Research Article

In-line preconcentration capillary zone electrophoresis for the analysis of haloacetic acids in water

Two in-line enrichment procedures (large volume sample stacking (LVSS) and field amplified sample injection (FASI)) have been evaluated for the CZE analysis of haloacetic acids (HAAs) in drinking water. For LVSS, separation on normal polarity using 20 mM acetic acid–ammonium acetate (pH 5.5) containing 20% ACN as BGE was required. For FASI, the optimum conditions were 25 s hydrodynamic injection (3.5 kPa) of a water plug followed by 25 s electrokinetic injection (–10 kV) of the sample, and 200 mM formic acid–ammonium formate buffer at pH 3.0 as BGE. For both FASI and LVSS methods, linear calibration curves ($r^2 > 0.992$), limit of detection on standards prepared in Milli-Q water (49.1–200 mg/L for LVSS and 4.2–48 mg/L for FASI), and both run-to-run and day-to-day precisions (RSD values up to 15.8% for concentration) were established. Due to the higher sensitive enhancement (up to 310-fold) achieved with FASI-CZE, this method was selected for the analysis of HAAs in drinking water. However, for an optimal FASI application sample salinity was removed by SPE using Oasis WAX cartridges. With SPE-FASI-CZE, method detection limits in the range 0.05–0.8 mg/L were obtained, with recoveries, in general, higher than 90% (around 65% for monochloroacetic and monobromoacetic acids). The applicability of the SPE-FASI-CZE method was evaluated by analyzing drinking tap water from Barcelona where seven HAAs were found at concentration levels between 3 and 13 mg/L.

Keywords: Direct UV-detection / FASI / Haloacetic acids / Stacking / Water samples

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1 Introduction

It is well known that chlorination of drinking water has considerably reduced the number of deaths occurring annually from the outbreak of waterborne diseases. However, the natural organic matter in the water can also react with chlorine, forming organohalogen compounds usually referred as disinfection by-products (DBPs) [1, 2]. In addition, high bromide levels in water reservoirs used as sources of drinking water can significantly contribute to the formation of brominated and mixed bromo/chloro-DBPs during chlorination [3, 4]. The presence of some DBPs in drinking water is a matter of concern for human health and may also cause an unpleasant organoleptic taste. One of the most prevalent classes of known DBPs is the haloacetic acids (HAAs), which have potential adverse health effects [5]. At the moment, the US EPA has established a maximum contamination level (MCL) of 60 mg/L for the sum of five HAAs: monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), monobromoacetic acid (MBAA) and dibromoacetic acid (DBAA) [6–8]. European legislation is less restrictive than the USA one, and in relation with DBPs only four trihalomethanes are proposed to be controlled [9]. The World Health Organization has published guideline values for TCAA (200 mg/L) and MCAA (20 mg/L), and a provisional guideline value for DCAA (50 mg/L) in drinking water [10].

The typical methods used to determine HAAs involve GC with electron capture detection (ECD) [11–15] or coupled to mass spectrometry (GC-MS) [16–19]. Ion chromatography (IC) has also been proposed [20] using fluorescence detection after post-column derivatization [21], conductometric detection [22] or coupled to MS [23–25]. Conductometric determination of HAAs in drinking waters has also been

Abbreviations: BCAA, bromochloroacetic acid; BDCAA, bromodichloroacetic acid; CDBAA, chlorodibromoacetic acid; DBAA, dibromoacetic acid; DBPs, disinfection by-products; DCAA, dichloroacetic acid; FASI, field-amplified sample injection; HAAs, haloacetic acids; LVSS, large volume sample stacking; MBAA, monobromoacetic acid; MCAA, monochloroacetic acid; TBAA, tribromoacetic acid; TCAA, trichloroacetic acid
recently described using molecularly imprinted polymer (MIP)-modified electrode sensors [26].

CZE has also been reported for the determination of HAAs using indirect UV detection [27–30]. However, many of the indirect UV buffers are expensive and may be prone to matrix interferences, thus CZE methods with direct UV detection have been developed [31–33]. Non-aqueous buffers have also been proposed for the analysis of HAAs in waters by capillary electrophoresis coupled to MS (CE-MS) [34], and recently microchip capillary electrophoresis has also been used for the analysis of DCAA and TCAA [35]. In general, to improve detection limits, preconcentration methods such as solid-phase extraction (SPE) [30, 32] or liquid–liquid extraction (LLE) [31] are usually employed. Today, many in-line CE preconcentration procedures such as isotachophoresis, field amplified sample injection (FASI), stacking and sweeping are described in the literature [36, 37], which allow proposing CE methodologies for the environmental analysis of many pollutants at the required legislated levels. For the analysis of HAAs in water samples by CZE at low ppb levels, only an in-line preconcentration method has been published [32] that was a stacking with sample matrix removal (employing NaOH solution as sample matrix) after an offline LLE step, although the method was only applied to the analysis of six HAAs.

The aim of this work is the evaluation of two in-line CZE enrichment procedures, FASI and stacking with sample matrix removal (without using NaOH solutions), also known as large volume sample stacking (LVSS), to improve detection in the analysis of nine HAAs (including the mixed bromo/chloro-HAAs not usually reported in the literature) by CZE. Parameters that can affect the performance of the in-line preconcentration, such as buffer concentration and pH, injection time and reversal time (in LVSS), among others, were optimized, and quality parameters were established. The best preconcentration method was applied to the analysis of HAAs in Barcelona tap water. Weak anion-exchange SPE was proposed to remove sample salinity before submitting the drinking water to the in-line CZE preconcentration method.

2 Materials and methods

2.1 Chemicals

The reagents, all of analytical grade, were obtained from the following sources: MCAA, DCAA, TCAA, MBAA, DBAA, tribromoacetic acid (TBA), bromochloroacetic acid (BCAA) and trifluoroacetic acid (TFA, used as internal standard) from Fluka (Buchs, Switzerland), chlorodibromoacetic acid (CDBAA) and bromodichloroacetic acid (BDCAA) from Supelco (Bellefonte, PA, USA). Hydrochloric acid (25%), sodium hydroxide, formic acid, acetic acid and ammonium acetate were purchased from Merck (Darmstadt, Germany), maleic acid from Carlo Erba (Milan, Italy) and ammonium formate from Fluka. Water was purified using an Elix 3 module coupled to a Milli-Q system (Millipore, Bedford, MA, USA).

Stock standard solutions of individual HAAs and the internal standards TFA and maleic acid (1000 mg/L) were prepared in Milli-Q water, stored in plastic vials and kept at 4°C. Working solutions were obtained by dilution with Milli-Q water. Buffers were prepared daily by dilution of stock solutions of formic acid and ammonium formate or acetic acid and ammonium acetate. All buffers and working solutions were sonicated and filtered through a 0.45-µm membrane filter before use.

2.2 Instrumentation

CZE-UV and FASI experiments were performed on a Beckman P/ACE MDQ capillary electrophoresis instrument equipped with a diode array. Electrophoretic separations were carried out using uncoated fused-silica capillaries with a total length of 60 cm (50 cm effective length) × 50 µm id (360 µm od). For CZE-UV, a capillary voltage of −20 kV (reversed polarity) was used. Sample introduction was performed by hydrodynamic injection (25 s, 3.5 kPa). FASI was performed as follows. The capillary was first filled with BGE (200 mM formic acid–ammonium formate buffer (pH 3.0)) and then a water plug (20 s, 3.5 kPa) was introduced. Samples were then introduced into the capillary by electrokinetic injection at −10 kV (reversed polarity) during 20 s. The electrophoretic separation was then performed by applying −25 kV (reversed polarity) through the capillary. The CE instrument was controlled using a Beckman P/ACE station software version 1.2.

LVSS experiments were performed on a Beckman P/ACE 5500 capillary electrophoresis instrument (Fullerton, CA, USA) modified to control the reversal of the electrode polarity and equipped with a diode array detector. Acquisition data were processed using the P/ACE Station software version 1.0. The electrophoretic separation was carried out using uncoated fused-silica capillaries of 57 cm (50 cm effective length) × 50 µm id (360 µm od). An optimal application of LVSS for the analysis of HAAs required an electrophoretic separation in positive polarity mode. For this purpose, a 20-mM acetic acid–ammonium acetate buffer (pH 5.5) containing 20% ACN as BGE and a capillary voltage of +25 kV (normal polarity) were employed. The application of LVSS involved several steps. The capillary was first filled with BGE and then a long plug of sample was introduced hydrodynamically by pressure (140 kPa) for 15 s. A high capillary voltage (~25 kV, reversed polarity) was then applied and the electric current was monitored to indicate when the sample matrix was almost removed from the capillary by the electroosmotic flow (EOF). When the current was 95% of the original BGE current value, the voltage was turned off and the electrodes were switched to the separation configuration (reversal time: ~1.7 min). Electrophoretic separation was then carried out by applying +25 kV (normal polarity).
All the experiments were performed by keeping capillary temperature at 25°C, and direct UV detection was carried out at 200 nm.

2.3 Capillary conditioning

New capillaries were pretreated by using 0.1 M hydrochloric acid for 30 min, Milli-Q water for 30 min, 0.1 M sodium hydroxide for 30 min and finally rinsed with Milli-Q water for 30 min. The capillary was conditioned daily by rinsing with 0.1 M sodium hydroxide for 30 min, Milli-Q water for 30 min and finally with the BGE for 30 min before the first run. Finally, the capillary was rinsed with BGE for 5 min between runs and stored after rinsing with water.

2.4 Sample clean-up and preconcentration step

In order to remove water sample salinity and enhance HAAs detection, a SPE step using Oasis WAX (150 mg) cartridges (Waters, Milford, MA, USA) was performed. The sample treatment was performed following the procedure described by Taniyasu et al. [38]. Briefly, the cartridge was washed with 4 mL of MeOH containing 0.1% ammonium hydroxide, 4 mL of MeOH and finally with 4 mL of Milli-Q water. Water samples of 100 mL were passed through the cartridge at a flow rate of 2–3 mL/min using a Visiprep System (Supelco). The cartridge was then washed with 25 mL of Milli-Q water (to remove salt content), 2 mL of MeOH and finally dried with air. Elution was carried out with 2 mL of MeOH containing 0.1% ammonium hydroxide, and the eluate was then evaporated to dryness under a N2 stream. Finally, the extract was re-dissolved in 1 mL of Milli-Q water and directly introduced into the CE system and analyzed by the FASI in-line preconcentration procedure.

3 Results and discussion

3.1 CZE-UV and FASI optimization

As a preliminary study, the electrophoretic separation of this family of compounds in negative polarity mode was optimized. Formic acid–ammonium formate buffers were chosen as background electrolytes for this purpose, and the effect of both buffer concentration and pH was evaluated. An important improvement on HAA signals and peak shapes was observed with the increase in buffer ionic strength, so high buffer concentrations were proposed as optimal. Since HAAs have pK values between 0.66 (TCAA and TBAA) and 2.88 (MBAA), pH values higher than 3.0 must be used in order to guarantee anionic species. However, as the separation of anions was performed with negative polarity, high pH values must be prevented because the increase in the EOF, in opposite direction than HAAs, interferes their separation as well as removes from the capillary those HAAs with low electrophoretic mobility (MCAA and MBAA). As a compromise, 200 mM formic acid–ammonium formate buffer at pH 3.0 was chosen as optimal BGE for the separation of this family of compounds. Hydrodynamic injection time was also optimized and an injection time of 25 s was selected as optimal since higher values produced peak broadening and the loss of electrophoretic separation. Under these conditions, limits of detection (LOD) around 1 mg/L were obtained for almost all HAAs, so preconcentration methods are mandatory to increase sensitivity.

Among in-line enrichment procedures, FASI is very popular since it is quite simple only requiring the electrokinetic injection of the sample after the introduction of a short plug of a high-resistivity solvent. In this study, the electrolyte previously optimized for the conventional CZE separation (200 mM formic acid–ammonium formate at pH 3.0) was used as BGE for FASI-CZE procedure.

Water was used as high resistivity solvent for FASI application. Injection times for both the plug of water (hydrodynamic mode) and the sample (electrokinetic mode) were simultaneously optimized. Hydrodynamic injection (3.5 kPa) of a water plug from 5 s to 30 s and electrokinetic sample injection (−10 kV) from 5 s to 30 s were tested. The best results were obtained with an injection time of 25 s for both the water plug and the sample. Obviously, when increasing injection time an enhancement of the response was observed; however, peak broadening occurred at sample injection times higher than 25 s affecting the electrophoretic separation. On the other hand, a reduction of the water plug produced a significant decrease on HAA signals. Once the sample was introduced by FASI, separation was performed by applying −25 kV as capillary voltage. As an example, Fig. 1 shows the electrophoretic separation of a 70-μg/L standard of HAAs (250 μg/L for MCAA) in Milli-Q water.

![Figure 1. Electrophoretic separation of HAAs (70 and 250 μg/L for MCAA) by FASI-CZE. BGE: 200 mM formic acid–ammonium formate buffer at pH 3.0; water plug injection time: 25 s (hydrodynamic injection at 3.5 kPa); sample injection time: 25 s (electrokinetic injection at −10 kV); sample matrix: Milli-Q water; capillary voltage: −25 kV; capillary temperature: 25°C; acquisition: λ 200 nm; peak identification: 1, DCAA; 2, BCAA; 3, TCAA; 4, DBAA; 5, BDCAA; 6, CDBAA; 7, TBAA; 8, MCAA; 9, MBAA.](image-url)
3.2 LVSS optimization

The anionic nature of HAAs makes necessary to develop an electrophoretic separation in cathodic mode for an optimal application of LVSS, as EOF will help in the removal of sample matrix in a first step and then will produce the electrophoretic separation of HAAs in a second step. For this reason, relatively high pH buffer values are necessary in order to reach EOF mobilities able to carry out the analytes (with anionic electrophoretic mobilities) to the detector. The BGE previously optimized for the application of FASI cannot be applied in this case because of its low pH value, so for LVSS acetic acid–ammonium acetate buffers at higher pH values were evaluated. As an example, Fig. 2A (0% ACN) shows the electrophoretic separation obtained with a 200-mM acetic acid–ammonium acetate buffer at pH 5.5, and normal polarity mode (+25 kV). Under these conditions, almost all HAAs were baseline separated but DBAA and TCAA co-migrated (peaks 4 and 5). At lower buffer concentrations, separation worsened, and comigration of TBAA, CDBAA and BDCAA (peaks 1, 2 and 3, respectively) were observed, while higher concentrations did not improve separation of DBAA and TCAA. In order to achieve baseline separation of all HAAs, the use of BGE organic modifiers such as methanol and acetonitrile was evaluated. The addition of methanol did not improve the separation of HAAs, only a decrease in EOF and, consequently, higher analysis times were obtained. In contrast, ACN affected both, EOF and HAA electrophoretic mobilities, as it can be seen in Fig. 2A where the effect of ACN in the BGE (from 10 to 30%) is shown. When 20% ACN was added to BGE, separation of all HAAs in normal polarity mode was achieved. Higher ACN contents produced comigration of DCAA and MBAA, so 20% was proposed as optimal organic amount.

To apply the LVSS enrichment procedure, the capillary must be first almost filled with a sample (hydrodynamic injection (15 s, 140 kPa)) prepared in a low conductivity matrix. Then, a negative voltage is applied until the sample is pushed out from the capillary through the inlet side by the EOF. The reversal time (i.e. the moment when polarity must be switched) is critical and must be established at the beginning of every working day. In this work, reversal time was established by monitoring the capillary current (at 95% of BGE), being in this case 1.7 min. Figure 2B shows, as an example, the electropherogram obtained by LVSS-CZE of a 500-μg/L HAA standard prepared in Milli-Q water. The application of LVSS enrichment procedure did not produce a loss in electrophoretic separation although an increase in analysis time was observed because of the characteristics of the methodology used. However, it should be pointed out that reversal time strongly depends on sample salinity. For this reason, when samples with different matrices are analyzed, reversal time must be determined separately, increasing then the total analysis time and being a disadvantage in front of the FASI method previously described.

Finally, the presence of high concentrations of different co-ions between BGE and sample matrix in both FASI and LVSS procedures evaluated can result in another preconcentration effect such as transient isotachophoresis although no terminal electrolyte is used [39].

3.3 Quality parameters

Quality parameters of the proposed conventional CZE (hydrodynamic injection), LVSS-CZE and FASI-CZE
methods under optimal conditions were determined and are given in Table 1. The LODs, based on a signal-to-noise ratio of 3:1, were calculated using standard solutions prepared in Milli-Q water at low-concentration levels. The use of conventional CZE with hydrodynamic injection provided LODs around 1 mg/L for all HAAs except for MCAA (5 mg/L), in agreement with values previously described in the literature [31, 32]. When LVSS-CZE was applied, a 25-fold signal enhancement was achieved for all HAAs obtaining LODs around 50 μg/L except for MCAA (200 μg/L). These results are similar to those previously reported for six HAAs [32], although the method here proposed has the advantage of not needing a NaOH solution as the sample matrix. The best sensitivity for HAAs was observed using FASI obtaining LOD values between 4 and 6 μg/L for most of the compounds, except DCAA (11 μg/L) and MCAA (48 μg/L). This represents a signal enhancement higher than 80-fold and up to 300-fold in the best of the cases, providing a method sensitive enough for the analysis of these compounds.

Calibration curves based on peak area ratio (compound/internal standard) at a working range of 5–100 mg/L (CZE), 0.15–2.5 mg/L (LVSS) and 0.03–0.5 mg/L (FASI) were obtained and good linearity, with correlation coefficients ($r^2$) higher than 0.992, was obtained. Run-to-run and day-to-day precisions for HAA quantification were calculated at two concentration levels, a low level ($\sim 3 \times$ LOD) and a medium level (see values in Table 1). To obtain the run-to-run precision, a total of six replicate determinations for each concentration level were carried out, while for the day-to-day precision a total of 18 replicate determinations of each concentration level on three non-consecutive days (six replicates each day) were performed. The relative standard deviations (%RSDs) obtained at medium concentration level with conventional CZE were between 2.4 and 3.7% and between 3.2 and 6.5% for run-to-run and day-to-day precisions, respectively. The values were slightly higher for the low-concentration level, as it can be expected, although always RSD values were lower than 5.5 and 8.3% for the run-to-run and day-to-day

### Table 1. Quality parameters

| Compound | Method | LODs (µg/L) | Sensitive enhancement (SE$_c$)\textsuperscript{a)} | Run-to-run precision, % RSD ($n = 6$) | Day-to-day precision, % RSD ($n = 6 \times 3$) |
|----------|--------|-------------|-----------------------------------------------|--------------------------------------|-----------------------------------------------|
|          |        |             | Relative migration time\textsuperscript{b)} | Conc. (low level)\textsuperscript{c)} | Conc. (medium level)\textsuperscript{d)} | Conc. migration time\textsuperscript{b)} | Conc. (low level)\textsuperscript{c)} | Conc. (medium level)\textsuperscript{d)} |
| MCAA     | CZE    | 5000        | –                                              | 0.40                                 | 5.2                                           | 2.4                                   | 0.8                                   | 5.3                                           | 4.1                                           |
|          | LVSS   | 200         | 25                                             | 0.21                                 | 12.4                                          | 8.5                                   | 2.20                                  | 16                                            | 9.8                                           |
|          | FASI   | 48          | 104                                            | 0.10                                 | 11.0                                          | 4.5                                   | 1.25                                  | 11.4                                          | 10.7                                          |
| DCAA     | CZE    | 1200        | –                                              | 0.30                                 | 4.7                                           | 3.0                                   | 0.35                                  | 7.6                                            | 3.2                                           |
|          | LVSS   | 49.7        | 24.1                                            | 0.25                                 | 12.9                                          | 5.7                                   | 1.84                                  | 12.5                                          | 8.4                                           |
|          | FASI   | 11          | 109                                            | 0.15                                 | 5.1                                           | 3.2                                   | 0.98                                  | 13.8                                          | 10.1                                          |
| TCAA     | CZE    | 1300        | –                                              | 0.25                                 | 5.4                                           | 3.7                                   | 0.35                                  | 8.3                                            | 6.5                                           |
|          | LVSS   | 50.5        | 25.7                                            | 0.26                                 | 9.1                                           | 6.3                                   | 1.26                                  | 15.2                                          | 15.4                                          |
|          | FASI   | 15.8        | 82                                              | 0.15                                 | 6.7                                           | 3.7                                   | 0.78                                  | 7.7                                            | 5.5                                           |
| MBAA     | CZE    | 1200        | –                                              | 0.45                                 | 4.3                                           | 3.7                                   | 0.86                                  | 7.7                                            | 5.9                                           |
|          | LVSS   | 52.4        | 22.9                                            | 0.24                                 | 16.3                                          | 10.0                                  | 1.94                                  | 15.8                                          | 13.7                                          |
|          | FASI   | 6.2         | 194                                             | 0.14                                 | 10.4                                          | 4.4                                   | 1.81                                  | 13.3                                          | 11.9                                          |
| DBAA     | CZE    | 1300        | –                                              | 0.26                                 | 4.7                                           | 3.7                                   | 0.36                                  | 6.8                                            | 3.8                                           |
|          | LVSS   | 49.5        | 26.3                                            | 0.38                                 | 8.2                                           | 4.5                                   | 1.28                                  | 12.2                                          | 10.3                                          |
|          | FASI   | 4.2         | 310                                             | 0.13                                 | 8.0                                           | 5.1                                   | 0.81                                  | 13.4                                          | 11.2                                          |
| TBAA     | CZE    | 1200        | –                                              | 0.28                                 | 5.5                                           | 2.5                                   | 0.40                                  | 5.6                                            | 4.4                                           |
|          | LVSS   | 49.2        | 24.4                                            | 0.25                                 | 7.9                                           | 4.0                                   | 1.10                                  | 9.2                                            | 10.6                                          |
|          | FASI   | 5.8         | 207                                             | 0.12                                 | 5.5                                           | 4.1                                   | 0.68                                  | 13.3                                          | 11.4                                          |
| BCAA     | CZE    | 1200        | –                                              | 0.27                                 | 4.8                                           | 3.5                                   | 0.32                                  | 4.9                                            | 5.8                                           |
|          | LVSS   | 51.1        | 23.5                                            | 0.25                                 | 6.7                                           | 9.0                                   | 1.31                                  | 15.5                                          | 14.7                                          |
|          | FASI   | 6.4         | 188                                             | 0.11                                 | 6.6                                           | 4.5                                   | 0.58                                  | 12.2                                          | 13.9                                          |
| BDCAA    | CZE    | 1300        | –                                              | 0.26                                 | 5.4                                           | 3.3                                   | 0.37                                  | 5.5                                            | 3.4                                           |
|          | LVSS   | 49.1        | 26.5                                            | 0.24                                 | 7.0                                           | 7.6                                   | 1.14                                  | 13.5                                          | 15.3                                          |
|          | FASI   | 6.5         | 224                                             | 0.13                                 | 6.1                                           | 3.6                                   | 0.51                                  | 12.6                                          | 11.6                                          |
| CDBAA    | CZE    | 1300        | –                                              | 0.27                                 | 4.5                                           | 3.4                                   | 0.37                                  | 6.3                                            | 5.9                                           |
|          | LVSS   | 50.1        | 25.9                                            | 0.25                                 | 9.3                                           | 7.9                                   | 1.12                                  | 11.4                                          | 14.8                                          |
|          | FASI   | 5.8         | 200                                             | 0.13                                 | 5.7                                           | 4.3                                   | 0.54                                  | 13.5                                          | 11.7                                          |

\textsuperscript{a)} SE$_c$ = LOD (CZE)/LOD (LVSS or FASI).

\textsuperscript{b)} analyte migration time/internal standard migration time.

\textsuperscript{c)} × LOD.

\textsuperscript{d)} CZE: ~25 mg/L; LVSS: ~600 µg/L; FASI: ~350 µg/L.
3.4 Analysis of water

Although FASI-CZE provided LODs in Milli-Q water lower than the values established by legislation [6–8, 10], this sensitivity is difficult to be achieved when analyzing real water samples since in-line preconcentration procedures based on modifications in electrophoretic conditions are strongly dependent on sample salinity. To evaluate the performance of FASI-CZE method for the analysis of real water, LODs were determined in two bottled mineral water samples of different salinity content (water 1 of 663 μS/cm and water 2 of 1187 μS/cm) free of HAAs which were spiked at very low concentration levels. As expected, an increase in sample salinity produced a decrease in the FASI signal enhancement achieved, resulting in LODs between 39 and 530 μg/L (water 1) and between 92 and 1200 μg/L (water 2), which are 9–25 times higher than those observed in Milli-Q water. As a result, the removal of matrix salinity from real water samples is mandatory for a suitable application of this in-line enrichment procedure.

To remove sample salinity, the use of Oasis WAX cartridges (150 mg), specifically proposed for preconcentration of acidic species, was evaluated [38]. The breakthrough volume was determined using a water sample free of HAAs (water 2) spiked at several concentration levels with the sample amount kept constant (200 ng for each HAA). Therefore, sample volume was increased (2–250 mL) and the concentration of HAAs was decreased (100–0.8 μg/L). Sample volumes higher than 250 mL were not studied because the total analysis time would be too long. After preconcentration, the FASE-CZE method was applied. Recoveries were then calculated by comparing the peak areas with those obtained from a control sample at a concentration representing 100% recovery (200 μg/L). Recoveries higher than 90% were obtained for all compounds except MBAA and MCAA that showed a recovery around 65% (Table 2). A decrease on the recoveries was observed when volumes higher than 100 mL were used, so this volume was chosen as optimum. LODs were determined using a water sample free of HAAs (bottled mineral water 2) and values between 0.05 and 0.2 μg/L were obtained for almost all HAAs (0.8 μg/L for MCAA) (Table 2), which represents a enhancement between 6250 and 26 000-fold when compared with conventional CZE values. However, it should be pointed out that robustness of the proposed method will be strongly dependent on sample salinity, observing higher LOD values for samples with important salinity content such as the case of some drinking tap waters. Nevertheless, these LODs were always below the maximum contaminant levels stipulated by the EPA (60 μg/L for the sum of five HAAs) [6–8] and the WHO (20–200 μg/L for some HAAs) [10] for drinking water. Therefore, the combination of SPE using Oasis WAX cartridges and FASI-CZE for in-line enrichment can be proposed for the analysis of HAAs in drinking waters at the levels established by present legislation. With the proposed method, the total sample treatment time per sample is about 2 h (preconcentration, evaporation and redissolution), but 12 samples can be treated simultaneously using the Visiprep System from Supelco. Hence, the total sample throughput per day could be higher than 48 samples.

A tap water from Barcelona (Spain) was analyzed using the proposed method. Figure 3 shows the electropherogram obtained when 100 mL were preconcentrated by SPE and analyzed by FASI-CZE. All HAAs except MCAA and MBAA were detected. Quantitation using standard addition calibration was performed, and the concentration levels found are given in Table 2.

Concentrations in the range 3–13 μg/L were found for the individual compounds being DCAA, TCAA and DBAA the HAAs present at higher concentration (11 ± 0.9, 12 ± 0.9 and 13 ± 1.1 μg/L, respectively). Brominated and mixed (chlorinated/brominated) species represent an important fraction (60%) of the total HAAs. The presence of these compounds has been described in Barcelona tap water [16, 40, 41] and can be explained because the raw water used in the drinking water treatment plant (DWTP) is rich in bromide [40, 42]. The concentrations found were similar to those described in previously reported analysis of Barcelona tap water [16, 40, 41]. Despite the presence of HAAs in the

| Compound | MLLODs (μg/L) | Sensitive enhancementa | Recoveries (%) | Barcelona tap water (μg/L) |
|----------|--------------|------------------------|----------------|---------------------------|
| MCAA     | 0.8          | 6250                   | 64             | n.d.                      |
| DCAA     | 0.1          | 12 000                 | 91             | 11 ± 0.9                  |
| TCAA     | 0.2          | 6500                   | 92             | 12 ± 0.9                  |
| MBAA     | 0.1          | 12 000                 | 67             | n.d.                      |
| DBAA     | 0.05         | 26 000                 | 91             | 13 ± 1.1                  |
| TBAA     | 0.07         | 17 140                 | 90             | 9 ± 0.8                   |
| BCAA     | 0.06         | 20 000                 | 93             | 3 ± 0.3                   |
| BDCAA    | 0.08         | 16 250                 | 90             | 6 ± 0.5                   |
| CDBAA    | 0.07         | 18 570                 | 90             | 4 ± 0.3                   |

a) LOD (CZE method)/LOD (SPE-FASI-CZE method).
tap water, the total concentration for the sum of the five HAAs legislated by USEPA (MCAA, DCAA, TCAA, MBAA and DBAA) was 36 μg/L, which is lower than the MCL (60 μg/L) established by the USEPA [6–8]. Therefore, this drinking water is suitable for consumption.

4 Concluding remarks

Two in-line enrichment procedures (LVSS and FASI) were evaluated to enhance sensitivity in the analysis of HAAs by CZE. LODs ~25-fold (LVSS) and between 82- to 310-fold (FASI) lower than those achieved by CZE without preconcentration were obtained for standards in Milli-Q water. Since better detection limits were obtained for the FASI-CZE method, it was proposed for the analysis of HAAs in water samples. To remove sample salinity and improve sensitivity when dealing with real water samples ion-exchange SPE is recommended. Good results for drinking water were obtained with the SPE-FASI-CZE method, with LODs down to 0.05–0.8 μg/L and recoveries, in general, higher than 90% (~65% for MCAA and MBAA). The method was applied to the analysis of Barcelona (Spain) tap water and seven HAAs were found, with concentrations ranging from 3 to 13 μg/L. The results of this study showed that the combination of SPE with Oasis WAX cartridges and FASI-CZE in-line enrichment can be used for the analysis of HAAs in drinking water samples at the levels established by current legislation.

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