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Identification of Benzalkonium Chloride in Commercial Grapefruit Seed Extracts

GARY R. TAKEOKA,* LAN T. DAO, ROSALIND Y. WONG, AND LESLIE A. HARDEN

Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 800 Buchanan Street, Albany, California 94710

Commercial grapefruit seed extracts (GSE) were extracted with chloroform. The solvent was evaporated, and the resulting solid was subsequently analyzed by high-performance liquid chromatography (HPLC), electrospray ionization mass spectrometry (ESI/MS), tandem mass spectrometry (ESI/MS/MS), and elemental analysis (by proton-induced X-ray emission analysis). Three major constituents were observed by HPLC and were identified as benzyldimethyldodecylammonium chloride, benzyldimethyltetradecylammonium chloride, and benzyldimethylhexadecylammonium chloride. This mixture of homologues is commonly known as benzalkonium chloride, a widely used synthetic antimicrobial ingredient used in cleaning and disinfection agents.

KEYWORDS: Benzalkonium chloride; grapefruit seed extract; electrospray ionization mass spectrometry; proton-induced X-ray emission (PIXE) analysis; antimicrobial activity

INTRODUCTION

Grapefruit seed extract (GSE) is promoted as a natural product that has reported antibacterial and antiviral properties. It is reported by the manufacturer to be safe and effective to use internally and externally for a wide variety of conditions such as acne, allergies, athlete's foot, body odor, candida, colds, cold sores, gastrointestinal infections, gingivitis, impetigo, parasitic infection, sinusitis, sore throat, and thrush (*1*, *2*). It is sold in health food stores and is widely available on the Internet.

There is recent evidence that some commercial GSE is adulterated with synthetic preservatives and that these additives are solely responsible for the antimicrobial activity of these products. Sakamoto et al. (*3*) found the preservative agents methyl 4-hydroxybenzoate (methyl paraben) and 2,4,4′-trichloro-2′-hydroxydiphenyl ether (triclosan) in commercially available GSE but not in ethanol extracts of grapefruit seeds. The identities of the preservatives were confirmed by comparing their high-performance liquid chromatography (HPLC) retention times and absorption spectra with those of authentic standards, whereas triclosan was additionally confirmed by liquid chromatography−mass spectrometry (LC-MS) using negative ion electrospray ionization. The presence of methyl paraben and triclosan was later confirmed by von Woedtke and co-workers (*4*), who also identified benzethonium chloride (**Figure 1**) in these products. Using thin-layer chromatography (TLC) as their analytical method these researchers reported that five of the six commercial GSE contained between 1.25 and 10% benzethonium chloride, whereas levels of 0.0125 and 0.025% triclosan were present in three samples. Methyl paraben was detected in three GSE samples, although no concentrations were reported. Five of the commercial GSE samples that contained one to three preservative substances showed high growth inhibition against *Bacillus subtilis* SBUG 14, *Micrococcus flavus* SBUG 16, *Staphylococcus aureus* SBUG 11, *Serratia marcescens* SBUG 9, *Escherichia coli* SBUG 17, *Proteus mirabilis* SBUG 47, and *Candida maltosa* SBUG 700. Dose−response curves of the three preservative agents on the seven microbes tested revealed that benzethonium chloride was responsible for most of the antimicrobial activity observed. Only one of the six commercial GSE samples was preservative-free, and this sample did not exhibit any antimicrobial activity. The authors made their own GSE samples by hot and cold extraction of grapefruit seeds and juiceless pulp using glycerol, water, and ethanol as well as mixtures of these solvents. These samples also did not have any antimicrobial activity. It was concluded that the potent antimicrobial activity attributed to GSE is due to the synthetic preservative agents, with benzethonium chloride being responsible for the majority of activity. One of the manufacturers later claimed that their product does not contain benzethonium

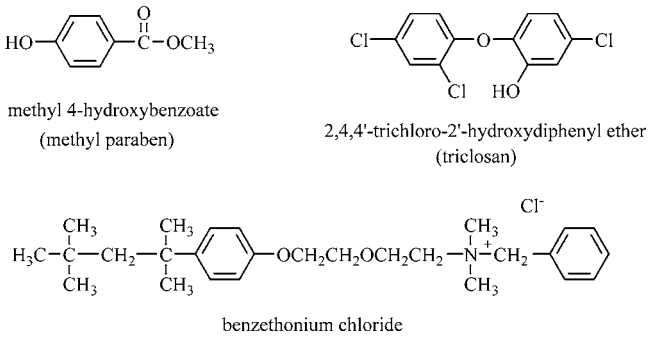

Figure 1. Synthetic antimicrobial agents previously identified in commercial grapefruit seed extracts.

* Author to whom correspondence should be addressed [telephone (510) 559-5668; fax (510) 559-5828; e-mail grt@pw.usda.gov].

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chloride and that the error was due to the similarity in molecular weight of the quaternary ammonium compound (said to be formed in the proprietary manufacturing process) with that of benzethonium chloride. However, the presence of 8% benzethonium chloride in commercial GSE was conclusively demonstrated by the use of HPLC, one- and two-dimensional NMR, proton-induced X-ray emission (PIXE) analysis, and electrospray ionization mass spectrometry (*5*). Since the appearance of the later publication we have been repeatedly asked by manufacturers who use GSE as an ingredient to analyze commercial GSE for the presence of benzethonium chloride. During our recent investigation of commercial GSE we observed three peaks in HPLC analysis for which the retention times did not match those of benzethonium chloride, triclosan, or methyl paraben. The goal of this research was to identify these unknown constituents in commercial GSE.

EXPERIMENTAL PROCEDURES

Materials. Various batches of grapefruit seed extract from one company were analyzed.

Chemicals. Benzyldimethyldecylammonium chloride (purum), benzyldimethyldodecylammonium bromide (puriss.): ^{1}H NMR ($CDCl_3$) δ 0.88 [t, J = 6.8 Hz, 3H [CH_3(CH$_2$)$_{11}$N]]; ^{13}C NMR δ 14.11, 1.25 [m, 18H [CH$_3$(CH_2)$_9$CH$_2$CH$_2$N], 31.91, 29.59 (2), 29.46, 29.40, 29.33, 29.28, 26.35, 22.69], 1.80 [m, 2H [CH$_3$(CH$_2$)$_9$CH_2CH$_2$N], 22.99], 3.31 [s, 6H [Bz(CH_3)$_2$N(CH$_2$)$_{11}$CH$_3$], 49.70 (2 CH$_3$)], 3.56 [m, 2H [CH$_3$(CH$_2$)$_9$-CH$_2$CH_2N], 63.82], 5.11 [s, 2H (PhCH_2N), 67.39 (C at 1-position of the phenyl ring) 127.46], 7.43 [m, 2H (2Hs at 3- and 5-positions of the phenyl ring), 129.22], 7.45 [m, 1H (H at 4-position of the phenyl ring), 130.73], 7.69 [m, 2H (2Hs at 2- and 6-positions of the phenyl ring), 133.30]. Benzyldimethyltetradecylammonium chloride (puriss anhydrous): ^{1}H NMR ($CDCl_3$) δ 0.88 [t, J = 6.8 Hz, 3H [CH_3-(CH$_2$)$_{13}$N], 14.11], 1.26 [m, 22H [CH$_3$(CH_2)$_{11}$CH$_2$CH$_2$N] 31.93, 29.68, 29.65 (2), 29.59, 29.46, 29.41, 29.35, 29.28, 26.39, 22.69], 1.80 [m, 2H [CH$_3$(CH$_2$)$_{11}$CH_2CH$_2$N] 22.98], 3.32 [s, 6H [Bz(CH_3)$_2$N(CH$_2$)$_{13}$-CH$_3$] 49.66], 3.55 [m, 2H [CH$_3$(CH$_2$)$_{11}$CH$_2$CH_2N] 63.72], 5.10, [s, 2H (PhCH_2N) 67.42 (C at 1-position of the phenyl ring) 127.69], 7.42 [m, 2H (2Hs at 3- and 5-positions of the phenyl ring) 129.16], 7.44 [m, 1H (H at 4-position of the phenyl ring) 130.62], 7.69 [m, 2H (2Hs at 2- and 6-positions of the phenyl ring) 133.33]. These chemicals as well as benzyldimethylhexadecylammonium chloride (purum) were obtained from Fluka (Sigma-Aldrich Corp., Milwaukee, WI). Triclosan was purchased from RIC Chemicals, Inc. (Armonk, NY), whereas methyl 4-hydroxybenzoate (methyl paraben) and benzethonium chloride were supplied by TCI America (Portland, OR). A sample of benzalkonium chloride (BAC) USP/NF (batch 203753; 65.4% C_{12}, 34.3% C_{14}, and 0.3% C_{16}) was supplied by FeF Chemicals A/S (Køge, Denmark). Solvents were of HPLC spectroscopic grade. Purified water was obtained from a Milli-Q Plus water purification system (Millipore, Billerica, MA).

Extraction. Approximately 1 g of GSE DF-100 was mixed with 3 mL of water and extracted with 40 mL of chloroform in a separatory funnel. The chloroform layer was collected, and the milky residue was extracted with another 40 mL aliquot of chloroform. The combined chloroform extract was evaporated under a stream of nitrogen. The resulting semisolid was dried in a desiccator containing $CaSO_4$. The solid was dissolved in HPLC mobile phase and filtered through a disposable membrane filter before injection.

High-Performance Liquid Chromatography. The HPLC system consisted of an HP 1100 quaternary pump, a manual injector (model 7725i, Rheodyne, Rohnert Park, CA) equipped with a 20 μL sample loop, and an 1100 diode array detector. The instrument was controlled and the data were processed by an HP ChemStation [version (A.08.03)]. The analytical column was a Phenomenex Luna C18(2) (250 $\times$ 4.6 mm i.d., 5 μm, 100 Å, 17.8% carbon load; Phenomenex Inc., Torrance, CA) protected by a Supelguard LC-18-DB (Supelco, Inc., Bellefonte, PA) guard column. The mobile phase consisted of methanol/water (9:1, v/v) containing 0.1 M sodium perchlorate. The pH was adjusted to 3 by the addition of phosphoric acid (*6*). The flow rate was 1.0 mL/min, and the detector was set at 215 nm.

Preparative HPLC. The preparative HPLC system consisted of Gilson model 305 and 306 pumps, a Gilson 806 manometric module, a Gilson 811C dynamic mixer (Gilson Inc., Middelton, WI), a manual injector (Rheodyne model 1725) fitted with a 900 μL sample loop (PEEK tubing), and a Gilson 115 UV detector. A C18 reversed phase Varian Dynamax preparative column (250 $\times$ 21.4 mm i.d., 8 μm, 100 Å; Varian Associates, Walnut Creek, CA) was directly coupled to a guard column (50 mm $\times$ 21.4 mm i.d.) containing the same packing material. The flow rate was 8 mL/min. The fractions of interest were collected and purified by using a C18 Strata SPE cartridge (55 μm, 70Å, 1 g/6 mL, Phenomenex, Torrance, CA), previously activated with 1 volume of methanol followed by 1 volume of water. The cartridge was washed with 50 mL of water. The sample was eluted with 1 volume of methanol containing 0.1% sodium chloride.

Electrospray Ionization/Mass Spectrometry (ESI/MS). An HP 1100 liquid chromatograph equipped with a manual injector (Rheodyne, model 1725) fitted with a 20 μL sample loop and an HP 1100 diode array detector (DAD) was coupled to an HP 1100 mass selective detector (single quadrupole). Electrospray ionization (ESI) was utilized with the following operating conditions: positive ion mode; gas temperature, 350 °C, with a nitrogen flow rate of 10 L/min; nebulizer pressure, 30 psi; capillary voltage, 4000 V; fragmentor voltage, ramped from 90 V at mass 100 to 350 V at mass 1800. The mobile phase was 50:50 methanol/water (v/v) at a flow rate of 0.5 mL/min. The instrument was controlled and the data were processed by an HP ChemStation [rev. A.07.01 (682)].

ESI/MS/MS. An Applied Biosystems (Foster City, CA) quadrupole time-of-flight mass spectrometer (Q/TOF) equipped with an electrospray ionization probe operated in positive ion mode was used. The mobile phase consisted of 50:50 acetonitrile/water (v/v) with 0.15% trifluoroacetic acid (TFA). Structural identification was performed by MS/MS experiments after the parent ion was selected and fragmented.

Nuclear Magnetic Resonance (NMR) Spectroscopy. NMR spectra were obtained at 293 K from samples in $CDCl_3$ with tetramethylsilane (TMS) as an internal standard on a Bruker model ARX400 spectrometer at frequencies of 100.62 MHz for carbon and 400.13 MHz for proton.

Elemental Analysis. PIXE analysis was performed by Elemental Analysis Corp. (Lexington, KY). PIXE is an X-ray spectroscopic technique that provides simultaneous elemental analysis for the elements from sodium to uranium. The X-ray spectrum is initiated by energetic protons interacting with the electrons to create inner shell vacancies in the atoms of the sample material. The energies of the X-rays that are emitted when these vacancies are filled again are characteristic of the elements from which they originate, whereas the number of X-rays of a certain energy is proportional to the mass of the corresponding element found in the sample.

RESULTS AND DISCUSSION

Chloroform extraction of DF-100 GSE followed by solvent evaporation produced a yellow solid that comprised 22.16 $\pm$ 0.88% (n = 3) of the original extract. HPLC analysis of the solid revealed three major peaks that eluted at 5.0, 7.1, and 11.1 min (**Figure 2**). A small artifact peak eluted at 4 min. These retention times did not match those of methyl paraben (3.3 min), benzethonium chloride (5.5 min), or triclosan (6.7 min), preservative agents previously identified in commercial GSE. However, the retention times and on-line UV spectra of these three unknown constituents in DF-100 GSE did closely match those of a benzalkonium chloride USP/NF standard (FeF Chemicals A/S) containing benzyldimethyldodecylammonium chloride (C_{12} BAC), benzyldimethyltetradecylammonium chloride (C_{14} BAC), and benzyldimethylhexadecylammonium chloride (C_{16} BAC). Addition of individual standards of benzalkonium chloride (C_{12}, C_{14}, and C_{16} BAC) into the DF-100 GSE extract produced single peaks of each homologue by HPLC.

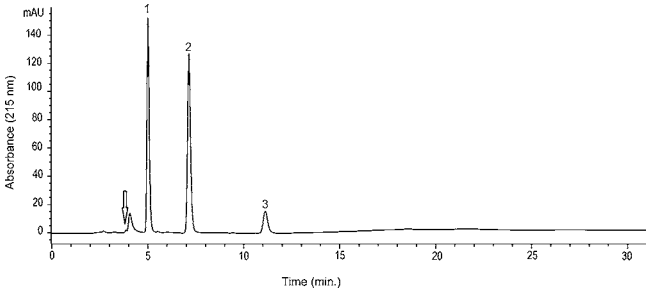

Figure 2. HPLC chromatogram (detection at 215 nm) of chloroform-extracted solid from commercial grapefruit seed extract (DF-100). Peaks: 1, benzyldimethyldodecylammonium chloride (C_{12} BAC; 41%); 2, benzyldimethyltetradecylammonium chloride (C_{14} BAC; 49%); 3, benzyldimethylhexadecylammonium chloride (C_{16} BAC; 10%). The arrow points to a peak containing a trace amount of benzyldimethyldecylammonium chloride (C_{10} BAC). Conditions: column, Phenomenex Luna C18(2), 250 × 4.6 mm i.d., 5 μm, 100 Å; mobile phase, methanol/water (9:1, v/v) containing 0.1 M sodium perchlorate (the pH was adjusted to 3 by the addition of phosphoric acid); flow rate, 1 mL/min.

The percentages of homologues were calculated using the formula

$$100A/B$$

in which *A* is the product of the area obtained for the homologue multiplied by its molecular weight and *B* is the sum of all of these products (*7*). The DF-100 GSE extract consisted of 41% C_{12} BAC, 49% C_{14} BAC, 10% C_{16} BAC, and a trace of C_{10} BAC.

The extracted solid was subjected to preparative HPLC to purify individual constituents. Purified fractions were treated by SPE to remove sodium perchlorate, which was found to interfere with subsequent mass spectrometric analysis. The fractions were analyzed by ESI-MS in the positive ion mode. The first purified fraction displayed its base peak at *m*/*z* 304.5. Smaller ions at *m*/*z* 276.5, 212.4, and 184.4 were observed. The second fraction had its base peak at *m*/*z* 332.6 and a smaller ion at *m*/*z* 240.4. The third fraction had its base peak at *m*/*z* 360.7 and a smaller ion at *m*/*z* 268.5. On the basis of these results and the strong positive ion signals under neutral pH conditions, it was suspected that the constituents were a homologous series of quaternary ammonium compounds. Their mass spectra were compared to standards of benzyldimethyldecylammonium chloride (C_{10} BAC, MW − 311.93, M^+ − 276.27), C_{12} BAC (MW − 339.99, M^+ − 304.30), C_{14} BAC (MW − 368.04, M^+ − 332.33), and C_{16} BAC (MW − 396.09, M^+ − 360.36). Excellent agreement was found between the mass spectra of the unknown constituents and the standards. For all of the homologues the molecular ion was the base peak, whereas the secondary fragment ions (i.e., *m*/*z* 184.4, 212.4, 240.4, and 268.5) resulted from loss of toluene (*8*). For further structural confirmation the purified fractions and standards were subjected to tandem MS/MS experiments. For these experiments the molecular ions were selected by the quadrupole analyzer and passed into a hexapole enclosed in a gas collision cell. The gas pressure in the hexapole was increased to fragment the molecular ions by collisions with gas molecules. The ions were passed into the time-of-flight analyzer to produce the final mass spectrum. Selecting and fragmenting the molecular ions under MS/MS conditions provide specific fragmentation information that confirms constituent identification. MS/MS experiments were performed by selecting the base peak from the three purified fractions. **Figure 3A** shows the mass spectrum of the first purified fraction obtained by selecting and fragmenting the base peak at *m*/*z* 304.3, whereas **Figure 3B** shows the corresponding mass spectrum of the C_{12} BAC standard obtained according to the same procedure. There is a very similar fragmentation pattern between the purified unknown and the C_{12} BAC standard. The peak at *m*/*z* 304.30 is the molecular ion, whereas the peak at *m*/*z* 212.24 results from the loss of toluene. The *m*/*z* 91.05 ion is the stabilized tropylium ion ($C_7H_7^+$) formed from rupture of the benzylic bond. The same fragment ions were observed for C_{12} BAC using ion trap LC-MS and LC-MS/MS (*9*). **Figure 4A** shows the mass spectrum of the second purified fraction obtained by selecting and fragmenting the base peak at *m*/*z* 332.33, whereas **Figure 4B** shows the corresponding mass spectrum of the C_{14} BAC standard. There is again excellent agreement between the mass spectra of the purified unknown and the C_{14} BAC standard. Similarly, the peak at *m*/*z* 332.33 is the molecular ion, whereas the peak at *m*/*z* 240.27 results from the loss of toluene and the peak at *m*/*z* 91 is the tropylium ion. The same two fragment ions were reported for C_{14} BAC by ion trap LC-MS, although only the ion at *m*/*z* 240 was obtained using LC-MS/MS (*9*). **Figure 5A** shows the mass spectrum of the third purified fraction obtained by selecting and fragmenting the base peak at *m*/*z* 360.36, whereas **Figure 5B** shows the corresponding mass spectrum of the C_{16} BAC standard. There is close agreement between the mass spectra of the purified unknown and the C_{16} BAC standard. The peak at *m*/*z* 360.36 represents the molecular ion, whereas the peak at *m*/*z* 268.30 results from loss of toluene and the *m*/*z* 91 ion is the tropylium ion resulting from the rupture of the benzylic bond.

BAC is a mixture of alkylbenzyldimethylammonium chlorides with the general formula $[C_6H_5CH_2N(CH_3)_2R]Cl$, where R = *n*-C_8H_{17} to *n*-$C_{18}H_{37}$ (**Figure 6**). The major homologues in the mixture are those possessing C_{12}, C_{14}, and C_{16} straight-chain alkyls. Interestingly, the homologues possess different microbiological activities. It has been reported that the C_{12} homologue is most effective against yeasts and fungi, the C_{14} homologue against Gram-positive bacteria, and the C_{16} homologue against Gram-negative bacteria (*10*). The proportion of these homologues in the mixture determines its effectiveness as a preservative and disinfectant. BAC is widely used as disinfectants and sanitizers in hospitals, food plants, homes, and many public places. Other reported uses include preservatives and antiseptics in health care products (ophthalmologic, skin, and nasal) (*11*) and pharmaceutical preparations (particularly aerosols) (*12*) and as antistatic, emulsifiers, and preservatives in the coatings industry (paints, wood treatments, and electronics). These quaternary ammonium salts are effective bacteriostats in very high dilutions due to their ability to inhibit certain bacterial enzymes, especially those involved in respiration and glycolysis (*13*). The bactericidal effect of quaternary ammonium salts appears to be due to their ability to cause release of bacterial cell contents into the surrounding medium (*13*). BAC can affect biological membranes and induce cytolysis that may lead to organ destruction and subsequent death (*14*). LD_{50} values in rats of 234−525 and 14 mg/kg were reported for oral and intravenous administration, respectively (*14*−*18*). Oral administration of 250 mg/kg of BAC to rats produced highly variable responses (*19*). About half of the rats (from a total of 30) appeared to be normal throughout the experimental period, whereas the other half had symptoms such as sneezing, diarrhea, or difficulty in breathing. Rats that aspirated BAC into their lungs had some systemic symptoms and higher blood and tissue concentrations of BAC. The researchers suggested that only a

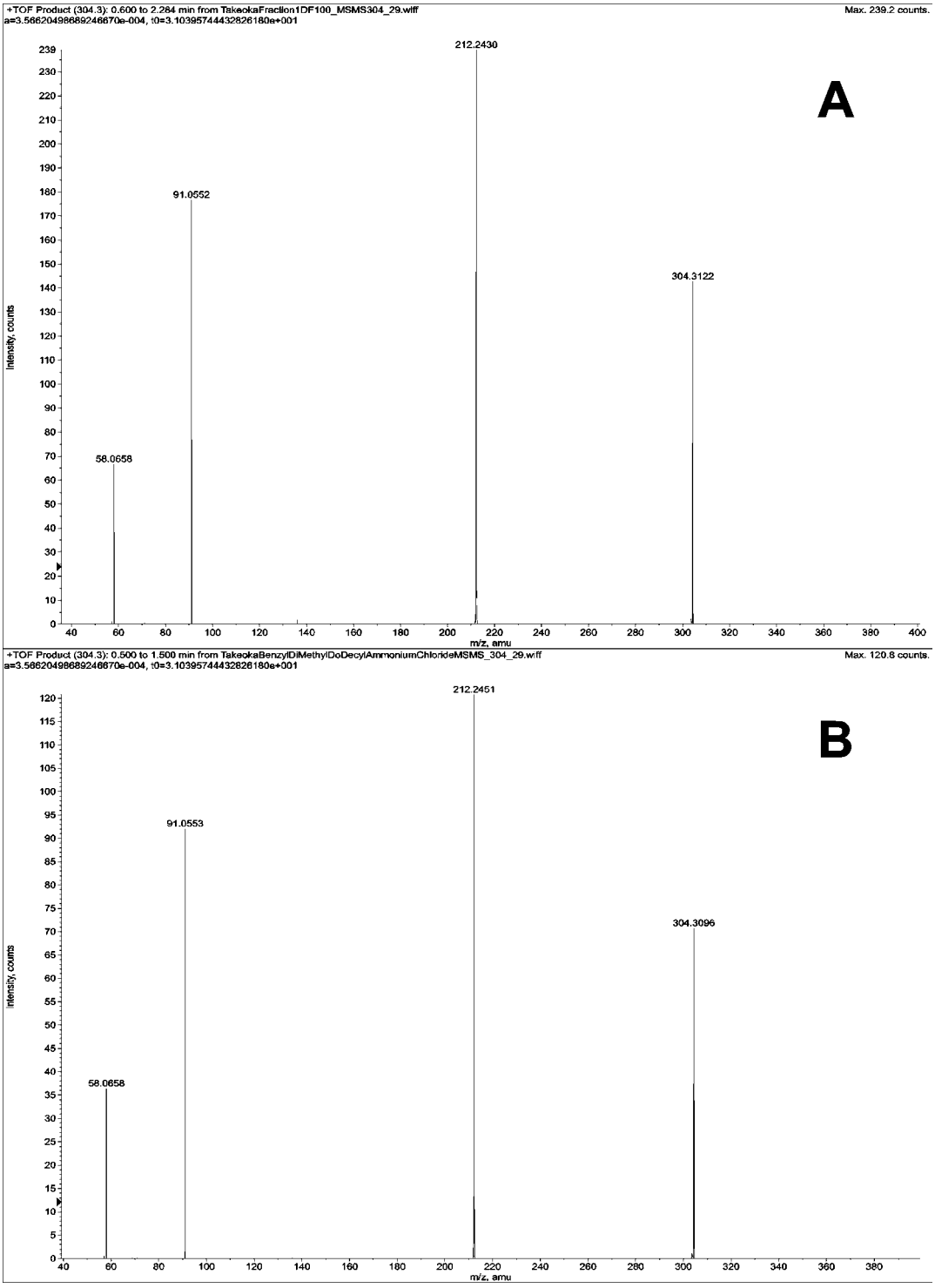

Figure 3. Positive ion electrospray MS/MS product ion analysis of *m*/*z* 304.30 obtained from (**A**) the first HPLC purified fraction from GSE extract and (**B**) benzyldimethyldodecylammonium chloride (C_{12} BAC) standard. The fragment ions at *m*/*z* 212.24 and 91.05 result from breaking the carbon–nitrogen bond between the quaternary amine and the benzyl substituent.

small amount of BAC was systemically absorbed by the stomach or intestinal mucosa. This was consistent with a report that BAC is categorized as a hard drug that is not readily metabolized by animals or humans and is excreted in its active form (*20*). Oral administration of BAC to rats resulted in 10-fold higher concentrations in lungs and kidneys than in blood, suggesting that these organs are reservoirs and possible targets of BAC (*19*). Human poisonings or fatalities have occurred from accidental or intentional ingestion of products containing large concentrations (≥10%) of BAC (*21*−*25*). An oral dose of 100−400 mg/kg is thought to be fatal to humans (*26*). Benzalkonium chloride is used in many nasal sprays and metered dose inhalers. Its use by asthmatic patients can produce significant bronchoconstriction. In a study of 28 asthmatic patients a significant decrease in pulmonary function tests was observed after BAC inhalation (*27*). The effect was greatest after 1 min of exposure and lasted for up to 60 min. The response was blocked by concomitant application of cromolyn, suggesting an allergic

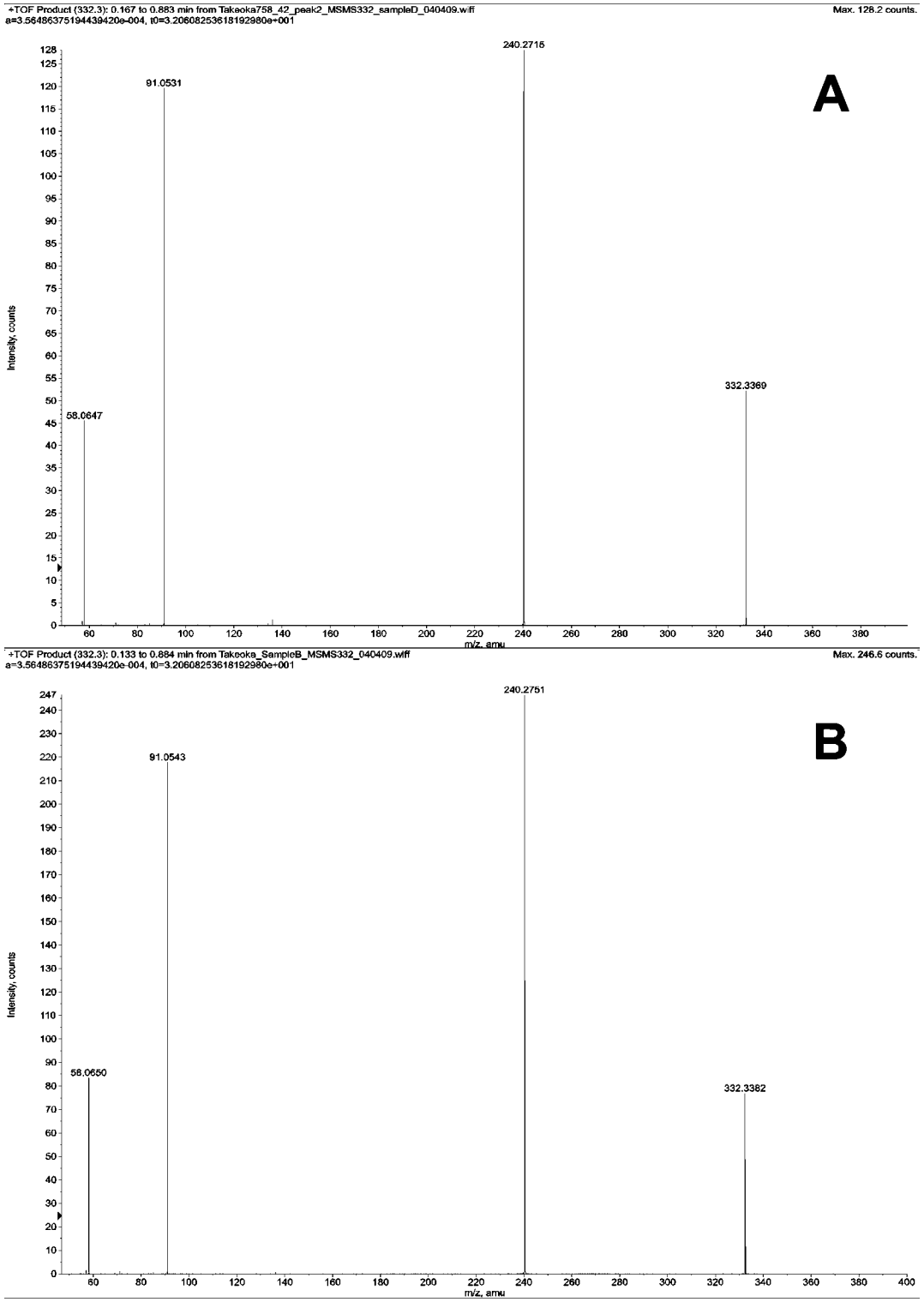

Figure 4. Positive ion electrospray MS/MS product ion analysis of *m*/*z* 332.33 obtained from (**A**) the second HPLC purified fraction from GSE extract and (**B**) benzyldimethyltetradecylammonium chloride (C_{14} BAC) standard. The fragment ions at *m*/*z* 240.27 and 91.05 result from breaking the carbon–nitrogen bond between the quaternary amine and the benzyl substituent.

mechanism. The use of nasal sprays containing BAC has been linked to nasal congestion and mucosal damage (*28*, *29*). Allergenic responses to BAC as a result of skin exposure have also been reported (*30*, *31*).

The presence of chloride in the sample was confirmed by PIXE analysis. The weight fraction of chlorine in the unknown was 8.7%, whereas the weight fraction expected on the basis of the homologue composition was 9.9%.

This work has demonstrated for the first time the presence of benzalkonium chloride in commercial GSE samples. It seems unlikely that the high concentrations of BAC (22%) arise from contamination or that the preservative is formed during the extraction and/or processing of grapefruit seeds and pulp. The presence of BAC in a commercial product designated for internal and external use by humans is troubling in light of its toxicity and allergenicity. This research adds to the list of synthetic

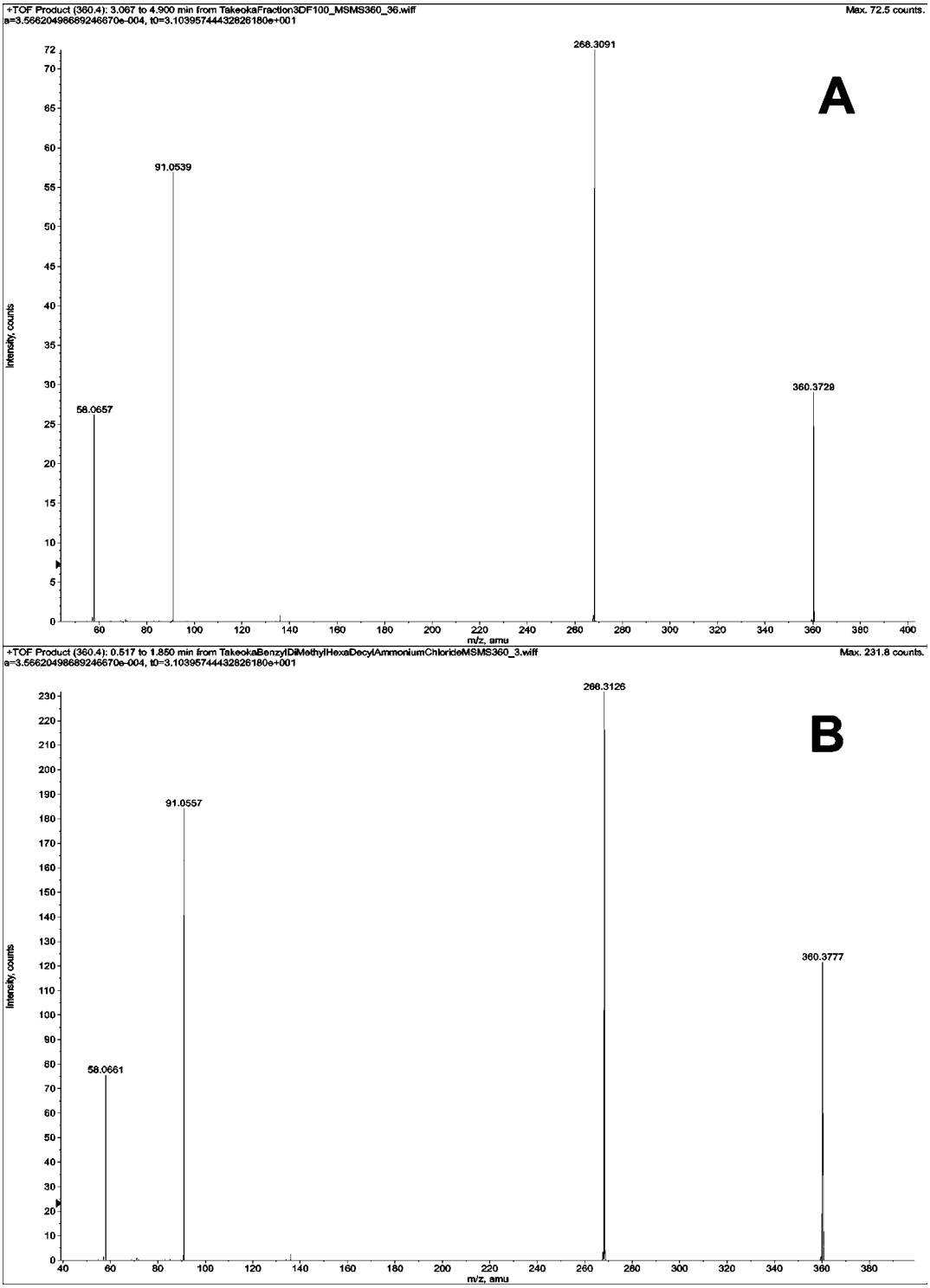

Figure 5. Positive ion electrospray MS/MS product ion analysis of *m*/*z* 360.36 obtained from (**A**) the third HPLC purified fraction from GSE extract and (**B**) benzyldimethylhexadecylammonium chloride (C_{16} BAC) standard. The fragment ions at *m*/*z* 268.30 and 91.05 result from breaking the carbon–nitrogen bond between the quaternary amine and the benzyl substituent.

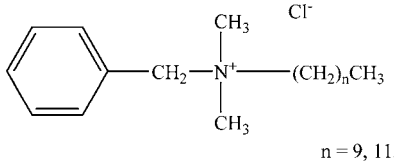

Figure 6. Structure of benzalkonium chloride.

preservatives such as benzethonium chloride, methyl paraben (methyl 4-hydroxybenzoate), and triclosan that have been identified in commercial GSE samples.

LITERATURE CITED

(1) Sachs, A. *The Authoritative Guide to Grapefruit Seed Extract*; LifeRhythm: Mendocino, CA, 1997; 125 pp.

(2) Sharamon, S.; Baginski, B. J. *The Healing Power of Grapefruit Seed*; Lotus Light Publications: Twin Lakes, WI, 1997; 152 pp.

(3) Sakamoto, S.; Sato, K.; Maitani, T.; Yamada, T. Analysis of components in natural food additive "grapefruit seed extract" by HPLC and LC/MS. *Bull. Natl. Inst. Health Sci.* **1996**, *114*, 38−42.

(4) von Woedtke, T.; Schlüter, B.; Pflegel, P.; Lindequist, U.; Jülich, W.-D. Aspects of the antimicrobial efficacy of grapefruit seed extract and its relation to preservative substances contained. *Pharmazie* **1999**, *54*, 452−456.
(5) Takeoka, G.; Dao, L.; Wong, R. Y.; Harden, L. Identification of benzethonium chloride in commercial grapefruit seed extracts. *J. Agric. Food Chem*. **2001**, *49*, 3316−3320.
(6) Abidi, S. L. Retention behavior of long chain quaternary ammonium homologues and related nitroso-alkylmethylamines. *J. Chromatogr*. **1985**, *324*, 209−230.
(7) *United States Pharmacopeia 24/National Formulary 19*; U.S. Pharmacopeial Convention: Rockville, MD, 2000; pp 2419−2421.
(8) Balasanmugam, K.; Hercules, D. M. Quantitative analysis of mixed benzalkonium chlorides by laser mass spectrometry. *Anal. Chem*. **1983**, *55*, 145−146.
(9) Ferrer, I.; Furlong, E. T. Identification of alkylbenzylammonium surfactants in water samples by solid-phase extraction followed by ion trap LC/MS and LC/MS/MS. *Environ. Sci. Technol*. **2001**, *35*, 2583−2588.
(10) Merianos, J. J. In *Disinfection, Sterilization, and Preservation*; Block, S. S., Ed.; Lea and Febiger: Philadelphia, PA, 1991; pp 225−255.
(11) Elrod, L., Jr.; Golic, T. G.; Morley, J. A. Determination of benzalkonium chloride in eye care products by high-performance liquid chromatography and solid-phase extraction or on-line column switching. *J. Chromatogr*. **1992**, *625*, 362−367.
(12) Dudkiewicz-Wilczyñska, J.; Tautt, J.; Roman, I. Application of the HPLC method for benzalkonium chloride determination in aerosol preparations. *J. Pharm. Biomed. Anal*. **2004**, *34*, 909−920.
(13) Gump, W. Disinfectants and Antiseptics. In *Kirk-Othmer Encyclopedia of Chemical Technology*, 3rd ed.; Wiley: New York, 1979; Vol. 7, pp 793−832.
(14) Gloxhuber, C. Toxicological properties of surfactants. *Arch. Toxicol*. **1974**, *32*, 245−270.
(15) Alfredson, B.; Stiefel, J.; Thorp, F.; Baten, W.; Gray, M. Toxicity studies on alkyldimethylbenzylammonium chloride in rats and in dogs. *J. Am. Pharm. Assoc*. **1951**, *40*, 263−267.
(16) Cummins, L.; Kimura, E. Safety evaluation of selenium sulfide antidandruff shampoos. *Toxicol. Appl. Pharmacol.* **1971**, *20*, 89−96.
(17) American College of Toxicology (ACT). Final report on the safety assessment of benzalkonium chloride. *J. Am. Coll. Toxicol*. **1989**, *8*, 589−625.
(18) Kamiya, A.; Oie, S. *Manual of Disinfectants: Characteristics, Usages and Precautions of Disinfectants*, 2nd ed.; Kenei Pharmaceutical Co.: Osaka, Japan, 2000; pp 96−98.
(19) Xue, Y.; Hieda, Y.; Saito, Y.; Nomura, T.; Fujihara, J.; Takayama, K.; Kimura, K.; Takeshita, H. Distribution and disposition of benzalkonium chloride following various routes of administration in rats. *Toxicol. Lett*. **2004**, *148*, 113−123.
(20) Thorsteinsson, T.; Loftsson, T.; Masson, M. Soft antibacterial agents. *Curr. Med. Chem*. **2003**, *10*, 1129−1136.
(21) Adelson, L.; Sunshine, I. Fatal poisoning due to a cationic detergent of the quaternary ammonium compounds type. *Clin. Pathol.* **1952**, *22*, 656−661.
(22) Ohta, K.; Yamanaka, Y.; Ubukata, Y.; Fujii, K. A case report of pharyngolaryngitis and esophagitis due to Osvan solution drunk by accident. *J. Nissei Hosp*. **1984**, *12*, 93−96.
(23) Berkel, V. M.; Wolff, F. A. D. Survival after benzalkonium chloride poisoning. *Hum. Toxicol*. **1988**, *7*, 191−193.
(24) Hitosugi, M.; Maruyama, K.; Takatsu, A. A case of fatal benzalkonium chloride poisoning. *Int. J. Legal Med*. **1998**, *111*, 265−266.
(25) Akutsu, I.; Motojima, S.; Ogata, H.; Fukuda, T.; Ikemori, R.; Makino, S. A case of acute benzalkonium chloride intoxication. *Jpn. J. Intern. Med*. **1989**, *78*, 1613−1614.
(26) Tiess, D.; Nagel, K. H. Beitrag zur Morphologie und analytik der inverseifenintoxikation zwei akut-tödliche vergiftungen durch perorale anfnahme des desinfetionsmittels C_4. *Arch. Toxikol*. **1967**, *22*, 333−348.
(27) Zhang, Y. G.; Wright, W. J.; Tam, W. K.; Nguyen-Dang, T. H.; Salome, C. M.; Woolcock, A. J. Effect of inhaled preservatives on asthmatic subjects. II. Benzalkonium chloride. *Am. Rev. Respir. Dis.* **1990**, *141*, 1405−1408.
(28) Hallen, H.; Graf, P. Benzalkonium chloride in nasal decongestive sprays has a long-lasting adverse effect on the nasal mucosa of healthy volunteers. *Clin. Exp. Allergy* **1994**, *25*, 401−405.
(29) Berg, O. H.; Lei, K.; Steinvag, S. The effects of topical nasal steroids on rat respiratory mucosa in vivo with special reference to benzalkonium chloride. *Allergy* **1997**, *52*, 627−632.
(30) Cox, N. H. Allergy to benzalkonium chloride simulating dermatomyositis. *Contact Dermatitis* **1994**, *31*, 50.
(31) Stanford, D.; Georgouras, K. Allergic contact dermatitis from benzalkonium chloride in plaster of Paris. *Contact Dermatitis* **1996**, *35*, 371−372.

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