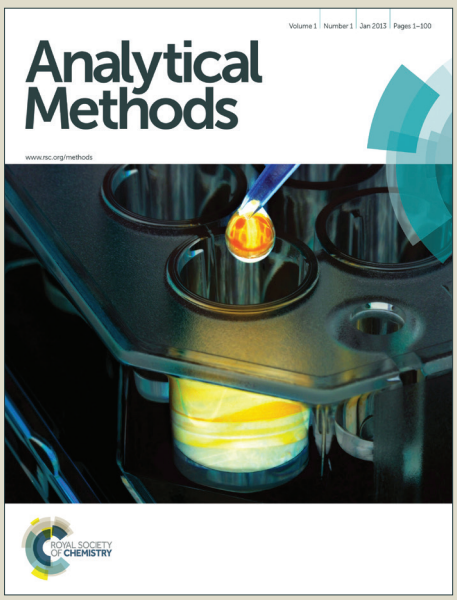
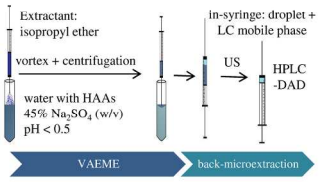
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods



Graphical Abstract
209x148mm (300 x 300 DPI)

Vortex-assisted emulsification microextraction followed by in-syringe ultrasound-assisted back-microextraction to determine haloacetic acids in waters

Priscilla Rocío-Bautista, Verónica Pino, Bárbara Delgado, Ana M. Afonso and Juan H. Ayala*

Departamento de Química Analítica, Nutrición y Bromatología, Universidad de La Laguna (ULL), La Laguna (Tenerife), 38206 Spain

Abstract

We have evaluated a vortex-assisted emulsification microextraction (VAEME) procedure followed by in-syringe ultrasound-assisted back-microextraction for the determination of nine haloacetic acids in waters of different nature, using high-performance liquid chromatography with diode array detection. The optimized method requires 600 µL of isopropyl ether as extractant solvent and 5 mL of the water sample containing: Na_2SO_4 (45%, w/v) and a low pH value (< 0.5). After emulsification assisted by vortex for 5 min, the droplet is separated from the water sample after centrifugation (5 min, 3500 rpm) using a syringe. This droplet is then back-microextracted in the syringe by mixing it with a low volume (50 µL) of an aqueous solution of $(NH_4)_2SO_4$ (0.2M), to ensure compatibility with the HPLC mobile phase. After 5 min of sonication, the aqueous solution containing HAAs is directly injected in the chromatograph. The method is characterized by (a) average relative recoveries of 77.7-89.0%, depending on the spiked level, (b) average enrichment factors of ~10 for the VAEME and of ~21 for the overall method, (c) precisions of the overall method (expressed as relative standard deviations) between 5 and 23%, and (d) average extraction efficiencies of ~88% for the VAEME method.

Keywords*:* Microextraction / Vortex-assisted emulsification / Haloacetic acids / Drinking Waters / Preconcentration / Back-microextraction

*Corresponding author: Tel. +34 922318044. Email: jayala@ull.es

1 Introduction

Chlorination is probably the most common worldwide treatment of waters intended for human consumption, because it efficiently removes pathogenic microorganisms like those responsible of malaria or typhus. However, the chlorine used in the process can react with the natural organic matter already present in waters, mainly humic and fulvic acids, originating disinfection byproducts (DBPs).[1] The extent of such formation is also dictated by the bromine content of waters as well as by the acidity.[1-3] Among disinfection byproducts, haloacetic acids (HAAs) and trihalomethanes (THMs) can be cited.

HAAs are highly polar compounds. Their structure is based in that of acetic acid molecule, but the alpha carbon adjacent to the carbonyl contains one, two or three halogens atoms (Br or Cl). There is a growing concern about the presence of HAAs in waters due to health issues.[4,5] Current in vivo studies with mice have proved toxic and carcinogen effect.[6] Indeed, the International Agency of Research on Cancer (IARC, http://monographs.iarc.fr/ENG/Classification/ClassificationsAlphaOrder.pdf) has classified four HAAs as possible human carcinogenic: dichloroacetic acid, trichloroacetic acid, dibromoacetic acid and bromochloroacetic acid; whereas the Environmental Protection Agency of United States (US-EPA, http://water.epa.gov/drink/contaminants/#List) classifies one HAA (trichloroacetic acid) as possible human carcinogen and another HAA (dichloroacetic acid) as probable human carcinogen.

In this sense, there is a need of analytical methods for the efficient determination of HAAs in waters. Current analytical methods take use of gas-chromatography (GC), high-performance liquid chromatography (HPLC), and ion-chromatography (IC), all in combination with a variety of detection systems. When GC is utilized, a derivatization step is needed to decrease the polarity and also to increase the volatility of HAAs, forming esters in the majority of cases.[7,8] Afterwards, the extraction is normally conducted using headspace (HS)[9] or headspace solid-phase microextraction (HS-SPME).[10,11] When HPLC is used, the most common strategy is to employ hydrophilic interaction liquid chromatography (HILIC),[12,13] generally in combination with a supported liquid membrane microextraction (SLMME) method.[14,15] When IC is used (with or without a suppressor column),[16] the extraction strategy normally involves solid-phase extraction (SPE).[17,18]

The utilization of microextraction procedures in sample preparation has been a hot topic in analytical chemistry during the past years,[19,20] because the elimination or minimization of the organic solvent consumption during the sample extraction step lies within the requirements of green analytical chemistry.[21,22] Among microextraction techniques, those based of liquid-phase microextraction (LPME) have been quite successful in its different performance modes.[23-26]

Despite this, to the best of our knowledge SLMME is the only LPME mode that has been used for the HPLC determination of HAAs.[14,15,27,28]

Thus, the main purpose of this work is the utilization of LPME to determine a group of 9 HAAs in different water samples. Specifically, it is intended the employment of the novel LPME mode vortex-assisted emulsification microextraction (VAEME),[29] which is based in the utilization of a low amount of extractant solvent, which is dispersed in the sample solution with the aid of vortex to assist the emulsification.[30-33] Furthermore, the method will be combined with an in-syringe ultrasound-assisted back-microextraction step to increase the sensitivity of the overall method and facilitate the compatibility with the HPLC mobile phase.

2 Materials and methods

2.1 Chemicals, reagents and materials

9 HAAs were used in the study (purity in %): chloroacetic acid (MCAA) (99%), bromoacetic acid (MBAA) (97%), bromochloroacetic acid (BCAA) (97%), dichloroacetic acid (DCAA) (≥99%), dibromoacetic acid (DBAA) (97%), trichloroacetic acid (TCAA) (≥ 99.5%), tribromoacetic acid (TBAA) (99%), chlorodibromoacetic acid (CDBAA) (94.6%), and bromodichloroacetic acid (BDCAA) (99.9%). DCAA, MCAA and TCAA were purchased from Sigma-Aldrich (Steinheim, Germany). BDCAA and CDBAA were supplied by Supelco (Bellefonte, PA, USA). The remaining HAAs were obtained from Aldrich (Steinheim, Germany). The EPA 552.3 acid calibration mix (with HAAs concentration of 200-2000 $\mu g \cdot L^{-1}$ in methyl *tert*-butyl ether) was purchased from Supelco.

All experiments were carried out using deionized water (Milli-Q ultrapure grade) obtained by a water purification system A10 MilliPore (Watford, UK). Acetonitrile (ACN) and acetone were of HPLC grade (Chromasolv®), from Sigma-Aldrich. Octanol, pentane, trichloromethane and methyl *tert*-butyl ether (MTBE) were of pro-analysis purity grade, and obtained from Sigma-Aldrich. Decanol was purchased from Aldrich. Isopropyl ether was supplied from Panreac (Barcelona, Spain), with pro-analysis purity grade. Hexane was obtained from Merck (Darmstadt, Germany).

Sodium sulfate and ammonium sulfate were obtained from Scharlau (Barcelona, Spain). Sulfuric acid (97%) was purchased from Sigma-Aldrich.

The standard solutions of HAAs were prepared in different solvents: $(NH_4)_2SO_4$ 0.2 $mol \cdot L^{-1}$, decanol, MTBE, octanol, or isopropyl ether, and stored at -18ºC.

Treated water was collected from a desalination plant in Santa Cruz de Tenerife. Swimming pool water was sampled in a local pool (La Orotava). Two tap waters were also taken (La

Orotava and La Laguna). Treated water and swimming pool water samples were kept at -18 ºC until analysis. All water samples were filtered using Chromafil® Xtra PET-45/25, purchased from Panreac.

2.2 Instruments

The HPLC used was a L-2130 HITACHI model purchased from Merck, with an analytical column C18 (5 μm, 150x4.6 mm) obtained from Varian (Palo Alto, USA), and a Rheodyne 7725i injection valve obtained from Supelco, with a loop of 20 μL. A diode array detector (DAD) Varian ProStart 330 was used, and the quantification wavelength was 210 nm. For the separation, the flow rate was linearly varied from 0.3 $mL \cdot min^{-1}$ to 1 $mL \cdot min^{-1}$ in 10 min, whereas the mobile phase, composed of ACN and $(NH_4)_2SO_4$ 0.2 $mol \cdot L^{-1}$, was also linearly varied from 0 to 10% (v/v) in ACN in 10 min, and then kept for 5 minutes.

A centrifuge model 5720 Eppendorf (Hamburg, Germany), a vortex from Reax-Control Heidolph GMBH (Schwabach, Germany), and an ultra sound bath KM (Shenzhen Codyson Electrical Co., Ltd. Shenzhen, China) were also utilized.

2.3 Procedures

The VAEME procedure was optimized using different organic solvents and volumes to find the maximum enrichment factor (E_F) and droplet reproducibility. This procedure was carried out altogether with the back microextraction, necessary to ensure compatibility between the organic solvent containing HAAs (obtained in VAEME) and the chromatographic mobile phase used. Furthermore, the ultrasound-assisted back-microextraction was carried out *in situ* in the syringe used to sample the VAEME droplet, and optimized in order to incorporate an extra preconcentration step in the method. In this sense, organic solvents nature and volume, vortex time, centrifugation time, sample requirements, conditions for the in situ preconcentration, and so on were carefully optimized using a factor by factor procedure.

The optimum conditions for VAEME in combination with in-syringe ultrasound-assisted back-microextraction were: 5 mL of water sample were placed in a centrifuge tube of 15 mL volume, containing 2.25 g of ammonium sulfate and 130 μL of concentrated sulfuric acid (to ensure pH < 0.5). Then, 600 μL of isopropyl ether were added, followed by 5 min of vortex to ensure the efficient formation of droplets in the absence of a dispersive solvent. The tube was then centrifuged during 5 min at 3500 rpm. The obtained phase of isopropyl ether after VAEME (containing extracted HAAs) was collected with a Hamilton syringe (of 1 mL). Then, 50 μL of mobile phase $(NH_4)_2SO_4$ 0.2 $mol \cdot L^{-1}$ (ratio 8:1 with the isopropyl ether, to increase the preconcentration factor) were also introduced in the same syringe (already containing HAAs in isopropyl ether) to perform the in-syringe ultrasound-assisted back-microextraction. The syringe was subjected to ultrasounds during 5 min, and the organic phase was discarded. Thus, the

aqueous phase (0.2 mol·L^{-1} $(NH_4)_2SO_4$) containing HAAs was directly injected in the HPLC system avoiding this way compatibility problems.

2.4 Assessment of the method performance

The relative recovery was calculated as:

$$RR(\%) = 100 \cdot \frac{C_{found}}{C_{initial}} \quad (1)$$

being C_{found} the calculated concentration of the HAAs using the overall method (VAEME-back microextraction-HPLC-DAD) calibration, and $C_{initial}$ the spiked concentration of HAAs in water. In general, for microextraction methods it is expected the obtaining of relative recoveries around 100% if the precision of the method is acceptable.

The enrichment factor of the overall method is given by:

$$E_F = \frac{C_{droplet}}{C_{initial}} \quad (2)$$

being $C_{droplet}$ the concentration of HAAs obtained in the final droplet that is injected in the HPLC, and so it can be calculated with the chromatographic calibration.

The overall extraction efficiency (E_R) of the method can be calculated by:

$$E_R = 100 \cdot \frac{E_F}{E_{Fmax}} \quad (3)$$

being E_{Fmax} the maximum preconcentration that would be achieved if all HAAs (initially present in the water sample) were successfully transferred to the final droplet that is injected in the HPLC. This value can be estimated from the ratio $V_{initial}/V_{droplet}$, being $V_{initial}$ the initial aqueous sample volume (5 mL).

The extraction efficiency only associated to VAEME was calculated indirectly, taking into account the overall extraction efficiency and the extraction efficiency solely associated to the in-syringe ultrasound-assisted back-microextraction step. The same comments are applied to the calculations of the E_F only associated to VAEME.

3 Results and discussion

3.1 Chromatographic determination

This work intended the determination of HAAs using HPLC with a conventional C18 column, and so the method is based on a modification of the work of Chen e*t al.*,[14] but using lower content of ammonium sulfate, and with the help of ACN to decrease the analysis time. Under these conditions (see section 2.2.), adequate resolution of HAAs was achieved in 13 min (see Fig. S1 of the supplementary material). Table S1 of the supplementary material also includes several quality analytical parameters of the calibrations obtained.

3.2 VAEME optimization

In order to adequately extract HAAs using a micro-volume of an organic solvent in VAEME (or in any liquid-phase microextraction technique), HAAs should be in nonionic form. HAAs present quite low pK_a values and so it is necessary to work at low pH values (pH < 0.5), as it is also suggested by the EPA (www.epa.gov/ogwdw/methods/pdfs/methods/met552_3.pdf) This is achieved employing concentrated sulfuric acid (130 µL to 5 mL of water sample). Water sample volume was fixed to 5 mL simply considering the volume capacity of our centrifuge.

Furthermore, it is also advisable to work with high ionic strengths to take advantage of the salting out effect. In this case, Na_2SO_4 is used to adjust the ionic strength up to 45% (w/v) and not NaCl to avoid the artifact formation of HAAs containing chlorine. Other contents of Na_2SO_4 were tried and best performance was achieved at 45% (data not shown).

We conducted a simple optimization factor by factor with VAEME given the *a priori* relatively low number of variables to study: nature and volume of the extractant solvent and extraction time (vortex stirring).

The studied extractant solvents were: MTBE, isopropyl ether, decanol, octanol, hexane, trichloromethane and pentane, fixing their volumes to 100 µL (with pH and ionic strength as abovementioned), and applying vortex for 1 minute and centrifugation at 3500 rpm. To ensure an adequate microdroplet formation, different centrifugation times were tested (from 1 to 8 min) and 5 min was selected. Thus, hexane, trichloromethane and pentane were discarded due to lack of reproducibility in the microdroplet obtained after centrifugation. To evaluate the extraction efficiency of the remaining solvents for HAAs in VAEME it is necessary to perform a solvent exchange step, to ensure the compatibility of the final solvent with HPLC. In this sense, we decided to carry out a back-microextraction rather than other possible solvent-exchange steps.

3.2.1 In-syringe ultrasound-assisted back-microextraction optimization

Once HAAs were extracted in the micro-droplet of organic solvent obtained by VAEME, it is sampled with a micro-syringe. Then, such micro-droplet is mixed within the syringe with an equivalent volume (1:1 ratio) of initial HPLC mobile phase (0.2 $mol \cdot L^{-1}$ $(NH_4)_2SO_4$, pH = 5.18). Thus, neutral HAAs present in the organic solvent pass to this aqueous phase in ionic form, and are ready to HPLC injection. Fig. 1 (A) schematically shows this step. Obviously, it is important to optimize this strategy that takes place *in situ* in the syringe, and to evaluate which organic solvent suits better for this back-microextraction.

The sonication time to which the syringe is subjected to ultrasounds in the back-microextraction step was studied from 2 to 10 min. For all solvents studied, there were not significant differences when sonication times were higher than 5 min, and so this time was selected. Fig. S2 of the supplementary material shows the results of such optimization when

using isopropyl ether as organic solvent, and a concentration of 5 $mg \cdot L^{-1}$ for the HAAs. In these studies, the volume of organic solvent in the syringe was 100 µL.

The extraction efficiency of the back-microextraction itself was then evaluated, also using 100 µL of organic solvent in the syringe (and mixed in a 1:1 ratio with the aqueous salty phase), and a known concentration of HAAs. Table 1 shows the extraction efficiencies achieved in each case. Higher efficiencies were obtained with MTBE and isopropyl ether, with average values of 77.8 and 67.2%, respectively. For decanol and octanol, lower efficiencies were obtained. In spite of low recoveries for decanol and octanol, they were not yet discarded, until the overall method was not tested. For all solvents, RSD values (in %) for the back-microextraction step were adequate, ranging between 2.7 and 17%. These values can be considered acceptable because it must be taken into account that this microextraction step takes place *in situ* in the micro-syringe, followed by HPLC injection, and so no further losses will be added to the method.

3.2.2 Overall VAEME and in-syringe ultrasound-assisted back-microextraction optimization

The overall VAEME and back-microextraction optimization was focused on the following parameters: VAEME vortex time, nature of the extractant solvent, and volume of extractant solvent.

Different vortex times were studied, between 1 to 10 min. In the majority of cases, the best option was a vortex time of 5 min. Fig. S3 of the supplementary material shows the effect of the vortex time for some HAAs (as examples) using 200 µL of isopropyl ether as extractant solvent.

The selection of the extractant solvent was carried out once fixed the vortex time to 5 min. Thus, MTBE, isopropyl ether, decanol and octanol were evaluated using the same volume (200 µL). Decanol was discarded at this point due to low recoveries. Fig. 2 shows the results obtained. In the majority of cases, the best extractant solvent was isopropyl ether, generating higher extraction efficiencies, and therefore it was selected in further experiments.

The last study was the selection of the optimum volume of isopropyl ether. Volumes between 300 to 700 µL were tested, as it is shown in Fig. 3. For the overall VAEME and back-microextraction procedure in combination with HPLC-DAD, higher extraction efficiencies (>75% for the majority of HAAs) were obtained when using 600 µL of isopropyl ether (generating a microdroplet in VAEME of ~500 µL).

In summary, optimum conditions imply the use of 5 mL water containing 2.25 g Na_2SO_4 and 130 µL H_2SO_4 conc., which are mixed with 600 µL of isopropyl ether (extractant solvent), subjected to 5 min vortex, and then centrifugation at 3500 rpm for 5 min. The in-syringe ultrasound-assisted back-microextraction step is then performed with a 1:1 ratio with the mobile phase and applying 5 min of ultrasounds.

3.2.3 Inclusion of a preconcentration step within the back-microextraction

All experiments abovementioned have been carried out using a 1:1 ratio in the back-microextraction step. In order to increase the overall sensitivity of the extraction method, we decided to use other ratios. Given the fact that 500 µL of isopropyl droplet are obtained in the optimum method after VAEME, 400 µL are sampled in the syringe. This implies mixing 400 µL of isopropyl ether with 400 µL of the aqueous salty solution for the 1:1 ratio, 400 µL of isopropyl ether with 200 µL of aqueous salty solution for the 2:1 ratio, and so on. All experiments were carried out using a concentration of 5 $mg \cdot L^{-1}$ for HAAs. Table S2 of the supplementary material shows the extraction efficiency of the overall method when using different ratios in the back-microextraction (1:1, 2:1, 4:1 and 8:1). Higher extraction efficiencies (almost quantitative) were obtained with the 1:1 ratio. On the other hand, worse extraction efficiencies were obtained when using the 8:1 ratio. Despite the obtaining of worse extraction efficiencies with the 8:1 ratio, we selected it because it is accompanied by an important preconcentration step, as it is clearly shown in Fig. 4 and it is pursued the achieving of low limits of detection. It is important to highlight the difficulty in achieving extraction efficiency values close to 100% in microextraction procedures. Indeed, these values are valid as long as the performance of the method fulfills the requirements of a given application.[34]

3.3 Quality analytical parameters of the optimum VAEME, in-syringe ultrasound-assisted back-microextraction and HPLC-DAD method.

The optimum conditions of overall method are depicted in Fig. 1 (B). Calibrations were undertaken using standards of HAAs dissolved in deionized water, which were subjected to the overall method (VAEME, back-microextraction, and HPLC). Table 2 includes several quality analytical parameters of the overall method: microextraction procedures and chromatographic separation. Correlation coefficients for the overall calibration method were higher than 0.990 for all HAAs, except for MCAA (with R being 0.986). LODs and LOQs were calculated on a signal to noise ratio of three and ten, respectively; and verified by preparation of standards spiked at such levels and subjected to the overall method. Thus, LODs ranged from 1.02 $\mu g \cdot L^{-1}$ for TBAA and 9.95 $\mu g \cdot L^{-1}$ for DCAA, whereas higher values were obtained for MCAA and MBAA (being 44.1 and 60.1 $\mu g \cdot L^{-1}$, respectively).

Given the fact that there are no other published works of VAEME for HAAs and HPLC, the comparison of the present mode with other literature data will be mainly carried out with other LPME modes, such as SLLME, in combination with HPLC. Thus, LODs for the 9 HAAs using SLLME and HPLC-UV have been reported to vary between 0.10 and 6.84 $\mu g \cdot L^{-1}$ (without calibration performance),[14] 0.02 and 2.69 $\mu g \cdot L^{-1}$ (being the calibration range 0.4-20 $\mu g \cdot L^{-1}$),[27] 2.23 and 107 $\mu g \cdot L^{-1}$ (being the calibration range 20-20000 $\mu g \cdot L^{-1}$),[28] and down to 0.072-40.3 $ng \cdot L^{-1}$ when utilizing electromembrane extraction with a SLM containing toluene (being the

calibration range 5-200 μg·L^{-1}).[15] These literature methods require extraction times ranging from 15 to 60 min (~17 min in the present work), samples volumes between 23 and 100 mL (only 5 mL in the present work), and solvents such as dihexyl ether and toluene (being isopropyl ether in the present work).[14,15,27,28]

Other works in the recent literature also included different microextraction steps and other determination techniques, such as single-drop microextraction with derivatization in combination with GC-mass spectrometry (MS)[35], getting LODs between 0.33 to 1.5 μg·L^{-1}, liquid-liquid microextraction with derivatization in combination with HS-GC-MS[36], with LODs between from 0.02 to 0.4 μg·L^{-1}; IC coupled with SPE[37] being the LODs between 1.89 and 11.8 μg·L^{-1}, or not coupled with a SPE step[38], being the LODs between 0.37 and 31.64 μg·L^{-1}; and also HPLC in HILIC mode in combination with MS[13], with LODs between 0.18 and 71.5 μg·L^{-1}.

The performance was assessed by the extraction efficiency of the overall method (E_R in %), the efficiency only associated to the VAEME step (E_R' in %), the enrichment factor of the overall method (E_F), the enrichment factor only associated to the VAEME step (E_F'), the precision of the overall method (as RSD in %), and the relative recovery of the overall method (RR in %).

In this work, E_{Fmax} (theoretical value) has a value of 11.4 only considering VAEME, and of 91.2 if considering the overall method.

Table 3 includes the results obtained for the extraction performance of the method according to these parameters. This study was accomplished using deionized waters spiked at two different levels (100 and 700 μg·L^{-1}), and subjected to the overall method (n = 4). E_R' is calculated considering E_R as well as the losses obtained in the in-syringe ultrasound-assisted back-microextraction using the 8:1 ratio (Table S2).

In all cases, it can be observed adequate relative recoveries, with average values of 89.0 and 77.7% for the higher and the lower spiked level, respectively. RSD values ranged from 7.5% for DCAA and 22% for MCAA for the higher spiked level, and from 5.2% for DCAA and 23% for MCAA for the low spiked level.

Average enrichment factors only associated to VAEME (E_F') were 10.1 for the lower spiked level and 10.0 for the higher spiked level. Both values are quite close to the E_{Fmax} (11.4), which already indicates that the extraction efficiency only associated to VAEME (E_R') is almost quantitative. Indeed, average E_R' values ranged from 88.5% for the lower spiked level to 87.7% for the higher spiked level.

Regarding the overall method, the overall extraction efficiency is lower, mainly to the losses associated to the back-microextraction step when using the 8:1 ratio, with average values of 22.6% for the lower spiked level and 22.9% for the higher spiked level. It must be highlighted that it is difficult to achieve E_R values close to 100% in any microextraction

procedure, and so E_R values are valid as long as the LODs, E_F values, and reproducibility of the method are sufficient for a given application.

The overall method is characterized for average E_F values of 20.6 for the lower spiked level and of 20.8 for the higher spiked level. Thus, despite the losses in the back-microextraction, adequate reproducibility, low detection limits, and overall preconcentration factors of ~21 are obtained using HPLC with a conventional C18 column. Comparing with other SLLME-HPLC-UV works, E_F values of 500,[14] 300-3000,[27] and 10-65;[28] and E_R values of 54%,[15] and 16-50%,[28] have been reported.

3.4 Analysis of different water samples using the optimized method

Several waters have been analyzed with this methodology as a preliminary study to test its validity with more complex samples. Tap waters from La Laguna University and from La Orotava, pool water from a local pool in La Orotava, and re-mineralized water from a desalination plant (Santa Cruz de Tenerife) were analyzed in order to assess the applicability of the method with real samples. Samples were filtered, and their pH was adequately adjusted before carrying out the overall optimum procedure. Each sample was analyzed by triplicate. Results from this study are shown in Table 4. Furthermore, accuracy and precision studies were performed with tap waters from La Laguna, spiked at 400 $\mu g \cdot L^{-1}$ level, getting RSD values lower than 18.4% (as intra-day precision) and average relative recovery of 79.3%.

Tap waters were shown to be free of HAAs, as well as the pool water. However six HAAs were detected in waters coming from the desalination plant (MCAA, MBAA, BCAA, DBAA, CDBAA and TBAA), and four of them were quantified, being for example the content of MCAA up to 244 ± 22 $\mu g \cdot L^{-1}$. Ongoing work is carried out to utilize this methodology as a monitoring technique with real samples.

4 Conclusions

A VAEME method followed by in-syringe ultrasound-assisted back-microextraction has been developed for first time and applied for the determination of a group of nine haloacetic acids in real water samples. The VAEME method is quick, simply based on the extraction of HAAs using a low volume of isopropyl ether, being the emulsification assisted by vortex. Afterwards, an in-syringe back-microextraction is carried out to ensure the further compatibility with HPLC. Moreover, this in-syringe step is carried out incorporating a preconcentration strategy (by mixing the organic droplet containing HAAs with a much lower volume of an aqueous solution of Na_2SO_4), to increase the overall sensitivity of the method. Furthermore, the HPLC method with diode array detector is carried out with a conventional C18 column. Under optimized

conditions, average relative recoveries of 77.7 to 89.0%, depending on the concentration level considered, and precision values lower than 23% (as RSD) were obtained.

The overall method is therefore fast (~17 min for the overall microextraction procedure, and ~15 min for the chromatographic run), it does not use toxic organic solvents, and it results quite simple. The applicability of the procedure was verified by considering sample matrices of different complexities, and the performance of the method was still successful.

Acknowledgements

V.P. thanks the Spanish Ministry of Economy and Competitiveness (MINECO) for the Ramón y Cajal contract with the University of La Laguna (ULL). P.R.-B. acknowledges Fundación CajaCanarias for her PhD fellowship. A.M.A. acknowledges funding from a Bridge Project and a Consolidated Research Funding Project of ULL. J.H.A. thanks funding from the Project: ProID20100241 of Canary agency of Research, Innovation and Information Society (ACIISI) under the European Regional Development Fund (FEDER). J.H.A. and V.P. also thank funding from Fundación CajaCanarias ref. project AGUA05.

References

1 H. Shi, Z. Qiang and C. Adams, *Chemosphere*, 2013, **90**, 2485–2492.

2 H. Chang, C. Chen and G. Wang, *Water Res.*, 2013, **47**, 2729–2741.

3 C. Tian, R. Liu, T. Guo, H. Liu, Q. Luo and J. Qu, *Sep. Purif. Technol*., 2013, **102**, 86–93.

4 J. Lee, E. S. Kim, B. S. Roh, S. W. Eom and K. D. Zoh, *Environ. Monit. Assess*., 2013, **185,** 7675–7691.

5 C. H. Jeong, E. D. Wagner, V. R. Siebert, S. Anduri, S. D. Richardson, E. J. Daiber, A. B. McKague, M. Kogevinas, C. M. Villanueva, E. H. Goslan, W. Luo, L. M. Isabelle, J. F. Pankow, R. Grazuleviciene, S. Cordier, S. C. Edwards, E. Righi, M. J. Nieuwenhuijsen and M. J. Plewa, *Environ. Sci. Technol*., 2012, **46**, 12120−12128.

6 R. L. Melnick, A. Nyska, P. M. Foster, J. H. Roycroft and G. E. Kissling, *Toxicology*, 2007, **230**, 126–136.

7 P. Varanusupakul, N. Vora-Adisak and B. Pulpoka, *Anal. Chim. Acta*, 2007, **598**, 82–86.

8 B. Álvarez-Sánchez, F. Priego-Capote and M. D. Luque de Castro, *J. Chromatogr. A*, 2008, **1201**, 21–26.

9 M. J. Cardador and M. Gallego, *J. Agric. Food Chem.*, 2012, **60**, 725−730.

10 N. Rastkari, R. Ahmadkhaniha and M. Yunesian, *Int. J. Environ. Anal. Chem.*, 2012, **92**, 1650–1665.

11 B. Hammami and M. R. Driss, *J. Anal. Chem.*, 2013, **68**, 671−679.

12 S. Kim, L. B. Collins, G. Boysen, J. A. Swenberg, A. Gold, L. M. Ball, B. U. Bradford and I. Rusyn, *Toxicology*, 2009, **262**, 230–238.

13 C. Y. Chen, S. N. Chang and G. S. Wang, *J. Chromatogr. Sci.*, 2009, **47**, 67–74.

14 X. Wan, D. Kou and S. Mitra, *J. Chromatogr. A*, 2005, **1089**, 39-44.

15 K. Alhooshani, C. Basheer, J. Kaur, A. Gjelstad, K. E. Rasmussen, S. Pedersen-Bjergaard and H. K. Lee, *Talanta*, 2011, **86**, 109–113.

16 B. Paull and L. Barron, *J. Chromatogr. A*, 2004, **1046**, 1–9.

17 M. C. Bruzzoniti, R. M. de-Carlo, K. Horvath, D. Perrachon, A. Prelle, R. Tófalvi, C. Sarzanini and P. Hajós, *J. Chromatogr. A*, 2008, **1187**, 188–196.

18 S. Ying-xue and G. Ping, *J. Environ. Sci.*, 2007, **19**, 885–891.

19 F. Pena-Pereira, I. Lavilla and C. Bendicho, *Trends Anal. Chem*., 2010, **29**, 617–628.

20 C. Duan, Z. Shen, D. Wu and Y. Guan, *Trends Anal. Chem.*, 2011, **30**, 1568–1574.

21 M. Farré, S. Pérez, C. Gonçalves, M. F. Alpendurada and D. Barceló, *Trends Anal. Chem*., 2010, **29**, 1347–1362.

22 S. Armenta, S. Garrigues and M. de la Guardia, *Trends Anal. Chem.*, 2008, **27**, 497–511.

23 M. R. Ganjali, H. R. Sobhi, H. Farahani, P. Norouzi, R. Dinarvand and A. Kashtiaray, *J. Chromatogr. A*, 2010, **1217**, 2337–2341.

24 A. Jain and K. K. Verma, *Anal. Chim. Acta*, 2011, **706**, 37–65.

25 L. Kocúrová, I. S. Balogh and V. Andruch, *J. Sep. Sci.*, 2013, **36**, 3758–3768.

26 H. Lin, J. Wang, L. Zeng, G. Li, Y. Sha, D. Wu and B. Liu, *J. Chromatogr. A*, 2013, **1296**, 235–242.

27 D. Kou, X. Wang and S. Mitra, *J. Chromatogr. A*, 2004, **1055**, 63–69.

28 X. Wang, C. Saridara and S. Mitra, *Anal. Chim. Acta*, 2005, **543**, 92–98.

29 V. Andruch, M. Burdel, L. Kocúrová, J. Šandrejová and J. S. Balogh, *Trends Anal. Chem*., 2013, **49**, 1–19.
30 C. K. Zacharis, C. Christophoridis and K. Fytianos, *J. Sep. Sci.*, 2012, **35**, 2422–2429.
31 Y. Zhang and H. K. Lee, *J. Chromatogr. A*, 2012, **1249**, 25–31.
32 C. Jia, X. Zhua, J. Wang, E. Zhao, M. He, L. Chen and P. Yu, *J. Chromatogr. A*, 2010, **1217**, 5868–5871.
33 E. Yiantzi, E. Psillakis, K. Tyrovola and N. Kalogerakis, *Talanta*, 2010, **80**, 2057–2062.
34 M. J. Trujillo-Rodríguez, P. Rocío-Bautista, V. Pino and A. M. Afonso, *Trends Anal. Chem.*, 2013, **51**, 87–106.
35 M. Saraji and A. A. H. Bidgoli, *J. Chromatogr. A*, 2009, **1216** 1059–1066.
36 M. J. Cardador, A. Serrano and M. Gallego, *J. Chromatogr. A*, 2008, **1209,** 61–69.
37 L. Barron and B. Paull, *Anal. Chim. Acta*, 2004, **522**, 153–161.
38 Y. Liu and S. Mou, *J. Chromatogr. A*, 2003**, 997**, 225–235.

Figure Captions

Fig. 1 A) Scheme of the in-syringe ultrasound-assisted back-microextraction procedure using a 1:1 ratio. B) Scheme of the overall procedure under optimum conditions: VAEME followed by in-syringe ultrasound-assisted back-microextraction using a 8:1 ratio, and HPLC injection.

Fig. 2 Effect of the solvent nature on the extraction efficiency (as peak area) of HAAs when applying the overall method by triplicate (VAEME, back-microextraction and HPLC-DAD), and using 200 µL of extractant solvent. Rest of conditions as described in the text.

Fig. 3 Effect of the isopropyl ether volume on the extraction efficiency of HAAs (spiked concentration of 5 $mg \cdot L^{-1}$) using the overall method by triplicate (VAEME, back-microextraction and HPLC-DAD), and 5 min of vortex. Rest of conditions as described in the text.

Fig. 4 Effect of different ratios of the back-microextraction step (HAA concentration of 5 $mg \cdot L^{-1}$) on the sensitivity of the overall method.

447 **Table 1** Extraction efficiencies when using different organic solvents solely associated to the back-microextraction step.

HAAs	MTBE[a] Obtained concentration ($mg \cdot L^{-1}$) ± SD[c]	Extraction eficiency (%)	Decanol[b] Obtained concentration ($mg \cdot L^{-1}$) ± SD[c]	Extraction eficiency (%)	Octanol[b] Obtained concentration ($mg \cdot L^{-1}$) ± SD[c]	Extraction eficiency (%)	Isopropyl ether[b] Obtained concentration ($mg \cdot L^{-1}$) ± SD[c]	Extraction efficiency (%)
MCAA	3.59 ± 0.58	62.7	2.07 ± 0.34	41.4	2.94 ± 0.36	58.8	3.37 ± 0.37	67.3
MBAA	2.67 ± 0.19	68.8	2.09 ± 0.32	41.7	2.95 ± 0.24	59.0	3.13 ± 0.45	67.0
DCAA	6.85 ± 0.60	116	3.19 ± 0.38	63.7	4.03 ± 0.34	80.5	3.38 ± 0.13	67.6
BCAA	4.06 ± 0.41	99.9	2.83 ± 0.46	56.7	3.62 ± 0.34	72.4	3.54 ± 0.19	70.7
DBAA	1.97 ± 0.11	92.9	2.73 ± 0.42	54.6	2.80 ± 0.25	56.0	3.34 ± 0.09	66.8
TCAA	1.57 ± 0.22	76.9	2.23 ± 0.24	44.6	1.05 ± 0.18	21.1	2.31 ± 0.25	46.1
BDCAA	2.33 ± 0.24	53.4	2.51 ± 0.16	50.3	1.36 ± 0.11	27.1	3.52 ± 0.28	70.4
CDBAA	7.24 ± 0.95	72.2	3.11 ± 0.23	62.2	3.28 ± 0.50	65.6	2.48 ± 0.29	49.5
TBAA	12.7 ± 0.75	57.6	2.81 ± 0.21	56.2	3.31 ± 0.29	66.1	5.96 ± 0.81	99.3
Average		77.8		53.4		56.3		67.2

[a]MTBE containing the following concentration of HAAs ($mg \cdot L^{-1}$): 5.73 for MCAA, 3.88 for MBAA, 5.90 for DCAA, 4.70 for BCAA, 2.12 for DBAA, 2.04 for TCAA, 4.36 for BDCAA, 10.03 for CDBAA and 22.0 for TBAA (obtained by dilution of the EPA 552.3 acid calibration mix)

[b]HAAs concentration: 5 $mg \cdot L^{-1}$

[c]standard deviation (n = 6)

Table 2 Quality analytical parameters of the calibration for the overall method (VAEME, back-microextraction with 8:1 ratio and HPLC-DAD determination).

HAAs	Linearity range ($\mu g \cdot L^{-1}$)	Slope ± $t_{n-2} \times SD$[a]	Intercept ± $t_{n-2} \times SD$[a]	R	LOD[b] ($\mu g \cdot L^{-1}$)	LOQ[b] ($\mu g \cdot L^{-1}$)
MCAA	150 - 300	158 ± 49	-14015 ± 9047	0.986	44.1	147
MBAA	200 - 1000	729 ± 43	-18671 ± 20904	0.997	60.1	200
DCAA	100 - 1000	608 ± 157	-18252 ± 31882	0.992	9.95	33.2
BCAA	25 - 1000	962 ± 99	6405 ± 43399	0.993	4.95	16.5
DBAA	28 - 1000	1610 ± 123	9090 ± 61961	0.997	8.36	27.9
TCAA	100 - 1000	641 ± 90	19228 ± 38463	0.990	9.06	30.2
BDCAA	25 - 1000	1233 ± 142	3420 ± 69413	0.991	1.46	4.89
CDBAA	10 - 1000	1515 ± 120	-15700 ± 52896	0.991	2.95	9.84
TBAA	10 - 1000	1579 ± 125	38407 ± 55209	0.991	1.02	3.39

[a]confidence level ($\alpha = 0.05$) for n = 14, except MCAA and MBAA with n = 6

[b]calculated as described in the text

Table 3 Extraction efficiency and precision study for the overall method (VAEME, back-microextraction with 8:1 ratio and HPLC-DAD determination) and performance solely associated to VAEME.

HAA	Spiked level: 100 μg·L^{-1} (n = 4)						Spiked level: 700 μg·L^{-1} (n = 4)					
	RR[a] (%)	RSD[b] (%)	E_R'[c] (%)	E_R[d] (%)	E_F'[e]	E_F[f]	RR[a] (%)	RSD[b] (%)	E_R'[c] (%)	E_R[d] (%)	E_F'[e]	E_F[f]
MCAA	62.8	23	80.2	11.3	9.19	10.3	78.1	22	71.8	12.9	8.23	11.8
MBAA	63.4	21	80.9	17.4	9.27	15.9	83.8	12	82.0	18.5	9.39	16.9
DCAA	125	5.2	98.0	29.5	11.2	26.9	96.3	7.5	94.5	26.0	10.8	23.1
BCAA	77.0	6.7	83.6	20.1	9.58	18.3	89.8	8.8	84.8	21.3	9.72	19.4
DBAA	83.7	10	93.5	23.7	10.7	21.6	91.1	7.8	91.8	22.0	10.5	20.1
TCAA	63.4	11	105	22.7	12.0	20.7	102	8.7	106	24.1	12.2	22.0
BDCAA	80.7	9.2	86.7	22.6	9.94	20.6	80.3	9.2	86.9	22.8	9.96	20.8
CDBAA	76.0	11	91.1	28.1	10.4	25.6	99.9	8.6	95.0	32.0	10.9	29.2
TBAA	67.3	8.8	77.8	28.3	8.91	25.8	80.1	8.9	76.1	26.6	8.72	24.3
Average	77.7		88.5	22.6	10.1	20.6	89.0		87.7	22.9	10.1	20.8

[a]relative recovery of the overall method

[b]relative standard deviation of the overall method

[c]extraction efficiency only associated to VAEME

[d]extraction efficiency of the overall method

[e]enrichment factor only associated to VAEME

[f]enrichment factor of the overall method

Table 4 Analysis of real water samples using the overall optimized procedure.

HAA	Tap water 1 (La Laguna)			Tap water 2 (La Orotava)	Swimming pool water (La Orotava)	Desalination plant water (Santa Cruz)
	non spiked	spiked (400 μg·L^{-1})		non spiked	non spiked	non spiked
	Conc[a] ± SD[b]	RSD[c] (%)	RR[d] (%)	Conc[a] ± SD[b]	Conc[a] ± SD[b]	Conc[a] ± SD[b]
MCAA	nd	16.4	67.4	nd	nd	224 ± 22
MBAA	nd	12.6	116	nd	nd	nq
DCAA	nd	15.5	64.9	nd	nd	nd
BCAA	nd	18.4	64.0	nd	nd	31.3 ± 5.6
DBAA	nd	12.2	88.5	nd	nd	nq
TCAA	nd	10.2	63.9	nd	nd	nd
BDCAA	nd	7.43	110	nd	nd	nd
CDBAA	nd	17.0	79.3	nd	nd	21.3 ± 3.8
TBAA	nd	5.06	60.0	nd	nd	6.27 ± 4.02

[a]concentration in μg·L^{-1}

[b]standard deviation (n = 3)

[c]relative standard deviation (n = 3)

[d]relative recovery (n = 3)

nd: non-detected

nq: non-quantified

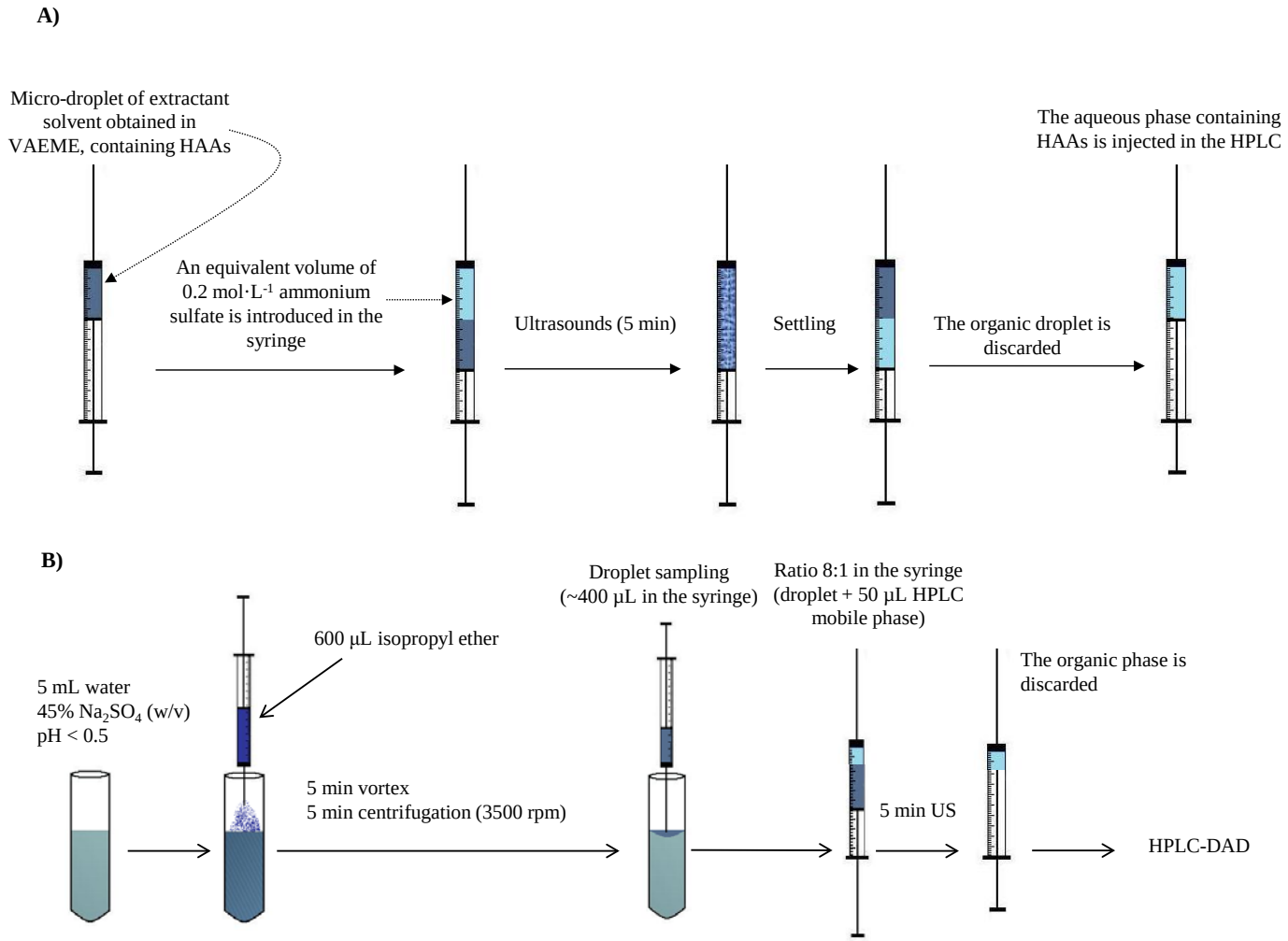


Figure 1

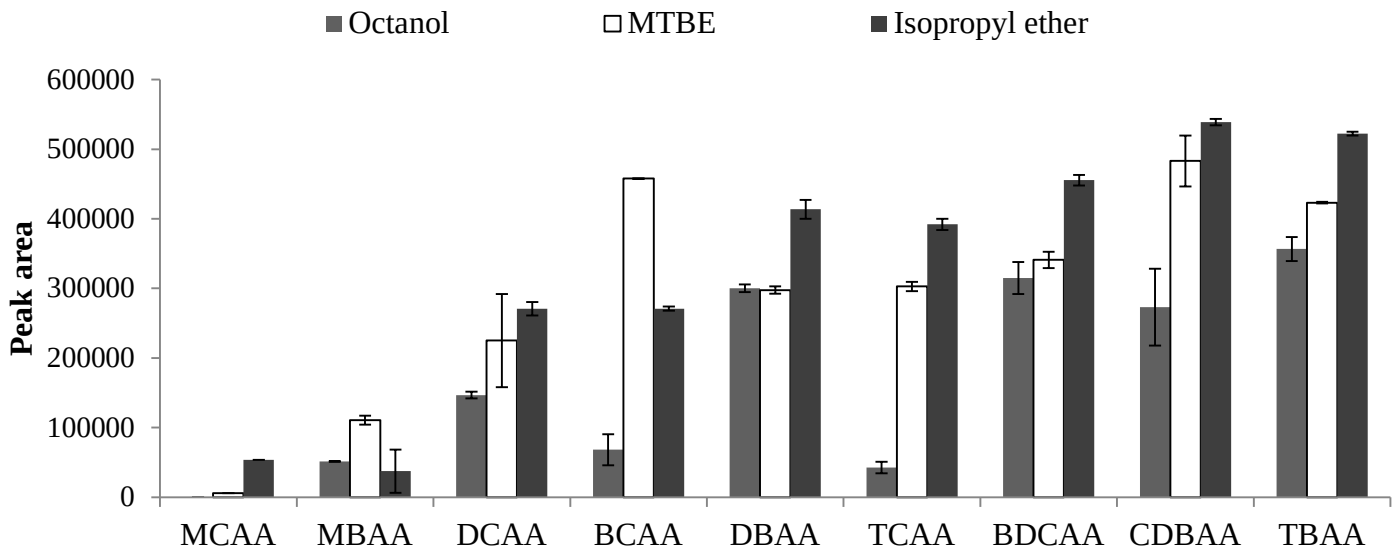


Figure 2

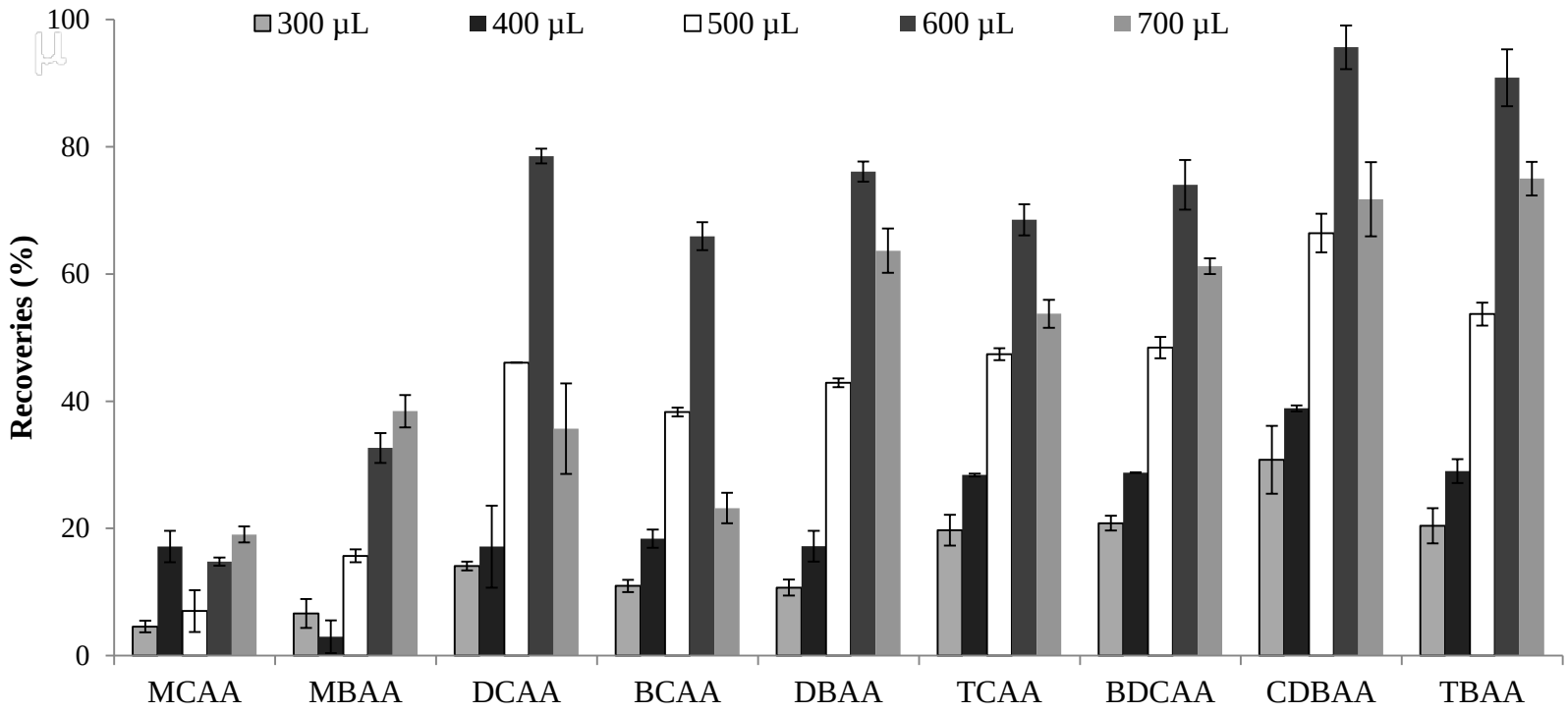


Figure 3

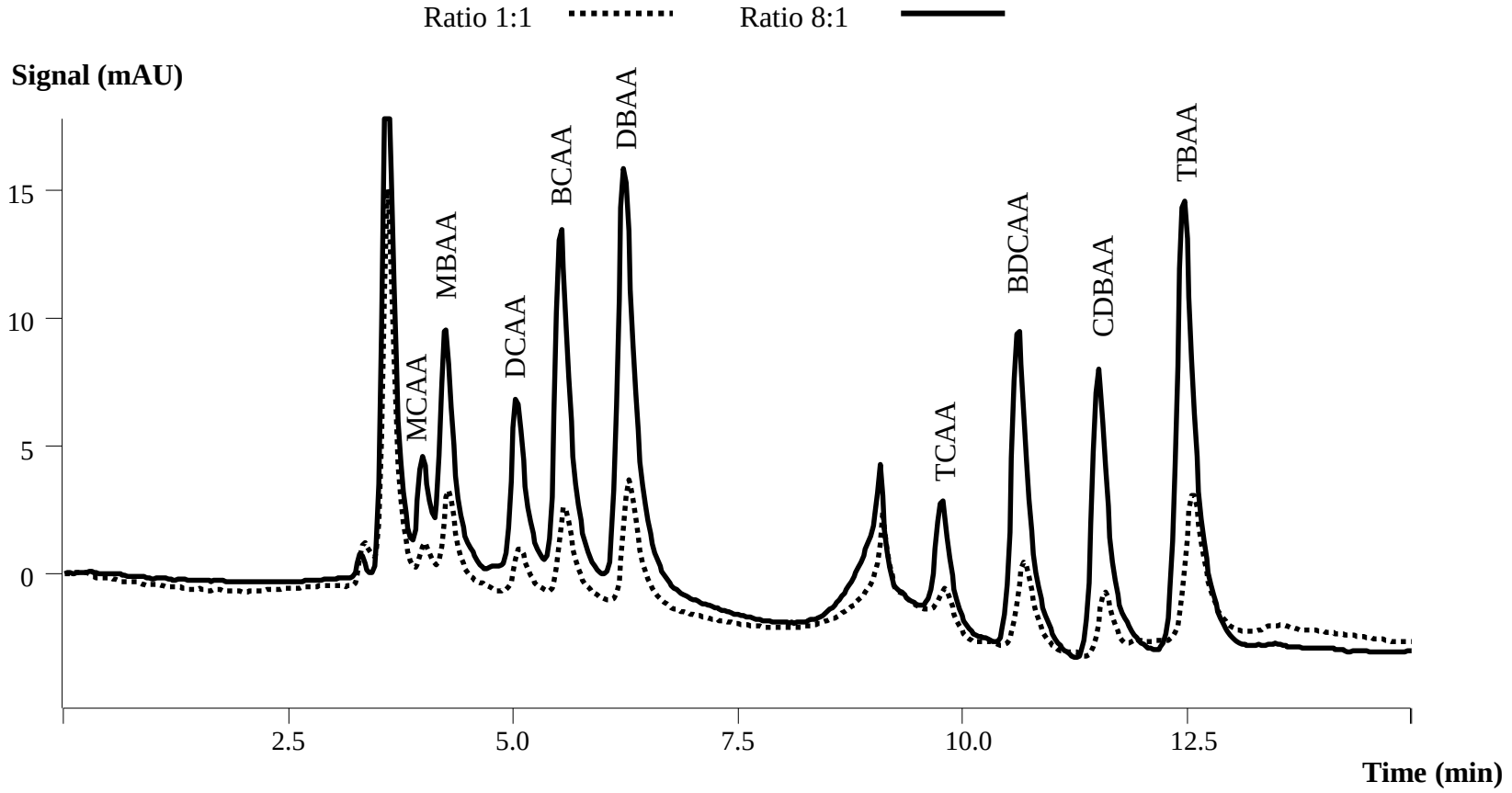


Figure 4