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Preconcentration and separation of haloacetic acids by ion chromatography

Corrado Sarzanini*, Maria Concetta Bruzzoniti, Edoardo Mentasti

Department of Analytical Chemistry, University of Turin, Via P. Giuria 5, 10125 Turin, Italy

Abstract

A comparative study was made of the chromatographic behaviour of five haloacetic acids (mono-, dibromoacetic and mono-, di-, trichloroacetic acids). The techniques investigated included reversed-phase ion interaction chromatography with UV detection, suppressed and non-suppressed anion-exchange chromatography. The systems are discussed studying the retention as a function of the mobile phase parameters and the stationary phases used (LiChrospher 100 RP-18, IonPac AS9, AS10 and AS11). A preconcentration step, performed on different substrates, namely LiChrolut-EN and activated vegetal carbon, has been optimized in order to reduce the method detection limits. Results obtained for drinking water samples are shown.

Keywords: Water analysis; Sample handling; Haloacetic acids; Organic acids

1. Introduction

In the last several years particular attention has been focused on environmental contaminants in various compartments, for example drinking waters. Together with trihalomethanes, halo-derivatives of alkanes/alkenes, nitriles, ketones, esters, aldeydes, amines, ethers and aromatics, haloacetic acids can be found in trace amounts in drinking waters as chlorination by-products after disinfection processes [1,2]. The haloacetic acids are formed by the chlorination of natural organic (humic and fulmic) matter. Utilities using chlorine as a water disinfectant generate haloacetic acids, usually as the second most prevalent group of known disinfection by-products, the primary group being usually formed by trihalomethanes.

For the sake of completeness, other fields in which haloacetic acids are involved will be briefly cited. Mono- and dichloroacetic acids are supposed to be the product of the hydrolysis of chlorinated acyl chlorides in the troposphere chemical reactions [3]. Mono- and trichloroacetic acids are not only used as intermediates for the manufacture of drugs, dyes and chemicals, but also as herbicides [4], while dichloroacetic acid is used in pharmaceutical syntheses.

Haloacetic acids (di-, trichloro-, bromochloro-, di-, tribromoacetic acid) were found to be carcinogenic at low concentrations [5], and therefore federal regulation for their monitoring is being considered. The standard method for their determination [6] requires quite a long procedure based on a liquid–liquid extraction with methyl terbutyl ether, followed by esterification with diazomethane and gas chromatographic analysis. The long procedure required for haloacetic acids analysis is compensated by the good quantitation limits achieved ($\approx$0.5 μg/l).

Due to the presence of electronegative atoms in their molecular structure, specific detectors such as

*Corresponding author. Tel.: +39-11-6707628; fax: +39-11-6707615.

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electron-capture and mass spectrometry have been used in the gas chromatographic analysis of haloacetic acids [7–9].

Since haloacetic acids determination is quite a new environmental concern, few references are available dealing with development of liquid chromatographic analytical methods for their analysis and with applications to drinking waters.

Anion-exchange chromatography has been used by Fuchs and Bächmann [3] for the determination of mono- and dichloroacetic acids in atmospheric precipitation samples. Even if applied to the determination of monochloroacetic acid only, Simon et al. [10] used an ion-exclusion technique coupled to a sorption on Florisil sorbent to analyse airborne aliphatic carboxylic acids. Mono-, di- and trichloroacetic acids have been determined by reversed-phase liquid chromatography and detected by UV spectroscopy [11,12] and applied to the analysis of process effluent of a monochloroacetic plant. Two chromatographic methods have been described by Nair et al. [13] for mono- (MCA), di- (DCA), trichloroacetic (TCA), mono- (MBA), dibromoacetic (DBA) acids based on the anion-exchange and ion-exclusion mechanisms. The first, coupled with a suppressed conductometric detection, gave detection limits ranging from 8 to 80 μg/l and was applied for the determination of DCA, DBA and TCA in a drinking water sample. Ion-exclusion separation, coupled with UV detection at 210 nm, provided detection limits ranging between 5 and 90 μg/l and was applied to the determination of DCA and TCA in a herbicide sample.

Recently, a liquid chromatographic method coupled with negative ionization electrospray mass spectrometry has been proposed by Hashimoto and Otsuki [14]. The method, applied to the the nine haloacetic acids containing bromine and chlorine, requires an extraction of the analytes with methyl *tert*-butyl ether and a concentration under a controlled stream of dry nitrogen. Although the good limits of detection reached (0.003–0.070 μg/l for 200 ml of water samples, sample loop 25 μl) few details on chromatographic separation of mixture of analytes are provided. Moreover coelutions among dichloro-, bromochloro-, tribromo-, chlorodibromoacetic acids are not resolved.

A method which couples amperometry as a detection system after anion-exchange separation has been presented by Akhtar et al. [2]. In this work, conducting polymer modified (polypirrole/sulfobenzoic acid) microelectrodes have been used as sensors for haloacetic acids. The electroactivity of the polymer modified electrode is associated with the incorporation and expulsion of the anions of the acids. The detection limits, determined for MCA, DCA, TCA and DBA, ranged from 1 to 100 μg/l. The most interesting aspect concerning the conducting polymer sensors is the selectivity tunable through the manipulation of the composition of the polymer and of the potentials applied.

Specially constructed all-solid-state tubular flow-through ion selective electrodes have been used for the on-line detection of MCA in industrial processes for the production of zwitterionic surfactants in excess of chloride ions up to 1 *M* [15]. The system consists of a separation step from chloride through an anion-exchange column. Although the determination is simple, the technique can be applied for MCA concentration included within 10^{-4} and 10^{-1} *M* in real samples. Titrimetric, potentiometric and polarographic techniques have also been employed for TCA determination in water [11].

The aim of this work has been the study and the optimization of chromatographic procedures (based on the ion-interaction and anion-exchange mechanisms) for the separation of MBA, DBA, TBA, DCA and TCA acids in the analysis of drinking waters. An ion-interaction chromatographic (IIC) separation coupled with UV detection has been optimized studying the effect of mobile phase composition in the presence of different pairing ions (tetrabutylammonium and cetyltrimethylammonium). The anion-exchange separations have been carried out on various stationary phases (IonPac AS9, AS10 and AS11) and coupled with different detection systems (spectrophotometric and conductimetric). According to the detection system used, different mobile phase components have been used and their effect on the separation of haloacetic acids has been studied.

The methods have been compared and optimized in the presence of nitrate and chloride anions which commonly occur in drinking water.

In order to improve detection limits, an off-line preconcentration method, based on the reversed-phase (RP) mechanism has been used in non-sup-

pressed IC. The preconcentration step, performed in acidic medium, has been chosen in order to reduce the interference of inorganic anions (Cl^- and NO_3^-), using hydrophobic interactions between haloacetic acids and the RP resin. Detection limits obtained either with or without a preconcentration step are applicable to drinking water samples. The results obtained after preconcentration were compared with those obtained using carbon as preconcentrator substrate. Each method developed has been applied to the determination of haloacetic acids in drinking water spiked samples.

2. Experimental

2.1. Instrumentation

For ion interaction chromatography, a Varian LC 9010 liquid chromatograph (Varian, Walnut Creek, CA, USA) equipped with a Rheodyne injection valve (100-μl sample loop), a Kontron (Kontron Instruments, Milan, Italy) UV–Vis spectrophotometric detector and an Axxiom Chromatography 727 Data System (Axxiom Chromatography, Calabasas, CA, USA) were used. The separation column was a 10 μm LiChrospher 100 RP-18 endcapped (250×4 mm I.D.) coupled with a LiChroCART 100 RP-18 (4×4 mm I.D.) both purchased from Merck (Darmstadt, Germany).

For non-suppressed anion-exchange chromatography, a 4000i gradient pump (Dionex, Sunnyvale, CA, USA) equipped with a 100-μl loop injector and a VDM-II variable-wavelength UV–Vis detector (Dionex) was used. For suppressed IC, a QIC ion chromatograph (Dionex) equipped with a 200-μl sample loop was employed. Detection was performed using a micromembrane suppressor (AMMS-II) and a conductivity detector. Suppression solution was 12.5 m*M* H_2SO_4. The analytical columns were IonPac AG9 (4×4 mm I.D.) and IonPac AS9 (250×4 mm I.D.), IonPac AG10 (4×4 mm I.D.) and IonPac AS10, IonPac AG11 (4×4 mm I.D.) and IonPac AS11. OnGuard-Ag cartridges were from Dionex.

The eluent flow-rate was 1.0 ml/min and experiments were performed at room temperature. Retention times were the means from triplicate injections. The dead volume of the columns was measured by injection of water.

For the preconcentration step, LiChrolut-EN cartridges (Merck) and vegetal activated carbon (Carlo Erba) were used. Before use, LiChrolut-EN cartridges were conditioned with 3 ml CH_3OH and 3 ml high purity water. During the conditioning, the cartridge should not be allowed to dry out. In order to eliminate soluble species in aqueous and organic solvents, carbon has been washed with HF, water, NaOH, water, and CH_3OH and finally dried at 80°C.

Data manipulation and the operation of all components in the system were controlled by AI-450 chromatographic software (Dionex) interfaced via an Advanced Computer Interface ACI-2 (Dionex) to an IBM personal computer. A Hitachi (Tokyo, Japan) 150-20 spectrophotometer was used for spectrophotometric measurements. For pH measurements, an Ion Analyzer EA 920 (Orion Research) has been employed.

2.2. Reagents and solutions

Dichloroacetic, trichloroacetic, bromoacetic, dibromoacetic, tribromoacetic acids, sodium carbonate, sodium hydrogencarbonate, sodium iodate, sodium bromide, sodium iodide and potassium bromate were purchased from Sigma-Aldrich (Milan, Italy). Cetyltrimethylammonium chloride (CTACl), tetrabutylammonium chloride (TBACl) were from Fluka (Buchs, Switzerland).

Ammonium chloride, sodium chloride, sodium sulphate and sodium hydroxide were from Carlo Erba (Milan, Italy). Acetonitrile, methanol, hydrochloric acid and ammonia solution (25% NH_3) were from BDH (BDH Italia, Milan, Italy).

Eluents and standard solutions were prepared with high-purity water obtained from a Milli-Q System (Millipore, Bedford, MA, USA). Before use, eluents were filtered through GSWP 0.22 μm filters (Millipore).

3. Results and discussion

3.1. Ion interaction chromatography

In order to minimize the interference of chloride

ions commonly occurring in drinking waters, a detection wavelength of 210 nm has been selected. For the IIC method optimization, two ion pairing reagents, CTACl and TBACl have been chosen for their different chemical and selectivity properties. The optimization has been performed through a detailed study of the mobile phase parameters affecting k'. Depending on the pairing ion, a different organic modifier has been used. In fact, in order to compensate the different lipophilicity of the two reagents, CH_3OH has been used with TBACl, whereas CH_3CN, having higher elution power, has been used with CTACl.

3.2. TBACl–CH_3OH mobile phase

3.2.1. Effect of CH_3OH

The effect of organic modifier has been studied in the range 35–80%, keeping constant the mobile phase composition at pH 5.0, and 20 m*M* TBACl. The range investigated allowed a sensitive variation of the polarity of the eluents, still maintaining a good elution power and allowing to act both on the partition and on the dynamic ion-exchange equilibria. The effect of methanol on k' is shown in Fig. 1, where it is possible to note that the typical exponential dependence is very enhanced for TCA and TBA and less stressed for the mono- and di-halo substituted acids. For CH_3OH content less than 40%, the baseline is unstable and TCA and TBA present tailed peaks. For the following measurements 50% CH_3OH has been chosen as a compromise for a good peak shape, reproducibility of the system and analysis time, and a good separation among analytes.

3.2.2. Effect of TBACl

The range investigated was included between 10 and 50 m*M* TBACl, keeping constant the eluent pH

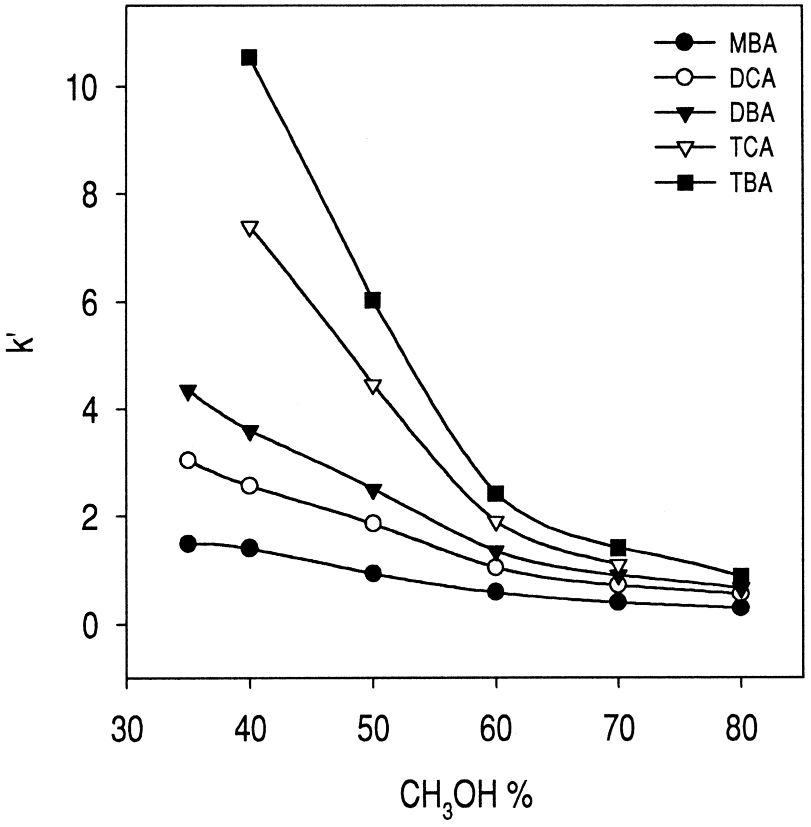

Fig. 1. Effect of CH_3OH on k' of haloacids by ion interaction chromatography. Column: LiChrospher 100 RP-18 endcapped (250×4 mm I.D.). Eluent: 20 m*M* TBACl, pH 5.0. Methanol as shown.

(5.0) and the methanol content (50%). The typical increase of k' when the pairing ion concentration is increased has not been observed as indicated from the data on Table 1. Comparing the chromatograms obtained as a function of baseline stability, resolution and peak shape, a 50 m*M* TBACl has been chosen as optimal concentration.

3.2.3. Effect of NaCl

When a salt is added to the mobile phase, ionic strength is increased, influencing the retention behavior of analytes through partition and ion-exchange equilibria. The effect of NaCl has been studied in the range 0–45 m*M* with eluents at pH 5.0 containing 10 m*M* TBACl and 50% CH_3OH. The results obtained (Fig. 2) show that k' has an exponential dependence on salt concentration typical of the counter-ion competition equilibria. Experimental data clearly show that separation can be successfully performed when the mobile phase does not contain NaCl, without compromising the shape of the peaks.

3.2.4. Effect of pH

In reversed-phase IIC, eluent pH can affect retention of ionizable analytes in a relevant way. Considering the stability of the silica support, and the dissociation constants for the haloacetic acids [16,17] $pK_{DCA}=1.30$, $pK_{TCA}=0.70$ and $pK_{MBA}=2.87$, the pH range studied in this work is 2.8–5.0. The results obtained for k' are shown in Table 2. Differently from the behaviour of haloacetic acids observed in IC mode (see further), IIC separations of analytes is little influenced by pH. Surprisingly, k' of stronger ionizable acids seems more affected by eluent pH. Nevertheless, eluent pH significantly influences the symmetry of the more retained peaks (TCA and TBA).

Table 1
Effect of TBACl concentration on k' of haloacetic acids[a]

TBACl (m*M*)	k'				
	MBA	DCA	DBA	TCA	TBA
10	0.87	1.63	2.21	3.74	5.10
12	0.81	1.54	2.05	3.49	4.68
15	0.77	1.42	2.01	3.50	4.72
20	0.74	1.41	1.92	3.42	4.50
25	0.72	1.31	1.76	2.94	3.88
30	0.66	1.23	1.66	2.83	3.72
35	0.64	1.24	1.67	2.91	3.82
40	0.63	1.23	1.64	2.94	3.85
45	0.61	1.20	1.57	2.78	3.69
50	0.62	1.28	1.71	3.21	4.24

[a] Eluent: pH 5.0, 50% CH_3OH.

3.3. CTACl–CH_3CN mobile phase

Considering the results obtained with the TBACl–CH_3OH system, eluent pH has been kept at 5.0 and no ionic strength modifier has been added to the mobile phase. The use of CH_3CN reduced the background noise of the baseline, improving the sensitivity of the detection. Anyway, differently from the system previously studied, more than 1 h was necessary to equilibrate the chromatographic column. Using this system, determination of tribromoacetic acid was not feasible, due to a split of its chromatographic peak interfering with the determination of the other haloacetic acids. Difficulties in the determination of TBA and TCA were also encountered with the subsequent IC separations (see below).

3.3.1. Effect of CTACl

The effect of ion pairing reagent on k' of haloacetic acids and NO_3^- has been studied in a range 1.2–3.5 m*M* at pH 5.0 and 50% CH_3CN and the results are shown in Table 3. From the results obtained, 3.5 m*M* CTACl has been chosen for the separation of the analytes considered. Since the content of the organic solvent has been considered suitable for the separation, its effect on k' was not investigated.

The two IIC systems investigated evidenced that the retention order is: MBA<DCA<DBA<TCA<TBA. Since the retention order is the same of what observed in IC systems (see further), we can state that in these IIC separations, electrostatic interactions play a more relevant role than the lipophilic ones.

According to the results obtained two mobile phase compositions have been selected in order to separate haloacetic acids:

- Eluent 1. 50% CH_3OH, 50 m*M* TBACl, pH 5.0
- Eluent 2. 50% CH_3CN, 3.5 m*M* CTACl, pH 5.0.

At these conditions, the quantitation limits, defined as three times of signal noise, have been evaluated (Table 4). It must be mentioned the lower limits reached with the Eluent 2.

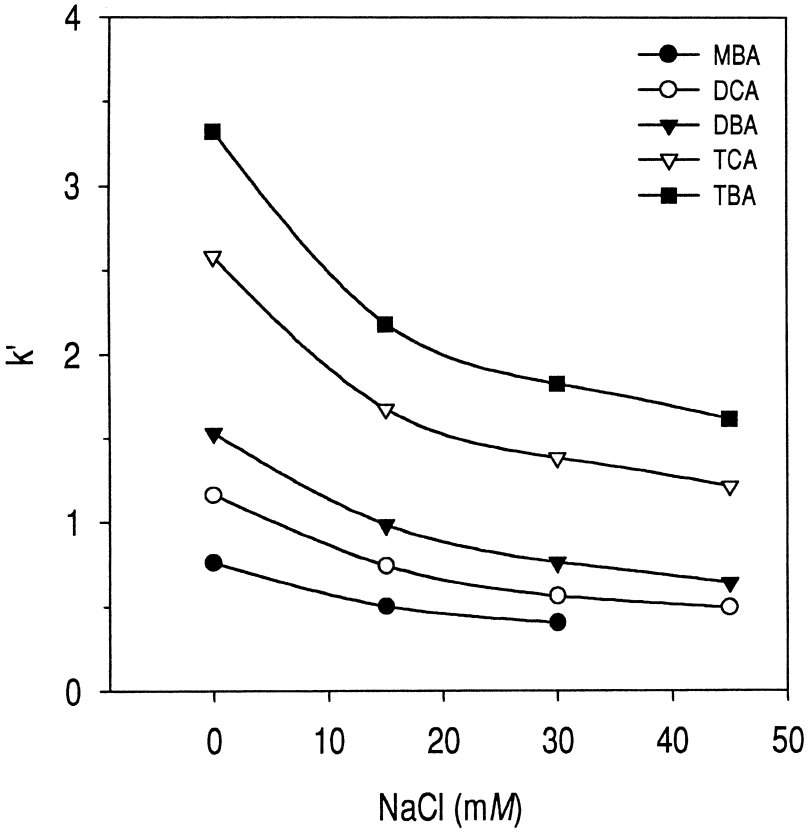

Fig. 2. Effect of NaCl on k' of haloacids by ion interaction chromatography. Column: LiChrospher 100 RP-18 endcapped (250×4 mm I.D.). Eluent: 50% CH_3OH, 10 m*M* TBACl. Aqueous pH 5.0. Sodium chloride as shown.

3.4. IIC. Analysis of tap water

A tap water sample from our laboratory has been filtered with 0.22 μm filters and injected as such (blank) or spiked with 30 mg/l of MBA, DBA, TCA and 25 mg/l DCA at the two mobile phases optimized. The results for the spiked samples are shown

Table 2
Effect of eluent pH on k' of haloacetic acids[a]

pH	k'				
	MBA	DCA	DBA	TCA	TBA
2.8	0.72	1.35	1.79	3.02	4.03
3.3	0.76	1.13	2.37	2.97	3.93
3.7	0.79	1.22	1.61	2.74	3.58
4.1	0.79	1.13	1.55	2.62	3.38
5.0	0.76	1.16	1.53	2.58	3.34

[a] Eluent: 50% CH_3OH, 10 m*M* TBACl.

Table 3
Effect of CTACl concentration on k' of haloacetic acids[a]

CTACl (m*M*)	k'				
	MBA	NO_3^-	DCA	DBA	TCA
1.2	1.29	1.60	1.74	1.90	2.65
2.0	1.42	1.78	2.05	2.10	3.06
3.5	1.62	2.00	2.26	2.47	3.79

[a] Eluent: 50% CH_3OH, pH 5.0.

Table 4
Quantitation limits determined with the IIC mobile phases optimized[a]

Eluent	Quantitation limit (mg/l)				
	MBA	DCA	DBA	TCA	TBA
1	3.0	1.5	2.0	3.0	1.5
2	0.4	0.05	0.2	0.15	–

[a] Eluent compositions; see text.

in Fig. 3(A) and (B) for elution by eluents 1 and 2, respectively. Comparing the peak areas of haloacetic acids in spiked tap water samples with standard solutions containing the same concentration of analytes, a recovery higher than 99% has been calculated for all acids but MBA. From these results, it can be stated that matrix effect is negligible. Due to the partial coelution with nitrate ion, the recovery for MBA acid has not been calculated. The standard deviation of the method developed is 4.5% ($n=3$ replicates).

3.5. Suppressed IC

The separation of haloacetic acids by suppressed IC has been optimized through the study of their chromatographic behavior using IonPac AS11 and IonPac AS9 and carbonate-based buffers or NaOH as

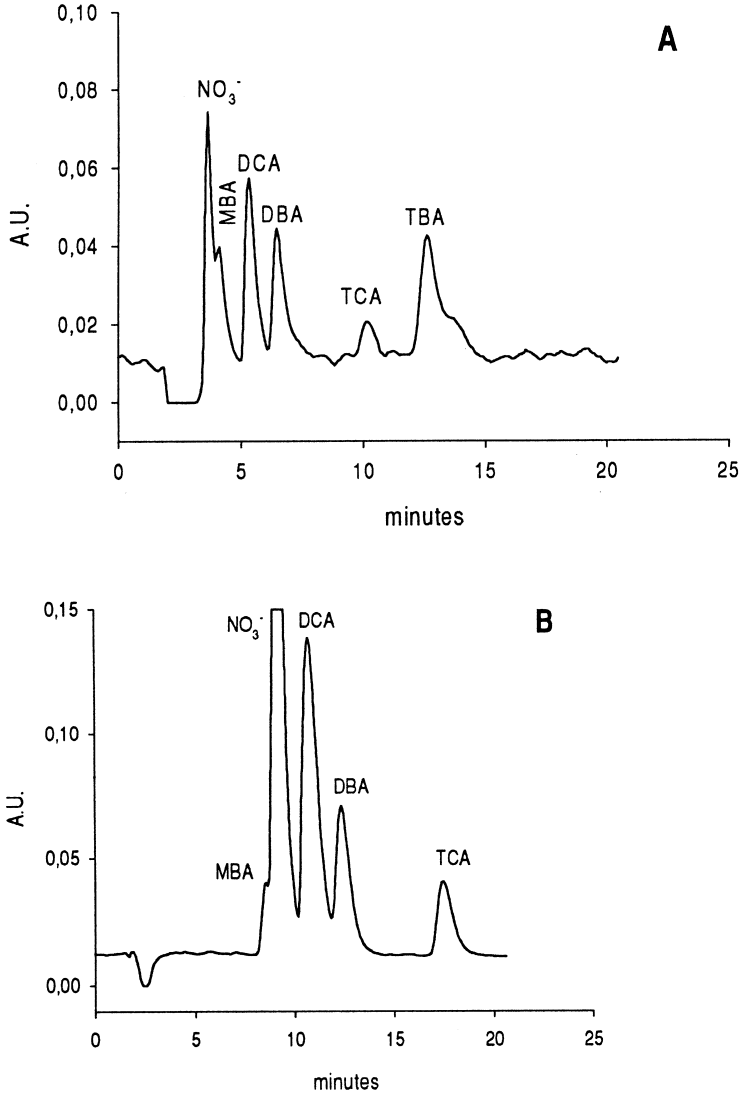

Fig. 3. Determination of haloacids in tap water by IIC. Column: LiChrospher 100 RP-18 endcapped. Detection wavelength: 210 nm. Eluent (A): 50% CH_3OH, 50 m*M* TBACl, pH 5.0. Eluent (B): 50% CH_3CN, 3.5 m*M* CTACl, pH 5.0. Sample: tap water spiked with 30 mg/l of MBA, DBA, TCA, TBA and 25 mg/l DCA.

mobile phases. The performance of the different columns has been evaluated in the presence of further other common anions (Br^-, BrO_3^-, I^- and IO_3^-).

3.5.1. IonPac AS11 and eluents

Since this column is characterized by a high hydroxide selectivity, NaOH solutions (15–50 m*M*) have been considered as eluents. In the range evaluated, the coelutions between Cl^-–MBA and DCA–NO_3^- can not be baseline resolved. Therefore, we select as eluents carbonate solutions, studying the effect on k' of the carbonate concentrations (0.3–0.9 m*M*) in eluents buffered at pH 10.2. Moreover, keeping constant at 0.5 m*M* the carbonate concentration, the effect of pH (between 9.6 and 10.2) on k' has also been studied. Though the eluent composition has been changed from NaOH to carbonate solutions, complete separation between Cl^-–MBA and DCA–NO_3^- was not obtained, meaning that selectivity is unaffected either by the competing ion or by mobile phase pH. Considering these results, it seemed necessary to alter selectivity by changing the stationary phase.

3.5.2. IonPac AS9 and eluents

This kind of stationary phase is not compatible with NaOH eluents and, therefore, only carbonate-based buffers have been used. Whenever possible, for a direct comparison, eluents have been prepared with the same concentrations as those used with IonPac AS11 column. IonPac AS9 proved to be suitable for the separation of haloacetic acids and moreover, differently from IonPac AS11, selectivity of some of the analytes considered (e.g. DBA–Br^-, TBA–I^-) can also be altered by simply changing eluent concentration. With IonPac AS9, due to its lower capacity, retention of analytes is lower than IonPac AS11, and therefore, a wider range of pH has been investigated (8.6–10.1). Even with changing pH, inorganic analytes were subjected to changes in their selectivity.

The optimization study has shown that the best separation of haloacetic acids in the presence of inorganic anions (mainly Cl^- and NO_3^-) can be achieved using a 0.3 m*M* [Na_2CO_3]+[$NaHCO_3$], (3:1) (Eluent 3) and an IonPac AS9 column.

3.6. IC. Analysis of water samples

After optimization of stationary phase and eluent composition, a feasibility study of haloacetic acids determination in drinking water samples has been performed.

A commercial mineral water and a tap water sample have been filtered through 0.22 μm filters. Although separation between Cl^- and MBA was obtained, both samples have been passed through OnGuard-Ag cartridges to reduce Cl^- content. By means of addition of known amounts of haloacetic acids before and after the pretreatment on the ion-exchange cartridges, it has been verified that the sample pretreatment does not affect the recovery of each haloacetic acid considered. The sample was then injected as such and spiked with known amounts of analytes. As an example of typical chromatograms obtained, Fig. 4(A) shows the results of injection of the mineral water sample added with 100 μg/l MBA, 200 μg/l DCA, 500 μg/l DBA, 500 μg/l TCA and 1 mg/l TBA.

In Fig. 4(B) the chromatogram of the tap water sample subjected to the same pretreatment and added with 1 mg/l of each haloacetic acid is shown. In this matrix, the detection limits (calculated as three times the background signal) were: 25 μg/l MBA, 54 μg/l DCA, 75 μg/l DBA, 56 μg/l TCA and 207 μg/l TBA.

Standard deviations, evaluated by elution of the same water samples spiked in different periods of time, averaged in a 2.0% for peak area and 0.2% for retention times.

3.7. Non-suppressed IC

The anion-exchange separation was performed in a non-suppressed mode, using spectrophotometric detection at 206 nm. The column used was a high capacity, solvent compatible stationary phase, IonPac AS10. The eluent strength of the mobile phase has been studied and optimized through the study of the effect of different salts and organic modifiers on k' and eluent pH.

3.7.1. Effect of pH

The effect of eluent pH on k' has been studied in the range 3.5–8.0 at 200 m*M* NaCl. It has been

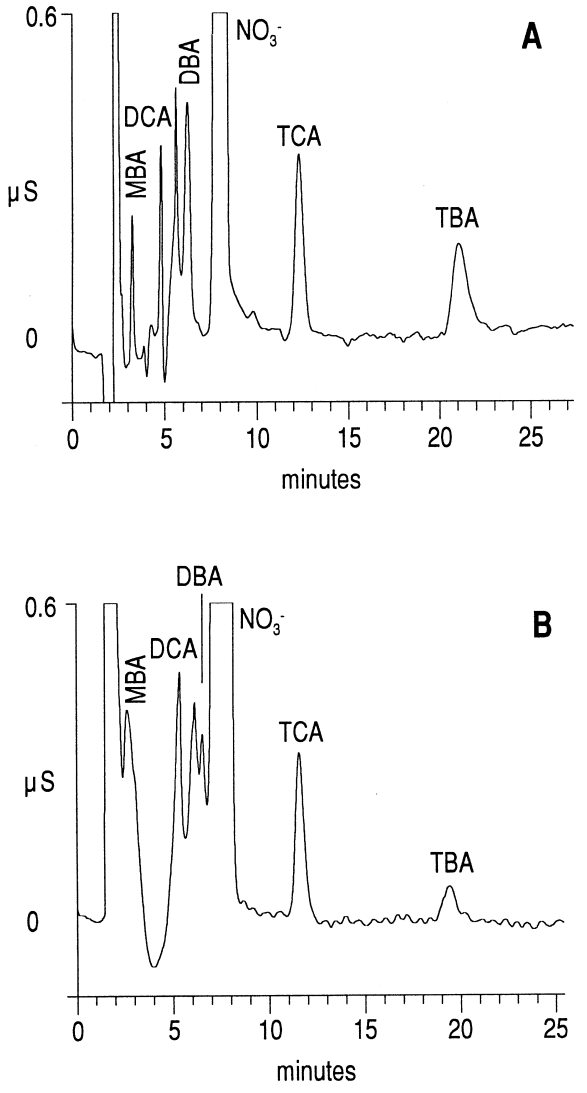

Fig. 4. Determination of haloacids in drinking waters by suppressed IC. Column: IonPac AS9 (250×4 mm I.D.). Eluent: 0.3 m*M* [Na_2CO_3]+[$NaHCO_3$], (3:1). Sample pretreatment: OnGuard-Ag cartridges. Sample: (A): mineral water added with 100 μg/l MBA, 200 μg/l DCA, 500 μg/l DBA, 500 μg/l TCA and 1 mg/l TBA. (B): tap water spiked with 1 mg/l of each analyte.

observed that at acidic pH values, an inversion in the retention order of MBA and DCA takes place and a decrease of the signal-to-noise ratio occurs. Up to pH values of 8.0 retention of all analytes but TBA (for which a slight increase of k' has been observed) is unaffected by pH changes and no significant gain in the signal-to-noise ratio has been noted. A pH value of 5.7 (the pH of the ammonium chloride solution) was considered suitable for haloacetic separation.

3.7.2. Effect of NaCl

The effect of salt concentration on k' (Fig. 5) has been evaluated using mobile phases of 18 m*M* NH_4Cl at pH 5.7. Retention order of haloacetic acids is the same as that of IIC separations. Experimental data showed that separation among analytes is feasible at short analysis time still at 200 m*M* NaCl even in the presence of common interfering anions. The long retention times of TBA and TCA can not be reduced even using Na_2SO_4 in the eluent at concentrations up to 150 m*M*. Moreover, this mobile phase caused baseline instability when preconcentration and elution by an organic solvent is performed.

As cited, the preconcentration step requires the use of an organic solvent to elute the haloacetic acids from the preconcentration resin. For this reason, the chromatographic behaviour of CH_3OH and CH_3CN has also been studied. The use of CH_3CN as a solvent for the haloacetic acids resulted problematic. In fact, standard solutions of analytes in CH_3CN do not give any chromatographic peak for TCA and TBA. A pseudo nucleophilic addition has been proposed to take place between the solvent and the haloacids [18], leading to species with different chromatographic and/or spectrophotometric behavior. The extent of the reaction could be related to the acidic strength of the acids and thus justifying the non-reactivity of less halosubstituted analytes. The injection of CH_3OH showed that the higher interference with the peaks of haloacetic acids was obtained at 200 m*M* NaCl, where the organic solvent signal covered the peaks of MBA, DCA and DBA. After verifying that methanol retention was influenced slightly by counter ion concentration, a gradient elution has been optimized in order to allow the prior elution of methanol and then the elution of analytes. The optimal gradient composition (Eluent 4) resulted to be:

$t=0$ to $t=11$ min: 18 m*M* NH_4Cl, 10 m*M* NaCl.

$t=11$ to $t=12$ min: 18 m*M* NH_4Cl, 200 m*M* NaCl.

3.7.3. Effect of organic solvent

To improve analysis time an organic solvent has

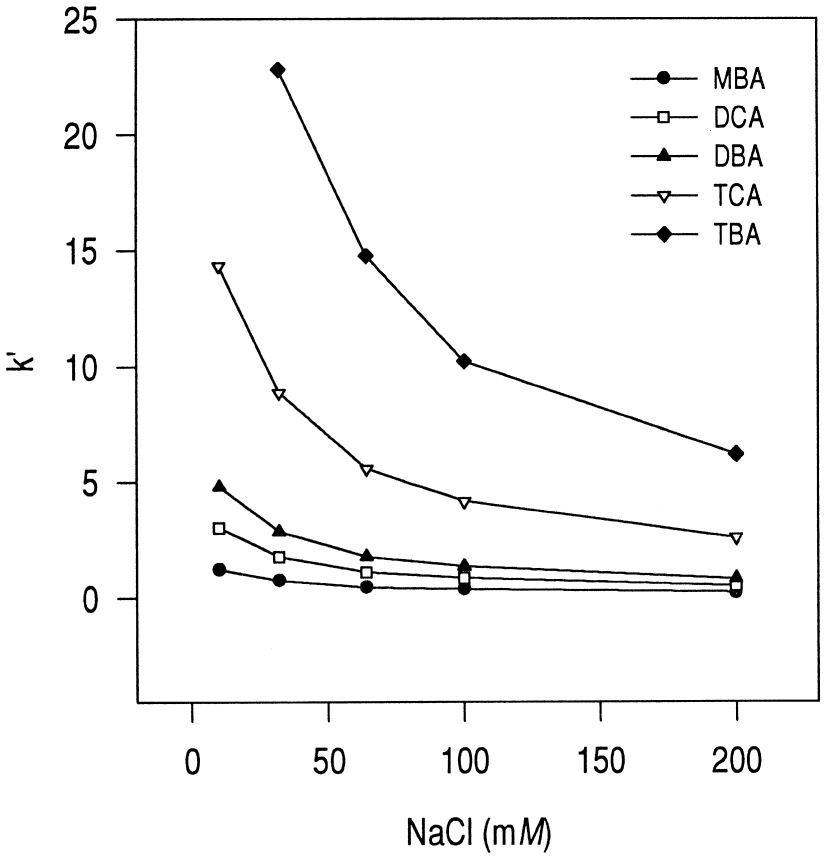

Fig. 5. Effect of NaCl on k' of haloacids by non-suppressed IC. Column: IonPac AS10 (250×4 mm I.D.). Eluent: 18 m*M* NH_4Cl, pH 5.7. Sodium chloride as shown.

been added in the mobile phases. For its higher UV transparency, acetonitrile (30–40%) has been chosen and has been added in 18 m*M* NH_4Cl, 10 m*M* NaCl eluents. Fig. 6 shows as retention is reduced throughout hydrophobic interactions. In the same figure, retention of nitrate ion is also shown. In prevision of analyzing preconcentrated analytes recovered with methanol, methanol has been injected in this kind of eluent, verifying that it does not interfere with haloacetic acids determination. A mobile phase (Eluent 5) containing 35% CH_3CN, 18 m*M* NH_4Cl, 10 m*M* NaCl was considered optimal for haloacids determination.

3.8. Preconcentration

In order to improve detection limits for haloacids, a method for the preconcentration of analytes and a simultaneous reduction of interfering species has been studied. Among the solid substrates tested, we report the results obtained with two phases: a high capacity reversed-phase material (LiChrolut-EN) and activated carbon.

3.8.1. Lichrolut-EN-non-suppressed IC

According to manufacturer informations, LiChrolut-EN cartridges have a 10-fold higher capacity than the conventional reversed-phase materials. Moreover, considering the polymeric characteristics of the resin, differently from silica-based materials, it can be used at extreme acidic pH conditions. The preconcentration procedure was primarily based on the hydrophobic interactions between the substrate

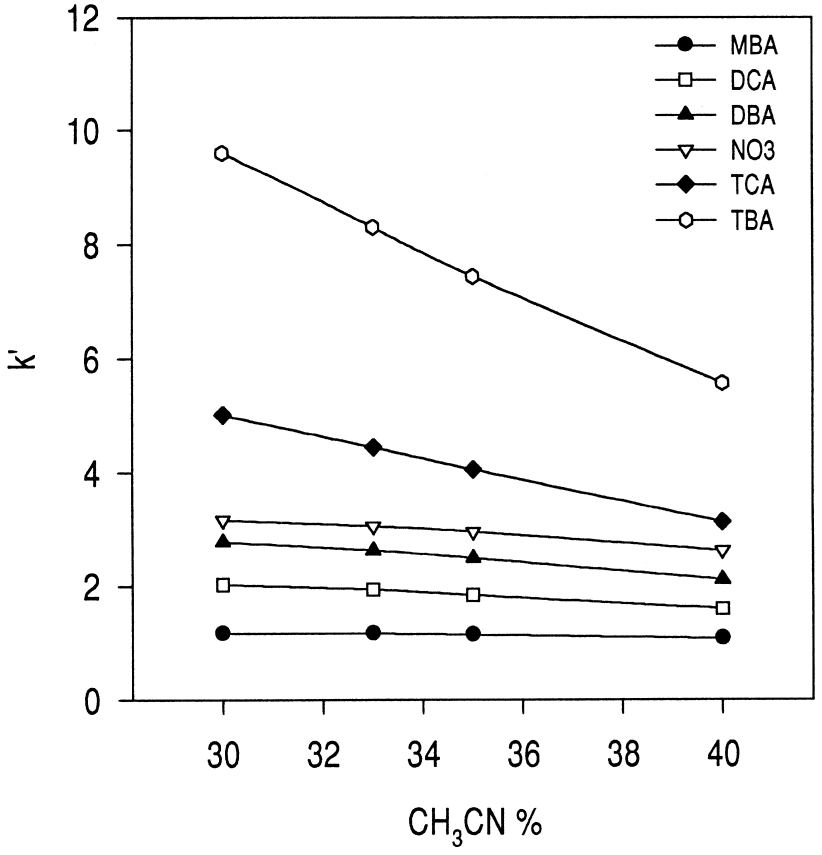

Fig. 6. Effect of CH_3CN on k' of haloacids by non-suppressed IC. Column: IonPac AS10. Eluent: 18 m*M* NH_4Cl, 10 m*M* NaCl. Acetonitrile as shown.

and the protonated haloacids in acidic solutions. This operation should considerably reduce the interference from Cl^- and NO_3^- that, being strongly ionized, should poorly interact with the reversed-phase preconcentration material.

Preconcentration experiments have been performed on acidified (pH 1.0 with H_2SO_4) tap water samples from our laboratory. Aliquots of 100 ml of tap water (as such or spiked with known amounts of haloacids) have been preconcentrated on LiChrolut-EN cartridges and eluted with 2 ml CH_3OH. The eluates have been injected and eluted by Eluent 4 and 5 (Fig. 7(A) and (B)). Recoveries and reproducibility of the enrichment step, evaluated by Eluent 4, have been calculated by standard addition and are shown in Table 5. Quantitation limits (defined as three times of signal noise) have been evaluated in tap water samples and are shown in Table 6.

As predicted, the preconcentration procedure allowed us to reduce the interference of nitrate anions during the IC separation. In fact the initial content of 20 mg/l NO_3^- has been reduced to 5 mg/l after preconcentration.

3.8.2. Activated carbon-suppressed IC

When the preconcentration step by LiChrolut-EN cartridges has been applied to suppressed IC (Eluent 3), the background noise strongly interfered with the detection of ppb of analytes. Activated carbon has been studied as a further substrate for the preconcentration of haloacetic acids.

Aliquots of 0.5 g of carbon have been stirred with tap water samples as such or spiked with known

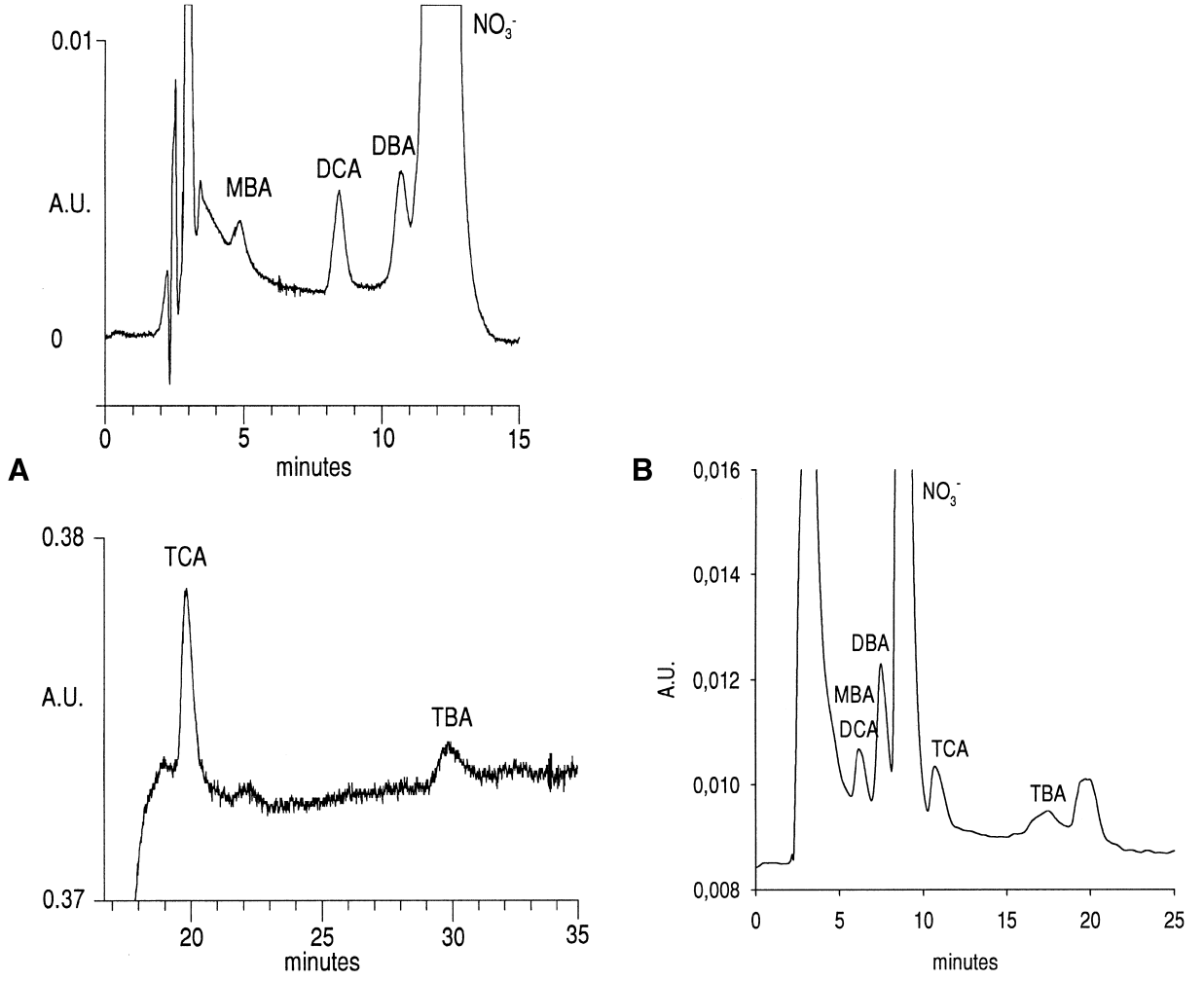

Fig. 7. Preconcentration and separation of haloacetic acids in acidified spiked tap water. Preconcentrator: LiChrolut-EN cartridges. Column: IonPac AS10. Eluent: (A): gradient $t=0$ to $t=11$ min: 18 m*M* NH_4Cl, 10 m*M* NaCl, $t=11$ to $t=12$ min: 18 m*M* NH_4Cl, 200 m*M* NaCl. (B): 35% CH_3CN, 18 m*M* NH_4Cl, 10 m*M* NaCl. Detection wavelength: 206 nm. Sample preconcentrated: 100 ml, sample eluted: 2 ml. Analytes concentration: 50 μg/l each.

amounts of analytes. In order to optimize the procedure, preliminary experiments have been performed on acidified and non-acidified tap water samples. These preliminary results demonstrated that higher recoveries can be obtained on acidified samples and therefore, this preconcentration procedure has been investigated in-depth.

In order to avoid a further addition of anions that can interfere in the chromatographic separation of the analytes, tap water has been acidified throughout the use of a AMMS-II micromembrane. Cations commonly present in tap water (Ca^{2+}, Mg^{2+}, Na^{+}) can be exchanged in the membrane flowed with a 0.1 *M* H_2SO_4 suppression solution which promotes the protonation of haloacids. Aliquots of 50 ml of tap water as such or spiked with 1 mg/l of each haloacid have been acidified. The analytes retained on the carbon were eluted with 5 ml CH_3OH and injected in the chromatographic system. Typical chromatograms for tap water and tap water spiked with 0.5 ppm of each analyte after elution with Eluent 3 have been shown in Fig. 8. Recoveries, evaluated by the

Table 5
Recovery of 50 μg/l of haloacetic acids in a 100 ml tap water sample after preconcentration on LiChrolut-EN cartridges (preconcentration factor 50)[b]

Analytes	Recovery (%)[a]
MBA	80.3±8.4
DCA	83.7±13
DBA	73.1±13
TCA	81.8±11
TBA	77.7±3.0

[a] $n=3$ replicates.
[b] Recovery has been evaluated by non-suppressed IC and by elution with Eluent 4.

Table 6
Quantitation limits determined, after preconcentration on LiChrolut-EN cartridges, with the non-suppressed IC mobile phases optimized[a]

Eluent	Quantitative limit (μg/l)				
	MBA	DCA	DBA	TCA	TBA
4	20	7.0	10	10	40
5	60	15	6	10	25

[a] Eluent compositions; see text.

standard addition method, are shown in Table 7. Considering the incomplete recovery of the spiked tap water sample, the fraction of spiked tap water after elution through carbon has been injected in the

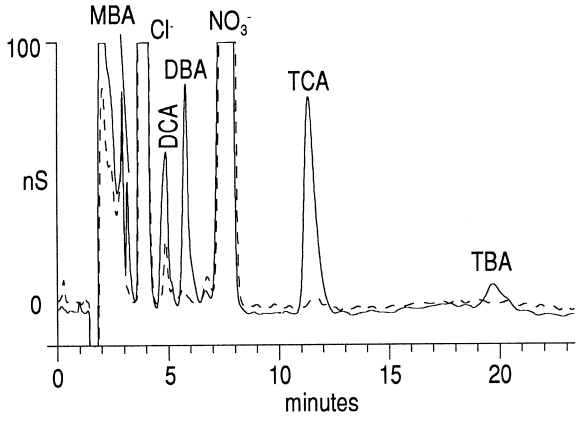

Fig. 8. Preconcentration and separation of haloacetic acids in acidified spiked tap water. Sample acidification performed by AMMS-II micromembrane flowed with 0.1 *M* H_2SO_4. Preconcentrator: activated vegetal carbon. Column: IonPac AS9. Eluent: 0.3 m*M* [Na_2CO_3]+[$NaHCO_3$], (3:1). Sample preconcentrated: 50 ml, sample eluted: 5 ml. Analytes concentration: 500 μg/l each.

Table 7
Recovery of 500 μg/l of haloacetic acids in a 100 ml tap water sample after preconcentration on activated carbon (preconcentration factor 20)[b]

Analytes	Recovery (%)[a]
MBA	5.4±0.9
DCA	22.9±8.2
DBA	34.9±6.1
TCA	55.7±16
TBA	25.8±10

[a] $n=3$ replicates.
[b] Recovery has been evaluated by suppressed IC and by elution with Eluent 3.

chromatographic system, in order to verify if incomplete sorption on carbon occurs. The great amount of Cl^- ions prevents the determination of chromatographic peaks of MBA and DCA, while there is no evidence of DBA, TCA and TBA peaks, meaning that these analytes are strongly retained by carbon and only partially eluted by methanol.

Although recoveries are not quantitative, the preconcentration-matrix removal step allows a reduction of interference of Cl^- and NO_3^- whose concentrations at the end of the pretreatment are 1/3 and 2/5 of the initial ones, respectively. Detection limits (three times background signal) have been evaluated in tap water: 150 μg/l MBA, 44 μg/l DCA, 31 μg/l DBA, 16 μg/l TCA and 86 μg/l TBA. Except for MBA, the limits of detection for haloacetic acids have been lowered with respect to those previously obtained without preconcentration. A tap water sample spiked with haloacids at concentrations corresponding to detection limits has been preconcentrated as optimized and injected, confirming the applicability of the method (Fig. 9).

4. Conclusions

In this paper we have developed and compared the performance of different analytical techniques for the separation and determination of haloacetic acids based on different chromatographic mechanisms (reversed-phase ion interaction and anion-exchange chromatography). Each method developed has been applied for haloacids determination in drinking water samples.

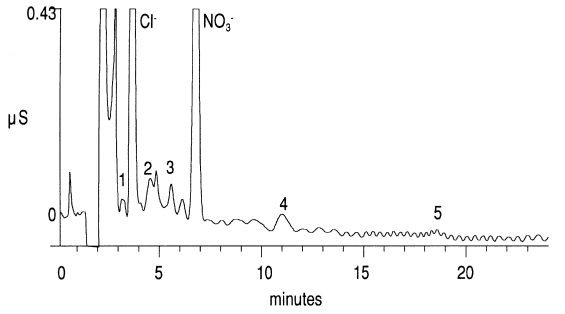

Fig. 9. Preconcentration and separation of haloacetic acids in acidified spiked tap water. Analytes concentrations corresponding to detection limits. 1. MBA 150 μg/l, 2. DCA 44 μg/l, 3. DBA 31 μg/l, 4. TCA 16 μg/l, 5. TBA 86 μg/l. Other conditions as Fig. 8.

The ion interaction-based method coupled with UV detection can be performed using an eluent containing either tetrabutylammonium–methanol or cetyltrimethylammonium–acetonitrile and a silica-based C_{18} column. The lower detection limits (50–400 μg/l) have been achieved with the cetyltrimethylammonium–acetonitrile eluent. The advantage of such a method is the removal of interference by chloride ion when drinking waters have to be analyzed. The anion-exchange-based method has been coupled with suppressed conductivity and with UV detection. Suppressed conductivity detection has been performed in different eluent conditions (carbonate-based buffers and NaOH mobile phases) and in different chromatographic columns (IonPac AS9 and IonPac AS11). After optimization of separation conditions, a carbonate-based eluent and the IonPac AS9 column were considered suitable for the separation and the determination of haloacetic acids. Due to the strong conductivity of chloride ions, the method requires a pretreatment step with OnGuard-Ag cartridges for chloride removal before analysis of drinking water samples. For this method, good detection limits, ranging from 25–207 μg/l have been obtained. The anion-exchange separation coupled with UV detection has been performed and optimized with a IonPac AS10 column. Two eluent systems were considered suitable for haloacetic acids separation. In order to reduce detection limits and matrix interferences, preconcentration techniques with different substrates (reversed-phase and activated carbon) have been developed. The control of sample pH during the preconcentration step allows the reduction of the interferences by Cl^- and NO_3^- ions. When reversed-phase substrate and the UV detection are used, detection limits range from 7 to 40 μg/l. When activated carbon substrate and suppressed conductivity detection are used, detection limits of 16–150 μg/l are obtained. The apparently higher detection limits are due to the incomplete elution of preconcentrated analytes from the carbon substrate. The choice of the substrates as well as the proper pH during the enrichment step allowed a significant reduction of interferences by chloride and nitrate ions present in drinking waters.

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