LC-HRMS Based Analytical Platform to Determine Nitrosamines in Pharmaceuticals: Modern Analytical Techniques To Meet Regulatory Needs

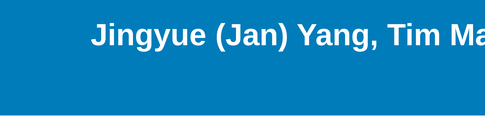
Jingyue (Jan) Yang, Tim Marzan, Raghavi Kakarla, Wei Ye, Cynthia (Cindy) Sommers, Jason Rodriguez, and David Keire
FDA/CDER/OPQ/OTR, 645 Newstead Ave., Saint Louis, MO 63110

Abstract

Since 2018, various types of nitrosamine impurities have been found in several medicines. Because most nitrosamines are probable or possible human carcinogens, the FDA developed and validated multiple mass spectrometry based analytical methods to test the affected drug products and identify batches with unacceptably high levels of nitrosamines. Among these methods are those based on liquid chromatography-high resolution mass spectrometry (LC-HRMS), a novel analytical technique for quantitation in a regulated environment. These LC-HRMS methods are capable of detecting and quantitating as many as eight different nitrosamine impurities in various drug products with quantitation limits as low as 0.005 ppm (0.005 µg of nitrosamine per gram of API). The high selectivity has allowed for the differentiation of nitrosamine-like impurities from actual nitrosamines, ensuring the reliability of test results. These LC-HRMS methods have been used by the FDA to test for nitrosamines in hundreds of drug product lots and have also been published on the Agency's website to provide method development examples for industry. Applying LC-HRMS techniques to evaluate nitrosamine contamination exemplifies the benefit of adopting modern analytical techniques to meet regulatory needs.

Introduction

- N-Nitrosamines are probable or possible human carcinogens and belong to a 'cohort of concern' as defined in ICH M7 (ref. 1). Contamination by N-nitrosamines has led to recalls of ARB drugs and metformin as well as market withdrawal of ranitidine.



ARB (Angiotensin II receptor blockers) (blood pressure medication) | Ranitidine (Zantac) (treatment of acid reflux) | Metformin (diabetes medication)

Figure 1. Drugs that were affected by the contamination of nitrosamines, including ARB, Ranitidine (Zantac), and Metformin

- N-Nitrosodimethylamine (**NDMA**), N-Nitrosodiethylamine (**NDEA**), N-Nitroso-N-methyl-4-aminobutyric acid (**NMBA**) and N-nitroso-methylphenylamine (**NMPA**) have been found in medications by FDA. Several other N-nitrosamines are also suspected and were screened, including N-ethyl-N-nitroso-2-propanamine (**NEIPA**), N-nitroso-diisopropylamine (**NDIPA**), N-nitroso-di-n-butylamine (**NDBA**), and N-nitroso-di-n-propylamine (**NDPA**)

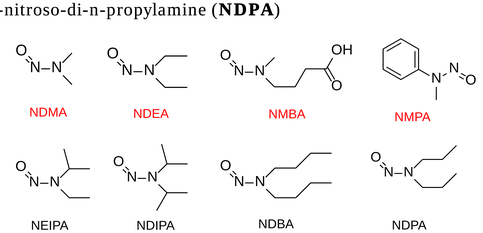


Figure 2. Chemical structures of N-nitrosamines. Those in red have been found in tested drug products.

Introduction

- Low resolution mass spectrometers (MS) with single or triple quadrupole mass analyzers have been predominantly used for the detection and quantitation of trace level N-nitrosamines.
- 'MS resolution' measures the ability of a mass spectrometer to differentiate two ion peaks with slightly different mass-to-charge ratios (m/z). A resolution of > 10,000 is considered high resolution mass spectrometry (HRMS) (ref. 2).

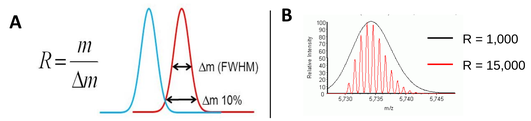


Figure 3. A: MS Resolution (R) can be expressed as $\frac{m}{\Delta m}$, where Δm is the width of the ion peak at a height which is a specified faction of the maximum peak height (IUPAC, USP <1736>). **B**: the mass spectrum of insulin is observed as a group of separated isotopic ion peaks at a resolution of 15,000, but as a single ion peak at a resolution of 1,000.

- TOF and Orbitrap are the most common mass analyzers for HRMS.

Mass Analyzer	TOF (time of flight)	Orbitrap
Resolution	30,000 – 40,000	500,000
Mass Range (m/z)	Up to 100,000	Up to 8,000
Mass Error (ppm)	< 3	< 3

(ref. 2 with update; resolution varies depending on the actual model)

- HRMS provides enhanced selectivity, accurate mass measurement for chemical structure elucidation, high sensitivity in full MS acquisition, and allows retrospective analysis.

Materials

- HPLC or UHPLC systems
- High resolution mass spectrometer:
  - Orbitrap Q Exactive™, HF, or HF-X hybrid quadrupole-orbitrap
  - Orbitrap Exploris 480
- HPLC columns
- N-nitrosamine reference standards
- Drug substances and drug products: valsartan, losartan, ranitidine, metformin, chloroquine, hydroxychloroquine

Results and Discussion

LC-HRMS Platform for Nitrosamine Detection and Quantitation

LC conditions	
Column	Suitable for the separation of active ingredient and nitrosamines
Mobile phase	0.1% formic acid in water
	0.1% formic acid in methanol
Column Temperature	30 – 40 ˚C
Flow rate	0.3 – 0.6 mL/min
Gradient	Suitable for the separation of active ingredient and nitrosamines
Injection volume	3 – 5 µL

Results and Discussion

LC-HRMS Platform for Nitrosamine Detection and Quantitation

HRMS conditions

Ionization: ESI or APCI
Scan conditions:
- Mode: Selected ion monitoring (SIM) or product ion scan (MS2)
- Resolution: 60,000 or 70,000 for SIM scan, and 35,000 or 45,000 for MS2 scan

Nitrosamine	Monoisotopic mass (Da)	Scan polarity	Scan mode
NDMA	74.0480	(+)	MS2 of m/z 75.0553
NDEA	102.0793	(+)	SIM of m/z 103.0866
NEIPA	116.0950	(+)	MS2 of m/z 117.1022
NDIPA	130.1106	(+)	SIM of m/z 131.1179
NDPA	130.1106	(+)	SIM of m/z 131.1179
NMPA	136.0637	(+)	SIM of m/z 137.0709
NDBA	158.1419	(+)	MS2 of m/z 159.1549
NMBA	146.0691	(-)	SIM of m/z 145.0619

Data Processing

Extracted ion chromatograms (EIC) of the *m/z* values in the table are performed with a mass extraction window of +/- 15 ppm

Nitrosamine	*m/z* to be extracted	Nitrosamine	*m/z* to be extracted
NDMA	75.0553	NDPA	131.1179
NDEA	103.0866	NMPA	137.0709
NEIPA	75.0553	NDBA	103.0866 or 57.0703
NDIPA	131.1179	NMBA	145.0619

LC-HRMS platform has been used for nitrosamine determination in various medicines

Application	Nitrosamine Analyzed	LOD and LOQ	Availability
Valsartan, losartan and other ARBs	NDMA, NDEA, NEIPA, NDIPA, NDBA, NMBA	0.003 – 0.01 ppm 0.05 ppm	FDA Website
Ranitidine	NDMA	0.01 ppm 0.03 ppm	FDA Website
Metformin	NDMA	0.01 ppm 0.03 ppm	FDA Website
Metformin	NDMA, NDEA, NEIPA, NDIPA, NDPA, NDBA, NMPA, NMBA	0.001 – 0.005 ppm 0.005 – 0.02 ppm	FDA Website
Chloroquine & Hydroxychloroquine	NDMA, NDEA, NEIPA, NDIPA, NDPA, NDBA, NMPA	0.003 – 0.006 ppm 0.02 ppm	In house

HRMS provides high selectivity that differentiates nitrosamine-like impurities from actual nitrosamines (ref. 3)

- *N*,*N*-Dimethylformamide (DMF), a common residual solvent in drug products, has a similar chemical structure and mass as NDMA.

DMF
Chemical Formula: C_3H_7NO
Monoisotopic Mass: 73.0528

NDMA
Chemical Formula: $C_2H_6N_2O$
Monoisotopic Mass: 74.0480

- DMF and NDMA also have similar fragmentation patterns when subject to MS fragmentation.
- Quantitation by low resolution MS generally employs a multiple reaction monitoring scan (MRM) which selectively detects an analyte by scanning the transition of the parent ion to a specified fragment. DMF interferes with the MRM transition for NDMA (m/z 75.1 → 43.1) when both compounds are present in a sample, and are not chromatographically separated, leading to false positive results of NDMA. (Figure 4)

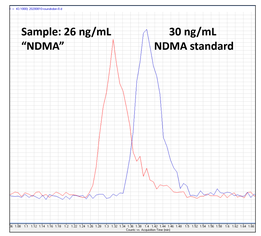


Figure 4. 26 ng/mL "NDMA" (false positive) is found in a valsartan drug product sample containing both DMF and NDMA by MRM (m/z 75.1 → 43.1) performed on a triple quadrupole mass spectrometer (resolution < 7500), while only 1 ng/mL of NDMA is actually present in the sample

- HRMS resolves the ^{15}N isotopic ion peak of DMF (*m/z* 75.0570) from the monoisotopic ion of NDMA (*m/z* 75.0553) (Figure 5), allowing NDMA to be detected and quantitated selectively without interference from DMF.

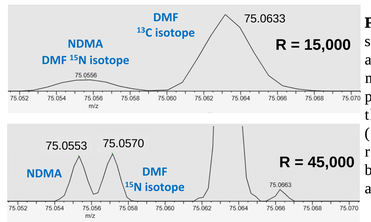


Figure 5. Mass spectra of a sample containing both NDMA and DMF acquired at different mass resolutions. The ^{15}N isotope peak of DMF (m/z 75.0570) and the NDMA monoisotopic peak (m/z 75.0553) are resolved at a resolution of 45,000 (bottom), but remain as one broad ion peak at a resolution of 15,000 (top).

Conclusion

Analytical methods based on LC-HRMS techniques have been developed and used by the Agency to analyze nitrosamines in various pharmaceuticals, highlighting the benefit of modern techniques for regulatory testing.

References

1. ICH Guideline M7 (R1) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk
2. Wood, M, Chapter 14 – High-Resolution Mass Spectrometry: An Emerging Analytical Method for Drug Testing, Critical Issues in Alcohol and Drugs of Abuse Testing (2nd edition), Academy Press, 2019, 173 - 188
3. Yang, J, Marzan, TA, Ye, W, Sommers, CD, Rodriguez, JD, Keire, DA, A Cautionary Tale: Quantitative LC-HRMS Analytical Procedures for the Analysis of N-Nitrosodimethylamine in Metformin, AAPS J. 2020; 22(4) 89

Disclaimer The ideas, findings, and conclusions in this presentation have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.