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Determination of halonitromethanes in treated water

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ABSTRACT

As halonitromethanes (HNMs) have begun to play an increasingly important role as disinfection by-products, the development of a highly sensitive method for their analysis has become a priority. The mass spectrometric behavior of the 9 HNMs revealed that trihalonitromethanes are more unstable than di- or monohalonitromethanes under common chromatographic conditions. The absence of a comprehensive method for HNMs has given rise to the development of the first method for the whole array of these species, involving the selection of a solventless technique. Single drop microextraction in the headspace mode (HS-SDME) was selected as it is inexpensive and easy to operate. Comparative measurements through EPA liquid–liquid extraction (LLE) method for halogenated volatile compounds, show this approach to be superior to the manual LLE procedure (the average limits of detection (LODs) for the 9 HNMs were 0.5 and 1 μg/L for the HS-SDME and EPA methods, respectively), adequate precision (8.2 and 7.0% for HS-SDME and EPA methods, respectively) and does not consume excessive solvent since the total extract (~2 μL) was injected completely into the GC–MS instrument. The method was used to measure HNMs in treated water and the results were compared to the EPA method in parallel.

1. Introduction

Water is a scarce commodity and a fundamental resource for the human being since it plays a decisive role in health. Since the end of the XIX century, water disinfection has been used to reduce the incidence of illnesses. Chemical disinfectants (chlorine, chloramines, ozone, chlorine dioxide, etc.) are effective in killing harmful microorganisms in drinking water, but they also oxidize organic matter that forms disinfection by-products (DBPs) [1–4]. Although more than 600 DBPs have been reported in the literature, only 11 are currently regulated [1,5]. Among the unregulated DBPs, the 9 halonitromethanes (HNMs) receive special attention because of their potentially high toxicity and their occurrence in final waters at some treatment facilities [6]. Chloropicrin (trichloronitromethane, TCNM) has been the most commonly measured example in this class followed by bromonitromethane (BNM) and bromopicrin (tribromonitromethane, TBNM), which are a potential concern for toxicity [7,8]. Average concentrations in treated water containing bromide [9] have been reported between 0.1 and 10 μg/L for some HNMs and between 0.9 and 1.5 μg/L for TCNM in wastewater treatment plant effluents [10]. Despite the increasing amount of the literature on HNMs, there has been little systematic research reported on a whole array of HNM species due to the lack of commercial chemical standards in all the species, which only became available in the early 2000s. For this reason it has only recently become possible to establish the formation and speciation characteristics of HNMs as well as the factors controlling their formation in drinking waters [10–12]. In these studies, liquid–liquid extraction has been employed as a preliminary step, using the EPA methods proposed to determine halogenated volatile organic compounds (VOCs) in water [13], in order to determine some HNMs by gas chromatography–electron capture detection (GC–ECD) [9–12] or by GC mass spectrometry (MS) [14]. Other alternatives for VOCs (including TCNM) such as solid phase microextraction (SPME) with GC–MS and Purge&Trap-GC–MS [15] have also been used. Chloropicrin or TCNM (the first HNM identified as a DBP) in a mixture of other chlorine VOCs, prepared with distilled water, has been determined for headspace (HS)-SPME–GC–ECD between 0.1 and 2.5 μg/L [16] and by HS and manual injection into a GC–MS [17] or GC–ECD [18] with limits of detection (LODs) of 0.5 or 2.5 μg/L, respectively. However, these methods heat the samples in the injector at 175–250 °C, which favor up to 50% decomposition of TCNM [14]. In summary, neither have proper methodologies been developed for the 9 HNMs nor has any study of the chromatographic temperatures of the GC–MS been found to minimize/eliminate the decomposition of the 9 compounds. Only BNM and TCNM have been found in the NIST (No. 69) or Wiley spectral library database, although mass spectral ions of the 9 HNMs have been reported in the bibliography [6].

As outlined above, to date the EPA methods (for halogenated VOCs) using GC to determine some HNMs in water require liquid–liquid extraction with methyl tert-butyl ether (MTBE) which

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implies great solvent consumption and cost. To overcome such problems, recent research activities have been oriented toward the development of miniaturized sample preparation techniques like SPME [19] and liquid phase microextraction (LPME) [20–23]. In DBPs, LPME has been used for the determination of trihalomethanes (THMs) and more recently for haloacetic acids [24] but never for HNMs. In order to find precedents applying LPME methods to HNMs, we have to refer to the 4 THMs which are also volatile DBPs. Two similar methods based on single drop microextraction (SDME)–GC–ECD are proposed for the 4 THMs with similar LODs (0.2–0.4 μg/L) using 1-octanol [25] or *n*-hexane [26] as extractant. Direct hollow fiber (HF)-LPME–GC–ECD uses 25 μL of 1-octanol at 35 °C, providing LODs of 0.01–0.2 μg/L [27]. The most recent method to determine the 4 THMs by LPME–GC–MS [28] is based on the solidification of a floating organic microdrop (7 μL of 1-undecanol) with enrichment factors up to 480-fold (LODs, 0.03–0.08 μg/L), but it requires drastic extraction conditions (15 min at 60 °C) which are related to low recoveries.

Taking into consideration the foregoing, the aims of this work have been: (i) to propose the first method for the whole array of HNM species in water because 7 HNMs, although still very expensive, are now commercially available (in addition to the 2 HNMs, TCNM and BNM, that have always been on the market); (ii) to develop a solventless technique in which only one drop of an organic solvent is employed, as occurs in LPME; (iii) to avoid/minimize the decomposition of HNMs during heating in the injection port of GC and/or hot transfer line/ion source of the MS, which can complicate their identification in treated water; and (iv) to obtain enough sensitivity to determine the 9 HNMs at ng/L levels in treated water samples. The proposed HS-LPME–GC–MS method consists of a simple and fast extraction stage using a microdrop of organic solvent at the tip of a commercial microsyringe to extract the 9 HNMs from the water sample under soft conditions. The method is nearly solvent-free since the total extract was injected into the GC–MS instrument. For the first time a rigorous study has been tackled on the impact of 9 HNM decomposition in the injection port of the gas chromatograph as well as of the ion source of the mass spectrometer on the mass spectra for all 9 HNMs, since only four of them had been studied previously.

2. Experimental

2.1. Chemicals

Chloronitromethane (CNM, 90–95%), dichloronitromethane (DCNM, 95%), bromochloronitromethane (BCNM, 85–90%), bromodichloronitromethane (BDCNM, 90–95%), dibromonitromethane (DBNM, 90%), dibromochloronitromethane (DBCNM, 90–95%) and tribromonitromethane (TBNM, 90–95%) standards were supplied by Orchid Cellmark (New Westminster, Canada), while trichloronitromethane (TCNM, 99%), and bromonitromethane (BNM, 90%) were purchased from Sigma–Aldrich (Madrid, Spain) and the internal standard, fluorobenzene, from Fluka (Madrid, Spain). The solvents, 1-octanol, o-xylene, decane and 1-hexanol were purchased from Sigma–Aldrich. Ethyl acetate, methyl tert-butyl ether (MTBE) and sulfuric acid were supplied from Merck (Darmstadt, Germany). Potassium chloride, sodium chloride, anhydrous sodium sulfate, anhydrous magnesium sulfate and ammonium sulfate (dechlorinating agent) were purchased from Panreac (Barcelona, Spain). Stock standard solutions containing 1 g/L of individual halonitromethane and cumulative solutions (0.1 g/L) were prepared in ethyl acetate and stored frozen in amber glass vials at −20 °C. More dilute cumulative solutions were prepared daily in mineral water (free of DBPs) at the microgram per liter level.

2.2. Apparatus

Sample analysis was performed with a Fisons 8000 GC instrument interfaced to a Voyager mass spectrometer and controlled by a computer running MASSLAB software (Thermo, Madrid, Spain). The gas chromatographic separation was achieved on a 30 m × 0.25 mm i.d., 0.25 μm film TRB-5 capillary column coated with a stationary phase of 5%-phenyl–95%-methylpolysiloxane and supplied by Teknokroma (Barcelona, Spain). All injections were made in the split mode (1:20 split ratio) by setting the injector temperature at 170 °C. The GC oven temperature program was: 40 °C (3 min) and then raised at 40 °C/min to 140 °C (2 min) and 180 °C (3 min). The helium carrier gas (6.0 grade purity, Air Liquid, Seville, Spain) was set at 1 mL/min. The mass spectrometer was used in the following conditions: ion source temperature, 200 °C; transfer line temperature, 200 °C; electron impact ionization mode, 70 eV; scan range from *m*/*z* 30–255; time for solvent delay, 2 min. Optimization experiments were conducted in total ion chromatography (TIC) mode at 3.5 scans/s. The ions selected for identification and quantification of HNMs (SIM mode) are listed in Table 1; *m*/*z* values for fluorobenzene (IS) were: 50, 70, 96 (base peak).

2.3. Sample collection and preservation

Water samples were collected in amber glass bottles of 125 mL with poly(tetrafluoroethylene) screw caps. The bottles, containing 1.7 g of ammonium sulfate as the quenching reagent of residual chlorine [29], were completely filled to avoid evaporation of volatile compounds. To validate the sampling protocol for the analysis of HNMs, the storage time of the sample at 4 °C was studied using mineral water fortified with 5 μg/L of HNMs (except to TBNM, 10 μg/L). The studies were conducted over 10 days; the results indicated that the concentrations of CNM, DCNM, TCNM, BNM and BCNM remained constant for 7 days, whereas DCBNM, DBNM, DBCNM and TBNM only for 1 day. Thus, samples were stored at 4 °C and analyzed within 1 day of collection. For analysis, 10 mL of water sample (prepared as described below) was placed in 15 mL glass vials.

2.4. HS-SDME–GC–MS procedure

A 5 μL GC microsyringe model 87925 from Hamilton (Teknokroma, Barcelona) was used to perform the SDME experiments. Ten milliliter water samples or mineral water containing between 0.2 and 300 μg/L of each halonitromethane and 20 μg/L of fluorobenzene (IS) were placed in a 15 mL glass vial containing 3 g (2.1 mol/L) of Na_2SO_4 and the pH was adjusted at ∼3.2 by adding 30 μL of 0.1 mol/L H_2SO_4. A stirring bar (1.3 cm long) was added to the vial, which was closed immediately with a screw cap equipped with a silicon septum. Afterward the vial was stirred in a vortex mixer for 2 min in order to dissolve the salt and then placed in a water bath. A 2.5 μL volume of 1-hexanol was withdrawn into the microsyringe, the needle tip was inserted through the silicone septum and the 2.5 μL drop of extractant exposed to the headspace of the sample stirred at 600 rpm for 20 min at 30 °C. After extraction, the drop was retracted back into the microsyringe and the total extract (∼2 μL) injected into the GC instrument.

2.5. LLE procedure (EPA method 551.1)

Liquid–liquid extraction for the determination of HNMs in water was performed in triplicate following the EPA method 551.1 [13] proposed for the determination of halogenated VOCs. Samples were collected in 62 mL amber bottles with a poly(tetrafluoroethylene) screw cap containing 0.8 g of ammonium sulfate and without headspace to avoid evaporation of VOCs. A 12 mL aliquot was

Table 1
Mass spectral ions selected for identification and quantification (boldfaced) of halonitromethanes.

Compound	Mol wt.	*m/z* (relative abundance) → [fragment ion]
CNM	95	**49** (100) → $[CH_2Cl]^+$, 51 (42) → $[CH_2Cl]^+$, 46 (12) → $[NO_2]^+$
BNM	139	**93** (100) → $[CH_2Br]^+$, 95 (95) → $[CH_2Br]^+$, 46 (10) → $[NO_2]^+$
DCNM	129	**83** (100) → $[CHCl_2]^+$, 85 (71) → $[CHCl_2]^+$, 46 (8) → $[NO_2]^+$
DBNM	217	**173** (100) → $[CHBr_2]^+$, 171 (67) → $[CHBr_2]^+$, 46 (10) → $[NO_2]^+$
BCNM	173	**129** (100) → $[CHClBr]^+$, 127 (87) → $[CHClBr]^+$, 46 (9) → $[NO_2]^+$
TCNM	163	**117** (100) → $[CCl_3]^+$, 119 (96) → $[CCl_3]^+$, 46 (1) → $[NO_2]^+$
TBNM	295	**251** (100) → $[CBr_3]^+$, 253 (98) → $[CBr_3]^+$, 46 (6) → $[NO_2]^+$
BDCNM	207	**163** (100) → $[CCl_2Br]^+$, 161 (70) → $[CCl_2Br]^+$, 46 (20) → $[NO_2]^+$
DBCNM	251	**207** (100) → $[CClBr_2]^+$, 209 (77) → $[CClBr_2]^+$, 46 (11) → $[NO_2]^+$

withdrawn from the sample bottle and discarded and the pH was adjusted at 4.5–5.5 with diluted H_2SO_4. Fifty μicrolitre of a 10 mg/L standard solution of fluorobenzene (IS), 3 mL of extracting solvent (MTBE), 20 g of Na_2SO_4 and 1 g of copper sulfate were added to the remaining sample (50 mL) and the vial was stirred for 4 min; once the HNMs were extracted, the vial was left to stand for 2 min in order to separate both phases. Then, 1 mL of the upper MTBE layer was transferred to a 2 mL glass vial and 0.1 g of sodium sulfate was added to dry the extract. Finally 2 μL of the extract was injected into the GC–MS instrument.

3. Results and discussion

Gas chromatography/mass spectrometry (GC–MS) has been the primary analytical tool used to identify DBPs in drinking water. A few trihalomethyl compounds partially decompose in the injection port of GC (forming mainly haloforms) or the GC–MS transfer line (the resulting mass spectra are a mixture of the native compound and decomposition products) [14]. Among LPME techniques, SDME is the most popular because it is inexpensive, does not require any equipment and is easy to operate; also the headspace mode provides the best resolution for VOCs [30]. Factors that influence extraction efficiency should be established, such as the organic solvent, sample pH, salting-out effect, and physical parameters.

3.1. Gas chromatography/mass spectrometry optimization conditions

Instrumental and analytical conditions can have a significant effect on determining halonitromethanes. The HNMs are thermally unstable and can decompose under temperatures commonly used in the injection port, hot transfer line and in the ion source during GC–MS analysis. A study of the behavior of some HNMs (mainly bromopicrin) in GC–MS analysis is carried out by Chen et al. [14]. To date there is no information either on the influence of temperatures on the GC–MS in the determination of mono- and dihalonitromethanes or on the mass spectrometer ion source temperature for the 9 HNMs. That is why this paper embarks on a rigorous study of the influence of the temperature in the GC injection port and the mass spectrometer ion source. For this purpose, 1 μL of a standard solution containing 50 μg/mL of each HNM in ethyl acetate was injected into the GC at different injection port temperatures between 150 and 250 °C. This parameter affects trihalonitromethanes in a different way than it does mono- and dihalonitromethanes. In fact, trihalonitromethanes decompose above 170 °C, their peak areas at 250 °C were 45% relative to values obtained at 150 °C, which is in agreement with the above study [14]. The major decomposition products are haloforms, which are probably formed by hydrogen abstraction from solvents due to the trihalomethyl radical. On the other hand, neither mono- nor dihalonitromethanes decomposed in the interval of temperatures assayed. This can be observed clearly in Fig. 1 where the peak areas of trihalonitromethanes decreased at a GC injection port temperature of 250 °C relative to 170 °C, whereas for mono- and dihalonitromethanes the analytical signals remain constant in both instances. This fact can hinder the identification and quantification of other DBPs also present in treated water, such as THMs. For example, the formation of chloroform and bromoform as the main decomposition products of TCNM and TBNM, respectively (with GC injection port temperatures above 170 °C) could contribute to overestimations of chloroform and bromoform concentrations in treated water samples, possibly allowing the presence of TCNM and TBNM to go undetected in the original drinking water.

In the present study the transfer line of the GC–MS was heated to a temperature (200 °C) similar to the highest temperature in the GC program (180 °C). Thus, only the effect of the mass spectrometer ion source temperature was checked for the 9 HNMs in the range 200–250 °C. None of the 9 species showed a decrease in the peak area, nor were halomethanes detected. So, it can be concluded that there was no evidence of decomposition in the 9 compounds up to 250 °C (since their mass spectra confirmed their identities), although they probably could decompose at higher temperatures. In conclusion, the selected temperatures were 170 °C for the injection port and 200 °C for both the transfer line and the ion source of the mass spectrometer, to avoid/minimize HNM decomposition.

Finally, after obtaining the mass spectra in the best chromatographic conditions for the 9 HNMs, the most significant ions for unequivocal identification were selected. For this purpose, the criteria employed were sensitivity (selecting the most abundant peak, base peak) and selectivity (selecting the characteristic ions of each compound). Table 1 shows the three ions selected for the identification of HNMs (quantification one in boldface print), their relative abundance and their corresponding fragments. None of the halonitromethanes show molecular ions in their mass spectra and their base peaks correspond to molecular weight less 46 Da, which is a consequence of losing a nitro group $[M–NO_2]^+$ [6]. In the ion source, when an electron impacts on a neutral molecule, the molecule is ionized and gives off an extra electron. When a molecule loses an electron, it acquires a positive charge and an unpaired electron and therefore the ion becomes a cation-radical. When the atom is highly electronegative, it will tend to gain the electron and will remain as a radical. The nitro group and the halogens are electronegative both capturing the electron to form radicals. The mechanism of fragmentation of these compounds (TCNM as model) is as follows:

$$[Cl_3CNO_2] + e^- \rightarrow [Cl_3CNO_2]^{+\bullet} + 2e^-; [Cl_3CNO_2]^{+\bullet} \rightarrow [Cl_3C]^+ + NO_2^\bullet$$

$$[Cl_3C]^+ \rightarrow [Cl_2C]^{+\bullet} + Cl^\bullet; [Cl_2C]^{+\bullet} \rightarrow [ClC]^+ + Cl^\bullet$$

Taking into account that the remaining fragments contained different combinations of halogen atoms (chlorine and/or bromine)

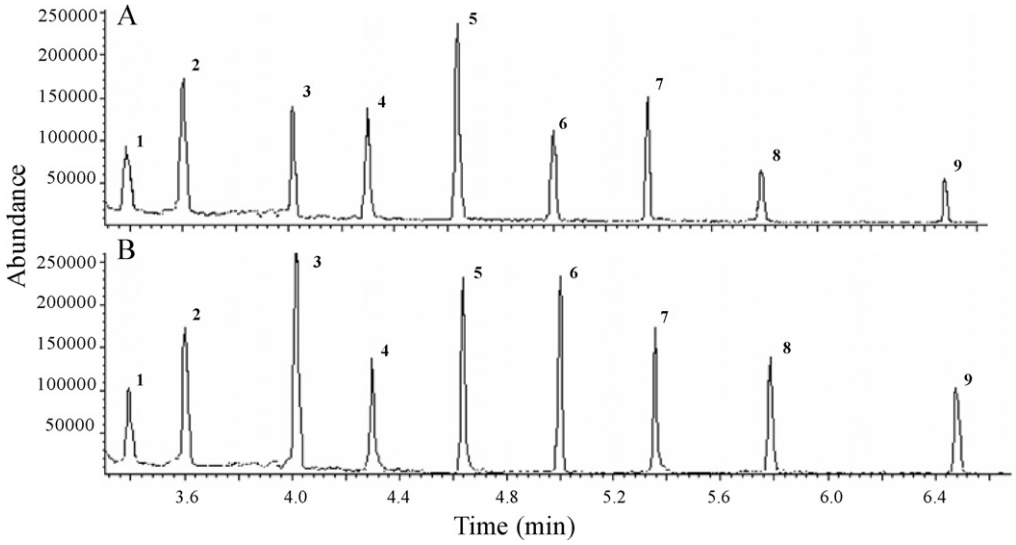

Fig. 1. GC–MS total ion chromatograms for the 9 HNM standards using different injection temperatures (A) 250 °C and (B) 170 °C. Peak identification: CNM (1); DCNM (2); TCNM (3); BNM (4); BCNM (5); BDCNM (6); DBNM (7); DBCNM (8); TBNM (9).

which presented specific mass spectra due to their isotopic abundance ratios [31], the fragments selected for the unequivocal identification of each compound were chosen based on the different isotopic signals provided for each analyte. Chlorine and bromine atoms have two stable isotopes of 35 and 37 amu, and 79 and 81 amu, respectively. Thus, molecules that contain chlorine and/or bromine atoms provide M+2 peaks related with their isotopes. By way of example, for the identification of trichloronitromethane (whose electron ionization mass spectrum appears in Fig. 2), the *m*/*z* ratios 117 (100% abundance) and 119 (96% abundance) were selected because the three chlorine atoms in the fragment could be identified due to the isotopic relative abundance of both ions. The nitro group (*m*/*z* 46) was selected as the third fragment ion for the identification of halonitromethanes in spite of its low abundance since this *m*/*z* ratio was specific for nitro derivatives suggesting the presence of a NO_2 group, so it could be used for the unequivocal identification of HNMs versus THMs and other halogenated hydrocarbons.

3.2. Selection of extraction solvent and droplet volume

It is essential to select a proper organic solvent for the establishment of a HS-SDME method, which is related to the chemical nature of the target compounds. As there is no study to date on this variable for HNMs, a variety of water-immiscible organic solvents were considered as the possible extractant. The uncertainty associated with the LPME technique, mainly the partial evaporation of the drop, was corrected by the use of an internal standard. Preliminary experiments with 2 μL of drop were examined at 30 °C using 10 mL of spiked mineral water samples at a concentration of 100 μg/L of the 9 HNMs and 20 μg/L of fluorobenzene (IS) containing 3 g of NaCl (in vials of 15 mL) under the following conditions at an extraction time 15 min and stirring rate of 600 rpm. All the extraction experiments were performed by measuring the relative peak area of each halonitromethane to the internal standard using the average of three replicate measurements (after that the different peak areas of fluorobenzene between solvents were normalized). After extraction, the drop was retracted and 1 μL of the extract injected into the GC–MS instrument. As can be seen in Fig. 3, 1-hexanol provided the best extraction efficiency for 5 HNMs (CNM, BNM, BCNM, DBCNM and TBNM) whereas 1-octanol (the most commonly used in LPME techniques) only provided slight advantages for 3 HNMs (DCNM, BDCNM and DBNM); it did not extract to TBNM and scarcely did so to CNM and BNM. In addition 1-octanol required higher temperatures (BP ~200 °C) in the injection port, chromatographic column and mass spectrometer ion source than 1-hexanol (BP ~160 °C), which, as mentioned above, is related to the decomposition of the trihalonitromethanes (see Fig. 1) to halomethanes among other compounds. Decane and o-xylene provided the poorest results and therefore were discarded. In conclusion, 1-hexanol showed the best extraction efficiency and adapted itself to the temperatures established for GC–MS analysis. The relative peak areas

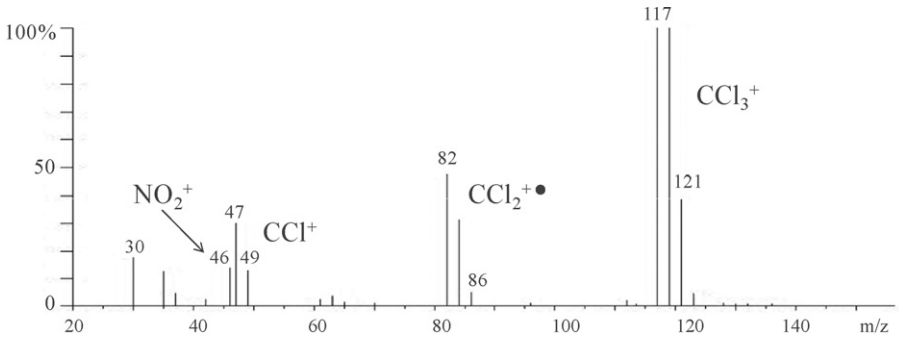

Fig. 2. Electron ionization mass spectrum of trichloronitromethane (TCNM).

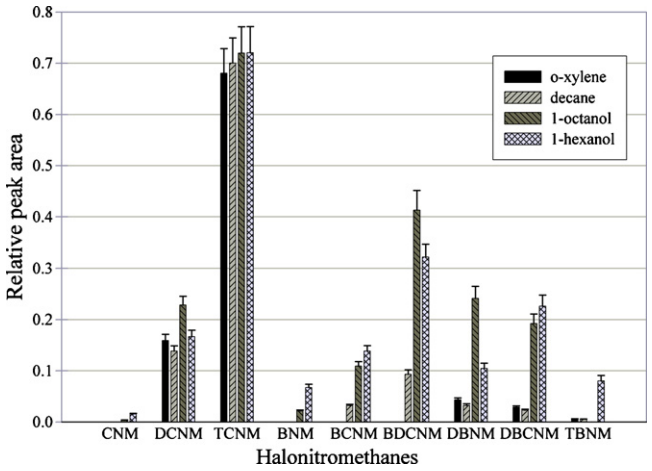

Fig. 3. Effect of selection of solvent on HS-SDME technique for 100 μg/L of each HNMs using 2 μL of extractant. Error bars are the standard deviation for three measurements.

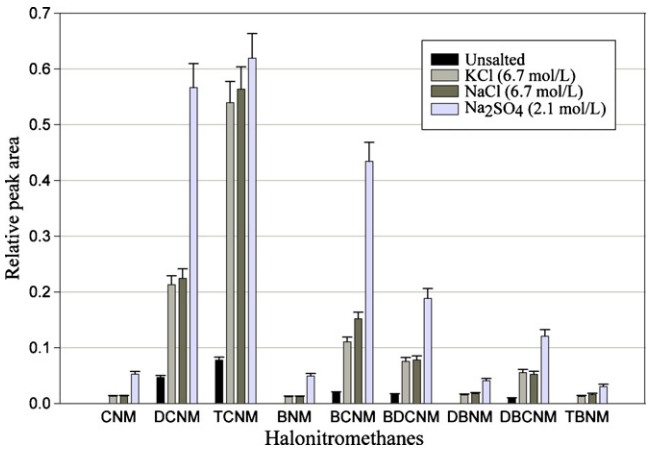

Fig. 5. Feasibility for using different salts at variable concentrations on the extraction of the 9 HNMs. Error bars are the standard deviation for three measurements.

increased with increasing solvent volume although when the drop exceeded 2.5 μL, its manipulation was more elaborate and less reliable. Furthermore, large injection volumes resulted in a more extensive band broadening in capillary GC. Considering these factors, 2.5 μL of 1-hexanol (~2 μL of extract) was selected as the extractant since it provided the best extraction efficiency, good reproducibility (RSD ~10%) and has a boiling point of 157 °C (vapor pressure, 0.947 Torr at 25 °C), which prevented its retention (or is insignificant) either into the column or in the mass spectrometer ion source at optimal conditions.

3.3. Effect of chemical variables

The only documentation about the influence of chemical parameters on HNMs is related with bromopicrin (TBNM) which is destroyed by common dechlorination agents (e.g. ascorbic acid) and requires a 3.5–4.0 pH to minimize base-catalyzed hydrolysis in water [14]. Therefore, the first chemical variable studied was the sample pH for the 9 HNMs because no information was available for these compounds. A few drops of diluted sulfuric acid solutions were used to adjust the pH of the aqueous sample in the acid region. As displayed in Fig. 4, chloropicrin (TCNM) was not affected by the sample pH in any interval assayed and only minimally by DCNM, BCNM and BNM, whereas BDCNM, DBCNM, CNM and DBNM were influenced by the sample pH, especially TBNM. Since the pH of the sample was related to extraction efficiency, the best results for the simultaneous extraction of the 9 HNMs were obtained at

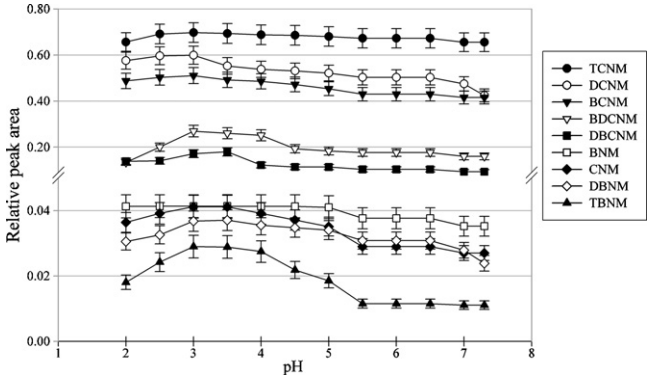

Fig. 4. Influence of pH on the volatilization/extraction of the 9 HNMs from aqueous samples with 1-hexanol. Error bars are the standard deviation for three measurements.

pH 3.0–3.5. To minimize sample manipulation, the aqueous sample was adjusted at pH ~3.2 by adding 30 μL of 0.1 mol/L H_2SO_4 per 10 mL of sample. A series of extraction experiments were carried out with a 1-hexanol drop (2.5 μL) by adding different salts (NaCl, KCl, Na_2SO_4 and $MgSO_4$) from 0 to 5 g to 10 mL of spiked mineral water samples (100 μg/L) at 30 °C for all HNMs. Better conditions for the extraction of all HNMs were achieved by adding salts. In the first approach, anhydrous $MgSO_4$ was discarded since during the initial extraction, a good bit of heat is generated by exothermic hydration after the addition of the salt (3 g, 2.5 mol/L) to the samples. In one sense, this heat generation aids the extraction speed or efficiency of HNMs while, on the other hand, too much heat may lead to a loss of the droplet [32]. Fig. 5 shows that KCl as well as NaCl provided the poorest peak areas even at high concentrations (~7 mol/L) whereas Na_2SO_4 was the better choice. The data confirmed that sodium sulfate increased the extraction efficiency for the 9 HNMs to double the amount for some compounds (CNM, DCNM, BNM, BCNM, BDCNM and DBCNM). Thus, the best conditions for the 9 HNMs extraction were performed with 3 g of Na_2SO_4 per 10 mL of sample.

The influence of the water sample and headspace volume was examined from 5 to 10 mL in 15 mL sample vials (headspace volume from 10 to 5 mL). In all experiments the amount of each HNM was 0.5 μg whereas the volume of the sample changed. The experimental results showed that the extraction efficiency increased as the sample volume grew since the volume of gaseous phase (headspace) was minimized, which was in agreement with other HS-LPME methods [33]. A sample volume of 10 mL (in 15 mL vials) was adopted considering that when 3 g of salt was added and agitated using stirrer, the volume increased to ~11.5 mL; this ensured that the drop of extractant would not come into contact with the aqueous sample during the extraction step.

3.4. Optimization of physical parameters

The variation in extraction efficiency as a function of extraction time was studied with a 1-hexanol drop in the interval 5–40 min. The HS-SDME experiments of over 40 min extraction time could not be used due to the evaporation of the solvent in the air, which seriously influenced the accuracy of the results. The relative peak areas increased as the extraction time (~20%) rose to 15 min, above which it remained constant. To ensure maximum extraction, an extraction time of 20 min was selected for further experiments. For volatile analytes, the extraction temperature had a double impact on HS-SDME. At a higher temperature, diffusion coefficients in both water and headspace were higher and the

extraction time could be shorter, but the partition coefficients for the analyte between the organic solvent and the gaseous phase were lower [30]. The effect of temperature was studied by exposing the 1-hexanol drop for 20 min in the headspace of 10 mL HNMs working solutions, in triplicate, between 25 and 40 °C. For temperatures over 40 °C, there was a faster solvent evaporation of the drop. As expected, the amounts of HNMs extracted increased at 30–35 °C, above which the relative signals decreased by ~10%. The last optimized study was the stirring rate. Agitation of the sample solution enhanced the mass transfer in the aqueous phase, induced convection in the headspace, and consequently reduced the time for reaching a thermodynamic equilibrium. At stirring rates above 800 rpm resulted in the instability of the vials causing the dislodgement of the organic drop from the needle. At stirring rate lower than 500 rpm the extraction efficiency decreased. Therefore, a stirring rate of 600 rpm was adopted in the method proposed.

Finally, fluorobenzene was selected in terms of volatility as internal standard to correct the uncertainty associated with the LPME technique and the injection of the extract into the GC instrument.

3.5. Efficiency of the HS-SDME process

The HS-SDME theory indicates that an organic compound initially present in the aqueous phase is volatilized and then a dynamic equilibrium is established between the concentration of the compound in the headspace and that of the analyte in the organic solvent drop. The yield of the volatilization/extraction process was jointly evaluated using an aqueous solution containing 15 μg/L of each HNM (except TBNM, 30 μg/L) in 2.1 mol/L Na_2SO_4 at pH ~3.2. In this experiment, five consecutive extractions of the same sample were carried out with a fresh drop of 2.5 μL of 1-hexanol, and the percentage of analytes extracted was calculated. The relative extraction yield was calculated using a normalization method in which the sum of the analytical signals obtained in the five sequential extractions was assigned a value of 100%. From these results, about 35% and 25% of the HNMs were extracted in the first and second extraction, above which the relative extraction yield decreased slowly. The results of this study showed that the highest fraction of the 9 HNMs was obtained in the first extraction. On the basis of the above considerations, although there was a lot of carry-over, only one extraction step was recommended in order to increase the sensitivity of the method because two sequential extractions provided a higher quantity of residues at the expense of a lower signal.

The following study focused on the average yield of the HS-SDME method by comparing the traditional LLE processes in the 9 HNMs. First, 1 mL of mineral water containing 60 μg (except TBNM, 120 μg) of each HNM and 30 μg of fluorobenzene in 2.1 mol/L Na_2SO_4 at pH ~3.2 was extracted with 1 mL of 1-hexanol in quintuplicate; the extraction efficiency of the manual LLE was calculated through calibration curves constructed with standards prepared directly in 1-hexanol. The average efficiency of the manual extraction after 5 min of agitation was 75% (for TCNM, BDCNM, DBCNM and TBNM), 65% (for DCNM and BCNM) and 40% (for CNM, BNM and DBNM); the other fractions of analytes were extracted in subsequent extractions in the remaining aqueous phase. The results were compared to those obtained with 10 mL of an aqueous solution containing 150 ng of individual halonitromethanes (except TBNM, 300 ng) prepared in the above conditions in quintuplicate, using the HS-SDME method and 2.5 μL of 1-hexanol. In these conditions the theoretical concentration in both extracts (from LLE and HS-SDME methods) was similar (60 μg/mL for 8 HNMs and 120 μg/mL for TBNM). The extraction efficiency of the HS-SDME method related to the LLE one was ~20% (for TCNM, BDCNM, DBCNM and TBNM), ~10% (for DCNM and BCNM) and ~3% for (CNM, BNM and DBNM). The results obtained showed that both in the manual and in the microextraction techniques, trihalonitromethanes were the most favorably extracted compounds due to their higher solubility in 1-hexanol, taking into account their lower polarity. Therefore the pre-concentration factor of the method proposed ranged between ~120 and ~800 for monohalonitromethanes and trihalonitromethanes, respectively.

3.6. Quantitative calibration and reproducibility

Several analytical curves for standards in mineral water over the concentration range 0.2–300 μg/L of HNMs were obtained by plotting the analyte to the internal standard peak area against the analyte concentration. The 12-point calibration curve for each halonitromethane throughout the experimental concentration range showed good linearity with the correlation coefficients (r) of ≥0.991. The limits of detection were defined as the concentration of the analyte that provided a chromatographic peak area equal to three times the regression standard deviation ($S_{y/x}$) divided by the slope of the calibration graph [34], ranging from 0.06 μg/L for TCNM to 1.2 μg/L for TBNM. The reproducibility of the method proposed (analyzing 11 mineral water samples spiked with 5 μg/L of each HNM; 10 μg/L for TBNM) was good, with average RSD values of 8.2 ± 1.7% (within-day) and 9.3 ± 1.8% (between-day). As can be seen in Table 2, the HS-SDME method was very sensitive and allowed the determination of DCNM, TCNM, BCNM and BDCNM at ng/L levels; the brominated compounds and CNM were those that presented the least sensitivity. The high degree of sensitivity achieved for TCNM was noteworthy (chloropicrin) since it is the compound usually detected in drinking water.

3.7. Validation of HS-SDME with EPA method 551.1

A comparison was carried out between the proposed method and that of EPA 551.1 in order to validate the alternative proposal; in this case the best pre-concentration factor for the manual EPA alternative was used [ratio aqueous volume (50 mL)/organic volume (3 mL) = 17]. All quantitative parameters were determined as previously mentioned; for the reproducibility study, 11 mineral water samples spiked with 10 μg/L of each HNM (except TBNM, 30 μg/L) were analyzed and the results are listed in Table 2. The EPA method 551.1 employed in this study using GC–MS was not as sensitive as the LOD value reported (0.014 μg/L) only for TCNM using GC–ECD [13] due to the higher sensitivity achieved with ECD in halogenated compounds. In the framework of comparison, the EPA method 551.1 was slightly more precise than that of HS-SDME with average RSD values of 7.0 ± 1.5% (within-day) and 8.0 ± 1.5% (between-day), but the sensitivity as the slope of the calibration graphs was lower than that achieved by the HS-SDME method proposed (except for CNM, BNM and DBNM). As can be seen in Table 2, the HS-SDME method provided lower LODs (average LODs, 0.5 μg/L) than those obtained by the EPA 551.1 (average LODs, 1 μg/L) for five HNMs (DCNM, TCNM, BDCNM, DBCNM and TBNM); it is necessary to highlight that TCNM was the one generally found in drinking water. With respect to reproducibility, the EPA method 551.1 provided lower RSD values than HS-SDME; although this difference was negligible when the error introduced by the miniaturization of the LLE technique was taken into account.

In the same vein, the recoveries of both methods were also calculated using a tap water that was fortified by two different concentrations of each HNM in quintuplicate. HS-SDME method recoveries were calculated by spiking 2 and 10 μg/L for DCNM, TCNM, BCNM, BDCNM and DBCNM or 5 and 20 μg/L for the other compounds (CNM, BNM, DBNM and TBNM). In the EPA method, tap

Table 2
Analytical figures of merit for the determination of HNMs by HS-SDME and EPA 551.1 methods.

Compound	HS-SDME					EPA 551.1				
	LOD (μg/L)	Linear range (μg/L)	Recovery (%)[a]	RSD (%)		LOD (μg/L)	Linear range (μg/L)	Recovery (%)[a]	RSD (%)	
				Within-day	Between-day				Within-day	Between-day
CNM	0.9	3.0–300	90,92	8.5	9.4	0.3	1.0–300	90,96	6.6	7.8
DCNM	0.07	0.3–300	94,95	6.5	7.6	0.09	0.3–300	95,98	5.7	6.8
TCNM	0.06	0.2–300	95,98	6.2	7.1	0.1	0.4–300	94,99	5.9	6.8
BNM	0.9	3.0–300	91,96	8.6	9.5	0.3	1.0–300	89,97	6.8	8.0
BCNM	0.08	0.3–300	94,96	6.8	7.8	0.09	0.3–300	92,96	5.4	6.5
BDCNM	0.2	0.7–300	95,97	8.0	9.3	0.5	1.7–300	90,97	7.4	8.2
DBNM	0.9	3.0–300	91,93	8.6	9.8	0.2	0.7–300	94,95	6.6	7.7
DBCNM	0.3	1.0–300	94,97	8.8	9.8	1.0	3.3–300	93,98	8.4	9.2
TBNM	1.2	4.0–300	90,93	12.0	13.2	6.0	20–300	90,94	10.1	11.2

[a] The first and second data corresponds to the average percent recoveries for the low and high amount levels, respectively.

water was fortified with 2 and 10 μg/L (omitting BDCNM, DBCNM and TBNM which were spiked at 20 and 40 μg/L levels). All waters contained TCNM at detectable levels and, in this case, its concentration in the spiked samples was quantified and compared to those calculated as the sum of the native concentration in unspiked samples and spiked concentration. As can be listed in Table 2 in the HS-SDME method, all compounds were determined with average recoveries between 93 and 95% for the low and the high amount levels, respectively, whereas the recoveries of the EPA method ranged from 92 (at low levels) to 97% (at high levels). The good agreement between the two methods demonstrated the reliability of the proposed microextraction method.

3.8. Analysis of water samples

Recent studies examining the potential of HNM formation in drinking waters under different oxidation conditions showed that ozonation–chlorination produced the highest HNM yields, followed in the order of chlorination by ozonation–chloramination and chloramination [11]. In order to verify the effectiveness of the proposed HS-SDME method in the application of interest, 20 treated water samples (tap and swimming pool) were analyzed, including samples subjected to oxidative treatment with ozone in addition to chlorination. In the waters analyzed, only chloropicrin (TCNM) was found; the others were either not found or were beneath detection limits. Table 3 lists the TCNM concentrations found in water treated by chlorination (samples 1–12, and all swimming pool waters) or ozonation plus chlorination (samples 13–15). The results obtained were compared to those provided by the EPA method 551.1, also listed in Table 3. The two methods provided similar results, although TCNM remained undetected in some water samples using the EPA method 551.1, which corroborated the good performance of the proposed HS-SDME method. In practice, the concurrent oxidation process with ozone increased the TCNM concentration, which was in agreement with previous observations by several groups [5,9,11]. There were no significant differences between tap and swimming pool waters treated only by chlorination, although the concentration of residual chlorine and organic matter was higher in swimming pools than in tap waters; therefore, the ozonation step substantially increased the formation of TCNM. The concentration of TCNM found in tap waters ranged from <0.2 to 4.3 μg/L which was in agreement with Bougeard et al. [35] who reported TCNM concentrations from non-detected to 3.4 μg/L in chlorine drinking waters and those found in waste water treatment plant effluents (0.9–1.5 μg/L) [10].

Table 3
Analysis of water samples treated by chlorination (except to 13–15 tap waters, treated also by ozonation) by the proposed (HS-SDME) and the reference (EPA 551.1) methods ($n = 5$).

	Concentration of TCNM found ± standard deviation (μg/L)	
	HS-SDME	EPA 551.1
Tap 1	0.3 ± 0.1	0.4 ± 0.1
Tap 2	<0.2	n.d[a]
Tap 3	2.4 ± 0.2	3.0 ± 0.2
Tap 4	0.2 ± 0.1	<0.4
Tap 5	2.6 ± 0.2	3.3 ± 0.2
Tap 6	0.2 ± 0.1	<0.4
Tap 7	0.3 ± 0.1	<0.4
Tap 8	0.9 ± 0.1	0.8 ± 0.1
Tap 9	<0.2	n.d.
Tap 10	1.1 ± 0.1	0.8 ± 0.1
Tap 11	2.5 ± 0.2	3.0 ± 0.2
Tap12	0.3 ± 0.1	<0.4
Tap 13	4.0 ± 0.3	3.6 ± 0.2
Tap 14	4.3 ± 0.3	4.4 ± 0.3
Tap 15	3.9 ± 0.3	3.7 ± 0.3
Swimming pool 1	2.3 ± 0.2	1.6 ± 0.2
Swimming pool 2	0.4 ± 0.1	0.5 ± 0.1
Swimming pool 3	1.7 ± 0.2	1.3 ± 0.1
Swimming pool 4	1.2 ± 0.1	1.5 ± 0.2
Swimming pool 5	0.8 ± 0.1	0.6 ± 0.1

[a] n.d., not detected.

Table 4
Comparison of the HS-SDME–GC–MS method with other related methods for determination of chloropicrin (TCNM).

Method	LOD (μg/L)	Sample volume (mL)	Extractant volume (mL)	Linear range (μg/L)	RSD (%)	Reference
LLE–GC–ECD	0.014	50	3	0.1–15[a]	7.7	13 (EPA Method 551.1)
LLE–GC–MS	0.1	35	2	0.25–100[a]	3.5–18.1	17 (EPA Method 551.1 modified)
LLE–GC–ECD	0.04	35	2	0.25–100[a]	2.7–8.4	17 (EPA Method 551.1 modified)
HS-GC–MS	0.5	8	–	0.25–100[a]	–	17
HS-GC–ECD	0.4[b]–2.5	5	–	–	10	18
LLE–GC–MS	0.1	50	3	0.4–300	5.9	EPA Method 551.1, this work
HS-SDME–GC–MS	0.06	10	2.5×10^{-3}	0.2–300	6.2	This work

[a] Calibration range for a mixture of halogenated VOCs including chloropicrin.
[b] Data using splitless sample injection.

4. Conclusion

A comparison of the proposed method with other methods reported in the literature for the determination of chloropicrin (TCNM) in water samples by different techniques is given in Table 4. The proposed method was the most sensitive compared with LLE–GC–MS alternatives, omitting the LLE–GC–ECD methods due to the higher sensitivity achieved with ECD in halogenated compounds. All LLE methods require large volume of extractant; in that way, the proposed HS-SDME method offers advantages since all extract ($\sim$2 μL) was injected into the instrument without residues. Current research on halonitromethanes (HNMs) in water (viz. toxicity study, factors controlling their formation in water) uses methods optimized for the determination of halogenated VOCs, normally EPA method 551.1. However, these methods have some pitfalls when applied to unknown analytes like HNMs. In the absence of a comprehensive method, it has been necessary to develop an alternative to take into account such important variables for the analyte as the sample pH, the type of extractant and salt, and GC–MS conditions, among others. For the first time a method has been developed to determine the whole array of 9 HNMs, taking into consideration some very important current concerns like miniaturization and environmental aspects. Moreover, the method does not substantially increase sample processing time compared to reported EPA methods, but does provide higher sensitivity and similar reproducibility to the EPA method 551.1. Therefore, the proposed HS-SDME method may be of practical utility in both sample screening and analysis.

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