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# Determination of benzalkonium chloride in wet wipes by using a validated capillary electrophoresis method

GÜNEŞ YILDIRIM and EBRU TÜRKÖZ ACAR, *Department of Analytical Chemistry, Faculty of Pharmacy, Yeditepe University, Istanbul, Turkey.*

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**Synopsis**

Benzalkonium chloride (BAC), which is a mixture of $C_8$ and $C_{18}$ alkylbenzyldimethylammonium chlorides, has an important biocide character and is used in many cosmetics, especially wet wipes, as a preservative and/or antibacterial agent. The concentration range of BAC is 0.005–0.5 % and it is an irritant substance when used at high concentrations. Thus, the concentration of BAC should be carefully monitored in commercial products intended for skin use. In this study, a capillary electrophoresis (CE) analysis method for BAC quantification was developed. The quantitative analysis was carried out by the external standard method. The electrophoretic separation was performed by using 75 mM (pH 6.0) phosphate buffer solution containing 30% acetonitrile as the electrolyte. The separation voltage was 10 kV and the temperature was held at 18°C. Samples were introduced into the capillary column hydrodynamically using 50.0-mbar pressure over a 3-s period. The developed method was validated and applied on samples prepared by wringing out antibacterial wet wipes containing BAC without any further extraction. The linearity of the method was controlled by applying the Mandel test. The limit of detection (LOD) values for the developed method were 0.313 and 0.309 µg/ml and the limit of quantification (LOQ) values were 1.042 and 1.029 µg/ml for $C_{10}$ and $C_{12}$ derivatives, respectively.

## INTRODUCTION

The use of wipes is becoming very popular for the purpose of general house cleaning or make-up remover, as well as for personal hygiene both in adults and babies. Besides this growth, wet wipes are used more than once daily due to practical usage. Different materials are used to provide cleaning efficiency in the wetting solution of wipe tissues. These materials contact with skin especially when the products are used for cosmetic or personal hygiene/cleaning, and can lead to different skin disorders. According to the literature, there are some cases reported declaring usage of wet wipes caused allergic contact dermatitis (ACD) (1–4). Reason of ACD in these cases was from some preservatives used in wetting solution of wipe tissues. One of these preservatives is benzalkonium chloride (BAC).

BAC is a quaternary ammonium cationic surfactant that is widely used as a biocide and/or a preservative in cosmetics, skin disinfectants, or ophthalmic preparations (5–6) (Figure 1).

Address all correspondence to Ebru Türköz Acar at ebruturkozacar@gmail.com.

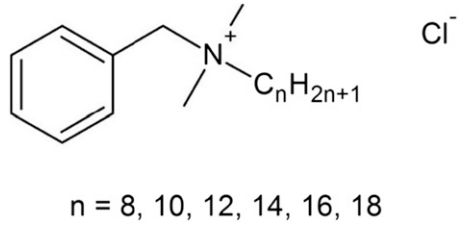


**Figure 1.** The molecular structure of BAC.

Nevertheless, BAC has also been demonstrated to be a significant skin irritant causing ACD. Indeed, Oiso *et al.* (7) reported an ACD case due to a shampoo containing BAC at a concentration of 0.1% (w/v). Also, results of studies carried out by Kanerva *et al.* (8) using patch testing showed an irritant response of BAC when used at concentrations of 0.1% (w/v). Since BAC concentrations in wet wipes vary from 0.05% to 0.5 % (w/v) (9), these products are susceptible to cause contact dermatitis and/or ACD on skin, especially on babies skin as wipes can be used several times a day for cleansing their bottoms. Therefore, the BAC concentration should be carefully monitored in wet wipes and should not exceed a concentration of $IC_{50}$ (ACD)/10 in baby products to provide a suitable safety factor.

BAC can be monitored via titrimetric methods (10–12), ultraviolet–visible (UV-Vis) spectroscopy (13,14), liquid/gas chromatography (15–21), or capillary electrophoresis (CE) methods (22–26) as reported in studies that investigate BAC concentrations in some pharmaceuticals and cosmetics. However, to the best of our knowledge, there is no data concerning the monitoring of BAC content in wet wipes. Another point at this stage, used detector in instrumental techniques is generally a mass spectrometer, which is very expensive and needs a qualified person. Thus, this study aims to develop a simple, accurate, and reproducible analytical method for BAC monitoring in antibacterial wet wipes that does not require any sample pretreatment. For this purpose, the CE technique was used, since it is very powerful for separating alkyl halides, even with a UV detector.

## MATERIALS AND METHODS

All chemicals used in experiments were of analytical or better grade. Standard material of BAC was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). Chemicals used for supporting electrolyte; acetonitrile (ACN), methanol, disodium hydrogen phosphate, and sodium dihydrogen phosphate were also obtained from Sigma-Aldrich Chemical Company.

Antibacterial wet wipe samples containing BAC, dry wipe tissues, and solution to wet wipe tissues were kindly supplied from Kardeşler Uçan Yağlar Ltd. Şti (Gebze- KOCAELİ, Turkey). Properties of these wipe tissues were 40 g/m$^2$ 20% viscose 80% polyester.

Ultrapure water (18.2 MW.cm at 25°C) was obtained by using Millipore Simplicity UV apparatus (Darmstad, Germany).

pH measurements were made with Oakton pH 2100 Series digital pH-meter (Eutech Instruments, Singapore).

Large-volume sample stacking CE technique was applied on an Agilent 3D Capillary Electrophoresis system (Germany) equipped with a diode array detector (DAD). Separation was achieved on an uncoated fused silica capillary, which was 75 µm I.D. and 47 cm in

length purchased from Agilent and the effective length of the column was 38.5 cm. The wavelength selected was 214 nm, due to the maximum absorption in spectrums obtained by the DAD detector of the CE system. The capillary was thermostatically controlled at 18°C during all experiments. The background electrolyte (BGE) was 75 mM (pH 6) phosphate buffer mixed with ACN at a ratio of 70:30.

A washing program was applied for new capillaries through the following steps: flushing with 1.0 M NaOH (20 min), flushing with 0.1 M NaOH (20 min), and flushing with water (20 min). After activation of the column, a short washing procedure was applied for each working day by flushing 1.0 M NaOH (20 min). Besides these washing steps, the capillary was conditioned by flushing with 1.0 M NaOH (1.5 min), 0.1 M NaOH (1.5 min), water (3 min), and BGE (5 min) between each measurement. After the conditioning step, samples were injected hydrodynamically by using 50-mbar pressure for 3 s at the cathodic end of the capillary and separation was carried on by applying +10 kV separation voltage. At the end of the measurement, the capillary was rinsed with methanol for 3 min to flush all residues from the capillary.

Stock solution of BAC was prepared by dissolving weighed amount of the standard substance in methanol at a concentration value of 5 mg/ml BAC. Standard solutions were obtained by diluting stock solution with methanol. According to the certificate of analysis, BAC standard consists of $C_{10}$ derivative (65.4%) and $C_{12}$ derivative (32.8%). Calculations to determine concentration of each species in standard solutions were made based on this information. Quality control solutions of BAC were prepared as standard solutions at different concentration values from calibration curve points. Low, medium, and high concentration values were selected as 0.0075, 0.06, and 0.2 mg/ml BAC. All solutions were stored in a refrigerator at 4°C.

Wet wipe solutions were used without any sample treatment for analysis. For this purpose, five wet wipe tissues were taken from the packet and were wrung out to obtain a solution containing BAC. This solution (100 µl) was diluted by the addition of 1400 µl of methanol. After the diluting step, this solution was injected to the capillary column for analysis.

### ADSORBED AMOUNT OF BAC ON WIPE TISSUES

Although these studies were performed, it was understood that wipe tissues adsorbed a part of BAC included in wetting solution. A set of experiment was designed to reveal the adsorbed amount of BAC onto the tissues as three repetitive, three different sets. For this purpose, dry tissue samples were wetted by using a solution containing 0.1% BAC in ratio of 2.80 (w/w). This application is used to moisture the dry wet wipes in commercial products in industrial area. After wetting of dry tissues, they were kept at room temperature for a week. Then five wet wipes were wringed and 100 µl of obtained solution is diluted by adding 1400 µl methanol. Fifteen different solutions were obtained by this way due to repetitions and all solutions were used directly for measurement. Adsorbed amount of BAC was calculated by comparing measured amount with total BAC amount. It was seen that dry wipes adsorbed a big amount of BAC species that measured $C_{10}$ value was 85.64% ± 9.68% of added amount and measured $C_{12}$ value was 67.06% ± 9.68% of added amount. The percentage of the adsorbed amount of BAC was used to analyze BAC in the solution of wet wipes.

## RESULTS AND DISCUSSION

### OPTIMIZATION OF CE CONDITIONS

Different buffer solutions such as acetate and phosphate buffers were tried to monitor BAC. According to the certificate of analysis of BAC standard, it contains two types of BAC with $C_{10}$ and $C_{12}$ derivatives. Due to this situation, two peaks were observed during analysis of standard material and these peaks were investigated simultaneously. When the buffer solution was selected, obtained peaks, shape of peaks, and also peak areas were compared. Phosphate buffer solution was selected as supporting electrolyte after comparing the obtained results.

A set of experiments was carried out to optimize electrolyte concentration using 25, 50, 75, 100, 200, and 300 mM phosphate buffer as supporting electrolyte. All electrophoregrams, obtained peaks, peak shapes, and peak area values were compared. There was no peak at a concentration value of 300 mM. Hence, obtained electrophoregrams were investigated for other concentration values. Since regular peak shapes were observed, only peak area values were used to compare electrophoregrams versus supporting electrolyte concentration. Peak area values increased with increasing buffer concentration up to 75 mM. However, area values decreased at higher buffer concentrations. For analytical purposes, 75 mM concentration value was selected as phosphate buffer concentration value due to the highest peak area value.

pH is very important parameter for CE. Because the pH value of supporting electrolyte solution changes the capillary inner wall, it provides power of the separation system: electroosmotic flow. Effect of pH value on determination of BAC was investigated between pH 5 and 8 values. Peaks of $C_{10}$ and $C_{12}$ derivatives closed up with increasing pH value. It means that pH value of phosphate buffer solution was effective on resolution of peaks. Beside the peak shape, peak area values were compared and it was seen that the highest peak area value were obtained at pH 6 buffer solution. pH 6 value was selected as supporting electrolyte pH value.

Temperature affects the mobilities of the substances and also analysis. Different temperature values were investigated by comparing obtained electrophoregrams between 18° and 28°C. It was seen that peak area values decreased when temperature increased to 24°C. But, peak areas increased with increasing temperature after this value. The mean higher temperature values could be selected, but in this situation, Joule effect affecting separation quality cannot be eliminated. As expected, repeated experiments showed that there was a repeatability problem at higher temperature values. Thus, low temperature values were more appropriate. Due to all these reasons, 18°C was selected as the working temperature.

Starting experiments showed that usage of only buffer solution did not achieve the separation of BAC species. Consequently, an organic additive was necessary to perform analysis. First of all, methanol was tried as organic additive, but remarkable results were not obtained, then another solvent ACN was tried. Due to separation of both BAC species, different ACN percentages were investigated between 10% and 60% and recorded electrophoregrams were evaluated. The separation of the peaks was not satisfactory at low ACN percentages of up to 30% due to shapes of the peaks. Peak shapes and peak resolutions were better at a ACN percentage of 30%. But the resolution value deteriorated with increasing ACN percentage. For these reasons, ACN percentage of 30% was selected to determine BAC.

Applied voltage is separation power of CE beside other parameters. For this purpose, different separation potentials between 5 and 20 kV were investigated to analyze BAC. When the obtained results were evaluated, it was seen that peak areas decreased with increasing separation potential. But at the same time, peak shapes changed and there was no separation at high potential levels. When the potential decreased, although relatively good peak area values were obtained, peaks were not symmetrical and well shaped. It was necessary that an optimum separation voltage should be selected to see the regular and symmetrical shape of peaks and obtain higher peak area values. According to the results, 10 kV separation potential was selected to analyze BAC.

At the end of the preliminary studies, optimum electrophoretic conditions were determined as follows—the BGE: 75 mM (pH 6) phosphate buffer mixed with ACN at a ratio of 70:30; temperature: 18°C; injection condition: 50 mbar pressure for 3 s; separation potential: 10 kV. A typical electrophoregram of BAC at these optimized conditions is presented in Figure 2. We surmise that the first peak belongs to the $C_{10}$ derivative of BAC and the second one belongs to the $C_{12}$ derivative of BAC because we assume that, of the two molecules only differing by two $CH_2$ groups, the molecule with the larger molecular mass has the lower electrophoretic mobility.

### METHOD VALIDATION

Validation of the method was made according to USP (United States Pharmacopeia) guidelines for this study.

### SYSTEM SUITABILITY

System suitability test was applied for the developed method. According to the literature, system suitability test should contain some parameters like capacity factor, resolution, etc. For this purpose, a standard solution of BAC at a medium concentration was injected to the CE system for six times. Results of the system suitability test for this study is

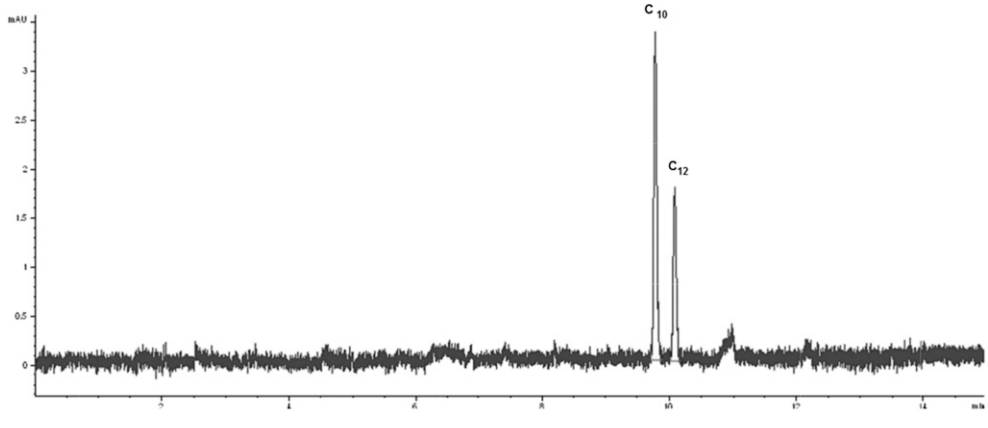


**Figure 2.** Electrophoregram of standard BAC solution in 75 mM (pH 6) phosphate buffer mixed with ACN at a ratio of 70:30 at 18°C; injection condition = 50 mbar pressure for 3 s; separation potential = 10 kV. Detection by absorbance at 214 nm.

**Table I**
System Suitability Test Results (n = 6)

| Parameter | $C_{10}$ derivative | $C_{12}$ derivative |
|---|---|---|
| Capacity factor ($k'$) | 2.700 | 2.837 |
| Resolution ($R$) | 1.605 | 1.605 |
| Number of theoretical plates ($N$) | 34,803.54 | 37,240.78 |
| Tailing factor ($T$) | 1.100 | 1.050 |
| Relative standard deviation (RSD) | 0.56 | 0.46 |

presented in Table I. According to these results, the system of the developed method was appropriate to analyze BAC species.

### SELECTIVITY

Selectivity of the developed method was investigated by comparing standard solution of BAC, wetting solution obtained by wrinkling of the wet wipe samples, and wetting solution without BAC. All electrophoregrams showed that BAC peaks were observed clearly in all electrophoregrams and there was no interference.

### LINEARITY AND CALIBRATION

When the preexperiments were carried on, a trend was observed that peak areas of both peaks were increased linearly with concentration. When the calibration curves were constructed between 0.003 and 0.25 mg/ml of BAC, it was seen that the curve was linear according to the correlation coefficients (0.999 and 0.989 for $C_{10}$ and $C_{12}$ derivatives, respectively). However, linearity of the method developed should be controlled and for this purpose, a Mandel test was used. Mandel test evaluates the change of the data sets by comparing constructed linear graph with polynomial graph statistically and at the end of the calculations, $F$ values are compared with theoretical $F$ values. If the calculated $F$ value is lower than theoretical $F$ value, the change of signal versus concentration is linear. For studied data set, theoretical $F$ value was 5.59 at 95% confidence interval. Calculated

**Table II**
Calibration Curve Parameters for the CE Method Developed to Determine BAC

| Parameter | $C_{10}$ derivative | $C_{12}$ derivative |
|---|---|---|
| Dynamic range (μg/ml) | 1.96–165.50 | 0.98–82.00 |
| Slope | 1.216 | 1.231 |
| Intercept | 2.779 | 1.986 |
| $F_{theorethical}$ (95% CI) | 5.59 | 5.59 |
| $F_{calculated}$ | -0.98 | 0.01 |
| LOD (μg/ml) | 0.313 | 0.309 |
| LOQ (μg/ml) | 1.042 | 1.029 |
| $R^2$ | 0.999 | 0.989 |

CI: confidence interval.

*F* values were -0.981 and 0.011 for $C_{10}$ and $C_{12}$ derivatives, respectively, by using Mandel test. This situation showed statistically that peak area values of BAC derivatives increased linearly with increasing concentration.

In light of this information, calibration curve parameters were evaluated. Resulted parameters are presented in Table II.

### ACCURACY AND PRECISION

Accuracy and precision of the developed method were evaluated at means by recovery and relative standard deviation, respectively. For this purpose, quality control solutions of BAC were investigated. Recovery studies were made by using wetting solution without BAC. Known amount of standard BAC solutions were added to this solution at three different concentration levels and analyzed. Recovery values and relative standard deviations for intraday and interday studies are shown in Table III.

According to Table III, there were some differences between recovery values. The reasons may be due to little deviations from calibration curve at high and low concentration values. Although these concentration values seem in the linear range according to the Mandel test, in view of recovery studies there were some differences. But this situation did not affect the wet wipe analysis because analysis of the samples was applied at medium concentration value.

To evaluate the precision of the developed method, standard solutions of BAC at three different levels were investigated. Retention time and peak area values were compared for both intraday and interday precision study. All results met the validation conditions.

### ROBUSTNESS AND RUGGEDNESS OF THE METHOD

Robustness and ruggedness of the method were investigated. For this purpose, some parameters of the method were changed deliberately as declared in USP between ±10% rate. These parameters were percent of ACN in supporting electrolyte, pH value of supporting electrolyte, and wavelength of the detector. Measurements were done in triplicate on different days. All results obtained are presented in Table IV.

**Table III**
Calculated Recovery and RSD Values for Known Concentration of BAC at Different Concentrations

| | Intraday | | | | Interday | | | |
|---|---|---|---|---|---|---|---|---|
| | $C_{10}$ derivative | | $C_{12}$ derivative | | $C_{10}$ derivative | | $C_{12}$ derivative | |
| Concentration Level | *R* | RSD | *R* | RSD | *R* | RSD | *R* | RSD |
| Low | 109.34 | 3.15 | 112.25 | 4.34 | 106.26 | 3.05 | 108.34 | 4.26 |
| Medium | 98.81 | 0.52 | 98.67 | 0.74 | 98.95 | 1.03 | 98.32 | 1.01 |
| High | 105.91 | 1.20 | 107.89 | 4.44 | 103.05 | 2.77 | 104.66 | 3.08 |

*R*: recovery %; RSD: relative standard deviation of the results.
Low, medium, and high concentration values were selected as 0.0075, 0.06, and 0.2 mg/ml BAC.

**Table IV**
RSD Values of Recovery Studies of the Robustness and Ruggedness Test for the Developed Method

| | ACN (%) | | pH | | Wavelength | |
|---|---|---|---|---|---|---|
| Concentration level | 28 | 32 | 5.8 | 6.2 | 212.2 | 216.2 |
| | | | $C_{10}$ | | | |
| Low | 1.16 | 3.50 | 2.55 | 2.28 | 3.05 | 4.40 |
| Medium | 2.50 | 3.49 | 6.12 | 5.80 | 1.93 | 1.97 |
| High | 3.08 | 3.88 | 5.81 | 3.35 | 5.33 | 4.95 |
| | | | $C_{12}$ | | | |
| Low | 1.60 | 2.78 | 3.42 | 1.37 | 4.02 | 6.40 |
| Medium | 2.48 | 3.82 | 1.50 | 5.82 | 2.09 | 1.96 |
| High | 3.41 | 3.39 | 6.24 | 3.55 | 5.41 | 4.89 |

RSD: relative standard deviation.

### ANALYSIS OF THE WET WIPES

For this purpose, different wet wipes were bought from the market. Commercial wet wipe solutions were analyzed by using the developed CE method for the determination of BAC. Every sample was analyzed by using calibration curve obtained in same day and amount of BAC was calculated according to the recovery values of quality control samples. Recovery values for BAC were different for each day due to different surface effect of the wipe tissues. Hence, there was no constant value to calculate the real amount of BAC in samples. Obtained results were presented in Table V.

## CONCLUSIONS

In this study, the CE method used for the quantitative determination of BAC has been developed and validated. Satisfactory validation data were achieved for sensitivity, linearity, accuracy, precision, robustness, and ruggedness. The developed method has some advantages by comparing literature. Other studies reported LOD values between 1 and 10 μg/ml which were higher than the reported value in this study. Only one study declared an LOD value as 0.01 μg/ml because they investigated the BAC in environmental samples and used a liquid chromatography–mass spectrometry/mass spectrometry (MS) device which makes the analysis more complex. The LOD values of the developed method were

**Table V**
Analysis Results of Some Commercial Wet Wipe Samples

| | Claimed amount of BAC (mg/ml) | | Found amount of BAC (mg/ml) | |
|---|---|---|---|---|
| Sample | $C_{10}$ | $C_{12}$ | $C_{10}$ | $C_{12}$ |
| Wipe 1 | 0.280 | 0.115 | 0.269 ± 0.04 | 0.111 ± 0.02 |
| Wipe 2 | 0.280 | 0.115 | 0.267 ± 0.05 | 0.127 ± 0.02 |
| Wipe 3 | 0.280 | 0.115 | 0.285 ± 0.06 | 0.114 ± 0.02 |

0.313 and 0.309 µg/ml for $C_{10}$ and $C_{12}$ derivatives, respectively. These values were good comparing the literature values and it was enough to achieve the analysis of BAC in wet wipe samples. Another advantage of the developed method was separation power. Theoretical plate number was declared as 10,688 in a high-performance liquid chromatography (HPLC) study for BAC analysis (18), whereas this number was almost 35,000 for the developed method. Furthermore, there was only one BAC peak in this HPLC study and this situation showed that this HPLC method could not separate the BAC homologues. In another study (20), the BAC homologues could be separated, but the detector used was an MS detector, not a UV-Vis detector. BAC homologues were separated by using the developed method and there was no need for an extra detection system like MS. The developed method is the first method for determination of BAC in wet wipes by using the CE with DAD detector. Identification and quantitation of homologs of BAC in wet wipes were done by using this method. The method is suitable for that aim.

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