Talanta 88 (2012) 284–289

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Pressurised hot water extraction followed by headspace solid-phase microextraction and gas chromatography–tandem mass spectrometry for the determination of *N*-nitrosamines in sewage sludge

Anna Llop, Francesc Borrull*, Eva Pocurull

Department of Analytical Chemistry and Organic Chemistry, Rovira i Virgili University, Sescelades Campus, Marcel lí Domingo s/n, Tarragona 43007, Spain

ARTICLE INFO

Article history:
Received 20 July 2011
Received in revised form 13 October 2011
Accepted 16 October 2011
Available online 18 November 2011

Keywords:
N-nitrosamines
Sewage sludge
Pressurised hot water extraction
Solid-phase microextraction
GC–MS–MS
Central composite design

ABSTRACT

A method for the quantitative determination of the nine EPA *N*-nitrosamines in sewage sludge was developed by using pressurised hot water extraction (PHWE) followed by headspace solid-phase microextraction (HS-SPME) and gas chromatography coupled to chemical ionization ion trap tandem mass spectrometry (GC–CI-MS–MS).

The pressurised hot water extraction was optimized using a central composite design with regard to operational parameters such as temperature, extraction time and pH of water as extracting solvent. The optimum conditions were: water at pH 7.5 as extracting solvent, temperature of 125 °C and extraction time of 5 min. The sewage sludge extract was automatically analyzed by HS-SPME using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber and GC–CI-MS–MS.

The limits of detection of all compounds were lower than 0.15 μg/kg of dry weight (d.w.) of sewage sludge. The repeatability and reproducibility between days (10 μg/kg d.w.) expressed as relative standard deviation were lower than 16 and 19%, respectively. The method was applied to determine the *N*-nitrosamines in sewage sludge from urban and industrial sewage treatment plants (STPs) and from a potable water treatment plant. Some *N*-nitrosamines were determined in the samples and *N*-nitrosodiethylamine (NDEA) and *N*-nitrosodi-*n*-butylamine (NDBA) showed the highest values (371 and 305 μg/kg (d.w.), respectively) in sewage from industrial STPs.

1. Introduction

Many *N*-nitrosamines, especially *N*-nitrosodimethylamine (NDMA), are considered probable human carcinogens. They have been found in many food products [1], beer [2], cosmetics [3,4], tobacco smoke [5], soils [6], water from chlorinated swimming pools [7], tap water, wastewater, treated wastewater, groundwater and drinking water [7–9]. Polymers, plasticisers, batteries, rocket fuel (incomplete oxidation of hydrazines), and other industrial products are the main anthropogenic sources of the *N*-nitrosamines [10]. They are usually formed by nitrosation or oxidation reactions of amine precursors [11,12]. However, research in recent years has shown that nitrosamines, particularly NDMA, can be generated in water and wastewater treatment systems by chlorine-based disinfection processes, making them an important group of potentially hazardous disinfection by-products (DBPs) [10,13–15].

Currently, *N*-nitrosodimethylamine (NDMA), *N*-nitrosomethylethylamine (NMEA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosodipropylamine (NDPA) and *N*-nitrosodibutylamine (NDBA), are included in the Unregulated Contaminant Monitoring Regulation (UCMR 2), listed in the recently proposed Contaminant Candidate List 3 (CCL 3) by the U.S. EPA. This organization has also established ng/L control levels in drinking water [7,8,16]. Several *N*-nitrosamines have been detected at concentrations between 1 and 2 orders of magnitude higher than their cancer risk levels in the effluents of water and wastewater treatment plants (WWTPs) [13,14,16–19]. The determination of these compounds in environmental samples is therefore of great interest due to the possible reuse of both water and sludge. However, to date, few studies have investigated the occurrence of *N*-nitrosamines in sewage sludge [13].

Several techniques have been used for the analytical determination of *N*-nitrosamines, the most common of which are liquid chromatography (LC) and gas chromatography (GC). LC has been used with UV, fluorescence, mass spectrometry (MS) and tandem mass spectrometry (MS–MS) detection [20–22]. Although LC–MS–MS may also be applied to determine *N*-nitrosamines, the sensitivity for most compounds was lower than that of GC–MS–MS

* Corresponding author. Tel.: +34 977559560; fax: +34 977558446.
E-mail address: francesc.borrull@urv.cat (F. Borrull).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.10.042

[21,23]. GC is often the preferred choice as it provides good resolution and it is easy to couple with sensitive and selective detectors, such as thermal energy analysis (TEA), nitrogen–phosphorus, nitrogen chemiluminescence and MS [24–26]. When GC–MS detection is used, the low molecular weight of *N*-nitrosamines makes them susceptible to chemical interferences with electron ionization (EI) [1,18,26], which leads to poor sensitivity and selectivity, because EI is a hard ionization process and produces nondistinctive fragmentation patterns. This can be counteracted by using positive chemical ionization (CI) either with GC–MS or with GC–tandem mass spectrometry (GC–MS–MS), which is a softer ionization process that produces less molecular fragmentation [9,18,27].

Due to the low levels *N*-nitrosamines are present in environmental samples; an extraction/preconcentration technique is necessary in order to determine them. Pressurised hot water extraction (PHWE) is an environmentally friendly organic solvent free technique in which water is used as the extraction solvent at elevated temperature and under pressure to keep water in liquid state. PHWE has been recently used by our group to extract primary aliphatic amines from different sewage sludges [28]. However, PHWE extracts are relatively dilute aqueous solution and a subsequent preconcentration technique such as liquid–liquid extraction (LLE) [29], solid-phase extraction (SPE) [18,27,30,31] and solid-phase microextraction (SPME) [2,9,26] should be used. SPME is a solvent free technique that is simple to perform and easy to automate, among other advantages.

The aim of this study is to develop a novel and environmentally friendly method to determine the nine EPA *N*-nitrosamines in sewage sludge from various urban and industrial wastewater treatment plants and from a potable water treatment plant. This method is based on pressurised hot water extraction of sewage sludge followed by headspace solid-phase microextraction (HS-SPME) and gas chromatography–tandem mass spectrometry detection system using chemical ionization (HS-SPME–GC–CI-MS–MS).

2. Experimental

2.1. Safety considerations

N-nitrosamines are potential or actual carcinogens and must be handled with extreme care inside a fume hood with ventilation. They must not be inhaled or come into contact with the skin, and appropriate personal protective equipment must be used (latex gloves, lab coat, and safety glasses).

2.2. Reagents and solutions

A standard solution containing 2000 mg/L of the 9 *N*-nitrosamines in methanol was purchased by Sigma–Aldrich (Buchs SG, Switzerland) in the highest purity available (EPA 8270/Appendix IX Nitrosamine Mix Catalog No. 502138): *N*-nitrosodimethylamine (NDMA), -methylethylamine (NMEA), -diethylamine (NDEA), -di-*n*-propylamine (NDPA), -morpholine (NMOR), -pyrrolidine (NPYR), -piperidine (NPIP), di-*n*-butylamine (NDBA), and di-*n*-phenylamine (NDPhA). Working standard solutions of 10 mg/L were prepared in methanol weekly. All solutions were stored in darkness in the freezer.

Methanol and acetone were purchased from SDS (Peypin, France) and were of gas chromatography (GC) grade. Sodium chloride was supplied by Sigma–Aldrich. Sodium hydroxide and hydrochloric acid were obtained from Scharlau Chemie (Barcelona, Spain). Ultrapure water was obtained using a Milli-Q purification system (18.2 MΩ cm) (Millipore, Bedford, MA, USA). Helium and nitrogen of 99.995% purity were obtained from Carburos Metálicos (Tarragona, Spain). Cellulose filters of 20 mm obtained from Tecknokroma (Barcelona, Spain) and diatomaceous earth (95%) from Sigma–Aldrich were used for PHWE.

2.3. Sampling and sample pre-treatment

Several types of sewage sludge samples were collected from an urban wastewater treatment plant (A), three industrial wastewater treatment plants (B, C and D) and a potable water treatment plant (E). All these plants are located on the outskirts of Tarragona (Spain). These sludge samples had different origins and matrix complexities and had also undergone different treatment processes, such as conventional activated sludge (CAS) treatment or membrane bioreactor (MBR) treatment.

The urban WWTP A is a CAS treatment plant that uses reverse osmosis after secondary treatment. It treats water from a population of approximately 140,000 inhabitants. The industrial WWTP B is a CAS treatment plant that treats a mixture of wastewater from three different chemical plants that make products of various types, such as surfactants, vinyl acetate and plastics (isocyanides, polyurethanes and ABS). The industrial WWTP C is an MBR treatment plant that uses ultrafiltration membranes to treat wastewater from any industrial plants. The industrial WWTP D is an MBR treatment plant that uses ultrafiltration membranes to treat wastewater from the distillation of used oil. The potable water plant E is a CAS treatment plant that uses carbon filters in the last process to obtain a high-quality effluent.

We had two types of sludge depending on the WWTPs: digested and activated sludge. The digested sludge samples were taken in WWTP A, B and E, and it is the total sludge collected in the WWTP dehydrated and digested. The activated sludge samples were taken in WWTP C and D, and this sludge come from the biological reactors of the secondary treatment in the WWTPs. All samples were frozen after collection.

Each frozen sludge sample was lyophilized using the freeze-dry system (Labconco, Kansas City, MO, USA) and sieved through a 125 μm screen.

The spiked sewage sludge was prepared by adding a dilution of the working standard solution in acetone. To optimize the PHWE, 20 μL of the working standard solution were diluted in 100 mL of acetone and adding 25 mL of this solution to 5 g of dry sewage sludge. Subsequently, the solvent was slowly evaporated at room temperature under frequent homogenization.

2.4. Pressurised hot water extraction

PHWE extraction was performed on a Dionex (Sunnyvale, CA, USA) ASE 200 instrument. One cellulose filter followed by 1 g of diatomaceous earth was placed at the bottom of each 33 mL stainless steel extraction cells. After loading 5 g of the pretreated sludge previously mixed with diatomaceous earth, the remaining volume in the cell was filled with diatomaceous earth.

Each sample was extracted using Milli-Q water at pH 7.5. The operating conditions were as follows: extraction temperature, 125 °C; extraction pressure, 1500 psi; preheating period, 6 min; static extraction, 5 min; number of cycles, 2; flush volume, 60% of extraction cell volume; final extraction volume, ~44 mL; and nitrogen purge, 60 s. The extraction temperature, extraction time and pH of water as extracting solvent were the parameters optimized by a central composite design. For this optimization the experimental design matrix and data analysis were performed using the Statgraphics statistical computer package "Statgraphics Plus 5.1" (Manugistics Inc., Rockville, MD, USA).

2.5. Headspace solid-phase microextraction (HS-SPME)

The PHWE extract was diluted with water to a final volume of 50 mL. A solution of 10 mL was taken and poured into a 20 mL headspace vial, which contained 3.6 g of sodium chloride and a magnetic stirring bar. We then performed a headspace solid-phase microextraction (HS-SPME), applying our previous method [9] to the aqueous sludge extract.

We used a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber purchased from Supelco. Before use, the fiber was thermally conditioned in accordance with the manufacturer's recommendations by inserting it into the GC injector port. The used fibers were cleaned by heating them at 250 °C for 10 min prior to extraction and a blank test was performed to check for possible carry-over. The entirely automated SPME extractions were performed by a commercial autosampler CombiPAL (CTC Analytics, Zwingen, Switzerland) mounted on the GC–MS system.

The 20 mL headspace vial containing the PHWE extract was placed in the tray for SPME. When the temperature of the heat/stir accessory reached 45 °C, the vial was automatically transported there and stabilized for 1 min. The fiber was then introduced through the septum and kept in the headspace of the vial for 60 min at 45 °C. During extraction, the sample was magnetically stirred at 750 rpm. The fiber was then withdrawn into the SPME syringe needle, which was then pulled out of the sample vial and immediately inserted into the GC injection port at 250 °C for 18 min (chromatographic time) for the desorption and clean-up of the fiber in order to prevent carryover.

2.6. Chromatographic analysis

The compounds were analyzed by GC–CI-MS–MS. The chromatographic instrument was a Varian 3800 gas chromatograph (Walnut Creek, CA, USA) connected to a Varian 4000 ion trap mass detector. The GC was equipped with a 1079 programmable temperature vaporizing (PTV) injector, a Merlin high-pressure microseal and a 0.8 mm i.d. insert liner (Varian). A fused silica capillary column (3 m × 0.25 mm i.d.) from Supelco (Bellefonte, PA, USA) was used as a guard column connected to a ZB-5 analytical column (30 m × 0.25 mm i.d.; 0.25 μm film thickness) from Torrance (CA, USA). Helium was used as a carrier and collision gas at a flow rate of 1 mL/min. Varian Workstation software was used for instrument control and data processing.

The injector temperature was set at 250 °C and the analyses were done in splitless mode. The column oven was programmed as follows: 40 °C hold for 2.10 min, ramp to 100 °C at 25 °C/min, hold for 4.50 min, and then ramp to 280 °C at 20 °C/min hold for 2 min. All compounds were separated within 18 min. The transfer line, manifold and trap temperatures were 280, 60 and 200 °C, respectively. A filament-multiplier delay of 3 min was established in order to prevent instrument damage. The analytes were ionized by positive chemical ionization using methanol. The CI–MS–MS process was carried out by collision-induced dissociation (CID) using a resonant waveform type. The GC–CI-MS–MS parameters were optimized for each compound in a previous paper [9].

2.7. Quality assurance and quality control

In order to confirm the presence of the compounds in the sewage sludge samples, two factors were considered: (1) retention time and (2) relative abundance of the parent ion and products ions in the spectra. For the quantification of the compounds, the area of the product ion most abundant was used. Spiked sewage sludge samples were also analyzed between the analyses to check the method performance. As sewage samples without the presence of the analytes were not found, areas of those compounds that appear in blanks were substracted from those obtained in spiked samples. The method was validated with sludge samples from potable water plant E by determining linear ranges, LODs, LOQs, repeatability and reproducibility between days.

Table 1
Matrix for central composite design.

Assay (block)	Temperature (°C)	Time (min)	pH of water
1 (1)	50 (−1)	15 (1)	11 (1)
2 (1)	50 (−1)	5 (−1)	4 (−1)
3 (1)	75 (0)	10 (0)	7.5 (0)
4 (1)	100 (1)	15 (1)	4 (−1)
5 (1)	100 (1)	5 (−1)	11 (1)
6 (2)	100 (1)	5 (−1)	4 (−1)
7 (2)	50 (−1)	15 (1)	4 (−1)
8 (2)	100 (1)	15 (1)	11 (1)
9 (2)	75 (0)	10 (0)	7.5 (0)
10 (2)	50 (−1)	5 (−1)	11 (1)
11 (3)	117 (1.67)	10 (0)	7.5 (0)
12 (3)	33 (−1.67)	10 (0)	7.5 (0)
13 (3)	75 (0)	10 (0)	1.6 (−1.67)
14 (3)	75 (0)	18 (1.67)	7.5 (0)
15 (3)	75 (0)	10 (0)	13.4 (1.67)
16 (3)	75 (0)	10 (0)	7.5 (0)
17 (3)	75 (0)	2 (−1.67)	7.5 (0)

3. Results and discussion

3.1. PHWE optimization

For the rapid and efficient extraction of the *N*-nitrosamines from the sewage sludge samples using PHWE, several instrumental parameters need to be optimized: temperature, pressure, extraction time, number of cycles, flush volume and purge time; and a suitable pH of water as extraction solvent should be used. As previous studies on the extraction of *N*-nitrosamines from sewage sludge or sediments using PHWE or PLE do not exist, we selected the initial conditions according to previous experience by our research group to extract organic compounds in solid matrices. We tested water as an extraction solvent instead of organic solvents because makes the extraction more environmentally friendly.

The initial conditions selected were: preheating period of 5 min, pressure of 1500 psi, 2 cycles, purge time of 60 s, flush volume of 60% and 5 g of dry sample. The most critical variables affecting the extraction efficiency in PHWE were the pH of water as the solvent extraction, the extraction time and the extraction temperature [28,32,33]. We chose a central composite design (with $\alpha = 1.67$) in three orthogonal blocks using a surface response to optimize the pH of water as the extraction solvent (from 4 to 13), the extraction temperature (from 50 to 100 °C), and the extraction time (from 5 to 15 min). We used the Statgraphics statistical package to generate the experimental matrix and calculate the standardized main effects of the factors considered. The complete design consisted of 17 randomly performed experiments (values are listed in Table 1). All experiments were conducted by extracting 5 g of a sewage sludge sample of potable water treatment plant spiked at 10 μg/kg (d.w.) of *N*-nitrosamines. The individual chromatographic peak areas of each compound were recorded as experimental responses for optimizing.

Pareto charts were used to identify the most influential factors. The data obtained in each central composite design were evaluated by ANOVA at the 5% significance level. These results are shown in bar chart format, with the effects sorted in rank order. For instance, Fig. 1 shows the Pareto chart for the area of NDMA extracted by PHWE followed by HS-SPME and GC–CI-MS–MS. The tendency observed is similar to that in the Pareto chart for the other compounds. In all the cases, the extraction temperature was the

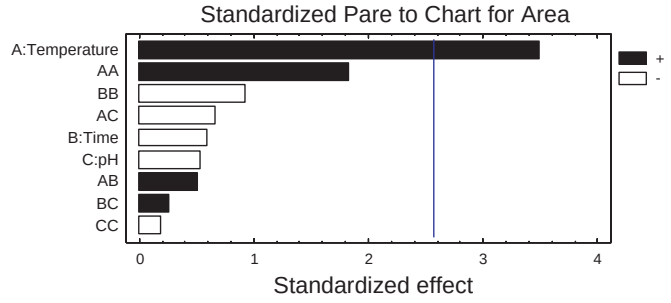

Fig. 1. Standardized Pareto chart of the main effects in the central composite design affecting the PHWE of NDMA. The line represents the significant limit.

most important parameter in the PHWE process. The chromatographic peak areas were largest when the temperature was the highest (100 °C). For the majority of the compounds, the time was the second most important factor and the areas were largest when the time was at the lowest level (5 min), so we selected 5 min as the optimum extraction time. The pH of water was the least influential factor and the peak areas of NDMA, NMEA and NDEA were largest when the pH was the lowest (pH 4), but for NDPA, NMOR, NPIP, NDBA and NDPhA, the areas were largest when the pH was the highest (pH 11). These results agree with the fact that we study a group of NAs with different polarities and have different affinity for water. Because pH of water was the least influential factor and because had different behaviour in compounds, we selected as pH of water 7.5 as a compromise. As we have mentioned, high temperatures (100 °C) improved the extraction of NAs from sludge. Fig. 2 shows the response surface graph when the extraction time is plotted against the extraction temperature for a pH of water of 7.5 for NDMA. The largest areas were found for 5 min and 100 °C. In order to confirm whether these were the optimum values, we decided to test other high temperatures (125 and 150 °C) using the last fixed conditions. We have to mention that using 125 and 150 °C as the extraction temperatures, the PLE software program fixed preheating extraction times of 6 and 7 min, respectively. When 150 °C was used, the PHWE extract obtained was much brownish compared to those which were obtained at lower temperature, which indicate the coextraction of additional compounds. As a result, using 150 °C, the chromatographic peak areas of the main NAs were lower. However, by the use of 125 °C as extraction temperature, the resulting peak areas of the majority of the compounds increased substantially compared with those using 100 °C. For instance, raising the extraction temperature from 100 to 125 °C, the peak areas of NDMA, NMEA, NDEA, NPYR and NDPA increased between 41 and 61%, and peak areas of NDPA, NPIP, NDPhA and NMOR increased,

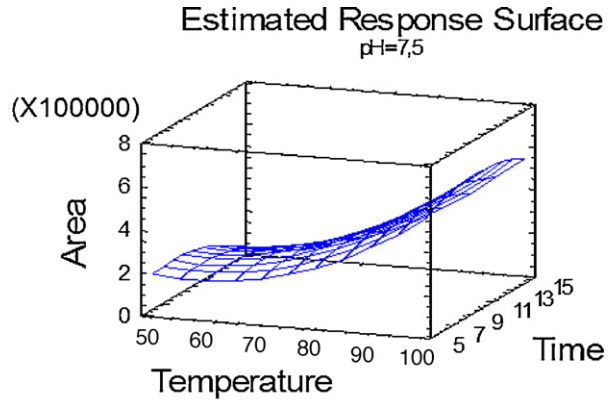

Fig. 2. Estimated response surface for NDMA obtained using the central composite design and plotting the extraction time against the extraction temperature in PHWE.

respectively, by 15, 22, 8 and 95%. However, when the extraction temperature was 150 °C, some interfering compounds were observed and in most of cases the peak areas of the compounds decreased. As an example, raising the extraction temperature from 100 to 150 °C, peak areas of NDMA, NDPhA and NDBA decreased from 12 to 23%. For the rest of compounds, when temperature increases from 100 to 150 °C, it was observed a slightly increment of the area compared with those using 125 °C (from 5 to 21%). Although the other parameters (time and pH) were also tested using values different to the initial conditions, the extraction did not improve. Therefore, the optimized conditions were an extraction temperature of 125 °C and 5 min extraction with water (pH 7.5).

Following the PHWE extraction of the sewage sludge under optimum conditions, we preconcentrated 10 mL of the aqueous extract by HS-SPME with 50/30 μm DVB/CAR/PDMS fiber, and then performed determination with GC–CI-MS–MS [9].

3.2. Method validation

The method developed to determine the nine EPA *N*-nitrosamines in sewage sludge was validated by establishing the linear ranges, limits of detection (LODs), limits of quantification (LOQs), repeatabilities and reproducibilities between days.

Sewage sludge from a potable water treatment plant was used as sample matrix to validate the method. Five samples of this sewage sludge were analyzed and a small peak of NDBA appeared in the chromatogram. Then, the averaged peak area of this compound was subtracted from the corresponding peak area of each spiked sample.

The linear range of the method was obtained by analysing the sewage sludge of the potable water treatment plant at concentrations between 100 ng/kg and 500 μg/kg of *N*-nitrosamines. All the compounds showed acceptable determination coefficients ($r^2 > 0.994$, 6 data points) and each linear range is shown in Table 2.

The LODs were defined as the concentration of analytes in the sewage sludge of the potable water treatment plant which caused a peak with a signal-to-noise ratio higher than 3 for the compounds that did not appeared in this sample. For NDBA, the LOD was defined as the concentration that gave a signal average of plus three times the standard deviation of the sample signal. Limits of quantification (LOQs) were defined as the lowest point of the calibration curve. LODs and LOQs ranged from 30 to 150 ng/kg, and from 100 to 500 ng/kg, respectively, and they are also shown in Table 2.

The repeatability and reproducibility between days were determined by spiking five replicates of the sewage sludge of the potable treatment plant at 10 μg/kg, and the results obtained, expressed as %RSD, were lower than 16% for repeatability and 19% for reproducibility.

3.3. Method application

The developed method was used to determine the nine EPA *N*-nitrosamines in different types of sewage sludge samples collected in one urban WWTP, three industrial WWTPs and one potable water treatment plant (see Section 2.3). The PHWE efficiency was tested with these different types of sludge samples and results were comparable to those obtained with sludge from the potable treatment plant.

For instance, Fig. 3 shows the PHWE–HS-SPME–GC–CI-MS–MS chromatograms of sewage sludge from an industrial WWTP C sample. NDEA, NDBA and NDPhA appeared in the chromatograms at concentrations of 371, 0.2 and 26 μg/kg, respectively.

Table 3 shows the results of the average concentrations of the *N*-nitrosamines found in each type of sewage sludge sample ($n = 3$). NDMA, NMEA, NDPA, NMOR and NPIP did not appear in any of the

Table 2
Method linear ranges, LODs, repeatability and reproducibility between days (%RSD, $n = 5$, 10 μg/kg) for the analysis of *N*-nitrosamines by PHWE followed by HS-SPME and GC–CI-MS–MS in sewage sludge. See text for other conditions.

Compound	Linear range (μg/kg)	LOD[a] (μg/kg)	Repeatability (%RSD)	Reproducibility (%RSD)
NDMA	0.50–250	0.12	8	10
NMEA	0.50–500	0.10	15	18
NDEA	0.20–500	0.05	6	11
NPYR	0.30–500	0.07	16	19
NDPA	0.20–500	0.05	9	12
NMOR	0.40–500	0.08	10	14
NPIP	0.50–500	0.15	11	13
NDBA	0.10–500	0.03	5	8
NDPhA	0.50–500	0.14	12	15

The analytical validation was performed using sewage sludge from a potable water plant.
[a] Limits of detection were defined as S/N = 3.

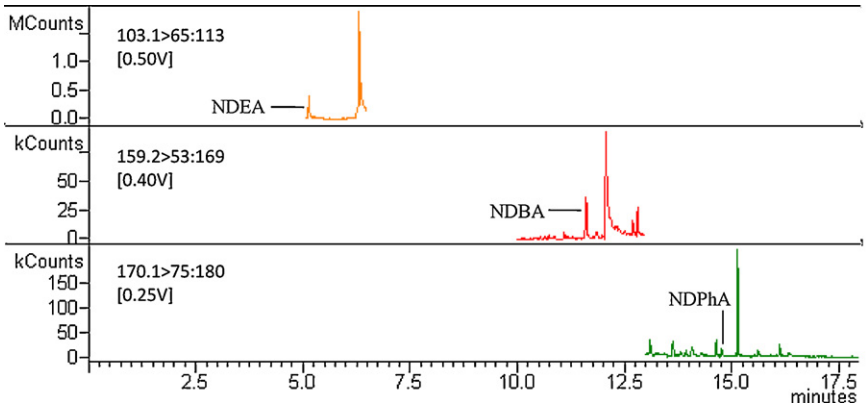

Fig. 3. PHWE–HS-SPME–GC–CI-MS–MS chromatograms of *N*-nitrosamines of a sewage sludge from the industrial WWTP C.

Table 3
Concentration (μg/kg) (d.w.) of *N*-nitrosamines in sewage sludge samples ($n = 3$, RSD < 20%).

Compound	WWTP A[a]	WWTP B[a]	WWTP C[a]	WWTP D[a]	Potable water treatment plant
NDEA	–	–	371	52	–
NPYR	–	60	–	–	–
NDBA	2.6	–	0.2	305	31
NDPhA	–	–	26	–	–

–: Below limit of detection.
[a] WWTP A is an urban WWTP; WWTP B, C and D are industrial WWTPs.

samples analyzed, thus they were excluded from Table 3. These results agree with that we expected, because NDMA and NMEA are the most polar *N*-nitrosamines and they are more hydrophilic. Samples from industrial WWTPs showed the highest concentration of *N*-nitrosamines, although their presence was not homogeneous. Thus, sludge from WWTP C showed the presence of three *N*-nitrosamines being NDEA the compound that showed the highest concentration (371 μg/kg d.w.) among all samples analyzed, while sludge from WWTP B only showed the presence of NPYR. Sludge from the urban WWTP A and the potable water treatment plant only showed the presence of one nitrosamine, NDBA, at low μg/kg levels.

Among all *N*-nitrosamines studied, NDBA was the compound that gave more positive results in the samples analyzed and being its highest concentration in the sludge from WWTP D (305 μg/kg d.w.).

It should also be remark that activated sludge (WWTPs C and D) showed the presence of more *N*-nitrosamines and at much higher concentration than digested sludge.

Little information has been reported about the presence of *N*-Nitrosamines in sewage sludge samples. Padhye et al. [13] determined NDMA and NPYR in primary sludge supernatant, in activated sludge and in anaerobic digester mixed liquor samples of three municipal WWTPs and found concentrations ranging from 57 to 994 ng/L. NDEA, NMEA, NDPA and NDBA were not detected. It should be noted that the analysis of those samples was carried out using the sludge filtrates followed by a SPE and GC/MS/MS and not a sludge extraction technique as we used in the present work.

4. Conclusions

This study shows for the first time the determination of the nine EPA *N*-nitrosamines at μg/kg levels in sewage sludge samples by the use of PHWE followed by HS-SPME–GC–CI-MS–MS. The method is automated with the exception of the PHWE steps, it is easy to perform and because it avoids the use of toxic organic solvents it is environmentally friendly. This method also provides LODs between 0.03 and 0.15 μg/kg, and moreover, the use of CI-MS–MS instead of single CI-MS detection provides high selectivity and sensitivity for the determination of nine *N*-nitrosamines in such highly complex sewage sludge samples from WWTPs.

The most important parameters involved in the PHWE were evaluated using a central composite design and the optimum conditions were an extraction temperature of 125 °C and 5 min with water (pH 7.5).

Some of the nine EPA *N*-nitrosamines appeared in the sewage sludge samples analyzed at concentrations ranging between 0.2 and 371 μg/kg. The most detected compound was NDBA, which appeared in all the samples excepting the industrial WWTP B. NDMA, NMEA, NDPA and NMOR were not find in any sample, which could be due to their hydrophilic character.

Acknowledgements

The authors wish to thank the personnel of the various water treatment plants for their cooperation in all aspects of this study. This study was financially supported by the Directorate-General for Research of the Spanish Ministry of Science and Technology; project CTM2008-06847-C02-01/TECNO and by Departament d'Innovació, Universitats i Empreses de la Generalitat de Catalunya; project 2009SGR223. A. Llop gratefully acknowledges the financial support of the Department of Innovation, Universities and Enterprise of the Catalan Government, as well as the European Social Fund (2007 FI00026).

References

[1] S. Ventanas, J. Ruiz, Talanta 70 (2006) 1017–1023.
[2] D. Méndez, G. González, E. Botello, E. Escamilla, J.F.J. Alvarado, Food Chem. 107 (2008) 1348–1352.
[3] R.C. Schothorst, H.H.J. Somers, Anal. Bioanal. Chem. 381 (2005) 681–685.
[4] C. Flower, S. Carter, A. Earls, R. Fowler, S. Hewlins, S. Lalljie, M. Lefebvre, J. Mavro, D. Small, N. Volpe, Int. J. Cosmet. Sci. 28 (2006) 21–33.
[5] H.L. Lee, C. Wang, S. Lin, D.P.H. Hsieh, Talanta 73 (2007) 76–80.
[6] X. Pan, B. Zhang, S.B. Cox, T.A. Anderson, G.P. Cobb, J. Chromatogr. A 1107 (2006) 2–8.
[7] S.D. Richardson, Anal. Chem. 81 (2009) 4645–4677.
[8] S.D. Richardson, Trend Anal. Chem. 22 (2003) 666–684.
[9] A. Llop, F. Borrull, E. Pocurull, J. Sep. Sci. 33 (2010) 3692–3700.
[10] US EPA Contaminant Candidate List 3 (CCL 3), as of January 2009. http://www.epa.gov/OGWDW/ccl/ccl3.html.
[11] P. Andrzejewski, B. Kasprzyk-Hordern, J. Nawrocki, Desalination 176 (2005) 37–45.
[12] A. Llop, E. Pocurull, F. Borrull, J. Chromatogr. A 1217 (2010) 575–581.
[13] L. Padhye, U. Tezel, W.A. Mitch, S.G. Pavlostathis, C.H. Huang, Environ. Sci. Technol. 43 (2009) 3087–3093.
[14] W.A. Mitch, J.O. Sharp, R.R. Trussell, R.L. Valentine, L. Alvarez-Cohen, D.L. Sedlak, Environ. Eng. Sci. 20 (2003) 389–404.
[15] Y.Y. Zhao, J.M. Boyd, M. Woodbeck, R.C. Andrews, F. Qin, X.F. Li, Environ. Sci. Technol. 42 (2008) 4857–4862.
[16] B. Jurado-Sánchez, E. Ballesteros, M. Gallego, J. Chromatogr. A 1216 (2009) 1200–1205.
[17] US EPA, N-nitrosodimethylamine (CASRN 62-75-9), Integrated Risk Information System (IRIS) as of January 2009. http://www.epa.gov/ncea/iris/subst/0045.html.
[18] C. Planas, O. Palacios, F. Ventura, J. Rivera, J. Caixach, Talanta 76 (2008) 906–913.
[19] D.L. Sedlak, R.A. Deeb, E.L. Hawley, W.A. Mitch, T.D. Durbin, S. Mowbray, S. Carr, Water Environ. Res. 77 (2005) 32–39.
[20] H. Kodamatani, S. Yamazaki, K. Saito, A. Amponsaa-Karikari, N. Kishikawa, N. Kuroda, T. Tomiyasu, Y. Komatsu, J. Chromatogr. A 1216 (2009) 92–98.
[21] Y.Y. Zhao, J. Boyd, S.E. Hrudey, X.F. Li, Environ. Sci. Technol. 40 (2006) 7636–7641.
[22] M. Krauss, J. Hollender, Anal. Chem. 80 (2008) 834–842.
[23] M. Plumlee, M. López-Mesas, A. Heidlberger, K.P. Ishida, M. Reinhard, Water Res. 42 (2008) 347–355.
[24] M.W. Byun, H.J. Ahn, J.H. Kim, J.W. Lee, H.S. Yook, S.B. Han, J. Chromatogr. A 1054 (2004) 403–407.
[25] J.E. Grebel, I.H. Suffet, J. Chromatogr. A 1175 (2007) 141–144.
[26] J.E. Grebel, C.C. Young, I.H. Suffet, J. Chromatogr. A 1117 (2006) 11–18.
[27] J.W.A. Charrois, M.W. Arend, K.L. Froese, S.E. Hrudey, Environ. Sci. Technol. 38 (2004) 4835–4841.
[28] A. Llop, F. Borrull, E. Pocurull, Anal. Chim. Acta 665 (2010) 231–236.
[29] A. Raksit, S. Johri, J. AOAC Int. 84 (2001) 1413–1419.
[30] J.W. Munch, M.V. Bassett, J. AOAC Int. 89 (2006) 486–497.
[31] M. Krauss, P. Longrée, F. Dorusch, C. Ort, J. Hollender, Water Res. 43 (2009) 4381–4391.
[32] V. Fernández-González, E. Concha-Graña, S. Munategui-Lorenzo, P. López-Mahía, D. Prada-Rodríguez, J. Chromatogr. A 1196–1197 (2008) 65–72.
[33] A. Nieto, F. Borrull, R.M. Marcé, E. Pocurull, J. Chromatogr. A 1216 (2009) 5619–5625.