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Analysis of benzalkonium chloride and its homologs: HPLC versus HPCE[1]

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Abstract

Benzalkonium chloride (BAK) is a mixture of alkylbenzyldimethylammonium chloride homologs with n-$C_{12}H_{25}$, n-$C_{14}H_{29}$, and n-$C_{16}H_{33}$ comprising a major portion of the alkyl groups present. An analytical method for BAK must differentiate and quantitate the homologs in the BAK mixture. Reversed-phase high performance liquid chromatography (HPLC) separates compounds based on their affinity for a nonpolar column, which is a direct correlation to the compounds' polarity. High performance capillary electrophoresis (HPCE), however, separates compounds in an electric field according to their charge and size. The BAK homologs are suitable for separation by either of these methods because their polarity and sizes differ significantly. The HPLC method employed a mobile phase of 60% acetonitrile and 40% 0.1 M sodium acetate buffer pH 5 pumped at 1.0 ml min^{-1}, a 4.6 × 250 mm cyano column with 5 μm packing, and UV detection at 254 nm. The HPCE method utilized a run buffer of 30% acetonitrile and 70% 0.05 M sodium phosphate pH 3.06, a 50 μm × 20 cm open silica capillary, 7.5 kV electric field and UV detection at 214 nm. Both HPLC and HPCE demonstrated good linearity in the range of 0.025 to 0.8 mg ml^{-1} with r^2 values of approximately 0.99. The HPLC method produced good separation of the homolog peaks with a total analysis time of 25 min. HPCE run time was less than 5 min and demonstrated good separation of the three homologs. The HPLC method, however, was superior to HPCE in the areas of sensitivity and precision. The HPLC has been extensively used in the routine quantitation and qualitation of benzalkonium chloride concentrations in various products; however, long analysis times make this method inefficient. The HPCE method produced comparable results to the HPLC method but with much shorter analysis times. An HPCE analysis method, as presented here, may prove to be a much more useful and efficient method for the analysis of benzalkonium chloride and its homologs.

Keywords: Benzalkonium chloride; High performance liquid chromatography; High performance capillary electrophoresis

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1. Introduction

Benzalkonium chloride (BAK) is a bactericidal antimicrobial agent used as a preservative in over 65% of the ophthalmic products currently available on the market [1]. It is used to preserve otic, nasal and parenteral formulations as well. It possesses surfactant properties and is also used as a topical antiseptic and medical equipment disinfectant. BAK is an unusual compound in that it is composed of a mixture of straight chain homologs which possess different physical, chemical and microbiological properties. Therefore, the proportions of these homologs in the mixture determine its effectiveness as a preservative and disinfectant.

The BAK is a mixture of alkylbenzyldimethylammonium chlorides with the general formula $[C_6H_5CH_2N(CH_3)_2R]Cl$ where $R = n\text{-}C_8H_{17}$ to $n\text{-}C_{19}H_{39}$. The C_{12}, C_{14} and C_{16} chains comprise the major portion of the alkyl mixture. The homologs do not possess identical bactericidal activity; therefore, the USP/NF sets forth guidelines for the content of Benzalkonium Chloride NF as follows [2]:

> On the anhydrous basis, the content of the $n\text{-}C_{12}H_{25}$ homolog is not less than 40%, and the content of the $n\text{-}C_{14}H_{29}$ homolog is not less than 20%, of the total alkylbenzyldimethylammonium chloride content. The amounts of the $n\text{-}C_{12}H_{25}$ and $n\text{-}C_{14}H_{29}$ homolog components comprise together not less than 70% of the total alkylbenzyldimethylammonium chloride content.

In general, the C_{12} homolog is most effective against yeast and fungi, the C_{14} homolog against gram-positive bacteria and the C_{16} homolog against gram-negative bacteria [3].

The BAK is a difficult compound to analyze due to its multicomponent nature. The assay used must be both quantitative and qualitative to be able to identify and distinguish the different homolog components and the level of each in the mixture. The method should be sensitive and efficient enough to detect all of the homologs present in a reasonable amount of time. High performance liquid chromatography (HPLC) has been used extensively and successfully to separate and quantitate the benzalkonium chloride homologs [2,4–14]. The analysis times, however, typically range from 15 to 30 min. These lengthy analysis times can be quite troublesome to those doing routine QA/QC checks of ophthalmic products containing BAK where there is a large number of samples. High performance capillary electrophoresis (HPCE) is an analytical method which separates compounds on the basis of their charge and size. This method will produce adequate separation of the homologs because of their incremental sizes and because the quaternary ammonium molecules carry a positive charge, as shown by Althia et. al. in a study of the analysis of BAK and histamine acid phosphates mixture by using HPCE [15]. The purpose of this study was to compare the analysis of BAK using HPLC to analysis using HPCE.

2. Materials and methods

2.1. Solvents and chemicals

The BAK was supplied as a 50% aqueous solution from Spectrum (Gardena, CA). The C_{12}, C_{14}, and C_{16} homologs were purchased from Sigma (St. Louis, MO). The acetonitrile and sodium acetate were purchased from Fisher Scientific (Fair Lawn, NJ), and the sodium phosphate, monobasic was purchased from Mallinckrodt (Paris, KY). All other chemicals used in the analytical methods were HPLC grade. Water was purified by using a Milli-Q water system from Millipore (Bedford, MA).

The composition of the BAK sample from Spectrum Chemicals used for HPLC analysis was 64.64% C_{12}, 27.49% C_{14}, and 7.86% C_{16} homolog. In comparison, the BAK sample prepared in the laboratory from the individual homologs used for HPCE analysis consisted of 41.84% C_{12}, 32.65% C_{14}, and 25.51% C_{16} homolog. A sample concentration of 0.1 mg ml^{-1} was used for determination of precision, and the concentrations for determination of linearity were 0.025, 0.05, 0.1, 0.2, and 0.4 mg ml^{-1}, with the exception of the BAK sample used in the HPLC method. The

BAK concentration used for HPLC precision was 0.2 mg ml^{-1}, and the concentrations used for linearity were 0.05, 0.1, 0.2, 0.4, and 0.8 mg ml^{-1} due to the low concentration of the C_{16} homolog in the BAK mixture.

2.2. Equipment

The HPLC system consisted of a Waters M-6000A Solvent Delivery System, Micromeritics 728 Autosampler, Rheodyne 7010 Electrically Actuated Valve, Waters Model 441 Fixed Wavelength Absorbance Detector and Waters 745 Data Module. A Beckman P/ACE System 2100 was used for the HPCE analysis and Beckman System Gold software was used for data collection.

2.3. Experimental conditions

The column used for the HPLC method was a 250 × 4.6 mm i.d. stainless steel column filled with Bakerbond cyano 5 μm packing and was kept at room temperature. The mobile phase consisted of acetonitrile–sodium acetate (pH 5.0; 0.1 M) (60:40, v/v), and the pH of the buffer was adjusted by adding glacial acetic acid. Elution was performed at a flow rate of 1.0 ml min^{-1} and the injection volume was 20 μl. The absorbance was monitored at 254 nm.

The capillary used for the HPCE method was a Beckman P/ACE System/eCAP 50 μm i.d. × 26 cm open silica capillary with a 20 cm effective length maintained at 20°C. The run buffer was acetonitrile-sodium phosphate, monobasic (pH 3.06 adjusted using phosphoric acid; 0.05M) (30:70, v/v), and the applied voltage was 7.5 kV. All samples were injected into the silica capillary by 5 s of pressure at 0.5 ψ, a volume of approximately 12.5 nl, and detected by absorbance at 214 nm.

3. Results and discussion

Both the HPLC and HPCE methods used for analysis of benzalkonium chloride solutions produced adequate resolution of the three major homolog peaks corresponding to the C_{12}, C_{14}, and C_{16} homologs (Figs. 1 and 2). The HPLC method produced an analysis time of 25 min for all three homologs, whereas the HPCE method produced an analysis time of only 5 min. The sensitivities of the HPLC and HPCE methods were approximately 2 and 5 μg ml^{-1}, respectively. The HPCE method was less sensitive due to a shorter path length in the detector and a smaller injection volume (12.5 nl for HPCE vs. 20 μl for HPLC).

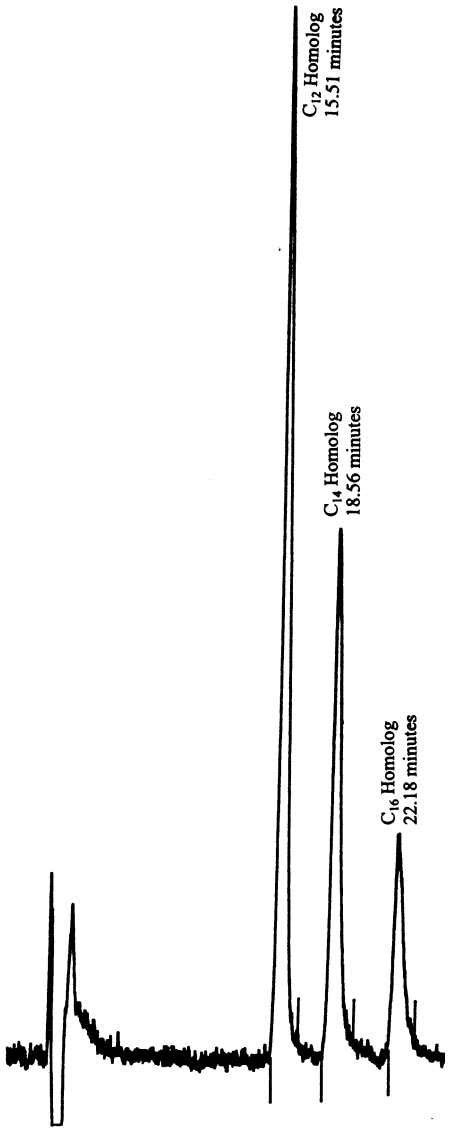

Fig. 1. Chromatogram of benzalkonium chloride 0.2 mg ml^{-1}.

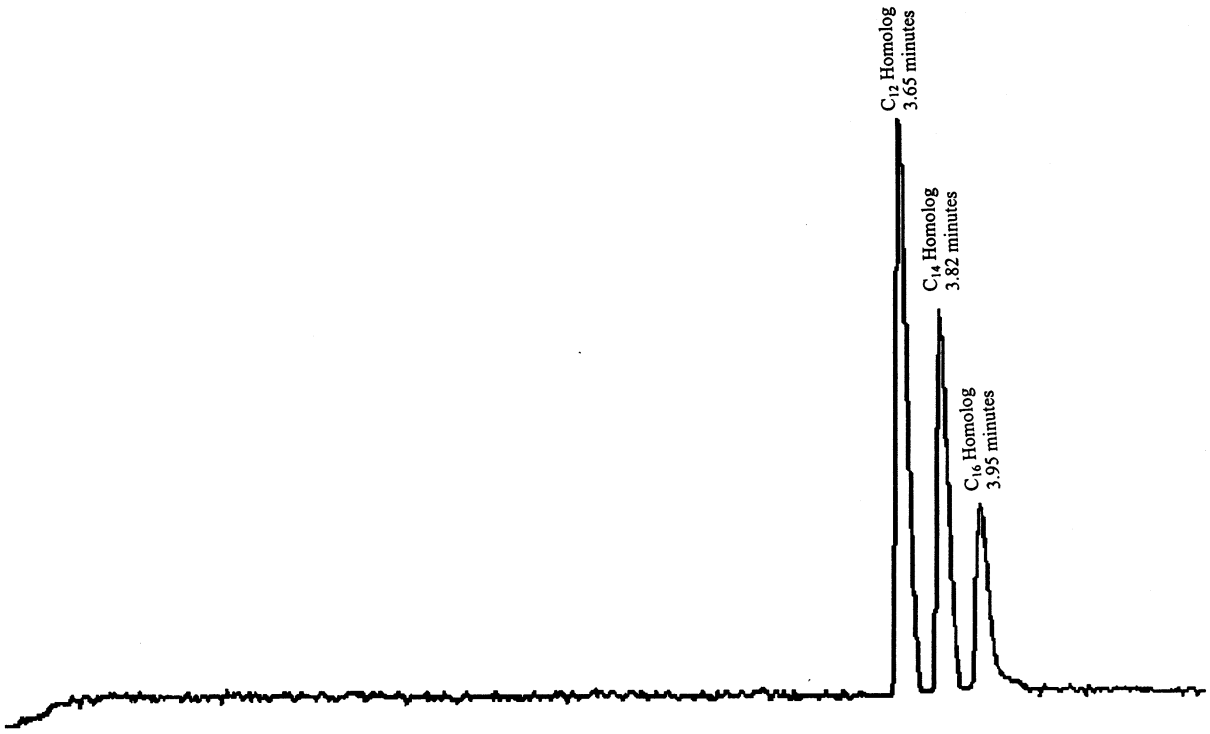

Fig. 2. Electropherogram of benzalkonium chloride 0.1 mg ml^{-1}.

The sensitivity for both methods is adequate, however, because the minimum concentration of BAK in ophthalmic formulations is 40 μg ml^{-1} (0.004%).

For both HPLC and HPCE systems, the precision was determined by the intraday variation of five injections of each homolog and BAK mixture and the interday variation for 5 days (Table 1). The precision for HPLC was higher than for HPCE in most instances. This was probably due to problems encountered with capillary degeneration, which was somewhat resolved by reconditioning the column after every five sample runs. The day 5 HPCE results for the C_{16} homolog and BAK samples are not reported because the capillary became unusable and reconditioning efforts failed; therefore, the data could not be obtained. This problem may have been due to adsorption of the C_{16} homolog to the silica capillary. The main causes of adsorption to the silica capillary are ionic interactions between cationic solutes and the negatively charged wall and hydrophobic interactions [16]. Because the C_{16} homolog is more hydrophobic than the C_{12} or C_{14} homologs, it exhibited a higher degree of hydrophobic interactions. Furthermore, all benzalkonium chloride homologs are large cations, so all demonstrated ionic interactions with the negatively charged wall to various degrees. Adsorption to the silica capillary walls can be minimized by increasing buffer concentration which will reduce solute interactions, by using buffers at the extremes of pH and also by coating the capillary wall [16]. The 0.05 M sodium phosphate buffer system used in this study was at low pH (3.06), but a higher buffer concentration exceeded the maximum suggested operating current.

The results for the standard curves obtained by the HPLC and HPCE methods are given below:

HPLC

C_{12} Homolog: peak area = 5132 + 314897 concentration ($r^2 = 0.9988$)

C_{14} Homolog: peak area = 5230 + 330413 concentration ($r^2 = 0.9991$)

Table 1
Intraday and interday variation for benzalkonium chloride and its homologs using HPLC and HPCE

	Day 1	Day 2	Day 3	Day 4	Day 5	Interday
	HPLC					
C_{12}	2.38[a]	2.26	2.78	1.74	3.85	0.50
C_{14}	3.31	2.77	5.91	2.92	3.86	1.16
C_{16}	4.87	3.73	2.99	2.53	3.34	1.62
BAK	2.59	1.97	0.99	3.30	1.13	0.93
	HPCE					
C_{12}	11.06	12.46	3.37	8.10	8.30	3.57
C_{14}	0.96	1.82	4.02	5.19	9.37	19.86
C_{16}	6.63	6.47	6.55	9.20	—	9.01
BAK	4.67	2.30	3.40	5.90	—	21.01

[a] RSD%, $n = 5$.

C_{16} Homolog: peak area = 6768 + 310245 concentration ($r^2 = 0.9942$)
BAK mixture: peak area = 2874 + 303956 concentration ($r^2 = 1.0000$)

HPCE
C_{12} Homolog: peak area = $-6.371 \times 10^{-3} + 6.205 \times 10^{-3}$ concentration ($r^2 = 0.9995$)
C_{14} Homolog: peak area = $-1.795 \times 10^{-3} + 5.062 \times 10^{-3}$ concentration ($r^2 = 0.9981$)
C_{16} Homolog: peak area = $-2.654 \times 10^{-2} + 5.400 \times 10^{-3}$ concentration ($r^2 = 0.9960$)
BAK mixture: peak area = $4.537 \times 10^{-3} + 5.125 \times 10^{-3}$ concentration ($r^2 = 0.9997$)

The linearity for both the HPLC and HPCE methods is comparable; therefore, both methods are useful for the accurate quantitation of benzalkonium chloride as well as the individual homologs in the mixture.

4. Conclusion

A high performance capillary electrophoresis assay for qualitation and quantitation of benzalkonium chloride and its homologs has been investigated. The primary advantage of the HPCE method, when compared to HPLC, is a much shorter analysis time. The linearity and sensitivity of both methods is comparable; however, the precision for the HPLC method was better than for HPCE. This problem may have been due in part to sorption of the C_{16} homolog to the capillary wall.

In summary, HPCE has the potential to be a more efficient and useful method for BAK analysis than the established HPLC methods.

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