Analytical Methods

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Analysis of nine N-nitrosamines using Liquid Chromatography-Accurate Mass High Resolution-Mass Spectrometry on a Q-Exactive instrument

Arnaud Djintchui Ngongang, Sung Vo Duy, Sébastien Sauvé*

Department of Chemistry, Université de Montréal, CP 6128 Centre-ville, Montréal, H3C 3J7 Québec, Canada.

*Corresponding author
Phone: +1 514 343-6749. Fax: +1 514 343-7586. E-mail: sebastien.sauve@umontreal.ca.

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

A selective and robust methodology for the analysis of nine N-nitrosamines (NA), N-nitrosodimethylamine (NDMA), N-nitrosomorpholine (NMor), N-nitrosomethylethylamine (NMEA), N-nitrosopirrolidine (NPyr), N-nitrosodiethylamine (NDEA), N-nitrosopiperidine (NPip), N-nitroso-n-dipropylamine (NDPA), N-nitrosodi-n-butylamine (NDBA) and N-nitrosodi-n-phenylamine (NDPhA) was developed and validated. This method is based on ultra-high-performance liquid chromatography (UHPLC) coupled to mass spectrometry using heated electrospray ionization (HESI) in positive ionization mode with a Q-Exactive mass spectrometer. After the selection of a suitable column for NA separation, the mobile phase and the injection volume as chromatography parameters were optimized. Mass spectrometry operating parameters, including sheath gas, auxiliary gas, spray voltage, S-Lens RF Level, resolution, automatic gain control (AGC) target and maximum injection time were also optimized in order to maximize the instrument analytical signal response. The method was optimized and validated in HPLC grade water, drinking water and wastewater matrices with satisfactory results. For accurate quantification, NDMA-d_6 and NDPA-d_{14} were used as internal standards. The extraction recoveries in real matrices ranged from 68-83% for eight of the nine target nitrosamines, except for NDPhA with values of 22-31%. The detection limits ranged from 0.4 to 12 ng/L. Analytical results revealed trace concentration of NDPhA (1.2 ng/L) in one of the analyzed water matrices. This work demonstrates that nitrosamines can be analyzed using LC-MS, on a Q-Exactive instrument, offering a faster alternative to the traditional GC-MS methods. The use of the high resolution accurate mass spectrometry helps to obtain good selectivity for the detection of both GC-detectable and GC-undetectable compounds.

Keywords: N-nitrosamines, water analysis, high resolution accurate mass, HRMS, Q-Exactive instrument.

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1. Introduction

Because of their high carcinogenic and mutagenic potential at nanogram per liter (ng/L) concentration level[1,2], N-nitrosamines (NA) are receiving special attention from environmental and analytical chemists[3,4]. Country-wide maximum contaminant level for NDMA in drinking water has not yet been established in North America, but some limits have been already set in jurisdictions such as in California at 10 ng/L[5] and in Ontario at 9 ng/L[6]. N-nitrosamines compounds are usually produced by industrial activities such as food (meats) and cosmetics processing, dye and rubber manufacturing, leather tanning and metal casting. Despite the treatment done by industries and water treatment plants, these compounds can still be found in the air, wastewater as well as in drinking water. N-nitrosamines such as NDMA are reported as by-products formed after the disinfection of wastewater effluent by chlorine and the drinking water chlorination and chloramination processes in the presence of nitrogen-containing organic matter[7-10]. N-nitrosamines might equally pose a risk to water resources and given their potential adverse effects on human health, the presence of these compounds is of more concern in drinking water than in wastewater. So far, only a few papers have been published on the analysis of nitrosamines in wastewater. The occurrence of NA in treated drinking water from several sites in the North America, particularly in Canada and U.S.A., has been investigated[11,12-15]. Similar work has also been done by Brisson et al.[14] on the presence of NA in the water supply systems in the province of Quebec. Gas chromatography coupled with different detection techniques such as mass spectrometry (GC–MS)[16-18], tandem mass spectrometry (GC–MS/MS)[19-21] or high resolution mass spectrometry (GC–HRMS)[22,23] have so far been the most common analytical techniques used to detect NA in water samples[16,19,24-26]. Nevertheless, a liquid chromatography approach has the advantage of detecting both thermally stable and unstable NA [27]. In addition, liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) has been widely used for NA analysis [28-31.] Given their hydrophilic and polar characters, the detection of low levels of NA and their extraction from water is always a challenge. For these reasons, solid-phase extraction (SPE) which is a cost effective method, allows shorter processing times and higher sample throughput compared to liquid-liquid extraction (LLE) which is very labor

Analytical Methods Accepted Manuscript

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

intensive and requires the use and disposal of large volumes of solvent[32]. Moreover, using a solid-phase extraction (SPE) step prior to the mass spectrometry analysis helps to achieve lower detection limits. The optimization of mass spectrometry operating parameters was performed using a one-factor-at-a-time (OFAT) approach[33]. The present work has demonstrated that using liquid chromatography coupled to a Q-Exactive high resolution-mass spectrometry (QE-HRMS) provides highly specific separation, identification and quantification of volatile NA with good selectivity and sensitivity. To the best of our knowledge, this is the first time that a selective, sensitive and robust analytical method for NA analysis is implemented on a Q-Exactive. In addition to a good chromatographic separation of the target NA, the use of high resolution-mass spectrometry allows the detection of NA without background interference despite their low molecular weights ($m/z < 200$).

Our goal in the current study was to develop and validate a simple and robust method with demonstrated validity and application for the determination of nine N-nitrosamines using a Q-Exactive mass spectrometer instrument. To demonstrate the applicability of the developed method, the occurrence of the selected target NA was evaluated in drinking water samples from Montreal, Laval and Trois-Rivières and a wastewater sample from Repentigny, all cities in the province of Quebec, Canada.

2. Materials and Methods

2.1. Apparatus

The chromatographic separation was performed with a Dionex Ultimate 3000 RS including an ISO-3100RS Pump, a WPS-3000RS autosampler and a TCC-3000 thermostated column compartment. The Chromeleon Xpress DCMSLink for Xcalibur (version 2.12) was the software used to perform the chromatography set up. The mass spectrometer was a Thermo Fisher Scientific Q-Exactive with heated electrospray ionization (HESI) interface. The software used for data analysis was Xcalibur (version 2.2 SP1).

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

2.2. Chemicals, Reagents and stock solutions

A mixture (2000 mg/L in MeOH) of nine nitrosamine reference standards (NDMA, NMEA, NPyr, NDEA, NPip, NMor, NDPA, NDBA and NDPhA) was purchased from Supelco, Bellefonte, PA, USA. Isotope-labelled standards (NDMA-d_6 and NDPA-d_{14}) (≥ 98%, 1000 mg/L in methylene chloride-d_2) were supplied by Cambridge Isotope Laboratories, Inc. (Andover, MA, U.S.A.). Chemical structures and formula of the studied nitrosamines are shown in Figure 1.

Reagent-grade formic acid (> 95%) was purchased from Sigma Aldrich (St. Louis, MO, USA). HPLC-grade submicron filtered water (H_2O), HPLC-grade methanol (MeOH) were purchased from Fisher Scientific (New Jersey, NJ, U.S.A.). Cartridges employed for off-line SPE experiments were coconut charcoal from EPA method 521 (2 g, 6 mL) and purchased from Restek (Bellefonte, PA, USA).

Preparation of working solutions and standard solutions

A primary stock solution of 2000 mg/L of the nine nitrosamines (NDMA, NMor, NMEA, NPyr, NDEA, NPip, NDPA, NDBA, NDPhA) in methanol (Supelco) was used for the preparation of working solutions. Intermediary stock solutions of nitrosamines mix (10 mg/L) and isotope-labelled standards (NDMA-d_6 and NDPA-d_{14}, 2 mg/L,) were prepared in MeOH and stored in a freezer at -20°C. Working mix solution of nitrosamines were prepared daily from stock solutions at the desired concentrations prior to LC-MS analysis. All organic solvents and water used for dilutions were of HPLC grade purity.

Water samples collection

Wastewater samples were collected in 4-L pre-cleaned amber glass bottles from the wastewater treatment plant (WWTP) of the city of Repentigny (Quebec, Canada), vacuum filtered through 2.6 μm and 0.3 μm glass microfiber filters (Sterlitech) and stored at 4 °C prior to their analysis within 48 hours. Drinking water samples were collected prior to the analysis in volumetric flasks from a tap at the Université de

Analytical Methods Accepted Manuscript

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

Montréal, Montreal, Quebec, Canada and in 4-L pre-cleaned amber glass bottles in the cities of Laval and Trois-Rivières (Quebec, Canada) with the extraction done within a 24-h period.

2.3. Solid-phase extraction (SPE)

Sample pre-treatment and N-nitrosamine extraction was carried out based on the US EPA Method 521[18]. The carbon-based charcoal cartridges were previously conditioned with 6 mL of methylene chloride, 12 mL of methanol and 15 mL of HPLC grade water before being air-dried under high vacuum. The analytes absorbed on the SPE cartridges were eluted using 15 mL of methylene chloride. The elution solvent was collected in 15 ml graduated centrifuge conical tubes and concentrated down to 1 mL under a high purity and moderate nitrogen stream at room temperature. The sample should not be evaporated to dryness. By doing this, a significant amount of the target nitrosamines could be lost. The extract was transferred to 2-mL autosampler vials and the internal standard mixture solution (NDMA-d_6 and NDPA-d_{14}, 20 µg/L) was added prior to the LC-MS analysis, for quantification. Using the approach of adding the internal standards after the SPE step, this required multiplying the concentration of each compound obtained after the SPE by its recovery rate determined beforehand, in order to find its initial concentration in the sample. Given the complexity of wastewater samples and to avoid overloading cartridges, 250 mL were used instead of 500 mL for drinking water samples. A blank sample was always provided at each SPE-run by passing a volume of HPLC grade water through the coconut cartridge to confirm the specificity of the procedure.

2.4. Method optimization

2.4.1. Chromatography parameters optimization

Choice of column. N-nitrosamine standard sample (20 µg/L) prepared in HPLC grade water was used to evaluate the performance of six different chromatography columns: Hypersil Gold C18, Hypersil Gold C8, Pentafluorophenyl (PFP) and Hypercab all from Thermo Fisher Scientific, TSKgel Amide-80 and Kinetex HILIC 100Å (from Phenomenex). Ultimately, the column giving the best performance was the Hypersil Gold

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

C18 column. The detailed characteristics of the tested columns are presented in Table 2. The various chromatograms are presented in the supporting information (Fig. S1).

Mobile phase. Different mobile phases were evaluated. For example ACN + 0.1% HCOOH /H_2O + 0.1% HCOOH; MeOH + 0.1% HCOOH / H_2O + 0.1% HCOOH; MeOH + 0.3% HCOOH / H_2O + 0.3% HCOOH; H_2O + Ammonium bicarbonate/ACN; H_2O/ACN + MeOH 50/50 were tested, only mobile phases which showed relevant chromatography results were selected for further experiments. Some tests have been conducted on the columns at the same flow rate of 0.5 mL/min with two mobile phases (H_2O/ACN) and (H_2O/MeOH). Similar results have been obtained for both mobile phases. H_2O/MeOH has been selected for further tests as it is more cost effective than acetonitrile. Analytical tests were then carried out with H_2O/MeOH by gradually adding small amounts of HCOOH as modifier. Five of the six columns have therefore been tested using the mobile phase H_2O + 0.1% HCOOH and MeOH + 0.1% HCOOH. The Kinetex Hilic (100 Å) column was tested using a buffer of 100 mM ammonium formate diluted with HPLC grade water and adjusted to pH 4.0 with HCOOH and ACN with 0.1% HCOOH. To obtain the best peak separation of the analytes, the methanol percentage in the mobile phase (H_2O/MeOH, 0.1% HCOOH) was linearly changed. Thus the final retained gradient was the following: 0 min, 5%; 1 min, 10%; 1.5 min, 90%; 4.5 min; 95%; 4.51 min, 5% and 6.50 min 5%. The total analysis run time was 6.5 min. The column was kept at 40°C and the sampler was maintained at 8°C.

Injection volume. In order to get adequate separation and very fine peaks, experiments were conducted by progressively increasing the sample injection volume. The maximum volume was reached when the peaks become broader. The NA mass spectrometry data i.e. the mean area response, recorded for different injection volumes, were compared. The final chosen volume was 100 µL for HPLC grade water samples (Fig. 3). The LC-MS method was developed in a preliminary experiment where the mass spectrometry and chromatography parameters were optimized in HPLC grade water using nitrosamine standards. This method could be used for the determination of NA in matrices containing huge amount of these compounds or for direct injection (without SPE) of a matrix into the instrument. In the case of environmental samples which was

Analytical Methods Accepted Manuscript

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

later optimized, the injection volume of methylene chloride samples was evaluated and set to 5 μL to avoid a modification of the mobile phase gradient.

2.4.2. Mass spectrometry optimization parameters

Mass spectrometry operating parameters, including sheath gas, auxiliary gas, spray voltage and S-Lens RF Level, resolution, automatic gain control (AGC) target and maximum injection time were optimized using the one-factor-at-a-time (OFAT) method[33]. The influence of the parameter variation on the analytical signal response was evaluated and the value of the parameter showing a higher signal response was retained for further experiments. The selected values for all the parameters are given in Table 3.

Acquisition mode comparison. Experiments using different acquisition modes comprising, MS/MS and full scan were performed. The same sample was used for all the experiments and in the same analytical conditions. The signal response were recorded and the target precursor of analytes and the fragment ions were identified depending on the acquisition mode. The full scan acquisition mode was chosen as it provided more sensitivity and selectivity.

2.5. Method validation

The validation was performed to evaluate the NA analytical method in terms of the following parameters: linearity, precision, accuracy (% bias), instrumental detection limit, method detection limits and quantification limits. The recovery of the extraction procedure was also calculated for the nine target nitrosamines. The precision of the instrument were assessed for five injections performed the same day (intra-day) and for fifteen injections from three different days (inter-day), and evaluated at two concentration levels (6 μg/L and 60 μg/L, n =5) of nitrosamine standards spiked in HPLC grade water. For drinking water and wastewater matrices, the two quality control (QC) concentration levels were QC1 at 12 ng/L and QC2 at 120 ng/L (n = 5), these values were chosen in order to get a better signal response and the best calibration curve for all target

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

nitrosamines within a realistic concentration range. A single run consisting of a calibration curve of eight concentration levels and three replicates of both low and high QC samples was processed to establish linearity, LODs and LOQs. The calibration curve and QC samples were prepared from intermediary solutions (1.2, 12, 120 and 1200 µg/L) by spiking calculated volumes of HPLC grade water or the matrix. The eight point of the calibration curve were from 0.01 to 100 µg/L corresponding to 0.02 to 200 ng/L considering the pre-concentration factor of 500-fold for HPLC grade water and drinking water and 250-fold for wastewater. The same procedure was repeated for the validation of the method in the environmental matrices i.e. drinking water and wastewater. Instrumental and method detection limits and quantification limits were calculated by multiplying by 3.3 and 10 the error on the y-intercept and dividing by the slope of the regression line equations, respectively. All the quality control standards were prepared as three replicates. The validation process was performed using the criteria's from the International Conferences of Harmonization (ICH), more specifically the Q2 (R1) guidelines [34].

Recovery of the extraction procedure. The recovery of the extraction process (RE) of the NA was determined by spiking drinking water and wastewater samples, resulting in a final concentration of 100 µg/L with nitrosamines standard solution and at 20 µg/L for the internal standards. Samples were prepared as three replicates and loaded onto the coconut SPE cartridges for extraction. Recoveries were calculated by comparing the mean area response ratio of extracted sample (spiked before extraction) to that of the post-extracted spiked sample (spiked after extraction) as defined by Equation 1.

$$RE = \frac{(SB - US)}{(SA - US)} \times 100 \qquad (Eq. 1)$$

SB: sample spiked before the extraction
SA: sample spiked after the extraction
US: unspiked sample

2.6. Application of the method

To prove the applicability of the developed SPE-LC-HRAMMS method, the occurrence of the studied N-nitrosamines was examined in drinking waters from Montreal, Laval and

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Trois-Rivières and in a wastewater sample from Repentigny, considered to be susceptible to these emerging disinfection by-products. For this purpose, level of the target NA in the analyzed matrices was evaluated using the standard addition method. The goal was not a complete exploration of the quality of the surrounding water as this type of work had already been done. For instance, Brisson et al. [14] have scrutinized one hundred and ninety-five samples from seven drinking water supply systems of the province of Quebec while Boyd et al.[11] have examined the presence of nine N-nitrosamines in thirty-eight drinking water systems in Canada and the U.S.A.

3. Results and discussion

3.1. Chromatographic parameters optimization

Among the tested columns for N-Nitrosamines analysis, the Hypersil Gold C18 column (from Thermo Fisher Scientific) was selected for best performance and a short analysis time of about 4.25 min has been recorded. In fact, this column gave the best results when considering peak width, peak tailing and separation and moreover, all compounds showed very fine and completely separated peaks.

In testing the mobile phase composition, an important increase in the signal intensity was observed for five of the nine N-nitrosamines when 0.05 or 0.1% of HCOOH was added to the mobile phase H_2O/MeOH 95/5 except for NDPhA which has shown a loss of signal (-32%) compared to results with the mobile phase without acid. At higher acid concentrations 0.3%, (Figure 2) the response intensities were systematically lower when compared to 0.05 and 0.1% of HCOOH. The use of HCOOH for several compounds allowed for better reproducibility than without HCOOH. Thus, the presence of acid also helps for the stabilization of the signal (Fig. 2).

The on-column injection volumes were also evaluated. The higher the injection volume, the stronger was the signal intensity. A higher injection volume certainly increases the signal and decreases the limit of detection of the method although the injection of more complex samples can give different results. Also, the size and the maximum capacity of the column should be considered before increasing the injection volume. Thus for the

Analytical Methods Accepted Manuscript

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

selected Hypersil Gold C18 (1.9 µm, 100 x 2.1 mm) column, the injection volume of 100 µL appeared to be the maximum. For these injection volumes, the peaks widths remained acceptable (Fig. 3).

3.2. Mass spectrometry parameters optimization

The one-factor-at-the-time approach was used for mass spectrometry operating parameters optimization and the following results were observed. The greater the value of the sheath gas (SG), the higher was the signal intensity for all the nine analyzed nitrosamines. As there was no significant variation of the signal intensity for SG 75 and 80, the value of 75 was selected to avoid any inconvenient by using 80 which is the maximum value. For auxiliary gas (AG) parameter, there was no significant influence of the variation of the AG on the signal intensity. Therefore the middle value AG 25 was chosen as a compromise for all analytes. Lower values of the spray voltage (2.0 to 5.0 kV) gave weaker signals for three compounds NDMA, NMEA and NDEA. Only the signal response of NDPhA decreased with the increase of the spray voltage. The value of 5.5 kV was then retained for all the analyzed compounds. The choice was easier to make for the S-Lens RF level parameter since the value 55 (among 50, 55, 60, 65 and 70) gave the strongest signal for five of the nine analyzed nitrosamines. The variation of the S-Lens RF level parameter is illustrated in the graphic in Fig. 4. Regarding the resolution parameter optimization, the values 140000, 70000, 35000 and 17500 were tested. There was a substantial decrease in the signal for all analytes when the resolution value was increased. But no significant variation of the signal was observed for the values 70000, 35000 and 17500. Thus, the highest value of 70000 was selected for further tests since higher resolving power may improve the accuracy on the expected mass. Moreover, a higher resolution also helps to improve the specificity of the method. The automatic gain control (AGC) values $5e^6$, $1e^6$, $2e^5$ and $5e^4$ were explored. The value $5e^6$ showed a weak signal for 7 of the 9 N-Nitrosamines (NPyr, NDEA, NPip, NMor, NDPA, NDBA, NDPhA). A decrease of the signal with the increase of the AGC value was observed. But, the value $5e^4$ presented a weaker signal for NDMA, NMEA and NDEA compared to the

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

other three values. Comparing $1e^5$ and $2e^5$, $1e^5$ was preferred since it gave a stronger signal. Given that these results were acquired for relatively clean samples, and that more complex matrices will contain more interferences, the AGC value could be increased to $2e^5$ to make sure that a considerable amount of the target molecules enter into the trap. The values 20, 50, 100 and 200 ms of the maximum injection time (IT) were tested and there was no notable influence on the signal response, we opted for a conservative approach by selecting the value of 100 ms. The variation of only few of the optimized mass spectrometry parameters showed a significant influence on the analyte signal response. These parameters performed as independent. Thus, the interactions between factors were minimized without significant impact.

The full scan and MS/MS modes were evaluated and compared. The fragmentation of precursor ions was operated successfully. Given the instrumental limitation of the Q-Exactive that fragment ions with $m/z < 50$ can not be detectable, and knowing that some fragment ions of the studied NA are under this mass limit, we could not implement more experiments using the AIF and MS/MS modes. However, high resolution (R=70000) coupled with the selected full scan mode for the analysis of known samples on an accurate mass spectrometry device like Q-Exactive, led to the unambiguous identification of the target precursor ions with excellent specificity[35]. Experimental results are shown in the supporting information (Figs. S2 and S3).

3.3. Method validation

Linearity, precision (inter-day and inter-day), accuracy, limits of detection and limits of quantification for HPLC grade water, drinking water and wastewater were evaluated for the method validation (Tables 4, 5, 7 and 8). Linearity of the method was satisfactory up to 200 ng/L considering the pre-concentration factor of 500. The calibration curves showed good linearity in HPLC grade water as well as in all water matrices, with best fit of coefficients of determinations (R^2) higher than 0.992 (Table 7). The method was found to have satisfactory accuracy and precisions in HPLC grade water and as well as in water matrices with RSD < 20% (Table 5 and 8). In fact, the precision for all nine NA in HPLC grade water and the two analyzed water matrices for both QC1 and QC2 ranged between 0.98 and 19%. The accuracy (% bias) from the expected concentrations, was between

Analytical Methods Accepted Manuscript

0.09 and 8.2% for HPLC water (Table 5) and between 0.74 and 19% for both types of water matrices (Table 8). Recovery values ranged from 68 to 83% (Table 6). These values were higher for eight of the nine target NA compared to an overall extraction efficiency of 52% presented in the EPA Method 521[18]. Only NDPhA showed a low average recovery of 26%. However, this value was higher than the 23% obtained by Planas et al.[19]. This author attempted to explain this low value for NDPhA by the irreversible adsorption on the coconut charcoal EPA 521 cartridge. Also, this result can also be explained by the polar characteristic and the very low water solubility of NDPhA[35] in addition to matrix effects causing signal suppression in water samples. A Q-Exactive mass spectrometer instrument was used for data acquisition in a full scan mode.

The instrument response was determined as the ratio of the analyte area to that of the isotope-labeled internal standard. Examples of the chromatograms recorded in HPLC grade water, drinking water and wastewater matrices are illustrated on Figs. 5, 6 and 7. Fig. 5 shows the LC-MS chromatograms of a 20 µg/L nitrosamine standards sample in HPLC grade water where all analyte peaks were quite thin. For drinking water and wastewater samples spiked at 120 ng/L in methylene chloride, (Fig. 6 and 7) analyte peaks for NMEA, NPyr and NMor were somewhat broader.

The instrumental detection and quantification limits ranged from 0.01 µg/L (for NDBA) to 0.4 µg/L (for NMEA) and from 0.05 µg/L (for NDBA) to 1.0 µg/L (for NMEA) in HPLC grade water respectively (Table 4). The LC-MS method detection and the quantification limits ranged from 0.4 ng/L (NDPhA) to 9.1 ng/L (NMEA) and from 1.3 ng/L (NDPhA) to 28 ng/L (NMEA) in drinking water and from 2.7 ng/L (NDBA) to 12 ng/L (NMEA) and from 8.1 ng/L (NDBA) to 35 ng/L (NMEA) in wastewater respectively (Table 7). The instrumental detection and quantification limits are greater than the detection and quantification limits of the LC-MS method considering the concentration factors of 250-fold for wastewater and 500-fold for drinking water after the SPE step.

Although the method detection and quantification limits are both matrix and analytical technique dependent values, our results have been compared with some published works performed in analogous conditions. For example, Ripollés et al.[36]

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

asserted LODs ranging from 1 to 8 ng/L for NA analyzed by LC–MS/MS QqQ for a concentration factor of 500-fold after the SPE of 500 mL of drinking water samples. LODs ranged from 0.4 to 9.1 ng/L in drinking water with the same concentration factor of 500-fold has been obtained using our method. Whereas, Krauss et al.[28] presented LODs between 0.3 to 3.9 ng/L for the same (nine) target N-nitrosamines. These results were obtained for the analysis of 500 mL of wastewater, concentrated down to 1 mL i.e. 500-fold, and analyzed by LC/MS using a linear ion trap-orbitrap hybrid instrument at high mass resolution. These values were lower than ours which ranged between 2.7 and 12 ng/L obtained in wastewater. But it should be mentioned here that only 250 mL of wastewater, for a concentration factor of 250-fold, were used in our case. In all the reported NA analytical methods using LC-MS, none relied on the use of a Q-Exactive mass spectrometer instrument.

3.4. Method Application

Drinking water samples from the cities of Montreal, Laval and Trois-Rivières and a wastewater sample from Repentigny were analyzed. Only N-nitrosodiphenylamine (NDPhA), a thermally unstable nitrosamine, was quantified at 1.2 ng/L, which value was above its detection limit of 0.8 ng/L, in the drinking water sample from Trois-Rivières. The other target NA were not detected at a concentration level higher than their detection limit. In some of the published works on the evaluation of N-nitrosamines in drinking water samples using SPE-LC-MS/MS, NDMA was the most commonly detected. Charrois et al. reported the detection of NDMA together with NMEA, NMOR and NDPhA at concentration levels above the method detection limit in drinking water samples from Alberta (Canada)[11]. NDMA concentrations ranging from 54 ng/L to 118 ng/L and NDPhA at a concentration of 0.23 ng/L were reported by J.M Boyd[33] for NA analysis done on drinking water samples from Canada and U.S.A. Otherwise, the examination of some drinking water supply systems in Quebec by Brisson et al. using GC-MS, revealed that NDMA was found in few samples and at a maximum concentration of 3.3 ng/L which was lower than those observed elsewhere in Canada; moreover, no sample showed a concentration above the Ontario standard of 9 ng/L.

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

4. Conclusion

A selective and robust SPE-LC-MS method was developed and optimized for the analysis of N-nitrosamines in drinking water and in wastewater matrices. The sensitivity of our method was comparable with that of published GC/MS and LC-MS/MS based methods. Also, the use of an accurate mass high resolution-mass spectrometer, the Q-Exactive, helps for the identification and quantification of the target NA without any ambiguity. A good selectivity of the LC-MS/HRAMMS method was obtained with the Q-Exactive instrument thus eliminating any interference of matrix compounds. Although GC-MS can provide better sensitivity for N-nitrosamines analysis, the advantage of the use of LC-MS is the significant time savings given the longer retention times recorded in GC-MS. Furthermore, this helps for the detection of both GC-detectable and GC-undetectable such as NDPhA. This is the first report for the analysis of N-nitrosamines using SPE-LC-MS using HRMS on a Q-Exactive instrument.

Acknowledgments

The authors would like to thank the Natural Sciences and Engineering Research Council of Canada for financial support and the Canadian Foundation for Innovation (equipment).

†Electronic Supplementary Information (ESI) available: Chromatograms showing the performance of the six tested columns and Experimental results of the mass spectrometry optimization parameters.

Analytical Methods Accepted Manuscript

Analytical Methods Accepted Manuscript

References

1- USEPA (1993) N-Nitrosodimethylamine (CASRN 62-75-9) Integrated Risk Information System (IRIS) [online]. Available from http://www.epa.gov/iris/subst/0045.htm [cited 20th November 2014].

2- United States Environmental Protection Agency. Unregulated contaminant monitoring-regulation (UCMR) for public water systems revisions. Fed. Regist. 70: (August 22, 2005).

3- Health Canada (2011). Guidelines for Canadian Drinking Water Quality: Guideline Technical Document- N-Nitrosodimethylamine. Water, Air and Climate Change Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario. (Catalogue No H128-1/11-662E).

4- W.A. Mitch, and D.L. Sedlak. (2004). Characterization and fate of N-nitrosodimethylamine (NDMA) precursors during municipal wastewater treatment. Environ. Sci. Technol., 38(5):1445–1454.

5- CDHS. (2005) Notification levels overview [online]. Available from http://www.dhs.ca.gov/ps/ddwem/chemicals/AL/notificationoverview.pdf [cited 20th November 2014].

6- OMEO (2003) Ontario drinking-water quality standards; O. Reg. 169/03, Sched. 2; O. Reg. 268/03, s. 1; O. Reg. 248/06, s. 2; O. Reg. 242/07, s. 1. [online] Available from http://www.e-laws.gov.on.ca/DBLaws/Regs/English/030169-e.htm [cited 20th November 2014].

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

7- OMEO (1994). Removal of N-nitrosodimethylamine from the Ohsweken (Six Nations) water supply. Final report. Ontario Ministry of the Environment, Toronto, Ontario. November. 10 pp. appendix (ISBN 0-7778-3439-1).

8- S.D. Richardson, (2003). Disinfection by-products and other emerging contaminants in drinking water. Trends Anal. Chem., 22(10):666–684.

9- I. Kristiana, J. Tan, C. A. Joll, A. Heitz, U. Von Gunten, and J. W. Charrois. 2013. “Formation of N-nitrosamines from chlorination and chloramination of molecular weight fractions of natural organic matter.” Water Research 47: 535-546.

10- J.E. Grebel, C.C. Young, I.H. Suffet, J. Chromatogr. A 1117 (2006) 11–18.

11- J.M. Boyd, Y.Y. Zhao, M. Wagner, F. Qin, P. Levallois, C. Legay, J.W.A. Charrois, S.D. Richardson, S.E. Hrudey, X.F. Li, Occurrence of N-nitrosodiphenylamine and N-nitrosodimethylamine in 38 drinking water systems in Canada and the USA, IWA World Water Congress and Exhibition, 2010, p. 8.

12- S. Barrett, C. Hwang, Y. Guo, S.A. Andrews, R.L. Valentine, Occurrence of NDMA in drinking water: a North American Survey, 2001–2002, AWWA Annual Conference, 2003, p. 19.

13- J.W.A. Charrois, J.M. Boyd, K.L. Froese, S.E. Hrudey, J. Environ. Eng. Sci. 6 (2007) 103.

14- I.J. Brisson, P. Levallois, H. Tremblay, J. Sérodes, C. Deblois, J. W. Charrois, V. Taguchi, J. Boyd, X. F. Li, and M. J. Rodriguez. 2013. “Spatial and temporal occurrence of N-nitrosamines in seven drinking water supply systems.” Environmental Monitoring and Assessment 185: 7693-7708.

15- J.W.A. Charrois, M.W. Arend, K.L. Froese, S.E. Hrudey, Detecting N-nitrosamines in drinking water at nanogram per liter levels using ammonia positive chemical ionization, Environ. Sci. Technol. 38 (2004) 4835–4841.

16- McDonald, J. A., Harden, N. B., Nghiem, L. D. & Khan, S. J. (2012). Analysis of N-nitrosamines in water by isotope dilution gas chromatography-electron ionisation tandem mass spectrometry. Talanta, 99 146-154.

17- S. Yoon, N. Nakada, H. Tanaka, Occurrence and removal of NDMA and NDMA formation potential in wastewater treatment plants, J. Hazard. Mater. 190 (2011) 897–902.

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

18- J.W. Munch and M.V. Bassett. Determination of Nitrosamines in Drinking Water by Solid-Phase Extraction and Capillary Column Gas Chromatography with Large Volume Injection and Chemical Ionization Tandem Mass Spectrometry (MS/MS); National Exposure Research Laboratory, Office of Research and Development, United States Environmental Protection Agency: Cincinnati, Ohio, 2004; Method 521; available at http://www.epa.gov/nerlcwww/m_521.pdf.

19- A. Llop, F. Borrull, E. Pocurull, Fully automated determination of N-nitrosamines in environmental waters by headspace solid-phase microextraction followed by GC–MS–MS, J. Sep. Sci. 33 (2010) 3692–3700.

20- H.-W. Hung, T.-F. Lin, C.-H. Chiu, Y.-C. Chang, T.-Y. Hsieh, Trace analysis of N-nitrosamines in water using solid-phase microextraction coupled with gas chromatograph–tandem mass spectrometry, Water, Air, Soil Pollut. 213 (2010) 459–469.

21- Ontario MOE, Protocol of accepted drinking water testing methods version 2.0, E3388 – the determination of N-nitrosamines in water by gas chromatography-high resolution mass spectrometry (GC/HRMS), Laboratory Services Branch, Ontario Ministry of the Environment Ontario, 2010.

22- C. Planas, Ó. Palacios, F. Ventura, J. Rivera, J. Caixach, Analysis of nitrosamines in water by automated SPE and isotope dilution GC/HRMS: occurrence in the different steps of a drinking water treatment plant, and in chlorinated samples from a reservoir and a sewage treatment plant effluent, Talanta 76 (2008) 906–913.

23- Y.Y. Zhao, J. Boyd, S.E. Hrudey, and X.F. Li. Characterization of new nitrosamines in drinking water using liquid chromatography tandem mass spectrometry. Environ. Sci. Technol. 40: 7636–41 (2006).

24- Richardson, S. D. Environmental mass spectrometry: Emerging contaminants and current issues. Anal. Chem. 2012, 84, (2), 747-778.

25- M.H. Plumlee, M. López-Mesas, A. Heidlberger, K.P. Ishida, and M. Reinhard. N-nitrosodimethylamine (NDMA) removal by reverse osmosis and UV treatment and analysis via LC–MS/MS. Water Res. 42: 347–55 (2008).

Analytical Methods Accepted Manuscript

26- U.S. Environmental Protection Agency (EPA). http://water.epa.gov/scitech/drinkingwater/dws/ccl/ccl3.cfm#ccl3 (accessed date: 17th January 2011).

27- J.M. Boyd, S.E. Hrudey, X.F. Li, S.D. Richardson, Solid-phase extraction and high-performance liquid chromatography mass spectrometry analysis of nitrosamines in treated drinking water and wastewater, TrAC, Trends Anal. Chem. 30 (2011) 1410–1421.

28- M. Krauss and J. Hollender. Analysis of nitrosamines in wastewater: Exploring the trace level quantification capabilities of a hybrid linear ion trap/orbitrap mass spectrometer. Anal. Chem. 2008, 80, 834-842.

29- W. Wang, S. Ren, H. Zhang, J. Yu, W. An, J. Hu, M. Yang. Occurrence of nine nitrosamines and secondary amines in source water and drinking water: Potential of secondary amines as nitrosamine precursors. Water Research 2011, 45, 4930-4938.

30- Y. Zhao, J. M. Boyd, M. Woodbeck, R. C. Andrews, Q. Feng, S. E. Hrudey, X. Li, Formation of N-nitrosamines from eleven disinfection treatments of seven different surface waters. Environ. Sci. Technol. 2008, 42, 4857–4862.

31- Y. Zhao, X. Liu, J. M. Boyd, F. Qin, J. Li, X. Li, Identification of N-nitrosamines in treated drinking water using nanoelectrospray ionization high-field asymmetric waveform ion mobility spectrometry with quadrupole time-of-flight mass spectrometry. Chromatogr. Sci. 2009, 47, 92–96.

32- Banerjee, K., Savant, R.H., Dasgupta, S., Patil, S.H., Oulkar, D.P., Adsule, P.G.: Multi-residue analysis of synthetic pyrethroid pesticides in grapes by gas chromatography with programmed temperature vaporizing-large volume injection coupled with ion trap mass spectrometry. J. AOAC Int. 93, 368–379 (2010).

33- Anon, 1995. CPMP/ICH/381/95. European Medicines Agency (adopted by the CPMP in November 1994.

34- S. Ventanas, J. Ruiz, On-site analysis of volatile nitrosamines in food model systems by solid-phase microextraction coupled to a direct extraction device, Talanta 70 (2006) 1017.

35- H. Henry, H. R. Sobhi, O. Scheibner, M. Bromirski, S. B. Nimkar and B. Rochat, Comparison between a high-resolution single-stage Orbitrap and a triple quadrupole

Analytical Methods Accepted Manuscript

mass spectrometer for quantitative analyses of drugs, Rapid Commun. Mass Spectrom. 2012, 26, 499–509.

36- C. Ripollés, E. Pitarch, J. V. Sancho, F. J. López, and F. Hernández, Determination of eight nitrosamines in water at the ng/L levels by liquid chromatography coupled to atmospheric pressure chemical ionization tandem mass spectrometry, Analytica Chimica Acta 702 (2011) 62– 71.

37- Boyd J. M. (2012). Analytical and Toxicological Characterization of Novel Nitrogen Containing Disinfection Byproducts. Doctoral dissertation, University of Alberta, Alberta, Canada, pp. 115-116.

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Tables and figures

Table 1. Physico-chemical properties of the studied N-Nitrosamines.

Compound	Formula	Molecular Mass	Theoretical Precursor $(M+H)^+$	Experimental Precursor $(M+H)^+$	$\Delta(M+H)^+$ in ppm*	$logK_{ow}$ (**)
NDMA	$C_2H_6N_2O$	74.04801	75.05584	75.05599	2.0	-0.57
NMEA	$C_3H_8N_2O$	88.06366	89.07149	89.07150	0.1	0.04
NPyr	$C_4H_8N_2O$	100.06366	101.07149	101.07137	-1.2	-0.19
NDEA	$C_4H_{10}N_2O$	102.07931	103.08714	103.08714	0.0	0.48
NPip	$C_5H_{10}N_2O$	114.07931	115.08714	115.08697	-1.5	0.36
NMor	$C_4H_8N_2O_2$	116.05858	117.06640	117.06612	-2.4	-0.44
NDPA	$C_6H_{14}N_2O$	130.11061	131.11844	131.11830	-1.1	1.36
NDBA	$C_8H_{18}N_2O$	158.14191	159.14974	159.14928	-2.9	2.63
NDPhA	$C_{12}H_{10}N_2O$	198.07931	199.08714	199.08698	-0.8	3.13

* The mass error ranged from 0.0 to 2.0 ppm. This error was calculated using the mean of three injections. There was no drift in the mass error for all compounds during a sequence run.

** C. Hansch, A. Leo, D. Hoekman, 1995. Exploring QSAR: Hydrophobic, electronic, and steric constants. American Chemical Society, Washington, DC.

Table 2. Names and characteristics of the six tested chromatographic columns for nitrosamines analysis.

Column	Particles Size (µm)	Column Size
Thermo Fisher Scientific Hypersil Gold C18	1.9	100 x 2.1 mm
Thermo Fisher Scientific Hypersil Gold C8	3	100 x 2.1 mm
Thermo Fisher Scientific Pentafluorophenyl (PFP)	3	100 x 2.1 mm
Thermo Fisher Scientific Hypercab	3	100 x 2.1 mm
TSKgel Amide-80	5	250 x 2.0 mm
Kinetex HILIC 100Å	2.6	100 X 2.1 mm

Analytical Methods Accepted Manuscript

Table 3. Summary of the mass spectrometry optimized parameters.

Parameter	Value	Parameter	Value
▪ Sheath Gas flow rate	75	▪ Maximum IT	100 ms
▪ Auxiliary Gas flow rate	25	▪ Scan Type	full MS
▪ Ion Sweep Gas flow rate	2	▪ Scan Range	50-500 m/z
▪ S-Lens RF Level	55	▪ Injection Volume	100 µL
▪ Resolution	70000	▪ Detection Mode	Positive
▪ AGC Target	$1e^5$	▪ Lock Masses	Off

Table 4. Method validation results for linearity (R^2), limits of detection (LOD) and quantification (LOQ) for HPLC grade water.

	HPLC grade water[a]			
Compound	R^2 [b]	Instrumental detection limit [c] LOD (µg/L)	Instrumental quantification [c] limit LOQ (µg/L)	Linearity range (µg/L)
NDMA	0.9996	0.2	0.5	0.5-100
NMEA	0.9997	0.4	1.0	1.0-100
NPyr	0.9997	0.05	0.2	0.2-100
NDEA	0.9996	0.15	0.5	0.5-100
NPip	0.9991	0.015	0.05	0.05-100
NMOR	0.9985	0.05	0.2	0.2-100
NDPA	0.9996	0.01	0.05	0.05-100
NDBA	0.9969	0.01	0.05	0.05-100
NDPhA	0.9939	0.01	0.05	0.05-100

[a] As no SPE was performed for HPLC grade water, these values represent the instrumental detection and quantification limits.
[b] R^2 determined by internal standard calibration for spiked solution of analyte (with eight point calibration standards).
[c] The LOD, $(3.3 \times SD_{y\text{-intercept}})/m)$ and LOQ, $(10 \times SD_{y\text{-intercept})/m})$ were determined using the calibration curve of the analyte peaks.

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 5. Method validation for accuracy (% bias) and precision (intra-day and inter-day) for two concentration levels (QC1 6 µg/L and QC2 60 µg/L) for the analyzed N-nitrosamines in HPLC water.

Compound	HPLC grade water					
	QC1 6 µg/L			QC1 60 µg/L		
	Bias (%)	Intra-day	Inter-day	Bias (%)	Intra-day	Inter-day
NDMA	-3.7	1.3	8.6	-1.9	1.0	9.3
NMEA	8.2	2.8	8.5	-2.6	0.9	8.8
NPyr	-0.8	1.1	8.8	0.3	2.2	10.4
NDEA	-1.0	2.0	11.8	-3.0	3.2	11.2
NPip	1.7	1.7	9.0	3.5	4.0	10.6
NMOR	-3.0	2.3	6.4	-2.5	2.6	10.2
NDPA	-1.1	1.8	8.2	0.2	0.9	9.7
NDBA	8.5	1.2	8.8	5.5	1.7	9.9
NDPhA	0.1	2.4	10.4	7.9	2.1	9.2

*Precision (%) = RSD, relative standard deviation. The RSD was calculated based on the peak areas for five injections in the same day (Intra-day, n = 5) and fifteen injections for a period of three days (Inter-day, n = 15). Concentrations are given in µg/L.

Table 6. Solid phase extraction recovery rates of N-Nitrosamines (100 ng/L) in drinking water and wastewater (three replicates).

Compound	Recovery rates (%)	
	Drinking water	Wastewater
NDMA	82 ± 2	75 ± 3
NMEA	82 ± 5	74 ± 3
NPYR	83 ± 3	70 ± 3
NDEA	75 ± 4	77 ± 17
NPIP	83 ± 2	72 ± 8
NMOR	83 ± 3	73 ± 2
NDPA	81 ± 3	70 ± 5
NDBA	78 ± 3	68 ± 6
NDPhA	31 ± 5	22 ± 9

Analytical Methods Accepted Manuscript

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

Table 7. Method validation results for linearity (R^2), method detection (LOD) and quantification (LOQ) limits in drinking water and wastewater.

	Drinking water				Wastewater			
Compound	R^2	MLOD (ng/L)	MLOQ (ng/L)	Linearity Range (ng/L)	R^2	MLOD (ng/L)	MLOQ (ng/L)	Linearity Range (ng/L)
NDMA	0.9969	4.2	13	10-200	0.9984	7.6	23	5.0-200
NMEA	0.9920	9.1	28	10-200	0.9980	12	35	10-200
NPyr	0.9968	1.5	4.6	0.4-200	0.9975	11	35	0.4-201
NDEA	0.9955	2.5	7.4	0.1-200	0.9973	5.9	18	2.0-200
NPip	0.9973	2.3	7.0	0.1-200	0.9982	6.4	20	2.0-200
NMOR	0.9968	6.5	20	2.0-200	0.9954	4.8	15	0.02-200
NDPA	0.9961	2.4	7.2	2.0-200	0.9985	4.7	14	2.0-200
NDBA	0.9960	1.8	5.3	0.4-200	0.9972	2.7	8.1	0.02-200
NDPhA	0.9983	0.4	1.3	0.1-200	0.9991	2.8	8.4	0.4-200

Analytical Methods Accepted Manuscript

Table 8. Method validation for accuracy (% bias) and precision (intra-day and inter-day) are reported as RSD for two concentration levels (QC1 12 ng/L and QC2 120 ng/L) for the analyzed N-nitrosamines in drinking water and wastewater matrices. RSD values were calculated based on the peak areas for five injections in the same day (Intra-day, n = 5) and fifteen injections for a period of three days (Inter-day, n = 15). Concentrations are given in ng/L.

Compound	QC1 12 ng/L						QC2 120 ng/L					
	Drinking water			Wastewater			Drinking water			Wastewater		
	Bias (%)	Intra-day	Inter-day	Bias (%)	Intra-day	Inter-day	Bias (%)	Intra-day	Inter-day	Bias (%)	Intra-day	Inter-day
NDMA	3.5	1.2	11.3	-16.5	5.5	17.5	15.7	2.4	8.5	-5.0	7.7	8.0
NMEA	3.5	10.3	10.7	13.7	14.8	18.3	6.9	5.9	12.2	-1.4	8.7	8.6
NPyr	19.1	9.6	15.2	-4.1	3.2	15.0	8.4	3.2	9.2	1.8	8.1	10.[illegible]
NDEA	11.0	3.7	3.3	-4.3	5.5	18.8	14.7	4.1	5.0	1.7	7.0	6.4
NPip	10.1	3.9	6.6	7.0	13.8	16.8	9.2	3.6	4.7	0.7	5.6	5.8
NMOR	18.6	5.4	13.2	-16.8	19.6	17.4	7.2	3.8	9.3	-1.7	7.8	11.1
NDPA	17.7	2.3	8.5	-1.7	10.3	15.1	9.6	4.5	4.8	-1.9	6.0	5.7
NDBA	17.2	2.7	5.2	-12.6	14.8	6.5	10.3	2.5	4.7	-0.2	4.8	5.3
NDPhA	15.9	1.1	5.1	-1.7	17.9	11.8	12.3	4.8	5.8	-1.8	4.7	5.6

Analytical Methods Accepted Manuscript

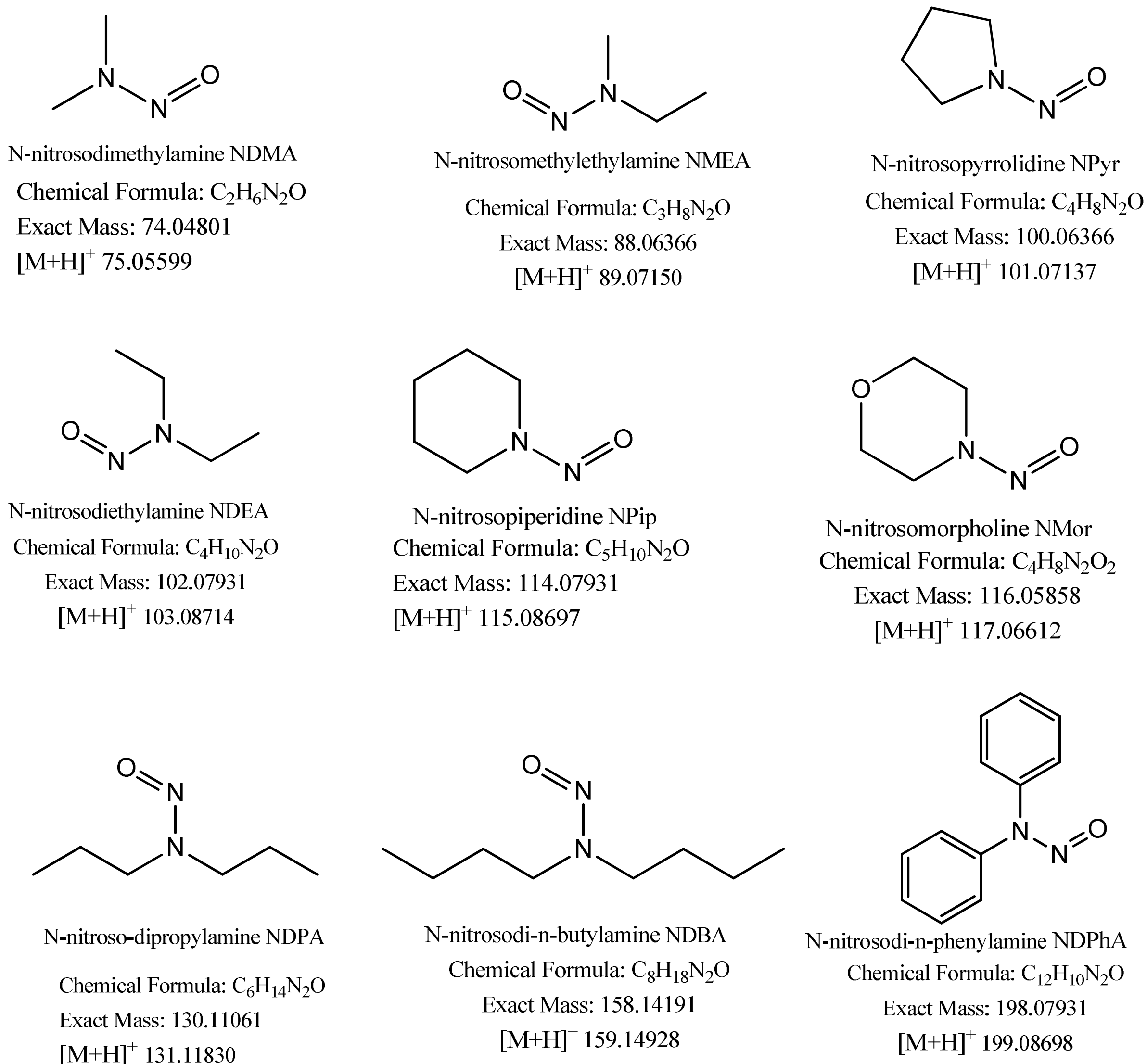

Fig. 1. Chemical structures and exact masses of the nine N-Nitrosamines studied.

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

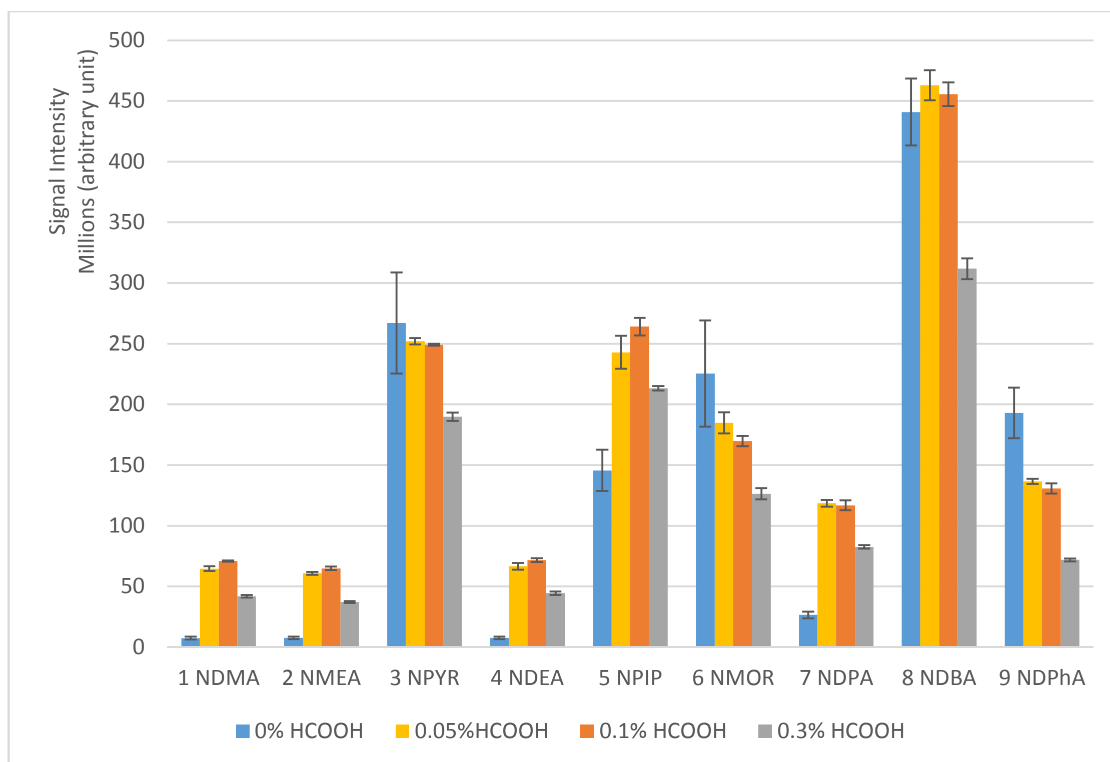

Fig.2. Experimental results obtained for different concentrations of HCOOH added to the H_2O/MeOH mobile phase. Injection volume was 25 µL and sample concentration was fixed at 100 µg/L. Errors bars are standard deviations of three replicates.

Analytical Methods Accepted Manuscript

Analytical Methods Accepted Manuscript

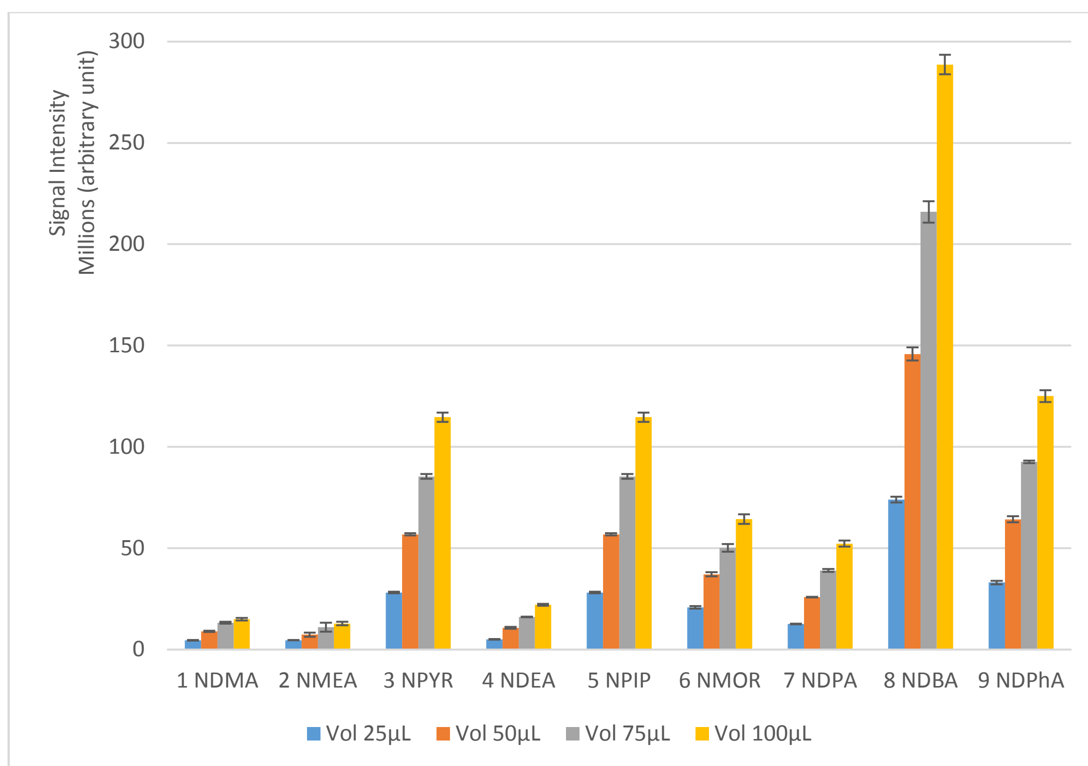

Fig. 3. Experimental results obtained with different injection volumes with the selected Hypersil Gold C18 (1.9 µm, 100 x 2.1 mm) column. The injection volume of 100 µL (HPLC grade water) appeared to be the maximum. Errors bars are standard deviations of three replicates.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

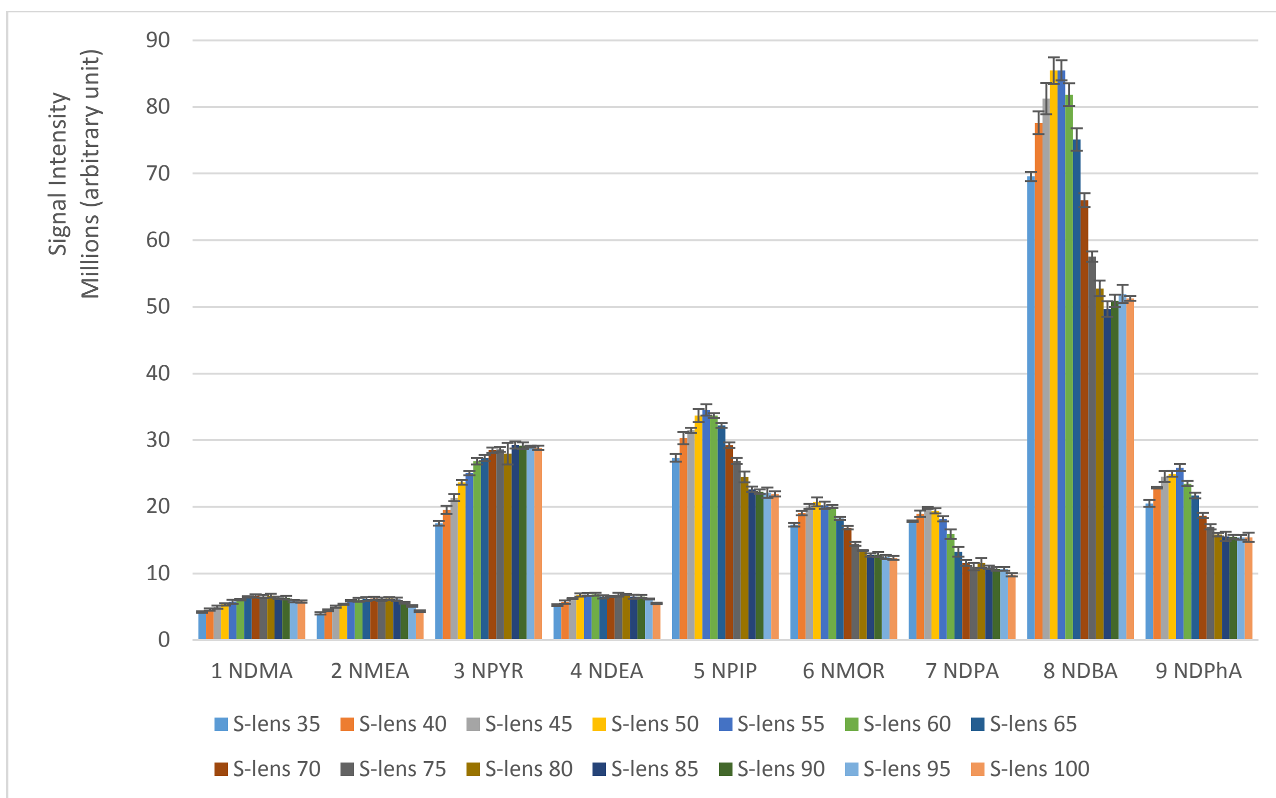

Fig. 4. Experimental results obtained by varying the S-Lens RF Level. The value of 55 (among 50, 55, 60, 65 and 70) showed the strongest signal for five of the nine analyzed nitrosamines. Errors bars are standard deviations of three replicates.

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

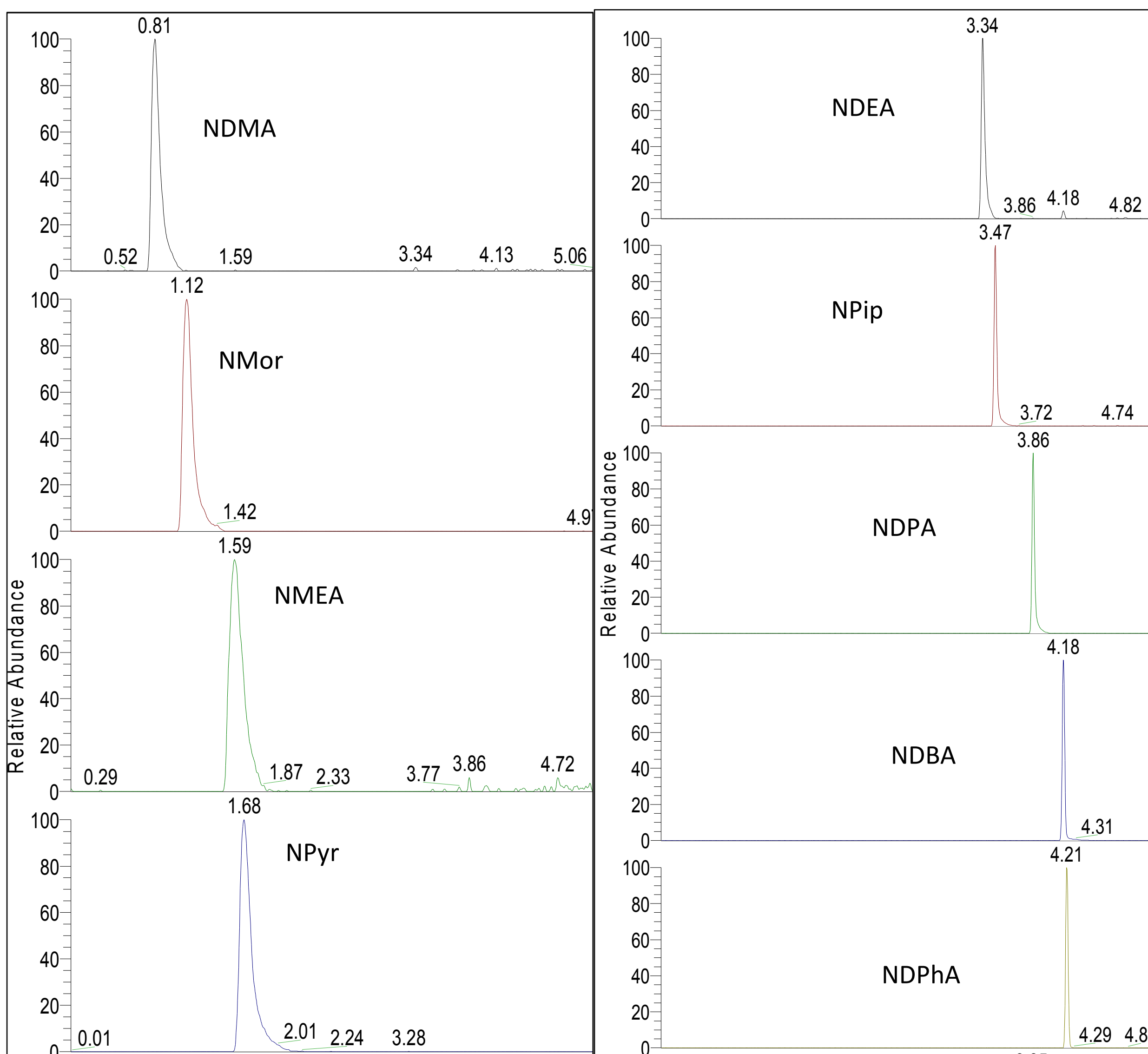

Fig. 5. Chromatogram of N-nitrosamines analyzed by LC-MS (with a Q-Exactive HRMS instrument) in HPLC water; Sample concentration 20 µg/L in HPLC grade water; Injection volume 5 µL.

Analytical Methods Accepted Manuscript

Fig. 6. Chromatogram of N-nitrosamines analyzed by LC-MS (with a Q-Exactive HRMS) in drinking water matrix; Sample concentration 120 ng/L in methylene chloride; Injection volume 5 µL.

Analytical Methods Accepted Manuscript

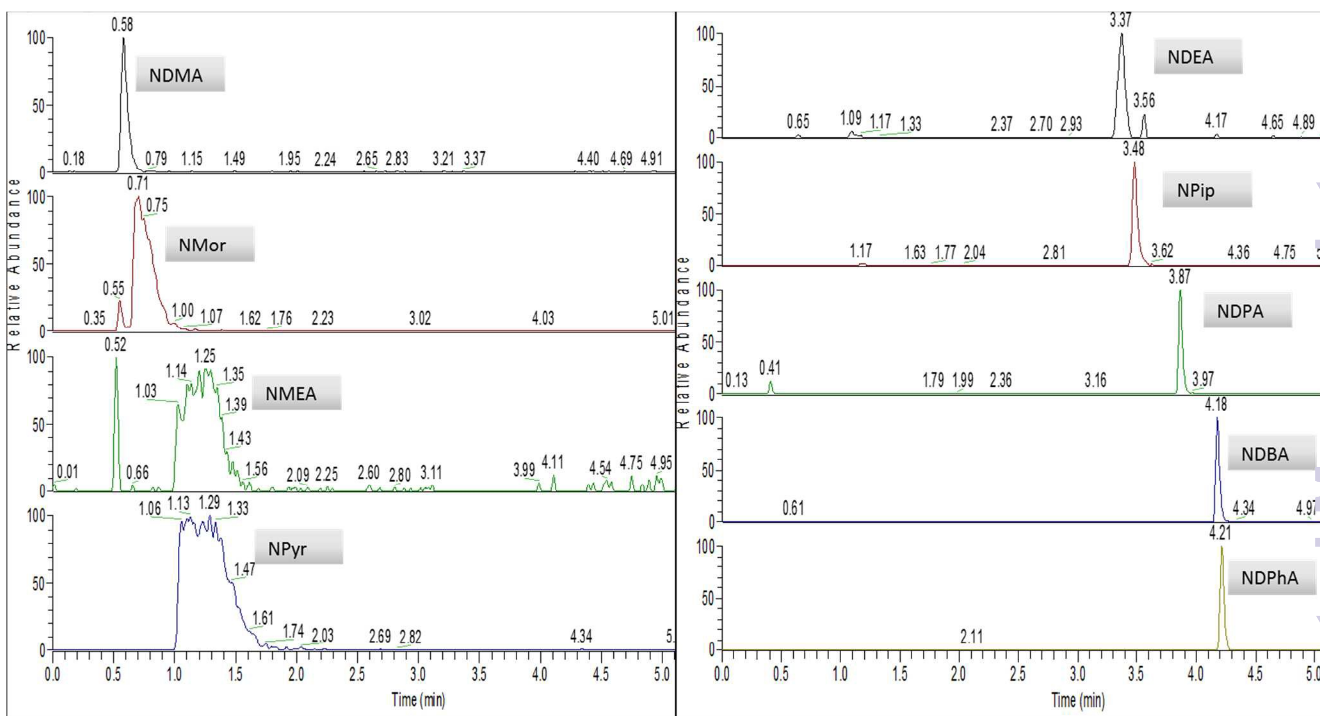

Fig. 7. Chromatogram of N-nitrosamines analyzed by LC-MS (with a Q-Exactive HRMS) in wastewater matrix; Sample concentration 120 ng/L in methylene chloride; Injection volume 5 µL.

Analytical Methods Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

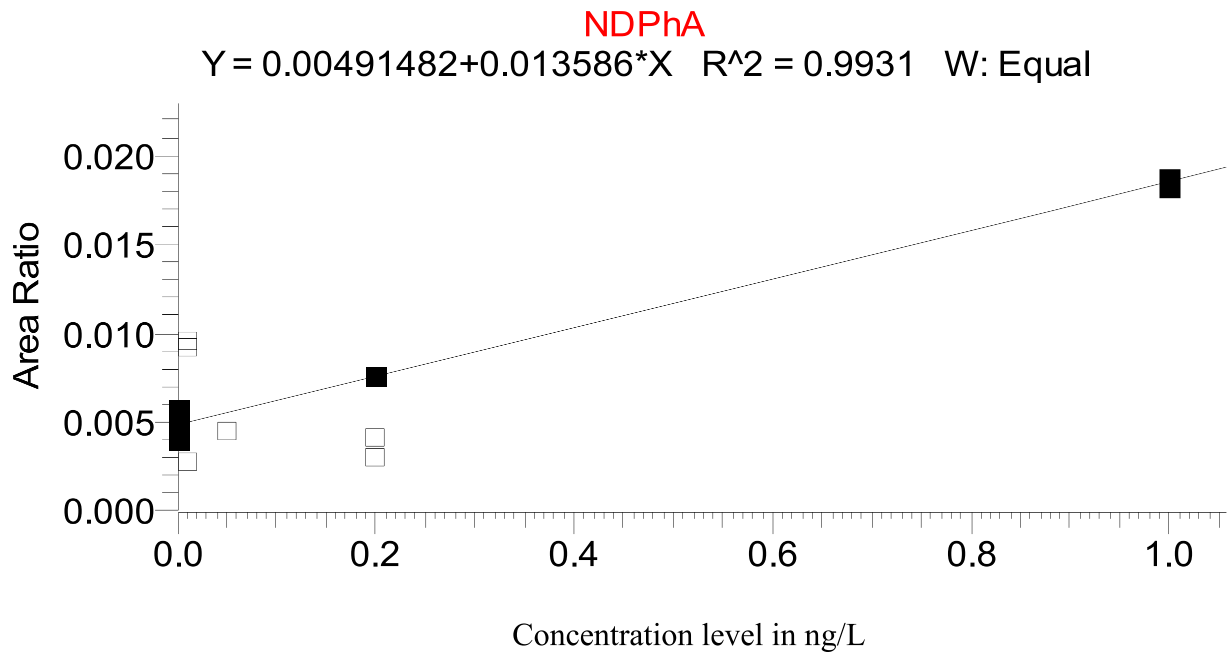

Fig. 8. Analytical results of N-nitrosodiphenylamine (NDPhA), the only N-nitrosamines quantified at a concentration level above the limit of detection. This compound was found in the drinking water sample collected in the city of Trois-Rivières, Quebec Canada.

Analytical Methods Accepted Manuscript