 0.02% of formic acid B: acetonitrile | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Gradient | <table border="1"> <thead> <tr> <th>Step</th> <th>Time (min)</th> <th>%A</th> <th>%B</th> <th>Curve</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>Initial</td> <td>95.0</td> <td>5.0</td> <td>Initial</td> </tr> <tr> <td>2</td> <td>0.50</td> <td>95.0</td> <td>5.0</td> <td>6</td> </tr> <tr> <td>3</td> <td>6.00</td> <td>5.0</td> <td>95.0</td> <td>6</td> </tr> <tr> <td>4</td> <td>7.00</td> <td>5.0</td> <td>95.0</td> <td>6</td> </tr> <tr> <td>5</td> <td>7.10</td> <td>95.0</td> <td>5.0</td> <td>6</td> </tr> <tr> <td>6</td> <td>10.5</td> <td>95.0</td> <td>5.0</td> <td>6</td> </tr> </tbody> </table> | Step | Time (min) | %A | %B | Curve | 1 | Initial | 95.0 | 5.0 | Initial | 2 | 0.50 | 95.0 | 5.0 | 6 | 3 | 6.00 | 5.0 | 95.0 | 6 | 4 | 7.00 | 5.0 | 95.0 | 6 | 5 | 7.10 | 95.0 | 5.0 | 6 | 6 | 10.5 | 95.0 | 5.0 | 6 |
| | Step | Time (min) | %A | %B | Curve | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1 | Initial | 95.0 | 5.0 | Initial | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 2 | 0.50 | 95.0 | 5.0 | 6 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 3 | 6.00 | 5.0 | 95.0 | 6 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 4 | 7.00 | 5.0 | 95.0 | 6 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 5 | 7.10 | 95.0 | 5.0 | 6 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 6 | 10.5 | 95.0 | 5.0 | 6 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Wash solvents | Purge: 50:50 water/acetonitrile Sample wash: 80:20 water/methanol Seal wash: 90:10 water/acetonitrile | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PDA detection | γ range: 210–400 nm, Derived at 245 nm Sampling rate: 10 pts/sec | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mass detection | ACQUITY QDa Detector Ionization mode: ESI+ Acquisition range: 50–500 m/z Diverter valve: switch flow to waste at 2.70 minutes | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Table 1. Instrument conditions for analysis of nitrosamine impurities.

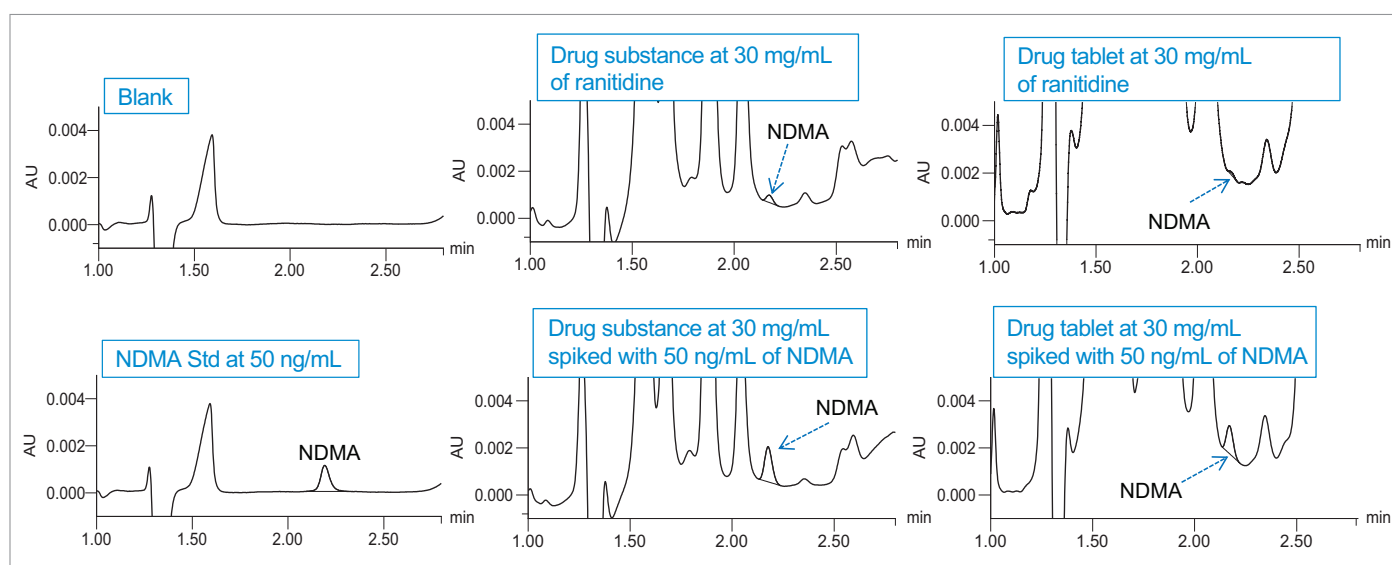


Figure 2. Representative chromatograms of solutions for NDMA analysis in ranitidine drug substance and drug tablet formulation.

The % recovery of NDMA spiked into the test samples was calculated by subtracting the already present amount of NDMA in the test samples and quantified against a calibration curve. The average % recovery of 50 ng/mL NDMA spiked into the test sample solutions was 85%.

Previously reported UV quantitation limits of nitrosamine impurities determined in valsartan and ranitidine drug substance are summarized in Waters Tech Brief [720006775EN](#).³

SUMMARY

The HPLC/UV method enabled quick and accurate analysis of NDMA in ranitidine drug substance and drug tablet products. The analysis was performed on the ACQUITY Arc System with 2998 PDA Detector, integrated with an ACQUITY QDa Mass Detector for quick and accurate peak identity confirmation. The XSelect HSS T3 Column, due to its unique polar chemistry, provided excellent retentivity for a polar nitrosamine impurity, NDMA. Overall, this method provides a quick and easy solution for the accurate determination of NDMA in medications containing ranitidine.

References

1. ICH M7 R1, Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, International Conference on Harmonization, March 2018.
2. <https://www.fda.gov/news-events/press-announcements/statement-alerting-patients-and-health-care-professionals-ndma-found-samples-ranitidine>
3. Maziarz M, Rainville P. Reliable HPLC/UV Quantitation of Nitrosamine Impurities in Valsartan and Ranitidine Drug Substances. Waters Technology Brief. [720006775EN](#). 2020.

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