WILEY-VCH ELECTROPHORESIS

Analysis of benzalkonium chloride by capillary electrophoresis-tandem mass spectrometry Running head: Analysis of BAC by CE-MS/MS

Journal:	*Electrophoresis*
Manuscript ID:	elps-2005-00716.R1
Wiley - Manuscript type:	Research Paper
Date Submitted by the Author:	n/a
Complete List of Authors:	Para, Bianca Veronica; University of Barcelona, Analytical Chemistry Núñez, Oscar; University of Barcelona, Analytical Chemistry Moyano, Encarnación; University of Barcelona, Analytical Chemistry Galceran, Maria Teresa; University of Barcelona, Analytical Chemistry
Keywords:	Biocides, benzalkonium chloride, Capillary electrophoresis, ion trap, Mass spectrometry

ANALYSIS OF BENZALKONIUM CHLORIDE BY CAPILLARY ELECTROPHORESIS-TANDEM MASS SPECTROMETRY.

Bianca Veronica Pară, Oscar Núñez, Encarnación Moyano and Maria Teresa Galceran*

Running title: ANALYSIS OF BAC BY AND CE-MS/MS.

Department of Analytical Chemistry, University of Barcelona.
Martí i Franquès, 1-11. E-08028, Barcelona, Spain.
Tel. 34 93 402 12 86 Fax. 34 93 402 12 33
e-mail: galceran@ub.edu

Keywords: Biocides, Mass spectrometry, Ion trap, Capillary electrophoresis, Benzalkonium Chloride

* Author to whom correspondence should be addressed.

Abstract

Conditions for the separation and determination of benzalkonium chloride (BAC) homologues by capillary electrophoresis with UV detection and capillary electrophoresis coupled to mass spectrometry (ion trap) using electrospray as ionization source were established. The separation was performed using fused silica capillaries of 50 μm i.d. and 100 mM acetic acid-ammonium acetate buffer solution at pH 4.5 with 80% of acetonitrile as carrier electrolyte. CE-MS coupling parameters were optimized and methanol-10 mM acetic acid (90:10 v/v) was selected as sheath liquid. Detection limits, based on a signal-to-noise ratio of 3:1, were calculated, and values between 0.8 and 1.3 mg/L with CE-ESI/MS and around 0.5 mg/L with CE-ESI/MS/MS, using hydrodynamic injection (15 s, 3.5 kPa), were obtained. Good run-to-run and day-to-day precisions on concentration were achieved with relative standard deviations lower than 8%. Quantitative analysis was carried out by the internal standard method and the calibration curves showed good linearities ($r^2 > 0.98$). The CE-ESI/MS/MS method was successfully applied to the analysis of BAC in different ophthalmic solutions, allowing the direct determination, identification and confirmation of the BAC homologues presented in these samples.

1 Introduction

Benzalkonium chloride (BAC) is a mixture of C_8 to C_{18} alkylbenzyldimethylammonium chlorides with an important biocide character [1]. It is widely employed as active substance in anti-bacterial and anti-fungal products, canning preservatives, pest control products, medical disinfectants, and ophthalmic or nasal formulations [2,3]. Each homologue in this family of compounds has different physical, chemical and biocidal properties related to the length of the alkyl chain. In general, the C_{12}-BAC homologue is the most effective one against yeast and fungi, the C_{14}-BAC homologue against gram-positive bacteria, and the C_{16}-BAC homologue against gram-negative bacteria [4]. As a consequence, the formulations require determination not only of the total amount of BAC but also of the ratio of its homologues.

The amount of BAC commonly found in the environment is considerable, due to the high number of products containing these compounds and the frequent leakage into surface waters from wastewater treatment facilities. Moreover, these compounds have been shown to be toxic to aquatic organisms even at low concentrations [5,6]. For instance, for fish LC_o=0.5-4 mg/L, LC_{100}=2-5 mg/L and for daphnids LC_0=0.1 mg/L, LC_{100}=1 mg/L [6,7]. Moreover, there is a lack of data about their degradation [8].

Liquid chromatography (LC) and capillary electrophoresis (CE) are the techniques most frequently used for the analysis of these cationic compounds, allowing the separation and determination of the most important BAC homologues. Reversed-phase liquid chromatography with UV-detection has been used for the determination of BAC in ophthalmic solutions [3,9-11], nasal sprays [12], and some biological samples such as blood and tissues [13]. Fluorescence detection has also been used for the analysis of some hospital effluents [14]. Other detection systems such as conductometric detection have also been used but only with standard solutions [15]. The cationic characteristics of BAC make CE a useful technique for the separation and determination of BAC homologues in drug formulations [16-19], in nasal and ophthalmic solutions [12,20,21], and in some studies using standards [22,23]. In all these cases, the concentration is high enough and the detection limits of the CE-UV methods do not present a problem. Micellar electrokinetic capillary chromatography (MECC) with UV detection

using deoxycholate micelles in the presence of large organic solvent concentrations has also been applied to the determination of this family of compounds in some commercial formulations such as disinfectants and spermicides [24].

Liquid chromatography coupled to mass spectrometry (LC-MS) has also been applied to the analysis of BAC [25-29]. Most of the publications use ion trap analyzers for the determination of these compounds [25-27,29], and only one study uses a quadrupole instrument [28]. In some cases single MS acquisition is performed, as happens, for instance, in the analysis of BAC in sewage sludge [25], environmental media and occupational hygiene samples [28], and water samples [26]. In this last-mentioned work, MS/MS fragmentation experiments were also performed but tandem mass spectrometry was only used for confirmation purposes. In order to improve sensitivity and selectivity, other authors have applied LC-MS/MS for the identification and determination of BAC homologues in several samples such as ophthalmic products, water [29] and sediments [27]. For the analysis of these compounds in environmental samples, enrichment procedures such as on-line solid phase extraction (SPE) for water samples and accelerated solvent extraction followed by on-line SPE preconcentration for sediments [26,27] have been proposed. Matrix-Assisted Laser Desorption Ionisation-Time-of-Flight Mass Spectrometry (MALDI-TOFMS) has been used for the confirmation of the presence of these compounds in different commercial formulations [30]. Nevertheless, to our knowledge, capillary electrophoresis coupled to mass spectrometry (CE-MS) has not been used for the analysis of benzalkonium chloride. So, the aim of this work was to develop a sensitive and rapid CE-MS method using an electrospray ionization source and an ion trap analyzer as complementary technique to LC-MS for the determination of BAC homologues.

2 Materials and methods

2.1. Chemicals

Benzyldimethyldodecyl ammonium bromide (C_{12}-BAC), benzyldimethyltetradecylammonium chloride (C_{14}-BAC),

benzyldimethylhexadecylammonium chloride (C_{16}-BAC) and N-Benzyl-N,N-dimethyloctadecylammonium chloride hydrate (90%) (C_{18}-BAC) were obtained from Sigma-Aldrich (Steinheim, Germany). Heptylviologen (1,1'-diheptyl-4,4'-bipyridinium ion, HV) purchased from TCI (Tokyo, Japan) was used as internal standard. The structures of all these compounds are shown in Figure 1.

HPLC-gradient grade methanol, acetonitrile (ACN) and water, acetic acid (100%), formic acid (98-100%), ammonium acetate, sodium hydroxide and hydrochloric acid (25%) were purchased from Merck (Darmstadt, Germany), and ammonium formate from Fluka (Buchs, Switzerland).

Stock standard solutions of BAC homologues and internal standard (1000 mg/L) were prepared in acetonitrile and water respectively. Working solutions were prepared by dilution of the stock standard solutions in acetonitrile:water 60:40% (v/v). Acetonitrile was also added to both ophthalmic solution and river water samples to achieve 60% acetonitrile content.

2.2. Instrumentation

The CE-UV experiments were performed on a Beckman P/ACE 5500 capillary electrophoresis instrument (Fullerton, CA, USA) equipped with a diode array detection system. Electrophoretic data were processed using the P/ACE Station software version 1.2. The electrophoretic separation was carried out using uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 67 cm (60 cm effective length) x 50 µm I.D. and a 50 mM acetic acid-ammonium acetate buffer solution (pH 4.5) containing 80% acetonitrile as carrier electrolyte. The separation was performed by applying a voltage of +20 kV (15 µA), and the temperature was held at 25 °C. The buffer was filtered through a 0.45 µm membrane filter, and degassed by sonication before use. Samples were introduced by pressure (3.5 kPa) using an injection time of 15 s. Direct detection was performed at 215 nm.

The CE-MS experiments were performed on a Beckman P/ACE MDQ capillary electrophoresis instrument (Fullerton, CA, USA) coupled to a Classic LCQ mass spectrometer (Finnigan, San Jose, CA, USA) equipped with a tricoaxial pneumatically assisted electrospray ionization source designed for the CE-MS coupling and with an ion trap as analyzer. The electrophoretic separations

were carried out using uncoated fused-silica capillaries of 80 cm x 50 µm I.D. and the same carrier electrolyte used for CE-UV experiments. A capillary voltage of +25 kV (21 µA) was applied. Samples were loaded applying two injections modes: hydrodynamic injection pressure assisted (3.5 kPa) with an injection time of 15 s and electrokinetic injection (10 kV, 15 s). When the injection was performed, the electrospray voltage was turned off in order to prevent electrokinetic introduction of the analytes. The CE instrument was controlled using a Beckman 32 Karat software version 5.0.

A solution of methanol-10 mM acetic acid (90:10 v/v) at a flow-rate of 5 µL min^{-1} was used as sheath liquid after degassed by sonication. The ESI was pneumatically assisted using nitrogen as sheath gas at a flow-rate of 10 arbitrary units (a.u.). The electrospray needle was set at +3.0 kV and the heated capillary temperature was held at 200 °C. The CE capillary protrudes from the electrospray needle 0.1 mm, and the distance to the heated capillary was 1.5 cm. Moreover 0.5-1 cm of the polyamide coating was eliminated from the end of the fused-silica capillary in order to improve the contact between both sheath liquid and electrophoretic flow.

CE-MS data acquisition was carried out in full scan mode from *m/z* 50-400 in centroid mode using a maximum injection time of 200 ms and performing 3 µscans. CE-MS/MS data acquisition was performed in product ion scan mode using a maximum injection time of 100 ms and 3 µscans. An isolation width of *m/z* 1.5 was used, the activation Q (AQ) was set at 0.4 and the Activation time (AT) was 30 s. The precursor ions, the product ion scan ranges, the diagnostic product ion and the normalized collision energy (NCE) used for each BAC homologue in the MS/MS experiments are indicated in Table 1. Mass spectrometry data were processed with a Xcalibur 1.3 software.

2.3. Capillary conditioning

New capillaries were pre-treated using 0.1 M hydrochloric acid for 45 min, water for 30 min, 1 M sodium hydroxide for 30 min, and finally rinsed with water for 30 min. At the beginning of each session, the capillary was rinsed with sodium hydroxide for 30 min, with water for 30 min, and with carrier electrolyte during 30 min. The conditioning method was carried out daily in order to prevent

adsorption of the quaternary ammonium biocides on the capillary wall. Finally, the capillary was rinsed with carrier electrolyte during 5 min between runs and stored after rinsed with water.

3 Results and discussion

Most of the publications that analyze BAC by capillary electrophoresis generally use non-volatile salts such as phosphate buffers as carrier electrolytes [16-22] or sulfonic electrolytes [12]. When coupling CE to mass spectrometry the use of volatile buffers is recommended in order to prevent deposition of non-volatile salts into the mass spectrometric system. Hence, in this study a 50 mM acetic acid-ammonium acetate (pH 4,0) buffer was used as preliminary carrier electrolyte. Under these conditions, only the C_{12}-BAC and C_{14}-BAC homologues were detected when performing the analysis of a standard solution using UV-detection. The other two homologues, C_{16}-BAC and C_{18}-BAC, gave small and wide peaks due to micelle formation. As the addition of organic solvents in the carrier electrolytes prevent micelle formation [22,23], we studied the effect on the separation of the addition of acetonitrile to both carrier electrolyte and samples. These experiments were performed in a CE-UV system using a fused silica capillary of 67 cm length and the working conditions indicated in instrumentation section. Figure 2 shows the electropherograms obtained for a standard solution (~20 mg/L of each BAC homologue in acetonitrile:water 30:70% v/v) when different amounts of acetonitrile (from 0 to 80%) were added to the carrier electrolyte (Figure 2a) and to the BAC standard solution (Figure 2b). As can be seen in Figure 2a, peak shape, especially for C_{16}-BAC and C_{18}-BAC homologues, improved considerable up to 40% acetonitrile and the highest signal enhancement was obtained with 80% acetonitrile. The addition of higher amounts ofacetonitrile produced a non-stable capillary current, so 80% acetonitrile was chosen as optimum. However, the addition of acetonitrile to the carrier electrolyte was not enough for a good micelle disruption when analyzing water samples. Figure 2b shows the electropherograms obtained using different amounts of acetonitrile added to the samples using 50 mM acetic acid-ammonium acetate, pH 4.0, containing 80% acetonitrile as carrier electrolyte for the separation. As can be seen, at least a 60% acetonitrile must be added to the samples before injection to

obtain a good micelle disruption for all BAC homologues. Acetonitrile concentration higher than 60% only produced a slight enhancement on the response, so to prevent a high dilution of the sample 60% of acetonitrile was chosen as optimum value.

3.2. Capillary electrophoresis-mass spectrometry

Different electrophoretic parameters such as buffer concentration, pH, and capillary voltage were optimized. Different buffer concentrations from 50 to 200 mM were tested and an improvement of peak shapes was observed increasing the ionic strength of the carrier electrolyte. As a compromise between separation resolution and the capillary current suitable for CE-MS, 100 mM was chosen as optimum buffer concentration. Different pH values from 3.0 to 4.5 (formic acid-ammonium formate buffer) and from 4.0 to 5.5 (acetic acid-ammonium acetate buffer) were evaluated. Good electrophoretic separation of BAC homologues was obtained in the full range of pH studied. When acetic acid-ammonium acetate buffers were used as carrier electrolytes lower analysis times (more than 2 min at the same pH) were obtained than with formic acid-ammonium formate buffers. An increase in the pH also produced an enhancement of the ionic strength and, consequently, in the capillary current giving as a result worse signal-to-noise ratios. As a compromise, a pH of 4.5 was chosen as optimum value. Then, a 100 mM acetic acid-ammonium acetate (pH 4.5) buffer containing 80% acetonitrile was selected as the best carrier electrolyte for the electrophoretic separation of BAC.

The effect of the capillary voltage (5-30 kV) on the BAC separation was also studied. Although the analysis time decreased with the increase of the capillary voltage, values higher than 25 kV produced a loss in the peak resolution between some BAC homologues and high spray currents in the electrospray source.

Some ESI-MS instrumental parameters such as sheath liquid flow-rate and composition, sheath gas flow-rate, electrospray voltage, temperature and CE capillary length protruding from the electrospray needle were optimized in order to obtain the highest response. For this purpose a standard solution of C_{12}-BAC (10 mg/L) prepared in carrier electrolyte was infused into the ESI source by

applying simultaneously a CE voltage of +25 kV and an overimposed pressure of 3.5 kPa on the CE inlet vial. In order to compensate the electrospray voltage (+3.5 kV) and to obtain similar electrophoretic separation to that obtained with CE-UV, a voltage of +25 kV was used.

Sheath liquid and sheath gas flow-rates correlated, so they have to be optimized simultaneously. For this purpose, these parameters were varied from 5 to 20 μL/min and from 3 to 22 a.u., respectively. It was observed that the signal increased with the sheath gas flow-rate up to 10 a.u. and at higher values the response decreased, probably due to electrospray instability. In order to maximize the response, sheath liquid and sheath gas flow-rates of 5 μL/min and 10 a.u., respectively, were used.

The composition of the sheath liquid is critical for the performance of the CE-MS coupling [31]. A high amount of an organic solvent can help in the ionization due to a better evaporation efficiency but the sheath liquid must be conductive enough to permit completing the electrical circuit between the inlet CE and the outlet (the ESI source). In this work, a 10 mM acetic acid solution was mixed with different amounts of methanol (from 50% to 90%) and the response increased when the amount of methanol was raised. A mixture of methanol-10 mM acetic acid (90:10 v/v) was then used as optimal sheath liquid composition.

The electrospray voltage was optimized from 1.5 to 4.5 kV since at voltages higher than 4.5 kV discharge occurred in the electrospray source. The response increased up to 3.5 kV and then a decrease in the signal was produced. The heated capillary temperature was also optimized from 100 to 350 °C. The higher response was observed at temperatures from 150 to 200 °C.

The distance that the CE capillary protrudes from the electrospray needle has an important effect on the signal. This position will affect the final response [32] because the mixing volume between CE flow and sheath liquid flow must be the minimum possible in order to prevent peak broadening [31]. However, it must be enough to allow a good contact between both solutions and to close the electrical circuit. In our case, the response was maximal when the CE capillary only protrudes 0.1 mm from the electrospray needle. At lower and higher values the response decreased due to the instability in the formation of the charged droplets in the electrospray.

Single MS spectra of BAC homologues were obtained by infusion into the mass spectrometer using the CE system. The molecular ion $[M]^+$ was the base peak in these spectra and no fragmentation or cluster formation was observed. For tandem experiments, the molecular ion was isolated with a *m/z* window width of 1.5 to obtain the maximum trapping efficiency of the precursor ion without any interference from isotopic species or matrix components. The magnitude (AA, activation amplitude) and the duration (AT, activation time) of the resonance excitation voltage applied to the endcap electrodes, and the magnitude of the trapping radio frequency voltage (AQ, activation Q) do not depend on the separation technique coupled to the ion trap instrument. So the values previously established with LC-MS/MS and the same ion-trap instrument [29] were used in this work.

The MS/MS fragmentation pattern of BAC was very simple and for all the homologues only two fragment ions, the loss of a $CH_3C_6H_5$ group and the tropylium ion at *m/z* 91, were observed. This fragmentation agrees with that described by other authors using LC-MS/MS [26,28,29].

3.3. Method performance

Quality parameters using the proposed CE-UV, CE-MS and CE-MS/MS methods were obtained and the values are given in Table 2. LODs based on a signal-to-noise ratio of 3:1 were lower than 1.3 mg/L when using both UV detection and the proposed CE-MS method. Nevertheless, the selectivity of tandem mass spectrometry allows a better signal-to-noise ratio, lowering the LODs values to 0.5 mg/L. The LODs obtained were similar or slightly lower than those reported for BAC by other authors using capillary electrophoresis [19-22] with UV detection, with the advantage of the direct confirmation provided by the MS/MS spectra.

Calibration curves based on peak area ratio ($A_{compound}/A_{internal\ standard}$) for BAC homologues at concentrations between 1 and 200 mg/L and using HV as internal standard were calculated. Good linearities were obtained with correlation coefficients higher than 0.98 for all homologues and methods.

For run-to-run precision, five replicate determinations of a standard solution of BAC homologues were carried out under optimum conditions, while

day-to-day precision was calculated by performing 15 replicate determinations of a standard solution at two concentration levels in 3 days (five replicates each day). Figure 3 shows, as an example, the CE-ESI/MS/MS electropherogram obtained for a standard solution of BAC homologues and internal standard (HV) (10 mg/L). In terms of migration times, relative standard deviations (RSDs) ranged from 0.8% to 1.1% for run-to-run precision and between 1.4% and 1.9% for day-to-day precision. The concentration RSDs values obtained for run-to-run and day-to-day precisions were always lower than 5% and 8%, respectively. Although for CE-MS and CE-MS/MS better precisions were obtained at the high concentration level evaluated (50 mg/L), the loss in reproducibility when working at low levels was not significant. In addition, bias was calculated and concentration relative errors ranging from 4.0% to 5.8% were found. The results obtained showed that the methods proposed in this work are good in terms of repeatability and reproducibility.

3.4. Application

To show the applicability of the CE-MS/MS method three ophthalmic solutions were analyzed. As an example, Figure 4 shows the CE-MS/MS electropherogram of one of these samples and the MS/MS spectra of the identified compounds. Only the C_{12}-BAC and C_{14}-BAC homologues were detected and confirmed in these samples. These two homologues are the most abundant and the most frequently used in this kind of samples. The determinations were carried out in triplicate using the internal standard method and the calculated concentrations are given in Table 3. The RSDs values obtained for the triplicate determinations were always lower than 2.5%. The sample Liquifilm lagrimas was also analyzed in a previous work using a LC-MS/MS method [29] and the quantitation results were 32.3±0.5 and 16.6±0.9 mg/L for the C_{12}-BAC and C_{14}-BAC homologues, respectively, showing that the CE-MS/MS method gave results comparable to those obtained by LC-MS/MS.

The applicability of the CE-MS/MS method for the analysis of BAC homologues in environmental samples was also evaluated. For this purpose, spiked river water samples (Llobregat River, Barcelona, Spain) were analyzed. LODs were estimated from the analysis of river water samples free of BAC

spiked at low levels (Table 4). The values obtained ranged from 0.4 to 0.6 mg/L and were similar to those obtained using standards prepared in Merck water. A spiked river water sample (~2 mg/L) was analyzed by triplicate using external calibration and HV as internal standard, and both target value and calculated value are given in Table 4. As can be seen, low relative errors, from 4.5 to 6.2%, were obtained. In relation to matrix effect it must be noted that the response of the spiked river water sample was quite similar to that obtained for standards containing analytes at the same concentration level and no significant migration time shifting was observed.

Due to the relatively high LODs obtained with the hydrodynamic injection the method is only applicable to the analysis of high contaminated river water samples. Nevertheless, to enhance sensitivity electrokinetic injection (15 s, 10 kV) was also evaluated in combination with the CE-MS/MS method. A small improvement on LODs (5 times lower) was observed, but the repeatability increased considerably and it was impossible to obtain good quantitative results.

4. Conclusions

CE-MS and CE-MS/MS methods using an electrospray ionization source and an ion trap analyzer were developed for the analysis of BAC homologues. The CE-MS/MS method provided LODs at the 0.5 mg/L level with a good linearity and good run-to-run and day-to-day precisions. The CE-MS/MS method was applied to the analysis of some ophthalmic solutions, and the results achieved showed that the method can be used for the identification and direct determination of BAC homologues in pharmaceutical samples. For environmental samples, the CE-MS/MS method can be proposed for the analysis of these compounds in relatively high-contaminated samples. However, further research to enhance sensitivity by using several enrichment procedures is being carried out.

Acknowledgements

This work was supported by the *Ministerio de Ciencia y Tecnología* of Spain and FEDER (project no. REN2003-02692/MAR). Bianca Veronica Pară

expresses her thanks to the European Union for a Socrates-Erasmus grant for a stay in Barcelona.

References

[1] Merck. Merck index, 13th ed. Whitehouse station: Merck Research Laboratories, 2001. p. 1060.

[2] Ambrus, G., Takahashi, L.T., Marty, P.A., *J. Pharm. Sci.* 1987, *76*, 174-176.

[3] Gomez-Gomar, A., Gonzalez-Aubert, M.M., Garces-Torrents, J., Costa-Segarra, J., *J. Pharm. Biomed. Anal.* 1990, *8*, 871-876.

[4] J.J. Merianos, in: S.S. Block (Ed.), Disinfection, Sterilization and Preservation, Lea and Kebiger, Pittsburg, PA, 1991, p. 225.

[5] Richards, R.M.E., Xing, D.K.L., *J. Pharm. Sci.* 1993, *82*, 1218-

[6] Sánchez-Leal, J., González, J.J., Kaiser, K.L.E., Palabrica, V.S., Comelles, F., Garcia, M.T., *Acta Hydrochim. Hydrobiol.* 1994, *22*, 13-

[7] Technical Information Sheet, Preventol R 50 and Preventol R 80, Bayer, Leverkusen, August 1995.

[8] Matthew, J.S., Malcolm, N.J., *Biochim. Biophys. Acta* 2000, *1508*, 235-

[9] Fan, T.Y., Wall, G.M., *J. Pharm. Sci.* 1993, *82*, 1172-1174.

[10] Parhizkari, G., Miller, R.B., Chen, C., *J. Liq. Chromatogr.* 1995, *18*, 553-563.

[11] Miller, R.B., Chen, C., Sherwood, C.H., *J. Liq. Chromatogr.* 1993, *16*, 3801-3811.

[12] Bernal, J.L., del Nozal, M.J., Martín, M.T., Diez-Masa, J.C., Cifuentes, A., *J. Chromatogr. A* 1998, *823*, 423-431.

[13] Xue, Y., Hieda, Y., Kimura, K., Nishiyama, T., Adachi, T., *Legal Medicine* 2002, *4*, 232-238.

[14] Kümmerer, K., Eitel, A., Braun, U., Hubner, P., Daschner, F., Mascart, G., Milandri, M., Reinthaler, F., Verhoef, J., *J. Chromatgr. A* 1997, *774*, 281-286.

[15] Shibukawa, M., Eto, R., Kira, A., Miura, F., Oguma, K., Tatsumoto, H., Ogura, H., Uchiumi, A., *J. Chromatgr. A* 1999, *830*, 321-328.

[16] Altria, K.D., Elgey, J., Howells, J.S., *J. Chromatogr. B* 1996, *686*, 111-117.

[17] Jimidar, M., Beyns, I., Rome, R., Peeters, R., Musch, G., *Biomed. Chromatogr.* 1998, *12*, 128-130.

[18] Taylor, R.B., Toasaksiri, S., Reid, R.G., *J. Chromatogr. A* 1998, *798*, 335-343.

[19] Prince, S.J., Mclaury, H.J., Allen, L.V., Mclaury, P., *J. Pharmaceut. Biomed. Anal.* 1999, *19*, 877-882.

[20] Heinig, K., Vogt, C., Werner, G., *Fresenius J. Anal. Chem.* 1997, *358*, 500-505.

[21] Hou, Y.H., Wu, C.Y., Ding, W.H., *J. Chromatogr. A* 2002, *976*, 207-213.

[22] Lin, C.E., Chiou, W.C., Lin, W.C., *J. Chromatogr. A* 1996, *722*, 345-352.

[23] Lin, C.E., Chiou, W.C., Lin, W.C., *J. Chromatogr. A* 1996, *723*, 189-195.

[24] Herrero-Martínez, J.M., Simó-Alfonso, E.F., Mongay-Fernández, C., Ramis-Ramos, G., *J. Chromatogr. A* 2000, *895*, 227-235.

[25] Merino, F., Rubio, S., Pérez-Bendito, D., *J. Chromatogr. A* 2003, *998*, 143-154.

[26] Ferrer, I., Furlong, E.T., *Environ. Sci. Technol.* 2001, *35*, 2583-2588.

[27] Ferrer, I., Furlong, E.T., *Anal. Chem.* 2002, *74*, 1275-1280.

[28] Ford, M.J., Tetler, L.W., White, J., Rimmer, D., *J. Chromatogr. A* 2002, *952*, 165-172.

[29] Núñez, O., Moyano, E., Galceran, M.T., *J. Chromatgr. A* 2004, *1058*, 89-95.

[30] Morrow, A.P., Kassim Folahan, O.O., Ayorinde, O., *Rapid. Commun. Mass. Spectrom.* 2001, *15*, 767-770.

[31] van Brocke, A., Nicholson, G., Bayer, E., *Electrophoresis* 2001, *22*, 1251-1266.

[32] Banks, J.F., Dresch, T., *Anal. Chem.* 1996, *68*, 1480-1485.

Figure captions

Figure 1. Molecular structures of the BAC homologues and the internal standard.

Figure 2. (a) Effect of ACN in the carrier electrolyte. Sample concentration: ~20 mg/L of each BAC homologue prepared in Merck water with 30% ACN. Carrier electrolyte: 50 mM acetic acid-ammonium acetate (pH 4.0). (b) Effect of ACN in the samples. Sample concentration: ~20 mg/L of each BAC homologue prepared in Merck water. Carrier electrolyte: as in (a) with 80% ACN. Other CE conditions for (a) and (b): capillary voltage, + 20 kV; injection mode and time: hydrodynamic injection during 15 s (3.5 kPa). Peak identification: 1, C_{12}-BAC; 2, C_{14}-BAC; 3, C_{16}-BAC; 4, C_{18}-BAC.

Figure 3. CE-ESI/MS/MS electropherogram of a mixture of BAC homologues and I.S. HV (~10 mg/L) prepared in Merck water:ACN (40:60 v/v). Carrier electrolyte, 50 mM acetic acid-ammonium acetate (pH 4.0) with 80% ACN; capillary voltage, +25 kV; hydrodynamic injection during 15s (3.5 kPa).

Figure 4. CE-ESI/MS/MS electropherogram of an ophthalmic solution containing 100 mg/L of BAC. MS/MS spectra of the identified compounds are also shown. Experimental conditions as figure 3.

Table 1. MS and MS/MS acquisition parameters.

Analyte	MS	MS/MS		
	Diagnostic ion, m/z[a]	Product ion scan m/z	Diagnostic product ion m/z	Normalized collision energy (NCE%)
C_{12}-BAC	304	150-350	212	41
C_{12}-BAC	332	145-350	240	43
C_{12}-BAC	360	155-400	268	43
C_{12}-BAC	388	170-400	296	45
I.S. (HV)	354	155-400	255	43

[a] Precursor ion for MS/MS experiments.

Table 2. Quality parameters using standards in Merck water.

Compound	CE-UV			CE-ESI/MS					CE-ESI/MS/MS				
	LODs (mg/L)	Concentration level (30 mg/L)		LODs (mg/L)	Low concentration level (3 mg/L)		High concentration level (50 mg/L)		LODs (mg/L)	Low concentration level (1.5 mg/L)		High concentration level (50 mg/L)	
		run-to-run (%RSD)	day-to-day (%RSD)		run-to-run (%RSD)	day-to-day (%RSD)	run-to-run (%RSD)	run-to-run (%RSD)		run-to-run (%RSD)	day-to-day (%RSD)	run-to-run (%RSD)	day-to-day (%RSD)
C_{12}-BAC	0.8	3.3	5.0	1.0	4.4	5.6	2.7	2.8	0.5	4.6	4.8	3.2	3.3
C_{14}-BAC	1.1	3.8	7.8	0.8	4.7	8.1	2.7	4.5	0.6	3.1	5.0	2.9	3.0
C_{16}-BAC	0.8	3.9	7.5	0.9	5.1	7.9	1.7	3.2	0.5	3.3	3.5	2.4	3.4
C_{18}-BAC	1.2	3.6	6.1	1.3	4.2	6.4	1.0	2.6	0.5	3.1	4.8	2.5	4.3

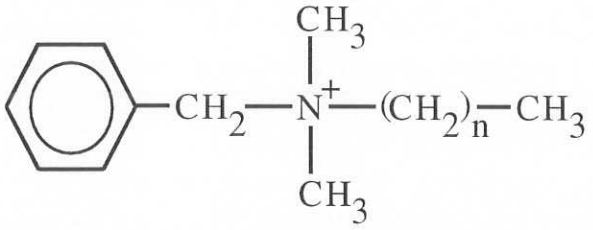
Table 3. Ophthalmic solution quantitation

Ophthalmic solution	Total amount of BAC	BAC homologues detected	
		C_{12}-BAC	C_{14}-BAC
		Concentration (mg/L)	Concentration (mg/L)
Tobrex (Alcon Cusí, S.A., Spain)	100 mg L^{-1}	51.3±0.5	48.8±0.8
Liquifilm Lagrimas (Allergan, S.A., Spain)	50 mg L^{-1}	32.0±0.4	17.2±0.7
Betagan 0.5% (Allergan, S.A., Spain)	40 mg L^{-1}	23.1±0.8	16.7±0.9

Table 4. River water sample.

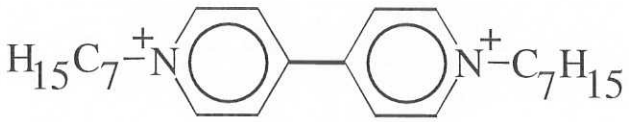
Compound	LOD (mg/L)	Target value (mg/L)	Calculated value (mg/L)	Relative error (%)
C_{12}-BAC	0.5	1.6	1.5±0.3[a]	6.2
C_{14}-BAC	0.5	1.8	1.7±0.4[a]	5.6
C_{16}-BAC	0.6	2.2	2.3±0.3[a]	4.5
C_{18}-BAC	0.4	1.9	2.0±0.4[a]	5.3

[a] $t_{student}(95\%) = 4.2$

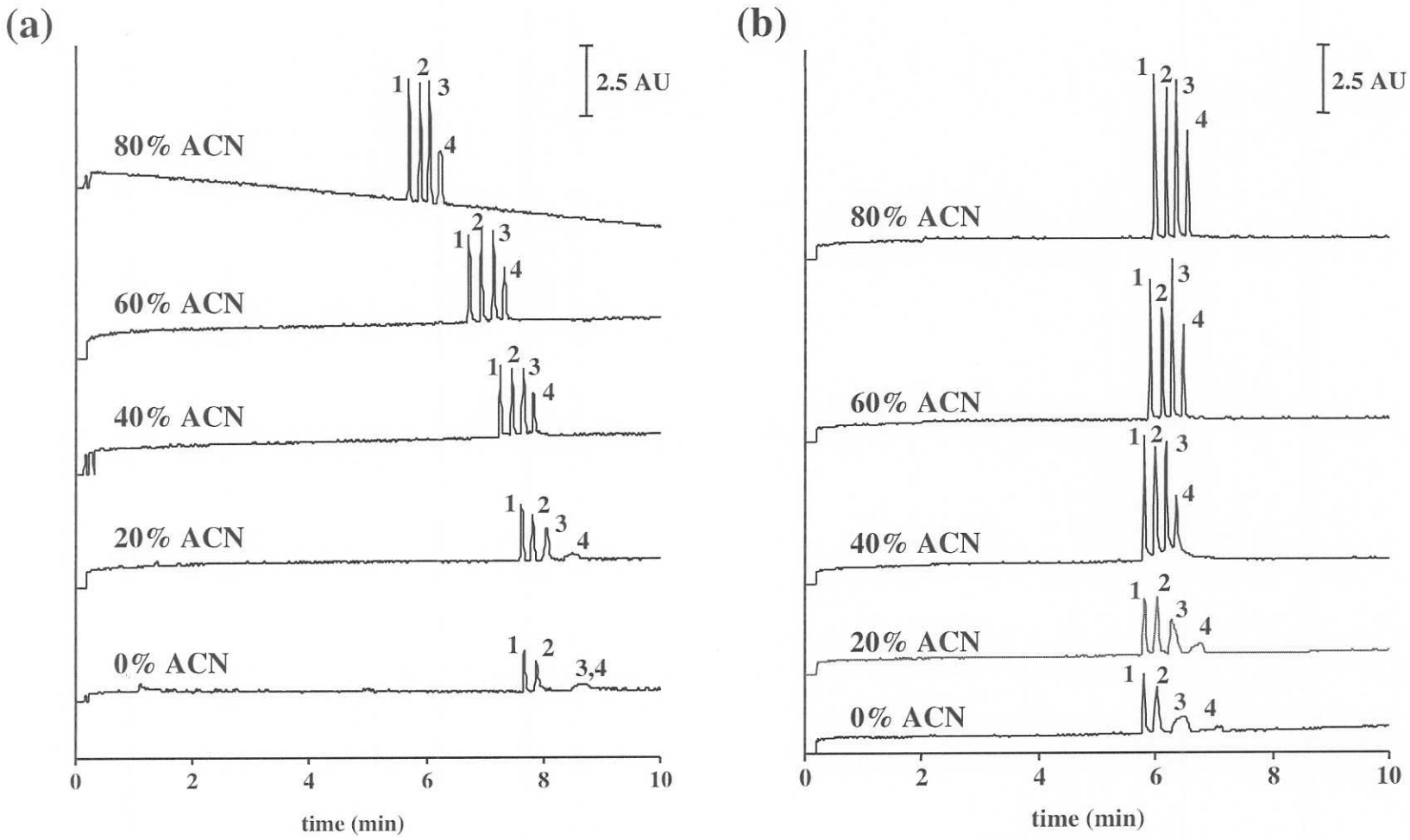


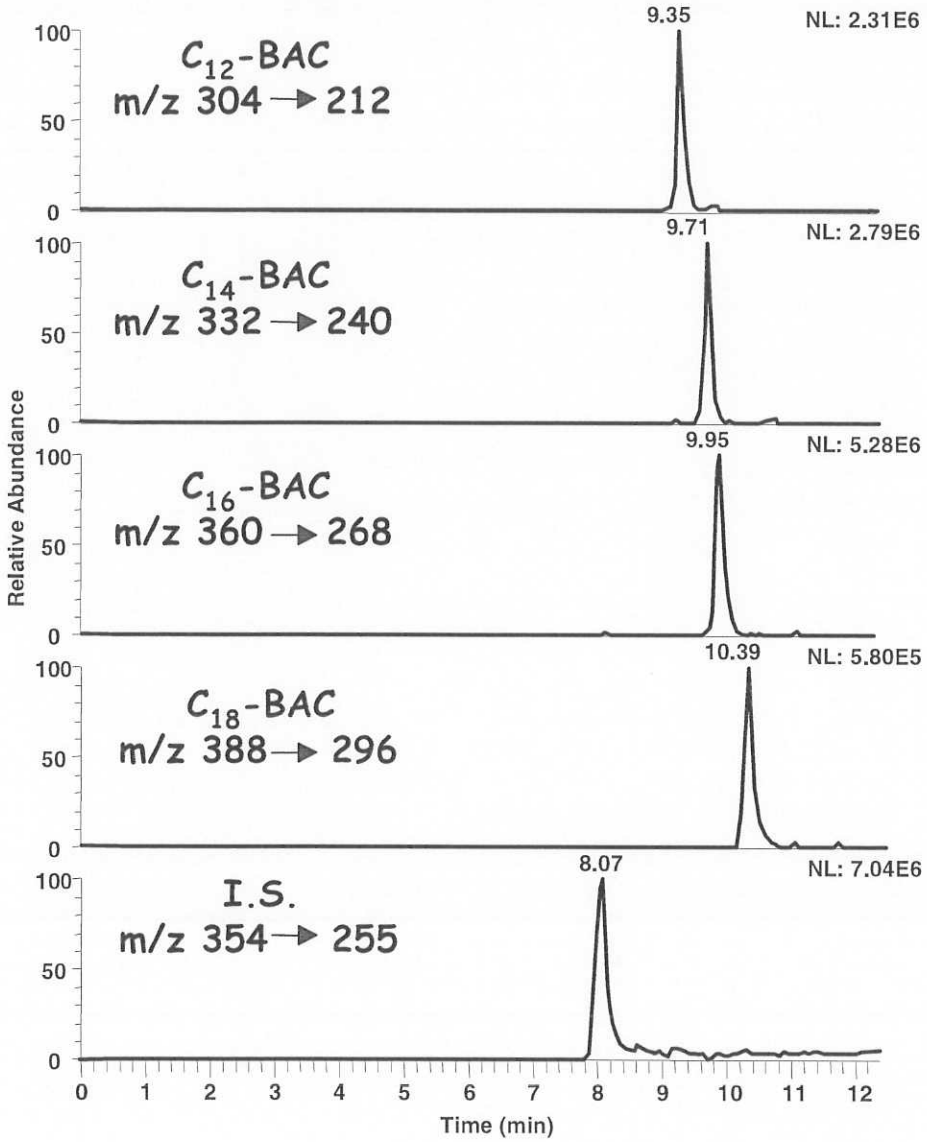
n=11, C_{12}-BAC
n=13, C_{14}-BAC
n=15, C_{16}-BAC
n=17, C_{18}-BAC

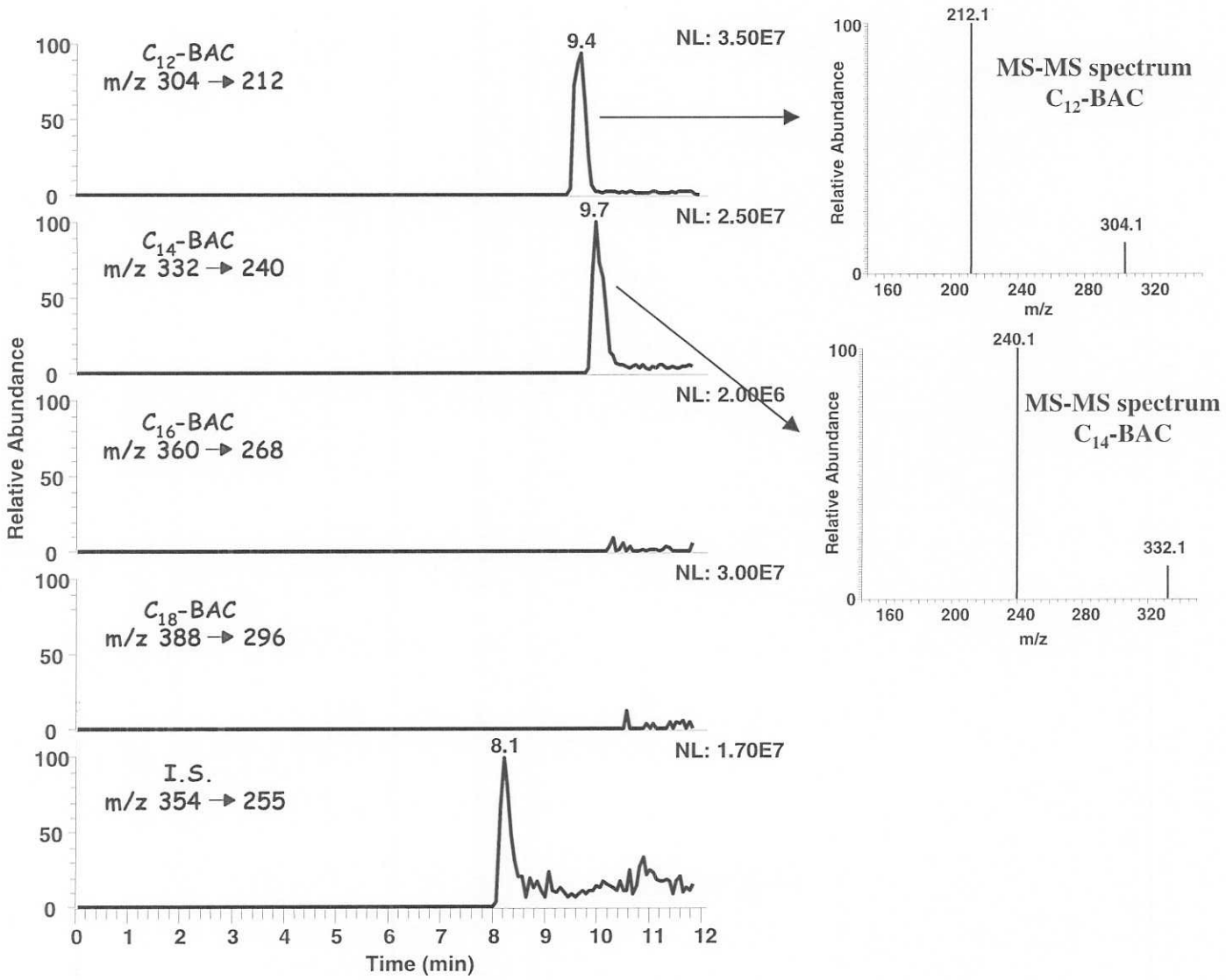
alquilbenzyldimethylammonium ion



I.S.: Heptylviologen (HV)


(a)
2.5 AU
80% ACN
60% ACN
40% ACN
20% ACN
0% ACN
1
2
3
4
3,4
0
2
4
6
8
10
time (min)
(b)
2.5 AU
80% ACN
60% ACN
40% ACN
20% ACN
0% ACN
1
2
3
4
0
2
4
6
8
10
time (min)


C_{12}-BAC
m/z 304 → 212
9.35
NL: 2.31E6
C_{14}-BAC
m/z 332 → 240
9.71
NL: 2.79E6
C_{16}-BAC
m/z 360 → 268
9.95
NL: 5.28E6
C_{18}-BAC
m/z 388 → 296
10.39
NL: 5.80E5
I.S.
m/z 354 → 255
8.07
NL: 7.04E6
Relative Abundance
Time (min)


C_{12}-BAC
m/z 304 → 212
9.4
NL: 3.50E7
C_{14}-BAC
m/z 332 → 240
9.7
NL: 2.50E7
NL: 2.00E6
C_{16}-BAC
m/z 360 → 268
NL: 3.00E7
C_{18}-BAC
m/z 388 → 296
NL: 1.70E7
I.S.
m/z 354 → 255
8.1
Relative Abundance
Time (min)
MS-MS spectrum
C_{12}-BAC
212.1
304.1
MS-MS spectrum
C_{14}-BAC
240.1
332.1
m/z