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Improving methodological aspects of the analysis of five regulated haloacetic acids in water samples by solid-phase extraction, ion-pair liquid chromatography and electrospray tandem mass spectrometry

M.C. Prieto-Blanco[a], M.F. Alpendurada[b,*], P. López-Mahía[a,c], S. Muniategui-Lorenzo[a], D. Prada-Rodríguez[a,c], S. Machado[b], C. Gonçalves[b,d]

[a] Departamento Química Analítica, Facultade de Ciencias, Universidade da Coruña, Campus da Zapateira, E-15071 A Coruña, Spain
[b] Instituto da Agua da Regiao Norte (IAREN), Rua Dr. Eduardo Torres, 229/4450-113 Matosinhos, Portugal
[c] Instituto de Medio Ambiente, Universidade da Coruña, Pazo de Lóngora, Liáns-Oleiros E-15179, Spain
[d] Laboratório de Hidrologia da Faculdade de Farmácia da Universidade do Porto, Rua Aníbal Cunha, 164/4050-047 Porto, Portugal

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ABSTRACT

Haloacetic acids (HAAs) are organic pollutants originated from the drinking water disinfection process, which ought to be controlled and minimized. In this work a method for monitoring haloacetic acids (HAAs) in water samples is proposed, which can be used in quality control laboratories using the techniques most frequently available. Among its main advantages we may highlight its automated character, including minimal steps of sample preparation, and above all, its improved selectivity and sensitivity in the analysis of real samples. Five haloacetic acids (HAA5) were analyzed using solid-phase extraction (SPE) combined with ion-pair liquid chromatography and tandem mass spectrometry. For the optimization of the chromatographic separation, two amines (triethylamine, TEA and dibutylamine, DBA) as ion pair reagents were compared, and a better selectivity and sensitivity was obtained using DBA, especially for monohaloacetic acids. SPE conditions were optimized using different polymeric adsorbents.

The electrospray source parameters were studied for maximum precursor ion accumulation, while the collision cell energy of the triple quadrupole mass spectrometer was adjusted for optimum fragmentation. Precursor ions detected were deprotonated, dimeric and decarboxylated ions. The major product ions formed were: ionized halogen atom (chloride and bromide) and decarboxylated ions. After enrichment of the HAAs in Lichrolut EN adsorbent, the limits of detection obtained by LC–MS/MS analysis (between 0.04 and 0.3 ng mL^{-1}) were comparable to those obtained by GC–MS after derivatization. Linearity with good correlation coefficients was obtained over two orders of magnitude irrespective of the compound. Adequate recoveries were achieved (60–102%), and the repeatability and intermediate precision were in the range of 2.4–6.6% and 3.8–14.8%, respectively. In order to demonstrate the usefulness of the method for routine HAAs monitoring, different types of water samples were analyzed. In swimming pool water samples the $\sum$HAAs were determined between 76 and 154 ng mL^{-1}.

1. Introduction

Haloacetic acids (HAAs) are an important group of by-products formed during the disinfection process of drinking water by chlorination. This treatment process is necessary for the removal of bacteria, viruses and pathogenic microorganisms present in water. In addition, the presence of free chlorine inhibits microbial growth in sedimentation basins, filters and the distribution system. More than 600 disinfection by-products belonging to 14 chemical families have been reported in the literature, some of them possessing carcinogenic, mutagenic and cytotoxic properties [1]. The formation of by-products (HAAs, trihalomethanes (THMs), haloacetonitriles (HANs), haloketones (HAKs) and other yet unidentified halogenated organics) is due to the reaction of dissolved organic matter (DOM) with free chlorine. The inorganic bromide present in ground and surface waters is also responsible for the formation of brominated by-products. Chlorine dosage, pH, contact time, temperature, bromide concentration, and type and concentration of DOM are the parameters controlled in the treatment process which have influence on HAAs formation [2,3].

The control and monitoring for HAAs is less extensive and stringent than for other by-products, such as THMs, which have an enforced maximum total limit of 100 μg L^{-1}. Nevertheless haloacetic acids are highly toxic to humans, plants and

* Corresponding author.
E-mail address: mfalpendurada@iaren.pt (M.F. Alpendurada).

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algae and some of them such as dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA) pose carcinogenic risks. TCAA used as a contact herbicide acts synergistically with chloroform, increasing the toxicity of the latter [4–6]. The US Environmental Protection Agency (EPA) has regulated a maximum contaminant level (MCL) of 60 $\mu g\,L^{-1}$ for the sum of five HAAs (HAA5), monochloroacetic acid (MCAA), DCAA and TCAA, and monobromoacetic acid (MBAA) and dibromoacetic acid (DBAA) [7,8]. A maximum contaminant level goal (MCLG) was also established for individual components: MBAA (70 $\mu g\,L^{-1}$), DCAA (zero), TCAA (20 $\mu g\,L^{-1}$). In addition, the World Health Organization (WHO) has established advisory levels of 50 $\mu g\,L^{-1}$ for DCA and 100 $\mu g\,L^{-1}$ for TCA [3].

Official analytical methods such as EPA methods 552.1, 552.2, 552.3 use gas chromatography (GC) with electron capture detection (ECD) [9–11] but mass spectrometry (MS) is also employed. GC methods require a treatment of derivatization, due to the acidic nature, high polarity and low volatility of HAAs. The most frequent derivatizing reagents are acidic methanol and diazomethane [12–15] used in non aqueous medium although dimethylsulfate provides good results [16]. Long analysis time and in some cases poor automation in sample preparation are disadvantages of these methods. In addition, reactions of thermal decomposition and hydrolysis of methylester derivatives of HAAs can take place in the GC injection port [14].

Numerous methods for analysis of HAAs have been reported based on ion chromatography (IC) [2,17–21]. The characteristics of these analytes (hydrophilic character and strong acidity) facilitate their separation by anion-exchange on hydroxide selective columns or even on new macrocycle-based column and gradient elution [17]. In general, detection limits by IC are usually higher than GC methods and inorganic anions can cause interference. Nevertheless, in EPA method 557 [22] similar detection limits to those reported in GC methods for the nine HAAs are achieved. In this method, chromatographic separation of haloacetic acids, bromate and dalapon is undertaken in 55 min by IC and using tandem mass spectrometry (MS/MS) for detection.

HAA analysis is also possible by high performance liquid chromatography (HPLC) using electrochemical detection [6] or UV detection at low wavelength. Ion-pair chromatography with quaternary ammonium compounds as ion-pairing reagents and UV detection has been also used [18]. The determination of HAAs by ion-pair liquid chromatography coupled to mass spectrometry (LC–MS) has been described [4,23]. Since non-volatile quaternary ammonium compounds can produce contamination of the interface, different amines such as triethylamine (TEA) [23], dibutylamine (DBA), tributylamine, and N,N-dimethylbutylamine (DBMA) [4] are employed. The use of buffers (ammonium acetate) in ultra-performance liquid chromatography (UPLC) and hydrophilic interaction liquid chromatography (HILIC) has also provided a good separation of ten HAAs [24]. Tandem mass spectrometry (MS/MS), used as detection mode, can improve HAA analysis since chemical noise could be reduced and several transitions could be monitored. Chen et al. analyzed HAAs in spiked tap water by UPLC–MS/MS but the detection limits were not comparable with GC. Also Meng et al. used UPLC–MS/MS, improving LODs of MCAA and MBAA. In both studies, only one transition was selected [24,25]. Solid-phase extraction (SPE) is generally the preconcentration method chosen when IC [17–20] and LC [2,19,26] are used. But in some studies a preconcentration step was not required [4,22]. Since coupling of IC with MS is not common in many laboratories, LC remains the technique of choice.

HAAs has been studied in the cycle of water disinfection and distribution: tap water [16,27,28], drinking water distribution system [28,29], water treated with different disinfection agents [30], treatment plants [26,29] and swimming pool water [16,23,31]. In addition, they have been found in several environmental matrixes (snow, groundwater, wastewater [27,28], lake water, precipitation [28] and seawater [32]).

Several improvements are still necessary in the analysis of HAAs. Fast and straightforward liquid chromatography methods for determination of HAAs compatible to MS which provide detection limits comparable with those obtained by GC and a good selectivity in real samples are necessary. Due to the low volatility of some amines, problems of contamination or ionization suppression have been reported by Loos and Barceló particularly with tributylamine [23]. TEA was considered by Takino et al. as unsuitable for the determination of MCAA, MBAA and DCAA [4]. Some troubles remain in the sample pretreatment, such as tedious steps of concentration and use of harmful chemicals which can be minimized by automation and use of simplified approaches. Identification problems, interferences and low sensitivity for MCAA observed in GC-ECD [33] can be improved using GC–MS. In LC methods, the low chromatographic retention and high detection limits can be solved using new stationary phases and mobile phase additives as well as a more selective detection.

This work proposes the use of ion-pair liquid chromatography–tandem mass spectrometry (LC–MS/MS) for the monitoring of HAA5. A comparison of DBA and TEA as ion-pair reagents using a column with a special phase (PAH Waters) is performed. In addition, the suitability of DBA as eluent for the HAAs preconcentrated on three SPE polymeric sorbents was examined. The applicability of the method was demonstrated in different matrixes of environmental and treated waters.

2. Experimental

2.1. Reagents

The ion-pair reagents TEA and DBA were obtained from Fluka (Buchs, Switzerland). Individual haloacetic acids, MCAA, DCAA and TCAA, were obtained from Riedel-de-Haën (Seelze, Germany) whereas MBAA and DBAA were obtained from Fluka (Buchs, Switzerland). The standard solutions were prepared in Milli-Q water at a concentration of 1 mg mL^{-1} and stored in the dark at 4 °C. Suitable diluted solutions were prepared using a mixture of 15 mM DBA in water and acetonitrile (95/5). Sulfuric acid was puriss. p.a. grade from Sigma–Aldrich (Seelze, Germany).

The solvents used as mobile phases were acetonitrile of HPLC grade purchased from Sigma Aldrich (Steinheim, Germany) and ultrapure water obtained from a Milli-Q system (Millipore, Molsheim, France).

2.2. Apparatus

The chromatographic analysis was performed on a Waters Alliance 2795 HPLC Separation Module equipped with a quaternary pump, automatic injector and thermostated column compartment connected to a Quattro Micro triple quadrupole mass spectrometer, equipped with a Z-spray electrospray ionization source (Waters Micromass, Manchester, UK). The mass spectrometry data handling was performed by the Mass Lynx software, version 4.0. Sample preconcentration was carried out in an off-line automated sample preparation system ASPEC XL from Gilson (Villiers-le-bel, France).

2.3. Collection and treatment of samples

The swimming pool water, river water and tap water samples were collected in Porto (Portugal) in August 2008 and stored in the refrigerator at 4 °C until analysis. Tap water and swimming pool water were pre-treated at the moment of collection with sodium thiosulfate (20 and 40 mg L^{-1}, respectively) for free chlorine removal.

Lichrolut EN (500 mg/6 mL, Merck) a crosslinked styrene-divinylbenzene polymer, Oasis HLB (200 mg/6 mL, Waters), a macroporous poly(divinylbenzene-co-N-vinylpirrolidone) copolymer and Isolute ENV+ (200 mg/6 mL), a styrene–divinylbenzene polymer were the cartridges tested for SPE extraction.

100 mL of water samples were acidified by addition of 4.5 mL of sulfuric acid, according to Barron and Paull [19]. The cartridges were conditioned prior to use with 5 mL of methanol and 5 mL of acetonitrile followed by 5 mL of 200 mM aqueous sulfuric acid. The samples were loaded onto the cartridges at 5 mL min^{-1} flow rate. Then, a washing step with 1 mL of Milli-Q water was performed. Extracted analytes were eluted in four steps with 2 mL of 15 mM DBA/acetonitrile mixture (95/5) at 2 mL min^{-1} flow rate. A concentration factor of 12.5 was achieved with this procedure.

2.4. Liquid chromatography–mass spectrometry

The separation was performed on a Waters (Milford, MA, USA) PAH column (250 mm × 3.0 mm, 5 μm) using a binary gradient. Acetonitrile and an aqueous eluent (5 mM dibutylamine adjusted at pH = 7 with acetic acid) were used. The final gradient is given in Table 1. The injection volume was 50 μL. A Polaris C_{18} 50 mm × 2.1 mm, 5 μm column was also tested.

Detection was carried out by negative electrospray ionization under the following source-dependent conditions: capillary – 4 KV, cone – 10 V and extractor – 3.0 V. The source temperature was 150 °C and the desolvation gas temperature was 400 °C. Gas flow rates were 675 L h^{-1} for the desolvation gas, and 60 L h^{-1} for the cone gas. Cone voltage optimized for each precursor ion and collision energy used in multiple reaction monitoring (MRM) mode are reported in Table 2. The naturally occurring chlorine-37 and bromine-81 isotopes were considered in the formation of the precursor and product ions. For quantification two options are possible, selection of the most intense transition or the isotopic transitions. Other minor transitions can be used for confirmation.

Table 1
Optimized conditions of HPLC and SPE for the haloacetic acids.

HPLC	
Column	Waters PAH column (250 mm × 3.0 mm, 5 μm)
Mobile phase	(A) 5mM dibutylamine (DBA), pH = 7 in 100% water and (B) acetonitrile
Gradient program	90–70% A in 5 min at 0.3 mL min^{-1}, 70–50% A and 0.3–0.2 mL min^{-1} until 10 min, 50–90% A and 0.2–0.3 mL min^{-1} until 15 min
SPE	
Cartridge	Lichrolut EN (500 mg/6 mL, Merck)
Eluent	15 mM DBA/acetonitrile mixture (95/5)
Conditioning	5 mL methanol, 5 mL acetonitrile, 5 mL of 200 mM sulfuric acid
Loading	100 mL acidified at 5 mL min^{-1} flow-rate
Washing	1 mL of Milli-Q water
Elution	In 4 steps with 2 mL at 2 mL min^{-1} flow-rate

3. Results and discussion

3.1. Optimization of MS–MS conditions

The electrospray source-dependent parameters (cone voltage, source and desolvation temperature, capillary voltage, extractor voltage and RF lens) were studied for maximum precursor ion accumulation. Desolvation temperature of 400 °C was chosen in order to obtain a more stable signal for bromoacetic acids although a temperature of 300 °C was sufficient for chloroacetic acids. This effect is more remarkable for DBAA than MBAA.

Three types of precursor ions (deprotonated $[M-H]^-$, dimeric $[2M-H]^-$ and decarboxylated $[M-COOH]^-$ ions) were detected. Differences between the five compounds were found to be a function of the degree of substitution and the nature of the halogen (chlorine or bromine). Deprotonated ions and dimeric ions were detected for all HAAs except for TCAA. Moreover [M−H]s produce a more intense signal than dimeric ions for all compounds. Decarboxylated ion $[M-COOH]^-$, formed by loss of carbon dioxide, is

Table 2
Optimized cone voltage for each precursor ion and collision energies used in selected MRM transitions for the haloacetic acids.

Compound	Precursor ion (*m/z*)		Product ion (*m/z*)		Cone voltage (v)	Collision energy (eV)
MCAA						
$[M-H]^-$	^{35}Cl	**93**	**$[X]^-$**	**35**	**20**	**5**
	^{37}Cl	95		37		
$[2M-H]^-$	$^{35}Cl, ^{35}Cl$	187	$[M-H]^-$	93	10	3
	$^{37}Cl, ^{37}Cl$	191		95		
MBAA						
$[M-H]^-$	^{79}Br	**137**	**$[X]^-$**	**79**	**20**	**3**
	^{81}Br	139		81		
$[2M-H]^-$	$^{79}Br, ^{79}Br$	275	$[M-H]^-$	137	10	3
	$^{79}Br, ^{81}Br$	277		137		
	$^{79}Br, ^{81}Br$	277		139		
	$^{81}Br, ^{81}Br$	279		139		
DCAA						
$[M-H]^-$	$^{35}Cl, ^{35}Cl$	**127**	**$[M-COOH]^-$**	**83**	**20**	**3**
	$^{35}Cl, ^{37}Cl$	129		85		
	$^{37}Cl, ^{37}Cl$	131		87		
DBAA						
$[M-H]^-$	$^{79}Br, ^{79}Br$	215	$[M-COOH]^-$	171	20	3
	$^{79}Br, ^{81}Br$	217		173		
	$^{81}Br, ^{81}Br$	219		175		
$[M-COOH]^-$	$^{79}Br, ^{81}Br$	173	**$[X]^-$**	79	**40**	**10**
	$^{79}Br, ^{81}Br$	173		81		
	$^{79}Br, ^{79}Br$	171		79		
	$^{81}Br, ^{81}Br$	175		81		
TCAA						
$[M-COOH]^-$	$^{35}Cl, ^{35}Cl, ^{35}Cl$	117	**$[X]^-$**	35	**20**	**5**
	$^{35}Cl, ^{35}Cl, ^{37}Cl$	119		35		
	$^{35}Cl, ^{35}Cl, ^{37}Cl$	119		37		
	$^{35}Cl, ^{37}Cl, ^{37}Cl$	121		35		
	$^{35}Cl, ^{37}Cl, ^{37}Cl$	121		37		

The most intense MRM transitions are given in bold.

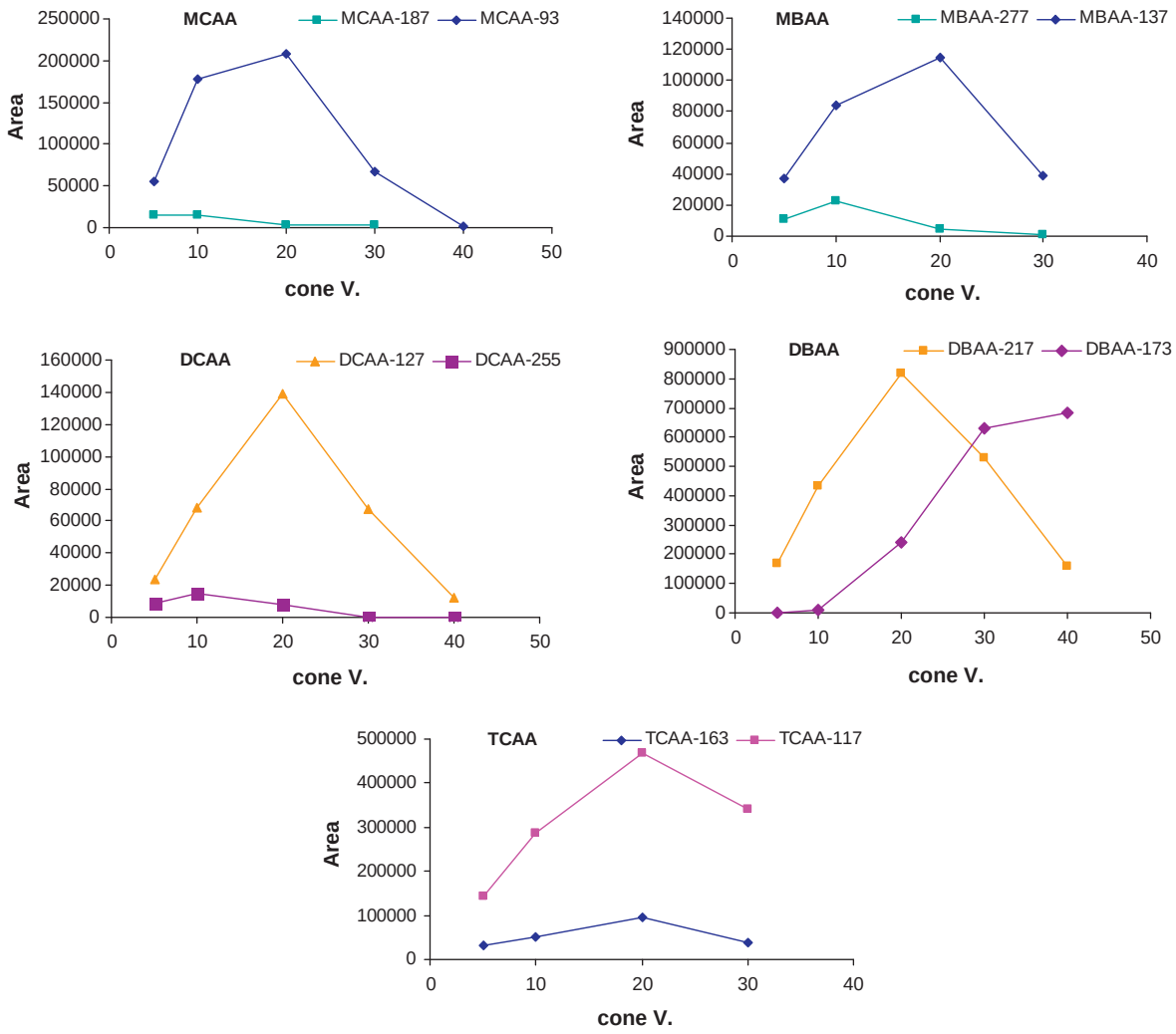

Fig. 1. Cone voltage effect on the formation of precursor ions of HAA5.

typical of compounds containing the carboxylic acid moiety. Only the HAAs with greater degree of substitution and more electronegative halogen, as DBAA and TCAA showed this type of precursor ion. For TCAA, $[M-H]^-$ was of lower intensity than $[M-COOH]^-$ being the latter the most intense precursor ion. Additionally, the dimeric ion of TCAA was not found, and the dimeric ion DBAA showed a lower signal than $[M-H]^-$ and $[M-COOH]^-$ ions. The formation of dimeric ion, possibly due to a hydrogen bond, is not favored in compounds with a high degree of substitution, such as trihalogenated acids, but this characteristic favors the loss of carbon dioxide and the formation in the source of $[M-COOH]^-$. In this case, the comparison with other authors' works [4,23,24] showed that, the relative intensities are more dependent on the operating conditions such as the type of mobile phase additives [34] than of the design of the ESI probes (orthogonal or Z-spray-ESI).

The naturally occurring chlorine-37 and bromine-81 isotopes are considered in the formation of the precursor ion, as shown in Table 2. The combinations of the two isotopes for each halogen (^{35}Cl, ^{37}Cl and ^{79}Br, ^{81}Br) were detected in the formation of the three types of ions for di- and trihalogenated acids, and in dimeric ions for monohalogenated acids. The cone voltage for each precursor ion was optimized in order to achieve the highest sensitivity (Fig. 1). For the HAA5, the most intense precursor ions were obtained for values of 20 and 10 V for deprotonated ions and dimeric ions, respectively. For $[M-COOH]$s of TCAA and DBAA, the cone voltages which provide the best sensitivity were 20 and 40 V, respectively.

The major product ions formed were: ionized halogen atom (chlorine and bromine) and $[M-COOH]$s. The formation of ionized halogen ion as product ion is reported in the literature for chlorinated compounds [35]. For each of the HAA5, the effect of collision energies on the selected transitions was studied (Table 2). Despite the fact that collision energies up to 25 eV were tested, maximum values of intensities of product ions were obtained at low collision energies (<10 eV).

For each precursor ion, several selected transitions were tested. The most intense are shown in Table 2. It is necessary to optimize more than just one transition, in order to avoid potential interferences, which may co-elute and additionally present the same MRM transition [36]. For instance, bromate and dichloroacetic acid have the same ion with m/z 127. Thus, other transitions of lower intensity were considered. Another alternative to ensure the correct identification of the analyte and to avoid false-positive values is monitoring the most intense isotopic transitions. Product ions with different combinations of the two isotopes were also observed. For TCAA, five isotopic transitions were found (Table 2).

3.2. Separation of five haloacetic acids

TEA and DBA as ion-pair reagents were tested in order to increase the HAAs' retention by the formation of the neutral species

with the HAAs. In preliminary studies, a C_{18} column with short dimensions (50 mm × 2.1 mm) was tested using a concentration of amine and pH (5 mM TEA, pH = 7) similar to those used by other authors [4,23] but a low retention of HAAs on the column was observed. Subsequently, the retention was increased using TEA in combination with a longer column and greater inner diameter and a special proprietary stationary phase Waters PAH. In SPE extraction, materials containing aromatic structures give good results in the retention of polar compounds such as HAAs. In reversed-phase liquid chromatography, columns used for the analysis of polycyclic aromatic hydrocarbons (PAHs) are silica-based usually functionalized (e.g. phenyl groups), so they promote π–π interactions with the solutes. Due to possible similarity between the two phases described, PAH column was tested.

The retention order is dependent on the degree of halogenation (mono-, di-, and tri-halogenated) and on the type of halogen (chloroacids elute before the bromoacids). The best separation between DCAA and monohalogenated acids is achieved using 5% acetonitrile. Nevertheless, the monohalogenated acids are only slightly affected by %acetonitrile and thus the chromatographic separation between MCAA and MBAA was not achieved although they can be separated by mass spectrometry specific transitions. Besides, two peaks are detected for each one of the monohalogenated acids, the first around 6 min and the second around 8 min (in Fig. 2A), possibly due to insufficient formation of ion-pair with TEA. This phenomenon is more evident if the sample is dissolved in water than in solution of TEA or DBA.

When dibutylamine was tested under the same conditions (Fig. 2B), the retention of all compounds was increased due to the longer alkyl chain of this amine. Monohalogenated acids can be separated between them and each one elutes in a single peak. Besides, an important increase of the peak area for all compounds with respect to the separation using triethylamine (8.6 for MCAA, 2.8 for MBAA, 1.5 for DCAA and TCAA, and 1.8 for DBAA) was observed. These results may be due to the different basicity of the amines, since a greater gas-phase affinity would increase the stabilization of negative ions. The percentage of acetonitrile has influence on all compounds, and the best separation conditions were obtained with 10% acetonitrile as starting gradient. If the first step of the elution gradient is enlarged to 10 min, an improvement of the selectivity of monohalogenated acids is accomplished. Finally, although initial conditions were tested at 0.2 mL min^{-1} flow-rate, using a gradient of flow rate from 0.3 to 0.2 mL min^{-1} results in a decrease in retention time for all compounds and, in consequence, a decrease in analysis time. The final optimized gradient is described in Table 1.

3.3. SPE optimization

HAA determination in water using LC–MS usually requires a pre-concentration step in order to achieve low limits of detection. A different strategy was adopted by Takino et al. [4] avoiding sample pre-concentration. These authors injected a large volume of sample and, in consequence, post-column addition of propan-2-ol was necessary to facilitate the HAA ionization in the detection step. In this work, we have chosen SPE because it is a technique readily available in environmental laboratories, which allows an automated extraction and it has been applied with good results for the pre-concentration of HAAs. Hydrophilic polymeric adsorbents and an eluent similar to the mobile phase (containing DBA) were tested. Various parameters, such as the adsorbent type; sample volume; volume, pH and additive concentration in the eluent; and steps of elution were studied.

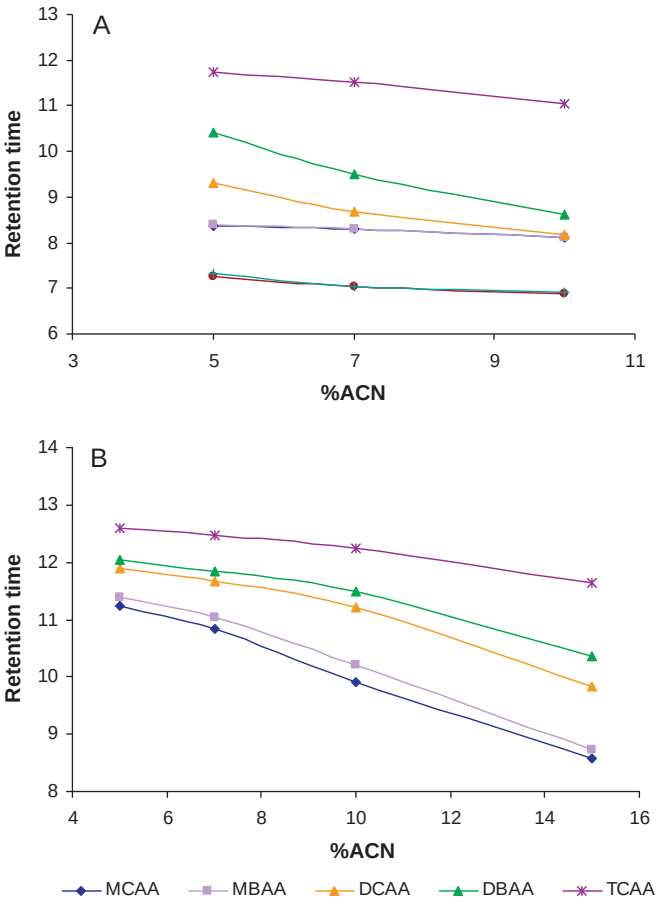

Fig. 2. Effect of %acetonitrile (%ACN) on retention of five haloacetic acids using TEA at 5 mM (A) and DBA at 5 mM (B). Gradient elution: initial %ACN to 50%ACN in 5 min and returning to initial conditions at 15 min. Flow rate 0.2 mL min^{-1}.

The extraction conditions at a sample concentration of 20 ng mL^{-1} were optimized by means of an automated SPE system which allows a strict control of flow rates and volumes. The concentration of the eluent was studied from 5 to 15 mM of DBA and the pH was adjusted to 7 and 11. An increase of pH and DBA concentration produced better recoveries for all compounds except for MCAA. Initially, only aqueous 15 mM DBA was used but latter the mixture with 5% acetonitrile has provided improvements in the

Table 3
Recoveries of five HAAs (20 ng mL^{-1} each one of five HAAs) from 100 mL Milli Q water using three commercial sorbents.

Compound	OASIS HLB		Isolute ENV+		Lichrolut EN	
	Recovery[a]	%RSD	Recovery[a]	%RSD	Recovery[a]	%RSD[b]
MCAA	27.3	3.0	44.6	1.8	76.2	9.7
MBAA	70.3	2.0	91.0	13.0	102.4	13.6
DCAA	76.1	11.9	46.5	2.8	60.1	8.5
DBAA	99.6	10.0	46.3	7.5	73.1	12.2
TCAA	90.6	15.9	58.1	2.2	87.9	3.1

[a] % average recovery of SPE cartridges.
[b] $n = 3$.

Table 4
Quality parameters of the method proposed: precursor ion, product ion, linear range and calibration curves, precision, limits of detection and quantification of HAA5.

Compound	Precursor ion (m/z)	Product ion (m/z)	Linear range ($\mu g\,mL^{-1}$)	Calibration curve	R^2	LOD[a] ($ng\,mL^{-1}$)	LOQ[a] ($ng\,mL^{-1}$)	Repeatability (%RSD) $n=2$		Reproducibility (%RSD) $n=5$	
								0.06 $\mu g\,mL^{-1}$	0.4 $\mu g\,mL^{-1}$	0.06 $\mu g\,mL^{-1}$	0.4 $\mu g\,mL^{-1}$
MCAA	93	35	0.01–1	$y = 1304.8x + 53.19$	0.9961			4.6	1.3	11.4	3.9[b]
MCAA	93 95	35 37	0.01–1	$y = 1833.9x + 56.07$	0.9988	0.30	0.99	5.3	6.6	13.9	6.3
MBAA	137	79	0.01–1	$y = 1680.3x + 28.12$	0.9986			3.7	1.9	3.7[b]	6.9
MBAA	137 139	79 81	0.01–1	$y = 3529.9x + 61.10$	0.9990	0.31	1.05	3.4	3.3	3.8	7.3
DCAA	127	83	0.01–0.6	$y = 3626.4x + 219.32$	0.9949			2.5	0.9	9.3	8.2
DCAA	127 129 131	83 85 87	0.01–0.6	$y = 4291.8x + 126.72$	0.9964	0.24	0.81	5.2	4.1	12.5	5.0
DBAA	173	79	0.01–0.6	$y = 6991.1x + 66.31$	0.9994			7.2	0.7	9.0	8.8
DBAA	173 173 171 175	79 81 79 81	0.01–0.6	$y = 38{,}275.0x + 255.58$	0.9996	0.04	0.12	2.9	2.4	6.5	9.4
TCAA	117	35	0.06–0.6	$y = 2079.0x + 123.96$	0.9965			6.5	3.4	7.7	14.6[b]
TCAA	117 119 119 121 121	35 35 37 35 37	0.06–0.6	$y = 3899.1x + 225.26$	0.9990	0.09	0.30	5.9	2.7	8.3	14.8

[a] LODs and LOQs after concentration, according to Ref. [37]. $LOD = 3 \times S_{(y/x)}/b$ and $LOQ = 3.3 \times LOD$.
[b] $n = 4$.

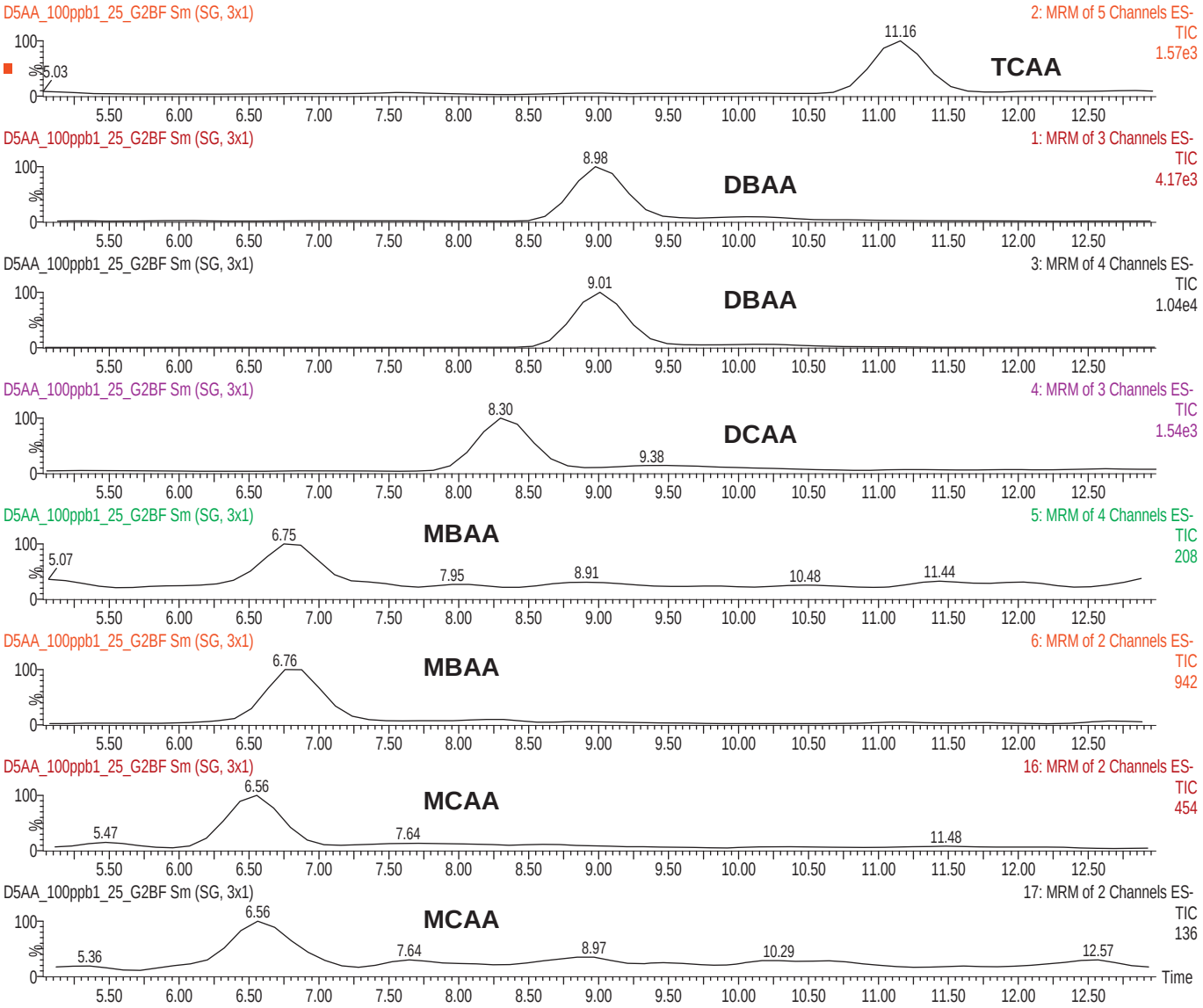

Fig. 3. LC–MS/MS chromatogram for standard solutions (individual HAA concentration of 100 ng mL^{-1}).

TCAA recovery. An eluent volume between 2 and 8 mL was tested. 8 mL proved to be the most suitable volume and the elution was more effective in four steps of 2 mL each rather than two steps of 4 mL, especially for TCAA (by a factor of 1.4). Then, sample volumes from 50 to 250 mL were tested; the optimal volume was 100 mL percolated at 5 mL min^{-1} flow-rate. Higher sample volumes cause a decrease of recoveries, especially of MCAA (from 76 to 28%) and MBAA (from 102 to 58%).

The recoveries and coefficients of variation obtained for the three adsorbents tested are shown in Table 3. Lichrolut EN was the most adequate adsorbent, with an average recovery rate of 80%. Isolute ENV, which was tested by other authors using MeOH as eluent [24], gave better results for monohalogenated acids than those reported by Loos and Barceló [23], nevertheless the average figure was 57.3%. Although the Lichrolut EN and Isolute ENV cartridges are both made of a styrene-divinylbenzene copolymer, the former adsorbent is highly crosslinked and the amount is 500 mg while the latter only contains 200 mg. Recoveries obtained with Oasis HLB cartridges containing 200 mg adsorbent gave an average value of 72.8%, higher than that obtained by Loos and Barceló; and Martínez et al. using cartridges of 60 mg, eluted with methanol [23,26]. However, recovery of MCAA was low and was influenced by sample volume (for 50 mL, MCAA recovery was 45.6%) as it was also reported by Martínez [26].

3.4. Analytical performance characteristics

Calibration equations were calculated for each compound at most intense transitions with correlation coefficient (r^2) greater than 0.995. In addition, isotopic transitions can be used not only for confirmation but also for the quantification. When the latter are used an increase of the slope was observed. Chloroacetic acids showed an increase lower than bromoacetic acids consistent with the greater chlorine isotope ratio ($^{35}Cl/^{37}Cl = 3$) than bromine ($^{79}Br/^{81}Br = 1$). The higher increase was observed for DBAA with a slope 5.5 times greater including the isotopic transitions.

Calibration was found to be linear in a range around two orders of magnitude and dependent of HAA type as can be seen in Table 4. Linear response between 0.01 and 1 μg mL^{-1} corresponding to 0.5–50 ng injected was obtained for monohalogenated acids. For di- and tri-halogenated acids the upper limit of calibration was 0.6 μg mL^{-1} corresponding to 30 ng injected, this decrease was also observed by Chen et al. [24] but more remarkable (0.05 μg mL^{-1} corresponding to 2.5 ng injected). Possibly, the ion-pairing amine

Table 5
Results of the analysis of water samples from different sources (ng mL^{-1}).

	TOC	MCAA	MBAA	DCAA	DBAA	TCAA	ΣHAA
Swimming pool water							
15104[a]	2.4	n.d.	n.d.	33.5	0.5	42.0	76.0
15109[b]	3.9	n.d.	n.d.	29.2	0.3	55.1	84.6
15106[b]	6.0	<LOQ	n.d.	43.5	0.5	76.3	120.3
15107[b]	6.2	<LOQ	n.d.	60.0	0.7	54.3	115.0
15108[b]	7.4	2.1	n.d.	54.7	0.7	29.3	86.8
15105[a]	6.5	2.7	n.d.	84.0	0.7	66.3	153.7
Tap water							
14543[b]	n.d.	n.d.	n.d.	n.d.	0.4	0.8	1.25
River water							
15160[b]	n.d.	n.d.	n.d.	n.d.	0.5	<LOQ	0.5

n.d. = not detected; <LOQ = response below limit of quantification, TOC = total organic carbon (mg L^{-1} C).
[a] $n = 4$.
[b] $n = 2$.

Table 6
Comparison of the values of HAA in swimming pool water provided by different authors and organizations and those obtained in this study.

	MCAA	MBAA	DCAA	DBAA	TCAA
Sarrión et al. [16]	4.2	n.d.	45.2	2.8	155
Loos and Barceló [23]	15–1000	n.d.	n.d.	n.d.	1000–1700
Martínez et al. [26]	24.7	7.1	68.8	15.2	42.1
WHO (2006)* [40]	2.6–81	<0.5–3.3	1.5–192	0.2–7.7	3.5–199
AFFSET (2010)* [40]	9.2–110	–	77–1000	<5–16.5	104–320
This study	n.d.–2.7	n.d.	29-84	0.3-0.7	29–76

Cited in [40].
AFFSET, 2010. Evaluation des risques sanitaires liés aux piscines Partie I: piscines réglementées: 244.
WHO, 2006. Guidelines for Safe Recreational Waters. vol. 2. Swimming Pools and Similar Recreational-water Environments. WHO, Geneve, p. 118.

used in the present work plays a relevant role in the ionization efficiency allowing a wider linear range.

The method provides an adequate precision, with an intra-day relative standard deviation (RSD, repeatability) ranging from 2.4 to 6.6% and an inter-day RSD (reproducibility) ranging from 3.8 to 14.8% at two concentration levels (Table 4).

The instrumental limits of detection (LOD) obtained based on Miller and Miller calculation method [37] were from 0.5 to 3.7 ng mL^{-1} and, after the treatment of preconcentration (samples concentrated for 12.5 times) LODs were from 0.04 to 0.3 ng mL^{-1} (Table 4). The limit of detection for MCAA without preconcentration was remarkably lower than that obtained by Chen et al. [24] using LC–MS/MS (3.7 ng mL^{-1} versus 71.5 ng mL^{-1}). Good LODs, after concentration, were achieved for DBAA and TCAA (0.04 and 0.09 ng mL^{-1}, respectively). In general, the LOD after concentration are comparable with those obtained by Sarrión et al. [16] using GC–MS with derivatization.

Several authors diverge in the occurrence of matrix effect in the analysis of this type of samples by LC–ESI–MS/MS [4,23–25,33]. Therefore, for reliable quantitation of real samples correction by recoveries, or preferentially use of isotopically labeled internal standards, is advised and will be further studied.

3.5. Application to water samples

The method was applied to several water samples: swimming pool water, river water and drinking water from the supply system. A control standard at 100 ng mL^{-1} was also analyzed (see Fig. 3). Natural water samples were analyzed without any pre-treatment while chlorine disinfected samples were added of sodium thiosulfate at the moment of sampling. Pepich et al. [38] investigated the formation of HAAs in samples stored at 6–10 °C containing high concentration of free chlorine and moderate total organic carbon (TOC) during 28 days. Their findings emphasize the need for chlorine removal since the concentration of DCAA, DBAA and TCAA increased 5.5, 2.5 and 3.4 times, respectively, after an elapsed time of 14 days. The HAAs concentrations found in the samples analyzed are given in Table 5. These results should be considered preliminary in the characterization of HAAs in such unusual matrixes as swimming pool water samples. Strict quality control procedures (such as: use of isotopically labeled surrogates, independent control standards and recovery criteria) would be needed to obtain definitive results, according to EN ISO/IEC 17025 accreditation requirements.

Swimming pool water samples are reported in the literature as potential sources of HAAs at levels of ng mL^{-1} [15,23] or even μg mL^{-1} [22]. In our work, a total HAA5 average concentration around 105 ng mL^{-1} was determined. The sum of 5 HAA is around 3 times lower than the values found by Sarrión et al. [16] in swimming pool water but exceeds the EPA limit of 60 ng mL^{-1} for drinking water. In river and tap water samples, out of the five HAAs analyzed only DBAA and TCAA were found and their concentrations were lower than 2 ng mL^{-1}. Dihaloacetic acid and TCAA were found in all samples, whereas MBAA was not detected in any sample analyzed and MCAA represents only 2.1% of total HAA quantified. These results are in general agreement with those reported by Sarrión et al. [16]. Nevertheless, these authors found that TCAA was the greatest fraction of the total HAAs, while in the present work, TCAA and DCAA are the major species. For comparison purposes, Table 6 indicates the concentration ranges of HAAs found by other authors and the guidelines enforced by AFFSET and WHO regarding the safety of recreational waters.

Additionally, two trihalomethanes (chloroform and dichlorobromomethane) were determined in the swimming pool water samples by SPME–GC–MS according to the procedure published by Guimarães et al. [39]. The concentration interval of chloroform ranged from 42 to 15.6 ng mL^{-1} while for dichlorobromomethane was from 0.9 to 1.9 ng mL^{-1}. A positive correlation ($r = 0.5$) was

found between TCAA and chloroform. Dichlorobromomethane showed a good correlation with DCAA and DBAA ($r = 0.68$ and 0.77 respectively). DCAA and DBAA showed a good correlation ($r = 0.8$) between them.

4. Conclusions

A straightforward method for the monitoring of HAA5 using available instrumentation in environmental laboratories is proposed. The method showed the following improvements:

- It affords high selectivity and does not require derivatization as in the GC methods; consequently, the work load and the time of analysis are considerably reduced and analytical errors are minimized.
- The detection limits obtained for MCAA, MBAA, DBAA and TCAA are better than those achieved by other LC–MS methods and comparable with GC–MS methods or EPA method 557, which is of primary importance considering the low concentrations found in water samples.
- SPE using an hydrophilic polymeric adsorbent Lichrolut EN and an eluent containing high percentage of DBA provided higher recoveries (60–102%) than other methods which use only organic solvent as eluent. A step of evaporation was not necessary.
- Although other studies have employed tandem mass spectrometry for HAAs analysis, in this method isotopic and minor MRM transitions were used to improve quantification and confirmation providing a better quality in the analytical results.
- A comparative study with other LC–MS methods highlighted the important role that the mobile phase plays on relative intensities of the three types of precursor ions (deprotonated, decarboxylated and dimeric ions) with respect to the instrumental design.
- The application of the proposed method to swimming pool water samples revealed that the concentration level of HAA5 is superior to the regulated maximum concentration by EPA (60 $\mu g\,L^{-1}$). Preventive measures such as a strict control of chlorination parameters or alternative treatments are necessary. Also, the study of different kinds of disinfection by-products in treated waters would be useful to understand their quantitative and qualitative formation during the course of water treatment.

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