

## APPLICATION NOTE

# Identification of Dimethyldioctadecylammonium Ion ( $m/z$ 550.6) and Related Species ( $m/z$ 522.6, 494.6) as a Source of Contamination in Mass Spectrometry

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Chemical contamination can be one of the more common problems encountered when performing trace-level analysis regardless of the analytical technique. Minimizing or eliminating background interferences can be a difficult task, so knowledge of the chemical composition of these contaminants can prove invaluable when it comes to identifying the source. Once the source is identified, proper steps may be taken to reduce or eliminate it. In this study, we report the identity of some commonly seen contaminants ( $m/z$  550.6, 522.6, and 494.6) in electrospray ionization (ESI) mass spectrometry (MS). Through MS, tandem MS, accurate-mass, and high-resolution measurements we have identified these background contaminants as being quaternary ammonium species that contain long-chain hydrocarbon groups, where  $m/z$  550.6 is a dimethyldioctadecylammonium ion ( $C_{18}$ ,  $C_{18}$ ) and  $m/z$  522.6 and 494.6 are similar in nature but have shorter alkyl-chain groups. The lipophilic nature of these compounds and the fact that they have molecular weights similar to lysophospholipids make them a frequent contaminant in lipidomic studies. The likely sources of these compounds are commonly used personal and household products. (J Am Soc Mass Spectrom 2008, 19, 666–670) © 2008 American Society for Mass Spectrometry

Ongoing advances in mass spectrometry allow for instrumentation having ever-increasing sensitivity. Unfortunately, the ultimate detection of analytes is generally limited by the chemical background present. Full-scan mass spectra often contain background peaks at essentially every  $m/z$  value [1]. For known analytes the use of tandem MS techniques, which monitor specific precursor-to-product transitions, can greatly improve the sensitivity provided there are no background ions that have the same transitions. However, the ability to screen for “unknown” compounds present at trace levels may be greatly hampered by chemical background noise. The background may originate from many sources such as impurities present in water and solvents, or it may be leached from the membrane disks commonly used to filter solvents before HPLC and mass spectrometry [2, 3]. Other sources include detergent residues [3, 4],

plasticizers [5, 6], volatiles present in ambient laboratory air [4], and compounds from mold release agents used in manufacture of laboratory plastic ware. Also, protonated solvent clusters or solvent clustered with  $Na^+$ ,  $K^+$ , or contaminant ions can contribute to chemical noise.

Here we report the identity of a frequently seen contaminant ( $m/z$  551) in mass spectrometry as being a dimethyldioctadecylammonium ion, which generally appears in mass spectra along with similar species at  $m/z$  523 and 495. The likely sources of these contaminants are commonly used personal and household products such as lotions, hair care products, detergents, and fabric softeners.

## Experimental

### Materials and Reagents

HPLC grade acetonitrile was obtained from EMD Chemicals Inc. (Gibbstown, NJ). A Milli-Q water purification system was used to generate water at  $18.2 \text{ M}\Omega \text{ cm}^{-1}$ ;  $<6 \text{ ppb TOC}$ . Dimethyldioctade-

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**Table 1.** Possible elemental formulas for contaminant ions

Mass $m/z$	Measured $m/z$	Formula	Calculated $m/z$	Mass accuracy (ppm)
298 Fragment	298.34671	C <sub>20</sub> H <sub>44</sub> N	298.346827	0.4
551	550.62894	C <sub>38</sub> H <sub>80</sub> N	550.628528	0.7
		C <sub>23</sub> H <sub>76</sub> N <sub>13</sub> O	550.629031	0.2
		C <sub>24</sub> H <sub>82</sub> N <sub>6</sub> O <sub>6</sub>	550.629036	0.2
523	522.59704	C <sub>36</sub> H <sub>76</sub> N	522.597228	0.4

cylammonium chloride (Fluka brand) was purchased from Sigma-Aldrich Corporation (St. Louis, MO). An ESI tuning mix (part no. G2431A) was obtained from Agilent Technology, Inc. (Wilmington, DE). Loperamide was a gift from Dr. Alastair Wood (Vanderbilt University).

### Instrumentation

Full scan and tandem mass spectra were acquired on a ThermoFinnigan TSQ 7000 triple quadrupole instrument (ThermoFinnigan, San Jose, CA). The system was equipped with electrospray ionization and was operated in the positive-ion mode with the electrospray needle maintained at 4.5 kV. Nitrogen was used for the sheath gas at a pressure of 50 psi. The heated capillary was operated at 20 V and 200 °C and the tube lens voltage was set to 100 V. For tandem mass spectra, ions were collisionally activated at an indicated argon pressure of 2.0 mTorr and collision energies ranging from –20 to –70 eV. The mass spectrometer was operated at unit mass resolution for both precursor and product ions. Full-scan and tandem mass spectra were acquired over the  $m/z$  ranges 10–1000 (2 s) and 10–600 (1 s), respectively. Samples were introduced into the mass spectrometer by direct-liquid infusion at 10  $\mu\text{L}/\text{min}$ .

ESI-FTMS accurate mass measurements were made using a Bruker Daltonics 9.4T Apex Qe with Apollo II Dual Source; –30 V was applied to the external collision cell to induce dissociation with no  $m/z$  filtering applied before ions entered the collision cell. Analyte solution was infused directly into the electrospray source at a rate of 3  $\mu\text{L}/\text{min}$ .

### Experimental Procedures

For accurate mass measurements, a solution of the contaminants in 50:50 acetonitrile: water was co-mixed with an Agilent ESI tune mix and loperamide, both of which served as internal mass calibrants.

For MS and tandem MS measurements, a stock solution of dimethyldioctadecylammonium chloride at 0.9 mg/mL was prepared in acetonitrile. Serial dilutions in acetonitrile were made from the stock as needed. A solution of the contaminants in 50:50 acetonitrile:water was also analyzed.

## Results and Discussion

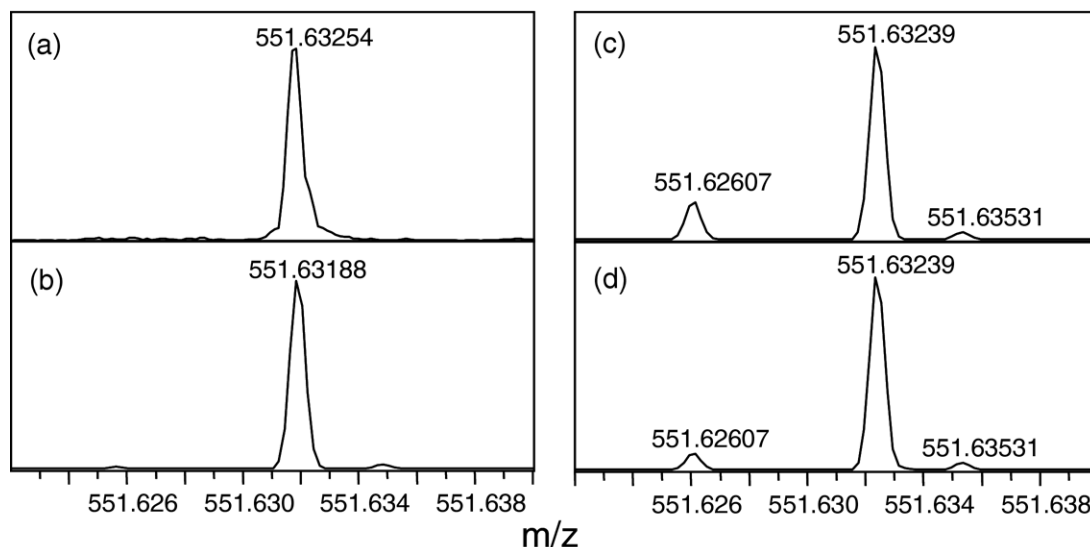
### Identification of Contaminant Species

Over the course of several years, while performing positive-ion ESI analysis, we have frequently observed background contamination at  $m/z$  551, 523, and 495. The background may be seen when infusing pure solvents such as methanol or acetonitrile and generally tends to decrease in intensity when acid and analyte are present. MS/MS fragmentation of the ion at 551 u yields a dominant ion at 298 u and smaller hydrocarbon-like ions at 43, 57, 71, and 85 u.

We used ESI-FTMS to obtain molecular formula information as a means to identify the contamination. A solution containing the contaminants along with internal calibrants loperamide ( $[\text{M} + \text{H}]^+$  477, fragment at  $m/z$  266) and the tune mix [hexamethoxyphosphazene at  $m/z$  322 and hexakis (2,2-difluoroethoxy) phosphazene at  $m/z$  622] were analyzed by ESI-FTMS. The data that contained CID fragments as well as precursors were internally calibrated using a quadratic function. The measured mass of each of the four internal calibrants agreed within 0.2 ppm of the theoretical value. The measured mass for the major contaminant ion was obtained at 550.62894, with the average mass resolution in the spectrum  $\sim 250,000$ . The measured masses for the contaminant ion at  $m/z$  523 as well as the fragment ion at  $m/z$  298 are given in Table 1.

Possible elemental formulas for the 551 peak, its associated fragment at 298, and the 523 peak are computed using criteria of accuracy  $<1$  ppm,  $> \text{C}_{23}$ ,  $> \text{C}_{16}$ , and  $> \text{C}_{20}$ , respectively, based on the  $A + 1$  peaks in the spectra. The elements C, H, N, and O were considered. For each ion, the possible elemental formulas are presented in Table 1.

A narrowband high-resolution ( $R = 950,000$ ) scan of the 550.6 ion envelope was acquired to see if the isotopic profile would eliminate one or more of the possible compositions. A portion of this spectrum showing the  $A + 1$  peak at  $m/z$  551.6 is shown in Figure 1. It compares the  $A + 1$  region of the measured contaminant spectrum (Figure 1a) with the theoretical isotopic distribution for the three possibilities. Two of the three possible compositions (Figure 1c and d) contain a sufficiently large number of nitrogen atoms that a significant  $^{15}\text{N}$  peak should be observed in a high-resolution scan. Because the  $^{15}\text{N}$  isotope has a negative mass defect relative to  $^{13}\text{C}$ , if a significant number of



**Figure 1.** Isotopic distribution for A + 1 peak of  $m/z$  550.5 for (a) contaminated sample and potential candidates, (b)  $C_{38}H_{80}N$ , (c)  $C_{23}H_{76}N_{13}O$  (d)  $C_{24}H_{82}N_6O_6$ .

nitrogens are present, there will be a peak present in the spectrum at a mass slightly less than that of the normal  $^{13}C$  A + 1 peak. From these data, the measured spectrum is consistent with a composition containing a few nitrogen atoms (Figure 1b), indicating the molecular formula of  $C_{38}H_{80}N$  is the likely one.

Based on this information, possible chemical compositions of compounds containing the molecular formula  $C_{38}H_{80}N$  and  $C_{38}H_{79}N$  (unprotonated) were explored using SciFinder Scholar (American Chemical Society, 2006). There were 29 possible compounds consisting of primary, secondary, tertiary, and quaternary amines. In most cases, the compound's structure was not consistent with producing the tandem mass spectrum we have observed (dominant ion at 298 u and smaller hydrocarbon-like ions at 43, 57, 71, and 85 u). Many of the structures had alkyl groups of different chain lengths attached to nitrogen and therefore would not produce one dominant ion at  $m/z$  298. Only four to five compounds had structures that could reasonably produce the observed spectrum. Of these only one, dimethyldioctadecylammonium chloride, appeared to be in wide commercial use and was readily commercially available. Because it contains two identical  $C_{18}$  side chains, its structure (Scheme 1) is consistent with producing a MS/MS mass spectrum whereby  $m/z$  551 fragments to produce a dominant  $m/z$  298 and it could

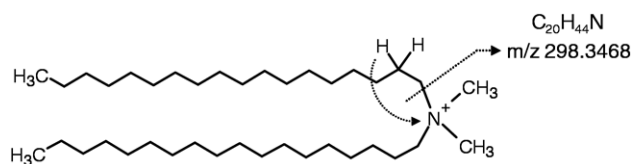
potentially produce low-mass hydrocarbon peaks at 43, 57, 71, and 85 u.

Dimethyldioctadecylammonium chloride (also known as distearyldimonium chloride) is a commonly used product in personal and household products. The ionic nature of the amine group coupled with the hydrocarbons gives the compound good surfactant properties. It may be found as an ingredient in cosmetics, hair products and fabric softeners in which it is added primarily for its antistatic softening properties [7, 8].

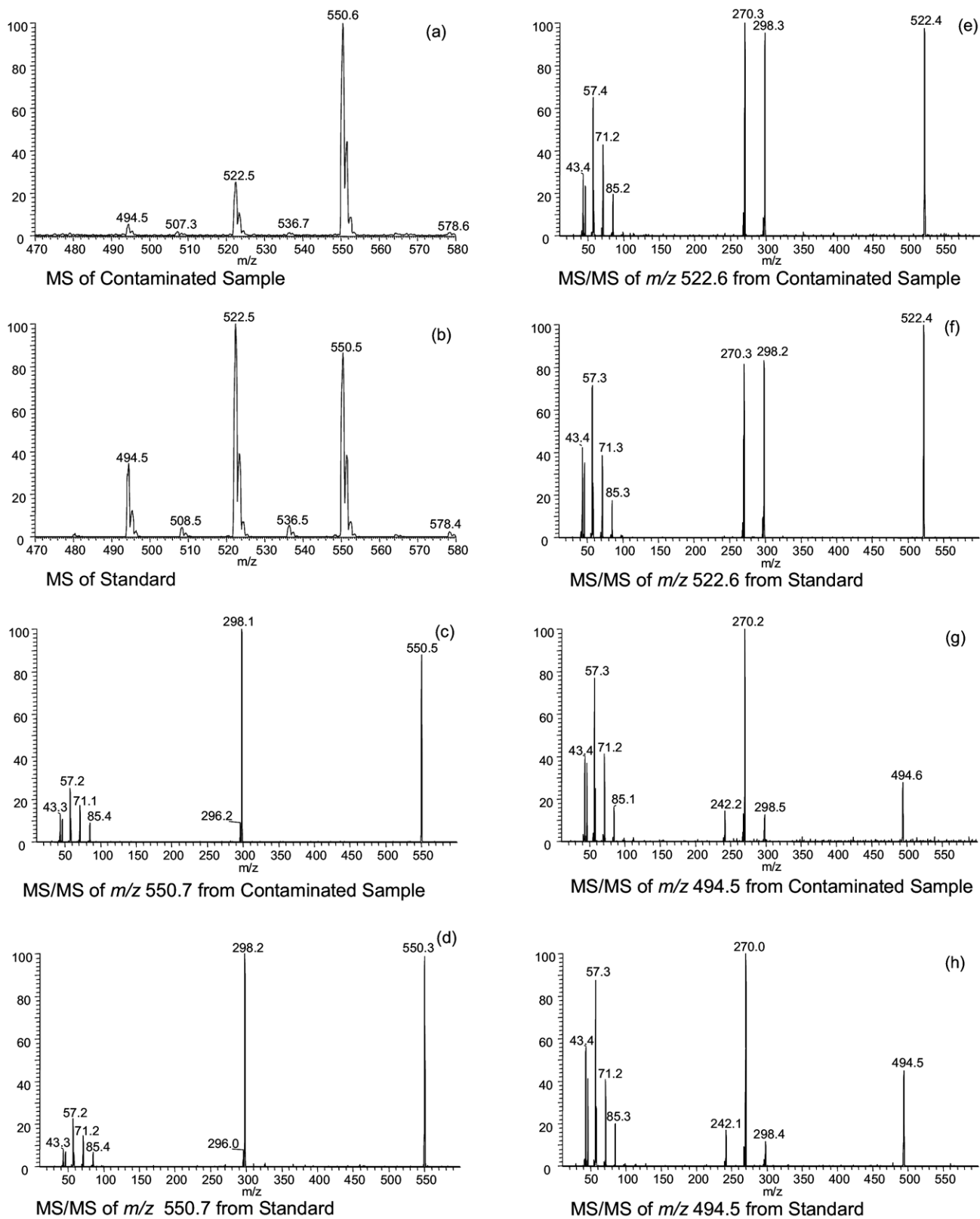
#### Comparison of Contaminants to Dimethyldioctadecylammonium Chloride Standard

A low-resolution full scan spectrum of acetonitrile containing the contaminant ions is shown in Figure 2a and a standard containing 570 ng/mL dimethyldioctadecylammonium ( $C_{18}$ ,  $C_{18}$ ) chloride in acetonitrile in Figure 2b. It is interesting to note the relative spectral intensity differences between the ions at 495, 523, and 551 u for the two samples. The ions at 495 and 523 u are more intense relative to 551 in the purchased standard than in the contaminated acetonitrile sample. MS/MS of  $m/z$  551 from the contaminated sample and standard shown in Figure 2c and d, respectively, show one dominant fragment at  $m/z$  298. Because MS/MS of 551 does not produce any fragment ions at  $m/z$  495 or 523 (even at lower collision energies), the ions in the MS spectrum at 495 and 523 u are likely impurities in the commercial standard we obtained and not a result of in-source fragmentation. This leads to some questions as to the purity of the purchased standard.

MS/MS fragmentation of ions at 523 and 495 u from the contaminated sample and standard are shown in Figure 2e–h. Because 523 fragments to give ions at both 298 and 270 u, the precursor ion likely is dimethylhexa-



**Scheme 1.** Fragmentation of dimethyldioctadecylammonium ion ( $m/z$  550.6).



**Figure 2.** MS spectrum of (a) acetonitrile contaminated with compounds at  $m/z$  550.6, 522.5, and 494.5 and (b) standard containing 570 ng/mL dimethyldioctadecylammonium chloride in acetonitrile. (c)–(h) MS/MS spectra of specified ions at a collision energy of  $-50\text{eV}$ .

decyloctadecylammonium ( $C_{16}$ ,  $C_{18}$ ). Because the ion at 495 fragments to give ions at 242, 270, and 298 u there is likely a combination of the dimethyldihexadecylammonium ( $C_{16}$ ,  $C_{16}$ ) and dimethyltetradecyloctadecylammonium ( $C_{14}$ ,  $C_{18}$ ) ions present. In Europe, mixtures of these compounds are commonly referred to as "tallowquats." These data are consistent with the findings of Fernandez et al. [8], who found similar spectral patterns from ditallowdimethylammonium surfactant extracted from sewage treatment plant sludge. Indeed, pure dimethyldioctadecylammonium chloride is not generally manufactured as a final product. It results from manufacture using fatty acids derived from fats and oils such as tallow, coconut, or palm. Production of nitriles from these fatty acids followed by catalytic hydrogenation generates a mixture of ammonium compounds containing long-chain hydrocarbon groups. Subsequent addition of methyl groups produces dialkyldimethyl quaternary amines [9]. The use of ditallowdimethylammonium chloride in Europe has decreased since 1991 when it was replaced by more easily biodegradable quaternary ammonium esters [10]. The product is still widely used in the United States in various consumer products. A search of patents list dimethyldioctadecylammonium chloride or ditallowdimethylammonium chloride as being used in things such as lotions, hair products, body washes, fabric softener sheets, and paper used for tissue products.

## Conclusions

Frequently seen background interferences in positive ion electrospray mass spectrometry at  $m/z$  495, 523, and 551 have been identified as ditallowdimethylammonium ions and likely result from use of personal and household products. It is reasonable that this contamination could also be seen by other mass spectrometry techniques. Because these quaternary ammonium compounds exist as charged species, they have good mass

spectrometry sensitivity even when no acid is present in the solution. This type of background should be of concern to those in the field of lipidomics because these ions are isobaric with some lipid species (e.g., lysoglycerophospholipids). Because the product is frequently used in body lotions, the contamination likely originates from handling tubing, fittings, syringes, etc. with bare hands.

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