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A highly sensitive liquid chromatography-tandem mass spectrometry method for the analysis of a toxic water disinfection by-product, *N*-nitrosomethylethylamine

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Recently, among the emerging contaminants, *N*-nitrosomethylethylamine has become of special concern because it is a potent human mutagenic and carcinogenic contaminant detected in chlorinated or chloraminated drinking waters and wastewaters. In this work a sensitive and robust method, which was based on solid-phase extraction followed by ultra-high-pressure liquid chromatography coupled with tandem mass spectrometry, was developed for the determination of *N*-nitrosomethylethylamine in water at ultra-trace levels. Chromatographic separation was performed on a C18 column. Quantification of *N*-nitrosomethylethylamine was achieved by using a triple quadrupole mass spectrometer that was equipped with an electrospray interface and was operated in positive ionization mode. Under optimized conditions, the calibration curve was linear from 0.1 to 100 μg L^{-1} ($r^2 \geq 0.999$). The precision of the intra- and inter-day values was found to be less than 2.5%, and the accuracy of the method was within ±3%. Moreover, an extraction efficiency greater than 86% was obtained at different concentration levels with relative standard deviation, RSD < 4.2%. Therefore, the experimental results showed that the proposed analytical method can be used successfully to determine *N*-nitrosomethylethylamine at ultra-trace levels (ng L^{-1}) in aqueous samples.

1 Introduction

A wide array of disinfection by-products, including *N*-nitrosamines, is formed during water treatment using chlorination and chloramination processes.[1,2] These compounds comprise a group of mutagenic chemicals that have been classified as probable human carcinogens.[3] In recent years, *N*-nitrosomethylethylamine (NMEA), which is a non-halogenated *N*-nitrosamine, has attracted considerable attention because it is frequently detected in drinking water in many countries around the world.[4,5]

The United States Environmental Protection Agency (U.S. EPA) has classified NMEA into the B2 group (probable carcinogenic effects on humans) and indicated that this compound produces an increased cancer risk at the 10^{-6} level at a very low concentration of 20 ng L^{-1}.[6] Consequently, sensitive and reliable analytical techniques to determine ultra-trace levels of NMEA in water are required. Due to the low concentration levels of this compound in environmental samples, extraction and pre-concentration steps are necessary. Solid-phase microextraction (SPME)[7,8] and solid-phase extraction (SPE)[9,10] have been used to preconcentrate NMEA in water samples. However, the SPME method has some limitations such as the possibility of sample contamination, low extraction recoveries, low preconcentration factor, and high detection limits. Furthermore, it is especially used for the extraction of volatile organic molecules and is particularly combined with gas chromatography. As an alternative method, SPE was successfully applied to the extraction of a wide variety of compounds such as volatile and non-volatile organic compounds from environmental water samples.

Several analytical techniques have been developed for the quantification of NMEA. They are based on liquid chromatography (LC) and gas chromatography (GC). The analyte has been analyzed in water samples by using the GC technique coupled with different types of detection methods, such as nitrogen chemiluminescence detection (NCD),[11] nitrogen phosphorous detection (NPD),[12] mass spectrometry (MS),[13–15] and tandem mass spectrometry (GC/MS/MS).[16,17] However, these techniques are limited to the analysis of volatile and thermally stable compounds. Moreover, liquid chromatography-tandem mass spectrometry (LC/MS/MS)[18,19] methods have also been reported for the determination of NDMA or other *N*-nitrosamines in water samples. To date, only a few and recent LC-MS/MS methods have been reported for the analysis of *N*-nitrosamines in water samples. Plumlee *et al.* in 2008 (ref. 18) described an optimized method only for NDMA determination. More recently, Ripollés *et al.* reported SPE-LC-MS/MS combined with a triple quadripole

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analyzer using an atmospheric pressure chemical ionization (APCI) mode for the analysis of NDMA and other *N*-nitrosamines in drinking water samples. For NMEA the achieved recoveries were only of 64–88% and the estimated limit of detection was found to be 5 ng L^{-1}.[19] However, to the best of our knowledge, the analytical method developed in this work is the first UHPLC/MS/MS method that has been proposed for the determination of NMEA at ultra-trace concentrations providing high sensitivity and high SPE recoveries (between 85 and 97%).

The scope of the research reported in this paper was to develop a simple, rapid, sensitive, and reliable analytical method for the analysis of ultra-trace levels of NMEA in water by combining solid-phase extraction with ultra-high-pressure liquid chromatography-electrospray ionization-tandem mass spectrometry (SPE-UHPLC-(ESI)-MS/MS).

2 Materials and methods

An NMEA standard solution (2000 mg L^{-1} in methanol) was purchased from LGC Standards (Wesel, Germany). All chemical reagents used in this work (for SPE procedure, solution preparation and LC/MS/MS measurements) were of the highest analytical purity grade. Acetonitrile and formic acid were obtained from J.T. Baker (Deventer, Netherlands). Methanol and dichloromethane, obtained from Fischer Scientific-Bioblock (Illkirch, France), were of LC-MS grade. Acetic acid was supplied by Acros Organics (Noisy-le-Grand, France).

Stock solutions (100 mg L^{-1} in methanol) for NMEA were prepared and stored at −20 °C for at least three months. The working solutions were freshly prepared by a series of dilutions with acetonitrile–ultrapure water (60 : 40, v/v). The ultrapure water was produced by an Elga Option-Q DV-25 system (Antony, France). Surface water samples were collected from a river (Britanny region, France) and stored at 4 °C until SPE extraction and analysis (within one week of collection).

Chromatographic separation was performed on an Acquity™ UHPLC H-Class system (Waters, Saint-Quentin en Yvelines, France), with a BEH C18 column (100 mm × 2.1 mm i.d., 1.7 μm; Waters) maintained at 45 °C. The mobile phase that was used consisted of formic acid in acetonitrile and water (60 : 40 : 0.1, v/v/v). The flow rate was 0.4 mL min^{-1} and the injection volume was 5 μL. The total run time was two minutes. The UHPLC system was connected to a Waters Acquity™ triple quadrupole mass spectrometer (MS/MS). Positive ionization tandem MS detection in multiple reaction monitoring (MRM) mode was used.

The analyte was extracted, purified, and concentrated from water samples using a Sep-Pak Plus®AC-2 cartridge (400 mg, 85 μm; Waters, Guyancourt, France). Then, the extract was evaporated to a final volume of approximately 100 μL in an N-Evap system (Organomation, Berlin, MA, USA) under a high-purity nitrogen stream. For the SPE procedure, several factors were optimized in order to obtain high recoveries in ultrapure water, *i.e.*, cartridge conditioning, pH values of the samples, loading rates, washing conditions, and elution volumes. Then the selected SPE technique was used to determine the recovery of NMEA in real water samples.

The performance and reliability of the proposed method were assessed by determining the regression equation, linear range, analyte detectability, precision, accuracy, and extraction recovery for the *N*-nitrosamine studied.

The linearity of the proposed method was assessed by direct injection of seven working solutions, prepared in ultrapure water in the concentration range from 1 to 100 μg L^{-1}. Each solution was analyzed in triplicate. The calibration curves were constructed by a least squares linear regression analysis. This method was used to determine the slope, intercept, and correlation coefficient (r^2) of the linear regression equation. The LOD and the LOQ values were determined at concentrations with a signal-to-noise ratio (S/N) of 3 and 10, respectively. The instrument limit of detection (LOD) is the lowest concentration of the analyte that the analytical process can reliably differentiate from background levels, while the instrument limit of quantification (LOQ) is the lowest concentration of analyte that can be quantified.

The intra-day and inter-day precision of the analyses was estimated in terms of repeatability. These parameters were expressed as relative standard deviation (RSD, %). The accuracy (RE, %) was expressed by 100 − [(mean observed concentration)/(spiked concentration)] × 100. 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.

The extraction recovery (R, %) was calculated using the following procedure: a sample spiked with the 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 an 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 = A/B) was evaluated by calculating the ratio of the peak area in the presence of the matrix (A: samples spiked after extraction) to the peak area in the absence of the matrix (B: pure standard solution). In this work, the matrix effect was estimated by using real environmental water samples.

3 Results and discussion

Different mobile phases (*i.e.*, acetonitrile–water and methanol–water) containing acetic acid or formic acid and mobile phase flow rates were tested and compared for NMEA analysis by UHPLC/MS/MS. Finally, acetonitrile–water containing 0.1% formic acid was used as the mobile phase due to its good separation and high sensitivity to NMEA. The results demonstrated that the flow rate of the mobile phase of 0.4 mL min^{-1} achieved satisfactory separation, limiting the dilution of the analyte chromatographic peak, and allowed a low solvent requirement. Moreover, the effect of column temperature was also examined. Column temperatures from 35 to 50 °C were assayed, and 45 °C was selected. Under these conditions, the analysis time was two minutes. Fig. 1 shows the typical UHPLC/MS/MS chromatogram obtained for ultrapure water spiked with NMEA obtained under optimized conditions.

For the MS/MS detection, the result showed that electrospray operation in positive ionization mode (ESI) was better and had excellent signal sensitivity. In order to achieve the quantification of NMEA, the mass spectrometric parameters, such as collision energy and cone voltage, were optimized to attain the maximum sensitivity for the detection of the analyte. The precursor ions and product ions were observed in the MS/MS spectra after infusing the standard solution (1 mg L^{-1}) into the mass spectrometer. In this work, two sensitive MRM transitions were selected for the *N*-nitrosamine that was studied. Different conditions of the cone voltage, source temperature, and the collision energy were tested. The optimized MS/MS transitions used for the UHPLC/MS/MS analysis, as well as specific cone voltage, source temperature, collision energy, and segment periods, are provided in Table 1.

SPE extraction and concentration of the analyte were achieved with the Sep-Pak Plus®AC-2 cartridge. To establish a SPE method for NMEA extraction the effects of several parameters influencing the extraction efficiency, such as organic solvents and their volume, pH of the samples, loading rates, washing conditions, and the elution volume, were investigated and optimized in detail in this study. The selected SPE enrichment conditions were sample conditioning with methanol, dichloromethane, acetonitrile (8 mL of each), and 5 mL of water. Then, 250 mL of the water sample spiked with the analyte and acidified with formic acid to pH 2 was loaded at the optimum flow rate (approximately 3 mL min^{-1}). After the sample solution had passed through, the cartridge was washed with 5 mL of ultrapure water adjusted to pH 2 to remove co-adsorbed matrix materials from the cartridge. Subsequently, the analyte retained on the SPE cartridge was eluted with 6 mL of dichloromethane, 4 mL of acetonitrile, and 2 mL of methanol at a flow rate that ranged from 2 to 3 mL min^{-1}. Solvents are carefully evaporated (at 20–25 °C) and concentrated under a high-purity nitrogen stream to a volume of 50 μL. The obtained extracts are brought up to a final volume of 100 μL using acetonitrile–ultrapure water (60 : 40, v/v). Finally, the extract was stored at 4 °C until further analysis was performed by UHPLC/MS/MS.

The calibration curves showed good linearity ($r^2 \geq 0.999$) over the concentration range of 1 to 100 μg L^{-1} for NMEA in water samples. The linear regression equation of the calibration curve was $y = 27.1761x + 23.4682$, where y represents the peak area and x represents the concentration of the analyte. The instrumental limit of detection (LOD) and the instrumental limit of quantification (LOQ) of NMEA were 1 and 2 μg L^{-1}, respectively.

Table 1 Optimized MS/MS parameters

Parameter	Value
Source temperature (°C)	120
Capillary voltage (kV)	3.0
Desolvation temperature (°C)	350
Desolvation gas flow (L h^{-1})	750
Cone gas flow (L h^{-1})	75
Quantification transition, *m*/*z*	88.7 > 60.7
Confirmation transition, *m*/*z*	88.7 > 42.8
Cone voltage (V)	25
Collision energy (eV)	10

The intra-day precision, inter-day precision, and accuracy of the method were evaluated by spiking NMEA in ultrapure water at three quality control levels (1, 2 and 20 μg L^{-1}). The intra-day precision and inter-day precision were less than 2.5% and 3% (RSD, %), respectively. The accuracy ranged from 100 to 103%. The detailed values of intra-day, inter-day precision, and accuracy are shown in Table 2. All the values are within the 15% acceptable range. Therefore, the UHPLC/MS/MS method proved to be precise and accurate.

The SPE extraction recoveries were established by analyzing spiked ultrapure water samples ($N = 6$) at three quality control concentration levels. The calculated extraction recoveries of the NMEA were greater than 86%, and the relative standard deviations were less than 4.3% (Table 3). Therefore, the SPE-UHPLC/MS/MS method that we developed allowed quantification limits in the range of ng L^{-1} (considering that the pre-concentration factor of the SPE method is 2500). Under these conditions the detection limit and the quantification limit of the overall analytical procedure were 0.4 and 0.8 ng L^{-1}, respectively. As illustrated in Table 3, these values are lower than the ones reported in the literature[1,19] for the LC-MS/MS methods confirming the performance of the developed procedure.

Table 2 Precision and accuracy of the method for the determination of NMEA using UHPLC/MS/MS

Spiked level (μg L^{-1})	Intra-day[a] (RSD, %)	(RE, %)	Intra-day[a] (RSD, %)	(RE, %)
1	1.25	100.61	2.16	101.93
2	1.38	101.52	2.17	102.25
20	2.23	102.36	2.47	102.77

[a] $N = 6$.

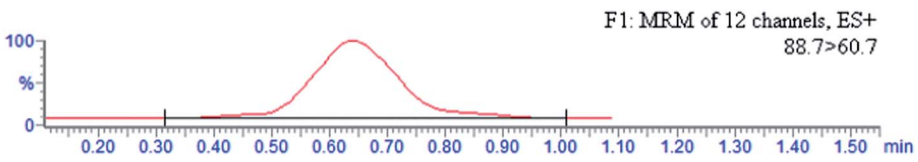

Fig. 1 UHPLC-(ESI^+)-MS/MS chromatograms obtained from the analysis of the NMEA standard at 5 μg L^{-1} (only quantification transition), retention time (min), and peak area (arbitrary units).

Table 3 Recoveries (*R*), relative standard deviations (RSD) and detection limits of NMEA at different concentrations ($N = 6$) for the presently developed method compared with literature reported data obtained for the LC-MS/MS methods

Spiked level ($\mu g\ L^{-1}$)	Detected level ($\mu g\ L^{-1}$)	*R* (%)	RSD (%)	LOQ method ($ng\ L^{-1}$)	LOQ[1] ($ng\ L^{-1}$)	LOQ[19] ($ng\ L^{-1}$)
1	0.43	86.00	3.23	0.8	2.5	5
2	1.97	98.62	3.63			
20	17.43	87.15	4.25			

Table 4 Determination of the matrix effect (ME) and relative standard deviations (RSD) of NMEA using the SPE/UHPLC/MS/MS method

	Matrix effect[a]			
	River water 1		River water 2	
Spiked level ($\mu g\ L^{-1}$)	ME (%)	Precision (RSD, %)	ME (%)	Precision (RSD, %)
1	85.12	3.22	84.89	3.13
2	97.14	4.12	96.71	4.15
20	89.11	3.67	88.79	3.37

[a] $N = 6$.

For the calculations, the matrix effect of NMEA was evaluated by analyzing spiked samples ($N = 6$) at three different concentration levels. Moreover, the presence of co-extracted matrix components may severely affect the quantification of the analyte by UHPLC-ESI-MS/MS. The matrix effect of NMEA was found to be within the acceptable range; all recovery values ranged from 85% to 97% and the relative standard deviations were less than 4.5% in the river water samples. The results, as well as the satisfactory recoveries of NMEA in river waters, are shown in Table 4.

4 Conclusion

In conclusion, the UHPLC-ESI-MS/MS method that was developed in this work showed good linearity, precision, and accuracy for the determination of NMEA in water. Furthermore, the SPE method using Sep-Pak Plus®AC-2 cartridges provided high recoveries for the extraction and concentration of the analyte from environmental water samples. The SPE-UHPLC-(ESI)-MS/MS analytical method can be considered to be a promising technique that has obvious advantages over conventional analytical techniques in this field of application. On the other hand UHPLC-ESI-MS/MS under positive mode of ionization provides high sensitivity for the determination and quantification of NMEA in real water samples at ultra-trace levels ($ng\ L^{-1}$).

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