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Determination of nitrosamines in water by gas chromatography/chemical ionization/selective ion trapping mass spectrometry

Romina Pozzi*, Paola Bocchini, Francesca Pinelli, Guido C. Galletti

Department of Chemistry "G. Ciamician", University of Bologna, via F. Selmi 2, I-40126 Bologna, Italy

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ABSTRACT

A gas chromatography/mass spectrometry (GC/MS) method for determination of nine N-nitrosamines (NAs) in water is described. Two ionization modes, electron impact (EI) and chemical ionization (CI) with methanol, as well as different ion analysis techniques, i.e. full scan, selected ion storage (SIS) and tandem mass spectrometry (MS/MS) were tested. Chemical ionization followed by SIS resulted the mass spectrometric method of choice, with detection limits in the range of 1–2 ng/L. Solid Phase Extraction (SPE) with coconut charcoal cartridges was applied to extract NAs from real samples, according EPA Method 521. Drinking water samples were collected from seven surface- and two groundwater treatment plants. Three surface water treatment plants were sampled before and after addition of O_3/ClO_2 to observe the effect of disinfection on NAs' formation. N-nitrosodiethylamine (NDEA), n-nitrosodipropylamine (NDPA), n-nitrosomorpholine (NMOR) and n-nitrosodibutylamine (NDBA) were found up to concentrations exceeding three times the risk level of 10 ng/L set by the California Department of Public Health. Because dermal adsorption has been recently indicated as a new contamination route of exposure to NAs for people who practice swimming activity, water samples from five swimming pools in the Bologna (Italy) area were collected. N-nitrosopyrrolidine (NPYR) was detected in all samples at concentrations larger than 50 ng/L, likely as a disinfection by-product from the amino acid precursor proline, a main constituent of skin collagen.

1. Introduction

In the last years, environmental scientists have been focusing their attention on emerging contaminants in water [1,2], such as disinfection by products (DBPs), which include nitrosamines (NAs) in addition to halogenated compounds. Besides water, NAs occur in soils and food and have been studied for over 30 years in order to understand their effect on human health. This considerable toxicological research has yielded important discoveries regarding carcinogenesis [3–5]: NAs are biomarkers of bladder cancer in humans [6,7] and are highly carcinogenic to bladder tissue [8,9]. Up to now, great attention has been paid on a particular NA, nitrosodimethylamine (NDMA), since it was first discovered in chlorinated drinking water from Ontario (Canada) in 1989 [10]. Mitch and co-workers [11] published a review in which they discussed NDMA toxicity issues, its formation as a DBP in drinking- and wastewaters and the treatments that can potentially be used to remove NDMA or its precursors. In 2006 a detailed study on NDMA was published by the World Health Organization [12]. Among the various water disinfection processes yielding NDMA as by-product [13], the reactions of monochloramine (NH_2Cl) with dimethylamine (DMA) [14], an ubiquitous precursor in surface waters [15,16], or with natural organic matter [17] are the most important. Monochloramine is purposely added as a disinfectant, but it may also be formed in chlorinated water in the presence of ammonia.

Other alkylamines or pesticides may decompose to give rise to potential precursors of NDMA [18]. In 2007, great concern was raised in Europe after the release of an EU Commission Decision imposing urgent measures to ensure that uses of plant protection products containing tolylfluanid do not lead to drinking water contamination [19]. Microbial decomposition of the fungicide tolylfluanid produces dimethylsulfamide which is likely to be found in soil, and in ground- and surface waters. This metabolite is converted into NDMA by a standard drinking water preparation process (ozonisation) [20].

Although NDMA is the most studied nitrosamine and may serve as a surrogate for nitrosamine exposure assessment, other NAs, such as n-nitrosopyrrolidine (NPYR), n-nitrosopiperidine (NPIP), n-nitrosomorpholine (NMOR), n-nitrosodipropyilamine (NDPA) and n-nitrosodiphenylamine (NDPhA) were detected in water in recent investigations [13,21–23]. This demonstrates that the natural organic matter and/or anthropogenic contaminants present in water may

* Corresponding author. Tel.: +39 051 2099580; fax: +39 051 2099456.
E-mail address: romina.pozzi4@unibo.it (R. Pozzi).

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contain different subunits that lead to the formation of various nitrosamines.

As of today, there are no laws setting NAs' concentration limits threshold in drinking water in Europe, Canada or United States. However, the U.S. Environmental Protection Agency has added NDMA, NPYR, nitrosodiethylamine (NDEA), nitrosodi-n-butylamine (NDBA), nitrosodi-n-propylamine (NDPA), and nitrosomethylethylamine (NMEA) to the Unregulated Contaminant Monitoring Rule 2 (UCMR-2) [24]. These nitrosamines represent 6 of 26 compounds included in the UCMR-2 list, signaling the importance of monitoring this class of compounds in drinking water. The Ontario Ministry of the Environment has ruled a maximum allowable concentration (MAC) of 9 ng/L for NDMA while the California Department of Public Health has established a notification level of 10 ng/L for NDMA, NDEA, and NDPA [25,26].

Ingestion of disinfected drinking water is not the sole important route of exposure to NAs. New researches are revealing that dermal absorption and inhalation, from bathing and other activities, can often provide equivalent or even greater exposures [27,28]; Mitragotri et al. demonstrated that NDMA is predicted to have the same skin permeability (10^{-4} cm/h) as hydrocortisone, the active ingredient in topical ointments used to treat skin illness [29]. Related to other research, involving alternative exposures to ingesting drinking water, swimming pool studies have shown a marked increase in the last years [30]; Walse et al. investigated, in 2008, public swimming pools, hot tubs and aquaria searching after NAs [31].

Analytical methods to determine NAs in water are generally based on two steps, namely (a) extraction from water by Solid Phase Extraction (SPE) [32–34], Solid Phase Micro-Extraction (SPME) [35,36], or liquid–liquid extraction [37], and (b) determination by Gas Chromatography (GC) with different detectors, such as a Thermal Energy Analyzer (GC/TEA) [38], Nitrogen Chemiluminescence Detector (NCD), Nitrogen–Phosphorus Detector (NPD) [37], or Mass Spectrometry (GC/MS) [35,36,39], tandem Mass Spectrometry (GC/MS/MS) [32,33] and High Resolution Mass Spectrometry (GC/HRMS) [34,23]. Liquid chromatography (LC) has also been applied to NAs' determination in water, using fluorescence detector [40] and tandem mass spectrometry (LC/MS/MS) [22,13]. Detection limits ranged from 0.1 to 1.7 ng/L as obtained by SPE followed by GC/HRMS [23], to 30–390 ng/L as obtained by SPME followed by GC with various detectors [35].

Of particular interest with regard to the determination of NAs in drinking water is the EPA Method 521 [32], published in 2004 (a related paper was published by Munch and Bassett [33]) and based on coconut charcoal SPE and GC/MS/MS, large volume injector and chemical ionization (CI) with methanol or acetonitrile. By this procedure, detection limits ranging from 0.26 to 0.66 ng/L can be reached.

In the present work, a simple GC/MS method was set up to determine NAs in water for human consumption and in the novel field of investigation represented by the swimming pools. Two ionization modes (Electron impact (EI) and chemical ionization (CI)) as well as different ion analysis modes were tested. Three spectra acquisition modes were compared, i.e. (a) full scan, (b) selected ion storage (SIS), a technique that enriches the sample ions relative to the unwanted matrix ions by ejecting the latter throughout ionization [41], and (c) tandem mass spectrometry (MS/MS).

2. Experimental

2.1. Chemical standards and solutions

A methanol solution (2000 mg/L each component) containing N-nitrosodimethylamine (NDMA), N-nitrosomethylethylamine (NMEA), N-nitrosodiethylamine (NDEA), N-nitrosodipropylamine (NDPA), N-nitrosomorpholine (NMOR), N-nitrosopyrrolidine (NPYR), N-nitrosopiperidine (NPIP), N-nitrosodibutylamine (NDBA) and N-nitrosodiphenylamine (NDPhA) was purchased from Sigma–Aldrich (Milan, Italy). Isotopically labelled standards [6-^{2}H] N-nitrosodimethylamine (NDMA-d_6, 98%) and [14-^{2}H] N-nitrosodipropylamine (NDPA-d_{14}, 98%) (1000 mg/L in dichloromethane) were obtained from Cambridge Isotope Laboratories (Andover, MA) and used as surrogate (SS) and internal (IS) standard respectively. All NAs solutions used for calibration and the set-up of GC/MS operating conditions were obtained by dilution of the original standard solutions with methylene chloride (Sigma–Aldrich, Italy). For the determination of method performance, NAs solutions were prepared in distilled water.

2.2. GC/MS

A Star 3400CX Varian gaschromatograph coupled with a Saturn 2000 Varian ion trap mass spectrometer was used. A Supelco (Milan, Italy) PTA-5 column, 30 m × 0.25 mm I. D. × 0.5 μm film thickness, was operated under the following oven temperature program: the initial temperature, 35 °C, was held for 4 min, raised first to 110 °C at 4 °C/min and then to 280 °C at 40 °C/min, holding this final temperature for 2 min (total run time 29 min). The injector was heated at 250 °C and was equipped with a deactivated Siltek™ (Restek, Italy) liner; injection volume was 5 μL in the splitless mode. Trap and transfer line temperatures were held constant at 150 °C and 170 °C, respectively.

Ionization modes were electron impact [EI] and chemical ionization [CI] with methanol (Sigma–Aldrich, Italy) as reagent gas. Three ion analysis modes were tested: full scan (range m/z 40–300), selected ion storage [SIS] (isolation window m/z 3, non-resonant waveform type) and tandem mass spectrometry [MS/MS]. Mass spectra were recorded at a filament emission current of 50 μA in all analysis modes; a scan rate of 0.5 s/scan was applied in the full scan and the [SIS] mode. In the tandem mass spectrometry mode all parameters were according the USEPA Method 521 [32].

2.3. Sample extraction

Water samples (1000 mL) were fortified with the surrogate NDMA-d_6 at the final concentration of 25 ng/L and extracted by passing them, at the flow rate of 1 L/h, through a SPE cartridge (Restek, Italy) filled with 2 g of coconut charcoal. The cartridge was conditioned with methylene chloride (Sigma–Aldrich), methanol (Sigma–Aldrich), and distilled water according to EPA Method 521 [32]. After extraction, the cartridge was dried by drawing air through it at full vacuum for 10 min and then eluted with approximately 12 mL methylene chloride. In order to eliminate residual water, the extract was passed through a drying column packed with approximately 7 g of anhydrous sodium sulphate (Sigma–Aldrich, Italy). The dried extract was concentrated to <1 mL volume. After addition of internal standard DPNA-d_{14} at the final concentration of 25 μg/L, the volume was adjusted to 1 mL with methylene chloride.

2.4. NAs' calibration and method's performance

Calibration was performed by direct injection of calibration solutions, prepared in the range from 1 to 200 μg/L (1, 2, 5, 10, 20, 50, 100, 200 μg/L). Surrogate standard NDMA-d_6 and internal standard DPNA-d_{14} were added to each calibration solution to obtain a final concentration of 25 μg/L. Calibration curves were calculated plotting the NA/IS area ratio against known NAs concentration (μg/L).

Detection limits (DL) were determined in two different ways, by the signal to noise (S/N) method and the standard deviation

Table 1
Retention times (min), molecular ions (*m*/*z*), protonated molecular/parent ions (*m*/*z*), MS/MS product ions (*m*/*z*) of the studied NAs.

	Ret. time (min)	Molecular ion $[M]^+$ (*m*/*z*)	Protonated molecular/parent ion $[M+H]^+$ (*m*/*z*)	MS/MS product ion (*m*/*z*)
NDMA-d_6 (SS)	7.05	80	81	46
NDMA	7.11	74	75	43
NMEA	10.28	88	89	61
NDEA	13.23	102	103	75
NPYR	20.40	100	101	55
NDPA-d_{14} (IS)	20.49	144	145	97
NMOR	20.56	116	117	86
NDPA	20.80	130	131	89
NPIP	22.21	114	115	69
NDBA	25.26	158	159	103
NDPhA	27.65	169[a]	170[a]	92

[a] For n-nitrosodiphenylamine (NDPhA), that decomposes at injector temperature, molecular and protonated molecular ion's *m*/*z* values of the product DPhA (diphenylamine) are reported.

(SD) method. In the signal to noise method, detection limit is set at the concentration corresponding to a signal three times the noise level of the background. Starting at 5 ng/L, water samples spiked at progressively lower concentrations were extracted using the procedure previously described and analyzed to determine DL for each NA. The standard deviation method is based on the standard deviation of the signal obtained at low concentration of the analyte and was applied according to EPA method 521 [32]. Seven NAs' water samples at the concentration of 3 ng/L were extracted and analyzed over a period of 3 days. To obtain the DL, the standard deviation of the seven replicates analysis was multiplied by the Student's *t*-number (3.143) for 99% confidence.

The data obtained at 3 ng/L were used to determine the method performance at low NA concentration. The method performance at high concentration was assessed by analyzing seven distilled water samples, spiked with 50 ng/L of each NA.

2.5. Sample collection

Twelve water samples were collected from treatment plants in an intensively cultivated area in the Italian North-East: three surface water samples were collected before and after disinfection with O_3/ClO_2, while four surface- and two ground water samples were collected after O_3/ClO_2 disinfection only. Pool water samples were obtained from five public indoor pools in Bologna disinfected with NaClO. Pools were 25 m × 6/7 lanes, 12 h/day open with about 100 swimmers/h, water temperature 24–30 °C, pH: 6.5–7.5, free available chlorine 0.7–1.5 mg/L, combined active chlorine ≤0.4 mg/L.

All samples were put in amber glass bottles avoiding headspace and sealed with Teflon lined caps after addition of 100 mg of $Na_2S_2O_3$ (Sigma–Aldrich, Italy) as a preservative. Samples were stored at 4 °C and analyzed within five days.

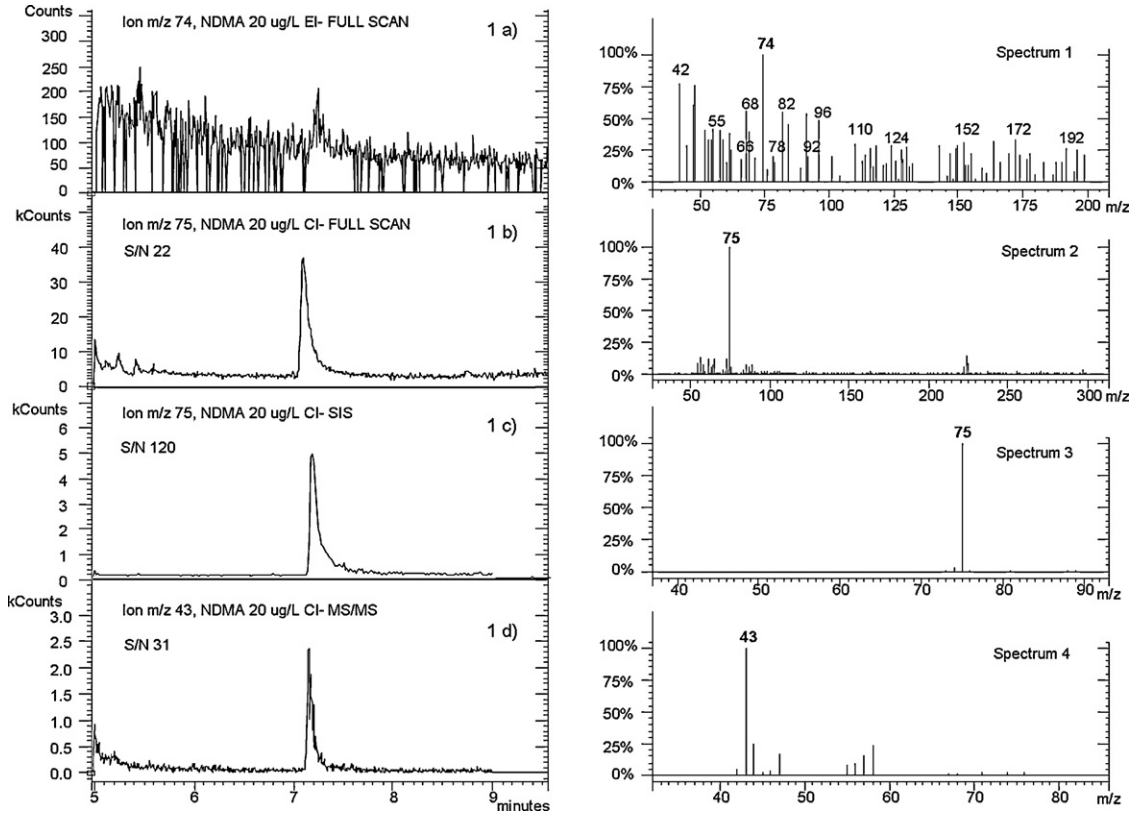

Fig. 1. Analysis of NDMA at 20 μg/L by the different ionization and ion analysis modes. (a) Signal of the molecular ion (*m*/*z* 74) acquired in the EI-Full scan mode. (b) Signal of the protonated molecular ion (*m*/*z* 75) acquired in the CI–full scan mode. (c) Signal of the protonated molecular ion (*m*/*z* 75) acquired in the CI–SIS mode. (d) Signal of the product ion (*m*/*z* 43) acquired in the CI–MS/MS mode. On the right, the corresponding mass spectra acquired at the apex of the peak.

3. Results and discussion

3.1. GC/MS set-up

Although a column recommended for amines' analysis was used, it was not possible to accomplish complete chromatographic separation and NMEA, the third eluting NA, produced a slightly broad shaped peak. However, close elution of NDMA and its isotopically labelled analogue NDMA-d_6 and incomplete separation of NPYR, NDPA-d_{14} and NMOR did not affect quantitative analysis, because NAs were identified and quantified by different ions. Table 1 reports the retention times and ion masses used for the analysis of the different NAs.

NAs most abundant mass spectral peaks were the molecular ion $[M]^{+\bullet}$ and the protonated molecular ion $[M+H]^+$ in the [EI] ionization mode and [CI] ionization mode respectively. For NDPhA, that decomposes at the injector temperature [42], the molecular and protonated molecular ions of the product diphenylamine (DPhA) were considered. DPhA itself is an environment contaminant, yet much less hazardous than NDPhA. Consequently, whenever DPhA is found in a sample, specific analysis should be performed to determine whether DPhA or NDPhA is present [42].

Fig. 1 shows the results for NDMA, the most important NA, produced by the analysis of the same 20 μg/L NAs' mixture in the different ionizations ([EI] and [CI]) and ion analysis (full scan, [SIS] and [MS/MS]) modes. The two different ionization modes, [EI] and [CI], were compared by matching the molecular $[M]^{+\bullet}$ and protonated molecular $[M+H]^+$ ion currents acquired in the full scan ion analysis mode. In Fig. 1a and b the molecular (m/z 74) and protonated molecular (m/z 75) ion currents for NDMA are displayed to show that, whereas in the [CI] mode a good peak (S/N = 22) was obtained (Fig. 1b), only a little spike, indistinguishable from the background noise, was recorded in the [EI] mode (Fig. 1a). Chemical ionization resulted the most efficient ionization mode and was therefore used to test the remaining ion analysis modes, [SIS] and [MS/MS].

As discussed previously, the close elution of few NAs did not cause any problem both in the [SIS] and in the [MS/MS] ion analysis mode, because the mass spectrometer allows selective storage and enrichment for up to ten analytes or parent ions at the same time.

Fig. 1c shows the results for NDMA produced by the analysis of the 20 μg/L NAs mixture under CI conditions, with spectra acquired in selected ion storage [SIS] mode. Compared to the signal acquired in the [CI] full scan mode (Fig. 1b), the peak produced in the [CI–SIS] mode (Fig. 1c) shows a less noisy signal and thus a better S/N ratio (S/N = 120). The selective ion trapping performed by SIS is better appreciable from the simple mass spectrum (spectrum 3, Fig. 1) recorded at the apex of the peak in which only the protonated molecular ion (m/z 75) of NDMA is present. Fig. 1d shows the signal current of the NDMA's product ion (m/z 43, the product ions for all NAs are listed in Table 1) obtained from dissociation of the protonated molecular ion (m/z 75) in the [CI–MS/MS] ion analysis mode. As expected, the MS/MS signal (S/N = 31) was 4-fold less intense than the signal registered in the [CI–SIS] mode and the noise was higher.

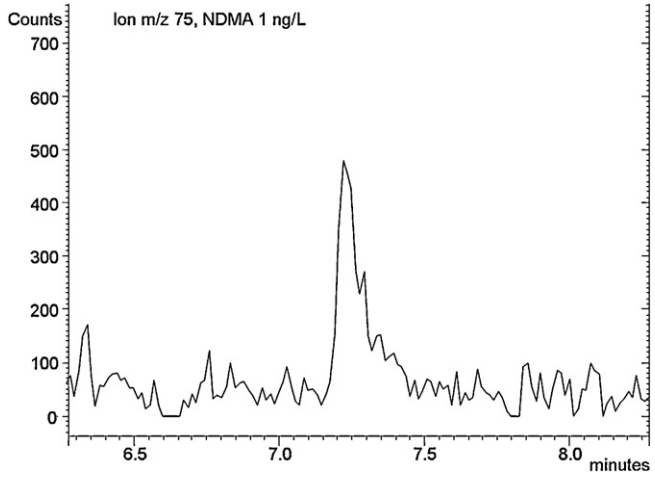

Fig. 2. Signal of the protonated molecular ion (m/z 75) of NDMA at the detection limit concentration of 1 ng/L.

The results showed for NDMA were comparable to those of all the other NAs, so [CI–SIS] resulted the method best suited to yield low detection limits thanks to the best S/N ratio. The potential advantage of [CI/MS/MS] in terms of selectivity was of no interest in the present work, because drinking and swimming pool waters are rather simple matrixes with a small risk of interferences. The [CI/MS/MS] was therefore used in this work only to confirm the presence of certain analytes after [CI–SIS] analysis.

3.2. Method's performance

Table 2 compares the detection limits for all NAs obtained by the S/N method and by the SD method. Detection limits obtained by the S/N method were 1 ng/L for NDMA, NDEA, NPYR, NMOR, NDPA, NPIP and NDBA. Higher detection limits (2 ng/L) were obtained for NMEA, likely due to its broad peak shape, and NDPhA, probably due to its determination as the thermal decomposition product DPhA, which is also the last eluting compound. Fig. 2 shows the signal for NDMA at the DL concentration of 1 ng/L. Although the S/N method is very intuitive it does not provide any information about reproducibility, as opposed to the SD method. The standard deviations obtained by the seven replicate analyses at 3 ng/L ranged from 0.3 ng/L for NDMA and NMOR to 0.8 ng/L for NDBA; the great variability observed for NDBA was probably due to memory effects noticed for this NA. NDBA was the compound with the highest detection limit (2.7 ng/L) while NMOR and NDMA were the NAs

Table 2
Detection limits (DL) obtained by the signal to noise (S/N) and by standard deviation (SD) method, in addition to standard deviations and mean percentage recoveries for all NAs calculated from distilled water samples fortified at 3 ng/L (n = 7) and 50 ng/L (n = 7) respectively.

	S/N method	SD method 3 ng/L (n = 7)		% Mean recovery (% SD)	
	DL (ng/L)	SD (ng/L)	DL (ng/L)	3 ng/L (n = 7)	50 ng/L (n = 7)
NDMA	1	0.3	1.0	96 (11)	97 (10)
NMEA	2	0.6	1.9	92 (20)	99 (7)
NDEA	1	0.4	1.4	73 (13)	74 (13)
NPYR	1	0.5	1.5	84 (17)	93 (7)
NMOR	1	0.3	0.8	75 (10)	76 (8)
NDPA	1	0.5	1.4	76 (15)	90 (7)
NPIP	1	0.4	1.1	81 (12)	98 (7)
NDBA	1	0.8	2.7	82 (27)	92 (6)
NDPhA	2	0.4	1.4	71 (15)	82 (11)

Table 3
Results for seven surface water treatment plants (1–7) and two ground water plants (8 and 9). (Raw: water before disinfection; Dis.: water after disinfection.).

	Surface waters (ng/L)										Ground waters (ng/L)	
	1		2		3		4	5	6	7	8	9
	Raw	Dis.	Raw	Dis.	Raw	Dis.	Dis.	Dis.	Dis.	Dis.	Dis.	Dis.
NDMA	–	–	–	–	–	–	–	–	–	–	–	–
NMEA	–	–	–	–	–	–	–	–	–	–	–	–
NDEA	–	–	–	–	–	–	–	–	10.3	8.9	18.7	30.7
NDPA	–	–	–	–	–	–	–	–	8.1	–	–	–
NMOR	–	–	–	83.7	–	–	–	–	–	–	–	–
NPYR	–	–	–	–	–	–	–	–	–	–	–	–
NPIP	–	–	–	–	–	–	–	–	–	–	–	–
NDBA	–	–	–	–	–	–	–	–	11.0	–	–	–
NDPhA	–	–	–	–	–	–	–	–	–	–	–	–

with the lowest DL, namely 0.8 ng/L and 1 ng/L. For all NAs, the mean percentage recoveries were between the limits (70–130%) allowed by EPA Method 521 [32], ranging from 71 to 96% (81% average). The fact that NDPhA showed the lowest recovery (71%), could further explain the higher detection limit (2 ng/L) obtained for this compound with the S/N method.

Although DLs obtained by the S/N were in the most cases slightly lower, the detection limits obtained by both methods were overall comparable and suitable for drinking water nitrosamine analysis.

Table 2 also reports the method's performance at high concentration, that was determined by seven replicate analyses of distilled water samples spiked with NAs, 50 ng/L each. The mean percentage recoveries, ranging from 74% for NDEA to 99% for NMEA (average 89%), are all slightly higher than those obtained at 3 ng/L, especially for NDPA (from 76% at 3 ng/L to 90% at 50 ng/L) and NPIP (from 81% at 3 ng/L to 98% at 50 ng/L). The percentage standard deviations are lower than 11% for all NA and generally better than those obtained at 3 ng/L, also for NDBA and NMEA that showed the worst % SD at low concentration. As expected, the memory effect that had affected NDBA's reproducibility at 3 ng/L was negligible at 50 ng/L, so that the % SD decreased from 27% to 6%. At the same manner, the broad peak shape that had hindered the proper quantification of NMEA at low concentration was not a problem at high concentration and % SD decreased from 20% at 3 ng/L to 7% at 50 ng/L.

For the present work, a 1-L sample volume was chosen in order to maximize the extraction yield. The recoveries obtained at low and high concentration (average 81 and 89%, respectively) compared well with those reported by Munch and Bassett [33], who showed average recoveries of 88% for a 0.5 L sample at 20 ng/L concentration and 75% for 1 L sample at 10 ng/L concentration.

3.3. Real samples analysis

NAs' concentration values in real samples were calculated from the calibration curves built in the range 1–200 μg/L ($R^2 > 0.999$ for all NAs) and were corrected for the surrogate recovery [43] taking into account SPE concentration factor (1000). Matrix effects were ruled out, because the average surrogate recoveries obtained by our real samples (83% for drinking waters and 85% for pool waters) were comparable to those obtained by the set of seven replicate analysis of samples prepared in distilled water at 3 ng/L (average surrogate recovery 85%) and 50 ng/L (average surrogate recovery 87%).

3.4. Drinking waters

The results for the analysis of surface and ground water samples for human consumption are shown in Table 3. Surface water treatment plants 1, 2 and 3 were sampled before and after disinfection with O_3/ClO_2. NAs were not found in the three raw water samples. For plant 2, the fact that NMOR (83.7 ng/L) was only detected in the sample collected after disinfection demonstrates that this NA is a by-product of the O_3/ClO_2 disinfection procedure, as established by Zhao et al. [13]. NDEA was found in two surface waters after disinfection, namely sample 6 (10.3 ng/L) and 7 (8.9 ng/L), and in the two ground waters after disinfection, namely sample 8 (18.7 ng/L) and 9 (30.7 ng/L). Except for sample 7, all concentrations were above the notification level of 10 ng/L set for NDEA by the California Department of Public Health. In sample 6, NDPA (8.1 ng/L) and NDBA (11.0 ng/L) were also detected. NDMA, one of the most dangerous nitrosamine, was not found both in raw and disinfected waters. The absence of NDMA in our samples excluded a contamination of the sampling area by an illegal use of tolylfluanide, prohibited in Italy as a fungicide since 2007 [19].

Comparing our results with previous findings, data about nil NDMA in raw waters were published by Charrois et al. [39,21] and Zhao et al. [22,13]. Regarding to disinfected waters, even if some disinfection procedures can lead to a greater production of NAs than others, an important role in NAs formation is also played by the kind and amount of organic matter present in the raw water [13] and a comparison becomes difficult. For instance, Zhao et al. found nil

Table 4
Results for five indoor swimming pools.

	Swimming pools (ng/L)				
	1	2	3	4	5
NDMA	–	–	–	–	–
NMEA	–	–	–	–	–
NDEA	–	–	–	–	–
NDPA	–	–	–	–	–
NMOR	–	–	–	–	–
NPYR	127.4	81.3	69.4	53.2	54.9
NPIP	–	–	–	–	–
NDBA	–	–	–	–	–
NDPhA	–	–	–	–	–

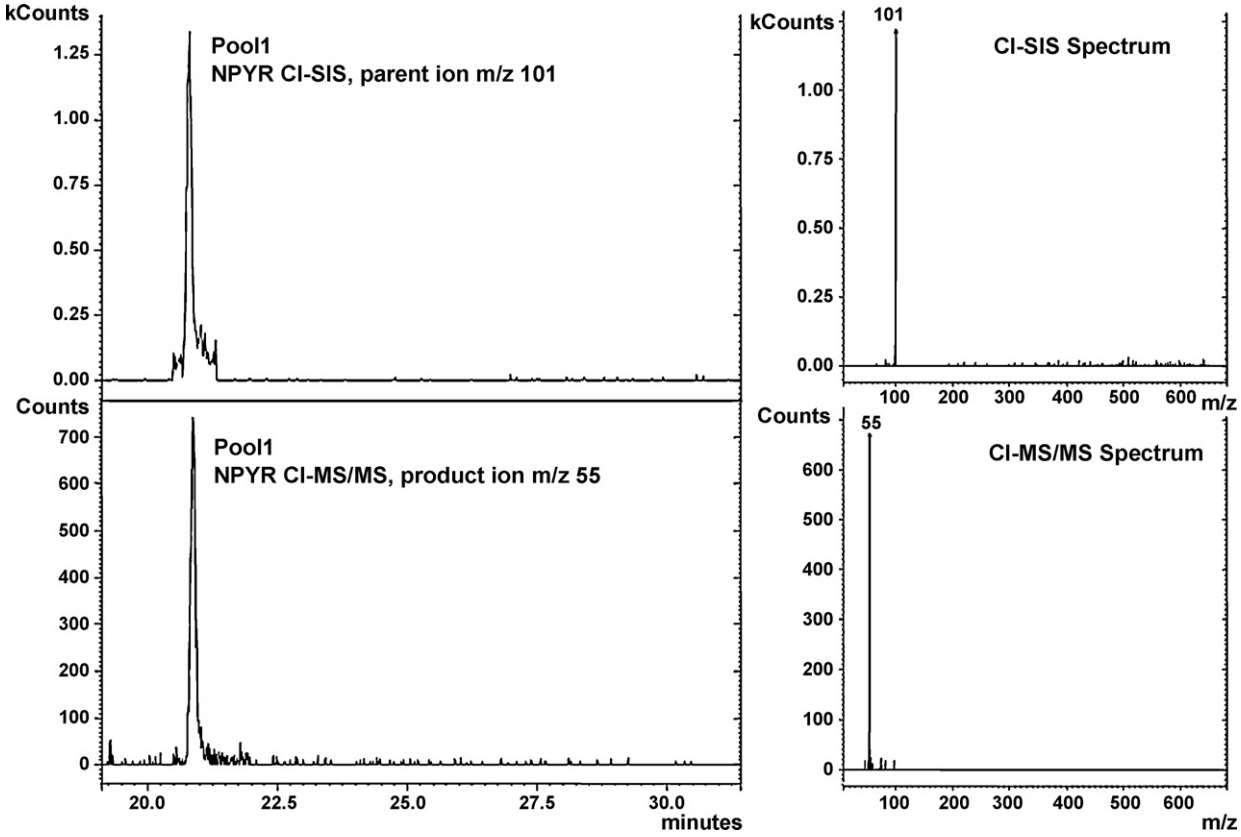

Fig. 3. Protonated molecular (parent) (m/z 101) and product (m/z 55) ion current acquired for NPYR in the CI–SIS and CI–MS/MS ion analysis modes (pool 1 sample). On the right side the corresponding mass spectra acquired at the apex of the peak.

NDMA [22] while Charrois et al. [39] found NDMA at concentrations >10 ng/L in water samples collected from plants disinfected with the same procedure, i.e. chloramination in combination with UV, one with the highest potential to produce NAs. Furthermore, Charrois et al. [39] found significantly different NDMA's concentrations, namely 14 and 67 ng/L, in the above mentioned plant collecting samples in two different periods of the year.

Although the majority of previous papers regards NDMA, other NAs were found, for instance (a) NMOR and NPYR up to 3 and 4 ng/L in a chlorinated drinking water distribution systems [21], and (b) NMOR (up to 11.5 ng/L), NDEA (up to 13.3 ng/L), NPYR (up to 5.4 ng/L), NPIP (1.3 ng/L) and NDPA (2.6 ng/L), in addition to NDMA (up to 11.5 ng/L), in a drinking water treatment plant after chlorination and ozonation [23].

3.5. Swimming pools

Table 4 displays the results obtained from the analysis of the five swimming pools samples. In all samples NPYR was found at concentrations higher than 50 ng/L. Presence of NPYR was confirmed for all samples by the less sensitive but more selective CI–MS/MS investigation selecting the NPYR product ion m/z 55 (Table 1). Fig. 3 shows, for pool 1 sample, the peaks produced by the protonated molecular ion (m/z 101, Table 1) in the CI–SIS mode and the product ion (m/z 55) in the CI–MS/MS mode. The protonated molecular- and the product ion of NPYR can be noticed in the mass spectra recorded at the apex of the peaks (Fig. 3). Previous works on swimming pools were published by Walse and Mitch [31] and by Jurado-Sanchez et al. [44]. Walse and Mitch found NDMA in all swimming pools, with a direct correlation with temperature, the lower concentrations being found in colder pools (approx. 24 °C): indoor- and outdoor pools roughly averaged 32 and 5.3 ng/L, respectively. By contrast, Jurado-Sanchez et al. found no NDMA in 12 out of 14 pool water sample; they found NDMA, NDEA and NPYR in one sample, and NDMA and NDEA in the other sample. If NDMA is due to the reaction of chlorinating agents with dimethylamine, a constituent of urine and sweat [31], NPYR might originate from pyrrolidine, the skeleton of proline, one of the main constituents of skin collagen. Such a protein might be the predominant NA precursor in our pools crowded with swimmers who practice for many hours. While urine and sweat are dependent on many factors, including personal (bad) habits, the exposure of skin to nitrosating agents is unavoidably correlated with the number of swimmers and the hours of practice.

4. Conclusions

Optimization of the GC–MS conditions allowed us to obtain an analytical method for NAs determination at ng/L levels. This procedure uses chemical ionization with methanol and selective ion trapping, is relatively simple, and does not require large volume injectors [32] and expensive high resolution mass spectrometers [34,23]. The detection limits were compatible with the amounts of NAs so far found in drinking water. Analysis of samples collected before and after disinfection showed that some NAs can be a by-product of O_3/ClO_2 disinfection treatment. NAs detected in drinking waters were NDEA, NDPA, NMOR and NDBA, at concentrations up to three times above the risk level established by the California Department of Public Health. These results raise concern when one considers that previous studies [22,39] report that NAs' content in water tend to increase from the plant exit to the final user.

With regard to swimming pools, NPYR was found in all water samples, probably as a disinfection byproduct stemming from proline, a main constituent of skin collagen. People regularly doing sport activities in swimming pools might therefore be subjected

to dermal adsorption of NAs, in addition to the more investigated inhalation and ingestion [31].

Up to now, great attention was paid on NDMA's formation and toxicity despite the fact that also other nitrosamines contaminate our environment in different ways. In the future, it would be important to study all the other NAs in the same manner and to understand the combined effect on human health of multiple exposure ways to different nitrosamines.

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