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Journal of Chromatography A, 808 (1998) 229–236

JOURNAL OF CHROMATOGRAPHY A

Capillary zone electrophoresis with indirect UV detection of haloacetic acids in water

D. Martínez[a], J. Farré[a], F. Borrull[a], M. Calull[a,*], J. Ruana[b], A. Colom[b]

[a]*Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili, Placa Imperial Tarraco 1, 43005 Tarragona, Spain*

[b]*Consorci d'Aigües de Tarragona, Car. Nac. 420, PK-883, Apt. 886, 43080 Tarragona, Spain*

Received 16 October 1997; received in revised form 2 February 1998; accepted 2 February 1998

Abstract

A capillary zone electrophoresis (CZE) system for determining haloacetic acids in water was optimized with indirect photometric detection. Two different carrier electrolytes, potassium hydrogenphthalate and sodium 2,6-naphthalenedicarboxylate, were evaluated in terms of sensitivity and two different electroosmotic flow modifiers, tetradecyltrimethylammonium bromide and hexadecyltrimethylammonium bromide, were tested. Parameters such as electrolyte concentration and pH, and the concentration of the electroosmotic flow modifiers, which affect the CZE separations, were investigated. The method was used to determine haloacetic acids in chlorine tap water using the liquid–liquid extraction process.

Keywords: Water analysis; Haloacetic acids; Carboxylic acids

1. Introduction

The practice of chlorination as a means for disinfecting drinking water has been found to be responsible for the production of chlorinated organic compounds [1]. The US Environmental Protection Agency (EPA) has promulgated regulations to control disinfection by-products (DBPs) [2]. The DBPs selected for control include several haloacetic acids (HAAs): monochloroacetic acid (MCAA), monobromoacetic acid (MBAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), bromochloroacetic acid (BCAA) and dibromoacetic acid (DBAA). They are formed by the oxidation of natural waters with chlorine except in the case of brominated species which require bromide [3]. These compounds are considered to be the second most prevalent group of known DBPs; the primary group is the trihalomethane compounds. Toxicological studies indicate that DCAA and TCAA are animal carcinogens [4]. The EPA has proposed the disinfectants/disinfection by-products (D/DBPs) rule, the first stage of which establishes maximum contaminant levels (MCLs) of 60 $\mu g\ l^{-1}$ for the sum of the five regulated HAAs (all except BCAA) [2,5,6].

Studying the formation and distribution of HAAs in an aquatic environment continues to be an important and challenging task [7]. Present methods, including EPA Method 552.1, involve extraction of the acids using organic solvents followed by derivatization using methyl esters for analysis by gas chromatography [3,8]. Derivatization steps are time

*Corresponding author.

0021-9673/98/$19.00
PII: S0021-9673(98)00101-0

consuming and many derivatization reagents including diazomethane are toxic, carcinogenic and explosive. Therefore, methods for directly analysing haloacetic acids without the need for derivatization are desirable. Reversed-phase ion-pair chromatography (RP-IPC) with indirect detection has also been used to determine some of these compounds using UV detection, but the analysis time was long and the detection limits were not very good [9].

Capillary electrophoresis (CE), including capillary zone electrophoresis (CZE), has been used to separate charged analytes [10,11]. In the environmental field, CE has become increasingly popular because it gives high resolution separations for certain pollutants [12–18]. In CE the migration time, resolution and separation efficiency depend on the applied voltage, the electrophoretic mobility of the charged species and the electroosmotic flow (EOF) [19,20]. The combined effects of other separation parameters, such as composition, pH and concentration of the background electrolyte must also be considered.

Most works published about the analysis of inorganic and organic anions use indirect detection because UV–Vis absorption detectors are still the most popular in CZE because of their versatility and simplicity [21–23]. If the appropriate electrolyte composition is selected for indirect absorption, sensitivity will be high. Various chromophore compounds based on chromate, benzoate, phthalate and other aromatic carboxylic acid salts have been characterized for the analyses of inorganic and organic anions by CZE in many real samples [11,24–27].

The aim of this work was to study whether CZE is suitable for separating the HAAs as an alternative to the gas chromatography EPA method for determining these compounds in tap water. We have compared the sensitivity obtained with two different carrier electrolytes, as potassium hydrogenphthalate and sodium 2,6-naphthalenedicarboxylate. Parameters such as concentration and electrolyte pH, which influence CZE separation, and various EOF modifiers have been investigated.

To analyze anionic species, like haloacetic acids at pH above their pK_a, it is necessary to modify the normal electrode configuration, because these species migrate towards the positive electrode (anode), that is, away from the detection end of the capillary. Their migration is therefore in the opposite direction to the EOF. While this approach is recommended for large, relatively immobile ions, such as proteins and peptides, it is not appropriate for mobile anionic solutes, such as haloacetic acids. The rate of migration of these latter solutes toward the anode exceeds the magnitude of the EOF, so they do not reach the detector. This situation can be rectified by addition to the electrolyte of a cationic surfactant, such as hexadecyltrimethylammonium bromide (CTAB) or tetradecyltrimethylammonium bromide (TTAB), which has the effect of reversing the direction of the EOF so that it flows from the negative electrode to the positive electrode. If the instrumentation is configured with the negative electrode (cathode) at the inlet end of the capillary and the positive electrode (anode) at the outlet (detector) end, then the anionic solutes will migrate in the same direction as the EOF and will reach the detector. This combination of reversed EOF and anodic detection results in rapid, high efficiency separations of a wide range of organic acids [28].

The drawback of CE is the small sample volume introduced into the capillary which gives poor detection limits and leads to a major problem when attempting to analyze relatively dilute analyte mixtures, particularly those from environmental sources. One way of circumventing poor limits of detection is to concentrate the analytes off line, and in this work we use off-line liquid–liquid extraction prior to separation to determine these compounds in tap water samples. This extraction system is the same as the one used to extract different DBPs [29–31] but the solvent had to be changed before CE analysis. The selectivity of this method was checked for tap water from Tarragona and Barcelona.

2. Experimental

2.1. Instrumentation

Measurements were made on a Hewlett-Packard Model ^{3D}CE instrument (HP, Waldbronn, Germany) equipped with a UV detector. Data were collected with the HP Chemstation version A.04.01 chromatographic data system. All CZE experiments were performed using uncoated fused-silica capillary tub-

ing (64.5 cm×75 μm I.D.) supplied by Supelco (Bellefonte, PA, USA). A detection window was prepared by burning off the polyimide coating 56 cm from the capillary inlet. Samples were introduced by hydrodynamic injection.

2.2. Chemicals

The HAAs studied were: (1) monochloroacetic acid (MCAA), (2) monobromoacetic acid (MBAA), (3) dichloroacetic acid (DCAA), (4) bromochloroacetic acid (BCAA), (5) dibromoacetic acid (DBAA) and (6) trichloroacetic acid (TCAA). Individual standards were obtained from Merck (Darmstadt, Germany) except BCAA, which was only available in a commercial standard solution of 1000 mg l^{-1} of all HAAs. This commercial solution was obtained from J.T. Baker (Deventer, Netherlands). An individual standard solution of 2000 mg l^{-1} of each compound, except BCAA, was prepared with water which had been purified by a Milli-Q system (Millipore, Bedford, MA, USA). Standard working solutions were prepared weekly or daily, depending on their concentration. All solutions were stored at 4°C in the refrigerator.

Potassium hydrogenphthalate (phthalate) and 2,6-naphthalenedicarboxylic acid dipotassium (NDC) were obtained from Aldrich (Milwaukee, WI, USA) and they were studied as electrolytes. CTAB (Sigma, St. Louis, MO, USA) and TTAB (Fluka, Buchs, Switzerland) were used as EOF modifiers. Sodium hydroxide (Aldrich) was used to adjust the electrolyte pH.

Methyl *tert*.-butyl ether (MtBE) (Merck) was used in the extraction step as an organic phase. Sodium sulfate (Probus, Badalona, Spain) and copper(II) sulfate (Probus) were used to increase the extraction efficiency. Concentrated sulphuric acid (Probus) was used to adjust the pH of the sample.

2.3. Extraction process

The off-line trace enrichment process was carried out using a liquid–liquid extraction step. This extraction process has been described elsewhere [7,8,27–29]. Before the extraction step, 30 ml of sample was adjusted to pH 0.5 with concentrated H_2SO_4 and the extraction efficiency was increased with 12 g of Na_2SO_4 and 3 g of $CuSO_4$. Three ml of MtBE was added as an organic phase to the sample and then the mixture was shaken for 15 min in an orbital shaker (Selecta, Abrera, Spain). 2.5 ml of organic phase was separated and concentrated through nitrogen current almost to dryness and then 100 μl of deionized water was added.

2.4. Electrophoretic conditions and system operation

A solution of 12 m*M* of phthalate and 0.5 m*M* of CTAB as EOF modifier at pH 6 adjusted with NaOH was chosen as CE electrolyte when phthalate electrolyte was used.

All NDC electrolytes were prepared daily from the stock solution which contained 20 m*M* NDC. The best resolution was achieved for a solution of 4 m*M* NDC and 0.5 m*M* CTAB with a pH of 7.5.

Separations were carried out by rinsing the capillary for 3 min with a background electrolyte immediately before the injection. At the beginning of each experimental day, the capillary was washed with 0.1 *M* NaOH for 15 min and then rinsed with deionized water (10 min) and the used electrolyte (5 min).

The detector was set at 254 nm and 235 nm for the phthalate and NDC electrolyte, respectively (indirect UV detection). The capillary temperature was kept constant at 25°C. For the phthalate electrolyte the injection was made hydrodynamically at a pressure of 40 mbar for 20 s, and the separation voltage applied was −20 kV. For the NDC electrolyte the injection was the same as the phthalate electrolyte, and the separation was made at −20 kV for 4.5 min and then a linear gradient to −15 kV in 0.5 min and finally the same potential for a further 3 min.

3. Results and discussion

Several electrolytes have been used to analyze ionizable compounds. In this work phthalate and NDC were chosen to separate HAAs because the ionic mobilities of the carrier electrolytes and the sample ions mismatched [32].

The effect of parameters such as electrolyte concentration, pH and various EOF modifiers were

investigated to determine the optimum conditions for separating haloacetic acids with CZE.

The electrolyte concentration was chosen to maximize indirect UV absorbance and minimize noise. The absorbance was increased by simply increasing the electrolyte concentration. However, at concentrations that were too high the signal noise usually increased beyond an acceptable level. When the electrolyte concentration increased, electrolyte conductivity rose and baseline noise due to additional Joule heating increased as well [19,20].

In this study, a standard solution of 20 mg l^{-1} of HAAs was introduced hydrodynamically into the capillary at 40 mbar for 20 s and separation was carried out at −20 kV when the phthalate electrolyte was used. Several concentrations of electrolyte between 5 and 12 m*M* at pH 6 were tested to give the best separation. In all cases CTAB was added at a concentration of 0.5 m*M*. The increase in phthalate concentration caused a rise in the absolute value of the current from −16 μA at 5 m*M* phthalate to −35 μA at 12 m*M* with a −20 kV separation voltage. The optimum phthalate value was found to be 12 m*M* which is the best compromise between peak separation and sensitivity. Higher electrolyte concentrations were studied but baseline noise were increased.

Under the same conditions, and using a phthalate concentration of 12 m*M*, the influence of the electrolyte's pH value on the separation was studied. Fig. 1 shows this influence on the migration time of HAAs in the range between 4 and 10. As can be seen there is no significant dependence on pH for values higher than 6 in the studied range. It might be due to the smaller influence of this variable in EOF for these values, since all the analyzed acids are ionized completely (pK_a values range 0.63–2.90). A pH value of 6 was chosen because a good resolution between peaks with a short analysis time could be obtained under these conditions.

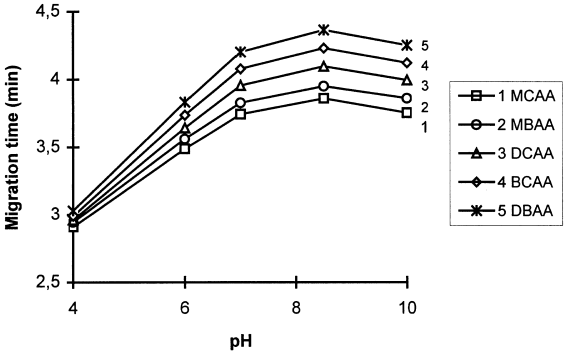

Fig. 1. Effect of the pH of the phthalate electrolyte on the migration time 20 mg l^{-1} of a haloacetic acid standard mixture. Electrolyte: 12 m*M* phthalate, 0.5 m*M* CTAB. Injection: 40 mbar, 20 s. Separation voltage: −20 kV.

Two different EOF modifiers (CTAB and TTAB) were tested. The results can be seen in Fig. 2a and Fig. 2b, respectively. These compounds are electrostatically attracted to the silanol groups on the inner wall of the capillary, and this results in a negative silica change being shielded which directly influences the direction and magnitude of EOF [10]. When TTAB was used, baseline distortion was higher in the first minutes of the analysis, and no significant differences in the analysis speed with the long alkyl group modifiers TTAB and CTAB were observed.

The EOF in fused-silica capillaries is directed toward the positive electrode, after adding CTAB or TTAB, and the voltage applied must have a negative

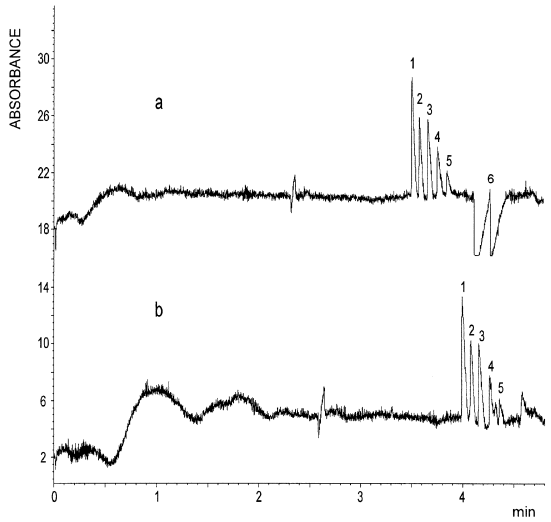

Fig. 2. Electropherograms of 20 mg l^{-1} of a haloacetic standard mixture obtained with the phthalate electrolyte with (a) 0.5 m*M* CTAB and (b) 0.5 m*M* TTAB. Other conditions as in Fig. 1. Peaks: 1=monochloroacetic acid (MCAA), 2=monobromoacetic acid (MBAA), 3=dichloroacetic acid (DCAA), 4=bromochloroacetic acid (BCAA), 5=dibromoacetic acid (DBAA), 6=trichloroacetic acid (TCAA).

polarity if these compounds are to be separated and detected. The voltage applied during the separation ranged between −15 to −25 kV and the best results were obtained for a voltage of −20 kV. TCAA could not be determined with this electrolyte system, because an interference coelutes with this compound (see Fig. 2a and Fig. 2b). A gradient voltage was studied in order to improve the separation of this compound, but results were no better.

We studied the NDC electrolyte system as a means for separating HAAs. It has a strong UV absorbance at 235 nm which enables these compounds to be detected. We paid attention to the change in their migration time with NDC concentration. As can be seen in Fig. 3, the differences in the range studied (2–10 m*M* NDC) were not significant.The electrophoretic conditions were the following: a standard solution of 20 mg l^{-1} of HAAs was introduced into the capillary at 40 mbar for 20 s using 0.5 m*M* of CTAB as EOF modifier at pH 9.5 with a separation voltage of −20 kV. We chose an NDC concentration of 4 m*M* because resolution was good, baseline noise low and analysis time short at this level of concentration. It should be mentioned that in all cases TCAA gave a poor sharp shape because of a negative interference, as in the phthalate electrolyte but in this case the separation of this compound improved when a gradient voltage was applied. The best results were obtained by applying −20 kV for 4.5 min followed by a linear gradient voltage to −15 kV in 0.5 min and finally the same voltage for the remainder of the analysis. Fig. 4 shows the improvement (Fig. 4a at a constant separation voltage of −20 kV and Fig. 4b applying the proposed linear gradient voltage). In this case the standard solution only contained five haloacetic compounds. That is to say, all of them except BCAA.

Studies were made of the pH and the EOF modifier with this electrolyte. The pH ranged from 6.5 to 10.5, and did not significantly depend on most of the compounds. The EOF modifier was studied in the same way as with the phthalate electrolyte and results show no significant differences between TTAB and CTAB. On the basis of the results reported above, 4 m*M* of NDC, a pH value of 7.5 and 0.5 m*M* of CTAB with a gradient separation voltage were used to separate the HAAs.

Fig. 5a shows an electropherogram of a standard solution of HAAs under the optimum conditions when the NDC electrolyte was used and Fig. 5b

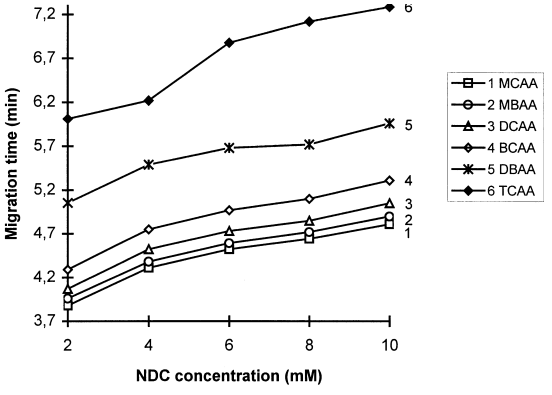

Fig. 3. Effect of NDC concentration on the migration time of 20 mg l^{-1} of a haloacetic acid standard mixture. Electrolyte: NDC, 0.5 m*M* CTAB, pH 9.5. Injection: 40 mbar, 20 s. Separation voltage: −20 kV.

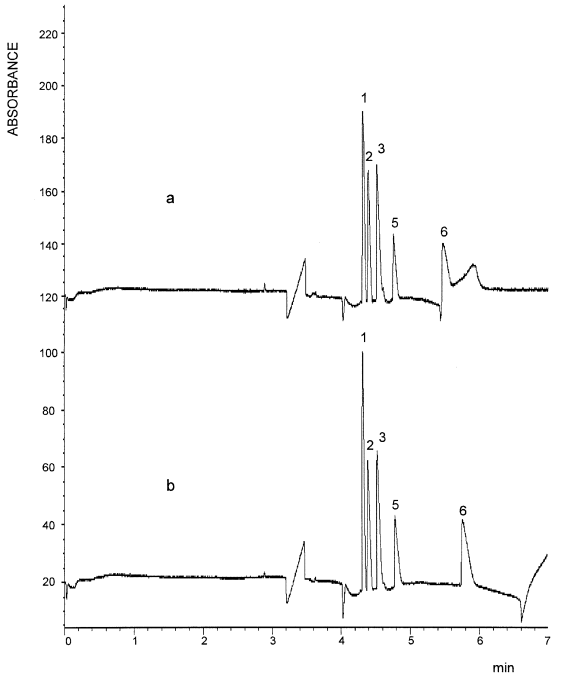

Fig. 4. Electropherograms of 20 mg l^{-1} of a haloacetic standard mixture obtained with the 4 m*M* NDC electrolyte at (a) a separation voltage of −20 kV and (b) a voltage gradient of −20 kV for 4.5 min followed by a linear gradient voltage to −15 kV in 0.5 min and finally the same voltage for the rest of the analysis. Other conditions as in Fig. 3.

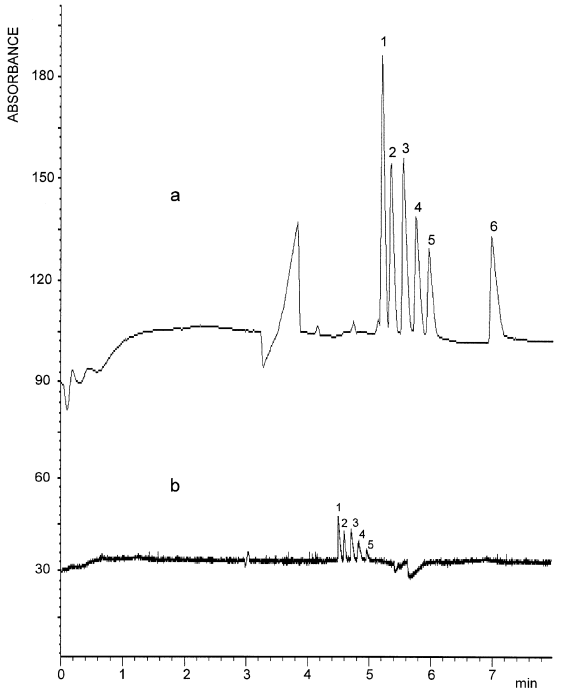

Fig. 5. Electropherograms of 20 mg l^{-1} of a haloacetic standard mixture obtained under optimum conditions with (a) the NDC electrolyte and (b) the phthalate electrolyte. See Section 2.4 for more details.

shows the same analysis using the phthalate electrolyte. These results confirm that when the NDC electrolyte was used, the signal was higher and, therefore, the detection limits lower. This may be due to the fact that NDC has higher molar absorptivity than phthalate. Moreover, with the NDC electrolyte TCAA was detected without interferences.

Quantitative determination was carried out using a hydrodynamic injection. The variation of the peak area with the amount of sample was investigated using both electrolyte systems. When this study was made with the phthalate electrolyte, the standard solution available only contained five haloacetic compounds (all except BCAA). The results are given in Table 1. Within the concentration range studied there was a good correlation between peak area and concentration for each compound. The limits of detection (LODs) were calculated using a signal-to-noise ratio of 3. In the phthalate electrolyte, the values were between 2 mg l^{-1} for MCAA and 5 mg l^{-1} for DBAA, and in the NDC electrolyte they were between 0.15 mg l^{-1} for DCAA and 0.9 mg l^{-1} for DBAA. As can be seen, when the NDC electrolyte was used detection limits were lower.

Repeatability was examined by performing 10 replicate injections of each compound at a concentration of 25 mg l^{-1} in the phthalate electrolyte and 15 mg l^{-1} in the NDC electrolyte and the results were similar in both cases. The area relative standard deviations (R.S.D.s) were between 1.1% for MCAA and 3.4% for DBAA in the phthalate electrolyte and between 1.1% for MCAA and 4.2% for BCAA in the NDC electrolyte.

We studied the proposed NDC method as a means for determining these compounds in tap water. Liquid–liquid extraction [29–31] was used prior to

Table 1
Study of the linearity of the response, LOD and precision using NDC and phthatale electrolytes

Peaks	Anion	NDC				Phthalate			
		Linear range (mg l^{-1})	r^2	LOD[a] (mg l^{-1})	R.S.D.[b] (%)	Linear range (mg l^{-1})	r^2	LOD[a] (mg l^{-1})	R.S.D.[c] (%)
1	MCAA	1–30	0.999	0.40	1.1	5–100	0.999	2.0	1.1
2	MBAA	2–30	0.998	0.75	2.0	5–100	0.999	2.5	2.0
3	DCAA	1–20	0.999	0.15	1.5	5–100	0.999	2.5	1.5
4	BCAA	2–20	0.999	0.50	4.2	–	–	–	–
5	DBAA	2–20	0.999	0.90	3.4	10–100	0.999	5.0	3.4
6	TCAA	2–20	0.999	0.50	1.9	–	–	–	–

[a] LOD is defined as three times the noise.
[b] Calculated for 10 consecutive runs at 15 mg l^{-1}.
[c] Calculated for 10 consecutive runs at 25 mg l^{-1}.

the CZE analysis which enabled these compounds to be detected at μg l^{-1} levels. The study of the recovery of the extraction process was made using standard compounds. In this case, the efficiency of this step was evaluated and the results obtained are shown in Table 2.

The linearity of the response for each compound, the correlation coefficient (r^2) and detection limits were studied using this liquid–liquid extraction step. Calibration graphs constructed for standard solutions in Milli-Q water showed that within the concentration range studied (10–60 μg l^{-1}) there was an acceptable correlation r^2 higher than 0.998 for all compounds except for MCAA, for which it was 0.980. Higher concentrations were tested but the resolution between peaks was poor. Limits of detection were between 3 μg l^{-1} for DCAA and 5 μg l^{-1} for the rest of the haloacetic compounds.

The method was tested with tap water. Fig. 6 shows the electropherograms for the analysis of 30 ml of standard solution of 30 μg l^{-1} (Fig. 6a), 30 ml of Tarragona tap water from the Ebro river (Fig. 6b) and 30 ml of Barcelona tap water from the Llobregat river (Fig. 6c). Several peaks with the same retention time as the HAAs appeared in the electropherograms. They were positively identified when this sample was compared with the same sample spiked with 15 μg l^{-1} of a standard solution. The presence of these compounds was confirmed by the GC–MS standard method. Table 3 shows the results obtained analysing the different samples. In the tap water from Tarragona DCAA, BCAA and TCAA were quantified and DBAA was identified but it could not, however, be quantified because its concentration was between the detection limit and the quantification limit of the method. The same occurred with the tap water from Barcelona where BCAA and DBAA were quantified and MBAA, DCAA and TCAA were only identified.

Table 2
Recovery study of the extraction process

Peak	Compound	Recovery (%)
1	MCAA	66
2	MBAA	60
3	DCAA	70
4	TCAA	80
5	BCAA	75
6	DCAA	70

[a] Calculated for a standard solution of 500 μg l^{-1}.

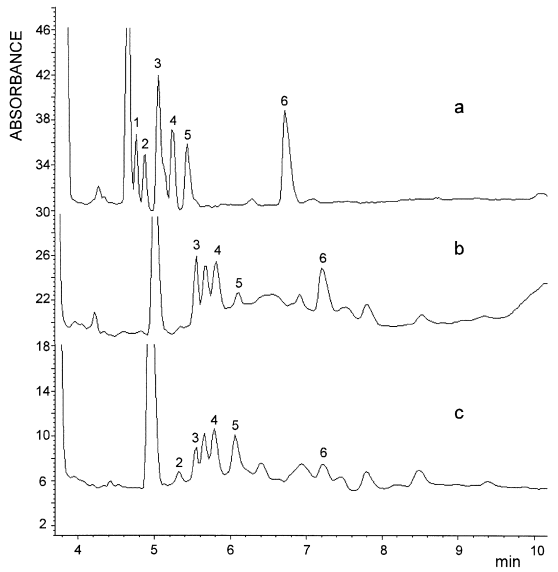

Fig. 6. Electropherograms obtained from the analysis of (a) 30 ml of standard solution of 30 μg l^{-1}, (b) 30 ml of tap water from Tarragona and (c) 30 ml of tap water from Barcelona. Electrolyte: 4 m*M* NDC, 0.5 m*M* CTAB, pH: 7.5. Injection: 40 mbar, 20 s. Separation voltage gradient: −20 kV for 4.5 min followed by a linear gradient voltage to −15 kV in 0.5 min and finally the same voltage for the rest of the analysis.

4. Conclusions

The method developed here for analyzing HAAs by CE is a good alternative to the standard GC method. Of the two electrolytes studied, phthalate and NDC, the latter has better sensitivity and selectivity for these compounds. On the other hand, the type and concentration of EOF modifier show no significant effect. The method enables HAAs to be determined in only 8 min. This shorter analysis time is an advantage over the GC method, where analyses of up to 30 min are normally required. Another important advantage is that no derivatization step is necessary to analyze these compounds.

The standard liquid–liquid extraction method was required to determine these compounds in natural

Table 3
Analytical results of different tap waters

Peak	Compound	Tap water from Tarragona[a]	Tap water from Barcelona[a]
1	MCAA	–	–
2	DBAA	–	<LOQ
3	DCAA	8	<LOQ
4	BCAA	16	18
5	DBAA	<LOQ	8
6	TBAA	14	<LOQ

LOQ=Limit of quantification.
[a] These values are defined in $\mu g\ l^{-1}$.

water. The overall method determines these compounds with low detection limits. The method was tested with tap water and some of the compounds studied were found and quantified.

Acknowledgements

We wish to thank the Consorci d'Aigües de Tarragona for financial support given.

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