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Water pollution

NEW LC-MS/MS METHOD FOR THE DETERMINATION OF EIGHT NITROSAMINES IN DRINKING WATER

T. GALAON*, L. CRUCERU, J. PETRE, L. F. PASCU, V. I. IANCU, M. NICULESCU

INCD-Ecoind, 71–73 Drumul Podu Dambovitei Street, Sector 6, Bucharest, Romania
E-mail: *tomagalaon@yahoo.com*

Abstract. A new sensitive, selective and accurate LC-MS/MS method with positive electrospray ionisation was developed to detect eight nitrosamines from drinking water. The method separates all nitrosamines using a Zorbax Eclipse Plus C18 column (100 × 2.1 mm, 3.5 µm) kept at 15°C and a mobile phase consisting of aq. 0.005% formic acid and acetonitrile in the ratio 90/10 (v/v). A strong gradient applied in 8 min up to 90% ACN allowed analyte separation. A low mobile phase flow-rate of 0.2 ml/min was used to enhance ESI ionisation. Collision energy, fragmentor and capillary voltages were optimised to enhance analyte S/N ratio. Optimisation of LC-MS parameters generated low instrumental LOQ values between 0.05–1.84 µg/l. Detector response was linear in the range 1–100 µg/l ($R^2 > 0.99$) for all nitrosamines. SPE using activated charcoal cartridges was used to concentrate nitrosamines from drinking water. Overall method LOQs were situated in the range 1.23–4.12 ng/l. LC-MS/MS method was fully validated with respect to specificity, linearity, precision, accuracy and LOQ, and provided good results. The method was tested on six tap water samples collected from different regions of Bucharest and the determined nitrosamine total content ranged between 2.8 and 14.8 ng/l.

Keywords: LC-MS/MS, nitrosamines, drinking water, solid-phase extraction, electrospray.

AIMS AND BACKGROUND

Nitrosamines are considered a highly genotoxic and carcinogenic class of organic compounds due to the presence of a nitroso moiety in their molecule. Most nitrosamines were proved to be carcinogenic and they can be found in cosmetics, plastics, tobacco, beer, smoked meat, rubber products, waste, surface and drinking water[1]. In the last decades, scientists discovered that nitrosamines (NA) occur in water treatment plants as water disinfection by-products (DBPs) during chloramination, ozonation or chlorination processes[2–4]. Although detected levels of NA in finished drinking water are generally quite low, several countries (Canada, USA, and Germany) introduced extremely low limits (max. 10 ng/l) for these compounds due to their high carcinogenic potential[5–8]. Highly sensitive detection methods are therefore required to determine NA at the low levels (few ng/l) found in source

* For correspondence.

and finished water. Literature data show that LC-ESI-MS/MS and GC-CI-MS/MS with SPE pre-concentration on polar adsorbents (Lichrolut EN, Oasis HLB, activated charcoal, etc.) are the most used techniques to detect NA due to their high specificity and sensitivity[9–13].

The present study was focused to develop, optimise and validate a sensitive, selective, and accurate LC-MS/MS method with positive electrospray ionisation able to determine eight nitrosamines from drinking water at ng/l levels. The investigated compounds are: N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodipropylamine (NDPA), N-nitrosodibutylamine (NDBA), N-nitrosopyrrolidine (NPYR), N-nitrosopiperidine (NPIP), N-nitrosomorpholine (NMOR) and N-nitrosodiphenylamine (NDPhA).

EXPERIMENTAL

Reagents and chemicals. HPLC grade methanol, acetonitrile and dichloromethane (DCM) were acquired from Merck (Darmstadt, Germany). Ammonium formate, ammonium acetate and formic acid p.a. were obtained from Sigma-Aldrich. Water for chromatography was obtained within the laboratory by means of a MilliQ instrument. High purity reference standards of nitrosamines: NDMA, NDEA, NDPA, NDBA, NPYR, NPIP, NMOR and NDPhA were obtained from Ultra Scientific (Rhode Island, USA). Deuterated nitrosamines used as surrogate internal standards: $[^2H]_6$-N-nitrosodimethylamine (NDMA-d6), $[^2H]_{14}$-N-nitrosodipropylamine (NDPA-d14) were obtained from Cambridge Isotope Laboratories (Andover, MA, USA). NDMA-d6 was used for NDMA, NDEA, NMOR, NPYR detection, while NDPA-d14 for detection of NPIP, NDPA, NDBA and NDPhA. SPE Coconut Charcoal (2 g/6 ml) cartridges were aquired from Supelco (Bellefonte, PA, USA). Individual stock standard solutions of NA were prepared at a concentration of 2000 µg/ml in ACN. Eight calibration solutions (1–100 µg/l) were obtained by successive dilutions from a 20 µg/ml mixed stock solution in ACN. All standard solutions were kept protected from light at 4°C.

LC-MS instrumentation and conditions. Experiments were performed using an Agilent 1260 series LC system (Waldbronn, Germany) consisting of: binary pump, thermostatted autosampler, thermostatted column compartment coupled with an Agilent 6410B triple-quadrupole mass analyser fitted with an ESI ionisation source. Data acquisition and analysis were performed using Agilent Mass Hunter software, revision B.04.01. All chromatographic runs were carried out on a single Zorbax Eclipse Plus C18 (100 × 2.1 mm, 3.5 µm) column from Agilent Technologies. Column was thermostatted at 15°C. All experiments were performed in gradient elution conditions at a flow-rate of 0.2 ml/min. Mobile phase composition was a mixture of aq. 0.005% formic acid (FA) and acetonitrile (ACN). The employed

gradient elution program and the re-equilibration step are given in Table 1. Sample injection volume was 50 µl.

Table 1. Gradient elution program used to separate the eight targeted nitrosamines

Time (min)	ACN (%)	Flow-rate (ml/min)	Gradient line purpose
0.00	10	0.200	analytical separation
8.00	90	0.200	
13.20	90	0.500	column re-equilibration
13.21	10	0.500	
17.40	10	0.500	
17.41	10	0.200	

Sample diluent was the same as initial mobile phase composition. Detection was achieved using Multiple Reaction Monitoring (MRM) acquisition mode. Retention time, MRM transitions, fragmentor voltages, collision energies and other MS parameters are given in Table 2. ESI ionisation source was operated in positive mode with drying gas temperature of 300°C and drying gas flow of 10 l/min. Analyte detection in separate timed acquisition windows allowed setting of different capillary voltages per segment in order to increase NA sensitivity (Table 2).

Table 2. Retention time (tR), MRM transitions, fragmentor voltage, collision energies, cell accelerator voltage, capillary voltage and timed acquisition windows for the target analytes

Compound	tR (min)	MRM transition	Fragmentor volt.	Collision energy	Cell accel. volt.	Cap. volt.	Aquisition window (min)
NDMA-d6	2.182	81.0 → 45.9	102	16	3	3700	1.5–2.65
NDMA	2.205	75.0 → 43.2	102	16	3		
NMOR	2.890	117.2 → 87.1	100	15	4	3200	2.65–5.0
NPYR	3.260	101.1 → 55.1	100	12	4		
NDEA	6.334	103.3 → 75.1	92	6	7	2800	5.0–7.3
NPIP	7.548	115.1 → 69.0	100	12	7	3700	7.3–9.0
NDPA-d14	10.537	145.1 → 50.1	100	10	7	3700	9.0–11.7
NDPA	10.657	131.1 → 43.2	100	10	7		
NDBA	12.384	159.1 → 57.1	100	10	6	3900	11.7–13.8
NDPhA	12.605	199.3 → 169.1	100	10	6		

RESULTS AND DISCUSSION

LC separation optimisation. The main purpose of the developed LC method was to obtain sharp peaks and good separation of NA in the shortest possible time. Although baseline separation is not a must in MS/MS detection, it is desirable because of the possibility to use different acquisition windows for different compounds.

This enhances method sensitivity due to reduced number of required MRM transitions. Considering the high polarity of some of the nitrosamines (lg K_{ow} NDMA/NPYR/NMOR = –0.57/–0.19/–0.44), a very polar initial mobile phase was required. Thus, a 90% aqueous component fraction of the mobile phase was chosen as the initial mobile phase composition with only 10% ACN. The highly aqueous mobile phase and the low column temperature (15°C) generated an acceptable retention ($k' \geq 2$) and separation of the first three eluting NA: NDMA, NMOR and NPYR (Fig. 1). The obtained separation of the compounds allowed a maximum number of 2 compounds included in an acquisition window. This was especially useful in detection of NDMA and NDEA, two compounds with low sensitivity due to their low molecular mass (M_{NDMA} = 74.1; M_{NDEA} = 102.1 g/mol). A ballistic gradient of 10%/min ACN increase in 8 min followed by an isocratic plateau at 90% generated a rapid and highly efficient elution of the rest of the nitrosamines, especially NDBA, NDPhA, NDPA whose instrumental LOQs were 0.05, 0.09 and 0.06 µg/l based on a S/N ratio of 10. The low mobile phase flow-rate of 0.2 ml/min was used to increase MS sensitivity of the NA keeping in mind that ESI ionisation is favoured by extremely low flow-rates.

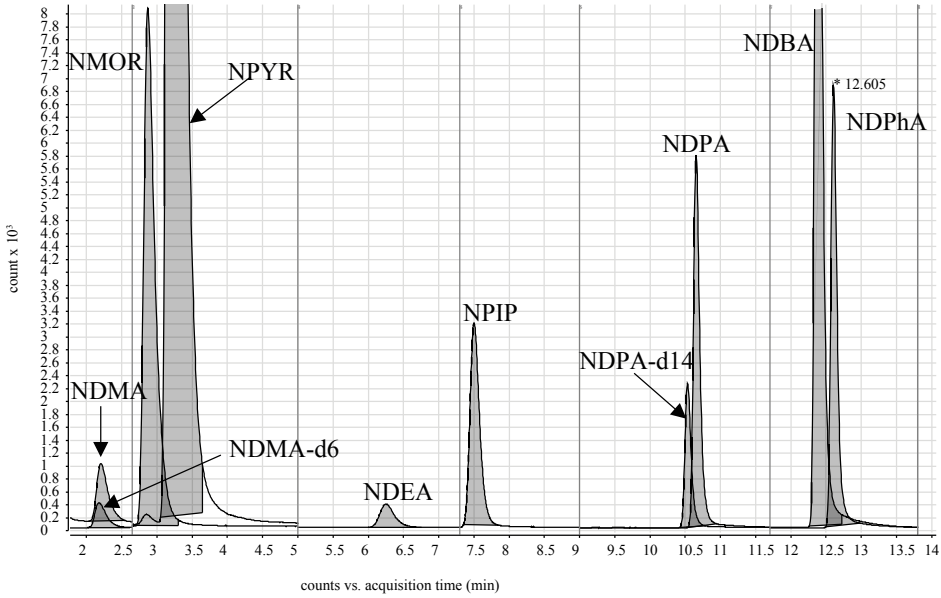

Fig. 1. MRM overlaid extracted ion chromatograms of a mixed 50 µg/l NDMA, NMOR, NPYR, NDEA, NPIP, NDPA, NDBA, NDPhA and 40 µg/l NDMA-d6 / NDPA-d14 solution

MS detection optimisation. Mass spectrometric detection parameters were optimised to obtain highest possible sensitivity when working in MRM mode for analyte quantitation. Influence of mobile phase nature on analyte MS response

was investigated. Ammonium formate, ammonium acetate and FA (0.0025–0.2%) were tested with both ACN and MeOH as organic solvents. The ammonium salts (5 mM) showed higher background noise and lower S/N ratio than FA and they were disregarded. FA proved to generate both good sensitivity and acceptable peak shape for all 8 NA. MeOH generated higher background noise and lower S/N ratio than ACN and much higher column backpressure and it was disregarded, too. Several FA concentrations were tested in the range 0.0025–0.2%. It was observed that decreasing FA concentration from 0.1 to 0.005%, a significant increase in S/N ratio for all NA is obtained. The increase was between 2.4 and 4.4 times and may be generated by ionisation suppression of NA in the presence of higher FA concentration. Further decrease of FA to 0.0025% generated a decrease in sensitivity for all NA. Collision energy (CE) applied in the collision cell (Q2) to the precursor ion to generate the product ion of the MRM transition was varied in the range 3–20 V (Fig. 2). Collision energies between 10–15 V generated highest dissociation yield for most NA. After this step a finer tuning of CE was done by varying this parameter between 80 and 120% of the previous optimised value. CE generating maximum S/N was chosen as the final method value (Table 2). The same procedure was applied to fragmentor voltage in the range 60–135 V. The obtained S/N ratio values are shown in Fig. 3. Fragmentor voltage of 100 V generates the highest transfer of NA precursor ions to first quadrupole (Q1). This was true for all NA except NDEA for which a lower voltage of 90 V was more efficient. For NDEA the most efficient CE was around 5 V. Other MS parameters such as capillary voltage (2500–4500 V), cell accelerator voltage (2–8 V), drying gas temperature (250–350°C) and drying gas flow-rate (5–12 l/min) were optimised with respect to NA sensitivity. After LC-MS optimisation, the obtained instrumental quantitation limits (IQL) were much lower than 1 µg/l (Table 3), except for NDMA and NDEA for which the obtained values were higher (1.84 and 1.02 µg/l).

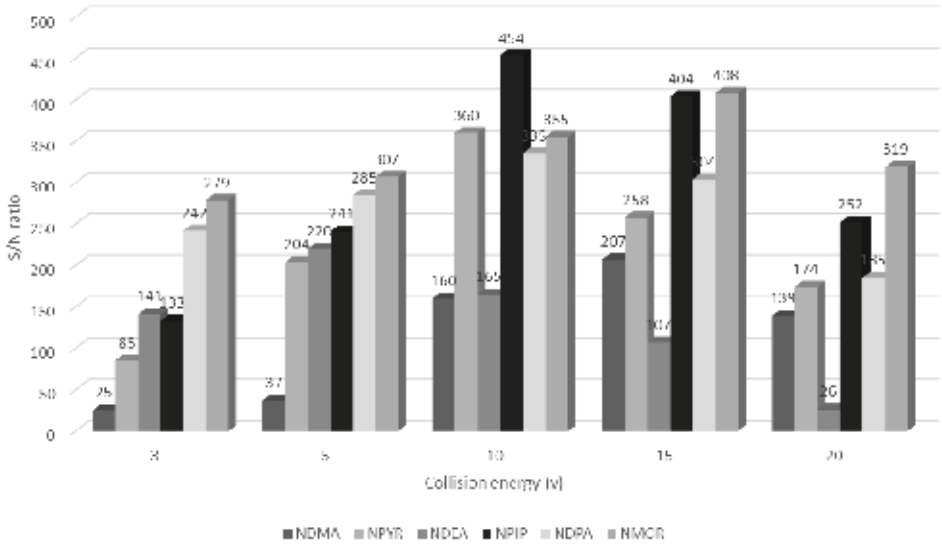

Fig. 2. S/N ratio variation with collision energy during MS method optimisation for 6 nitrosamines

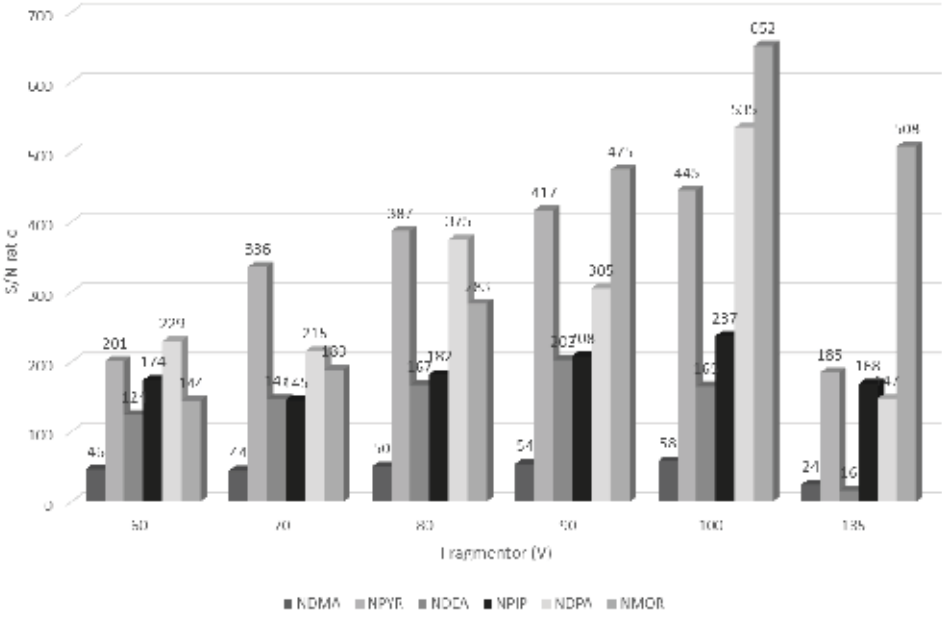

Fig. 3. S/N ratio variation with fragmentor voltage during MS method optimisation for 6 nitrosamines

Table 3. Correlation coefficients (R^2), intra-day and inter-day precision, absolute recovery, internal standard corrected recovery, instrumental and method LOQ

Analyte	R^2	Precision (%)		Absolute recovery (%)	Recovery with IS (%)	IQL (µg/l)	LOQ (ng/l)
		intra-day	inter-day				
NDMA	0.9998	9.8	11.7	78.2	110.4	1.84	4.12
NDEA	0.9992	5.3	10.1	80.5	83.8	1.02	2.78
NDPA	0.9997	4.4	6.2	85.6	95.9	0.06	2.56
NDBA	0.9983	6.7	11.4	91.4	92.5	0.05	2.07
NPIP	0.9989	8.1	9.4	84.8	100.8	0.18	1.43
NPYR	0.9988	8.6	10.5	77.2	109.0	0.24	1.23
NMOR	0.9983	7.7	9.2	73.3	103.6	0.16	1.82
NDPhA	0.9992	6.3	8.8	55.1	60.3	0.09	1.51

SPE extraction and concentration. Several SPE parameters were varied to determine the most efficient procedure to extract and isolate NA from drinking water. Thus, sample pH (7.4–8.5), elution solvent (DCM, MeOH, ACN), elution volume (6–12 ml) and sample loading flow-rate (3–5 ml/min) were modified. The final SPE parameters were chosen according to the highest recovery of NA and are described in the following. Drinking water samples were filtered under vacuum through 0.45 µm cellulose filters and then their pH was adjusted to 7.4 with $HCOONH_4$ (1M) in order to increase NA adsorption on the SPE cartridge. 500 ml drinking water sample were extracted using Coconut Charcoal (2 g/6 ml) cartridges. Before extraction, 1 ml of 40 µg/l internal standard solution (NDMA-d6/NDPA-d14) was added. The activated charcoal cartridges were previously conditioned with 2 × 3 ml DCM, 2 × 3 ml MeOH and then 5 × 3 ml water. Samples were loaded on the extraction cartridges under vacuum at a flow-rate of 4 ml/min. After adsorption the cartridges were dried in vacuum for 15 min and then the analytes were eluted 3 times with 3 ml DCM. The collected samples were spiked with 0.1 ml ACN and then they were concentrated under a very gentle N_2 stream at 25°C until the entire DCM volume is evaporated. The remaining volume of the sample (approx. 0.1 ml) is accurately measured with a micropipette and transferred to an injection vial. Finally, the sample is diluted to 1 ml with 0.005% FA. The extraction procedure results in a concentration factor of 500 which allowed NDEA and NDMA detection at concentrations down to 2.8 and 4.1 ng/l.

LC-MS/MS method validation. The optimised LC-MS/MS method was subjected to validation in order to account for its performance. Detector response proved to be linear in the range 1–100 µg/l with high correlation coefficients ($R^2 > 0.99$). Instrumental LOQs were determined by injecting decreasing concentrations of NA solutions until a S/N of approx. 10 was obtained (Table 3). Intra-day and inter-day precision were tested at 10 and 50 ng/l on 6 replicates. Results obtained after validation procedure are given in Table 3. Method proved to be precise with

RSD% values situated between 6.3–9.8% (intra-day) and 6.2–11.7% (inter-day). Analyte recovery was calculated with and without internal standard correction. Except for NDPhA (60%) whose adsorption in the activated charcoal cartridge is very strong, very good recoveries were obtained for the rest of NA (84–110%).

Drinking water sample analysis. The developed LC-MS/MS method was tested on several drinking water samples collected from six house-holds at different locations in Bucharest (Table 4). Sampling was done in 1-l amber glass bottles in October 2014. Taps were opened at maximum and were left to run for 5 min. Sampling bottles were rinsed with sample and then filled to capacity, transported to the lab immediately and kept at 4°C until extraction (max. 48 h). NDEA was the most detected NA (5 out of 6 samples) and showed the highest levels (3.3–7.9 ng/l). NDMA was detected in 2 locations with concentrations of 5.5 and 6.2 ng/l. NPIP and NPYR were also detected at somewhat lower levels from ND to 3.4 ng/l, whereas NDPA, NDBA, NMOR and NDPhA were not detected at all. Total content of NA detected in Bucharest distribution system was quite low with values ranging from 2.8 up to 14.8 ng/l.

Table 4. Nitrosamine concentrations (ng/l) detected in drinking water samples collected from six different locations in Bucharest

Water source	NDMA	NMOR	NPYR	NDEA	NPIP	NDPA	NDBA	NDPhA	Total NA
Tap 1	< LOQ	< LOQ	< LOQ	3.27	1.74	ND	ND	ND	5.01
Tap 2	5.48	< LOQ	1.73	5.47	<LOQ	ND	ND	ND	12.68
Tap 3	< LOQ	ND	3.44	7.86	2.92	ND	ND	ND	14.21
Tap 4	< LOQ	ND	ND	< LOQ	2.84	ND	ND	ND	2.84
Tap 5	< LOQ	ND	1.75	5.78	<LOQ	ND	ND	ND	7.53
Tap 6	6.17	< LOQ	2.04	6.63	<LOQ	ND	ND	ND	14.84
LOQ	4.12	1.82	1.23	2.78	1.43	2.56	2.07	1.51	–

CONCLUSIONS

A sensitive, selective and accurate LC-ESI(+)MS/MS method with SPE extraction was developed to detect eight nitrosamines from drinking water. Optimisation of the LC-MS method generated very low instrumental LOQs (0.05–1.84 µg/l). Drinking water samples were concentrated 500 times using SPE on activated charcoal which generated overall method LOQs between 1.23 and 4.12 ng/l. The LC-MS method was validated with respect to specificity, linearity, accuracy, precision and LOQ. Six tap water sources fed from Bucharest drinking water distribution system were analysed. NDPA, NDBA, NMOR and NDPhA were not detected in any of the analysed samples. Instead, low levels of NDEA (3.3–7.9 ng/l), NDMA (ND – 6.2 ng/l), NPIP (1.7–2.9 ng/l) and NPYR (ND – 3.4 ng/l) were determined showing a reduced contamination with these highly toxic compounds.

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