Low Level Detection of N-nitrosodimethylamine Using Gas Chromatography Coupled with Orbitrap-based Mass Spectrometry

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Introduction

N-nitrosodimethylamine (NDMA) is a semi-volatile organic chemical that belongs to nitrosamines, an emerging class of drinking water contaminants. NDMA is the main nitrosamine of concern and it is classified as a potent carcinogenic by the U.S. Environmental Protection Agency as it is known to induce tumors following administration by ingestion and inhalation.[1] NDMA is formed inadvertently as a by-product during industrial processes such as chloramination of wastewater and drinking water.[2] It is particularly important that NDMA is detected and accurately quantified in drinking water as even low levels of this chemical (e.g. 0.01 µg/L) can pose human cancer risks, being especially toxic to the liver.[1]

Traditionally, the analytical methodology used for NDMA detection and quantification employs either single or triple quadrupole GC-MS, or high resolution gas chromatography coupled with high resolution mass spectrometry (HRGC-HRMS). However, with these analytical instruments it is sometimes difficult to obtain high resolution and high sensitivity at the same time. Moreover, matrix and background chemical ions can strongly interfere with the NDMA ions and can lead to false positive detection and erroneous quantification of this compound. This is due to poor selectivity through insufficient resolving power of such instrumentation.

In this work, a sensitive and selective approach for NDMA detection and quantification using high resolution accurate mass GC-Orbitrap technology is described. Test samples were subjected to GC-MS analysis using a Thermo Scientific™ Q Exactive™ GC hybrid quadrupole-Orbitrap mass spectrometer and the quantitative performance of this novel analytical platform was tested for sensitivity, mass accuracy, repeatability and linearity of response.

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Experimental Conditions

In all experiments, a Thermo Scientific Q Exactive GC hybrid quadrupole-Orbitrap mass spectrometer was used. Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH Autosampler, and chromatographic separation was obtained with a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph system and a Thermo Scientific™ TraceGOLD™ TG-200 ms, 30 m × 0.25 mm × 0.25 µm film capillary column (P/N: 26084-1420). Additional details of instrument parameters are shown in Table 1 and Table 2.

Table 1. GC and injector conditions.

TRACE 1310 GC Parameters	
Injection Volume (µL):	2.0
Liner:	Single gooseneck carbofrit packed
Inlet (°C):	220
Inlet Module and Mode:	Surged Splitless
Surge pressure (kPa):	385
Surge duration (min):	1.0
Oven Temperature Program	
Temperature 1 (°C):	35
Hold Time (min):	1
Temperature 2 (°C):	130
Rate (°C/min):	25
Temperature 3 (°C):	230
Rate (°C/min):	125
Hold Time (min):	1

The Q Exactive GC was tuned and calibrated using perfluorotributylamine (PFTBA) to achieve mass accuracy of < 0.5 ppm. To ensure good selectivity data was acquired using 60,000 resolution (Full Width at Half Maxima, measured at *m/z* 200) (Table 2). This is particularly important when NDMA detection is targeted in complex matrices. These GC-MS settings ensured that chromatographic data was acquired with a minimum of 12 points/peak for consistent peak integration.

Table 2. Mass spectrometer conditions.

Q Exactive Parameters	
Transfer Line (°C):	260
Ionization Type:	EI
Ion Source (°C):	230
Electron Energy (eV):	70
Acquisition Mode:	full scan
Mass Range (*m/z*):	50–650
Mass Resolution (FWHM at *m/z* 200):	60,000
Lockmass (m/z):	207.03235

Sample Preparation

Typically, NDMA analysis starts with a solid phase extraction (SPE) of the water samples that will concentrate the NDMA up to 1000 times.[3] Taking this into account, the quantification performance of the Q Exactive GC-MS was tested using solvent standards prepared in dichloromethane (DCM) and spiked with native NDMA in DCM in a similar manner as for real water samples. The following concentration levels were used: 0.1, 1.0, 10, and 100 µg/L (ppb) which, assuming good NDMA recovery will translate into a limit of detection of >1 ng/L. Each standard was spiked with 20 µg/L deuterated d6-NDMA in DCM which was used as internal standard. In addition to these calibration standards, a procedural blank (DCM not spiked) was used (Table 3).

Table 3. Sample preparation.

Calibration Standard	Working Solution NDMA (ppb)	Volume	Final NDMA Conc. (ppb)	D6-NDMA 5 ppm (mL) Added Prior to Adjusting the Volume in the 10 mL Volumetric Flask	Final Conc. (ppb) d6NDMA
Cal6	5000	200 µL in 10 mL vol. flask (DCM)	100	40 µL	20
Cal5	5000	20 µL in 10 mL vol. flask (DCM)	10	40 µL	20
Cal4	5	2000 µL in 10 mL vol. flask (DCM)	1	40 µL	20
Cal3	5	200 µL in 10 mL vol. flask (DCM)	0.1	40 µL	20
Cal1	0	Blank No NDMA added (DCM)	0	40 µL	20

Data Processing

Data was acquired and processed using the Thermo Scientific™ TraceFinder™ software which allows for easy to set-up complete quantitative analysis workflow, including automated peak detection and integration, calculation of compounds concentration and recoveries as well as data reviewing and data reporting.

Results and Discussion

The objective of the analysis was to assess the use of GC-Orbitrap technology for the analysis of NDMA. For this, NDMA chromatography, sensitivity, linearity and peak area repeatability were evaluated using injection of solvent based standards.

Chromatography and Resolution

Using the GC conditions stated in the Table 1, fast GC separation with good chromatographic separation was obtained, allowing for a high sample throughput. An example of chromatography for NDMA in the lowest calibration standard (0.1 µg/L) is shown in Figure 1.

Accurate detection of NDMA molecular ion can be affected background ions such as silicone $Si(CH_3)_3$ of *m/z* 74.0469. To selectively separate these two ions, one will have to use a resolving power of at least 7000. In all experiments described here, the instrument resolving power was set to 60,000 (FWHM at *m/z* 200) and corresponded to a resolution of >100,000 at *m/z* 74, probably sufficient to achieve selective detection of NDMA target ions in matrix (Figure 2). This needs to be confirmed in future work using matrix samples.

Estimated Instrument Detection Limit (IDL) and Peak Area Repeatability

System sensitivity was assessed by calculating the minimum quantifiable limit or the instrument detection limit (IDL) for NDMA. This was done by using the peak area %RSD derived from n=9 repeat injections the lowest calibration standard 0.1 µg/L and taking into account the Student's-t critical values for the corresponding degrees of freedom (99% confidence). The results of this experiment showed that IDL derived from the QE GC data was 0.09 µg/L, close the lowest calibration standard used detectable in this experiments.

For any quantification work achieving reliable and robust instrumental response it is important, and this was demonstrated here, to assess the repeatability of NDMA quantification ion peak area (*m/z* 74). For this, each solvent standard was injected five times except for the 1.0 µg/L standard which was injected nine times. Absolute peak area repeatability was evaluated by looking at %RSD at each concentration level. Excellent %RSD values were obtained as shown in Figure 3.

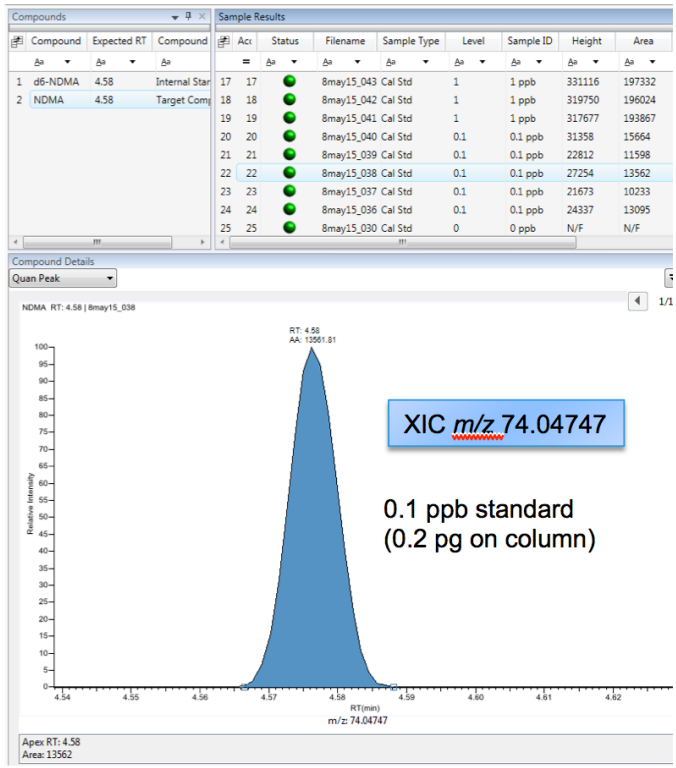


Figure 1. Extracted ion chromatogram (XIC) of the *m/z* 74 of NDMA at 0.1 ppb level (solvent standard). The absolute NDMA amount injected on column is shown.

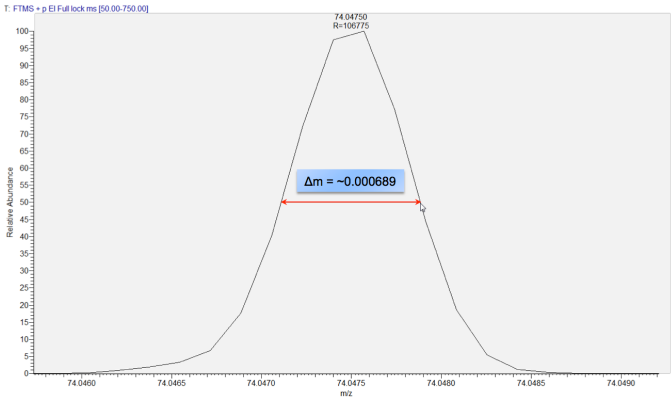
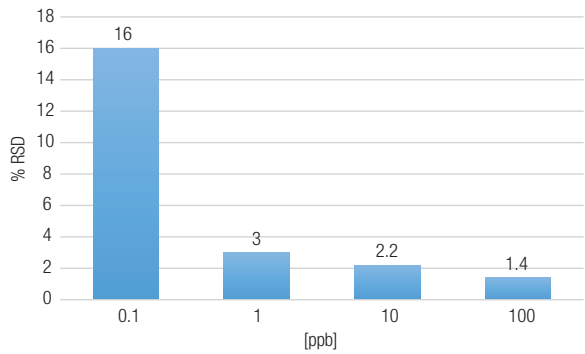


Figure 2. NDMA showing a mass resolution of › 100,000 (FWHM) for *m/z* 74 molecular ion. Data acquired in full scan, EI.



*n=5 injections per calibration standard were used except 1.0 ppb level; where n=9 inj. were used

Figure 3. Absolute peak area repeatability of NDMA at various concentration levels (n=5 for 0.1, 10 and 100 µg/L and n=9 for 1.0 µg/L). The average %RSD values at each level are indicated above each bar.

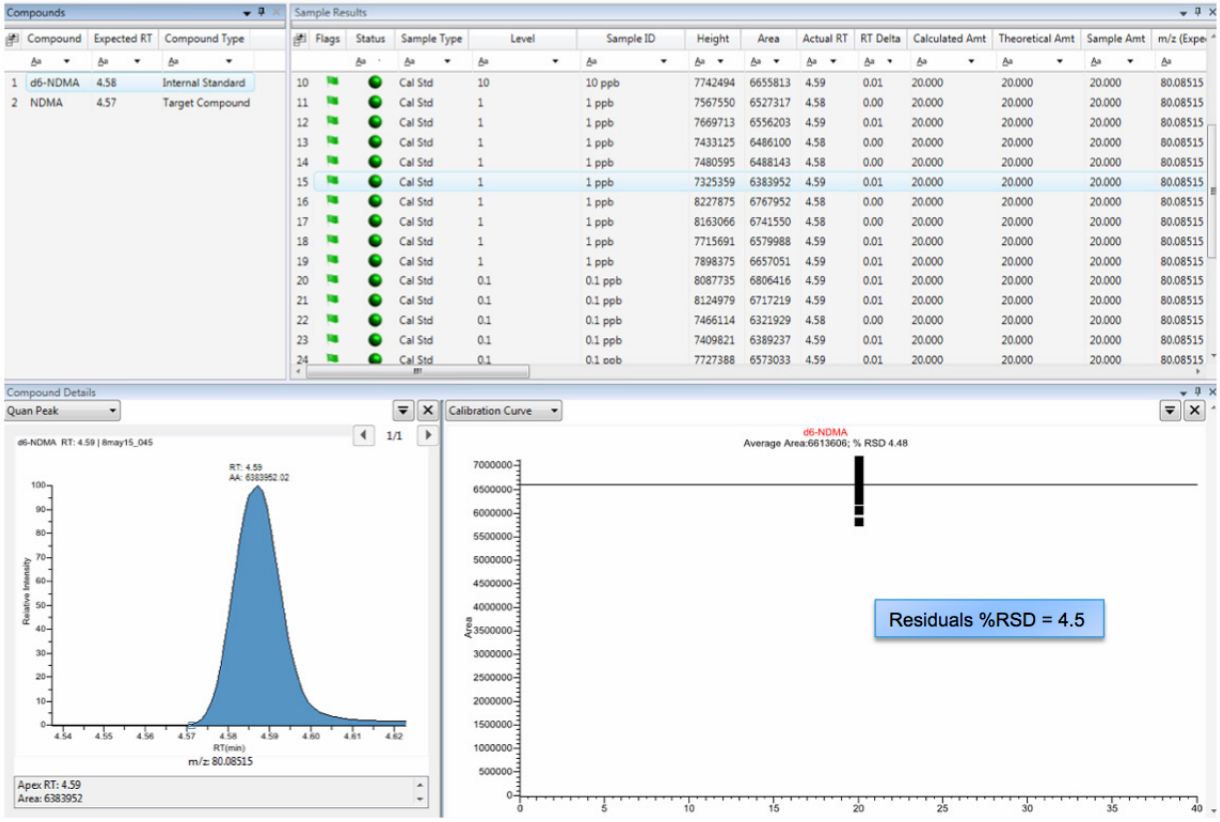


Figure 4. Peak area repeatability of d6-NDMA internal standard across n= 33 injections.

In addition, the %RSD calculated from the peak area response of the d6-NDMA internal standard across n=33 injections was <5 % demonstrating that the Q Exactive GC is able to generate consistent data across a sample batch (Figure 4).

Mass Accuracy

It is well known that selectivity increases with higher mass accuracy, therefore obtaining consistent sub ppm. Accurate mass information is important for achieving selectivity in difficult matrices and for confident characterization and confirmation of a target chemical. In this study, the mass accuracy for NDMA *m/z* 74.04747 was always <1 ppm at low and high levels as demonstrated in Figure 5.

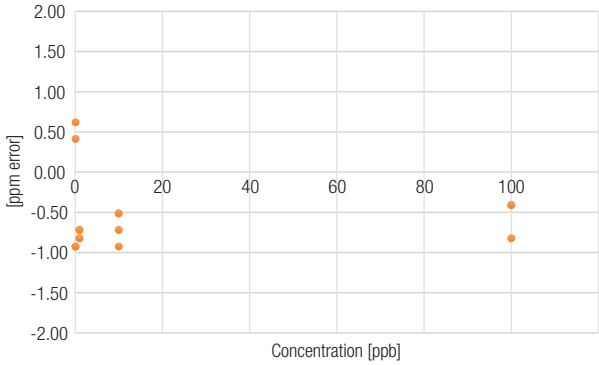


Figure 5. Mass accuracy measurements for NDMA quantitation ion *m/z* 74.04747. NDMA concentration levels (µg/L on the X-axis) as well as the corresponding mass error (ppm Y-axis) are shown. Each dot represents a separate injection.

Linearity of Response

Quantitative linearity was assessed across a concentration range of 0.1–100 µg/L (ppb) using five solvent calibration standards (including a solvent blank), each injected repeatedly five times. Calibration linearity assessed using a 1/x weighted linear regression showed that the coefficient of determination (R^2) was >0.999. Moreover, the % relative standard deviation (%RSD) of the relative response factors (RRF) was <16% across the NDMA concentration levels assessed (Figure 6).

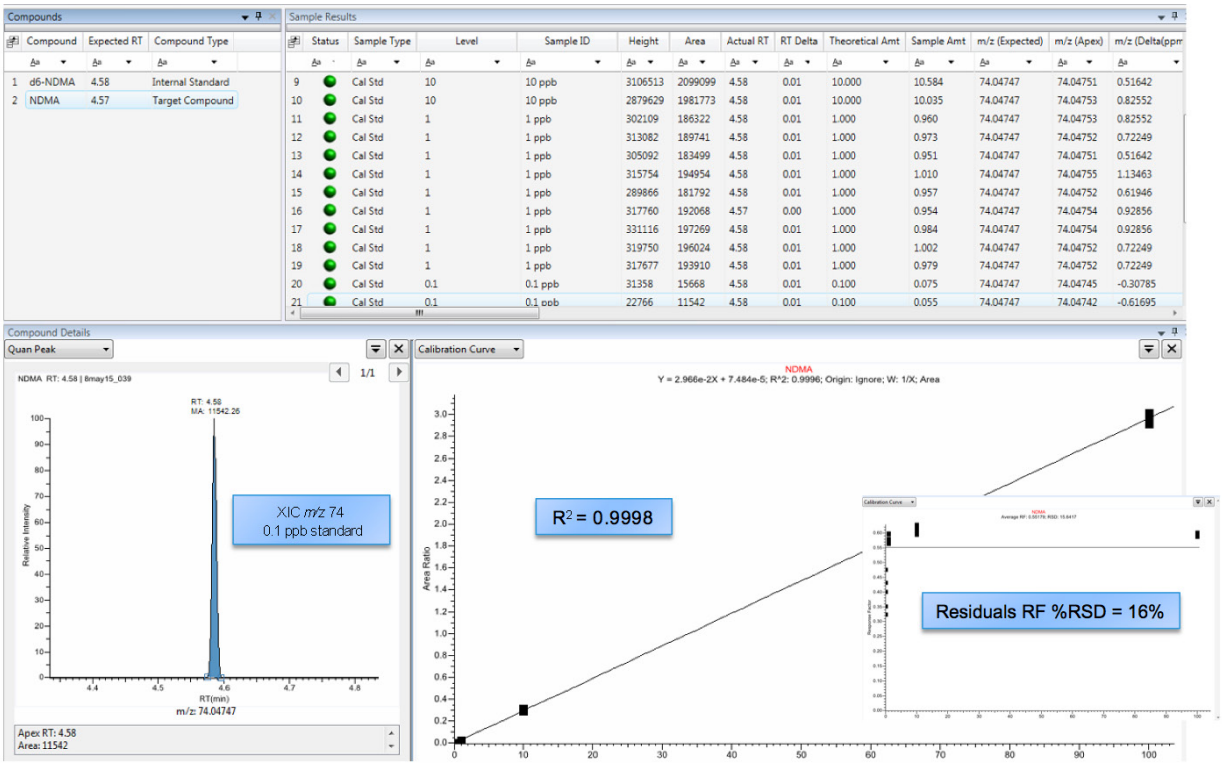


Figure 6. TraceFinder browser showing the quantification results table, the extracted ion chromatogram (XIC) of the NDMA quan ion *m/z* 74.04747 at the lowest detectable level (0.1 ppb) as well as the linear response across 0.1–100 ppb with the coefficient of determination R^2=0.9996 and residuals RF %RSD = 16%. Five (5x) repeat injection per calibration standard were used except for 1 ppb where nine (9x) repeat injection were used for repeatability purposes.

Summary

NDMA was detected at 0.1 ppb level, which, assuming 100% recovery will translate in a NDMA LOD of 0.1 ng/L using full scan acquisition and 60,000 resolution. Full scan acquisition will enable the detection of additional contaminants that may be present in the samples allowing for retrospective analysis of the data at a later date.

Besides state of the art sensitivity, excellent linear response across 0.1–100 µg/L was observed for NDMA with R^2 >0.999 and %RSD residuals <16.

Moreover, consistently low mass deviation from the theoretical NDMA mass was observed at all concentration levels.

Although a complete validation of these results using real water samples needs to be addressed in future experiments, the preliminary results described in this study demonstrate that the quantitative performance of the Q Exactive GC is well suited for the analysis of trace levels of NDMA.

References

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