APPLICATION NOTE 73472

High-resolution accurate-mass liquid chromatography mass spectrometry (HRAM LC-MS) methodology for the determination and quantitation of nitrosamine impurities in drug products

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Application benefits

- Panel of 11 nitrosamines in one run
- HRAM confirmation of impurities with high confidence
- Quantitation of nitrosamines in drug substance and finished drug product samples in line with currently expected regulatory guidelines
- Acquisition to reporting in GMP compliance-ready software with data integrity and 21CFR11 toolset

Goal

Develop and establish a simple and fast method, consistent with current FDA recommendations, for the quantitative analysis of the most common nitrosamines in finished drug product formulations and drug substances.

Introduction

The recent past has seen recalls of different small molecule drug products due to the risk of them containing unacceptable levels of nitrosamine impurities.[1] Nitrosamines belong to the larger class of genotoxic impurities (GTI), which are known to be carcinogenic and are potentially mutagenic. They may be formed either during synthesis, formulation, or even storage. The acceptable limits of GTIs

for pharmaceutics are stated in guidelines issued by the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) to keep the daily intake below the threshold of toxicological concern (TTC) of 1.5 µg.

This raises a clear need for analytical methods that enable the reliable and fast determination of the most commonly known nitrosamine impurities in an easy and cost-effective manner. High-resolution accurate-mass liquid chromatography-mass spectrometry (HRAM LC-MS) offers the platform of choice for fast and high-performance chromatographic separation impurities from the different components of the drug formulation, as well as for exceptionally sensitive, accurate, and highly selective mass spectrometric detection of these components for confident identification and reliable quantitation.

A method based on the use of a Thermo Scientific™ Vanquish™ Flex UHPLC system coupled to a Thermo Scientific™ Q Exactive™ Plus hybrid quadrupole-Orbitrap™ mass spectrometer was developed for the analysis of 11 nitrosamine components in drug substances and drug formulations. Sample preparation follows a fast, easy, and cost-effective approach. Extraction of nitrosamine impurities from drug substances and drug formulations was carried out as described in the FDA guideline FY19-177-DPA-S[2].

Experimental

Target analytes

- *N*-Nitrosodimethylamine $C_2H_6N_2O$ (NDMA)
- *N*-Nitrosodibutylamine $C_8H_{18}N_2O$ (NDBA)
- *N*-Nitrosodi-n-propylamine $C_6H_{14}N_2O$ (NDPA)
- *N*-Nitrosomethylethylamine $C_3H_8N_2O$ (NMEA)
- *N*-Nitrosodiethylamine $C_4H_{10}N_2O$ (NDEA)
- *N*-Nitrosopyrrolidine $C_4H_8N_2O$ (NPYR)
- *N*-Nitrosopiperidine $C_5H_{10}N_2O$ (NPIP)
- *N*-Nitrosomorpholine $C_4H_8N_2O_2$ (NMOR)
- *N*-Nitrosodiphenylamine $C_{12}H_{10}N_2O$ (NDPhA)
- *N*-Nitroso-ethylisopropylamine $C_5H_{12}N_2O$ (NEIPA)
- *N*-Nitroso-diisopropylamine $C_6H_{14}N_2O$ (NDIPA)

A standard mixture of nitrosamines was purchased from Sigma-Aldrich (Taufkirchen, Germany). NEIPA and NDIPA were purchased from LGC (Wesel, Germany).

Calibration standards

A stock solution with a concentration of 200 ng/mL for each of the nitrosamines in methanol was prepared from standard solutions. Methanol dilutions were used to generate calibration solutions with concentrations of 100, 50, 10, 5, 1, and 0.5 ng/mL.

Drug substance sample preparation

30 mg of the drug substance was weighed into a 10 mL glass vial, and methanol was added to produce a precise concentration of 30 mg/mL. The solution was mixed on a vortex mixer until all solids were fully dissolved.

Liquid chromatography

Parameter	Value		
HPLC column	Thermo Scientific™ Acclaim™ PolarAdvantage II, 100 × 2.1 mm, 2.2 µm (P/N 068990)		
Column temperature	40 °C		
Flow rate	0.5 mL/min		
Mobile phase A	Water + 0.1% formic acid		
Mobile phase B	Methanol + 0.1% formic acid		
Gradient	Time (min)	%A	%B
	0.0	95	5
	0.5	95	5
	8.0	5	95
	9.0	5	95
	9.1	95	5
	12.0	95	5
Injection volume	2 µL		

Mass spectrometry

Parameter	Value
Instrument	Thermo Scientific Q Exactive Plus mass spectrometer
Spray voltage	4000 V
Capillary temperature	300 °C
Sheath gas	60.0 arb
Aux gas	10.0 arb
Sweep gas	0.0 arb
Heater temperature	450 °C
Scan mode	PRM

Drug product sample preparation

Drug product tablets were crushed in a mortar and weighed accurately to take an amount of powder that contains the equivalent of 30 mg drug substance (or API). Methanol (1 mL) was added and the resulting mixture was shaken for 40 min using a mechanical wrist action shaker (speed 132; temperature 24 °C). The sample was centrifuged for 15 minutes at 4200 rpm. The supernatant was transferred into a 1.8 mL glass autosampler vial.

Method evaluation

Linearity was evaluated by collecting calibration curve data (n=3). Calibration was obtained on each day of analysis by plotting the peak area against the concentrations of the calibration standards. Calibration was performed in linear mode with a 1/× weighting.

Within a batch, precision was measured as percentage coefficient of variation (%CV), and accuracy was measured as the bias of the result, expressed as a percentage for the 10 repeat injections of a specific quality control sample spiked at a level of 5 ng/mL.

Data analysis

Data were acquired and processed using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, to meet modern regulatory requirements including United States Food and Drug Administration (US FDA) 21 CFR Part 11 and European Commission (EU) Annex 11.

Results and discussion

The analysis was carried out on a benchtop hybrid quadrupole Orbitrap high-resolution mass spectrometer using PRM scan mode with a resolution setting of 35,000 *@ m/z* 200, to achieve a highly sensitive and selective measurement.

For chromatographic separation, different column chemistries were tested, including the Thermo Scientific™ Hypersil GOLD™ C18 and Thermo Scientific™ Accucore™ aQ columns, but the polar embedded Acclaim PolarAdvantage II separation column gave the best combination of compound retention and peak shape for all nitrosamines analyzed. All compounds could be detected at the lowest calibration level of 0.5 ng/mL.

Figure 1 shows chromatograms of all compounds at this level. Intra-batch reproducibility was tested with a separate quality control sample spiked at a concentration level of

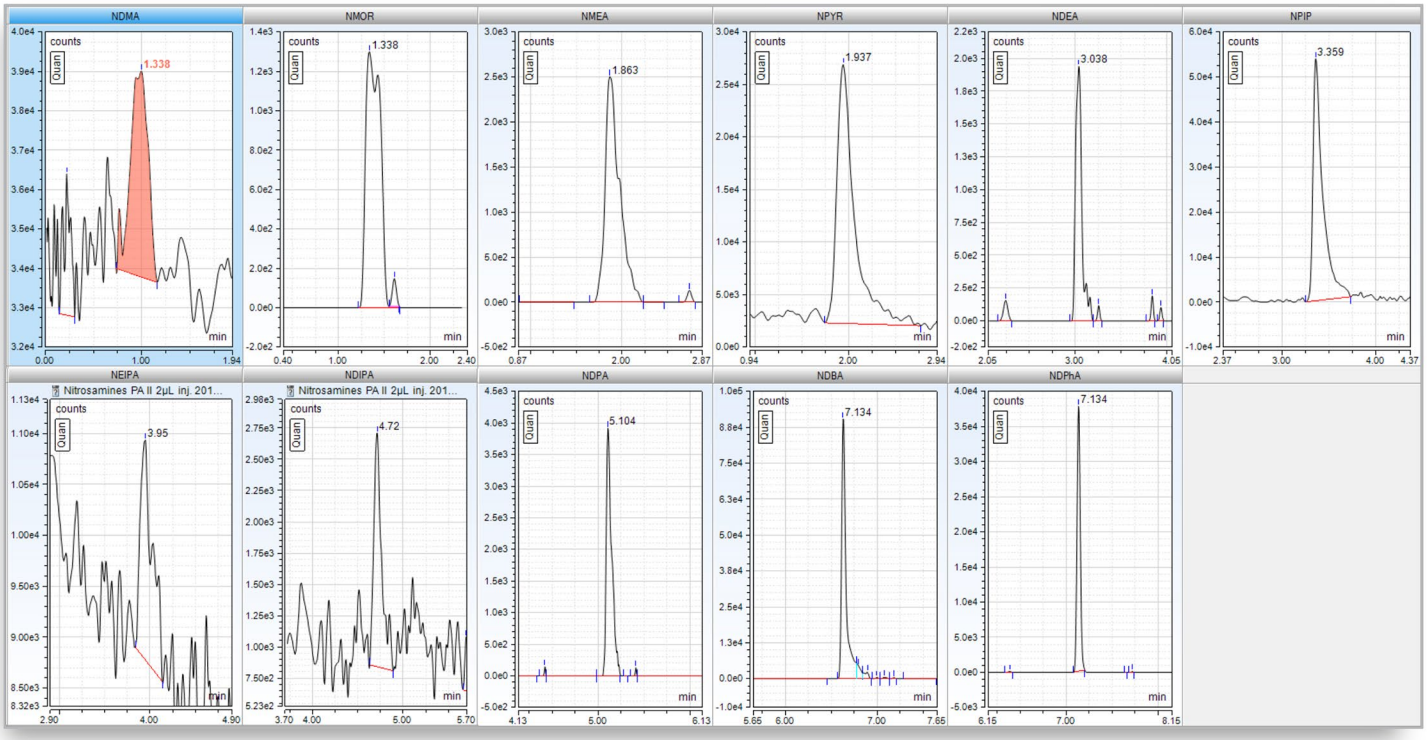

Figure 1. Extracted ion chromatograms of all compounds at 0.5 ng/mL

5 ng/mL and injected 10 times. Excellent reproducibility was achieved with relative standard deviations (RSD) between 0.32 and 3.15%—details shown in Table 1. Linearity of calibration was excellent for all compounds, the coefficient of correlation (r^2) is above 0.99 in all cases. Figure 2 shows calibration lines of the compounds analyzed. The sensitivity of the method was consistently high; Table 2 shows the instrument detection levels (IDL) calculated from the calibration data. The FDA requests a reporting threshold of 0.03 ppm of *N*-nitrosamine in the drug product. According to the published protocol for sample preparation,[2] this relates to a concentration of 1 ng/mL of *N*-nitrosamine in the injected sample. This can be easily met with the method described here.

Table 1. Reproducibility and accuracy data of 10 repeats of the check standard at 5 ng/mL

Compound	%Diff	%RSD	%CV
NDBA	-2.38	0.96	0.65
NDEA	1.74	0.96	1.06
NDIPA	3.07	2.22	2.73
NDMA	2.94	2.70	2.94
NDPA	-2.94	0.71	0.68
NDPhA	-3.60	1.16	1.14
NEIPA	0.97	2.40	2.92
NMEA	-3.82	3.15	3.39
NMOR	-3.29	1.60	1.60
NPIP	-1.56	0.32	0.32
NPYR	0.81	1.21	1.25

Table 2. Detection limits of the method

Compound	IDL (ng/mL)	IDL* (ng/mL)
NDBA	0.15	0.01
NDEA	0.15	0.01
NDIPA	0.35	0.02
NDMA	0.43	0.03
NDPA	0.11	0.01
NDPhA	0.18	0.01
NEIPA	0.38	0.03
NMEA	0.50	0.03
NMOR	0.25	0.02
NPIP	0.05	0.01
NPYR	0.19	0.01

* in relation to 30 mg of drug substance in the processed sample according to the FDA recommended sample preparation

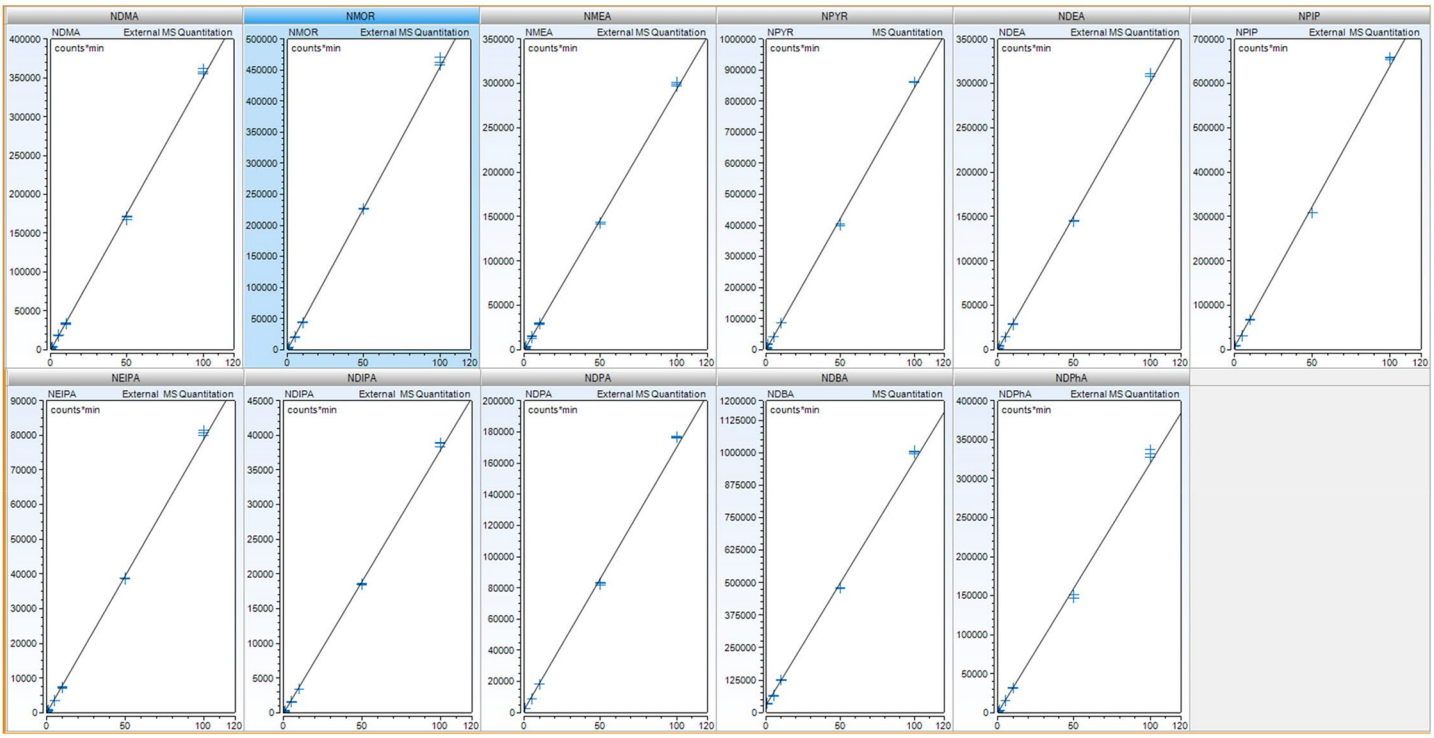

Figure 2. Calibration curves of all compounds

Additionally, commercially available standards of valsartan and ranitidine were analyzed. NDMA could be quantified at a level of 37 ng/mL in the valsartan standard, equaling 1.23 ppm NDMA in the drug substance. Likewise, NDMA could be quantified at a level of 25 ng/mL in the ranitidine standard, equaling 0.83 ppm NDMA in the drug substance (Figure 3). Drug products were also analyzed where a sample of the over-the-counter drug ranitidine, deliberately exceeding the use-by date by several years, showed a significant amount of NDMA. The detected peak area was roughly 400 times higher than the highest available calibration standard (100 ng/mL), which was outside of the linear range of calibration (Figure 4).

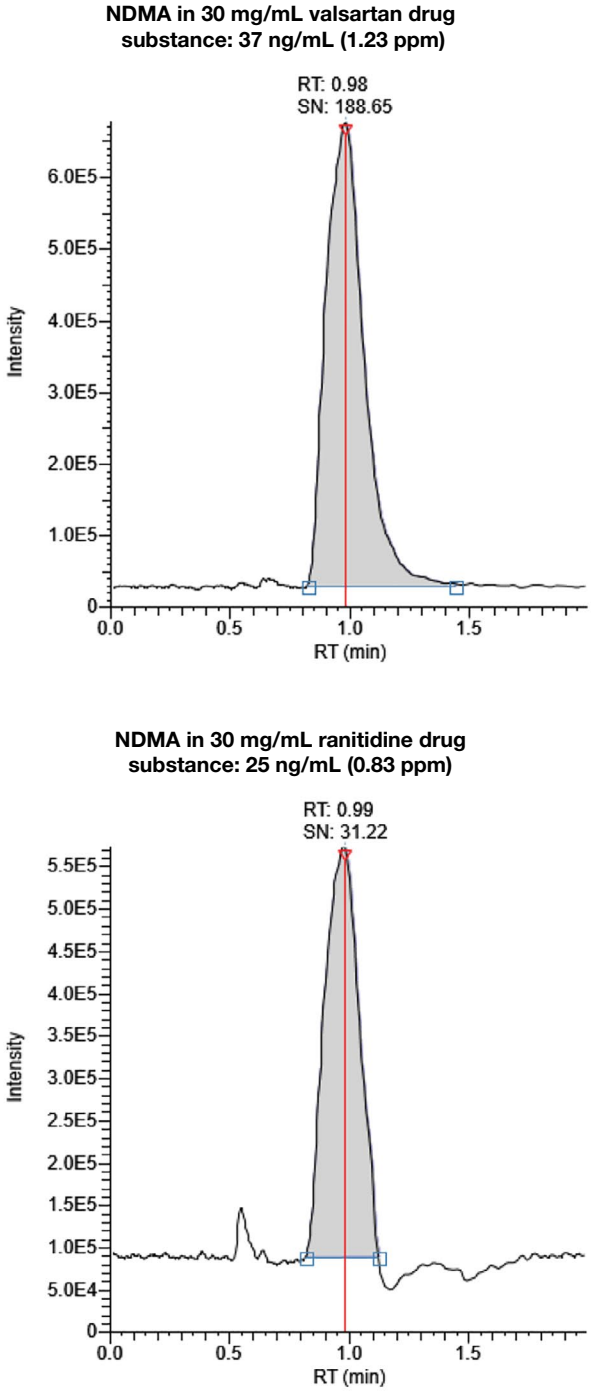

Figure 3. Example of NDMA content of selected commercially available drug standards

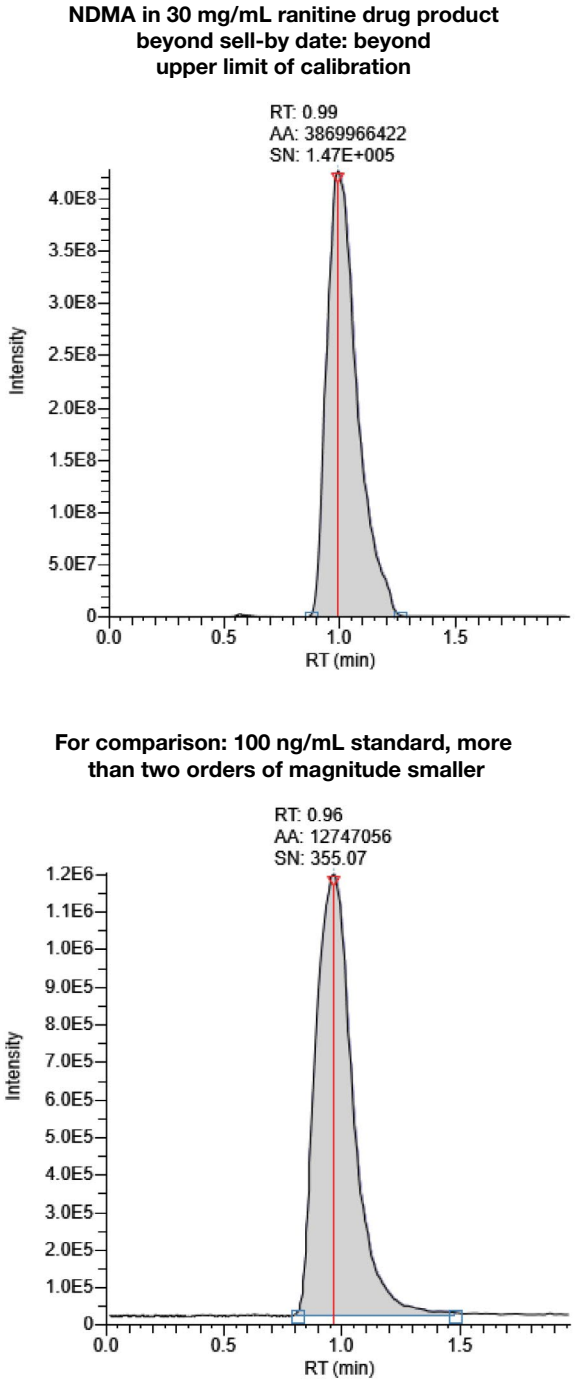

Figure 4. Example of NDMA content in an outdated sample of a ranitidin drug sample

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Conclusions

Eleven nitrosamines were analyzed on a Thermo Scientific Q Exactive Plus mass spectrometer using PRM scan mode. The lowest calibrator (0.5 ng/mL) was detected for all compounds. The linearity of the calibration was excellent with a coefficient of correlation (r^2) better than 0.99 for all compounds. Reproducibility of quality control (10 replicates) showed RSDs between 0.32 and 3.15%.

References

1. https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-angiotensin-ii-receptor-blocker-arb-recalls-valsartan-losartan
2. United States Food and Drug Administration (USFDA) (2019). 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. Accessed Oct. 10, 2019.

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