Detection and analysis of N-nitrosodimethylamine in ranitidine by using Q Exactive Focus High Resolution Mass Spectrometry

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Ranitidine, Genotoxic Impurity, NDMA, Orbitrap

Preface

Following the March 2019 discovery of genotoxic impurities, N-nitrosodimethylamine and N-nitrosodiethylamine contained in the API of valsartan, it was found that the content of N-Nitroso-N-methyl-4-aminobutyric acid in the hypotensor of losartan potassium exceeded the standard. In August 2019, the FDA extended its investigation into impurities in generic drugs and found that a ranitidine drug also contained N-nitrosodimethylamine impurities. In September 2019, the Food and Drug Administration (FDA) released a detection method for N-nitrosodimethylamine in ranitidine. The European Union subsequently issued a document to extend the assessment of N-nitrosodimethylamine to all chemical synthetic drugs. In just a few months, the supervisory laws and regulations have not only expanded the range of nitrosamine testing items, but are also covering all kinds of chemical drugs.

As a common type of genotoxic impurity nitrosamine compound, N-nitrosodimethylamine (abbreviation: NDMA) can directly or indirectly damage cellular DNA, producing mutagenic and carcinogenic substances.

This paper presents a highly sensitive detection method for the analysis of N-nitrosodimethylamine (NDMA) in ranitidine using the Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap MS. The detection and quantitative limits fully meet FDA requirements. This method can effectively separate the main components of ranitidine and NDMA, and permit NMDA detection with high sensitivity and reproducibility. This method is also the preferred method recommended by the FDA.

Experimental Section

Instruments and reagents

- Thermo Scientific™ Vanquish™ Flex Binary UHPLC system
- Q Exactive Focus hybrid quadrupole-Orbitrap MS
- Thermo Scientific™ Hypersil GOLD™ Phenyl HPLC Columns (P/N: 25903-104630)
- Thermo Scientific Hypersil GOLD Phenyl Guard Column (P/N: 25903-014001)
- Guard Column Holder (P/N: 850-00)
- Methanol, LCMS grade (Fisher A456-4)
- Acetonitrile, LCMS grade (Fisher A955-4)
- Water, LCMS grade (Fisher W6-4)
- Methanoic acid, LCMS grade (Fisher A117-50)
- Thermo Scientific™ Titan3™, 17 mm PVDF filter, 0.22 µm (P/N: 42213 PV)

Sample pretreatment

NDMA standard stock solution

The NDMA standard was prepared by using pure methanol diluted to 100 ng/ml with pure methanol as the standard stock solution.

Sample preparation of API

Accurately weigh 120 mg of API sample into a 15 ml centrifuge tube, add 4 ml of pure methanol solution, and fully vortex mix until dissolved.

Following extraction, centrifuge at 4500 rpm for 15 mins, take the supernate and filter it through a 0.22 µm PVDF filter membrane, then place 1 ml of the filtrate into a sample injection vial for LCMS analysis.

Sample preparation of finished medicine

Roughly weigh several tablets (capsules) into a 15 ml centrifuge tube, add an appropriate amount of pure methanol, then vortex mix for 1 min and vibrate for 40 mins. After extracting the solution with an API concentration of 30 mg/ml, centrifuge at 4500 rpm for 15 mins, take the supernate and filter it through a 0.22 µm PVDF filter membrane, then place 1 ml of the filtrate into a sample injection vial for LCMS analysis.

Chromatographic conditions

This experiment uses the Thermo Scientific Hypersil GOLD Phenyl HPLC analytical column which offers good retention of NDMA, which can be effectively separated from the main components of ranitidine. It is moderately hydrophobic, has enhanced pi-pi interaction with aromatic compounds, and an excellent peak pattern and sensitivity. The column is particularly suitable for the analysis of benzene rings and aromatic ring compounds, with outstanding retention ability and unique selectivity for aromatic ring analytes.

Table 1. Chromatographic conditions

HPLC Column	Hypersil Gold Phenyl 4.6x100 mm, 3 µm		
Column Temp.	30°C		
Flow Rate	0.5 mL/min		
Mobile Phase A	0.1% formic acid in water		
Mobile Phase B	0.1% formic acid in acetonitrile		
Gradient	Time (min)	A%	B%
	0	95	5
	1	95	5
	3	80	20
	7	0	100
	9	0	100
	9	0	100
	9.1	95	5
	14	95	5
Injection Volume	5 µl		
Autosampler Temp.	6		
Needle Wash	80: 20, Methanol: Water with 0.1% Formic Acid		

Mass spectrometry conditions

This experiment uses the Q Exactive Focus hybrid quadrupole-Orbitrap MS, which can simultaneously offer high resolution and high sensitivity. The PRM (parallel reaction monitoring) mode is used to quantify the target, which can effectively eliminate matrix or background interference. At the same time, the qualitative and quantitative method is used to ensure the accuracy and reliability of the results.

Table 2. Mass spectrometry conditions

Instrument parameters	
Sheath Gas Flow Rate	55 arbitrary units
Aux Gas Flow Rate	15 arbitrary units
Sweep Gas Flow Rate	0 units
Spray Voltage	3.5 kV
Capillary Temp.	400
Aux Gas Heater Temp.	350
Scan Type	PRM
Polarity	Positive
Divert Valve	0–5 to Mass
Scan Start-End(min)	0–4.9
m/z Isolate for PRM	75.0553
NCE	
Isolation Window	1.5 m/z
Scan Range	50–95 m/z
Microscans	3
Resolution	35,000
AGC target	2e5
Maximum IT	100ms

Experimental result

Spectrogram result

Using the above instrument method, NDMA is successfully separated from the API main peak, and exhibits a good peak pattern and sensitivity. The ultrahigh resolution of the Orbitrap MS can effectively eliminate impurity interference.

Figure 1. Blank methanol, LOD and LOQ sample chromatograms

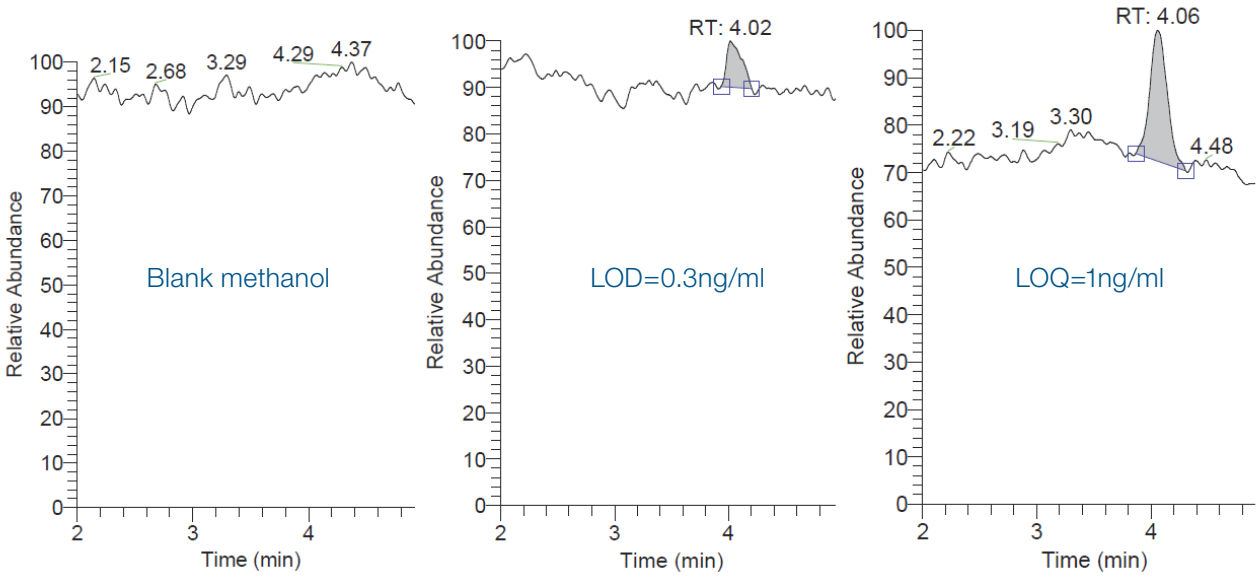
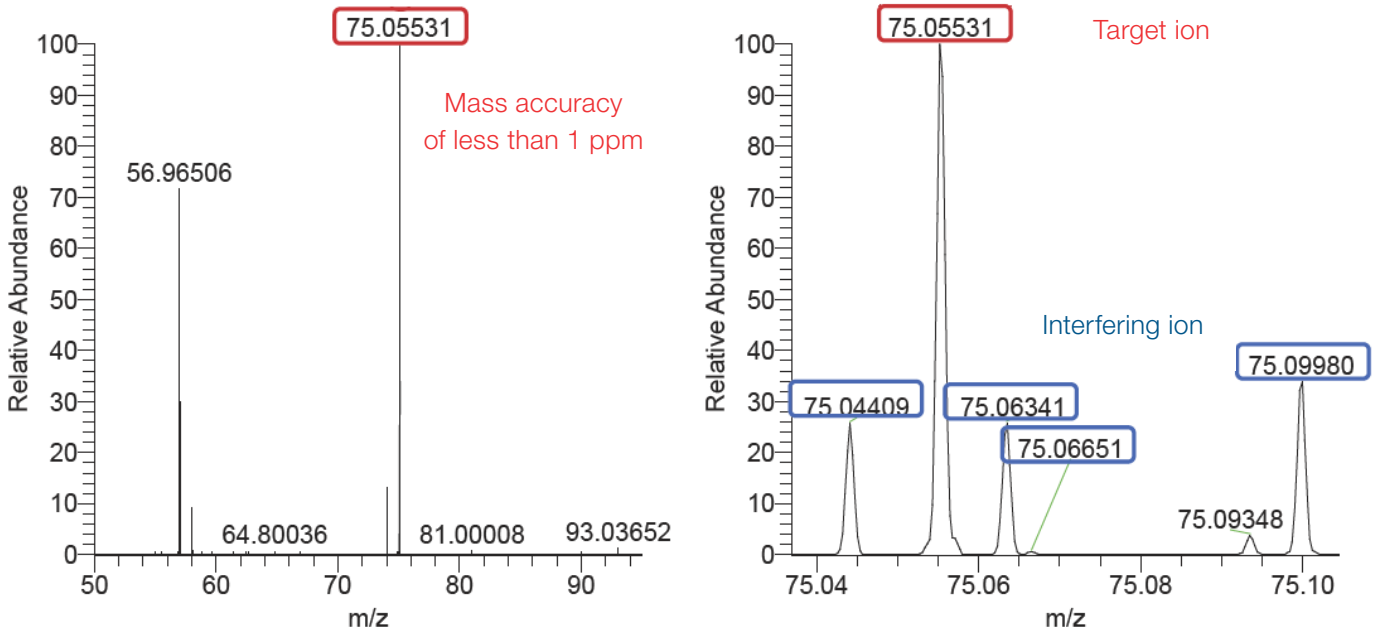


Figure 2. Mass spectrum of 1 ng/ml standard solution



Linear range and system adaptability test

Using the above instrument method, the linear correlation coefficient R2 of NDMA is greater than 0.99 when the concentration range is 1 ng/ml ~ 100 ng/ml. According to the requirements for sample injection sequence per FDA regulations, the relative standard deviation of the original 6 needles of standard solution (2 ng/ml) in the sequence is 2.05%. The relative standard deviation of standard solution in the entire sequence is 2.58%, and the retention time of NDMA in the sample deviates from the standard by less than 2%.

Figure 3. NDMA linear equation

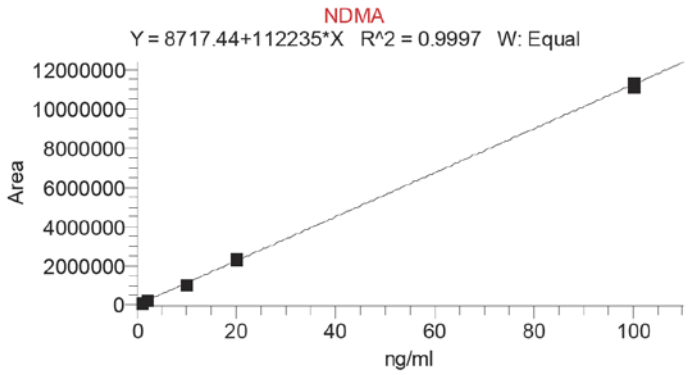


Figure 4. Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software

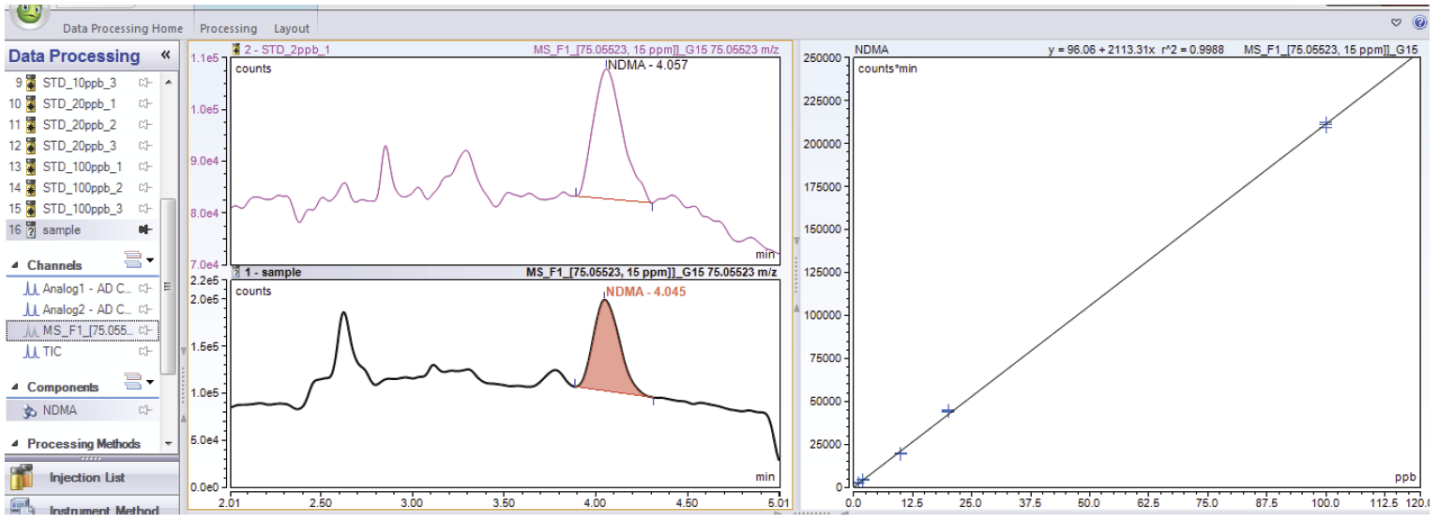
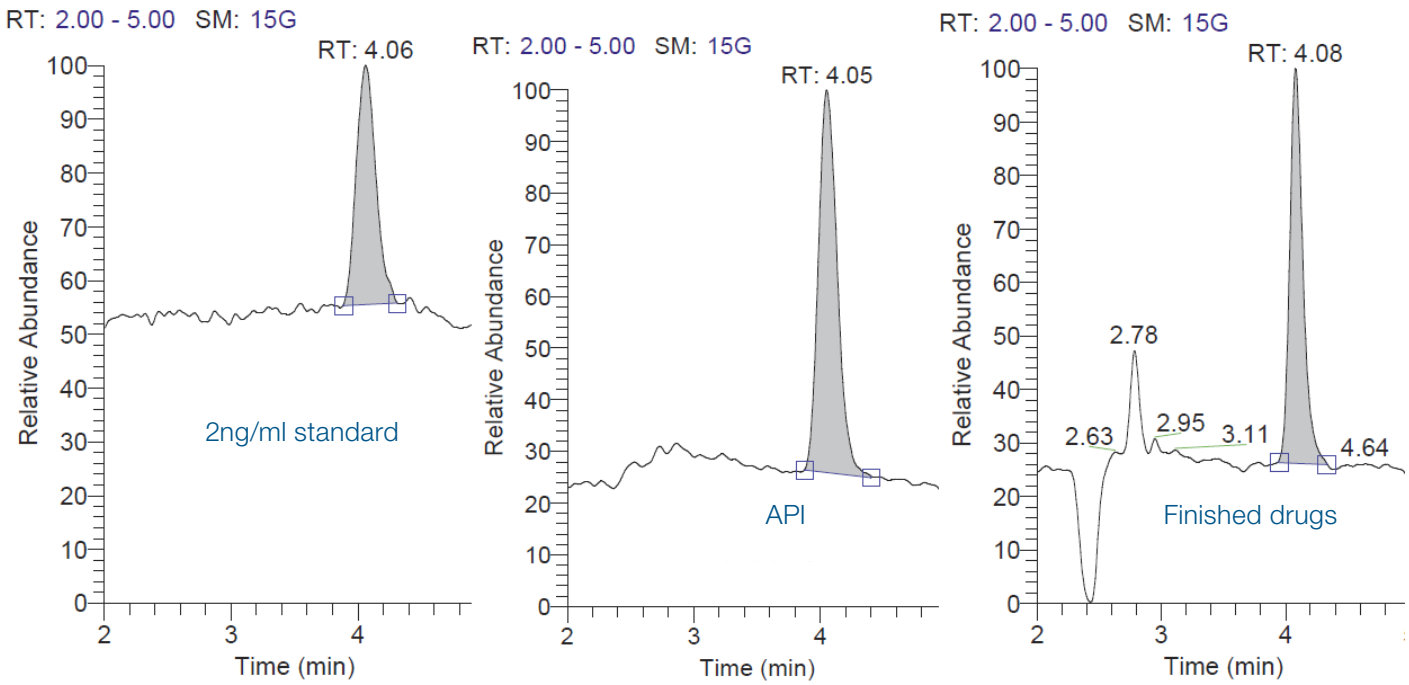


Table 3. Sample injection sequence and results

Sample injection sequence	Peak area	Retention time (min)
Blank_1	-	-
Blank_2	-	-
Standard_1	226766	4.05
Standard_2	221185	4.05
Standard_3	220338	4.06
Standard_4	227661	4.05
Standard_5	218950	4.06
Standard_6	230191	4.06
Blank_3	-	-
Sample_1-6	-	-
Standard_7	236567	4.06
Sample_7-12	-	-
Standard_8	227215	4.06
…	-	-

Figure 5. Sample chromatogram of NDMA in real samples



Conclusions

This paper presents a detection method for the analysis of genotoxic impurity N-nitrosodimethylamine (NDMA) in ranitidine using a Q Exactive Focus hybrid quadrupole-Orbitrap MS. The sensitivity and reproducibility of this method fully meet the FDA requirements. The Thermo Scientific™ Xcalibur™ and TraceFinder™ applications are suitable for routine testing requirements, along with the fully compliant Thermo Scientific Chromeleon Chromatography Data System (CDS) software, providing comprehensive solutions for a wide variety of users.

Reference Materials

1. FDA FY19-107-DPA-S_ Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of Six Nitrosamine Impurities in ARB Drugs (https://www.fda.gov/media/125478/download)
2. FDA FY19-177-DPA-S_ Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of NDMA in Ranitidine Drug Substance and Drug Product (https://www.fda.gov/media/130801/download)