Rapid Determination of Benzalkonium Chloride in a Cosmetic

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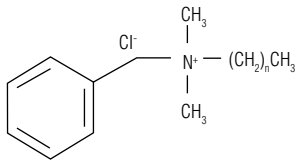
Key Words
Quaternary Ammonium Salt Analysis, Cationic Surfactant, Antiseptic Analysis, Acclaim Surfactant Plus Column

Goal
Develop an efficient high-performance liquid chromatography (HPLC) method for the rapid and sensitive determination of benzalkonium chloride in a cosmetic sample using a Thermo Scientific Acclaim Surfactant Plus column. This new method enables faster analysis and simpler separation conditions when compared to an existing method using the Acclaim Surfactant column.



n = 11, C_{12}-BKC homolog
n = 13, C_{14}-BKC homolog
n = 15, C_{16}-BKC homolog
n = 17, C_{18}-BKC homolog

Figure 1. Structures of benzalkonium chloride.

Introduction
Benzalkonium chloride (BKC), a typical quaternary ammonium salt, is often used as an antiseptic. Its structure is shown in Figure 1, and the C_{12} homolog is the major species in a benzalkonium chloride preparation.[1] The mode of antiseptic action of quaternary ammonium compounds appears to be associated with their effect on the cytoplasmic membrane that controls cell permeability, and the C_{12} homolog is most effective against yeast and fungi.[2]

Although HPLC has been used to separate and quantify benzalkonium chloride homologs,[3-7] it is difficult to obtain sharp and symmetrical peaks because of the strong ion-exchange interaction between the quaternary analyte and residual silanols on the surface of silica-based reversed-phase columns. A previous HPLC methodology used an Acclaim™ Surfactant column for the determination of benzalkonium chloride homologs in samples of sterile elastic strips and eye drops.[2] Although that approach yielded excellent peak shapes for this cationic surfactant, the previous method required a ternary mobile phase (formic acid solution/water/acetonitrile-water) with a 15 min gradient.

In the work shown here, the Acclaim Surfactant Plus column is used for the rapid and sensitive determination of benzalkonium chloride in a cosmetic sample using simpler separation conditions. The surface chemistry of the Acclaim Surfactant Plus column effectively deactivates the surface silanol activity so that cationic surfactants elute as symmetrical and efficient peaks compared to those obtained on conventional reversed-phase columns.[9]

Equipment
- Thermo Scientific Dionex UltiMate 3000 Rapid Separation LC system, including:
  - DGP-3600RS Pump
  - SRD-3600 Integrated Solvent and Degasser Rack
  - WPS-3000TRS Wellplate Sampler, Thermostatted, with 100 µL sample loop
  - TCC-3000RS Thermostatted Column Compartment
  - DAD-3000RS UV-vis Diode Array Detector with 5 µL flow cell
- Thermo Scientific Dionex Chromeleon Chromatography Data System software version 6.80, SR9 or higher
- Thermo Scientific MSQ Plus Single Quadrupole Mass Detector
- Thermo Scientific Orion 2-Star Benchtop pH Meter
- Kudos SK3200LH Ultrasonic Generator (Shanghai Kudos Ultrasonic Instrument Co., Ltd., China)
- Fisher Scientific MS2 Minishaker

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Reagents and Standards

- Deionized (DI) water, 18.2 MΩ-cm resistivity
- Methanol (CH_3OH), HPLC grade (Fisher Scientific P/N AC610090040)
- Acetonitrile (CH_3CN), HPLC grade (Fisher Scientific P/N AC610010040)
- Potassium dihydrogen phosphate (KH_2PO_4), analytical grade (SCRC, China)
- Phosphoric acid (H_3PO_4), analytical grade (SCRC, China)
- Ammonium acetate (NH_4Ac), analytical grade (SCRC, China)
- Alkyldimethyl ammonium (benzalkonium) chloride, 98% (Adamas-beta, China)

Preparation of Standards and Samples

Working Standard Solutions for Calibration

Weigh 0.1 g of benzalkonium chloride standard and dilute in a 10 mL volumetric flask with water. The concentration of the stock standard solution is 1%, w/v.

Prepare seven working standard solutions for calibration by adding defined volumes of the stock standard solution and diluting with water. The concentrations of benzalkonium chloride are 5, 10, 20, 50, 100, 200, and 1000 µg/mL, respectively.

Sample Preparation

The sample used in this study was a skin cream intended for skin cleansing and protection.

Mix 0.5 g of skin cream with 20 mL acetonitrile/water (1:1, v/v) in a 25 mL volumetric flask. Vortex this mixture for 1 min and ultrasonically extract at 150 W for 45 min. Allow the sonicated sample to cool to room temperature, then add water to the mark (25 mL). Prior to injection, filter the sample through a 0.22 µm filter.

Chromatographic Conditions

Column:	Acclaim Surfactant Plus, 3 µm Analytical, 3.0 × 150 mm (P/N 078951)
Mobile Phase:	75 mM KH_2PO_4 (pH 3.0, with H_3PO_4)/acetonitrile (1:1, v/v)
Flow Rate:	0.425 mL/min
Inj. Volume:	2 µL
Temperature:	30 °C
Detection:	UV absorbance at 215 nm

Results and Discussion

Because the benzalkonium chloride molecule contains a quaternary ammonium cation that remains as a charged species during reversed-phase chromatography, the ionic strength and pH value of the mobile phase will impact retention and possibly peak shape. Therefore, appropriate levels of mobile phase ionic strength and acidity are necessary for the separation of benzalkonium chloride on a reversed-phase column.

Figure 2 illustrates a series of benzalkonium chloride chromatograms with concentrations from 5 to 200 µg/mL using the Acclaim Surfactant Plus column and a 3.75 mM phosphate buffer, pH 3/50% acetonitrile mobile phase. The calculated peak purity match factor for the benzalkonium chloride peak is 1000 (the corresponding value for 100% purity), and the nominal monoisotopic mass of the benzalkonium cation obtained by an MSQ Plus™ Single Quadrupole Mass Detector is 304 *m/z*, indicating the peak is the C_{12}-BKC homolog.

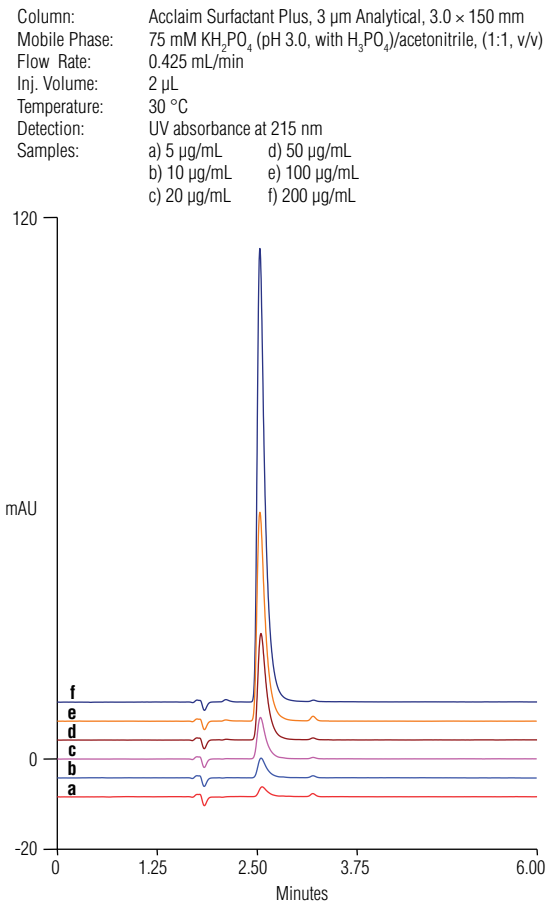

Figure 2. Overlay of chromatograms of the benzalkonium chloride standard with different concentrations.

Compared to the previous method using a ternary mobile phase (formic acid solution/water/acetonitrile-water) with a 15 min gradient,[8] sharp and symmetrical peaks with reasonable retention are still observed. Furthermore, the separation time is shortened to 4 min using an isocratic elution. The method is extended an additional 2 min to elute other sample components.

Method reproducibility was estimated by making seven consecutive injections of a benzalkonium chloride standard at a concentration of 200 µg/mL. The relative standard deviation was 0.07 for retention time and 1.86 for peak area. Calibration linearity for UV detection of benzalkonium chloride was investigated by making three consecutive injections of a standard prepared at seven different concentrations (i.e., 21 total injections). The external standard method was used to establish the calibration curve and quantify the analytes in the cosmetic sample.

As shown in Figure 3, excellent linearity was observed from 5 to 1000 µg/mL when plotting the concentration versus the peak area. The coefficient of determination was 0.9996. The method detection limit (MDL) of benzalkonium chloride for UV detection was calculated using the single-sided Student's *t* test method (at the 99% confidence limit). Seven consecutive injections of the skin cream sample mixed with benzalkonium chloride standard (20 µg/mL) were used to determine the standard deviation value for calculating MDL; the result was 1 µg/mL.

Figure 4 shows the chromatograms of the skin cream sample and the same sample spiked with a benzalkonium chloride standard. The UV spectra of the analyte collected in the standard and skin cream sample are highly consistent. The calculated peak purity match factor for benzalkonium chloride separated from the skin cream sample extract was 987 (a value of 1000 suggests 100% purity). The detected concentration of benzalkonium chloride in the sample solution was 210 µg/mL, equivalent to 1% of the skin cream. Recovery of benzalkonium chloride in the sample was 90%, suggesting good method accuracy.

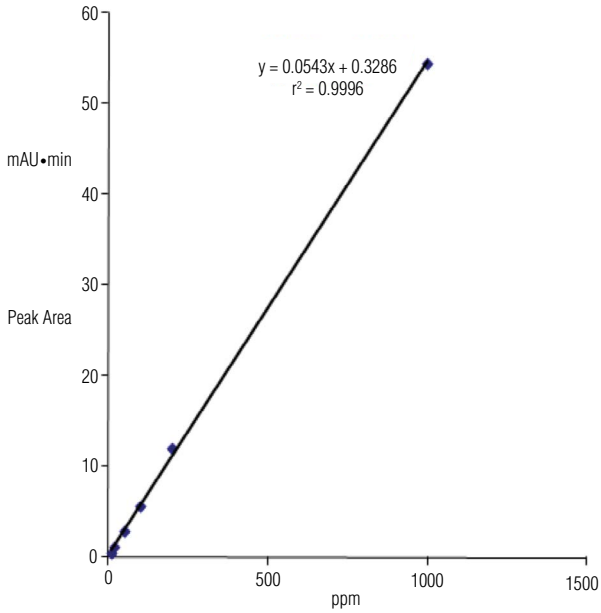

Figure 3. Calibration curve for benzalkonium chloride.

Column:	Acclaim Surfactant Plus, 3 µm Analytical, 3.0 × 150 mm
Mobile Phase:	75 mM KH_2PO_4 (pH 3.0, with H_3PO_4)/acetonitrile, (1:1, v/v)
Flow Rate:	0.425 mL/min
Inj. Volume:	2 µL
Temperature:	30 °C
Detection:	UV absorbance at 215 nm
Samples:	a) blank (water) b) benzalkonium chloride standard (100 µg/mL) c) cosmetic sample d) cosmetic sample spiked with a benzalkonium chloride standard (200 µg/mL)
Peak:	1. C_{12}-BKC

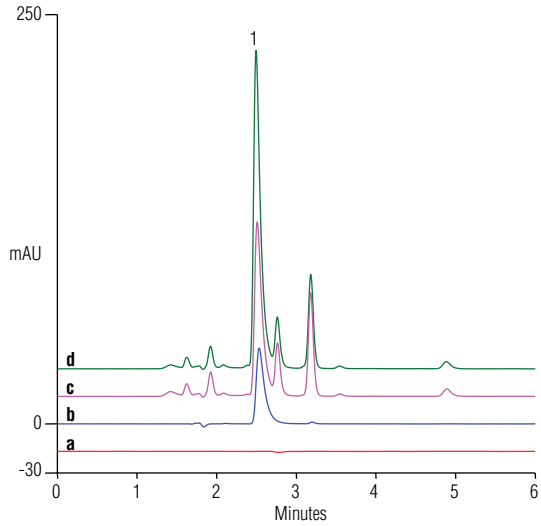

Figure 4. Chromatograms of the benzalkonium chloride standard and cosmetic sample.

Conclusion

This work describes an HPLC method with UV detection for the rapid and sensitive determination of benzalkonium chloride in cosmetic samples using an Acclaim Surfactant Plus column with a phosphate buffer (pH 3)/CH_3CN mobile phase. The separation requires only 6 min and the MDL of benzalkonium chloride is 1 µg/mL.

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