Article

Use of Direct Aqueous Injection and Solid Phase Extraction Coupled with Hydrophilic Interaction Chromatography to Analyze Haloacetic Acids in Drinking Water Samples

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In this study, two analytical methods were evaluated to determine haloacetic acids (HAAs) in drinking water samples. Direct aqueous injection (DAI) and solid phase extraction (SPE) were evaluated and determination was performed by liquid chromatography tandem mass spectrometry (LC-MS/MS) with a hydrophilic interaction chromatography (HILIC) analytical column. Limits of quantification (LOQ) were between 10 and 500 µg L^{-1} by DAI and, considering a 125-fold pre-concentration step, between 0.08 and 2.0 µg L^{-1} by SPE. Five HAAs exhibited good linear correlation coefficients, accuracy (70-120%) and precision (≤ 20%) using DAI, while accuracy (50-120%) and precision (≤ 20%) were reached for SPE, with the exception of monobromoacetic acid (MBAA), which showed accuracy < 50%. DAI showed to be a simple, fast and promising technique that reduces operators' exposure and may replace methods that require a derivatization process, reaching LOQs below those established by the regulations for most analytes. SPE using polymeric cartridges and 2 mL of acetonitrile as elution solvent showed to be an interesting alternative for samples with low levels of HAAs. After evaluating the techniques, DAI was successfully employed to determine HAAs in drinking water samples and DCAA was detected in samples in concentrations between 15.3 and 33.6 µg L^{-1} and DBAA in concentration below 10 µg L^{-1}.

Keywords: haloacetic acids, method validation, direct aqueous injection, solid phase extraction, HILIC

Introduction

Disinfection by-products (DBPs) are considered harmful compounds for humans[1] since some of their potential health risks are bladder cancer and reproductive effects during pregnancy.[2] One of the most common groups of DBPs which is easily detected in water samples is composed of haloacetic acids (HAAs).[3] They are non-volatile compounds formed by replacing hydrogen atoms in acetic acid with halogens, such as chlorine, bromine and iodine.[3] There are nine brominated and/or chlorinated HAAs: monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA),

monobromoacetic acid (MBAA), dibromoacetic acid (DBAA), tribromoacetic acid (TBAA), bromochloroacetic acid (BCAA), bromodichloroacetic acid (BDCAA) and chlorodibromoacetic acid (CDBAA).[3]

Formation of HAAs is influenced by some factors, such as natural organic matter, water pH, contact time with disinfecting agents, temperature and anthropogenic organic contaminants.[4,5] Their influence on the formation of this type of compound depends on the environment in which they develop. Some studies explain the existence of HAAs in several environments, such as disinfected water as the main environment,[6-8] hospital wastewater,[9] seawater swimming pools,[10] and swimming pool water.[11]

HAAs are considered important DBPs because of their occurrence and toxicity.[12,13] They have attracted considerable attention regarding their occurrence, monitoring,

concentrations and toxicological effects in animals and humans in the last decades. HAAs are polar compounds, with low molecular weight, high solubility in water and strong hydrophilic characteristics, which make them a group of organic compounds of great analytical challenges in terms of sample preparation and quantification techniques.

The United States Environmental Protection Agency (USEPA) has established 60 µg L^{-1} as the maximum limit of contamination (MLC) of five HAAs (MCAA, DCAA, TCAA, MBAA and DBAA) in drinking water samples.[14] Different methods have been used to determine them in water samples. Most of them are based on liquid-liquid extraction (LLE)[15,16] and gas chromatography (GC). One of the official methods established by the USEPA is based on LLE and determination by GC with an electron capture detector (ECD).[15] However, these methods require long extraction steps and derivatization, because compounds are found in their dissociated form as haloacetate ions and need to be converted into volatile species which are susceptible to GC analysis and have good thermal stability and sensitivity to be detected.[16,17]

To overcome limitations, different studies have focused on the use of other chromatographic techniques and on the development of appropriate extraction and pre-concentration techniques, such as solid phase extraction (SPE).[18] SPE is a conventional sample preparation technique which has been used for extracting HAAs with the advantages of providing a high enrichment factor and allowing determination of analytes at trace level by chromatographic methods.[19] In addition, SPE improves selectivity, specificity and reproducibility, since its operation is easy and it uses less volume of organic solvents than LLE.[20,21]

When direct aqueous injection (DAI) is used in combination with liquid chromatography (LC), neither derivatization of analytes in aqueous samples nor sample manipulation is needed, facts that mitigate the risk of sample loss and contamination during preparation steps. Recent studies[3,22-24] have focused on determination of HAAs by LC.

Kinani *et al.*[25] described and summarized different methods used for determining HAAs in water samples. Most of them focus on the use of GC due to much difficulty found in determination of HAAs by LC. One of the limitations of LC in the reverse phase mode is its low retention of polar or ionic molecules. The use of LC in the normal phase mode, whose stationary phases are more polar than mobile ones, can solve this limitation; however, solubility of polar molecules in nonpolar mobile phases is limited and restricts its applicability.[26]

Therefore, hydrophilic interaction chromatography (HILIC) arises as an interesting separation modality. HILIC is quite similar to normal phase LC; it uses an analytical column with a hydrophilic stationary phase, but also an eluent containing water or buffer and high concentration of organic solvent miscible with water, such as in the reversed phase.[27] In this case, the order of elution is directly proportional to the solute polarity and inversely to the mobile phase polarity, so the non-polar analytes will be weakly retained on the column than polar ones.[27]

This study reports two possibilities, using DAI or SPE, both coupled with liquid chromatography tandem mass spectrometry (LC-MS/MS) to analyze six brominated and chlorinated HAAs in drinking water samples. The study was carried out based on the principles of green analytical chemistry. The proposed method quantifies target analytes in 10 min, decreases analysis time (approximately 40 min) and does not require any derivatization process by comparison with USEPA Method 557[28] and other recent studies.[24,29] Besides, these methods do not require the use of ion-paring reagents which are used in other LC-MS/MS methods. The proposed method was used for determining HAAs in drinking water samples collected in Rio Grande do Sul (RS) state, Brazil. This study is considered a pioneer in the development of methods for the analysis of HAAs in South Brazil.

Experimental

Chemicals and reagents

Stock solution of HAAs mix, MCAA, DCAA, TCAA, MBAA, DBAA, TBAA, BCAA, DBCAA, DBCAA 1000 mg L^{-1} in methyl-*tert*-butyl ether (MTBE), was purchased from Sigma-Aldrich (São Paulo, SP, Brazil). High performance liquid chromatography (HPLC) grade acetonitrile (MeCN) and methanol (MeOH) were purchased from J. T. Baker (Mallinckrodt, NJ, USA) and all the other reagents were of analytical grade. Ultrapure water (18.2 MΩ cm^{-1}) was prepared by a Milli-Q Purification System Direct-Q UV3® Millipore (Bedford, MA, USA).

Stock standard solutions (40, 10, 1 and 0.5 mg L^{-1} in MeCN) of HAAs mix were prepared in the laboratory. All stock solutions were stored in the dark at 4 °C.

Sample preparation

Tap water samples were collected in the laboratory to validate the methods. A blank of these samples, without the addition of any HAAs standard solution, was analyzed to verify the presence of some analytes and eliminate false positives during extraction and instrument process.

Direct aqueous injection

Tap water samples were acidified to pH 4.1 with pure formic acid 99% from Merck (Rio de Janeiro, RJ, Brazil) and directly injected into the instrument for analysis. In the analysis of HAAs, validation and analysis standard addition method calibration was used.

Solid phase extraction

Tap water samples acidified to pH 1.0 with pure sulfuric acid 96% from Merck (Rio de Janeiro, RJ, Brazil) were extracted by SPE.[19] Extraction conditions were standardized and validated. HAAs were extracted with the use of Strata™-X cartridges (200 mg, 3 mL) from Phenomenex (Torrance, CA, USA) in triplicate.

Cartridges were conditioned with 3 mL MeCN and 3 mL ultrapure water at pH 1.0. Afterwards, 250 mL sample (pH 1.0) was passed through the cartridge at a flow rate of 5 mL min^{-1}. After sample loading, cartridges were dried under vacuum for 10 min to remove water excess. Finally, HAAs were eluted with 2 mL MeCN. Extracts were analyzed by LC-MS/MS in the HILIC mode.

Liquid chromatographic parameters

Chromatographic separations were carried out by an Alliance Separations Module 2695 Liquid Chromatography from Waters (Milford, MA, USA) equipped with an autosampler, quaternary pump, column oven and degassing system.

Separation was performed on a HPLC Luna HILIC 3 μm (3.0 mm × 100 mm, 200 Å) column from Phenomenex (Torrance, CA, USA). The mobile phase was composed of MeCN (A) and buffer solution ammonium formate pH 4.1 from Merck (Rio de Janeiro, RJ, Brazil) (B) in the isocratic elution mode (92% A and 8% B). Flow rate was 0.2 mL min^{-1}, injection volume was 10 μL and total analysis time was 10 min.

Mass spectrometer parameters

Mass spectrometer (MS) was equipped with an electrospray (ESI) source (Micromass® Quatro Micro™ API from Waters (Milford, MA, USA)) operated in the negative mode. The data acquisition system was obtained by MassLynx and QuanLynx 4.0 software[30] programs from Waters (Manchester, UK). The Peak Scientifics nitrogen generator system from Instruments Ltda (Scotland, UK) was used. Parameters of the MS system source were set as follows: capillary voltage at 3 kV, ionization source temperature at 120 °C; desolvation temperature at 400 °C; flow desolvation gas at 500 L h^{-1}; and flow cone gas at 50 L h^{-1}. Selected reaction monitoring (SRM) was applied to the detection. Dwell time was set at 0.1 s.

For each compound, optimum cone voltages and collision energies, which aimed at getting two characteristic transitions with the best signal intensity, were selected. Table 1 shows the optimized SRM transitions with their respective retention times (t_R).

A type of precursor ion (deprotonated $[M - H]^-$) was detected. Besides, isotopes of chlorine 37 and bromine 81 of natural origin were considered in the formation of precursor and product ions. During quantification, two transitions could be evaluated for each analyte, except MCAA and DCAA.

Table 1. Acquisition data for the analysis of HAAs by LC-MS/MS in the ESI negative and SRM mode

Analyte	t_R / min	Precursor ion (*m/z*)		Fragment ion (*m/z*)	Type of transition	Cone voltage / V	Collision energy / eV
MCAA	4.25	92.6[a]	^{35}Cl	34.6	$[M - H]^- \rightarrow Cl^-$	7	13
DCAA	4.28	126.6[a]	^{35}Cl	82.6	$[M - H]^- \rightarrow COO^-$	25	11
		128.9	$^{35}Cl\ ^{37}Cl$	84.9	$[M - H]^- \rightarrow COO^-$	25	13
MBAA	6.79	136.7[a]	^{79}Br	78.7	$[M - H]^- \rightarrow Br^-$	17	11
		138.7	^{81}Br	80.7	$[M - H]^- \rightarrow Br^-$	17	19
DBAA	4.34	216.8[a]	$^{79}Br\ ^{81}Br$	172.8	$[M - H]^- \rightarrow COO^-$	20	11
		216.8	$^{79}Br\ ^{81}Br$	81.0	$[M - H]^- \rightarrow Br^-$	17	37
BCAA	4.33	172.7[a]	$^{79}Br\ ^{37}Cl$	128.7	$[M - H]^- \rightarrow COO^-$	25	11
		172.7	$^{79}Br\ ^{37}Cl$	78.7	$[M - H]^- \rightarrow Br^-$	25	21
DBCAA	4.25	256.0[a]	$^{81}Br\ ^{37}Cl$	126.0	$[M - COOH]^- \rightarrow Br^-$	11	7
		256.0	$^{81}Br\ ^{37}Cl$	81.0	$[M - H]^- \rightarrow Br^-$	11	27

[a]Transitions used for quantification. *m/z*: mass/charge ratio; eV: electron-volt unit; t_R: retention time; V: voltage; MCAA: monochloroacetic acid; DCAA: dichloroacetic acid; MBAA: monobromoacetic acid; DBAA: dibromoacetic acid; BCAA: bromochloroacetic acid; DBCAA: dichlorobromoacetic acid.

Method validation

Analytical method parameters were validated in agreement with performance criteria established by INMETRO[31] and SANTE guidelines.[32] Selectivity was evaluated by the absence of a signal at the retention time of the analytes, by analyzing control blank samples. Limits of detection (LODs) and quantification (LOQs) were defined with the signal-to-noise ratio (SNR) in the analytical procedure. LOQs were required to have less than 20% error among replicates and had 10 times the area of any blank interference. Trueness is the average recovery of each spike level under evaluation; it must be between 50 and 120%. It was evaluated by equation 1,

$$\text{Recovery (\%)} = \left(\frac{A_1 - A_2}{A_3} \right) \times 100 \qquad (1)$$

where A_1 represents the area of the analytes in the fortified samples, A_2 represents the area in samples without fortification and A_3 represents the area of the analytes corresponding to the concentration added to the samples. Precision is defined as repeatability of each spike level under evaluation and relative standard deviation (RSD) must be $\leq 20\%$.

The matrix effect (ME) was also studied by comparing slopes of calibration curves in the solvent and in the extract, as shown by equation 2,

$$\text{ME (\%)} = \left(\frac{a_1 - a_2}{a_2} \right) \times 100 \qquad (2)$$

where a_1 corresponds to the slope of curves prepared in the solvent and a_2 corresponds to slopes of curves prepared in the extract.

Method validation was performed by representative matrices of the planned sampling campaign, namely tap water. In the method that employed DAI, the slope of the calibration curve prepared with tap water samples acidified to pH 4.1 was compared to a slope of the curve prepared with ultrapure water at pH 4.1. In the method that used SPE, the slope of the calibration curve prepared with the resulting SPE extract of a tap water sample acidified to pH 1 was compared with the slope of a calibration curve prepared with pure MeCN.

Regarding validation and analysis, external calibration was used by DAI, while dilution standard addition calibration (DSAC) was used as a practical calibration strategy for analysis of HAAs[33] by SPE using a fortification of 16 μg L^{-1}.

Quality control

Coefficients of determination for calibration were required to have minimum values of 0.99 with a linear regression line weighted with inverse concentration (1/X).

Reagent and ultrapure water blanks were analyzed every working day to check false positives during the analysis. Both mass-to-charge ratio transitions (*m/z*) were also used to guarantee identification and confirmation of analytes.

Applicability

Five water samples were collected in three cities in Rio Grande do Sul (RS) in December 2020. Three samples were collected in drinking water treatment stations (DWTS) in Rio Grande, Camaquã and Santa Vitória do Palmar. In Rio Grande, two other samples were collected directly from taps in two different neighborhoods (Carreiros (RG2) and Cassino (RG3)), besides the sample collected in the DWTS (RG1).

One-L drinking water samples were collected in amber glass bottles and sodium thiosulfate at 10 mg L^{-1} was added to them to remove residual chlorine.[8,20,34] Samples were kept cold in the dark, transported to the laboratory and analyzed on the same day they were collected.

Statistical analysis

Results were statistically evaluated. Before the statistical analysis, all data were assessed for normality and homoscedasticity by the Shapiro-Wilk's and Levene's tests, respectively. Recoveries obtained with the use of different adsorbent material were compared by Student's *t* tests while the type and volume of elution solvent were compared by parametric statistics (analysis of variance (ANOVA), followed by the Tukey's test). Data showed normal and homoscedastic distributions. Otherwise, the Kruskal-Wallis non-parametric test was used. Statistica 13.0 software from StatSoft, Inc.,[35] at 5% significance level for all tests ($p < 0.05$), was used.

Results and Discussion

Source and desolvation temperature

Source and desolvation temperature were optimized. Peak areas were monitored for HAAs at MS source at 80, 100 and 120 °C while 300 °C was the desolvation temperature. All compounds showed optimal performance (the highest peak areas) at source temperature of 120 °C,

except MCAA and DBCAA. Previously published methods have also used source temperatures of 120 °C[36] and 110 °C[23,37] also.

Peak areas were also monitored for HAAs at different desolvation temperatures, i.e., 300, 400 and 500 °C; 120 °C was the source temperature. All compounds showed optimal performance at desolvation temperatures of 400 and 500 °C. However, more stable results were found at 400 °C. In addition, most previous studies evaluated the desolvation temperature below 500 °C, due to the characteristics of these molecules,[29,36] as shown in Table S1 in the Supplementary Information (SI) section. Thus, this study chose 400 °C as the desolvation temperature. Capillary voltages were investigated from 3 to 5 kv. Responses presented better results with 3 kV (data not shown).

Optimization of chromatographic conditions

All chromatographic conditions were optimized by the standard mixture solution at 1 mg L^{-1}. HILIC has been reported for separation of polar molecules.[26,38] In this study, Kinetex C8 (50 mm × 3.0 mm, 2.6 μm), Sielsc Obelisc N (150 mm × 4.6 mm, 5 μm) and HPLC Luna HILIC (100 × 3.0 mm, 3.0 μm) columns were compared, as shown in Table S2 in the SI section. Poor retention of HAAs on the Kinetex C8 column, besides problems in the chromatographic profile peaks and separation profile on the Obelisc N column, was found. Only the HPLC Luna HILIC column provided both satisfactory peak shape and better interaction between analytes and the column. HILIC has been reported for separation of polar molecules.[26,38]

The greater polarity of the stationary phase used in the Luna HILIC column provided a stronger retention of HAAs by the different mechanisms that involve HILIC. Despite the higher retention factor of this column, some compounds such as TCAA and DCBAA have not yet been detected and DBCAA showed lower intensity in ESI(−) fragmentation, even using a buffer as a mobile phase. However, although the analytes eluted almost at the same time, except for MBAA, the MS/MS spectrometer used in this study helps with this problem of separation of HAAs.

Different combinations of ultrapure water acidified with acetic acid 0.1%, formic acid 0.1%, buffer solution of ammonium formate (pH 3.0; 4.1; 6.3; and 8.0), MeCN and ultrapure water were tested as mobile phases in this experiment. MeCN and buffer solution (ammonium formate pH 4.1) were chosen as the mobile phase in the isocratic mode. Good separation and good sensitivity were achieved, except TCAA and DBCAA, which did not exhibit chromatographic responses in the instrumental conditions.

Individual TCAA stock solution (0.5 and 1 mg L^{-1}) was injected to verify the absence of TCAA response in the chromatographic system. During direct infusion of TCAA in the MS system, medium intensity with precursor (160.9 *m/z*) and product ions (116.9 *m/z*) was obtained. Different precursor (162.9 *m/z*) and product (118.9 *m/z*) ions were also evaluated, as shown in Table 1. However, no chromatographic peak was observed after the injection of TCAA into a mobile phase with different elution strength in the chromatographic system. Nevertheless, only electronic noise was obtained, as shown in Figure 1. Therefore, the best chromatographic condition found for separation and quantification of HAAs was MeCN and aqueous buffer mixture. MeCN and aqueous buffer mixtures are mostly used in the HILIC mode, mainly because a hydrophilic stationary phase and less polar mobile phase would allow polar analytes to be sufficiently retained and well separated.[38]
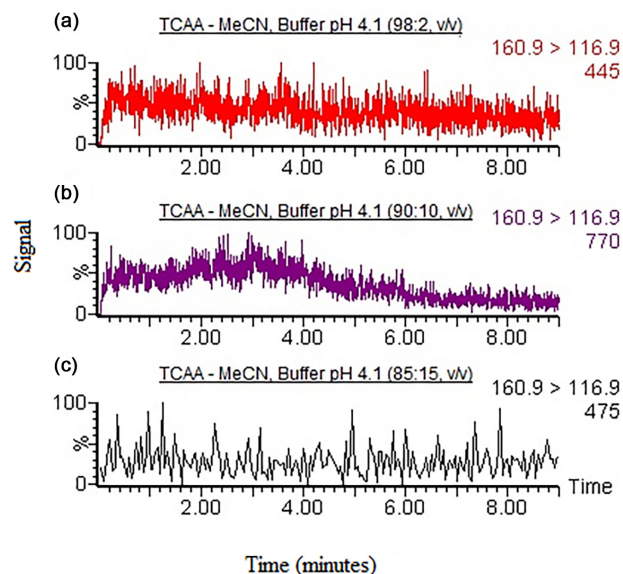

Figure 1. Chromatographic peaks of TCAA (1 mg L^{-1}) with the use of (a) weak mobile phase, (b) intermediate mobile phase and (c) strong mobile phase with Luna HILIC column.

Dwell time was also investigated in the range from 0.01 to 0.2 s by a stock solution of HAAs (1 mg L^{-1}). The best dwell time for integration and quantification of the chromatographic peak should have between 12 and 24 points *per* peak. This ideal condition took place at 0.1 and 0.2 s for the analytes, except TCAA and DCBAA. Thus, dwell time during SRM was 0.1 s in order to choose a suitable dwell time (Figure 2).

Direct aqueous injection optimization

During DAI optimization, tap water pH was investigated. A tap water sample collected without any pH adjustment
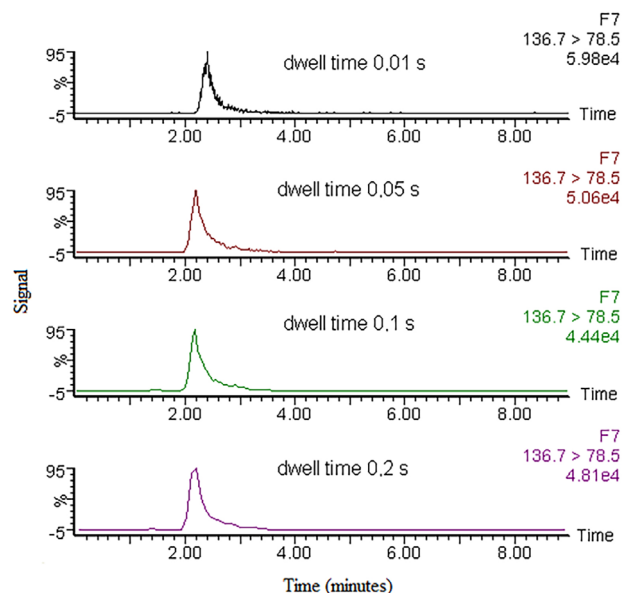
Figure 2. Chromatograms for MBAA at different dwell times under evaluation.

(pH was approximately 6.0) and a tap water sample adjusted to pH 4.1 (same pH of the mobile phase) were compared in terms of linear determination coefficient (R^2) and instrumental limits of quantification (LOQ_i), as shown in Table 2.

Determination coefficients above 0.99 were obtained for most compounds in both pH values under evaluation, as shown in Table 2. Besides, LOQi found for DCAA and MBAA in water samples at pH 4.1 were lower than those of samples without any pH adjustment. Thus, adjustment to pH 4.1 was chosen for the DAI analysis.

SPE optimization

SPE cartridges, type and volume of the elution solvent were tested. The tap water sample consisted of 250 mL (pH 1.0) while 3 mL MeCN and 3 mL

ultrapure water (pH 1.0) were used as the conditioning solvent.[20] Acidification of the sample is necessary since protonation of HAAs is mandatory to reach their successful preconcentration.[39]

SPE cartridges

Four types of SPE cartridges were tested by comparing recovery results: C18 SPE cartridge (500 mg, 6 mL) from Phenomenex (Torrance, CA, USA), Strata™-X (200 mg, 3 mL, 33 μm) from Phenomenex (Torrance, CA, USA), Oasis® HLB (500 mg, 6 mL) from Waters (Milford, MA, USA) and Cartridge Elut Plexa PAX (200 mg, 6 mL) from Agilent Technologies (California, CA, USA). C18 and Bond Elut Plexa (pH 1.0 and 4.0) cartridges showed low retention of HAAs, i.e., recoveries of compounds were < 50%. Strata™-X and Oasis HLB® cartridges showed recoveries between 50 and 120% of five and four analytes, respectively. Only MBAA showed significant differences ($p < 0.05$) in relation to analyte recovery when Strata™-X was used. In addition, recoveries of approximately 100% were found for DBAA and BCAA, as shown in Figure S1 in the SI section.

Due to difficulty in extraction and quantification of this type of molecules, some studies of the use of polymeric cartridges have been conducted. However, few used Strata™-X. Kinani *et al.*[40] have recently evaluated different cartridges, Oasis HLB, Bakerbond SDB, Strata SDB-L, LiChrolut EN, Bakerbond Carbon and Bakerbond C18, to extract HAAs. Recoveries between 44 and 92% were found with the use of the Strata SDB-L cartridge, except MCAA and MBAA, whose recoveries were 4 and 12%, respectively. Roumiguières *et al.*[41] determined a wide range of DBPs in aqueous samples by SPE and GC-MS. Best extraction results were reached by Bakerbond DDB-1 cartridges, followed by LiChrolut® EN and Strata® SDB-L while Oasis-HLB got lower recoveries.

Table 2. Linear range, determination coefficients (R^2), instrumental LOQs for the direct injection analysis

Analyte	Linear range / (μg L⁻¹)	Tap water (pH was approximately 6.0)		Tap water acidified (pH = 4.1)	
		R^2	LOQi / (μg L⁻¹)	R^2	LOQi / (μg L⁻¹)
MCAA	500-1000	0.98	500	1.00	500
DCAA	10-1000	0.98	50	0.99	10
MBAA	50-1000	1.00	100	0.99	50
DBAA	10-1000	1.00	10	1.00	10
BCAA	50-1000	1.00	50	0.99	50
DBCAA	500-1000	0.99	500	1.00	500

LOQi: instrumental quantification limit; R^2: determination coefficient; MCAA: monochloroacetic acid; DCAA: dichloroacetic acid; MBAA: monobromoacetic acid; DBAA: dibromoacetic acid; BCAA: bromochloroacetic acid; DBCAA: dichlorobromoacetic acid.

Type and volume of solvent elution

In this study, pure MeOH, MeOH 1% formic acid, pure MeCN, MeCN 1% formic acid, mixture of MeCN and MeOH (1% formic acid) and mobile phase (MeCN: buffer formate pH 4.1 (90:10, v/v)) were compared. MeCN and mobile phase mixture showed good recoveries of the same amounts of analytes (Figure S2 in the SI section). MCAA, MBAA and DBCAA showed statistically significant differences with the use of MeCN ($p > 0.05$), as seen in Figure S2 in the SI section. Additionally, resulting peak shapes with the use of MeCN were better than the mobile phase. Therefore, MeCN was chosen as the elution solvent for HAAs. MeCN is one of the aprotic solvents which is commonly used as a dipolar solvent. It is a medium polarity solvent that can break interactions between HAAs and the cartridge and reach the best extraction.[42] Although there are not many studies that evaluate pure MeCN as an elution solvent to extract HAAs by SPE, some involve MeCN as mobile phase or during cartridge conditioning. Prieto-Blanco *et al.*[20] used MeCN mixed with dibutylamine for the elution of five HAAs and reached good recovery results of MBAA, DCAA, DBAA and TCAA. In another study, MeOH was used for extracting DCAA and TCAA by SPE; extracts were evaporated and reconstituted with MeCN and ultrapure water.[37]

Volume of elution solvent is an important parameter in SPE since analytes are retained in the cartridges and are eluted with a small volume of solvent which is suitable for the analysis.[43] After sorbent drying and interference removal, interactions between analytes and material in SPE are interrupted by washing of small volumes of organic solvents, which leads to desorption of analytes from the solid phase.[44] Elution volume should be evaluated during SPE conditions, because larger elution volumes would produce a more diluted extract.[45]

The most suitable condition for HAAs extraction was found with 2.0 and 3.0 mL MeCN. However, 2.0 mL was chosen as the volume of solvent, because it did not show any significant differences for any analyte in terms of recovery ($p > 0.05$), as shown in Figure S3 in the SI section.

Validation of analytical method

In agreement with INMETRO[31] and SANTE guidelines,[32] calibration curves, linearity and linear range concentration were checked for DAI and SPE, as shown in Table 3. Calibration equations in the solvent and extracts of DAI and SPE found for each analyte, R^2, slope and more details are shown in Table S3 in the SI section. Five-to-ten-point calibration curves were constructed for standard solutions in a concentration range between 10 and 1000 µg L^{-1} by DAI, and between 0.08 and 8 µg L^{-1} by SPE, depending on the individual compound. Therefore, the regression model showed that the method is linear in the range of concentration under study, since it exhibited values of R^2 above 0.99 for analytical curves of HAAs, and MBAA showed $R^2 > 0.98$ in the solvent curve. Similar results were obtained by previous studies of DAI[9,46] and SPE.[22,37]

Concerning DAI, linear response between 10 and 1000 µg L^{-1} was obtained for di-halogenated acids DCAA and DBAA. In the case of MBAA and BCAA, it was from 50 to 1000 µg L^{-1} and for MCAA and DBCAA, it was from 500 to 1000 µg L^{-1}. Regarding SPE, linear response between 0.08 and 8.00 µg L^{-1} was found for DCAA and DBAA. For MBAA and BCAA, it was from 0.8 to 8.0 µg L^{-1} and for MCAA and DBCAA, it was between 2 and 8 µg L^{-1}.

Recoveries at the LOQ, middle and high concentration points of the calibration curve in tap water are shown in Table 4. Mean recoveries ranged from 70 and 120% in tap water matrices by DAI for all analytes, and 50 and 120%

Table 3. LODs, LOQs and linearity for both methods under evaluation, besides MCL by different regulatory

Analyte	DAI			SPE			EPA	Brazil	WHO
	Linearity / (µg L^{-1})	LOD$_m$ / (µg L^{-1})	LOQ$_m$ / (µg L^{-1})	Linearity / (µg L^{-1})	LOD$_m$ / (µg L^{-1})	LOQ$_m$ / (µg L^{-1})	MCL / (µg L^{-1})		
MCAA	500-1000	166.7	500	2.0-8.0	0.67	2.00	60[a]	80[a]	20
DCAA	10-1000	3.3	10	0.80-8.0	0.27	0.80			–
MBAA	50-1000	16.7	50	0.80-8.0	0.27	0.80			–
DBAA	10-1000	3.3	10	0.08-8.0	0.03	0.08			–
BCAA	50-1000	16.7	50	0.08-8.0	0.13	0.40	–	–	–
DBCAA	500-1000	166.7	500	2.0-8.0	0.67	2.00	–	–	–

[a]ΣHAAs$_5$: MCAA, DCAA, TCAA, MBAA and DBAA. DAI: direct aqueous inject; SPE: solid phase extraction; EPA: Environmental Protection Agency; WHO: World Health Organization; LOD$_m$: method limit of detection; LOQ$_m$: method limit of quantification; MCL: maximum concentration limit; MCAA: monochloroacetic acid; DCAA: dichloroacetic acid; MBAA: monobromoacetic acid; DBAA: dibromoacetic acid; BCAA: bromochloroacetic acid; DBCAA: dichlorobromoacetic acid.

Table 4. LOQs, average of recoveries and relative deviation standard (RSD) at LOQ, middle concentration (MC), and high concentration (HC) by DAI and SPE techniques (n = 3)

Analyte	Tap water						Tap water					
	DAI						SPE					
	LOQ / (μg L^{-1})	Recovery (RSD) / %	MC / (μg L^{-1})	Recovery (RSD) / %	HC / (μg L^{-1})	Recovery (RSD) / %	LOQ / (μg L^{-1})	Recovery (RSD) / %	MC / (μg L^{-1})	Recovery (RSD) / %	HC / (μg L^{-1})	Recovery (RSD) / %
MCAA	500	103 (5)	750	103 (5)	1000	105 (8)	2.00	105 (14)	4.00	105 (8)	8.00	104 (10)
DCAA	10	116 (16)	50	112 (10)	500	104 (3)	0.08	98 (13)	4.00	81 (6)	8.00	82 (11)
MBAA	50	116 (6)	500	90 (5)	1000	104 (5)	0.08	48 (18)	4.00	47 (12)	8.00	42 (8)
DBAA	10	127 (16)	50	107 (5)	500	101 (3)	0.08	118 (9)	0.40	78 (8)	8.00	74 (10)
BCAA	50	93 (9)	500	102 (4)	1000	100 (6)	0.50	94 (6)	2.00	76 (9)	4.00	53 (6)
DBCAA	500	99 (9)	750	101 (10)	1000	101 (11)	2.00	104 (13)	4.00	104 (6)	8.00	131 (10)

DAI: direct aqueous injection; SPE: solid phase extraction; LOQ: limit of quantification; MCAA: monochloroacetic acid; DCAA: dichloroacetic acid; MBAA: monobromoacetic acid; DBAA: dibromoacetic acid; BCAA: bromochloroacetic acid; DBCAA: dichlorobromoacetic acid.

by SPE for all spiked levels under investigation, except MBAA. In addition, relative standard deviations (RSDs) among replicates were below 20%; thus, mean recoveries were consistent. In a previous study[40] which aimed at improving recoveries reached by SPE, flow rate in the conditioning step was evaluated and the best recoveries were obtained for MBAA, a fact that may indicate that this analyte needs to be evaluated under other extraction conditions in order to reach higher recovery rates, due to its characteristic of pK_a (2.87) and low polarity.

Resulting LODs and LOQs (Table 3) were compared with MCL for HAAs.[14] Instrumental limits of detection (LOD$_i$) and LOQ$_i$ ranged from 3.33 to 83.33 and from 10 to 250 μg L^{-1}, respectively. Method limit of detection (LOD$_m$) and method limit of quantification (LOQ$_m$) varied between 3.33 and 166.67 and between 10 and 500 μg L^{-1}, respectively, by DAI. Concerning SPE, LODm ranged from 0.03 to 0.67 μg L^{-1}, while LOQm ranged from 0.08 to 2.00 μg L^{-1}. Resulting LOQs are in agreement with the maximum limit established by regulations, except MCAA when DAI was used, as shown in Table 4. Additionally, it should be mentioned that LOQs were much lower when SPE was used; thus, they are an alternative to be applied to samples with low levels of HAAs.

LODs found by SPE can be compared with those reported by Henson et al.[39] who used a fully-automated analyzer based on sequential injection analysis (SIA) with post-column reaction ion chromatography (IC), mainly for DBAA and BCAA where low LODs were obtained after preconcentration of DBAA and BCAA (0.03 and 0.13 μg L^{-1}, respectively). Resulting LOD by SPE for DBCAA (2 μg L^{-1}) was lower that the LOD obtained by Wang et al.[9] who used target and semi target screening with ultra high performance liquid chromatography (UHPLC) coupled with quadrupole orbitrap high resolution MS.

In general, LODs after preconcentration are comparable with those found by Prieto et al.[20] and Xue et al.[34] who used DAI and high performance ion chromatography tandem mass spectrometry (IC-MS/MS). They may also be compared to the ones reported by Postigo et al.[47] who used GC-negative chemical ionization (NCI) coupled with MS. However, a derivatization step is required.

Since the matrix effect was also considered an important factor in this analytical methodology,[48] it was evaluated in tap water. A matrix effect below 25% was observed as shown in Figure 3, for both DAI and SPE.
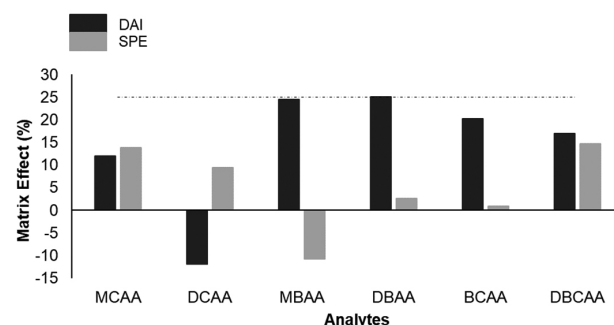
Figure 3. Matrix effect calculated for HAAs by DAI and SPE with LC-MS/MS in the HILIC mode.

Applicability

The proposed method was used for determining HAAs concentrations in drinking water samples from different DWTS in Camaquã, Rio Grande and Santa Vitória do Palmar, RS, Brazil. HAAs were determined by DAI as a sample preparation technique, mainly because good accuracy was observed during the method validation.

In addition, DAI seems to be a rapid and simple method with few preparation steps; thus, it avoids errors and long analytical time. Therefore, samples were injected into the

equipment after filtration and acidification. It was faster and consumed less reagent than SPE.

Detected HAAs species were DCAA in samples RG3 (33.6 μg L^{-1}) and RG1 (15.3 μg L^{-1}) and DBAA in RG3, at concentration below the LOQ of the method (< 10 μg L^{-1}). The other HAAs were not detected in drinking water samples under study. HAAs were mostly detected in drinking water samples collected from taps than in drinking water collected directly in DWTS. It seems that, along the distribution system, concentrations of HAAs tend to increase, a fact that is attributed to much contact time between the disinfecting agent and natural organic matter (NOM). Marcoux *et al.*[49] showed that, in some cases, 80% of DBPs measured in water samples had already been formed when water leaves the DWTS and enters the distribution system.[47]

Conclusions

Results show that the proposed method can meet requirements of HAAs determination in water samples considering their acidic and polar properties. It is an alternative to current methods that require a derivatization process, much time and more extraction steps to determine these analytes.

In the optimal extraction condition by SPE, the method reaches LOQ from 0.08 to 2 μg L^{-1}, as well as recoveries within the range recommended by the literature (50-120%), with values of RSD ≤ 20%. Regarding DAI, LOQs ranged between 10 and 500 μg L^{-1} and recoveries ranged from 70 to 120%. It should be highlighted that, even when higher limits were found when DAI was used, this method can be considered rapid and simple, with the only requirement of sample acidification to pH 4.1. Thus, it is a good alternative to perform fast evaluation of drinking water samples in laboratory routines.

Finally, this study shows that DAI and/or SPE in combination with determination by LC-MS/MS with a HILIC column are efficient tools to determine HAAs, since they are simple, fast and environmentally friendly for the evaluation of drinking water samples.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

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