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A RAPID AND SENSITIVE METHOD FOR THE MONITORING OF N-NITROSODIPHENYLAMINE AND N-NITROSODIMETHYLAMINE IN MULTIPLE WATER MATRICES

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Abstract: N-nitrosamines are probable human carcinogens that could seriously affect the safety of drinking water consumers. In this study, a rapid, sensitive and reliable solid-phase extraction method combined with ultra-performance liquid chromatography coupled with tandem mass spectrometry has been developed for the quantification of N-nitrosodiphenylamine and N-nitrosodimethylamine at ultra-trace levels in aqueous matrices. Chromatographic separation was performed using an Acquity UPLC BEH C18 column and a mobile phase consisting of acetonitrile, water, and formic acid (60:40:0.1, v/v/v) at a flow rate of 0.4 mL min^{-1}. The run time of the instrumental method was two minutes. Under optimized conditions the performance of the proposed method was studied in terms linearity ($r^2 \geq 0.998$), precision (< 3%), accuracy (between 99% and 103%), lower limit of detection (0.04–0.16 ng L^{-1}), and lower limit of quantification (0.08–0.32 ng L^{-1}). The obtained extraction recoveries of the target compounds were within the range of 85–101%, and the relative standard deviations were less than 4%. The matrix effect was within 84–99% at all quality control levels. These results showed clearly that, the developed method is rapid, sensitive efficient, and accurate for simultaneous determination of N-nitrosodiphenylamine and N-nitrosodimethylamine. In addition, the proposed method has been successfully applied for the screening of these molecules in real water samples.

Keywords: environmental monitoring, N-nitrosodiphenylamine, N-nitrosodimethylamine, solid-phase extraction, ultra-performance liquid chromatography, tandem mass spectrometry

1. INTRODUCTION

Drinking water is often chlorinated in an effort to disinfect the water prior to human consumption. However, the chlorination process leads to the formation of disinfection by-products (DBPs) (Gurzau et al., 2010; Thach et al., 2012). These chemicals originate from the reaction of chlorine with organic natural matter present in raw water. To date, more than 600 DBPs have been identified in drinking water (Krasner, 2009; Andrzejewski et al., 2008; Asami et al., 2009). One class of non-halogenated DBPs is made up of N-nitrosamines, which have been recently identified as by-products in chlorinated and chloraminated water (Nawrocki & Andrzejewski, 2011; Kristiana et al., 2013). These compounds are the subject of great recent concern because of their suspected effects on human health, especially of carcinogenic and genotoxic order, as a result of long-term consumption of chlorinated water, even at ng L^{-1} levels (Richardson, 2009; California Department of Public Health, 2013).

In the last years, the majority of studies of DBPs have focused on N-nitrosodimethylamine (NDMA) and N-nitrosodiphenylamine (NDPHe), because these are the most frequently detected in drinking water. The World Health Organization has set a guideline value for NDMA of 100 ng L^{-1} (World Health Organization, 2011). A provisional guide value of 12 ng L^{-1} was proposed for NDMA in Netherlands and a guide value of 10 ng L^{-1} for

NDMA in drinking water was recommended in Germany (Planas et al., 2008). The Ontario Ministry of the Environment has ruled a maximum allowable concentration (MAC) of 9 ng L^{-1} for NDMA (Ontario Ministry of the Environment, 2003). In addition, the United States Environmental Protection Agency (U.S. EPA) has classified NDMA and NDPHe into the B2 group (probable carcinogenic effects on humans) (United States Environmental Protection Agency, 2010) and indicated that these pollutants produce an increased cancer risk at the 10^{-6} level at the very low concentration of 20 ng L^{-1} for NDMA and 70 ng L^{-1} for NDPHe (Richardson, 2009).

Therefore, for water quality monitoring purposes, sensitive analytical methods able to detect N-nitrosamines at concentrations of few ng L^{-1} in different water matrices are needed.

As stated above, the N-nitrosamines concentration in water samples is in the range of ultra-trace level. Thus, prior to their instrumental analysis a preparation step is needed in order to extract the trace amount of analytes from the water sample, remove the interfering components from the matrix and to concentrate target compounds to a suitable concentration level.

In the literature, the most commonly used methods for sample preparation are solid-phase micro-extraction (SPME) (Hung et al., 2010) and solid-phase extraction (SPE) (Boyd et al., 2011; Munch & Basset, 2006). Several selective analytical techniques have been developed for the quantification of these molecules in different matrices. Most often N-nitrosamines have been analyzed in water samples by using gas chromatography (GC) coupled with different types of detectors, such as mass spectrometry (MS) (Pozzi et al., 2011) and tandem mass spectrometry (GC/MS/MS) (McDonald et al., 2012; Huang et al., 2013). However, the use these techniques have some limitations. Indeed, some N-nitrosamines may be undetectable by GC/MS/MS, because they are thermally unstable or non-volatile. For example, NDPHe is contained in the U.S. EPA Method 521 standard mix, but is not included in the GC/MS/MS EPA method, because it is thermally unstable and decomposes in the GC injector (Munch & Bassett, 2004). Moreover, some methods for N-nitrosamines analysis by high liquid chromatography-tandem mass spectrometry (HPLC/MS/MS) have been proposed by several workers (Boyd et al., 2011; Cha et al., 2006). Ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC/MS/MS) was used previously for the analysis of NDMA in water samples (Wang et al., 2010). More recently, a novel UPLC/MS/MS method has been developed for determination of NDMA in drinking water using an atmospheric pressure chemical ionization (Ripollés et al., 2011). However, these methods are less sensitive comparing to different guidelines proposed for N-nitrosamines in drinking water.

The aim of this study was to develop a rapid, sensitive and robust analytical method for the monitoring of NDMA and NDPHe in water samples using ultra-performance liquid chromatography–electrospray–tandem mass spectrometry (UPLC-ESI-MS/MS). Prior to analysis the N-nitrosamines were extracted, purified, and concentrated from different types of water samples in one step using a solid-phase extraction (SPE) method. Different analytical parameters were investigated and optimized and the developed method has been applied to real water samples. The obtained results demonstrated that the developed methodology is an interesting analytical tool for the monitoring of target analytes in multiple water matrices.

To the best of our knowledge, this is the first work reporting the analysis of NDMA and NDPHe at ultra-trace concentrations with high sensitivity and high SPE recoveries.

2. EXPERIMENTAL

2.1. Chemicals and preparation of stock solutions

All chemical reagents used in this work were of the highest analytical purity grade (suitable for trace analysis). The N-nitrosamines standard solutions (2000 mg L^{-1} in methanol) were supplied by LGC Standards (Wesel, Germany). Acetonitrile and formic acid were purchased by Baker (Deventer, Netherlands). Methanol and dichloromethane were obtained from Fischer Scientific-Bioblock (Illkirch, France). Acetic acid was supplied by Acros Organics (Noisy-le-Grand, France).

A standard stock solution of N-nitrosamines at 100 mg L^{-1} was prepared in methanol and stored at -20°C for at least three months. Working solutions were freshly prepared in acetonitrile/ultrapure water (60:40, v/v) by appropriate dilutions of the stock standard solution to reach the working concentration range. This composition ensured good stability of the analytes in water samples. The working solutions were used for spiking samples for the study of the extraction recovery and matrix effect and also for the preparation of calibration standards. The ultrapure water used for the preparation of the samples was produced by an Elga Option-Q DV-25 system (Antony, France).

2.2. Sampling

Sampling was carried out firstly to determine

the recovery of the target molecules in different matrices by applying the optimized analytical method and secondly to evaluate the application of the proposed analytical method.

For the first phase surface water samples were collected from different locations in France. The water samples were collected in baked, glass, 10-L amber bottles with Teflon lined caps to ensure sample integrity, filtered through a 0.45µm cellulose membrane in order to remove suspended particles and stored in the fridge at 4°C under light protection until analysis (within one week of collection). No additives were placed in the samples to prevent their contamination. For the application of the method step, the developed analytical strategy was used to determine the NDMA and NDPHe in drinking water samples. Samples were collected at two times periods (July and October 2013) from different areas, in France by using the same strategy as described above.

2.3. Apparatus and operation conditions

The chromatographic analyses were performed in isocratic mode, using an Acquity UPLC H-Class system, containing a binary pump, an auto-sampler and a thermostated column compartment (Waters, Saint-Quentin en Yvelines, France). Chromatographic separation of N-nitrosamines was carried out on Ethylene Bridged Hybrid (BEH) C18 column (100 mm × 2.1 mm, 1.7 µm) from Waters. The column in the chromatographic system was protected by an in-line filter unit purchased from Waters (Saint-Quentin en Yvelines, France). The analytical column compartment was maintained at 45°C. The auto-sampler was conditioned at 5°C. For the optimization of the chromatographic analysis and MS/MS characterization, standard solutions of each N-nitrosamine in mobile phase were used. The used mobile phase consists of 0.1% formic acid in acetonitrile, and 0.1% formic acid in water (60:40, v/v). The flow rate was 0.4 mL min^{-1}, and the injection volume was 5 µL.

Tandem mass spectrometry (MS/MS) determination was performed on a Quattro Premier Triple Quadrupole Mass Spectrometer (Waters, France) equipped with an electrospray ionization source (ESI). ESI experiments were carried out in positive ionization mode (ESI^+). The Quantitative analysis was carried out in Multiple-Reaction Monitoring mode (MRM). In addition, the cone voltage and the collision energy for NDMA and NDPHe were optimized in order to achieve best sensitivity.

The optimal MS/MS conditions selected for the analysis of target compounds were: capillary voltage 3 kV; cone voltage 40 V; source temperature 120°C and desolvation temperature 350°C. The cone and desolvation gas flows were 75 and 750 L h^{-1}, respectively. For collision-induced fragmentation, argon (99.99% purity, Air Liquid, Paris, France) was employed as collision gas at a flow rate of 0.12 mL min^{-1}. Dry nitrogen used as desolvation, nebulization and cone gas was produced by nitrogen generator (Peak Scientific, Inchinann, UK). The argon pressure in the collision cell was 3.52×10^{-3} mbar. Finally, the system control and data acquisition were processed using Masslynx software, version 4.1 (Waters, Saint-Quentin en Yvelines, France).

2.4. Sample preparation

The sample extraction procedure was performed by an off line SPE using a 12-port Visiprep SPE vacuum manifold obtained from Supelco (Bellefonte, PA, USA). The analytes were extracted, purified, and concentrated from water samples using Sep-Pak Plus®AC-2 cartridges (400 mg, 85 µm; Waters, Guyancourt, France). For the extraction procedure a sample volume of 250 mL was used. The SPE cartridges were sequentially pre-conditioned with 8 mL of methanol, 8 mL of dichloromethane, 8 mL of acetonitrile and 8 mL of ultrapure water. Then the sample was loaded with a flow rate of approximately 3 mL min^{-1}, and the cartridge was rinsed with 5 mL of ultrapure water adjusted to pH 2. Next, the analytes were eluted successively with 6 mL of dichloromethane, 4 mL of acetonitrile, and 2 mL of methanol at a flow rate ranging from 2 to 3 mL min^{-1}. After the extraction step, the organic eluent was collected in a 14 mL, conical graduated glass Pyrex® tube (VWR, Fontenay-sous-Bois, France).

The resulting extracts were evaporated in a water bath at 30°C and concentrated by evaporation under a high-purity nitrogen stream using a N-Evap system (Organomation, Berlin, MA, USA) to a final volume of 0.1 mL (concentration factor of 2.500). The obtained extracts are then reconstituted using acetonitrile/ultrapure water (60:40, v/v) and transferred to an injection vial. Finally, the extracts were stored at 4°C until further analysis was performed by UPLC/MS/MS system.

2.5. Quality parameters

2.5.1. Linearity, limit of detection, and limit of quantification

The linearity of the proposed instrumental method was studied from the calibration curves

established for seven concentrations levels of N-nitrosamines ranging from 0.1 to 100 µg L^{-1}. Each solution was analyzed in triplicate. The calibration curves were constructed by a least squares linear regression analysis and were not forced through the origin. This method was used to determine the slope, intercept, and correlation coefficient (r^2) of the linear regression equation. The sensitivity of the instrumental method was estimated by establishing the limits of detection and quantification. The limit of detection (LOD) is the lowest concentration of analyte that the analytical process can reliably differentiate from background levels, while the limit of quantification (LOQ) is the lowest concentration of analyte that can be quantified. The LOD and the LOQ values were estimated at concentrations with a signal-to-noise ratio (S/N) of 3 and 10, respectively. The noise at the retention time of each analyte was measured from six independent analyses.

2.5.2. Precision and accuracy

The precision of the method was evaluated in terms of repeatability (intra-day and inter-day precision). The repeatability values were expressed as relative standard deviation (RSD, %). The accuracy (RE, %) was expressed by:

$$RE,\% = \frac{\dfrac{\sum_{i=1}^{n}(observed\ concentrations)_i}{n}}{spiked\ concentration} \cdot 100 \quad (1)$$

where n is the number of replicates.

Moreover, the RSD calculated at each concentration level was not allowed to exceed 15% and the RE had to be within ±15% of the actual value.

2.5.3. Extraction recovery and matrix effect

The SPE recoveries and the matrix effect were determined quantitatively at low and high concentration levels. Recovery studies permitted the evaluation of the extraction efficiency of the SPE proposed method. The extraction recovery (R, %) was calculated using the following procedure: a sample spiked by a standard solution of analyte was extracted using the developed solid phase extraction procedure and the analysis result was compared to that of an unextracted standard which was prepared at the equivalent final concentration. So, the extraction recovery was calculated as the ratio between the resulting peak areas of the extracted and non-extracted samples.

The matrix effect (ME = C/D, %) was estimated for each compound by calculating the ratio of the peak area in the presence of the matrix (C: samples spiked for extraction) to the peak area in absence of the matrix (D: pure standard solution). In this study, the matrix effect was evaluated by using a real water samples. Then, the relative standard deviations (RSD, %) were also calculated.

3. RESULTS AND DISCUSSION

The aim of this work has been the development of a rapid and sensitive method for the simultaneous extraction and analysis of NDMA and NDPHe. Bearing in mind the lack of rapid, sensitive and robust methods for the analysis of these molecules in water samples, the developed methodology proposed in this work has been focused especially on the determination of these molecules at ultra-trace levels of concentration, relevant for environmental water samples. As stated above, the selection on the target molecules was based on their occurrence in environmental waters.

3.1. UPLC/MS/MS method development

Generally, the separation and ionization of analytes are affected by the composition of the mobile phase. Therefore, the effect of mobile phase composition and the additives on the separation of the target molecules was investigated in this work. Various compositions of the mobile phase (i.e., acetonitrile/water and methanol/water) modified with acetic acid, or formic acid (0.05, 0.1, 0.2, and 0.3%) were tested in order to achieve an efficient separation of N-nitrosamines by using BEH C18 column. The obtained results showed that the measured responses using acetonitrile/water (60:40, v/v), modified with formic acid were higher than those using methanol/water and acetonitrile/water containing acetic acid (data not shown). Indeed, the use of formic acid improves the ionization efficiency. Obtained data showed that a very low concentration of formic acid lead to a lack of protons while a high concentration produce a ion suppression. Thus, both conditions would reduce the analytical sensitivity (data not shown). Therefore, 0.1% of formic acid was chosen as additive for the mobile phase in this work. The effects of column temperature and flow rate were also studied. Column temperatures from 35°C to 50°C were assayed, and 45°C was selected as the working temperature. Flow rates from 0.2 to 0.5 mL min^{-1} were tested, and the results indicated that a flow rate of 0.4 mL min^{-1} significantly improves the resolution, peak shape, intensity of the response, and retention times. Under optimized analytical conditions the total run time was two minutes with retention times of 0.61 min for NDMA and 1.25 min for NDPHe (Fig. 1).

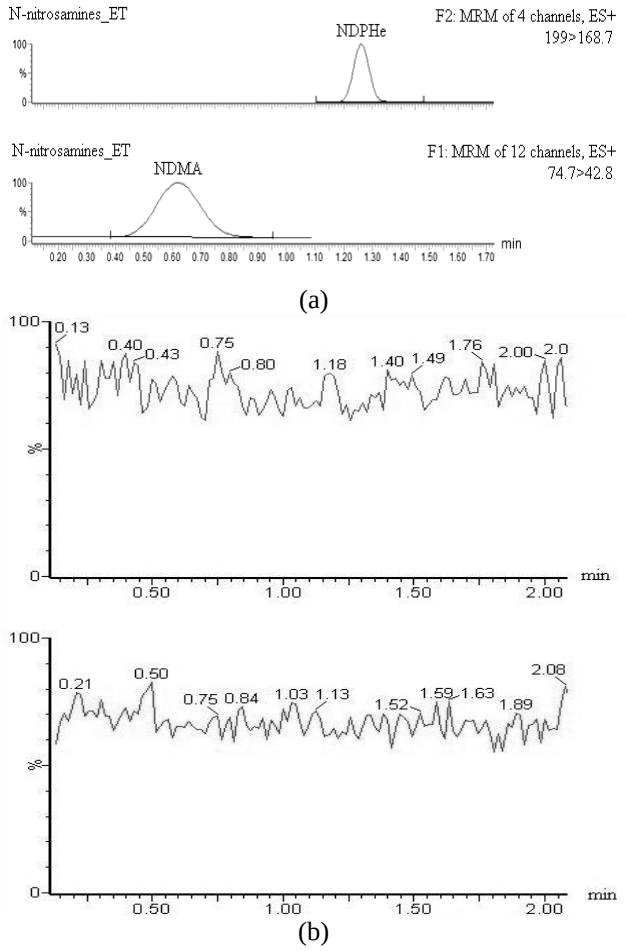

Figure 1. Typical UPLC/MS/MS chromatogram of ultrapure water sample: (a) spiked at 2 µg $L^{-1}$with a standard solution of N-nitrosamines, (b) unspiked sample.

In addition, a careful optimization of MS parameters was performed for each compound.

During MS/MS tuning, the positive and negative ESI modes were tested. The MS/MS detector was performed with an electrospray ionization interface, operating in positive ionization mode (ESI^+). The mobile phase consists of 0.1% formic acid was kept constant during the run using a UPLC mobile phase which resulted in better ionization of the analytes. The selection of MRM transitions and associated acquisition parameters (collision energy and cone voltage) were evaluated for best response under positive mode conditions (ESI^+) by direct infusion in the source of a standard solution of each compound (1 mg L^{-1}) into the mass spectrometer. In this study, two sensitive MRM transitions were selected for each N-nitrosamine according to the requirements regarding mass spectrometric confirmation defined by the EU Commission Decision 2002/657/EC. Two transitions have to be recorded for each analyte in order to get a sufficient number of identification points for a suitable confirmation. Thus, in this work, the peak area of the most intense transition was used for quantitative purposes and the less intense transition was used for the confirmation of each analyte. The cone voltage and the collision energy were also investigated. The optimized MS/MS transitions as well as specific cone voltage, collision energy, and segment periods for each N-nitrosamine, are shown in table 1. Thus, these parameters remained fixed during a single analysis.

3.2. Quality parameters

The performance of the proposed method was evaluated by determining the linearity, limit of detection, limit of quantification, precision, accuracy, extraction recovery, and matrix effect.

3.2.1. Linearity, limit of detection, and limit of quantification

Good linearity with high correlation coefficients ($r^2 \geq 0.998$) was observed over the concentration range of 0.4 to 100 µg L^{-1} and 0.1 to 100 µg L^{-1} for NDMA and NDPHe, respectively. The linear regression equation of the calibration curve was:

$$Y = 6.770X + 6.605 \quad (2)$$

and

$$Y = 221.3X - 273.1 \quad (3)$$

for NDMA and NDPHe, respectively; where Y represents the peak area and X represents the concentration of the analyte. Instrumental LOD values of 0.1 and 0.4 µg L^{-1} were obtained for NDPHe and NDMA, respectively. The estimated values of the instrumental LOQ were 0.2 and 0.8 µg L^{-1} for NDPHe and NDMA, respectively.

3.2.2. Precision and accuracy

Intra-day and inter-day assay studies for precision and accuracy of the instrumental method were carried out by replicate (n = 6) UPLC analysis of NDMA and NDPHe at two different concentrations (2 and 8 µg L^{-1}). The precision for all analytes was less than 3%.

The intra-day and inter-day standard deviations (RSD) calculated for NDMA and NDPHe ranged from 0.59% and 2.94%, respectively. Therefore, the RSD values were lower than 15% indicating high precision of the developed method. The intra- and inter-day accuracy results were between 99% and 103%. The detailed data of intra-day, inter-day precision and accuracy values for the instrumental method are shown in table 2. The analysis of the presented data clearly demonstrated that the UPLC/MS/MS method is precise and accurate.

Table 1. Optimized MS parameters used for the analysis of the each N-nitrosamines.

Analyte	MRM transition (m/z)	Cone voltage (V)	Collision energy (eV)	Segment (min)
NDMA	74.70 > 42.80[a]	25	10	0.00-1.10
	75.00 > 58.00[b]	25	10	0.00-1.10
NDPHe	199.0 > 168.7[a]	22	12	1.10-3.00
	199.0 > 103.6[b]	22	12	1.10-3.00

[a]Quantification transition
[b]Confirmation transition

Table 2. The inter-day and intra-day precision and accuracy values of the instrumental method in ultrapure water.

Analyte	Conc. spiked (µg L^{-1})	Intra-day (n = 6)		Inter-day (n = 6)	
		Precision (RSD, %)	Accuracy (RE, %)	Precision (RSD, %)	Accuracy (RE, %)
NDMA	2	1.19	100.38	2.21	100.05
	8	1.73	101.67	2.64	102.66
NDPHe	2	0.59	98.85	2.72	101.89
	8	0.74	100.77	2.94	102.87

3.2.3. Extraction recovery during solid-phase extraction and matrix effect

The sensitivity of the analytical system is not sufficient to direct analyze the target molecules in the range of concentration found in real water samples (ng L^{-1}). Thus, a preconcentration methodology by solid phase extraction was developed in this study.

The SPE extraction recoveries were determined by extracting and analyzing ultrapure water and mineral water samples spiked with each target molecule at two quality control concentration levels (2 and 8 µg L^{-1}). Six different sets of extractions (n = 6) for each sample were carried out.

The representative chromatograms of N-nitrosamines samples spiked with NDPHe and NDMA and their corresponding blank samples are shown in figure 1. The obtained chromatograms showed that the blank samples were free from interferences.

As shown in table 3 the extraction recoveries calculated for NDPHe and NDMA in spiked ultrapure water and mineral water were within the range of 85-87% and 98-101%, respectively. Moreover, the RSD values calculated for the both analytes ranged from 2.11% and 2.68% at the quality control levels.

Taking into account the instrumental quantification limits of the developed UPLC/MS/MS method (of 0.2 and 0.8 µg L^{-1} for NDPHe and NDMA, respectively) and the 2,500-fold concentration factor for all target analytes (sample volume, 250 mL to 0.1 mL).

The determined quantification limits of developed SPE-UPLC/MS/MS are in the range of ng L^{-1}. The obtained quantification limits of the SPE-UPLC/MS/MS method proposed in this work were 0.08 and 0.32 ng L^{-1} for NDPHe and NDMA, respectively. This results show clearly that, the proposed SPE-UPLC/MS/MS method is very sensitive and can be and useful tool for the survey of N-nitrosamines in water.

Signal suppression is one of common matrix effects in electrospray ionization. This phenomenon is due to the competition between the analyte and the matrix components. Indeed, the presence of co-extracted matrix components may severely affect the quantification of the analyte by UPLC-ESI-MS/MS. Lower amounts of organic matter after the SPE method resulted in less severe ion suppression.

Table 3. Extraction recoveries (R, %) and relative standard deviation (RSD, %) of the obtained with the proposed SPE methodology for each analyte at two different spiking levels.

Analyte	Conc. added (µg L^{-1})	Spiked ultrapure water: Recovery (n = 6)		Spiked mineral water: matrix effect (n = 6)	
		(R, %)	(RSD, %)	(ME, %)	(RSD, %)
NDMA	2	85.12	2.44	84.48	2.57
	8	87.00	2.53	84.37	2.68
NDPHe	2	97.92	2.11	99.12	2.26
	8	100.85	2.24	99.29	2.49

Table 4. Analysis of spiked river water samples using the developed SPE-UHPLC/MS/MS method.

Analytes	Conc. spiked ($\mu g\ L^{-1}$)	Conc. measured ($\mu g\ L^{-1}$)	Recovery (%)	RSD (%, n = 6)
Water sample 1				
NDMA	2	1.70	85.26	4.77
	8	6.94	86.84	4.42
NDPHe	2	1.85	92.54	3.54
	8	7.57	94.65	3.28
Water sample 2				
NDMA	2	1.98	99.17	3.78
	8	7.93	99.21	3.83
NDPHe	2	1.78	89.27	4.51
	8	7.30	91.36	4.14
Water sample 3				
NDMA	2	2.03	100.82	4.22
	8	8.19	102.39	4.85
NDPHe	2	2.02	101.05	4.43
	8	8.14	101.87	4.67

Thus, in this study, the matrix effect of the proposed method was also investigated by analyzing different river water samples spiked with the target molecules. The test were carried out in six replicates (n = 6) at two quality control concentration levels.

The obtained extraction recoveries of N-nitrosamines are shown in table 4. These data are very satisfactory. For the both analytes the calculated extraction recoveries were between 84% and 99%, with RSD below 3%. These values are very similar with the ones obtained in ultrapure and mineral water. Thus, the extraction recoveries were not affected by the change of type of water, which means no matrix effect for NDMA and NDPHe in this method.

The overall results presented in this paper confirm that the novel SPE-UPLC/MS/MS developed in this study technique is suitable for the simultaneous determination of N-nitrosamines in water samples.

4. APPLICATION OF THE PROPOSED METHOD

The applicability of the developed analytical method was assessed through the analysis of six tap water samples collected from different locations, in France, in July and October 2013. The samples were all extracted and analyzed under the optimum analytical conditions (6 replicates, n = 6) according to the procedures described in the Sections 2.3 and 2.4.

Table 5 lists the analyzed results of NDMA and NDPHe detected in the six analyzed samples. It can be remarked that both target molecules were detected at least one time in five of the collected water samples and the measured concentrations are at the low nanogram per liter level. As expected, the NDMA was most frequently detected in the collected samples, because this N-nitrosamine is one most detected in occurrence studies (Asami et al., 2009, Nawrocki & Andrzejewski, 2011).

Table 5. Concentration of N-nitrosamines detected in different drinking water samples collected from different areas situated in France.

Sampling point	N-nitrosamines detected ($ng\ L^{-1}$) ± RSD (%) (n = 6)[b]			
	July		October	
	NDMA	NDPHe	NDMA	NDPHe
Tap water 1	ND[a]	ND	ND	ND
Tap water 2	0.41 ± 0.92	ND	ND	ND
Tap water 3	ND	ND	0.54 ± 0.18	0.13 ± 0.83
Tap water 4	0.35 ± 0.53	ND	0.39 ± 0.12	0.11 ± 0.31
Tap water 5	ND	0.17 ± 0.26	ND	0.25± 0.11
Tap water 6	0.89 ± 0.15	ND	0.75 ± 0.23	ND

[a] ND means below the LOD

[b] six replicates

In sample 1 the detected concentrations for the both analytes are above the limit of detection of the proposed methodology. It suggests that N-nitrosamines contamination can be different depending on the location and also on the water source. In the other samples, the NDMA measured concentration ranged from 0.35 ± 0.53 to 0.89 ± 0.15 ng L^{-1} in July and from 0.39 ± 0.12 to 0.75 ± 0.23 ng L^{-1} in October. However, for NDPHe lower concentrations (ranging from 0.11 ± 0.31 to 0.25 ± 0.11 ng L^{-1}) were detected.

It will be noticed, that the all the measured concentrations are very lowest than different notification levels specified by different organizations over the world.

The obtained results clearly indicated that the proposed SPE-UPLC/MS/MS allowed the simultaneous identification and quantification of NDMA and NDPHe in different water matrices.

5. CONCLUSIONS

The analytical method developed in this study is highly sensitive for the determination of ultra-trace levels of NDMA and thermal labile N-nitrosamines, such as NDPHe. Good linearity, precision, accuracy, lower limits of detection, and limits of quantification were obtained. The used Sep-Pak AC-2 cartridges led to satisfactory recoveries and high pre-concentration factors of N-nitrosamines different environmental water samples. In addition, no significant matrix effect for NDMA and NDPHe was observed.

Moreover, the developed analytical technique provides low enough LOD to detect the N-nitrosamines according to the concentrations found in the water samples determined by many monitoring programs or established by different legislations.

In conclusion, this simple, rapid, low-cost, and reliable method is suitable for routine quantitative analysis of the target analytes at ultra-trace concentration levels (ng L^{-1}) in different types of water samples. This method can be a useful analytical tool for future toxicological and water quality surveillance studies or for the evaluation of the water disinfection processes.

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