Analysis of Residual Products in Benzalkonium Chloride by High-Performance Liquid Chromatography

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Abstract

A method for the separation of the homologous series of benzalkonium chloride (BAK), benzyl chloride, and possible byproducts is presented. Under recommended conditions, 2 mobile phases (isocratic and gradient elutions, depending on the impurities present) are proposed. A study of the kinetics of the removal of benzyl chloride under different conditions (catalyst, excess of amine, different solvents) allows the synthesis of BAK without residual benzyl chloride.

Introduction

Benzalkonium chloride (BAK), an antiseptic detergent, is a mixture of alkylbenzyldimethylammonium chlorides [C_6H_5 CH_2 $N(CH_3)_2$ R Cl] in which the alkyl groups (R) have a chain length from C_8 to C_{18} with C_{12} and C_{14} predominating. Commercially, it appears as a yellowish powder containing at least 95% BAK or in aqueous or alcoholic solutions at concentrations of 50% or more.

BAK is synthesized from benzyl chloride and alkyldimethylamine (Figure 1). Benzyl chloride is considered a toxic product (inh-hmm TCL_0 = 16 ppm/1M, oral-rat LD_{50} = 1231 mg/kg, orl-rat LC_{50} = 150 ppm/2h)(1). Thus, it would be advisable to remove residual benzyl chloride during the synthesis of BAK or to transform it into less toxic derivatives.

The characterization of BAK by high-performance liquid chromatography (HPLC) has focused extensively on the separation and quantitation of BAK homologues, and a linear correlation between the logarithmic capacity factor and the number of carbons in the alkyl chains has been established (2–4).

The more extensive bibliographical references are those that refer to the analysis of pharmaceutical formulations (5–9). In these cases, the major problems were interference with the active ingredients of high extinction coefficients or the low solubility of excipients in the eluents of the chromatographic separation. In order to resolve these problems and quantitate BAK at low concentrations (50–100 ppm), the authors used preconcentration techniques, derivatization, and detection at short wavelengths.

Several papers approach the dilution of BAK in the samples with specific techniques of preconcentration: salting-out without applying any particular matrix (10), an extraction column for determination in plasma (11), and solid-phase extraction and postcolumn derivatization of effluent samples from European hospitals (12). Also, BAK in cosmetic products has been determined by the direct analysis of heterophasic systems (13).

Another factor described in the analysis of BAK using HPLC involved the development of methods for the separation of surfactants (BAK and the other cationic surfactants). These methods refer to the separation of individual surfactants from each other or from respective homologues (14). In the environmental field, separations into the complex matrix (sewage water and activated sludge) have been investigated (15).

With respect to the analysis of residual products, no reports have been found in the literature in which HPLC methods are used for benzyl chloride determination in BAK or in any other matrix. Only a single report on the retention properties of chlorinated polycyclic compounds includes benzyl chloride (16). The other residual products (benzyl alcohol and benzaldehyde) have been determined in a pharmaceutical cream by HPLC (17).

In this study, a method was developed for the analysis of BAK that included the separation of the homologous chains, as well as

Figure 1. Formation reaction of BAK.

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possible residual products such as benzyl chloride. Two separation conditions are proposed: an isocratic elution and a gradient elution. The former is similar to that proposed by the U.S. *Pharmacopeia* (18) for homologous series of BAK but adapted for the analysis of residual products. The latter allows the separation in cases in which other unidentified impurities are present.

The procedure was applied to the determination of benzyl chloride and its derivatives (benzyl alcohol and benzaldehyde) during BAK synthesis. In addition, different reaction conditions that allow for the removal of benzyl chloride in a short period of time are discussed.

Experimental

Reagents and apparatus

A Hitachi-Merck (Tokyo, Japan) liquid chromatograph equipped with an L-500 gradient control, 655A-12 pump, 655A variable wavelength detector, and D-2000 chromato-integrator was used. The cyanopropyldimethylsilyl column (LiChrosorb, 250 × 4.0-mm i.d., 5-µm particle size) was purchased from Merck.

The solvents and reagents used (including acetonitrile, benzyl chloride, and benzaldehyde) were supplied by Merck (Darmstadt, Germany). Water was obtained from Scharlau (Barcelona, Spain), and benzyl alcohol was from Panreac (Barcelona, Spain). An eluent was prepared with 0.1M CH_3COONa (BDH, Poole, England); glacial acetic acid was used to adjust the pH to 5 and was filtered through 0.2-µm filters (MSI Separations, Westboro, MA). The reagents used for the synthesis were all purchased from commercial sources.

Preparation of standards and BAK samples

Stock solutions of benzyl chloride (3 mg/mL), benzaldehyde (2 mg/mL), and benzyl alcohol (2 mg/mL) were prepared in acetonitrile. Working solutions of these were made up by suitable dilution using a mobile phase. The BAK solution was diluted into the mobile phase to a concentration of 10 mg/mL on anhydrous basis. The first sample for kinetic analysis was diluted to 1 mg/mL.

Preparation of BAK

A mixture of alkyldimethylamine (110 g, 0.49 mol), benzyl chloride (62 g, 0.49 mol), and 172 mL of water or isopropyl alcohol was heated to 50°C. When this temperature was reached, heating was stopped, and the temperature increased to 80°C because of the exothermic reaction. The mixture became transparent, and the first sample was taken. The reaction was performed at 80°C, and several samplings were taken until the benzyl chloride was consumed. It was verified (different injections performed in several days) that the reaction had stopped when the samples were diluted to the concentration of analysis.

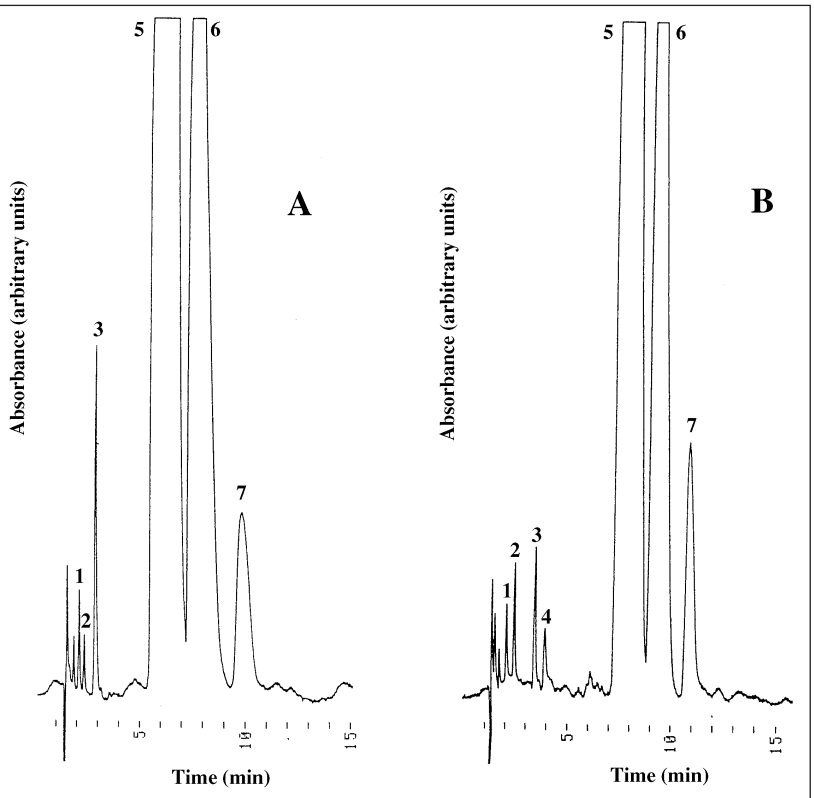

Figure 2. Chromatogram of BAK during the synthesis: isocratic elution in CH_3CN–0.1M CH_3COONa (55:45) mobile phase at pH 5.0 (A) and gradient elution (B). Conditions: column, LiChrosorb CN (250 × 4.0-mm i.d., 5-µm particle size); flow rate, 1.5 mL/min; detection, 220nm. Peaks: 1, benzyl alcohol; 2, benzaldehyde; 3, benzyl chloride; 4, unidentified impurity; 5, C_{12} homologue; 6, C_{14} homologue; 7, C_{16} homologue.

Chromatographic conditions

Chromatographic separation was carried out with the cyano column using an isocratic and gradient elution at a flow rate of 1.5 mL/min, injection volume of 20 µL, and detection at 220 nm.

The mobile phase for the isocratic elution consisted of a mixture of 45% acetate buffer (pH 5) and 55% acetonitrile (condition A). The gradient was as follows: initially isocratic elution with acetate buffer–acetonitrile (55:45, v/v) until 2.5 min, followed by a linear elution from 55:45 to 40:60 until 3 min, and another isocratic elution acetate buffer–acetonitrile (40:60, v/v) until 12 min. Finally, a linear gradient was required to reach the initial conditions from 12 to 15 min. Several minutes were required for mobile phase stabilization (condition B).

Results and Discussion

Chromatographic separation: optimization and identification of byproducts

Initially, the chromatographic separation was intended to separate benzyl chloride from the homologues of BAK in a short period of time. This goal is achieved under isocratic conditions (condition A described in the Experimental section) in an analysis time of less than 15 min. Byproducts such as benzyl alcohol and benzaldehyde were also separated (Figure 2). The purity of

peaks corresponding to byproducts was verified with a diode array detector (Waters, Milford, MA).

An unidentified impurity peak that eluted with a retention time near benzyl chloride appeared in several BAK samples. This peak overlapped with the benzyl chloride peak when isocratic conditions were used, making quantitation difficult. A study of the parameters influencing chromatographic separation (pH, buffer concentration) verified that the ratio of buffer to acetonitrile is decisive for a good separation of benzyl chloride. It was necessary to decrease acetonitrile from 55 to 45% (referring to condition A) to achieve good results. Nevertheless, this decrease caused an increase in the retention times of the BAK homologues and a decrease in resolution. This is caused by the increased hydrophobic interactions between BAK and the cyanopropyl group of stationary phase (8).

In order to improve the separation, a gradient with 2 isocratic steps was performed: the first, consisting of 45% acetonitrile, separated benzyl chloride (and its derivatives) from the impurity; the second, consisting of 60% acetonitrile, eluted the BAK homologues ($C_{12–16}$) in less than 15 min (Figure 2B).

Linearity, precision, and detection limit

Peak heights were linear in relation to benzyl chloride, benzyl alcohol, and benzaldehyde concentrations over the ranges of 0.4–70, 0.8–60, and 0.8–60 ppm (referring to the injected solution with 20-µL injections), respectively. The correlation coefficient was greater than 0.9990 (each regression point was the average of at least 2 injections for each standard).

The precision of benzyl chloride analysis was evaluated at 2 concentrations, and that of the byproduct at 1. The relative standard deviation (RSD) of benzyl chloride for 10 repetitions was 1.6% at 1.1 ppm and 0.23% at 28 ppm. The RSD of benzaldehyde (10 repetitions) was 1.8% at 9.8 ppm and benzyl alcohol, 1.7% at 3.8 ppm.

A detection limit (19) of 4 ppm (0.08 ppm referring to the injected solution) for benzyl chloride was obtained.

Application of the method in BAK synthesis

Reaction kinetics of benzyl chloride, benzyl alcohol, and benzaldehyde were followed under different conditions. The effects of inorganic catalyst, the excess of amine, alkyl chain length, and solvent in removing residual benzyl chloride were tested.

BAK at a concentration of 50% in water with a percentage of inorganic salt from 0 to 0.15% was synthesized. The molar ratio of tertiary amine to benzyl chloride was 1:1. A plot of residual benzyl chloride (for the different relationships) versus time is shown in Figure 3. The reaction without catalyst yields 800 ppm of benzyl chloride after 5 h, and the reaction with 0.1% catalyst yields 50 ppm. Benzyl chloride was not detected with 0.15% after 4 h. The action of the catalyst would only be possible in aqueous reaction media because of its insolubility in an alcoholic medium.

The evolution of the byproducts was also studied as shown in Figure 3B. Benzaldehyde formation occurs similarly for the 3 syntheses. A monoexponential kinetic equation (first order) decay with residual was confirmed with the experimental values. Benzyl alcohol behaves differently during the reaction with and without catalyst. With the former, the experimental values confirmed a monoexponential kinetic equation. With the latter, it was not possible to adjust to any equation, and final values

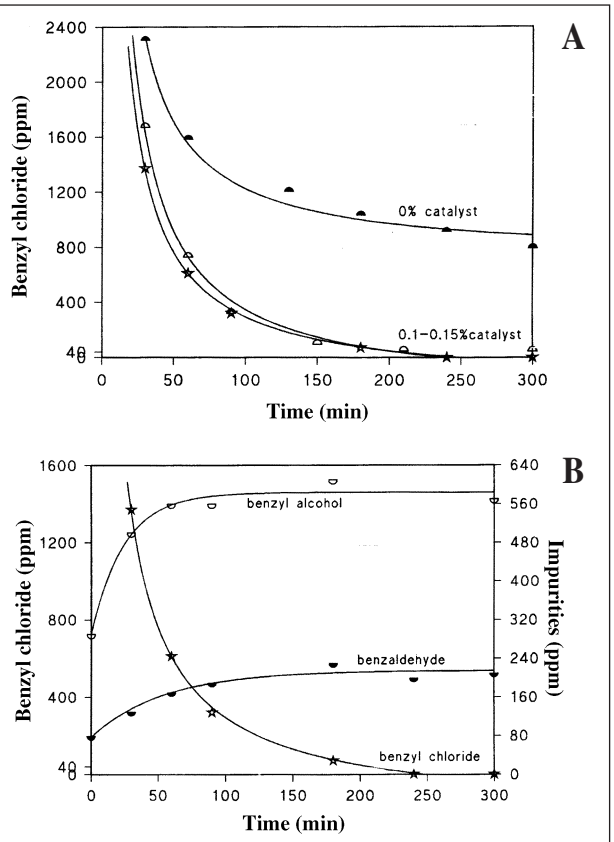

Figure. 3 Influence of catalyst on the removal of benzyl chloride during synthesis of BAK at 50% in water: kinetics of benzyl chloride with and without catalyst (A) and kinetics of benzyl alcohol, benzaldehyde, and benzyl chloride with 0.15% catalyst (B).

achieved 0.14% of benzyl alcohol.

The effect of excess amine on the removal of benzyl chloride at different molar ratios of benzyl chloride to amine (1:1.01, 1:1.02, 1:1.03) is shown in Figure 4A. Under those conditions, water was used as the solvent and the BAK concentration was 50%. One hour was necessary to remove benzyl chloride at molar ratio of 1:1.03, 1.5 h was necessary to remove benzyl chloride at molar ratio of 1:1.02 and 5 h was necessary to remove benzyl chloride at molar ratio of 1:1.01. This effect on the reaction velocity was stronger than that of the catalyst, but a molar ratio of 1:1.03 caused a large increase in alkalinity of BAK. Therefore, the molar ratio of 1:1.02 was chosen.

The effect of the alkyl chain length of the amine on the reaction was also studied. BAK at 50% in water at a molar ratio of 1:1.02 was synthesized with lauryldimethylamine ($C_{12}H_{25}$ > 95%) and myristyldimethylamine (C_{14} H_{29} > 95%). A small increase in the velocity of the reaction was observed with the decrease in chain length (Figure 4B). In polar solvents and water, C_{12} and C_{14} compounds form molecular aggregates at a work concentration (20) that depends on the hydrocarbon chain length. Therefore, the concentration of C_{12} compound that remains in the bulk polar solvent (not forming aggregates) is higher than that of the C_{14} compound. Consequently, the reaction is faster with the C_{12} compound than with the C_{14} compound. The alkyldimethylamine used in this work has a mixture of homologues: $C_{12}H_{25}$ (70%), $C_{14}H_{29}$ (27%), and $C_{16}H_{33}$ (3%).

In Figure 5, BAK at a concentration of 50% at a molar ratio of 1:1.02 in water and isopropyl alcohol (IPA) were compared. The results demonstrate that the reaction velocity in IPA was less than in water and also that the effect of excess amine was less remarkable (90 ppm benzyl chloride in BAK in IPA at 5 h are quantitated). When synthesis had finished, BAK, which contains residual benzyl chloride, was stored at room temperature. Nevertheless, the follow-up analysis of BAK stored at room temperature indicated that reaction continued. At 5 days, benzyl chloride was not detected.

Commercial formulations of BAK at 80% contain IPA. BAK at 80% in IPA with an excess of amine (1:1.02) was synthesized. In order to improve the removal of benzyl chloride, a small amount of water (5%) was added in synthesis to 80% (Figure 5B), but 4–5 days at room temperature was required. Benzyl alcohol was never detected in the synthesis of BAK in IPA. Benzaldehyde was detected, but it remained at constant values during the follow-up of the kinetics.

The values of benzyl chloride for all syntheses may be adjusted to a second-order kinetic equation with good correlation coefficients. In this equation, a residual negative term referring to concentration is present. This may be due to the impossibility of accurately determining the initial times (details in the Experimental section).

Conclusion

The proposed chromatographic methods allowed the simultaneous separation of homologues of benzalkonium chloride, benzyl chloride, benzyl alcohol, and benzaldehyde using isocratic and gradient elutions. Good linearity and precision was obtained for all residual products, and an acceptable limit of detection was obtained for benzyl chloride.

According to the kinetics study, residual benzyl chloride may be removed during BAK synthesis or storage (for several days). An inorganic catalyst or an excess of alkyldimethylamine produced a decrease of removal time, but the excess of amine had the greatest influence on the removal. The reaction of BAK in water is faster than in isopropyl alcohol.

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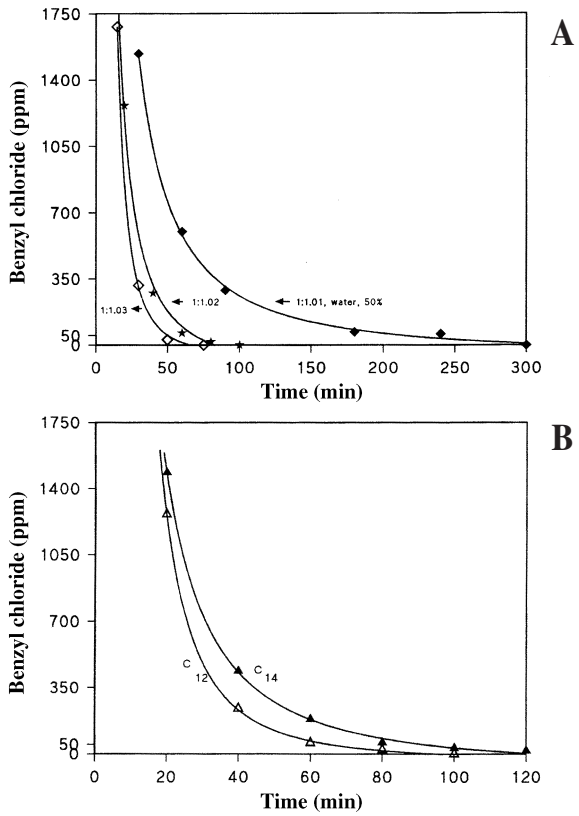

Figure 4. Influence of excess alkyldimethylamine on the removal of benzyl chloride during synthesis of BAK at 50% in water: kinetics of benzyl chloride at molar ratios of benzyl chloride to alkylamine of 1:1.01, 1:1.02, and 1:1.03 (A) and kinetics of benzyl chloride at a molar ratio of 1:1.02 with lauryldimethylamine (C_{12}) and myristyldimethylamine (C_{14}) (B).

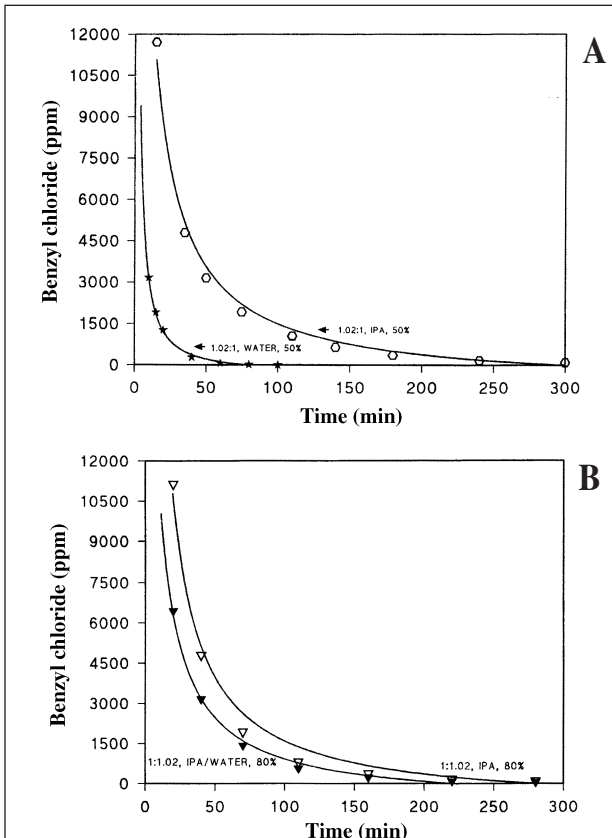

Figure 5. Influence of solvent on the removal of benzyl chloride during synthesis of BAK at 50% at a molar ratio of 1:1.02: kinetics of benzyl chloride in water and isopropyl alcohol (IPA) (A), Kinetics of benzyl chloride during the synthesis of BAK at 80% in IPA and water–IPA (B).

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