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Determination of quaternary ammonium biocides by liquid chromatography–mass spectrometry

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Abstract

In this study we have developed a method for the direct determination of benzalkonium chloride (BAC) homologues and didecyldimethylammonium chloride (DDMAC), generally used as biocides. The chromatographic separation was performed using a Luna C18 column and gradient elution. A 50 mM formic acid–ammonium formate buffer at pH 3.5 was used as aqueous phase to allow ion-pair formation with the quaternary ammonium biocides. The detection was carried out using an ion trap mass analyser and electrospray ionisation (ESI) source. Parameters such as the magnitude and duration of the resonant excitation voltage and the magnitude of the trapping RF voltage for full scan tandem mass spectrometry (MS–MS) experiments were studied to establish the optimal experimental conditions. Instrumental quality parameters of both liquid chromatography coupled to mass spectrometry (LC–MS) and LC–MS–MS methods were studied and good run-to-run and day-to-day precision values (relative standard deviations, RSDs lower than 11%) and LODs down to 0.1 $\mu g\,L^{-1}$ (LC–MS–MS) were obtained. Finally, the applicability of the LC–MS–MS method was demonstrated by analysis of a spiked water sample and some commercial products containing BAC.
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Keywords: Quaternary ammonium biocides; Water analysis

1. Introduction

Some quaternary ammonium compounds (QACs) containing at least one long alkyl chain have biocidal properties and are widely used as general fabric softeners, disinfectants, preservatives, pest-control and anti-fouling products. They frequently leak into surface waters from wastewater treatment plants, disturbing the ecological balance due to their toxicity to aquatic organisms [1]. Their fate in the environment is of concern since there is a lack of data on their degradation [2] and some of them are known to be toxic even at low concentrations [3]. The Biocidal Products Directive (BPD) aims to establish a single European market for biocides and to ensure that protection is provided for users as well as for the public and the environment [4]. For these reasons, reliable analytical methods for the determination of quaternary ammonium biocides are required for the effective monitoring of occupational and environmental exposure as well as in the quality control of manufactured products.

Benzalkonium chloride (BAC) is a mixture of alkylbenzyldimethylammonium chlorides in which the alkyl groups have a chain length from C_8 to C_{18} [5]. This mixture is widely used as an active substance in anti-bacterial and anti-fungal products, in can preservatives, medical disinfectants and ophthalmic systems [6,7]. The most commonly used homologues are C_{12}-BAC, C_{14}-BAC and C_{16}-BAC. Each homologue possesses different physical, chemical and biocidal properties. In general, the C_{12}-BAC homologue is most effective against yeast and fungi, the C_{14}-BAC homologue against gram-positive bacteria, and the C_{16}-BAC homologue against gram-negative bacteria [8]. Other quaternary ammonium compounds, such as dialkyldimethylammonium compounds, are also commonly used as biocides and some manufacturers of hospital disinfectant products have substituted BAC with didecyldimethylammonium chloride (DDMAC).

Due to their cationic character, liquid chromatography (LC) and capillary electrophoresis (CE) are the techniques

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most frequently used for the analysis of quaternary ammonium biocides. In this way, BAC has been analysed using LC with UV-detection in ophthalmic solutions [6,9–12] and CE with both direct [13–21] and indirect UV-detection [20,22] using standards or in nasal and ophthalmic solutions. Recently, liquid chromatography coupled to mass spectrometry (LC–MS) has also been applied to the analysis of BAC in several matrices [23–25]. The C_{12}-BAC and C_{14}-BAC homologues have been determined in water samples after preconcentration with on-line solid-phase extraction and using an ion trap analyser but, although MS–MS fragmentation studies were performed, LC–MS–MS was only applied for confirmation [24]. Nevertheless, for the determination of some BAC homologues in sediment samples, accelerated solvent extraction followed by an on-line preconcentration procedure and LC–MS–MS was necessary [25]. The same analyser was also used for the analysis of BAC and other cationic surfactants in sewage sludge [23]. Moreover, a triple quadrupole instrument was used for the analysis of these compounds in environmental media and occupational hygiene samples by LC–MS, also performing some MS/MS analysis [26].

The DDMAC biocide does not have chromophore groups, making MS the ideal technique for its analysis. Ford et al. [26] determined this compound simultaneously with some BAC homologues in environmental and occupational hygiene samples using a triple quadrupole instrument. The presence of these compounds in oral rinses and disinfectant formulations was also analysed by means of matrix-assisted laser desorption ionisation-time-of-flight mass spectrometry (MALDI-TOFMS) [27].

The aim of this work was to develop sensitive and rapid LC–MS and LC–MS–MS methods using an electrospray ionisation (ESI) source and an ion trap analyser for the analysis of BAC homologues and DDMAC in samples such as environmental samples, ophthalmic solutions and formulations.

2. Experimental

2.1. Chemicals

Benzyldimethyldodecylammonium bromide, benzyldimethyltetradecylammonium chloride, benzyldimethylhexadecylammonium chloride, *N*-benzyl-*N*,*N*-dimethyloctadecylammonium chloride hydrate (90%) and didecyldimethylammonium bromide (98%) were obtained from Sigma–Aldrich (Steinheim, Germany) and their structures are given in Fig. 1. HPLC-gradient grade acetonitrile and water, formic acid (98–100%), ammonia solution (25%) and hexadedyltrimethylammonium bromide (used as internal standard) were purchased from Merck (Darmstadt, Germany).

Stock standard solutions of quaternary ammonium biocides and internal standard (1000 mg L^{-1}) were prepared in acetonitrile. Working solutions were prepared by dilution of the stock standard solutions in HPLC Merck water.

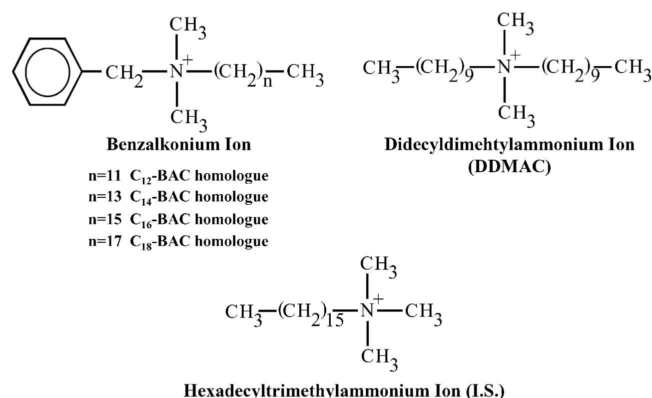

Fig. 1. Molecular structures of quaternary ammonium biocides and internal standard.

2.2. Chromatography

Chromatographic separation on a Luna C18 column (50 mm × 2.00 mm, 5 μm, Phenomenex) was performed using a Waters 2695 separation module (Milford, MA, USA) equipped with a quaternary solvent delivery system and autosampler. Acetonitrile post-column addition was carried out using an LKB-HPLC Pump 2248 from Pharmacia (Bromma, Sweden) and a Valco stainless-steel T-piece (Supelco, Alcobendas, Spain).

A gradient elution program was used for the separation of quaternary ammonium biocides; solvent A was formic acid–ammonium formate buffer (50 mM, pH 3.5) and solvent B was acetonitrile. The elution program started with an isocratic step of 2 min at 40% solvent B, a linear gradient from 40 to 70% in 3 min and then a 70% solvent B isocratic step. The flow rate was 300 μL min^{-1}, the column was maintained at room temperature and the injection volume was 20 μL. Post-column addition of acetonitrile was carried out at a flow rate of 150 μL min^{-1} as an increase in the response was observed. A switch valve was used before the mass spectrometer to divert the flow to waste for 1.5 min at the beginning of the chromatogram.

2.3. Mass spectrometry

Mass spectrometry was carried out using an LCQ Classic (Thermo Electron Corporation, San Jose, CA, USA) equipped with a pneumatically assisted electrospray ionisation source and an ion trap analyser, and controlled by Xcalibur vs. 1.3 software. A metallic capillary tube was used as electrospray sample inlet to avoid adsorptions of the biocides on the wall tube. The ESI was pneumatically assisted by nitrogen using a sheath gas flow rate of 70 a.u. (arbitrary units) and an auxiliary gas flow rate of 40 a.u. The heated capillary was held at 200 °C. A tube lens offset voltage of

Table 1
MS and MS–MS acquisition parameters

Segment	Time (min)	Analyte	MS (*m/z*)[a]	MS–MS		
				Product full scan (*m/z*)	Diagnostic product ion (*m/z*)	Normalised collision energy (%NCE)
1	0–7.0	C_{12}-BAC	304	150–350	212	46
2	7–10.5	C_{14}-BAC	332	145–350	240	46
		C_{16}-BAC	360	155–400	268	47
		DDMAC	326	140–350	186	55
		I.S.	284	140–350	270	56
3	10.5–15.0	C_{18}-BAC	388	170–400	296	48

[a] Precursor ion for MS–MS experiments.

1.0 V and an electrospray voltage of 1.0 kV were used. Single MS data acquisition was performed in scan mode from *m/z* 50 to 450 using a maximum injection time of 200 ms and 3 μscans. Moreover, the molecular ion $[M]^+$ was used as diagnostic ion for quantitation purposes. For LC–MS–MS the chromatogram was segmented in three windows (Table 1) and data acquisition was carried out in product ion scan mode. The *m/z* range acquired and the MS–MS transitions monitored for quantitation purposes are also given in Table 1. The activation Q (AQ) and the activation time (AT) were set at 0.40 and 30 ms, respectively. Moreover, an isolation width of *m/z* 1.5 was applied. The normalised collision energy (%NCE) selected for each analyte is also indicated in Table 1. The acquisition was performed with a maximum injection time of 150 ms and 3 μscans. Both single MS and MS–MS data acquisitions were performed in centroid mode.

3. Results and discussion

The chromatographic separation of quaternary ammonium biocides has been carried out by ion-pair liquid chromatography using as mobile phase acetonitrile:formic acid–ammonium formate buffer [23,25,26]. Buffer concentration and pH were optimised as they affect separation, peak shape and response. As a compromise, a buffer solution of 50 mM formic acid–ammonium formate at pH 3.5 which provided a stable spray and good sensitivity and chromatographic efficiency was used. Moreover, the application of a gradient elution (see Section 2) reduced both the analysis time (to less than 12 min) and peak widths.

The ESI–MS instrument parameters such as sheath gas and auxiliary gas flow rate, electrospray voltage and heated capillary temperature were also optimised in order to ob-

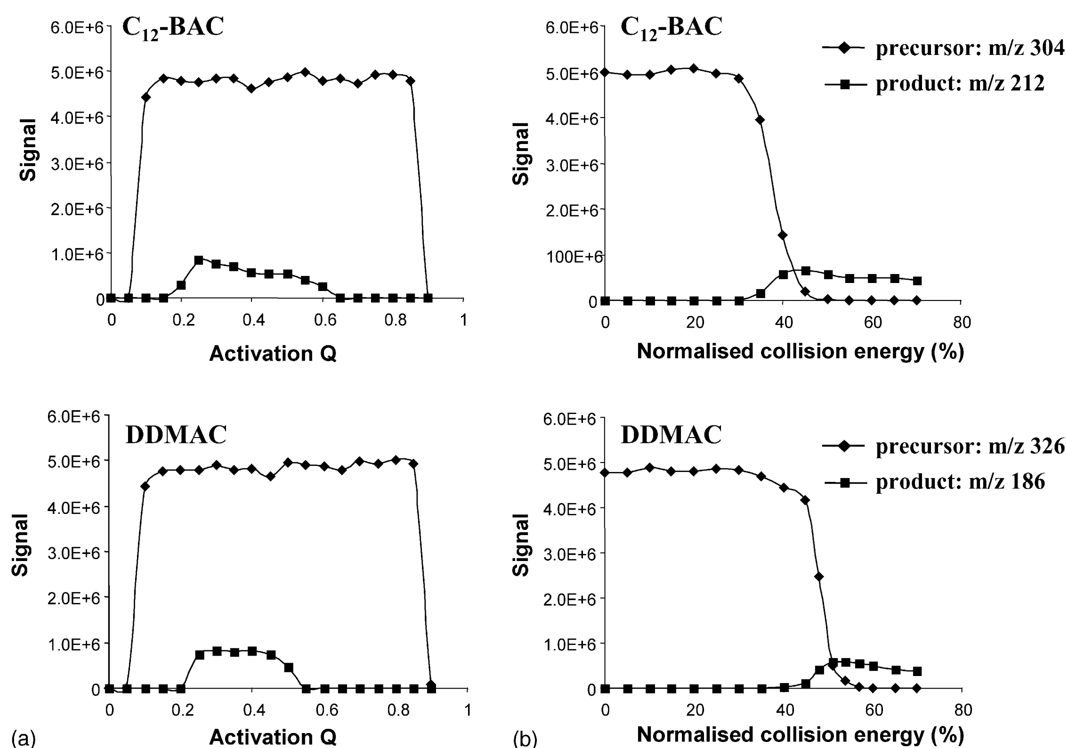

Fig. 2. (a) Variation of ion intensities vs. AQ for C_{12}-BAC and C_{14}-BAC homologues. (b) Effect of the normalised collision energy on both precursor and product ions for C_{12}-BAC and C_{14}-BAC homologues.

tain the highest response for the molecular ion $[M]^+$. Relatively high flow rate values for sheath and auxiliary gases (70 a.u. and 40 a.u., respectively) and a temperature of 200 °C were necessary to obtain the maximal response. Moreover, a spray voltage of 1.0 kV was enough to obtain the optimum signal.

3.1. Mass spectrometry

The single MS spectra (full scan) of quaternary ammonium biocides were obtained by infusion under the working conditions previously established. The molecular ion $[M]^+$ was the base peak in these spectra and no fragmentation or cluster formation was observed. To perform the MS–MS experiments, these molecular ions were used as precursor ions and an isolation width of *m/z* 1.5 was required to obtain the maximum trapping efficiency without interference from isotopic species or matrix components. Generally, when working with low molecular weight ions a precise control of activation Q voltage is necessary, as this parameter control the stability range for both precursor ion and product ion. The AQ voltage was varied from 0 to 0.98 and for this family of compounds a relatively wide stability range was observed (AQ from 0.25 to 0.6 for BAC homologues and from 0.25 to 0.5 for DDMAC) and as a compromise an AQ value of 0.4 was chosen as optimum for the five biocides (Fig. 2a).

The normalised collision energy necessary to fragment the biocides was studied and the value that produced the highest intensity product ion and at least 10% of the precursor ion was chosen as optimum (Table 1). For instance, the BAC homologues required an NCE higher than 45%, whilst the DDMAC fragmented at values higher than 55% (Fig. 2b). Under the optimised working conditions the MS–MS spectra of the five biocides were obtained. The fragmentation pattern was very simple and for all the BAC homologues only two fragment ions were observed. The base peak corresponded to the loss of a $CH_3C_6H_5$ group and this product ion was used as a diagnostic ion for quantitation purposes. The other fragment (*m/z* 91) showed abundance lower than 20% and corresponded to the tropylium ion. In contrast, the DDMAC biocide only gave one ion (*m/z* 186) due to the loss of a $C_{10}H_{20}$ group. These results agree with those obtained by other authors [24,26], although for DDMAC other fragments (*m/z* 85, 71 and 57) have also been reported using a triple quadrupole instrument [26]. These ions could be explained by multiple collisions in the triple quadrupole instrument since they only appeared in the MS^3 spectrum when working with the ion trap analyser. Fig. 3 shows the multistep fragmentation ion trap spectra for DDMAC.

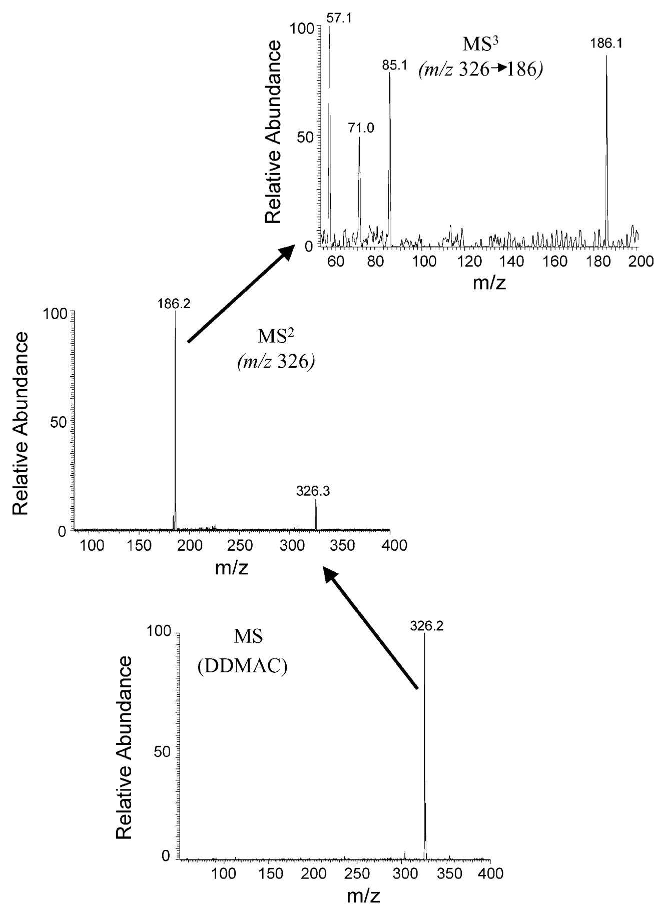

Fig. 3. MS^n fragmentation for DDMAC biocide.

3.2. Instrument quality parameters

Instrument quality parameters such as limits of detection (LODs) and run-to-run and day-to-day precisions were obtained for both LC–MS and LC–MS–MS methods and the

Table 2
Instrumental quality parameters

Compound	LC–MS				LC–MS–MS			
	LODs		Run-to-run	Day-to-day	LODs		Run-to-run	*Day − to − day*
	$\mu g\,L^{-1}$	pg injected	(%RSD, $n = 5$)	(%RSD, $n = 3 \times 5$)	$\mu g\,L^{-1}$	pg injected	(%RSD, $n = 5$)	(%RSD, $n = 3 \times 5$)
C_{12}-BAC	1.0	20	2.0	4.3	0.01	0.2	4.0	7.2
C_{14}-BAC	2.0	40	1.9	2.5	0.1	2	5.5	10.7
C_{16}-BAC	1.0	20	1.5	2.0	0.1	2	4.3	7.3
C_{18}-BAC	1.5	30	1.7	2.6	0.1	2	4.3	7.0
DDMAC	1.0	20	1.9	2.6	0.1	2	5.5	8.2

figures of merit are provided in Table 2. To obtain the LODs, different standard solutions of the five biocides prepared in Milli-Q water (Millipore, Bedford, MA, USA) were analysed. However, when non-spiked Milli-Q water was analysed, the C_{12}-BAC and C_{14}-BAC homologues were observed. This was due to contamination of the Milli-Q water with BAC as a result of the use of benzalkonium chloride as a disinfectant in the Milli-Q exchange resins and not to a memory effect of the LC–MS system. To overcome this problem, HPLC water from Merck was used for both mobile phase preparation and the determination of the instrument quality parameters. The LODs, based on a signal-to-noise ratio of 3:1, were ca. 1.0 $\mu g\,L^{-1}$ for LC–MS and dropped to 0.01–0.1 $\mu g\,L^{-1}$ with LC–MS–MS. The LC–MS LODs obtained for BAC homologues in this study were between two and four times lower than those reported by Ford et al. [26] using electrospray and a triple quadrupole instrument. Ferrer and Furlong [24] achieved LODs around 3–5 $ng\,L^{-1}$ by LC–MS with an ion trap instrument after an on-line preconcentration of 50 ml of water using PLRPs cartridges. In addition, both studies [24,26] used LC–MS–MS for identification but not for quantitation purposes.

Calibration curves based on the peak area ratio ($A_{compound}/A_{internal\ standard}$) for the five biocides at concentrations between 20 and 250 $\mu g\,L^{-1}$ for the LC–MS method and between 5 and 100 $\mu g\,L^{-1}$ for the LC–MS–MS method showed acceptable linearity ($r^2 > 0.996$ and $r^2 > 0.993$ for LC–MS and LC–MS–MS, respectively). Five replicate determinations of a standard solution that contained the five bioicides at a concentration level of ~50 $\mu g\,L^{-1}$ for LC–MS and ~30 $\mu g\,L^{-1}$ for LC–MS–MS method were carried out under the optimised conditions to determine run-to-run reproducibility. The relative standard deviations (RSDs) based on concentration ranged from 1.5 to 2.0% for LC–MS and were slightly higher for LC–MS–MS (4.3–5.5%). The day-to-day reproducibility was calculated from 15 replicate determinations of the same standard solutions mentioned above, performed on three different days (five replicates each day). The RSD values based on concentration ranged from 2.0 to 4.3% (LC–MS) and from 7.0 to 10.7% (LC–MS–MS), only slightly higher than those for the run-to-run precision. Moreover, the comparison of the calculated concentration of the replicates with the target value provided low relative errors ranging from 0.8 to 6.8% for LC–MS and 0.2 to 7.8% for LC–MS–MS.

3.3. Application

To assess the applicability of the LC–MS–MS method (which provided the lowest LODs) a spiked river water sample and some commercial samples (an ophthalmic solution and a BAC standard) were analysed. Fig. 4a shows the LC–MS–MS chromatogram of a spiked river water sample at a concentration level of ~10 $\mu g\,L^{-1}$, which is close to the values found in some environmental water samples [24]. As can be seen, the separation of all the biocides was good enough for their analysis in less than 12 min, but co-elution between the internal standard and the C_{14}-BAC homologue

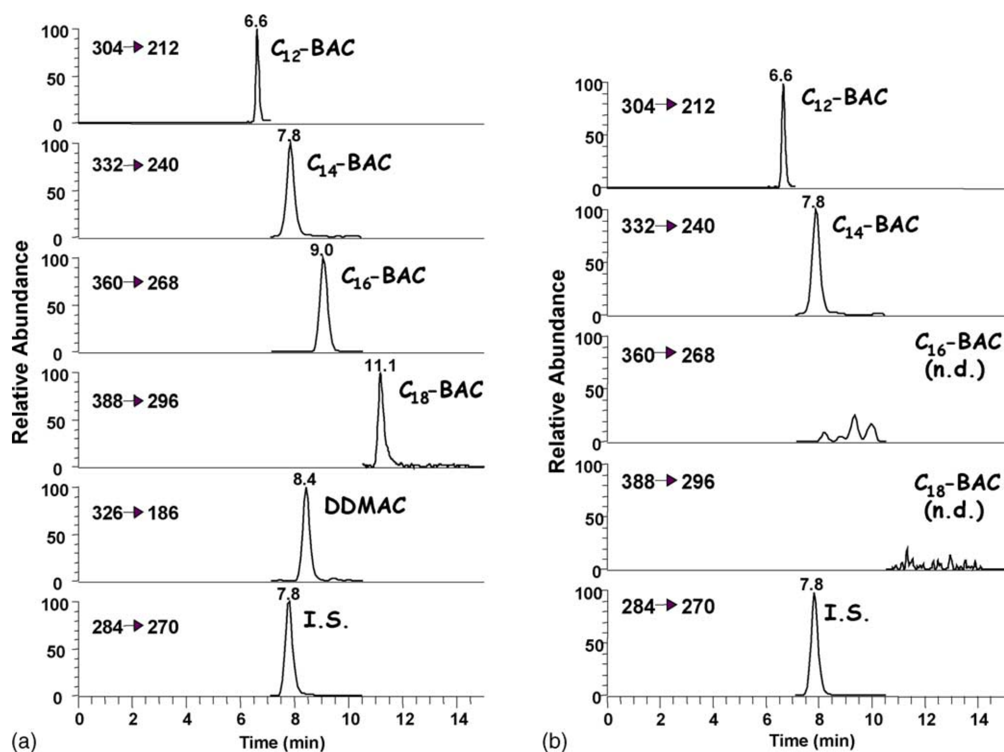

Fig. 4. LC–MS–MS chromatograms of (a) a spiked river water sample (~10 $\mu g\,L^{-1}$) for each biocide and (b) an ophthalmic solution containing 50 $mg\,L^{-1}$ of BAC (diluted 1:1000).

Table 3
Spiked river water sample by LC–MS–MS ($n = 3$)

Compound	LODs ($\mu g\,L^{-1}$)	Spiked value ($\mu g\,L^{-1}$)	Mean value ($\mu g\,L^{-1}$)
C_{12}-BAC	0.05	10.1	10.3 ± 0.4
C_{14}-BAC	0.1	10.3	10.8 ± 0.5
C_{16}-BAC	0.1	12.2	12.4 ± 0.6
C_{18}-BAC	0.1	10.2	10.4 ± 0.5
DDMAC	0.1	10.1	10.0 ± 0.4

occurred. Nevertheless, as no ionic suppression between the two species was observed, baseline chromatographic separation of both compounds was not required. LODs were estimated using a river water sample free of BAC and ranged from 0.05 to 0.1 $\mu g\,L^{-1}$ (Table 3). The values were similar to those obtained with standards, although for the C_{12}-BAC homologue the LOD increased five times probably due to ion suppression caused by the matrix. Moreover, these values are more than 10 times lower than the lowest concentration found in environmental samples (1–30 $\mu g\,L^{-1}$) [24], allowing direct analysis without on-line preconcentration. Analysis of the spiked river water samples was performed in triplicate by external calibration using hexadecyltrimethylammonium ion as internal standard and both target values and calculated values are given in Table 3. As can be seen, low relative errors, from 1.0 to 5%, were obtained.

The LC–MS–MS method was also applied to the analysis of two commercial products containing BAC, an ophthalmic solution (Liquifilm Lagrimas, Allergan, Madrid, Spain) containing 50 $mg\,L^{-1}$ of benzalkonium chloride, and a BAC standard from Sigma–Aldrich (Steinheim, Germany). Fig. 4b shows the LC–MS–MS chromatogram of the ophthalmic solution (diluted 1:1000). As can be seen, only the C_{12}-BAC and C_{14}-BAC homologues were found. These two homologues are the most frequently used in this kind of sample. The ophthalmic sample was quantified by external calibration ($n = 3$) and the concentrations found were 32.3 ± 0.5 and 16.6 ± 0.9 $mg\,L^{-1}$ for the C_{12}-BAC and C_{14}-BAC homologues, respectively (confidence interval using a $t_{student}$(95%) value of 4.3). The RSDs obtained for the three determinations were 0.74% and 2.49% for the C_{12}-BAC and C_{14}-BAC homologues, respectively. The commercial BAC standard was analysed in order to obtain the relative composition of each homologue in the sample. For this purpose, a normalization method with response factors to correct peak areas was used. Three replicate determinations of ca. 20 $\mu g\,L^{-1}$ of BAC were analysed by LC–MS–MS, obtaining the following results: C_{12}-BAC, 64.9%; C_{14}-BAC, 28.8%; C_{16}-BAC, 3.8% and C_{18}-BAC, 2.5% (RSDs of 1.7, 2.1, 1.9 and 1.6%, respectively).

4. Conclusions

LC–MS and LC–MS–MS methods using an electrospray ionisation source and an ion trap analyser have been developed for the analysis of biocides such as benzalkonium chloride homologues and didecyldimethylammonium chloride. The LC–MS–MS method provided LODs in the low ppb range with a very good linearity and good run-to-run and day-to-day precisions. The analysis of control samples provided satisfactory results with bias lower than 8% when compared with target values. The LC–MS–MS method was applied to the analysis of a spiked river water sample, an ophthalmic solution and a commercial product, and the results achieved showed that the method could be applied to the fast identification and direct determination of quaternary ammonium biocides in these kinds of environmental and pharmaceutical samples without the necessity to apply any additional preconcentration step and, in the same time, achieving acceptable limits of detection, being an important advantage over other existing methods.

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References

[1] R.M.E. Richards, D.K.L. Xing, J. Pharm. Sci. 82 (1993) 1218.
[2] J.S. Matthew, N.J. Malcolm, Biochim. Biophys. Acta 1508 (2000) 235.
[3] J. Sánchez-Leal, J.J. González, K.L.E. Kaiser, V.S. Palabrica, F. Comelles, M.T. Garcia, Acta Hydrochim. Hydrobiol. 22 (1994) 13.
[4] Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market, No. L123, 24 April 1998.
[5] Merck, Merck Index, 13th ed., Whitehouse station, Merck Research Laboratories, 2001, p. 1060.
[6] A. Gomez-Gomar, M.M. Gonzalez-Aubert, J. Garces-Torrents, J. Costa-Segarra, J. Pharm. Biomed. Anal. 8 (1990) 871.
[7] G. Ambrus, L.T. Takahashi, P.A. Marty, J. Pharm. Sci. 76 (1987) 174.
[8] J.J. Merianos, in: S.S. Block (Ed.), Disinfection, Sterilization and Preservation, Lea and Kebiger, Pittsburg, PA, 1991, p. 225.
[9] G. Parhizkari, G. Delker, R.B. Miller, C. Chen, Chromatographia 40 (1995) 155.
[10] G. Parhizkari, R.B. Miller, C. Chen, J. Liq. Chromatogr. 18 (1995) 553.
[11] T.Y. Fan, G.M. Wall, J. Pharm. Sci. 82 (1993) 1172.
[12] R.B. Miller, C. Chen, C.H. Sherwood, J. Liq. Chromatogr. 16 (1993) 3801.
[13] J.L. Bernal, M.J. del Nozal, M.T. Martín, J.C. Diez-Masa, A. Cifuentes, J. Chromatogr. A 823 (1998) 423.
[14] M. Jimidar, I. Beyns, R. Rome, R. Peeters, G. Musch, Biomed. Chromatogr. 12 (1998) 128.
[15] C.E. Lin, W.C. Chiou, W.C. Lin, J. Chromatogr. A 722 (1996) 345.
[16] J.M. Herrero-Martínez, E.F. Simó-Alfonso, C. Mongay-Fernández, G. Ramis-Ramos, J. Chromatogr. A 895 (2000) 227.
[17] K. Heinig, C. Vogt, G. Werner, Fresenius J. Anal. Chem. 358 (1997) 500.

[18] S.J. Prince, H.J. Mclaury, L.V. Allen, P. Mclaury, J. Pharm. Biomed. Anal. 19 (1999) 877.
[19] R.B. Taylor, S. Toasaksiri, R.G. Reid, J. Chromatogr. A 798 (1998) 335.
[20] C.S. Weiss, J.S. Hazlett, M.H. Datta, M.H. Danzer, J. Chromatogr. 608 (1992) 325.
[21] K.D. Altria, J. Elgey, J.S. Howells, J. Chromatogr. B 686 (1996) 111.
[22] K. Heinig, C. Vogt, G. Werner, J. Chromatogr. A 781 (1997) 17.
[23] F. Merino, S. Rubio, D. Pérez-Bendito, J. Chromatogr. A 998 (2003) 143.
[24] I. Ferrer, E.T. Furlong, Environ. Sci. Technol. 35 (2001) 2583.
[25] I. Ferrer, E.T. Furlong, Anal. Chem. 74 (2002) 1275.
[26] M.J. Ford, L.W. Tetler, J. White, D. Rimmer, J. Chromatogr. A 952 (2002) 165.
[27] A.P. Morrow, O.O. Kassim Folahan, O. Ayorinde, Rapid Commun. Mass. Spectrom. 15 (2001) 767.