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Nota de aplicación

Development of a Sensitive High Resolution Mass Spectrometry Method for Quantitation of *N*-Nitrosamines using Tof MRM on the Xevo G2-XS QTof

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Monitoring of *N*-nitrosamines in various matrices is of high interest, due to their potential carcinogenicity and detection in a variety of commodities and pharmaceuticals consumed by humans. These impurities require sensitive and selective detection in the sub-ng/mL range. The following method is capable of simultaneous detection and quantitation of six widely analyzed *N*-nitrosamines (NDMA, NDEA, NMBA, NDBA, NEIPA, and NDIPA) at or less than 0.1 ng/mL, using a highly sensitive and selective QTof acquisition mode. Referred to as Tof MRM, this acquisition mode provides selectivity through isolation of the analyte precursor *m/z* in the quadrupole, followed by a targeted increase in signal. Presented here are the UPLC chromatographic separation and LLODs, LLOQs, and linearity for the six *N*-nitrosamines analyzed using the Tof MRM method. Quantitative performance of this method is reported for NDMA and NDEA, demonstrating precise, accurate and repeatable measurement at both 1.25 and 12.5 ng/mL quality control (QC) levels. Furthermore, the use of the UNIFI Scientific Information System for acquisition and data processing offers a modern platform for GxP-compliant HRMS analysis of the investigated *N*-nitrosamines.

Benefits

- Sensitive, selective, and specific detection and quantitation of 6 high-profile *N*-nitrosamines using Tof MRM on the Xevo G2-XS QTof
- Robust chromatographic separation using the ACQUITY UPLC I-Class with HSS T3 Column
- Advanced informatics with the UNIFI Scientific Information System for compliant instrument control and data management

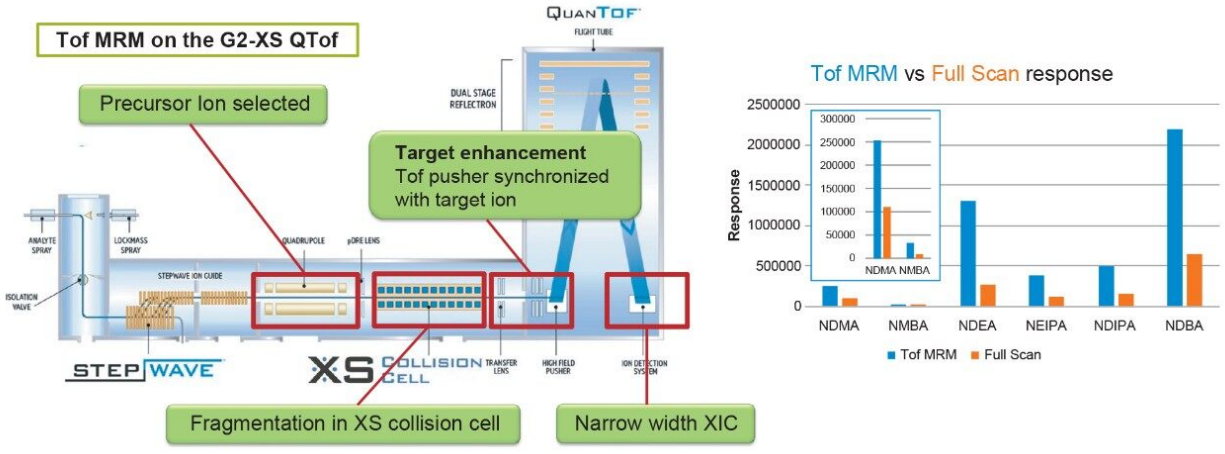
Introduction

N-nitrosamines are small molecule compounds generally formed as reaction products between nitrate and amines[1]. These compounds are known potential carcinogens[2], therefore warranting active monitoring to mitigate human exposure. They can be found occurring in natural water systems[2], tobacco products[2], and as impurities in pharmaceutical products[1,3,4]. Particular attention has been drawn to the 2019 detection of *N*-nitrosodimethylamine (NDMA) in ranitidine, a histamine-2 blocker available over the counter for the treatment of gastroesophageal reflux disease and stomach/intestinal ulcers[5]. NDMA, *N*-nitrosodiethylamine (NDEA) and *N*-nitroso-N-methyl-4-aminobutyric acid (NMBA) have also been found as impurities in

angiotensin II receptor blocker (ARB) drug products (the "-sartan" class of drugs) which are used to treat heart failure and high blood pressure[4]. It is suspected in the ARB drugs that three other *N*-nitrosamines, *N*-nitrosoethylisopropylamine (NEIPA), *N*-nitrosodiisopropylamine (NDIPA) and *N*-nitrosodibutylamine (NDBA) could also be present[4].

Active monitoring of these harmful compounds at relatively low concentrations (ng/mL or lower) is of high priority and relies on highly sensitive and selective analytical methods. Use of liquid chromatography-mass spectrometry (LC-MS) has been successfully applied for the confident identification and quantification of *N*-nitrosamines in a range of matrices[1,2]. Specifically, use of targeted acquisitions such as multiple reaction monitoring (MRM) on tandem quadrupole MS has proven to attain lower limits of quantification (LLOQ's) at or below 0.1 ng/mL for *N*-nitrosamines[6,7]. Methods using high resolution MS (HRMS) platforms for *N*-nitrosamines analysis have also been explored[4,8] and afford additional specificity through the use of accurate mass measurement.

Here, we demonstrate the ability to attain both sensitive and selective detection of six *N*-nitrosamines (NDMA, NDEA, NDIPA, NEIPA, NMBA, and NDBA) using Tof-MRM acquisition on the Xevo G2-XS QTof. This methodology isolates the known precursor molecule in the quadrupole, followed by targeted enhancement of a specified precursor or product ion(s). This combination produced increased selectivity and sensitivity of the *N*-nitrosamines over typical full scan MS, with the added benefit of specificity of accurate mass measurement inherent to HRMS.



Schematic describing the Tof MRM acquisition mode on the Xevo G2-XS QTof, which results in an increased response for targeted N-nitrosamine analytes over traditional full scan mode.

Results and Discussion

Analysis of NDMA, NMBA, NDEA, NDIPA, NEIPA, and NDBA was carried out using the ACQUITY UPLC I-Class and Xevo G2-XS QTof MS with the Ion Sabre II APCI probe. Data acquisition, processing and review was carried out using the UNIFI Scientific Information System, a comprehensive informatics platform which contains features for GxP compliance such as data traceability and advanced security[9]. Separation of the 6 nitrosamines (shown in Figure 1) was achieved using a previously described[6] LC method using the ACQUITY UPLC HSS T3 Column (p/n 186003539). Table 1 shows the LC-MS conditions employed for this analysis. Following retention time and optimal ion transmission settings determination, the development of a targeted QTof method was performed using Tof MRM acquisition. Tof MRM operates by time-segmented selection of the specified precursor mass, which is isolated in the quadrupole and then sent to the collision cell where either ion transmission or collision induced dissociation (CID) occurs. Then, user specified *m/z* value(s) are subjected to targeted signal enhancement through pusher synchronization in the Tof region[10]. Accurate mass measurement is then achieved for all ions, and an extracted ion chromatogram is generated by the UNIFI Software for the specified *m/z* values. For the *N*-nitrosamine compounds in this study, targeted enhancement was performed for NDMA, NMBA, NDIPA, NEIPA, and NDBA of both precursor and selected product ions. For NDEA, targeted enhancement was performed only for the precursor ion, owing to an abundance of signal. Figure 2 shows the final Tof MRM acquisition method, for which optimized collision energies were determined experimentally as part of method development.

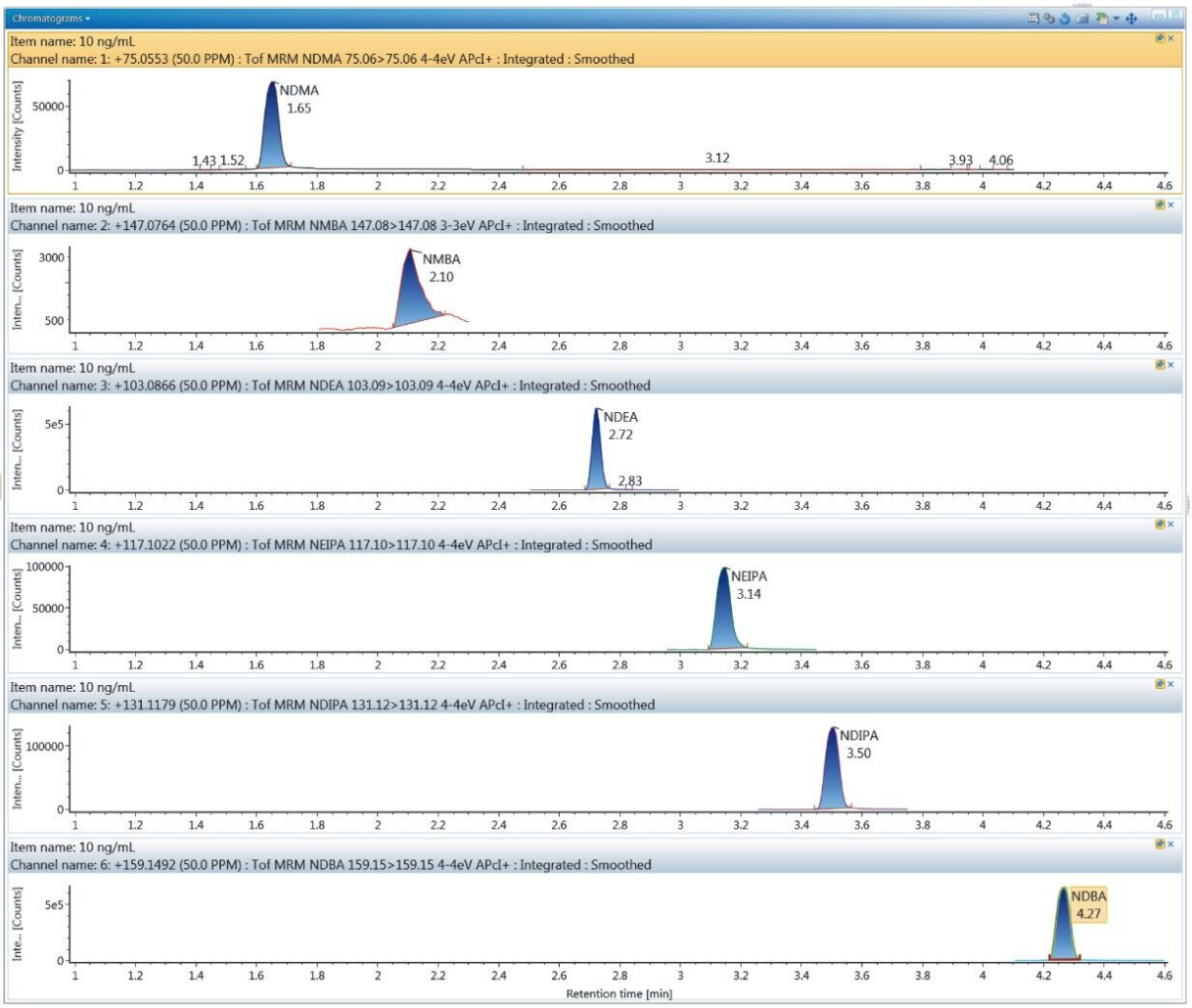
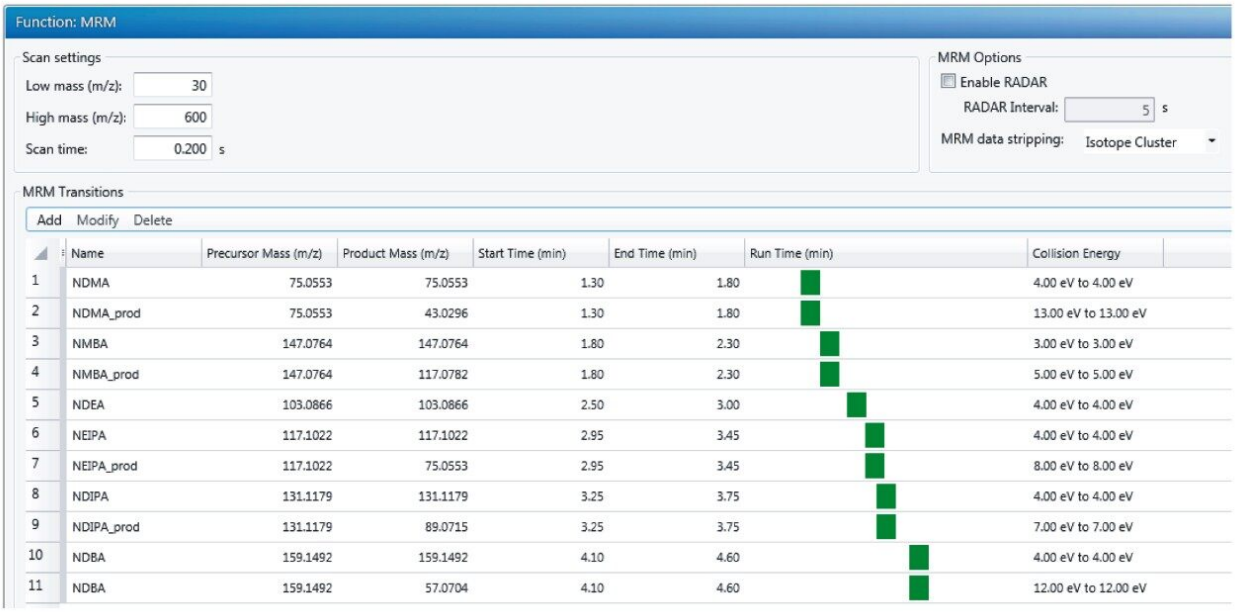


Figure 1. Extracted ion chromatograms at 10 ng/mL of all 6 nitrosamines.

LC system	ACQUITY UPLC I-Class (FTN)				
Column	ACQUITY UPLC HSS T3, 1.8 µm, 2.1 × 100 mm				
Column temp.	40 °C				
Flow rate	0.4 mL/min				
Injection volume	30 µL				
Mobile phase	A-5 mM ammonium formate and 0.1% formic acid in water				
	B-5 mM ammonium formate and 0.1% formic acid in methanol				
LC gradient	Time (min)	Flow rate (mL/min)	%A	%B	Curve
	Initial	0.400	98	2	Initial
	0.24	0.400	98	2	6
	4.00	0.400	5	95	6
	4.61	0.400	5	95	6
	5.00	0.400	98	2	6
	7.00	0.400	98	2	6
MS system	Xevo G2-XS QTof				
Ionization mode	APCI+				
Analyzer mode	Sensitivity				
Desolvation flow	1000 L/Hr				
Cone gas flow	50 L/Hr				
Source temp.	120 °C				
Corona current	2.1 µA				
Cone voltage	2.0 V				
Probe temp.	200 °C				
Lock mass	Leucine Enkephalin (278.1135; 556.2766 *m/z*)				
Data management	UNIFI Scientific Information System 1.9.4				

Table 1. LC-MS conditions employed for the analysis of N-nitrosamines.



	Name	Precursor Mass (m/z)	Product Mass (m/z)	Start Time (min)	End Time (min)	Run Time (min)	Collision Energy
1	NDMA	75.0553	75.0553	1.30	1.80		4.00 eV to 4.00 eV
2	NDMA_prod	75.0553	43.0296	1.30	1.80		13.00 eV to 13.00 eV
3	NMBA	147.0764	147.0764	1.80	2.30		3.00 eV to 3.00 eV
4	NMBA_prod	147.0764	117.0782	1.80	2.30		5.00 eV to 5.00 eV
5	NDEA	103.0866	103.0866	2.50	3.00		4.00 eV to 4.00 eV
6	NEIPA	117.1022	117.1022	2.95	3.45		4.00 eV to 4.00 eV
7	NEIPA_prod	117.1022	75.0553	2.95	3.45		8.00 eV to 8.00 eV
8	NDIPA	131.1179	131.1179	3.25	3.75		4.00 eV to 4.00 eV
9	NDIPA_prod	131.1179	89.0715	3.25	3.75		7.00 eV to 7.00 eV
10	NDBA	159.1492	159.1492	4.10	4.60		4.00 eV to 4.00 eV
11	NDBA	159.1492	57.0704	4.10	4.60		12.00 eV to 12.00 eV

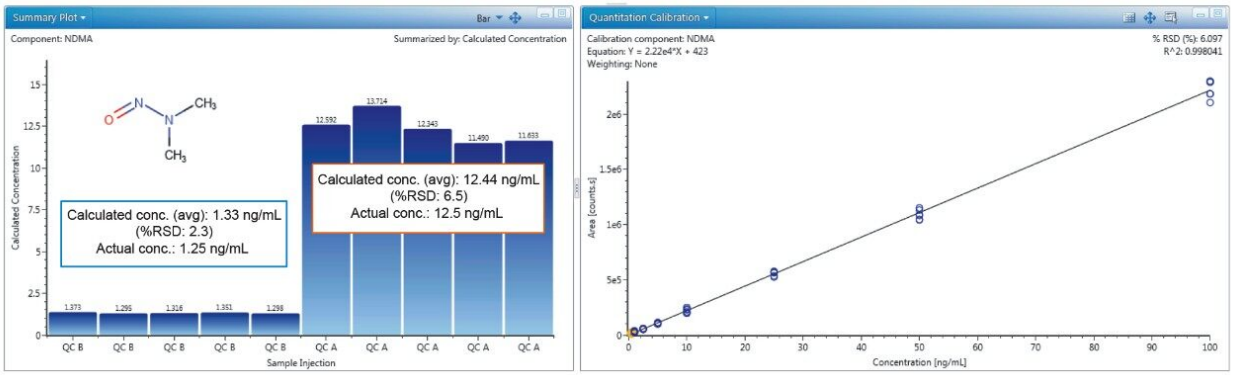
Figure 2. Final Tof MRM method for 6 N-nitrosamines. "Isotope Cluster" was chosen as the MRM data stripping method, which collects data in a 5Da region.

Quantitative performance using the developed method was assessed using two dilution series ranging from 0.01-100 ng/mL, with triplicate injections at each point (6 injections total). The resulting lower limits of detection and quantification (LOD/Q), linear range and R^2 values are described in Table 2, affording sub-ng/mL detection capability for the 6 N-nitrosamines studied here. Further measurement of two QC levels, 1.25 ng/mL and 12.5 ng/mL, are shown in Figure 3 for NDMA and NDEA. Here, both compounds are shown to have accurate and precise measurement at both levels. Calculated concentrations were averaged across the 5 replicate injections, and for both compounds are within 15% of the actual concentration (Figure 3a and b). Additionally, %RSDs were <7% for NDMA and NDEA at both QC levels, as based on calculated concentration. As this data demonstrates, reliable quantitation for these two high-profile *N*-nitrosamines is achieved using the developed UPLC-Tof MRM MS method.

N-nitrosamine	RT (min)	LLOD (ng/mL)	LLOQ (ng/mL)	Linear range (ng/mL)	R^2	Weighting
NDMA	1.65	0.10	0.50	0.5–100	>0.99	None
NMBA	2.10	0.25	0.50	0.5–100		
NDEA	2.72	0.025	0.05	0.05–25		
NEIPA	3.14	0.05	0.10	0.1–100		
NDIPA	3.50	0.05	0.10	0.1–100		1/x
NDBA	4.27	0.05	0.10	0.1–25		None

Table 2. Lower limits of detection/quantification (LLOD/Q), linear range, R^2, and weighting applied for the 6 N-nitrosamines. LLOD/Qs were determined based on S/N of 3 and 10, respectively.

3A



3B

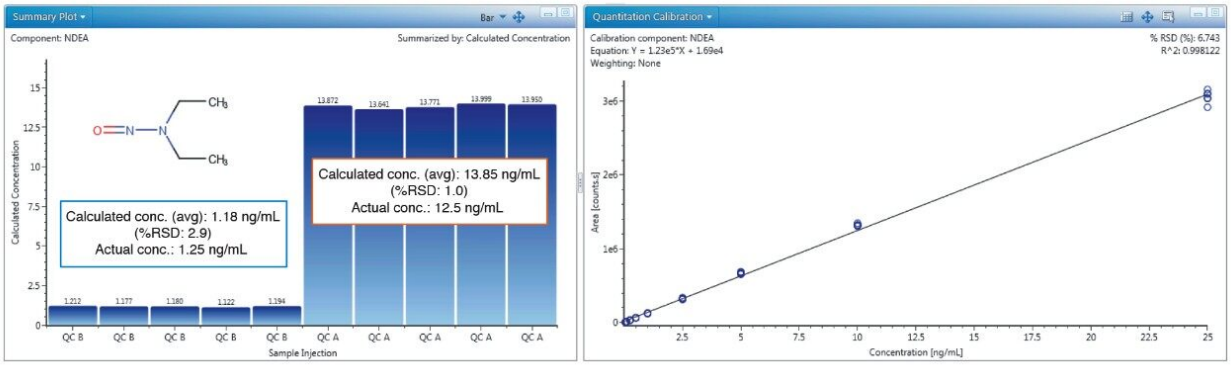


Figure 3a. NDMA summary plot of calculated concentrations (mean accuracy) at QC level A (12.5 ng/mL spike) and B (1.25 ng/mL spike), and calibration curve (0.1–100 ng/mL).

Figure 3b. NDEA summary plot of calculated concentrations (mean accuracy) at QC level A (12.5 ng/mL spike) and B (1.25 ng/mL spike), and calibration curve (0.025–25 ng/mL).

Conclusion

Methods for the analysis of potentially carcinogenic *N*-nitrosamines require sufficient sensitivity to accurately measure at sub-ng/mL concentrations. Here, we have demonstrated that using Tof MRM on the Xevo G2-XS QTof MS coupled with UPLC chromatographic separation, LLOQ levels at or below 0.5 ng/mL for NDMA, NDEA, NMBA, NDIPA, NEIPA, and NDBA were achieved. Quantitative testing of the method also demonstrated reliable and accurate measurement (within 15%) of two QC levels in the low ng/mL range for NDMA and NDEA. Overall, the

ACQUITY UPLC coupled to Xevo G2-XS QTof MS with the UNIFI Scientific Information System offers a modern platform for GxP-compliant, sensitive, selective, and specific HRMS quantitation of *N*-nitrosamines.

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