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Analysis of quaternary ammonium compounds in estuarine sediments by LC-ToF-MS: very high positive mass defects of alkylamine ions provide powerful diagnostic tools for identification and structural elucidation

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Abstract

A sensitive and robust method of analysis for quaternary ammonium compounds (QACs) in marine sediments is presented. Methods for extraction, sample purification, and HPLC-Time-of-Flight-MS analysis were optimized, providing solutions to problems associated with analysis of QACs, such as dialkyldimethylammonium (DADMAC) and benzalkonium (BAC) compounds experienced previously. Recognized in this study are the exceptionally high positive mass defects characteristic of alkylammonium or protonated alkylamine ions. No alternative and chemically-viable elemental formulas exist within 25.2 mDa when the number of double bond equivalents is low, effectively allowing facile discrimination of this compound class in complex mixtures. Accurate mass measurements of diagnostic collision induced dissociation fragment ions and heavy isotope peaks were obtained and also seen to be uniquely heavy compared to other elemental formulae. In the case of BACs, the ability to resolve masses of alkylamine fragment ions is greater than it is for molecular ions, opening up a wide range of potential applications. The power of utilizing a combination of approaches is illustrated with the identification of non-targeted DADMAC C8:C8 and C8:C10, two widely used biocides previously unreported in environmental samples. Concentrations of QACs in sewage-impacted estuarine sediments (up to 74 µg/g) were higher than concentrations of other organic contaminants measured in the same or nearby samples, suggesting further study is needed.

Introduction

Quaternary ammonium surfactants are high-production volume chemicals that constitute a large fraction of the cationic surfactant market. The salts of quaternary ammonium compounds (QACs) are used as active agents in detergent formulations, fabric softener products, microbicides, and personal care products, and they find application in a variety of industrial processes.^{1–3} As hydrophobic cation-exchangers, QACs sorb strongly to soils and sediments,⁴ and many tetra-alkylammonium QACs, including benzylalkyldimethylammonium compounds (BACs), alkyltrimethylammonium compounds, and dialkyldimethylammonium compounds (DADMACs), are persistent enough to be found at appreciable concentrations in wastewaters,^{5–7} sewage sludges,^{8–12} receiving waters,^{5, 13, 14} and sediments.^{6, 8–10, 14, 15}

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Early work on the analysis of QACs in the environment focused primarily on DADMACs with n-alkyl chain lengths of C14, C16, and C18. These relatively high-molecular weight DADMAC homologs are produced from a number of oleochemical feedstocks, and technical mixtures have commonly been referred to as ditallowdimethylammonium chlorides (DTDMACs). DTDMACs have primarily been used in fabric softeners and were voluntarily phased out in the early 1990's in some European countries when concentrations in sewage sludges were found to be extraordinary high (maximum concentration reported of 9,200 µg/g).⁸⁻¹² 16 DTDMAC concentrations in sludges from Switzerland were observed to decrease sharply after the phase-out,⁹ although use has continued in other regions of the world. In the same study, DTDMAC concentrations of 42.3-1,140 µg/g were reported in two sediments near Barcelona, and these remain the only reports of QACs in marine or estuarine sediments. Despite the relative paucity of data on QAC occurrence and fate in the aquatic environment, a recent ecotoxicological risk assessment that compares toxicological endpoints to measured levels in wastewater effluents suggests that DTDMACs, and especially other widely used QACs, are environmental contaminants deserving more attention.^{6, 17} Future studies on their occurrence and fate in the aquatic environment will require well tested and robust analytical methods of analysis.

QACs, as amphiphilic organic cations, are especially amenable to sensitive and selective detection using HPLC-electrospray ionization (ESI)-MS, yet relatively few studies have taken advantage of such approaches for compound-specific detection in environmental samples.^{5, 7, 10, 15} We have employed Time-of-Flight (ToF)-MS for the analysis of QACs, which proves to be an extremely valuable approach given the large number of alkyl homologs of interest within this class, the potential presence of non-targeted QACs of interest in environmental samples, and a uniquely high positive mass defect of QACs and alkylamine ions that enables high-resolution MS to provide diagnostic confirmation of known and non-targeted analyte identities.

The initial goal of this work was to develop and test a holistic method for the quantitative, trace level analysis of QACs present in highly complex sediment extracts. There are several problems that have been identified in trace level measurements of QACs. First, there have been few efforts to optimize methods for extracting QACs from sediments or other solid environmental phases. Fernandez et al.⁹ reported that supercritical fluid extraction (SFE) resulted in 30-40% higher DTDMAC concentrations in two marine sediment samples relative to those determined using a steaming acidic (1 M HCl) methanol method.^{8, 18} In other studies, tests of extraction efficiency have often relied upon recovery of QAC spiked to sediments, water, or sludge prior to extraction.^{8, 10, 15} Such techniques can overestimate extraction efficiency of QACs from field-aged soil or sediment, in which the QAC compounds may be more tightly sorbed.⁹ A second problem that has confounded trace level analysis of QACs in environmental samples is adsorptive losses of these compounds to surfaces used in extraction, purification, and separation steps (e.g., adsorption of more hydrophobic QACs to glass capillary columns).¹⁹ Glassware used in QAC analysis has often been pre-treated with QACs in order to minimize loss of analytes by adsorption to active sites.^{8, 9} A third limitation encountered has been ubiquitous instrumental contamination by more hydrophobic DTDMAC homologs during the LC-ESI-MS analysis of QACs and peptides, an important problem observed here and by others.^{20, 21} The methods reported have been developed for analysis of small 0.1 g sediment sample size, in order to minimize co-extracted matrix, sample size requirements, and materials used, and increase the speed of analysis. Special attention was paid to the efficiency of extraction methods, and an ultrasonically-assisted extraction method was developed here, leading to improved extraction when compared to two other previously-reported methods.^{8, 10} A related approach led to improved extraction of other amphiphilic sediment contaminants during development of a high temperature continuous-flow sonication extraction method.²²

The utilization of high-resolution LC-ToF-MS to provide accurate mass measurements for monoisotopic molecular, fragment ions, and even heavy isotope-containing ions for analyte identity confirmation purposes has been well reviewed.^{23, 24} The ability to resolve nominally-isobaric elemental formulas based on accurate mass measurements depends on the combined elemental mass defects of the atoms in the ion of interest as well as the mass-measurement accuracy achievable with a given instrument. A small number of elements contain positive defects, defined as the numeric difference between monoisotopic mass and integer or nominal mass (¹²C is defined by a mass defect of zero). For most organic molecules of interest in environmental samples, only H and N possess positive elemental mass defects. A unique property of protonated alkylamine and alkylammonium ions composed of only C, H, and N ($C_nH_{(2n+2 \times)}N^+$) and having a limited number of double bond equivalents (DBE; $-1 \leq x \leq 2$) is that the ion masses are larger than both the nominal mass and that of most other chemically-feasible isobaric ions with other elemental formulae. Therefore, QAC ions can be resolved or distinguished from other, nominally-isobaric compounds in complex mixtures by high-resolution MS (including HPLC-ToF-MS). Some of the other factors that favor the mass separation characteristic of these compounds, and their fragment ions, include the odd number of nitrogens present in these molecules and the fact that ions formed by ESI nearly always have even electron parity.

Others have reported that H-rich, saturated hydrocarbon ions have large enough positive mass defects, such that high-resolution MS^{25, 26} can readily resolve alternative elemental formulas possessing functional group or increasing number of double bond equivalents (DBE). Similarly, negative mass defects of rare elements can lead to more selective determination of phosphorylated peptides²⁷ and other peptides through the use of element-coded affinity tags²⁸ and fragment ion mass defect labeling.²⁹

In the present work, a comprehensive method based on HPLC-ToF-MS for analysis of QACs in sediments is developed that allows for not only quantitation of these important compounds, but also simultaneous qualitative identity confirmation. Of equal significance is the recognition and application of distinctive highly positive mass defects to determine elemental formulas and resolve QACs or alkylamine ions in high-resolution MS analyses of complex mixtures such as sediments. Unique masses of diagnostic CID fragment ions and heavy isotope peaks can also be obtained by HPLC-ToF-MS and are shown to provide additional confirmation of targeted compounds and tools to identify unknowns. The power of this combination of approaches is illustrated with the identification of non-targeted DADMAC C8:C8 and C8:C10, two widely used biocides previously unreported in environmental samples.

Experimental Section

Standards

Individual standards of the dialkyldimethylammonium bromides: didecyldimethylammonium bromide (C10:C10), didodecyldimethylammonium bromide (C12:C12), ditetradecylammonium bromide (C14:C14), dihexadecyldimethylammonium bromide (C16:C16), and dioctadecylammonium bromide (C18:C18) were purchased from Sigma-Aldrich (Milwaukee, WI). Benzyltetradecyldimethylammonium chloride (BAC 14) and benzylhexadecyldimethylammonium chloride (BAC 16) were purchased from Pfaltz & Bauer Inc. (Waterbury, CT), and the tertiary amine tridodecylamine was purchased from Acros Organics (NJ). A commercial mixture of DTDMAC (C14:C14 to C18:C18) was purchased from Chem Service (West Chester, PA). A commercial mixture of benzalkonium chloride mixture (BAC12 ~ 60%; BAC14 ~ 40%; traces of BAC 16 and 18) was purchased from Sigma-Aldrich (Milwaukee, WI).

Sediment samples

Surface sediments from four estuarine locations were collected and used for method development in this study. The samples were characterized by a range of QAC concentrations and organic matter contents. Sediment samples included: organic-rich sediments (0-5 cm) from sewage-impacted Jamaica Bay (JB) collected in 1998,³⁰ a sediment from Bowery Bay (BB), collected in 2004 proximate to LaGuardia International Airport, NY, and impacted by local inputs of sewage; a less organic matter-rich surficial fine-grain sediment from a central Long Island Sound (LIS) site located approximately 85 km east of BB; and two high organic carbon samples from a 2006-collected sediment core in the Forge River (FR), located on the north shore of Moriches Bay, NY. Samples analyzed from the latter site included a recently-deposited sediment from the upper 15 cm (FR-S); and a sample from deeper within the core (50-67 cm, FR-D), deposited before the advent of QACs as commercial chemicals. This sample was used in method blank and spike recovery experiments.

Sediment extraction and purification

Extraction—Method objectives were addressed via several modifications of the steaming acidic methanol extraction (referred to as steam extraction here) by Gerike and coworkers⁸ that included the addition of low-power ultrasonic energy, which led to an increased extraction efficiency. The volumes and sizes of reagents and apparatus were reduced to match the smaller samples (100 mg dry weight) analyzed in the present study. Frozen sediment samples were freeze-dried, ground and homogenized with mortar and pestle. 30 mL glass centrifuge tubes were used for extractions. DADMAC C12:C12 (10-300 ng) was added as a surrogate standard at concentrations that were high compared to levels (well under 1% of total QAC) in DTDMAC commercial products or standards. Glassware was combusted at 450°C before use. Sediment samples (100 mg) were extracted in a 60°C ultrasonic bath (Model 75HT, VWR) three times (1hr × 3) with 10mL acidic (1 M HCl) methanol (30mL total). Following centrifugation, combined extracts were collected in 30mL test tubes, and then evaporated to dryness under nitrogen flow.

Sequential extractions with different or more stringent conditions were used to test whether additional QACs could be extracted from contaminated sediments following the ultrasonically-assisted extraction method developed. Tests were conducted using both BB and LIS sediments. Extraction variables considered included: additional time of extraction (8 hr); effect of solvent polarity (1 M HCL in 1:1 methanol:dichloromethane); energy of ultrasonication (Cole Parmer, 4700 Series, 600 W ultrasonic probe for 5 min); and addition of a strong cation exchanger (0.1 M CsCl in methanol)^{31, 32} that competes with quaternary ammonium compounds for high energy cation exchange sites.³²

Sequential sediment extractions were also employed to determine how well two previously reported extraction methods recovered QACs from JB and LIS sediments. Following extraction of 5 g of sediment with previously reported Soxhlet,¹⁰ or more standard steam extraction⁸ methods, sediments were re-dried and 1 g portions extracted again by the ultrasonically-assisted extraction method. The Soxhlet extraction required 18 hr, and acidic methanol was also used as a solvent, albeit at lower (0.1 M) HCl concentrations. The steam extraction method involved 5 consecutive 1 hr extractions of the sediment in a beaker, also with 1 M HCl in methanol at an initial temperature of 68°C, which increased with evaporative loss of the methanol during the extractions. After extractions, equivalent amounts of each pair of extracts were then purified and analyzed with identical methods, as discussed below.

Sample purification—An important difference between the present method and those reported previously⁸ was the use of a single glass test tube throughout extract collection and multiple subsequent purification steps. This approach mitigated transfer losses of more hydrophobic DTDMACs by strong sorption to glassware^{8, 9} or residual sample matrix accumulating on glassware, while allowing for removal of salts and much of the co-extracted organic matrix. Samples were transferred into 60 mL separatory funnels for liquid-liquid extraction with 4 sequential washes with 5 mL of water, each time sonicating and vortexing to suspend dried sample matrix prior to transfer. The water was extracted with 10 mL chloroform three times, and the chloroform collected back into the original test tube to minimize losses of QACs due to adsorption to the test tube. Linear alkylbenzene sulfonate (LAS) has previously been added during liquid-liquid extraction to facilitate extraction of QACs into chloroform.^{8, 9, 18} However, we determined that the addition of LAS was not necessary for optimum recovery of QACs from estuarine sediment.

Anion exchange⁸ was then used to further reduce the organic matrix remaining in the N₂-dried extracts^{8, 9, 18}. Resin (AG 1-X2 resin, BIO-RAD, Hercules, CA) was conditioned overnight in methanol and ~3g of the resin was loaded into 6 mL glass SPE columns, held with Teflon frits (Supelco, Bellefonte, PA). The SPE columns were preconditioned with 50 mL of methanol. Extracts were reconstituted in methanol and QACs eluted with methanol at 3 mL/min to 15 mL volume, and recollected in the same test tube.

Chromatographic separation

HPLC-ToF-MS separation and analysis of QACs employed a Waters Alliance 2695 LC and LCT mass spectrometer with a Z-spray ESI source (Micromass, Manchester, UK) described elsewhere.³³ The sample volume was adjusted by concentration (1 mL under N₂ gas flow) or further dilution, as necessary (up to 300 mL), to account for expected concentration ranges and the dynamic range of the ToF analyzer used. An internal standard, tridodecylamine, was then added at a concentration of 5 ng/mL prior to 10 μ L sample injection.

Trace level analysis of more hydrophobic DADMACs (C16:C16, C16:C18; C18:C18) by reverse phase HPLC-MS is complicated by small and reproducible instrument blanks²⁰ that are magnified when mobile phase gradients are used. These broader peaks are especially important if initial HPLC mobile phase conditions contain a high fraction of aqueous buffer. This blank contamination is independent of HPLC column age. The cause of this blank contamination is as yet unknown, but it can become significant when injected DTDMAC masses are less than 2-10 pg. It is noteworthy that a similar problem was encountered in the analysis of surface active perfluorinated octanoic acid, in which case blank problems were overcome with an isocratic HPLC-MS method.³⁴ Two different HPLC methods were employed in this study. HPLC separation of QACs was initially modeled after the method reported by Martinez-Carballo et al.,⁷ utilizing a Luna C18 column (Phenomenex; 150 $\times$ 2.00 mm, 5 μ m). The first (Method 1) most closely resembled protocols reported⁷ and was utilized to better retain and provide good chromatographic separation of more soluble BAC and DADMAC homologs. DTDMAC blanks were reduced greatly by applying a different HPLC gradient (Method 2), which employed a shallower solvent gradient. Despite the broader chromatographic peak shapes produced by Method 2 and the much smaller (100 mg) sample size extracted in this work relative to previous work, the method detection limits for QACs using Method 2, reported below, are similar to, or lower than, those reported with other methods published to date.¹⁰

For Method 1, a gradient separation was achieved with solvent A, 20:80 acetonitrile:water with 1% acetic acid; solvent B, 95:5 acetonitrile:water with 10 mM ammonium acetate; and solvent C, isopropanol with 0.1% formic acid. Gradient conditions were initiated at 100% A

maintained for 2 minutes; linear gradient changed to 20% A and 80% C in 0.1 minute and held for 5 minutes; changed to 100% B in 2 minutes; changed to 90% B and 10% C in 0.1 minute and held for 6 minutes; then changed to 50% B and 50% C in 2 minutes, and maintained for 18 minutes before the column was re-equilibrated to initial conditions.

Method 2 overcame the DADMAC instrument blank. A gradient separation utilized only mobile phases B and C above, which consist of very low aqueous content. This proved critical in near elimination of DTDMAC instrumental blanks. Gradient conditions were initiated at 90% B and 10% C for 6.2 minutes, changed to 50% B and 50% C in 2 minutes, held for 12 minutes and changed back to 90% B and 10% C in 1 minute. Column oven temperature was held at 45 °C in both methods.

Mass spectrometry

ESI in positive ionization mode was conducted with capillary and cone voltages of 2800 V and 55 V, respectively. The approach for daily instrument mass calibrations, utilization of co-infused internal mass calibrant, and general accurate mass measurement methods are provided elsewhere.³³ Mass resolution of the ToF-MS was tuned to between 6000 and 6500; instrument manufacturer specifications for mass accuracy was 5 ppm for $m/z > 400$ and ± 2 mDa at lower m/z . As recently reported,³⁵ the MassLynx software mass calculator supplied with the Waters LCT does not account for the mass of an electron ($u = 0.00054$) when the elemental formula masses for even electron ions are calculated, which is the case for the quaternary ammonium or protonated alkylamine ions investigated here. This error was corrected here in the reporting of theoretical mass values and when reporting accurate mass measurements of sample peaks by subtracting the mass of an electron from the software-calculated masses of analytes, the instrument calibration standard peak masses, and that of the internal or co-infused internal mass calibrant ("lockmass") leucine enkephalin.

Identification of targeted QACs relied upon measurement of molecular ions (M^+ or $M+H^+$ in the case of the tridodecylamine internal standard) and chromatographic elution. Further confirmation of targeted and unknown QACs could be achieved by accurate mass measurements of both molecular ions and one (BACs: $(M-92)^+$) or two (de-alkylated DADMAC) in-source collision induced dissociation (CID) fragment ions (Table 1). The standard cone voltage of 55 V provided sensitive analysis for the analysis of molecular ions for all targeted QACs with a single mass spectrometric method, and allowed for additional confirmation through analysis of CID fragment ions in the case of BACs and lower molecular weight DADMACs. The cone voltages that were optimal for analysis of molecular and CID fragment ions increased with alkyl chain length within each homologous series studied. At a cone voltage of 55 V, there were no observed CID fragments for DADMACs with alkyl chain lengths above C10. Yet at increasing cone voltages, molecular ions, and then at higher voltages CID fragment ions, diminish in intensity for lower alkylated BACs and DADMACs. A mass spectrometric method, in which cone voltage was incrementally increased from 55 to 85 V as a function of run time, provided confirmation and accurate mass estimates for molecular and CID fragment ions for each of the four BACs and eight DADMAC analytes examined (Table 1). Further details of this method and accurate mass measurements and ion chromatograms for each parent and corresponding fragment ions are illustrated in the Supplement Information (Figure S-1).

Quantification

A six-point quantitative calibration series (typically 0.1-20 ng/mL in methanol) was analyzed daily, and the raw data files were processed using the all-file accurate mass measure function in MassLynx.³⁶ Analyte responses were normalized to the internal standard for quantification. The ESI-MS response factors of different DADMACs and of the

internal standard trioctadecylamine were within 20% of each other, whereas the quantitative response was lower for the more soluble BACs and decreased with decreasing BAC alkyl chain length. Concentrations of C14:C16 and C16:C18 DADMAC were estimated by interpolating very similar response factors of the most closely eluting DADMAC homologs, and the concentrations of BAC 12 and 18 were calculated assuming the response factors of BAC 14 and 16, respectively. Nontargeted DADMAC C8:C8 and C8:C10 concentrations were estimated from the response factor of DADMAC C10:C10.

Sediment analysis of QACs with the disulfine blue method

The disulfine blue active substances (DBAS) method has long been a standard method for detection of cationic surfactants in environmental samples but proved to be inadequate even for screening total QACs in estuarine sediment samples. The standard DBAS method³⁷ was tested by comparing it with HPLC-MS quantification of the same purified extract of two dissimilar sediments, BB and LIS. DBAS-based QAC concentrations of LIS and BB sediments were 60 and 300 µg/g, whereas concentrations of only 1.8 and 74 µg/g were determined by HPLC-MS, respectively. In prior work, comparisons of DBAS and HPLC methods for determining QACs were much more consistent when applied to extracts of wastewaters or sludges having high DTDMAC concentrations, whereas DBAS tended to overestimate QAC concentrations in sediments and soils samples with much lower DTDMAC levels.^{8, 12}

Results and Discussion

HPLC-ToF-MS separation and identification of target and nontarget QAC analytes

DADMAC and BAC homologues in sediment sample extracts were well separated by HPLC Method 2 (Figure 1). Selected ion chromatograms (mass window of 0.05 Da) of targeted analytes in samples showed excellent agreement with retention times and peak shapes of pure standards, or of components of the mixed BAC and DTDMAC standards in the cases of BAC 12 and DADMAC C14:C16 and C16:C18. The identification of BAC 18 was confirmed by accurate masses of molecular and CID fragment ions as well as the corresponding predicted retention time of both ions (Table 1; Figure S-1. and Table S-1). The average (RMS; root mean square) mass discrepancy between the measured and actual accurate masses for the 11 DADMAC and BAC analytes in the four sediments analyzed was 1.8 ± 1.1 mDa.

The utility of LC-ToF-MS to screen for and identify non-target, previously unreported QAC analytes is illustrated in Figures 2 and 3. Given the ease of detection of DADMAC C10:C10, the presence of two other DADMACs (C8:C8 and C8:C10) was also assayed. All three DADMAC homologs are used in a variety of current-generation mixtures of disinfectants and have been detected in personal care products,³⁸ but to our knowledge only DADMAC C10:C10 has been measured in the environment.^{6, 10} Analysis of a commercial DADMAC-containing product supported the identification provided by the HPLC-ToF-MS analysis of these unknowns (data not shown). The relative retention times of the putative DADMACs (Figure 2) were consistent with those of the DADMAC C10:C10 standard. The even mass parity of the even-electron ions detected for these compounds after electrospray ionization is indicative of an odd number of nitrogens in the formulae, according to the nitrogen rule.³⁹ Narrowing the m/z window from 0.5 to 0.05 Da (Figure 2B) largely eliminated isobaric interferences that were observed in nominal-mass chromatograms, especially for the lower abundance C8:C8 and C8:C10 homologs. Several of the interferences that were eliminated by narrowing the mass window were identified to be the M+1 heavy-isotope peaks from compounds having a molecular ion m/z one nominal mass unit lower. The measured accurate mass of a large isobaric interference with a base peak of 270.2438 was found to be

72.3 mDa less than the theoretical mass of DADMAC C8:C8; the most likely elemental formula for that ion is $C_{12}H_{32}NO_2$.

The accurate mass measurements of molecular ion peaks associated with DADMAC C8:C8 C8:C10 DADMAC homologs were 3.2 and 2.1 mDa greater than the respective theoretical masses. Figure 3A illustrates the mass spectrum measured at the retention time window corresponding to the DADMAC C8:C10 peak. Six peaks are highlighted. The corresponding mass measurement errors for the m/z 158 and 186 CID fragment ions were 1.9 and 0.3 mDa relative to theoretical values for the postulated formulae. Also shown in Figure 3A are the accurate mass measurements associated with the M+1 and M+2 heavy-isotope peaks, with corresponding mass measurement errors of -1.4 and 2.2 mDa, when compared to theoretical masses. The calculated m/z of heavy isotope peaks (M+1 and M+2) with this formula have $\Delta m/z$ 3.4 and 7.1 mDa heavier than the mono-isotopic peak, respectively. These differences are controlled by the masses and relative abundance of ^{13}C , with a minor contribution from 2H , as peaks containing these elements can only be resolved by higher resolution mass spectrometers. Thurman and Ferrer,²³ have provided illustrative examples suggesting the potential for complementary analyte confirmation through accurate mass measurements of heavier isotope peaks (e.g., M+1, M+2, etc.).

The reconstructed ion chromatograms for five ions associated with DADMAC C8:C10 illustrate the potential power associated with the resolution, accuracy, and full spectral sensitivity of LC-ToF-MS analysis (Figure 3B). By utilizing time varying cone voltage (Table 1), similar ion chromatograms of molecular and CID fragment ions for all QAC analytes in this sample were measured (Figure S-1). It is also shown in Figure 3A and B that the peak at m/z 228.2705 was associated with another compound having overlapping but distinct HPLC retention. The MassLynx elemental formula calculator indicated a unique match to the elemental formula $C_{15}H_{34}N$ (mass error of 2.0 mDa). Both that formula and the HPLC retention time turned out to match those of an authentic standard of dodecyltrimethylammonium (Figure 3B).

Positive mass defects of alkylamine and alkylammonium ions

Elemental mass defects change with atomic and isotope number due to changes in nuclear binding energies, and tend to become more negative with increasing atomic number for the lighter elements typically found in organic molecules (Figure 4A).²³ As mentioned above, the monoisotopic formulae of QACs and other alkylamine ions consist of the only elements in common organic molecules with zero (^{12}C) or positive elemental mass defects (0.00782 Da and 0.00307 Da for 1H and ^{14}N , respectively), making them unique among structures encountered in environmental samples that can be ionized readily during electrospray ionization. This combination of positive elemental mass defects leads to molecular ions with heavier masses (referred to here as positive ion mass defects) when compared to ions of alternate elemental formulae having the same nominal mass.

Figure 4B illustrates the remarkably large separation in masses of alkylammonium ions, or protonated alkylamine ions of the same formulae, from other nominally isobaric ions with chemically-viable elemental formulae, as well as elemental composition controls on the magnitude and uniqueness of the resulting mass differences. The elemental formula calculator provided in MassLynx 3.5 software was utilized to postulate a collection of elemental formulas with nearest mass to the selected $C_nH_{(2n+2x)}N^+$ alkylamine ions, as well as for caffeine, an example of a smaller molecule that with more hetero-atoms and DBE, but no elements with particularly large negative elemental mass defects (^{16}O has an elemental mass defect of only - 4.1 mDa). Because of the use of electrospray to generate ions in the present work, only even electron ions were considered. Very few parameter restrictions on elemental formula composition were specified in order to test the veracity of the results. The

number of atoms of each element was allowed to range up to 5 for N, O, S, Si, P, F, Cl, and Br. Formulae with a single Na, or K atom were considered in this analysis, as adduction of more than one alkali metal adduct in a small and singly charged ion is unlikely. Not shown in the calculations illustrated in Figure 3B was that inclusion of additional monoisotopes, corresponding to ^{13}C , ^{37}Cl , and ^{81}Br atoms, into the calculation did not affect any of the closest elemental formula matches.

The extent of the positive mass defect of alkyl amine ions, when compared to masses of other elemental formulas calculated may be unparalleled when DBE are relatively low. The example formulae provided (Figure 3B) correspond to those of DADMAC C18:C18; DADMAC C10:C10, and the latter with increasing number of DBE. The results for the BAC homologs are essentially the same as those shown for DADMAC C10:C10 with 4 DBE. Most striking is the observation that there are no alternative formulae within 25.2 mDa (replacement of C_2H_4 with N_2) for $\text{C}_n\text{H}_{(2n+2x)}\text{N}^+$ when DBE = 0, 1, or 2; and the next closest masses were 36.6 and 50.2 mDa lighter when DBE = 0 – 1 (replacements of CH_4 or C_4H_8 , with O or N_4 respectively). An additional elemental formula exists for DBE = 2 that is 38.8 mDa lighter (replacement of C_3H_3 with NaO). With respect to the very closest elemental formula matches, it is of great interest to note that increasing number of carbons (n) between DADMACs C10:C10 and C18:C18 does not affect the closest elemental formula matches. Other examples of constant yet smaller offsets in ion masses between nearest elemental formula as a function of alkyl chain length have been shown in examples that considered a smaller range of possible elemental substitutions.^{26, 39}

Increasing the degree of unsaturation of alkylamine ions further increases the number of alternate formulae within a given difference in mass. For DBE = 3 (Figure 3B) additional formulae (containing F atoms) that are 12.8 – 24.0 mDa lighter appear. When DBE = 4, there are three possible alternative formulas that are heavier in mass than the nominally isobaric alkylamine ion. Each of those alternative formulas is characterized by zero DBE (higher proportion of H) and having either F or N_5O element substitutions. Even in the example calculation for which DBE = 4 there is only one other elemental formula ($\text{C}_{19}\text{H}_{41}\text{NOF}$) with a mass difference (1.1 mDa), that is within 4.0 mDa (12.7 ppm) of the alkylammonium ion of interest. Thus, even when DBE = 4, there is a reasonable chance that accurate mass estimation by ToF, or other high resolution mass analyzers can provide elemental formulae with high confidence.

The results for the analysis of possible formulae corresponding to the nominal m/z of the [caffeine+H]⁺ ion illustrate a common problem encountered by the analytical chemist conducting trace analyses of polar molecules within highly complex sample matrices. There are a large number of candidate elemental formulas with masses very close to the theoretical mass of the formula for the caffeine ion. The [caffeine+H]⁺ ion contains 5 DBE, 2 oxygens, and an odd mass (even number of N in this case), opening up a much wider window of possible alternative formulae within a constant mass range.

Accurate mass measurements of CID fragments provide tools for improved confirmation of target compounds, as well as structural information important in identification of unknown compounds.²⁴ This is well illustrated here in the case of DADMAC C8:C10, where evidence includes both accurate mass of diagnostic fragment ions and agreement found between reconstructed ion chromatograms (Figure 3). Importantly, CID fragment ions (saturated protonated alkylamines in this study) possess the same characteristic ion mass defects calculated for the quaternary ammonium ions shown in Figure 4B. With a 25.2 mDa window between the next possible elemental formula, substantial reduction of isobaric interferences in ion chromatograms of CID fragments can be expected with the ToF-MS used, or virtually eliminated with higher resolution mass spectrometers. It can also be noted

that the accurate mass measurement of the BAC CID fragment ions (loss of protonated tropylium ion M-92) provide dramatically better elemental formula confirmation than does the molecular ion. The fragment ions are unsaturated alkylamine ions of formula $C_nH_{(2n+4)}N^+$ (with next closest elemental formula mass being 25.2 mDa lighter) whereas BAC molecular ions possess 4 DBE, such that masses of other possible elemental formulas do not differ nearly as much (Figure 4B). There may be situations in LC-ToF-based quantitative analysis of mixtures that fragment ions provide the separation from isobaric interferences that can not be achieved with analysis of molecular ions alone.

Accurate mass measurements of the M+1 and M+2 heavy-isotope ions (as shown in Figure 3) of alkylammonium and protonated alkylamine ions are also noteworthy as the average isotope elemental defects associated with isotope clusters can be diagnostic of formulae and the elements whose isotopes are responsible for high-abundance isotopic peaks at higher masses.²³ As discussed above (Figure 3), the mass accuracy in the present study (approximately 2 mDa) is just below the order of the expected mass defects of the M+1 ion relative to the monoisotopic molecular ion. When more precise mass accuracy is important, FTICR, Orbitrap, and more modern LC-ToF systems are capable of achieving mass accuracy < 1 ppm as well as much higher resolution.⁴⁰ Utilizing the elemental formula matching software, it was found that the M+1 and M+2 heavy-isotope ions of more saturated alkylamine ions are also appreciably higher in mass than all other feasible elemental formulas. For example when DBE = 0, as characterized by DADMAC C8:C10 (Figure 3), the elemental formulae with masses closest to the M+1 peak are 8.2, 19.4 and 33.3 mDa lighter. The potential for resolution of the M+2 ions is even greater, with nearest formulae 16.5, 27.8, and 41.7 mDa lighter. Thus, in the case of alkylamine ions, accurate mass measurements can readily distinguish elemental formulae of multiple peaks in isotope clusters, as well as in CID fragment ions. This combination provides greatly expanded possibilities for confirmation and identification of ions of interest.

Sequential extraction studies

The ultrasonically-assisted extraction method appears to be highly efficient. No additional recovery of QACs above 0.6 – 1.3% was observed when additional or more rigorous extraction was carried out on previously extracted sediment. As described above, additional extraction conditions tested the effects of time, sonication energy, solvent polarity, and strength of cation-exchanger in solution.

In contrast, extraction of sediments with the ultrasonically-assisted method sequentially following either Soxhlet¹⁰ or steam⁸ extraction resulted in additional recovery that was dependent upon the extraction method, sediment sample, and the analyte (Table 2). In this limited comparison, the Soxhlet method was the least efficient extraction method, most clearly seen in the case of the extraction of low QAC, low total organic carbon LIS sediment (Table 2 and 3). The amount of additional individual QACs determined during sequential re-extraction of LIS sediment were 33-130% of the amount determined by Soxhlet (i.e., the fraction of the total extracted QACs recovered in the re-extraction ranged from approximately 0.25 to 0.57; Table 2). In contrast, re-extraction of the higher QAC and total organic carbon JB sample with the ultrasonically-assisted extraction protocol recovered a lower fraction of the combined recovery of the sequential extractions (0.04 – 0.17). The steam extraction⁸ was more complete in the case of the LIS sediment; the fraction of the total recovered in the re-extraction ranged from approximately 0.08 to 0.21. However, the extraction efficiency of the Soxhlet and Steam extraction methods were quite similar in the case of the more contaminated JB sample (Table 2). The improvements in recovery provided by the sonication-assisted acidic methanol re-extraction are modest in the case of the JB sediment, but are appreciably greater than the additional recovery (always $\leq 1.3\%$) provided

by a variety of re-extraction approaches when the ultrasonically-assisted acidic methanol method was applied first (0.004-0.013 for re-extractions).

The difficulty of efficient extraction of “field aged” sediment-sorbed QACs is most likely due to less reversible sorption of QACs, following aging.⁴¹ More resistance to extraction observed with the lower QAC and TOC LIS sediment may be attributed to a combination of high sorption energies at lower concentrations (strongly nonlinear sorption isotherms),⁴ or more access to a greater fraction of stronger or less accessible binding sites,⁴² or differences in clay mineralogy (e.g., intercalation of organic cations). When an optimized SFE extraction method⁹ was compared to the standard steam extraction method of Gerike,⁸ there was no difference found between methods when applied to digested sewage sludge samples, but the SFE method led to an apparent 30-40% increase in extraction of DTDMAC from two sediments that were more mineral and clay-rich.

Method validation

The surrogate standard DADMAC C12:C12 was well recovered by the currently developed method. Average recoveries that include all baked sand (n=3), blank solvent (n=3), LIS (n=3), JB, BB, FR-S, and FR-D sediment samples was 99±10% (n=13). QACs were quantified in estuarine sediments from two sewage-impacted urban harbor sites (BB and JB) and sites (LIS and FR) less impacted by sewage (Table 3). The LIS sample was extracted in triplicate. This was the lowest-concentration sample analyzed and yet, the precision of analysis (Table 3) was good (4% relative standard deviation, RSD, for total QACs), although it was not as good for BAC 12 (20% RSD), which was found in very low abundance. There was no detection of QACs in the deeply buried FR-D sediment, and it was also analyzed in triplicate after spiking DADMACs C10:C10, C12:C12, C14:C14, C16:C16, and C18:C18 at small nominal concentrations (equivalent to 10 ng/g of spiked sediment). The recoveries of spiked analytes from these matrix-rich samples (TOC = 4.0 %) were uniformly good (98-104%), except for C10:C10 (118%), with RSD between 5 and 8%.

The sensitivity of this method is excellent given the small sample size extracted. Table 3 shows the calculated limits of quantification (LOQ; S/N = 10). LOQ for the C10 – C18 DADMACs (0.1 – 2.0 ng/g) and BAC 14 and 16 (2 -2.6 ng/g) were determined by spiked addition to FR-D, but at lower nominal concentrations (1 ng/g) than above. With injection of only 10 µL out of 1 mL extract, these LOQs are dramatically lower than those reported in earlier analysis of marine sediments that did not incorporate ESI-MS,⁹ and similar to or lower than the LOQ values reported by Martinez-Carballo et. al.¹⁰ (0.6-3 ng/g for much larger 5 g sediment samples). Ferrer and Furlong¹⁵ reported somewhat lower method detection limits for BAC 12 and BAC 14 (0.5 and 0.6 ng/g when corrected for the same S/N), again based on much larger mass of extracted sediment (10 g wet wt.).

Occurrence of QACs in estuarine sediments

The total QAC concentrations determined in these 4 estuarine sediments are much higher (1,800 – 74,000 ng/g) than those recently measured in freshwater sediments from Austria (12 – 5,100 ng/g, n=2110). The difference is largely the result of much greater concentrations of DADMAC C16:C16, C16:C18, and C18:C18 (DTDMAC) in this work, likely attributed to the extended use of DADMACs as fabric softeners in the U.S. It is also surely due to the location of our sample stations, two of which are located in more sewage affected areas of the highly urbanized New York Harbor complex. There have been very few reports of QACs in any sediments or sludges collected in the U.S., none of which were collected from marine or highly urbanized settings. The concentrations of BACs measured in this work (121 to 21,000 ng/g) were generally higher than those reported in another study 15 of four U.S. river sediments (78 – 571 ng/g), again most likely reflecting the concentrated sewage inputs of the

highly populated New York metropolitan area.³⁰ Finally, the concentrations of DTDMAC (1.7 – 52 µg/g) determined here, can be compared to concentrations of DTDMAC determined earlier in two estuarine sediments close to sewage discharge from Spain (42.3 and 1140 µg/g),⁹ and to HPLC measurements of DTDMAC in sewage affected Rapid Creek (South Dakota, USA) sediments (3.0 – 67 µg/g).¹⁴

The concentrations of QACs reported in sewage affected JB and BB sediments are high compared to those of more frequently monitored organic contaminants. Total QAC levels in JB sediment are greater than the sum of neutral metabolites of alkylphenol ethoxylates³⁰ and the combined sum of PCBs, DDT residues, and PAHs measured in splits of the same JB sample.⁴³ Finally, concentrations of total BAC and C10:C10 DADMAC disinfectants are greater than that of triclocarban (below 100 ng/g) and triclocarban (approximately 2,000 ng/g) reported in Jamaica Bay sediments at a site in very close proximity.⁴⁴

Conclusions

A sensitive and highly selective method is presented for the determination of a range of QACs in sediments. The comprehensive method developed provides more complete sample extraction of QACs, and solutions for problems associated with loss of DTDMAC to surfaces. Instrument blank problems for DTDMAC were greatly reduced by employing a much lower fractions of aqueous solvents in HPLC mobile phases. HPLC-ESI-ToF-MS has proven to be especially powerful in the analysis of both target QAC analytes and for the identification of nontargeted alkylammonium ions through a combination of accurate mass measurements, improved resolution of nominally isobaric ions with different elemental formulae, and detection of diagnostic CID fragment ions.

An important discovery in this work was the recognition, and insights into, the extraordinarily high positive mass defects associated with alkylammonium and protonated alkylamine ions. The heavy masses of molecular ions, diagnostic CID fragment ions, and heavy isotope peak ions allows for the unambiguous elemental formula identification by accurate mass measurements provided by LC-ToF-MS. The differences in ion masses with those of ions with other feasible elemental formulas may be uniquely large, and seen to greatly reduce isobaric interferences seen in HPLC-ToF-MS analysis of complex sediment extracts. The ion mass defects of alkylamine ions as a function of molecular weight and DBEs has also been explored, and indicates that positive ion mass defects of alkylamine ions are widespread, which has implications that extend beyond the analysis of alkylamine and alkylammonium compounds. As an example, more saturated alkylamine fragment ions of a wider range of compounds will have masses that are much easier to resolve than that of the parent or molecular ions. Thus analysis of alkylamine CID fragment or daughter ions could have broad applicability in analyte confirmation or discovery based identification studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Reconstructed ion chromatograms of targeted QACs obtained from sewage impacted estuarine sediment (BB). HPLC method 2 was employed. Note that only 10 μ L out of a 300 mL extract was injected, illustrating the high sensitivities that can be achieved in analysis of QACs in sediments. The elution pattern of the DADMAC homolog series as a function of alkyl chain length is explained by variable HPLC mobile phase gradients employed. The internal standard (IS) is tridodecylamine.

Figure 2.

HPLC-ToF-MS ion chromatograms of DADMACs (C8:C8, C8:C10, and C10:C10) in BB sediment with mass windows of 0.5 Da (A); and 0.05 Da (B). HPLC method 1 was utilized. 10 μ L out of a 15 mL extract was injected. Nominally isobaric interferences apparent with the larger mass window (A) are from ^{13}C isotopes of compounds with M-1 base peaks ($\dagger$), or an ion with likely elemental formula of $\text{C}_{12}\text{H}_{32}\text{NO}_2$ ($\dagger\dagger$).

Figure 3.

Mass spectrum of putative peak for DADMAC C8:C10 in sediment sample BB (A) corresponding to the peak in Figure 2, shown along with ion chromatograms (B) of the molecular ion; M+1 and M+2 heavy isotope peaks; and proposed fragment ions at nominal m/z of 158 and 186 (Table 1). Differences in measured accurate masses and theoretical values (Table 1) for these ions were -1.5, 1.8, 1.9 and 0.3 mDa, respectively. The mass spectral peak at m/z of 228.2711 corresponds to a formula of $\text{C}_{15}\text{H}_{34}\text{N}^+$ (2.0 mDa mass error), with HPLC elution corresponding to dodecyltrimethylammonium.

Figure 4.

An illustration of the change in mass defect with increasing atomic number for elements most commonly encountered in electrospray ionization of organic compounds in positive ionization mode modified from reference²³ (A) and the difference in mass between selected target ions and masses of nominally isobaric ions with alternate elemental formulas (B), illustrated for caffeine and DADMACs with different DBE (0-5); note that the relative differences in masses for BACs correspond to the example here for DBE = 4.

Table 1

Molecular and in-source CID ions detected, and the cone voltages used in a mass spectrometric method that provided abundant signals for each ion (illustrative results from chromatographic time-varying cone voltage are found in Figure S-1 and Table S-1).

QAC structure	Molecular ion (<i>m/z</i>)	CID fragment ions (<i>m/z</i>)	Cone voltage (V)
BAC12	304.2999	212.2377	55
BAC 14	332.3312	240.2659	65
BAC 16	360.3625	268.2939	65
BAC 18	388.3938	296.3278	65
DADMAC C8:C8	270.3156	158.1905	55
DADMAC C8:C10	298.3469	158.1905, 186.2230	55
DADMAC C10:C10	326.3782	186.2191	65
DADMAC C14:C14	438.5034	242.2811	75
DADMAC C14:C16	466.5347	242.2859, 270.3124	85
DADMAC C16:C16	494.5660	270.3124	85
DADMAC C16:C18	522.5973	270.3124, 298.3435	85
DADMAC C18:C18	550.6286	298.3435	85

Table 2

The fraction of QACs recovered by sediment re-extraction (re-extraction recovery/(initial extraction + re-extraction recovery)) using the ultrasonically-assisted acidic methanol method reported here. Samples were injected at same dilution, such that some low abundance analytes were not detected (n.d.) in the second extraction.

		BAC				DADMAC					
		C12	C14	C16	C18	10:10	14:14	14:16	16:16	16:18	18:18
Soxhlet method ¹⁰	LIS	n.d.	0.25	n.d.	0.39	0.52	0.57	0.48	0.38	0.40	0.31
	JB	0.06	0.09	0.06	0.07	0.17	0.07	0.06	0.05	0.04	0.04
Steaming acidic methanol method ⁸											
Steaming acidic methanol method ⁸	LIS	n.d.	n.d.	n.d.	0.17	0.08	0.18	0.19	0.19	0.21	0.21
	JB	n.d.	0.05	0.07	0.08	0.12	0.05	0.06	0.04	0.06	0.06

Table 3

Concentrations (ng/g) of BAC and DADMAC in estuarine sediments (RSD%)^a The limit of quantification (LOQ) was determined by spike addition to the FR-D sample at low levels (1 ng/g).

	TOC		BAC				DADMAC						Total	
	(%)	C12	C14	C16	C18	8:8	8:10	10:10	14:14	14:16	16:16	18:18		
LIS	1.6	6.2 (20)	19 (5)	23 (7)	73 (5)	n.d	n.d.	6.0 (2)	11 (4)	31 (7)	110 (8)	620 (6)	930 (2)	1800(4)
JB	5.1	64	210	420	590	5.4	14	130	300	440	3300	12000	18000	35000
BB	6.7	3700	7200	5900	4500	24	120	780	760	860	5100	19000	26000	74000
FR-S	7.9	17	60	84	57	n.d.	n.d.	7.4	28	87	470	1400	2700	4900
FR-D	4.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
LOQ	-	-	2.0	2.6	-	-	-	0.2	0.1	0.1	0.2	0.3	2.0	-

^aBased on triplicate samples measurement. RSDs given in percent.