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Determination of alkyl benzyl and dialkyl dimethyl quaternary ammonium biocides in occupational hygiene and environmental media by liquid chromatography with electrospray ionisation mass spectrometry and tandem mass spectrometry

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This paper is dedicated to the memory of Dr. Lee Tetler.

Abstract

A new method for the simultaneous qualitative and quantitative determination of alkyl benzyl and dialkyl quaternary ammonium compounds (QACs) has been developed. Analysis is by reversed-phase high-performance liquid chromatography coupled with electrospray ionisation mass spectrometry. QACs are extremely amenable to the electrospray ionisation technique (limit of detection of BAC C_{12} homologue 3 ng ml^{-1}). The selectivity of mass spectrometric detection allows simultaneous determination of benzyl and dialkyl dimethyl ammonium compounds. The method was successfully applied to the analysis of real samples (occupational hygiene sampling devices, products and swimming pool water). Structural information was obtained by MS–MS and cone voltage ion dissociation techniques. Ion dissociation enabled the structural elucidation of an unknown quaternary ammonium compound present in a commercial formulation.

Keywords: Mass spectrometry; Alkyl benzyl quaternary ammonium compounds; Dialkyl dimethyl quaternary ammonium compounds; Quaternary ammonium compounds

1. Introduction

The Biocidal Products Directive (BPD) [1] aims to establish a single European market for biocides and to ensure that a high level of protection is provided for users, the public and the environment. A biocide is defined as a product that is intended to kill or exert some controlling effect on harmful organisms by chemical or biological means. Biocidal products have a wide range of uses such as general disinfectant products, preservatives, pest control and anti-fouling products. There are currently many biocidal products on the UK market employing a variety of active substances (ASs) and preparations

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[2]. To aid potential enforcement of the Biocidal Products Regulations, in Great Britain, there is a requirement for analytical methods for the qualitative and quantitative determination of active substances used in biocidal products. Analysis is also required to monitor occupational and environmental exposure arising from biocide use.

Benzalkonium chloride (BAC) [3] is a mixture of *n*-alkyl benzyldimethyl ammonium chloride homologues varying in *n*-alkyl chain length, where *n* represents an even number of carbons from C_8 to C_{18}. The most commonly encountered homologues are C_{12}, C_{14} and C_{16} [4], the biocidal properties of the individual homologues are known to be different [5]. BAC is widely used as an active substance in a variety of applications including anti-bacterial products, anti-fungal products, in-can preservatives, timber treatments, masonary biocides [2], medical disinfectants and ophthalmic systems [6,7]. The preparations used, which vary in individual homologue content, also often contain other ingredients (e.g., amines, steroids, alcohols, etc.) and these can interfere with BAC determination. Other quaternary ammonium compounds (QACs) are also commonly used as biocides, and are collectively described as dialkyl dimethyl ammonium compounds. One example is didecyl dimethyl ammonium bromide (DDDMAB).

Several liquid chromatography (LC) methods for the determination of BAC have been described with separation achieved on cyanopropylsilica (CPS) [5–9], octadecylsilica (ODS) [10–13] or hydrophilic polymer [14] stationary phase columns. The phenyl substituent of BAC provides a suitable chromophore for UV–Vis analysis but the dialkyl dimethyl ammonium compounds such as DDDMAB have no chromophore and so cannot be readily detected by this method. Indirect UV detection has been described for dialkyl QACs but this is not a very specific method of analysis and can be affected by co-eluting compounds. The detector most commonly used for BAC has been UV–Vis but fluorimetric [14] and conductometric [15] methods have been reported. The conductometric [15] detector has the advantage of being able to detect QACs with no UV absorbance, QACs such as dodecyl trimethyl ammonium chloride. Mass spectrometry (MS) was applied to dialkyl dimethyl QACs by Radke et al. working in the 0.4–140 ng ml^{-1} range [13].

MS detection offers several advantages over the previously described methods of detection including increased sensitivity and specificity. The cationic nature of quaternary ammonium compounds makes them very amenable to positive ion liquid chromatography with electrospray ionisation (LC–ESI-MS). For BAC and dialkyl dimethyl ammonium compounds, LC–ESI-MS offers the possibility of a method of simultaneous determination and quantification.

This paper describes the application of LC–ESI-MS to the simultaneous determination of alkylbenzyl and dialkyl QACs. Structural determination is reported by the use of tandem mass spectrometry and cone voltage ion dissociation methods.

2. Experimental

2.1. Chemicals and reagents

Benzyldimethyl dodecyl ammonium bromide (97%), benzyldimethyl tetradecyl ammonium chloride (99%), benzyldimethyl hexadecyl ammonium chloride (99%) and didecyl dimethyl ammonium bromide (98%) were obtained from Sigma–Aldrich (Poole, UK). HPLC-grade acetonitrile and formic acid were obtained from Fischer (Loughborough, UK). Ammonium acetate (HiPerSolv) was purchased from BDH (Poole, UK). Milli-Q water was used in all the experiments where necessary.

Stock standard solutions were prepared by dissolving 1 mg of each standard compound in 1 ml of acetonitrile. Working solutions and calibration standards for the individual compounds were prepared by serial dilution of the stock standards with acetonitrile. Concentrations of 0.5, 1, 5, 10, 50 $\mu g\ ml^{-1}$ were prepared. Standards were analysed $\times 6$. A standard mixture was prepared using 1 ml of the 50 $\mu g\ ml^{-1}$ respective standards. Commercial products containing QACs were purchased from high street retail outlets and were diluted 1:100 with acetonitrile prior to LC–ESI-MS analysis. Swab (cotton pad) and other occupational hygiene samples were collected as part of the Health and Safety Executive (HSE)

routine programme of occupational hygiene monitoring and were desorbed with the mobile phase, as for this type of work [16,17]. One sample of swimming pool water was taken as part of HSE enforcement activity. The swimming pool water sample was injected directly onto the column as received. All solutions were stored in glass vials at 4 °C. Homogenisation of all samples was achieved by sonication of the sample at 25 °C for 5 min.

2.2. Instrumentation

The HPLC–UV–Vis system consisted of a Waters 610 plus solvent delivery system coupled with a Waters 717 autosampler and a Waters 996 diode array detector. Data was recorded on a Dell personal computer equipped with Waters Millennium software (Waters, Watford, UK). The diode array detection (DAD) system was set to acquire wavelengths in the 210–350 nm range with a sampling rate of 1, a resolution of 1.2 and a filter response of 1.0 (photo diode array settings are quoted in the arbitrary units of Millennium Software).

Direct infusion MS experiments were performed using a Harvard 11 syringe driver (Merck, Poole, UK) with a Hamilton 500 μl gas tight syringe (Supelco, Poole, UK). Polyether ether ketone (PEEK) fittings and tubing were purchased from Supelco and used throughout mass spectral data acquisition.

The HPLC–MS system consisted of a Jasco PU-980 Intelligent HPLC pump with an LG-980-02 ternary gradient unit (Jasco, Great Dunmow, UK), a VG Quattro I mass spectrometer (Fisons Instruments, VG Biotech, Altrincham, UK) equipped with a pneumattically assisted electrospray (ESI) interface and a Rheodyne injection valve mounted in a gas flow regulating unit. The mass spectrometer was operated in the positive ion mode with the following working conditions; capillary voltage 3.95 kV, HV lens voltage 0.3 kV, cone voltage 32 V, lens 3 potential 3 V, multiplier 550 V, source temperature was 95 °C. The nitrogen nebuliser and curtain gas (BOC, Guildford, UK) flows were 40 and 350 l h^{-1}, respectively. Data were recorded on a personal computer with Mass Lynx Software V2.0 (Micromass, Altrincham, UK). A flow-rate of 100 μl min^{-1} from the electrospray probe was achieved by means of a 10:1 post-column T-piece/PEEK tubing split (Supelco).

2.3. Analytical procedure

The chromatographic procedure employed was isocratic with a mobile phase of acetonitrile–100 m*M* ammonium formate acidified with formic acid, pH 3.7 (55:45, v/v). The columns used were a Jones Chromatography, Genesis CN, 4 μm, 100×4.6 mm (Phenomenex, Macclesfield, UK) and a Hypersil CN (CPS), 5 μm, 125×4.6 mm (Phenomenex). Chromatography was carried out with the column at room temperature. The flow-rate was 1.0 ml min^{-1} with an on-column injection volume of 25 μl for the UV–Vis work and 5 μl for the MS work. UV chromatograms were extracted at a wavelength of 262 nm, this wavelength was chosen to be the optimum for the working conditions by examination of the UV spectra of the homologues obtained using the UV–Vis detector.

Initial mass data acquisition was via sample infusion (50 μg ml^{-1}) performed at 5.0 μl min^{-1} using scan acquisition mode over a range of 50–400 *m/z*. Data acquisition was in centroid mode with a cycle time of 2 s and an interscan time of 0.1 s, the run time was 2 min. The formulations and swimming pool samples were analysed using the selected-ion recording (SIR) mode with a dwell time of 0.5 s and an inter channel delay of 0.02 s and a mass span of ±0.25 Da. The mass spectrometer conditions were optimised by tuning on the protonated molecules, $[M+H]^+$, of acetonitrile and formic acid 42 and 32 *m/z*, respectively. MS–MS acquisitions were performed via direct infusion of the standard material of DDDMAB and a diluted formulation. The product ion spectra were recorded with Q1 at low resolution allowing the highest number of precursor ions through into the collision cell. Q3 was optimised for unit resolution. Collision gas was introduced into Q2 to a density that reduced the precursor signal by approximately 50%. MS–MS data acquisition was in the continuum mode to provide improved sensitivity. Collision energy values of 100 were noted as being adequate to achieve structural eluding ion dissociation. Cone voltage bond dissociation was achieved

by increasing the value of the cone voltage whilst infusing the sample. Dissociation was monitored using the real time display of the tune page. Values of 60 V+ were found sufficient to produce ion dissociation for the compounds included in this report.

3. Results

3.1. Application of LC–ESI-MS

To demonstrate the applicability of the LC–ESI-MS technique, the standard mixture of the BAC homologues and DDMAB was analysed. Ions 304, 332, 360 and 326 m/z were monitored corresponding to the BAC C_{12}, BAC C_{14}, BAC C_{16} and DDDMAB cationic species. The order of elution is: BAC C_{12}, BAC C_{14}, DDDMAB and BAC C_{16}. The SIR method permits the previously unachievable simultaneous quantitative determination of all four compounds. Fig. 1 shows two chromatograms acquired from the analysis of a standard mixture of the benzyl and dialkyl QACs. Operating the mass spectrometer in SIR mode offers an increase in sensitivity and also permits the quantitation of the individual analytes without resolution ≥1 of the chromatographic peaks. Fig. 2 shows the single channels of a mass chromatogram recorded for a standard mixture of BAC C_{12}, BAC C_{14}, BAC C_{16} and DDDMAB, structures are shown. The ammonium formate in the mobile phase facilitated a low pH and improved peak resolution through its silanophilic interactions with the column packing. The BAC retention mechanism was greatly influenced by pH with low pH providing the optimum peak shape. The limits of detection (LODs) were determined as being 3, 4, 4 and 4 ng ml^{-1} ($S/N=3$) for C_{12}, C_{14}, C_{16} and DDDMAB, respectively. This is a sensitivity increase of three orders magnitude when compared to the UV–Vis method for the BAC homologues. For DDDMAB it allows

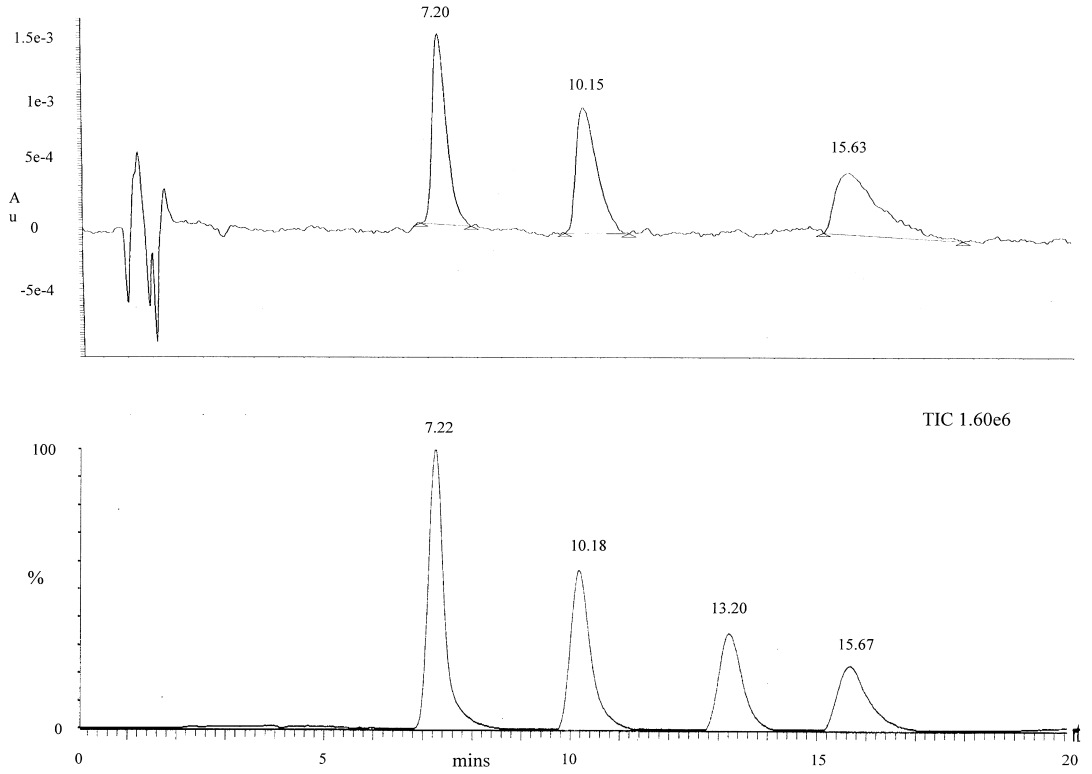

Fig. 1. Chromatograms obtained for a standard mixture of BAC C_{12}, C_{14}, C_{16} and DDDMAB. The upper trace was acquired with UV–Vis detection, the lower MS detection.

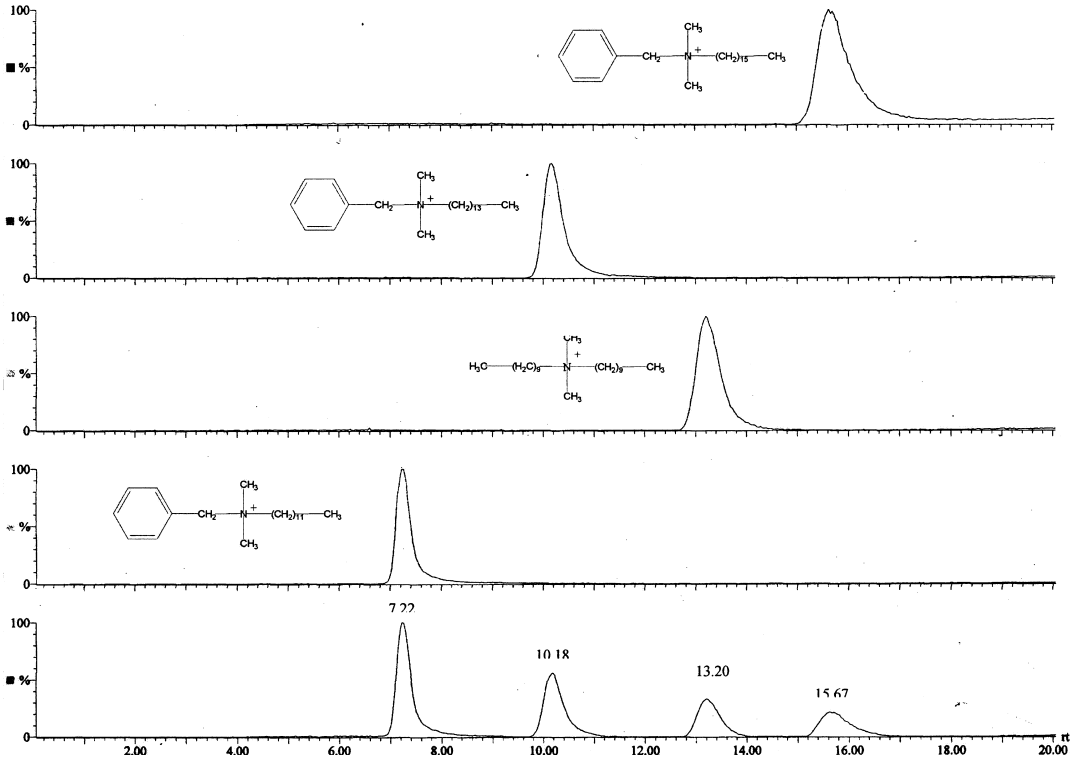

Fig. 2. A mass chromatogram acquired for a standard mixture of BAC C_{12}, C_{14}, C_{16} and DDDMAB peaks are annotated with the appropriate molecular structure.

the determination of a species that previously would not have been recorded by the UV–Vis method.

3.1.1. Evaluation of linear response of the LC–ESI-MS method

A linearity study was performed for the LC–MS method. A series of BAC, C_{12}, C_{14}, C_{16}, and DDDMAB standards were prepared at five concentrations (0.5, 1, 5, 10, 50 $\mu g\ ml^{-1}$). Six replicates were analysed at each concentration value. The Average relative standard deviation (RSD) calibration data was BAC C_{12}: 16.3% BAC C_{14}: 19.25% BAC C_{16}: 19.98% DDDMAB: 15.61%. Calibration curves were constructed by linear regression between peak area and compound concentration. Linear regression values were recorded; DDDMAB: 0.9966, BAC C_{12}: 0.9683, BAC C_{14}: 0.9519 and BAC C_{16}: 0.9878 and noted as being rather poor, this may be due to the use of manual injection (Rheodyne valve) in these experiments or because the LC–ESI-MS system used for this work is less reproducible than the LC–UV–Vis system.

3.1.2. Application of the LC–ESI-MS method to occupational hygiene, concentrates and forensic samples

Fig. 3 shows a mass chromatogram of a swab sample taken during occupational hygiene monitoring of a worker spraying a product known to contain BAC. The sampling procedures and other guidance on occupational hygiene monitoring have been published elsewhere [16,17]. No interferences effects were observed from either the desorption solvent. SIR was used to monitor six channels, corresponding to the C_8–C_{18} alkyl chain lengths of BAC, the six resolved peaks are clearly visible in the total ion chromatogram (TIC). The molecular mass information provided by MS is important for correctly identifying the preparation used as different BAC containing products contain different amounts of the

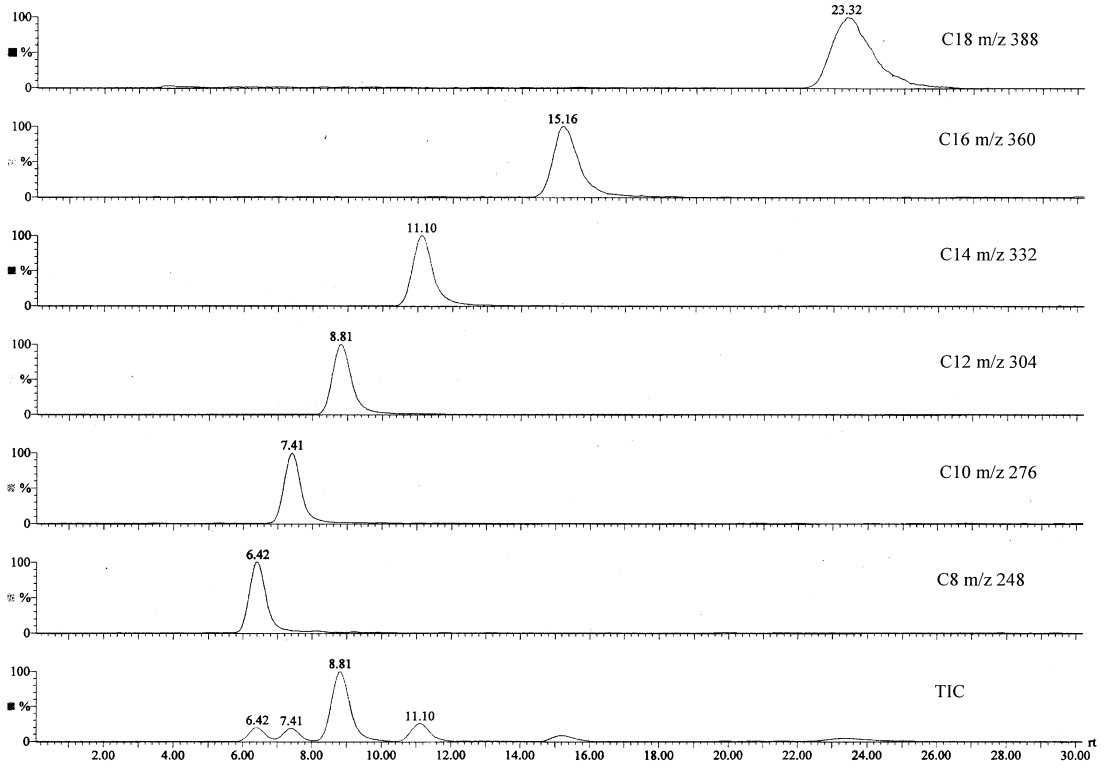

Fig. 3. A mass chromatogram showing SIR (six channels) and TIC data acquired for a diluted commercial formulation. Note: Differences in retention time between the commercial formulation and the standards caused by the use of the Jones Chromatography CPS (cyano), 100×2.1 mm column for standards and the Hypersil CN (CPS), 5 μm, 125×4.6 mm for formulations.

C_8 to C_{18} homologues. For some preparations a homologue may be absent. Identification is possible by UV–Vis but relies on retention time only and is much less sensitive than ESI/MS. Quantification of the C_{12}, C_{14} and C_{16} homologues was possible. The following concentrations were calculated for the BAC C_{12}, C_{14}, C_{16} homologues present in the formulation. BAC C_{12}: 6.41 mg ml^{-1}, BAC C_{14}: 2.25 mg ml^{-1} and BAC C_{16}: 1.66 mg ml^{-1}. The application highlights the sensitive and selective detection offered by LC–ESI-MS without time consuming sample clean up and pre-concentration methods.

Ingestion of swimming pool water treated with a BAC containing algaecide had been proposed as a cause of ill health amongst pupils at a school. Samples of the algaecide concentrate and the pool water were taken and analysed for BAC by both LC–UV–Vis and LC–ESI-MS. BAC C_{12} and C_{14} homologues were identified in the samples. Quantification of the individual peaks yielded C_{12} and C_{14} concentrations of 19.169 mg ml^{-1} and 2.163 mg ml^{-1} for the concentrated sample, and 13.036 μg ml^{-1} and 3.125ug ml^{-1} for the pool water, respectively. LC–ESI-MS provided a rapid sensitive and selective method of analysis for this sample.

3.2. Structural elucidation of quaternary ammonium compounds

Biocidal QACs are frequently reported as simply ‘‘dialkyl’’ on product labels. More information about the actual species present is required especially for

Table 1
Dissociation data obtained for didecyl dimethyl ammonium bromide

m/z Value	Fragment	Abundance (% full scale)	
		MS–MS	RSD
326	$CH_3(CH_2)_9N^+(CH_3)_2(CH_2)_9CH_3$	100	100
186	$CH_3(CH_2)_9N^+(CH_3)_2$	16	17
85	$C_6^+H_{13}$	2	2
71	$C_5^+H_{11}$	3	3
57	$C_4^+H_9$	19	18

forensic purposes. Initial work focused on the application of MS–MS to DDDMAB. Dissociation was noted about the quaternary nitrogen with the corresponding loss of a C_{10} alkyl chain (m/z 145). The alkyl chain values of m/z 57, 71 and 85 were also recorded, corresponding to C_4, C_5 and C_6 alkyl chain lengths, respectively. It was found that the bond dissociation produced by MS–MS could be reproduced on a single quadrupole by using an increased cone voltage. A cone voltage of 60 V was found sufficient to produce the same bond dissociation information achieved with MS–MS. Table 1 shows the data collected by cone voltage and collision induced dissociation techniques. Abundance values are quoted to the nearest integer.

3.2.1. Application of fragmentation methods to real samples

A sample of a commercially available surface biocide concentrate containing a dialkyl dimethyl QAC as the active substance was obtained. The dilute sample was initially analysed and the relative molecular mass (M_r) of the unknown QAC determined as 270. The sample was subjected to MS–MS and cone voltage bond dissociation; the data is given in Table 2. The unknown QAC cation was determined as being of the dioctyl dimethyl ammonium type.

4. Conclusions

The work shown here demonstrates a novel application of LC–ESI-MS to the determination of dialkyl and benzyl QACs. The sensitivity and selectivity advantages of MS detection are shown with respect to UV absorbing and non-UV absorbing QACs. Cone voltage and MS–MS ion dissociation methods have been applied to known and unknown dimethyl QAC showing the similarities in data obtained by the respective techniques. The unknown QAC was determined as being of the dioctyl dimethyl ammonium type. The LC–ESI-MS method developed has been successfully applied to the analysis of real samples with the requirement for minimum sample preparation demonstrated.

Table 2
Dissociation data obtained for an unknown dialkyl QAC

m/z Value	Fragment	Abundance (% full scale)	
		MS–MS	RSD
270	$CH_3(CH_2)_7N^+(CH_3)_2(CH_2)_7CH_3$	100	100
14	$CH_3(CH_2)_7N^+(CH_3)_2$	16	15
71	$C_5^+H_{11}$	2	2
57	$C_4^+H_9$	3	3

Acknowledgements

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