



## Determination of quaternary ammonium compounds in seawater samples by solid-phase extraction and liquid chromatography–mass spectrometry

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### ABSTRACT

A method for the simultaneous determination of two biocidal quaternary ammonium compounds; didecylmethylammonium chloride (didecylmethyl quat) and dodecylbenzylmethylammonium chloride (benzyl quat), in seawater by solid phase extraction (SPE) followed by liquid chromatography–mass spectrometry (LC–MS) was developed. The optimised procedure utilised off-line extraction of the analytes from seawater using polymeric (Strata-X) SPE cartridges. Recoveries ranged from 80 to 105%, with detection limits at the low parts-per-trillion (ng/l) level for both analytes. To demonstrate sensitivity, environmental concentrations were measured at three different locations along the North East coast of England with measured values in the range 120–270 ng/l.

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### 1. Introduction

Quaternary ammonium compounds (quats) are a broad range of antimicrobial chemicals which are used in applications ranging from disinfectants and preservatives to pest control [1]. These chemicals are believed to be more environmentally benign compared to their alternatives as they have a short degradation half life and do not bioaccumulate [2]. EU regulations are in place to evaluate the use of these chemicals, both the Biocidal Products Directive (BPD) [1] and Registration, Evaluation and Authorisation of Chemicals (REACH) [3], use risk assessment procedures to determine whether compounds are acceptable for use in Europe but do not offer a framework to enforce maximum acceptable environmental concentrations of approved chemicals. Currently these chemicals have not been assessed under the Water Framework Directive [4] which sets out Environmental Quality Standards (EQS), and as such environmental concentration limits have not been set or routinely monitored. Published studies dealing with environmental monitoring of quats have mainly focused on paraquat and diquat, two widely used herbicides in agriculture [5–7]. No studies have considered antimicrobial quat levels such as didecylmethylammonium or dodecylbenzylmethylammonium based compounds in solution in the marine environment,

though several studies however, have been published regarding the quantification of benzyl and didecylmethyl quats and their homologs in effluent and water treatment facilities or immediately downstream of them [8–11]. All these studies have reported results in the low  $\mu\text{g/l}$  concentration ranges for both analytes and their structural homologues. To follow the fate of these compounds further downstream, it is necessary to establish a sensitive quantitative technique to detect levels of these quats in sea water.

LC–MS is a widely accepted technique for analysing environmental samples for quats because of the greater detection sensitivity compared with alternative types of LC detection [12–15]. There is a much more limited number of examples of applications of this technique to analyse seawater samples for small organic molecules (e.g. [16]) in comparison to fresh water despite the oceans often being the ultimate depository for materials washed down rivers. Those methods often require some sample preparation prior to analysis which usually is in the form of solid phase extraction (SPE) [17–19], a technique which allows sample clean up and pre-concentration prior to analysis.

The aim of this paper is to develop a new analytical method using SPE–LC–MS for the determination of antimicrobial quats in seawater samples and demonstrate its applicability by analyzing environmental samples taken from three locations on the North East coast of England, near Newcastle upon Tyne.

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**Table 1**  
Final SPE protocol.

Step	Eluents
Conditioning	(i) 5 ml acetonitrile (ii) 10 ml distilled water
Load sample	10 ml sample at constant flow rate of 20 ml min <sup>-1</sup>
Wash	20 ml distilled water with 10% (v/v) acetic acid
Elution	8.5 ml 90(A):10(B); A – acetonitrile acidified with 10% (v/v) acetic acid B – distilled water with 10% (v/v) acetic acid
Post step	Addition of internal standard and extract made up to 10 ml with SPE eluent solution in volumetric glassware

## 2. Experimental

### 2.1. Chemicals

Analytical standard chemicals were purchased from the following companies: dodecyltrimethylammonium bromide 99%; didecyldimethylammonium bromide >98% and dodecylbenzyltrimethylammonium chloride >99% from Sigma–Aldrich (Gillingham, UK); tetra-*n*-butylammonium nitrate and ammonium acetate >97% from Alfa Aesar (Heysham, UK); acetonitrile HPLC Grade and Decon 90 from Fisher Scientific (Loughborough, UK) and acetic acid from VWR/BDH (Lutterworth, UK). Deionized, distilled water was obtained from a Milli-Q system (18 M $\Omega$  cm<sup>-1</sup>) and used throughout.

### 2.2. Artificial seawater preparation

Artificial seawater was prepared according to ASTM D1141-98 (2008) [20] omitting the heavy metal stock solution (stock no. 3).

### 2.3. Glassware pretreatment

Glassware and plasticware was purchased from Fisher Scientific (Loughborough, UK) apart from fluorinated plasticware which was obtained as a gift from Vitlab (Großostheim, Germany). All 100 ml borosilicate volumetric flasks were soaked overnight in non-cationic surfactant cleaning agent (Decon 90). Following rinsing 3 times with copious quantities of deionized water, the flasks were filled to overflowing with the surface treatment solution and left overnight. This solution was dodecyltrimethylammonium bromide in artificial seawater at 50,000  $\mu$ g/l concentration. After a final thorough rinse with deionized water the flasks were ready for sampling.

### 2.4. Sampling

Environmental seawater samples were taken in duplicate from three locations on the North East coast of England near the mouth of the River Tyne, near Newcastle upon Tyne. A blank sample was also freshly prepared using artificial seawater. Samples were collected by fully submerging each pre-treated 100 ml volumetric borosilicate glass flask in free flowing seawater until full; the sample flasks were then sealed and returned to the laboratory for analysis within 4 h without any pre-filtration.

### 2.5. Sample preparation: solid phase extraction

Off-line solid phase extraction (SPE) was performed by passing 100 ml of seawater through a Strata-X (200 mg/6 ml) cartridge from Phenomenex (Macclesfield, UK). The optimal SPE procedure is shown in Table 1.

The extraction liquor was then transferred to a 10 ml perfluoroalkoxy (PFA) volumetric flask and 100  $\mu$ l of internal standard (tetra-*n*-butylammonium nitrate), from a 5000  $\mu$ g/l stock solution

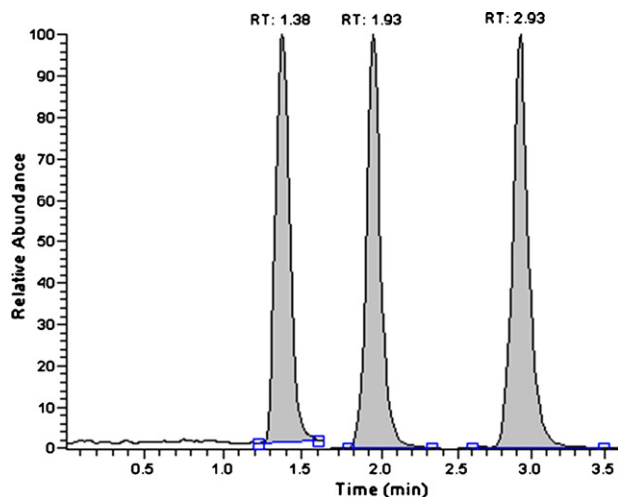
in the SPE elution solvent was added, and made up to the mark. An aliquot was then transferred to a 2 ml silanized autosampler vial (Chromacol, UK) and analysed.

### 2.6. Chromatography

Liquid chromatography (LC) was performed on a Thermo Surveyor HPLC equipped with a degasser, quaternary pump, autosampler and a C<sub>18</sub> Xterra MS column 50.0 mm  $\times$  4.6  $\times$  2.5  $\mu$ m at 20 °C with 50  $\mu$ l injection volume. The chromatography was performed using an isocratic method; 90(A):10(B) at 400  $\mu$ l min<sup>-1</sup>. Eluent (A) was acetonitrile acidified with 1% (v/v) acetic acid, while eluent (B) was aqueous 50 mM ammonium acetate solution acidified to pH 3.6 with acetic acid. The LC was coupled to an electrospray ion-trap mass spectrometer (MS) (Thermo LCQ Advantage) in positive ion configuration. Optimisation of the ion source was performed by the automatic optimisation function of the MS software (LCQ Tune) and assisted by direct injection of a 500  $\mu$ g/l methanolic solution of dodecylbenzyltrimethylammonium chloride. The electrospray ion source was operated at 280 °C. The other settings were as follows: sheath gas flow rate: 25; sweep gas: 12; ion spray voltage: 4.00 kV; capillary voltage: 4.0 V and tube lens offset: 5.0 V. Detection was *via* positive ion electrospray mass spectrometry, using an ion trap system to separate and, *via* collisionally induced dissociation, obtain unique ions for sensitive quantitation. The ion used for the benzyl quat cation determination is formed by the loss of toluene from the cation (Table 2). The ion used for the didecyldimethyl quat cation determination is formed by the loss of a decane molecule from the cation (Table 2). The internal standard was detected using SIM and the area ratios for the two analytes (benzyl and didecyldimethyl quats) detected using MS<sup>2</sup> used to create the calibration (Fig. 1).

## 3. Results and discussion

To discover the optimal SPE protocol, a broad range of hydrophobic and hydrophilic, cation exchange and polymeric phases SPE cartridges were compared. The best performing phases were cyano, weak cation exchange and two polymeric phases, styrene-divinylbenzene and Strata-X (a styrene-divinylbenzene polymer modified to contain cyclic amide moieties.) The latter phase was found to be optimum, and was used as the basis of the SPE methodology; details are outlined in Table 1.



**Fig. 1.** Composite normalized chromatogram showing internal standard cation with retention time 1.38 min, dodecylbenzyltrimethylammonium cation at 1.93 min and didecyldimethylammonium cation at 2.93 min.

**Table 2**  
Chromatographic and calibration details. Calibration range 50–100,000 ng/l ( $n = 10$ ).

Compound	Mass ion (m/z)	Quantification ion (m/z)	Retention time (min)	Calibration linearity $R^2$
Internal standard	242	242	1.38	–
Dodecylbenzyltrimethylammonium cation	304	212	1.93	0.9945
Didecyltrimethylammonium cation	326	186	2.93	0.9989

An initial SPE recovery test for quat samples in seawater and distilled water highlighted the need for the wash step to be included in this SPE protocol due to the presence of a fine precipitate in the recovered extracts from the artificial seawater samples. A further recovery test highlighted a discrepancy between samples subjected to the SPE protocol (quantified as 45  $\mu\text{g/l}$ ) and samples from the same 50  $\mu\text{g/l}$  benzyl quat artificial seawater stock solution, which were quantified without being subject to SPE (quantified as 18  $\mu\text{g/l}$ ). The origin of this discrepancy was ascribed to a mismatch between the sample matrices affecting electrospray ionization performance. Henceforth matrix matching of samples and standards was performed routinely.

The SPE protocol outlined above was evaluated for repeatability by measurement of successive recoveries of benzyl quats using intra-flask and inter-flask spiking at two different concentrations (5 and 50  $\mu\text{g/l}$ ) in artificial seawater. Analysis of the results indicated that the method was repeatable for inter-flask spiking, with recoveries of 98% (6.2% RSD,  $n = 5$ ) at the 50  $\mu\text{g/l}$  spike level and 95% (37% RSD,  $n = 5$ ) at the 5  $\mu\text{g/l}$  spike level. However, the results for the intra-flask spiking produced erratic recoveries and a significant deterioration with respect to repeat sampling from the same flask, as exemplified by the 50  $\mu\text{g/l}$  seawater samples changing by 20% over the five replicates. The major difference between these replicates was the time difference between when the sample stock solution was made and the time when the five aliquots were subjected to SPE suggesting a time dependent deterioration rather than a replication issue.

Support for this hypothesis came from a slight modification to the SPE process. Repeat duplicate artificial seawater samples (at 50  $\mu\text{g/l}$ ) were made in 10 ml volumetric flasks and their contents subject to the developed SPE methodology. However, for one set, prior to the addition of the 8.5 ml of eluent to the SPE cartridge this aliquot of eluent was first swirled around the inside of the 10 ml flask and then inserted directly into the SPE cartridge from the flask and the SPE method followed without further alteration. The results demonstrated an increased level of recovery for the washed flask sample (90%) compared to the unwashed glass (80%), suggesting that significant losses came from the adsorption of the analyte to the surface of the glassware.

In order to find a surface with a different adsorption profile for the analyte, several alternatives to standard borosilicate glassware were evaluated: three different types of plasticware (polypropylene, polymethylpentene and a perfluoroalkoxyl copolymer) and

two borosilicate glassware treated with different surface silanizing agents (Siliconize L-25 or Sigmacote SL-2) and a standard borosilicate as a control. Additionally, equivalent plasticware from two different manufacturers were tested. In all cases, there were significant losses which could be ascribed to adsorption, including silanized glassware and fluoro-polymer plasticware, though they adsorbed the least. It was also clear that the quats remained strongly adsorbed to a surface when rinsed with deionized water but could be desorbed using the SPE eluent.

To evaluate whether analyte quat could desorb into a sample solution over time, glassware which came into contact with the analytes was first soaked overnight in a solution of a non-cationic cleaning detergent, then soaked overnight in a 50,000  $\mu\text{g/l}$  solution of the benzyl quat in artificial seawater. This glassware was then thoroughly rinsed in deionized water and 5 and 50  $\mu\text{g/l}$  solutions of the benzyl quat in artificial seawater made up in them. Five repeat aliquots from each sample were then subject to the SPE protocol with resultant excellent recoveries at the 50  $\mu\text{g/l}$  spike level (109% (5.3% RSD,  $n = 5$ )) but lower recoveries at the 5  $\mu\text{g/l}$  spike level (64% (10.5% RSD,  $n = 5$ )), indicating the strong adherence of the adsorbed layer to the glassware under these conditions. The above process was repeated with this pre-saturation quat changed to the didecyltrimethyl quat. The results indicated a 95% recovery (13.3% RSD,  $n = 5$ ) and 92% recovery (7.2% RSD,  $n = 5$ ) of this quat at the 50  $\mu\text{g/l}$  level for intra-flask and inter-flask spiking, respectively. Similarly, the inter- and intra-spiking protocol was highly favourable resulting in 90% recovery (33.3% RSD,  $n = 5$ ) and 130% recovery (8.5% RSD,  $n = 5$ ) at the 5  $\mu\text{g/l}$  level for intra-flask and inter-flask spiking, respectively. It was concluded therefore, that pre-saturating the glassware with a strong quat solution was effective in enabling the analysis of quats in a seawater matrix; the principle had previously been shown to be effective by Fernández et al. for surfactant quats in sludge [21]. In addition, the saturation by a quat was stable on the surface of the glassware and did not cross contaminate the samples.

Considering that both analytes used the same chromatographic method but had different retention times, a single method for both analytes was possible. Additionally the presaturation quat was changed to dodecyltrimethylammonium bromide (which was shown to be an effective adsorbant pretreatment). Samples analysed using dodecyltrimethylammonium bromide to saturate the glassware and the newly developed combined SPE-LC-MS method gave the results shown in Table 3. These exhibit average recoveries

**Table 3**  
Recovery results for final SPE protocol for simultaneous LC-MS analysis of the two quats.

Repeat	Dodecylbenzyltrimethyl quat				Didecyltrimethyl quat			
	5 $\mu\text{g/l}$		50 $\mu\text{g/l}$		5 $\mu\text{g/l}$		50 $\mu\text{g/l}$	
	Inter-flask	Intra-flask	Inter-flask	Intra-flask	Inter-flask	Intra-flask	Inter-flask	Intra-flask
1	6.7	6.7	65.0	65.0	5.9	5.9	40.6	40.6
2	6.5	5.8	58.2	44.6	6.0	5.7	33.4	40.7
3	6.8	6.2	55.8	44.4	5.6	4.8	32.3	41.8
4	6.0	6.5	56.6	49.1	5.2	5.5	33.1	40.2
5	5.9	7.6	51.0	52.1	3.9	5.5	28.2	43.2
Average	6.4	6.6	57.3	51.0	5.3	5.5	33.5	41.3
RSD	6.4	10.3	8.8	16.5	16.0	7.6	13.4	3.0

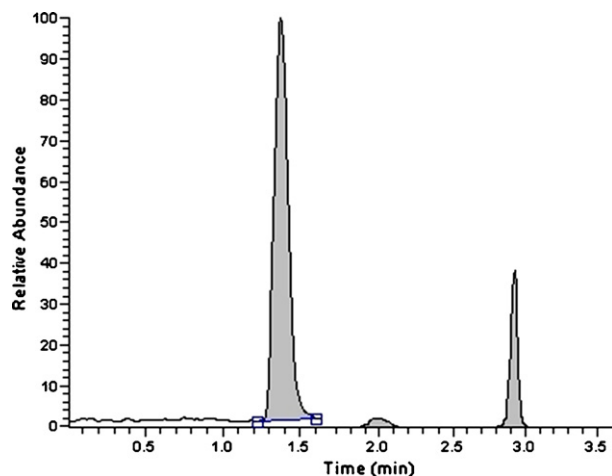


Fig. 2. Composite chromatogram of seawater, Tynemouth Sample 1 showing internal standard cation with retention time 1.38, minimal response for dodecylbenzylidimethylammonium cation at 1.93 min and didecyldimethylammonium cation at 2.93 min.

of 83–115% at 50  $\mu\text{g/l}$  in spiked seawater sample (3–17% RSD) and average recoveries of 106–132% at 5  $\mu\text{g/l}$  in spiked seawater sample (5–11% RSD) except for the intra-flask recoveries for the 50  $\mu\text{g/l}$  didecyldimethyl quat which had a poorer recovery of 67% (13.4% RSD).

## 4. Application

### 4.1. Analytical system

The calculated limits of detection (LOD) and quantification (LOQ) for the developed method are: LOQ (based on  $\times 10$  the standard deviation of the blank): 600 ng/l (benzyl quat) and 300 ng/l (didecyldimethyl quat); LOD (based on  $\times 3$  the standard deviation of the blank) are 200 ng/l (benzyl quat) and 100 ng/l (didecyldimethyl quat).

### 4.2. Application to environmental seawater samples

To demonstrate the suitability of the developed SPE-LC–MS method for monitoring very low levels of these quats in the marine environment, seawater samples were taken in duplicate from the North Sea coast close to Newcastle upon Tyne at three locations; the locations were selected for their ease of access to the marine environment and their decreasing proximity to the River Tyne, a possible source of quats into the marine environment. The first samples were taken at Tynemouth (a location as close to the mouth of the River Tyne as access allows); subsequent samples were taken at Cullercoats (2.5 km from the river mouth), and St Mary's Island (6.5 km from the river mouth). These samples were then analysed for both quats using this SPE-LC–MS method (Fig. 2). The results (Table 4) indicated levels below the LOD for the benzyl

**Table 4**  
Levels of biocidal quats in selected marine environments in North East England, near Newcastle upon Tyne.

	Dodecylbenzylidimethyl quat (ng/l) <sup>a</sup>	Didecyldimethyl quat (ng/l) <sup>a</sup>
Tynemouth	ND	195 (120, 270)
Cullercoats	ND	(130, ND)
St Mary's	ND	ND

ND = not detected.

Note: no quats were detected in the reagent blanks.

<sup>a</sup> Mean (individual values).

quat whereas the level of didecyldimethyl quat correlated with distance from the mouth of the Tyne. It is difficult to comment further regarding these data as, to the authors' knowledge, there have been no previous publications including environmental monitoring of these analytes in solution in the marine environment.

## 5. Conclusion

A new robust analytical method for the analysis of cationic surfactants in seawater has been developed based on SPE-LC–MS. The analytical protocol uses a method to deactivate the surface of glassware by soaking in an alternative quat thereby preventing loss of analytes. This procedure has been applied to the determination of cationic surfactants in seawater samples. The benzyl quat studied in this paper is the dominant homologue in the commercially available alkylbenzylidimethylammonium halides used in household biocidal products but it is likely that the established method could be applied to detect its other homologues.

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