

## Research Article



## Spectrophotometric Determination of Benzalkonium Chloride using Sulfonephthaleins

Yousry M. Issa<sup>1</sup>, Nora S. Abdel-kader<sup>1</sup>, Ahmed E. Zahran<sup>2</sup><sup>1</sup>Chemistry Department, Faculty of Science, Cairo University, Giza 12613, Egypt.<sup>2</sup>IVL Dhunseri Petrochem Industries Ltd., Suez, Egypt.\*Corresponding author's E-mail: [norabdelkader@sci.cu.edu.eg](mailto:norabdelkader@sci.cu.edu.eg)

Received: 12-02-2021; Revised: 22-04-2021; Accepted: 28-04-2021; Published on: 15-05-2021.

## ABSTRACT

A highly sensitive method for the quantitative determination of Benzalkonium chloride (BKC), in its pure form and pharmaceutical formulations, is described. The method involves four spectrophotometric ways for the determination of BKC via forming ion-associates with bromocresol green (BCG), bromophenol blue (BPB), bromothymol blue (BTB), and xlenol orange (XO). The study involves characterization of the ion-pairs formed between the BKC and the above-mentioned reagents Using UV-Visible and IR spectroscopy. In order to optimize the reaction conditions, the effects of pH, the quantity of reagent, time, and extracting solvent were studied. Statistical student's t-test and F test showed insignificant systematic error between proposed and official methods. The antibacterial disinfectant Zora C Lozenges<sup>®</sup> contains 0.01 mg Benzalkonium chloride, 1 mg Benzocaine, and 50 mg Vitamin C. as the active substance was analyzed at pH 8.5. The strategy was validated for linearity range, precision, accuracy, specificity, and limits of detection (LOD) and quantification (LOQ). Beer's law is obeyed over a wide concentration range (up to 145 µg/mL in case of BCG method). LOD and LOQ values reached 2.38 and 7.2 µg/mL, respectively, upon using BCG. The relative standard deviation (%RSD) was ≤1.33% while correlation coefficient values (r) were ≥ 0.998. High molar absorptivity values and low values of Sandell's sensitivity were calculated indicating that the proposed methods are highly sensitive. Applying the validated methods to the analysis of BKC in antibacterial disinfectant Zora C Lozenges<sup>®</sup> revealed that the drug was successfully resolved from the pharmaceutical formulation with recoveries ≥95.5%.

**Keywords:** Spectrophotometry, sulfonephthalein, Benzalkonium chloride, characterization, ion-pairs, pharmaceutical formulation.

QUICK RESPONSE CODE →

DOI:

10.47583/ijpsrr.2021.v68i01.009

DOI link: <http://dx.doi.org/10.47583/ijpsrr.2021.v68i01.009>

## INTRODUCTION

Benzalkonium chloride, also known as BZK, BKC, BAC, alkyl dimethyl benzyl ammonium chloride and ADBAC, is a type of cationic surfactant. It is an organic salt classified as a quaternary ammonium compound. It has three main categories of use: as a biocide, a cationic surfactant, and as a phase transfer agent.<sup>1</sup> ADBACs are a mixture of alkyl benzyl dimethylammonium chlorides, in which the alkyl group has various even-numbered alkyl chain lengths.

Spectrophotometric methods of analysis are still drawing the attention of chemists due to their simplicity and affordability.<sup>2-4</sup> In addition, they provide a fast and selective tool for routine analysis of pharmaceuticals making them suitable for rapid screening of raw materials. Moreover, spectrophotometric analyses do not require sophisticated instruments and/or well-experienced laboratory personnel compared to expensive and sophisticated chromatographic methods. Sulfonephthaleins belong to a class of organic compounds

known as arylsulfonic acids with a general formula containing three aromatic rings and a central carbon atom attaching them (triphenylmethane). These molecules are usually used as indicators in acid-base titrations and all of them are colored and absorb visible light. Therefore, many researchers used these compounds for color development with diverse pharmaceutical compounds followed by their subsequent spectrophotometric measurement.<sup>5,6</sup> Few analytical methods were developed for quantifying BKC in raw materials, tablets, eye drops, and serum samples. These methods include derivative spectrophotometric<sup>7-13</sup>, electrochemically<sup>14-16</sup> and titrimetrically.<sup>17-18</sup> In addition, HPLC was extensively used for the determination of BKC, which is considered an accurate but very expensive and sophisticated method of analysis.<sup>19-27</sup> It is obvious from the literature survey that, there is no previously published work devoted to the determination of BKC based only on sulfonephthalein dyes as coloring agents. Accordingly, the purpose of this work is to develop a new spectrophotometric method for the determination of Benzalkonium chloride (BKC) based on the ion-pair formation reaction to some sulfonephthalein dyes, namely Bromocresol green (BCG), Bromothymol blue (BTB), Bromophenol blue (BPB), and Xlenol orange (XO). This method relies on the extraction of the formed ion-pair in an organic solvent to eliminate blank interference. Other methods depend on the  $\beta$ -correction of the data obtained from the spectrophotometer due to the overlapping peaks



obtained in the absorption spectra of the ion-pair complexes.

## MATERIALS AND METHODS

### Instrumentations and Glassware

All spectral measurements were carried out using a double beam LAMBDA™ 25 UV/Vis Spectrophotometers, Perkin Elmer, USA equipped with glass or quartz cells of 1 cm optical path length. Micro burette, Borosil Glass Works Ltd., India, was used for transferring volumes of solutions. Mettler Toledo ML204 New Classic MF Analytical Balance 220 g x 0.1 mg, USA was used for weighing throughout the study. The conductometric titration curves were obtained with the aid of an Orion Model 162A Benchtop Conductivity Meter of 0.6 cm cell- Thermo Electron Corporation 166 Cummings Center, Beverly, MA 01915, USA. pH measurements were carried out on a digital pH meter (Mettler Toledo Seven Easy pH meter T138242 - USA) at room temperature (25.0±1.0 °C).

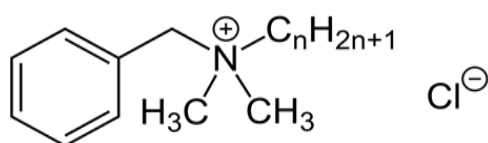
### Chemicals

The materials and reagents used in this work are highly pure chemical substances. The water used for solutions preparations and dilutions was bidistilled water prepared with the aid of a glass distillation instrument. Benzalkonium chloride (BKC) (98%) and the acid dye reagents; BCG, BPB, BTB and XO were obtained from Merck, Germany. Absolute ethanol, sodium dihydrogen phosphate (NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O), Chloroform (CHCl<sub>3</sub>), Dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>), and sodium hydroxide (NaOH) were bought from Sigma-Aldrich, Germany. The commercial tablets, Zora C Lozenges® (1 mg/tablet), were obtained from CID Pharmaceutical (Chemical Industries Development), Giza, Egypt.

### Solutions

#### Preparation of Benzalkonium chloride solutions (BKC)

An aqueous 1 x10<sup>-4</sup> mol/L stock solution of BKC, Fig. 1, 354.02 g/mol (average molecular weight as described in the Japanese pharmacopoeia) was prepared by accurately weighing the required amount of the powder (0.0354 g) and dissolving it in the least amount of bidistilled water. Thereafter, the solution was transferred quantitatively to a 1000 mL measuring flask and completed to the mark with bi distilled water. The stock 0.3 mol/L buffer (pH 8.2) solution NaH<sub>2</sub>PO<sub>4</sub> was prepared by dissolving 46.8 g of the solid salt in 1000 mL measuring flask and completed to the mark with bi distilled water.



$$n = 8, 10, 12, 14, 16, 18$$

Figure 1: Structural formula of Benzalkonium chloride

### Preparation of the tablet solution

For Zora C Lozenges® (1 mg/tablet), CID Pharmaceutical (Chemical Industries Development), Giza, Egypt, 10 tablets were accurately weighed and ground using a mortar. The average weight (equivalent to one tablet) was calculated. A series of solutions of concentration of 5, 10, 15, 20 mg BKC was prepared by weighing and dissolving the equivalent amounts of the ground tablets in bi distilled water.

### Preparation of reagent solutions

Standards stock solutions (4x10<sup>-3</sup> mol/L) of BCG, BPB, BTB and XO were prepared by dissolving 0.2792, 0.2679, 0.2497 and 0.269 g, respectively, in 10 mL of buffer. The resulting solutions were transferred quantitatively into 100 mL measuring flasks and completed up to the mark with a buffer solution of pH 8.2.

### Procedure for construction of calibration curves

Spectrophotometric determination of BKC using sulfonephthalein dyes has to be carried out within the linear concentration range of Beer's law. In order to detect this range, a series of solutions was prepared whereby reagent concentration was kept constant at the required volumes of 2.0 mL 1x10<sup>-3</sup> mol/L of BCG, BPB, BTB and XO, while that of the drug was regularly varying. The solution was then completed to 10 mL using bi distilled water. The optimum conditions (pH, wavelength, extracting solvent, and the reaction time) were enforced. Absorbance values of the resulting complexes were measured at the corresponding  $\lambda_{max}$ , against a blank solution prepared simultaneously without the drug being added and plotted versus drug concentration. The linear part of the absorbance-drug concentration plot represents the concentration range in which Beer's law is applicable. Determining Ringbom concentration range<sup>28</sup> enabled us to achieve more accuracy of the results; this was determined by plotting log drug concentration in  $\mu\text{g/mL}$  against % transmittance.

### Association constants and free energy changes

The Benesi-Hildebrand equation<sup>29</sup> defined the association constants of the formed complexes. The experimental procedures of this method rely on the state that the non-absorbing species of the two reactants must be added in large excess (more than 10 folds of the other component concentration). The drug solution was added to 60 mL separating funnels. Different volumes of BCG, BPB, BTB or XO solutions were added to the drug solution. The complexes formed are treated as described in the process of the calibration curve. The absorbance was measured at the corresponding wavelength against a blank solution prepared simultaneously. The relation was drawn according to the Benesi-Hildebrand equation.<sup>29</sup>

$$\frac{[A_0]}{A\lambda AD} = \frac{1}{\epsilon\lambda AD} + \frac{1}{Kc\epsilon\lambda AD} \cdot \frac{1}{[D_0]} \quad (1)$$

Where, [A<sub>0</sub>] and [D<sub>0</sub>] are the total concentration of the interacting species. By plotting [A<sub>0</sub>]/[A<sub>λ</sub><sup>AD</sup>] versus 1/[D<sub>0</sub>], a

straight line was obtained. The intercept of this line with the ordinate is  $(\epsilon^{AD})^{-1}$  and the slope equals  $Kc^{AD} \cdot \epsilon\lambda^{AD})^{-1}$

The standard free energy<sup>30</sup>  $\Delta G^\circ$ , of the complexation, is related to the association constant K or the stability constant by:

$$\Delta G^\circ = -2.303 RT \log K \quad (2)$$

Where, R is the universal gas constant  $1.987 \text{ cal mol}^{-1} \text{ degree}^{-1}$

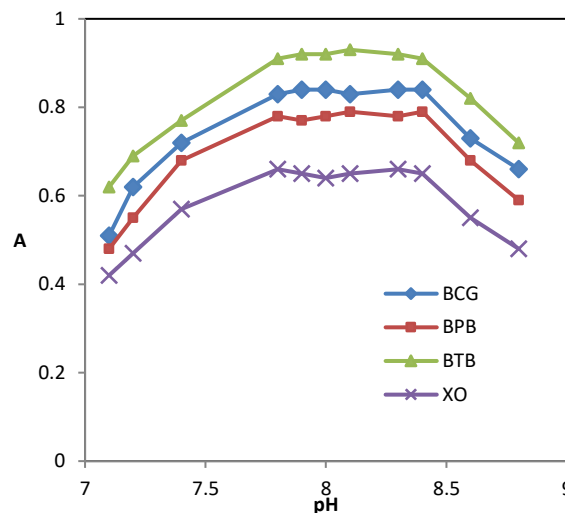
T is the temperature in Kelvin (T Kelvin = T Celsius + 273)

## RESULTS AND DISCUSSION

The spectrophotometric properties of the colored species, as well as the impact of different parameters on the color development, were studied to determine the ideal conditions for the complex formation. In this study, four anionic acid dyes (BCG, BPB, BTB and XO) were used. The stoichiometric ratios of the reactions were investigated using Jobs' method of continuous variation, molar ratio method and conductimetric titration. In all cases, the effect of reagent quantity and the effect of time on complex formation were studied. Furthermore, the association constants, formation constants and the free energy change were determined. The application was made for the proposed method to evaluate BKC in its formulation. The statistical analysis of the proposed methods was performed and the results obtained from the f- and t-tests were in good agreement with those in table (4). A literature survey was conducted to establish a correlation in the literature between the methods proposed and other methods.

### Effect of pH on extraction of the ion-pairs

The ion-pair formation with quaternary ammonium compounds may strongly depend on the pH. Therefore, the pH effect was investigated by using different buffer solutions. As shown in Fig. 2, the optimum pH range for the spectrophotometric measurements is pH 7.9 - 8.5. In this work, therefore, the ion associates were extracted at pH 8.2.



**Figure 2:** Effect of pH on the extraction of BKC-Reagent associates.

### Visible Absorption Spectra of the Formed Complexes in Aqueous Solutions

The visible absorption spectrum of BKC has no absorption maxima in the visible region (400-800 nm) because it is a colorless solution. The BCG, BPB, BTB, and XO reaction with BKC results in the formation of yellow-colored products. Table 1 shows the maxima of these products.

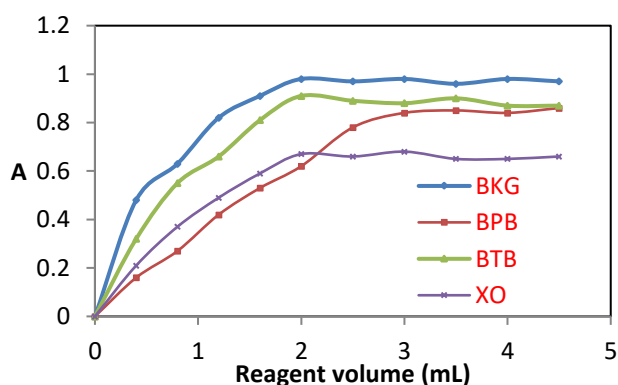
**Table 1:** The maxima of the BKC – complexes in the visible region

Complex	Solvent	Number of maxima	$\lambda_{\max}$ (nm)
BKC-BCG	Chloroform	2	413, <b>632*</b>
BKC-BPB	Chloroform	2	380, <b>591*</b>
BKC-BTB	Chloroform	2	435, <b>614*</b>
BKC-XO	Chloroform	2	430, <b>590*</b>

\*The bold numbers are the wavelengths selected for the absorbance measurement.

### Effect of the Reagent Concentration on the Complexes Formation

Fig. 3 shows the effect of the concentration of the four acid dyes on the complex formation. It is evident from the results that the optimum color intensity of the ion-pair was achieved with 2 mL of  $1 \times 10^{-3}$  mol/L of BCG, BPB, BTB, and XO for 1 mL of  $1 \times 10^{-3}$  mol/L BKC, and 1 mL of  $1 \times 10^{-3}$  mol/L of BPB solution for 1 mL of  $1 \times 10^{-3}$  mol/L BKC.

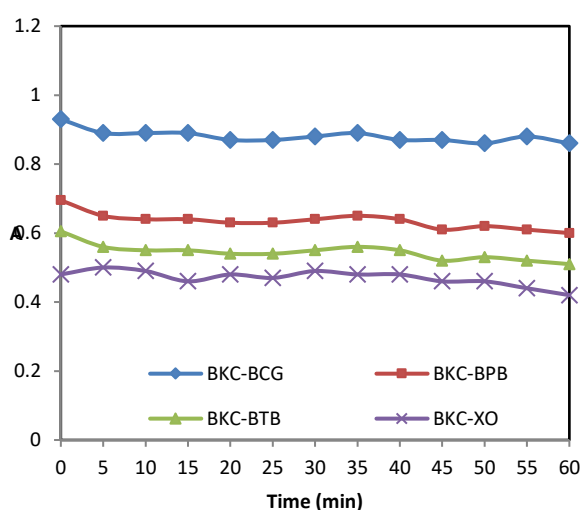


**Figure 3:** Effect of the reagent volume on the ion-pair formation



### Effect of Standing Time

The standing time effect on the formation of the complexes was studied to obtain the optimum conditions for the reaction. The absorbance values of the complexes of BKC with BCG, BPB, BTB, and XO were measured at different time intervals up to 60 minutes. From the measured absorbance values, it is obvious that all the reactions are spontaneous and the Time has almost no significant effect on the formation of the colored ion pairs. This reflects the rapid completion and spontaneity of the reaction confirmed by the negative  $\Delta G^\circ$  values in Table 2. Consequently, the absorbance of the test solutions can be measured after ion-pair preparation regardless of the time elapsed within 60 min. Furthermore, complexes had acceptable stability for a long period up to 60 minutes, Fig. 4.



**Figure 4:** Effect of standing time on the formation of complexes

### Stoichiometry of the formed complexes

#### Job's method of continuous variation<sup>31</sup>

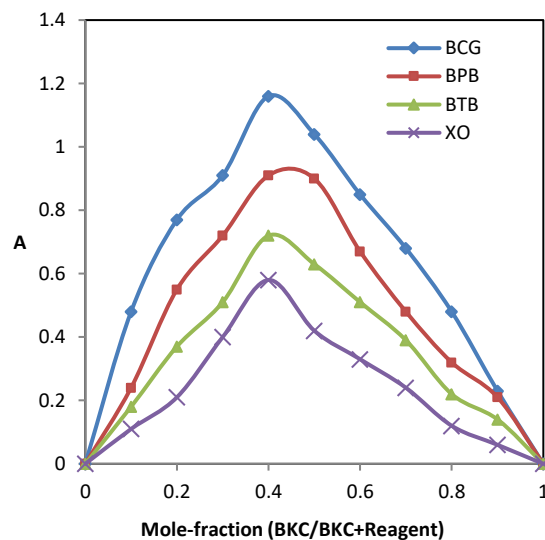
In case of extraction methods, a series of solutions with total molar concentration of  $1 \times 10^{-3}$  mol/L of BKC solution and  $1 \times 10^{-3}$  mol/L of BCG and BTB solution were made up comprising different complementary proportions (0.2:1.8, 0.4:1.6, ....., 1.6:0.4, 1.8:0.2) into 60 mL separating funnels. The resulting complexes were extracted into 5 mL chloroform in all cases by shaking for 2 minutes. The organic layer was separated and completed to 10 mL with chloroform. The absorbance of the solutions was measured at 632 and 614 nm for BCG and BTB, respectively, against a reagent blank prepared simultaneously. The absorbance values were plotted against the drug mole-fraction in the complex.

In the case of BPB, Job's plots are carried out twice using two different total concentrations. In the first case, a series of mL proportion of  $5 \times 10^{-4}$  BKC and BPB were made up by comprising different complementary proportions (1.8:0.2, 1.6:0.4, . . . . 0.4:1.6, 0.2:1.8) in 25 mL volumetric flask. The resulting solutions were completed to the mark using

bidistilled water. The absorbance of the formed complexes and blank solutions were measured at 380 and 591 nm against water blank. The corrected absorbance was calculated and plotted against the mole fraction of the drug in the complex. In the second case, a series of mL proportion of BKC and BPB were made up by comprising different complementary proportions (2.7:0.3, 2.4:0.6, . . . . 0.6:2.4, 0.3:2.7) in 25 mL volumetric flask. The prepared mixtures were completed to the mark using bidistilled water. The absorbance of the formed complexes and blank solutions of BPB were measured at the same wavelength and plotted against the drug mole-fraction.

In case of XO, a series of mL proportion of  $1 \times 10^{-3}$  mol/L of BKC and XO solutions were made up by comprising different complementary proportions (0.9:0.1, 0.8:0.2, . . . . 0.2:0.8, 0.1:0.9) in 10 mL volumetric flask. The resulting solutions were completed to the mark using bidistilled water. The absorbance of a blank solution of XO was measured at 590 nm against each solution of the prepared complexes.

The data attained from Job's method revealed that the stoichiometry of the reaction was 1:2 in all cases except for BKC-BPB complex, which was formed in 1:1 and 1:2 molar ratios, Fig. 5.

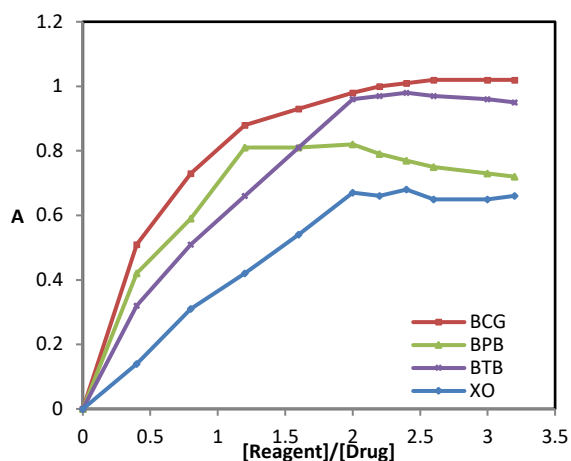


**Figure 5:** Job's method of continuous variation used for determination of the stoichiometry of the reaction of BKC with BCG, BPB, BTB, and XO.

#### Molar ratio method<sup>32</sup>

The molar ratio method is used to determine the stoichiometry of the reaction between BKC and the anionic dyes mentioned above. Constant concentration of the drug solution was taken, and the concentration of the reagent was varied successively [(0.2-2.4 mL of  $5 \times 10^{-4}$  mol/L (BPB), 0.2-1.8 mL of  $1 \times 10^{-3}$  mol/L (BCG), 0.2-2.0 mL of  $1 \times 10^{-3}$  mol/L (BTB) and 0.2-1.6 mL of  $1 \times 10^{-3}$  mol/L (XO)]. Fig. 6 shows two lines intersecting at the molar ratios 1:1 and 1:2 (BKC: BPB), 1:2 (BKC: BCG, BTB, and XO). It is clear that the data obtained from the molar ratio method are

compatible with those obtained from Job's method of continuous variation.



**Figure 6:** Molar ratio determination of stoichiometry of BKC reaction with BCG, BPB, BTB, and XO.

### Conductimetric titration method

In conductimetric titration, the completion of the titration is detected by a change in the conductivity of the solution. The results obtained from conductimetric titration (Fig. 7) elucidated that the reaction stoichiometry is 1:2 (Drug: Reagent) in all cases although the other methods revealed that the reaction stoichiometry between BKC and BPB was 1:1 and 1:2 (Drug: Reagent). To plot the relation between the volume of the standard solution added and the conductivity, the measured conductivity values have to be corrected to avoid the error that may arise from dilution.

50 mL of BKC solution of concentration  $1 \times 10^{-3}$  mol/L were transferred to a beaker where the conductivity electrode was immersed. Then  $1 \times 10^{-2}$  mol/L of BCG, BPB, BTB, or XO was added from a micro burette and the conductance was measured (at 25 °C) after each addition of the reagent after thorough stirring. Using formula (12), the conductance reading after each addition was corrected for dilution<sup>33</sup>. Stoichiometry was detected through constructing the conductimetric titration curves by plotting the molar ratio of one component against the corrected conductance ( $\mu\text{S}/\text{cm}$ ). The results showed that in all situations, BKC responds in a stoichiometric ratio of 1:2 with the colors under investigation.

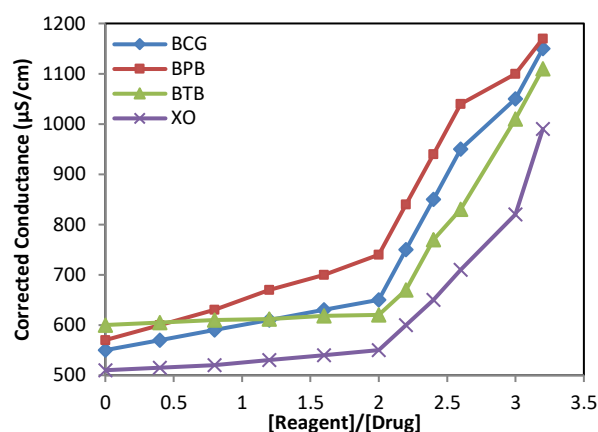
$$\Omega_{\text{Corr}} = \Omega_{\text{Obs}} [(V_1 + V_2) / V_1] \quad (3)$$

Where  $\Omega$  is the electrolytic conductivity,  $V_1$  is the initial volume and  $V_2$  is the volume of the added reagent. (Corr.; corrected, Obs.; observed).

### Selectivity and Interference Studies

The capacity of analytical techniques to specifically detect the response of the analyte among other species in the solution is called selectivity (in our case the drug additives and excipients expected to be present in the pharmaceutical formulation). During recovery studies, the effect of common materials and excipients were examined. The recovery values obtained from the

experiments showed that the common excipients of the drug did not interfere in case of BCG, BPB, BTB, and XO methods.

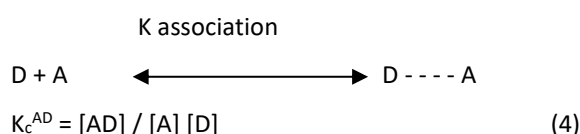


**Figure 7:** Conductimetric titration curves for determination of reaction stoichiometry between BKC and (a) BCG, (b) BPB, (c) BTB, and (d) XO.

The usual tablet diluents such as glucose, lactose, and starch did not interfere. Also, aromatic and aliphatic amines such as procaine, ephedrine, methyl ephedrine, papaverine, eserine, triethylamine at the  $6 \times 10^{-5}$  mol/L level and chlorpheniramine, diphenhydramine and dibucaine at  $3 \times 10^{-5}$  mol/L level did not interfere. However, chlorpheniramine caused a slight positive error. Quaternary ammonium salts such as tetraethylammonium, tetramethylammonium and neostigmine did not interfere. The effects on Benzalkonium determinations were similar.<sup>34</sup>

### Association and formation constants and free energy change

The association constant and the free energy change can be calculated using the Benesi-Hildebrand method.<sup>29</sup> This method is based on the condition that the concentration of one of the reactants is much higher than the other reactants in the solution. The absorbance of the formed complexes was measured as a function of varied [A] when  $[A] \gg [D]$ . The deviation of Benesi-Hildebrand equation is as follow:



Where:

$$[D]^0: \text{Total D (uncomplexed and complexed)} = [D] + [DA]$$

$$[A]^0: \text{Total A (uncomplexed and complexed)} = [A] + [DA]$$

Substitution into equation (31):

$$K_c^{AD} = \frac{[DA]}{([D]^0 - [DA])([A]^0 - [DA])} \quad (5)$$

In the proposed procedures, this was conducted through using variable concentrations of 0.2-1.4 mL of  $4.0 \times 10^{-5}$  mol/L BCG, BPB, BTB, or XO respectively, to which 1.0 or

2.0 mL of  $1.0 \times 10^{-3}$  mol/L of BKC was added. Then the experiment followed as described in the general procedures; and the absorbance was measured at the optimum wavelength for each ion-pair.

The absorbance of the resulting ion-associates were utilized for estimation of the association constant through Benesi-Hildebrand equation<sup>29</sup>

$$[A_o] / [A_{\lambda}^{AD}] = 1/\epsilon_{\lambda}^{AD} + (1/K_c^{AD} \cdot \epsilon_{\lambda}^{AD}) 1/ [D_o] \quad (6)$$

Where  $[A_o]$  and  $[D_o]$  are the total concentration of the interacting species,  $A_{\lambda}^{AD}$  and  $\epsilon_{\lambda}^{AD}$  are the absorbance and the molar absorptivity of the ion-pairs at 632, 591, 614, and 590 nm for BCG, BPB, BTB, and XO respectively.  $K_c^{AD}$  is the association constant of the ion-pair.

A straight line was obtained on plotting values of  $[A_o]$  versus  $1/[D_o]$ , Fig. 8 illustrated by the below equations:

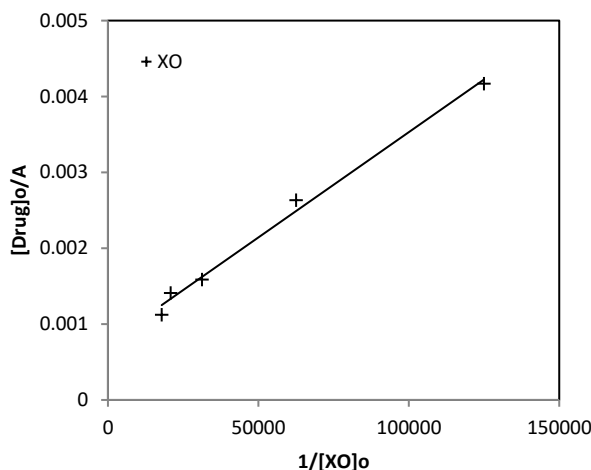
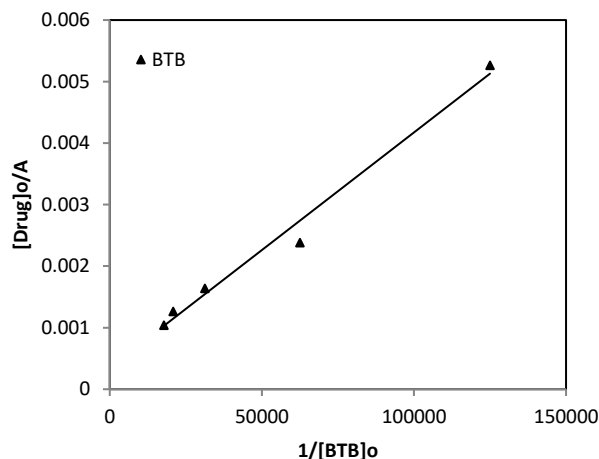
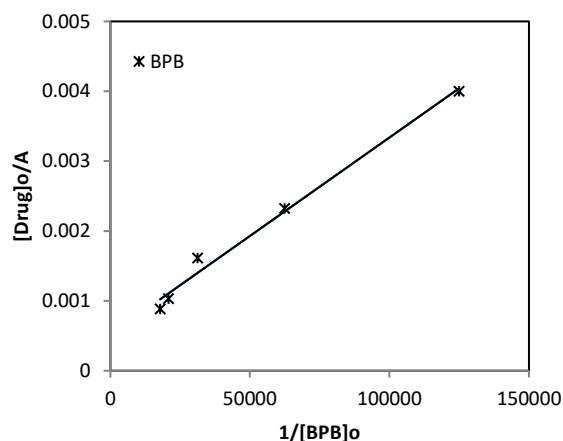
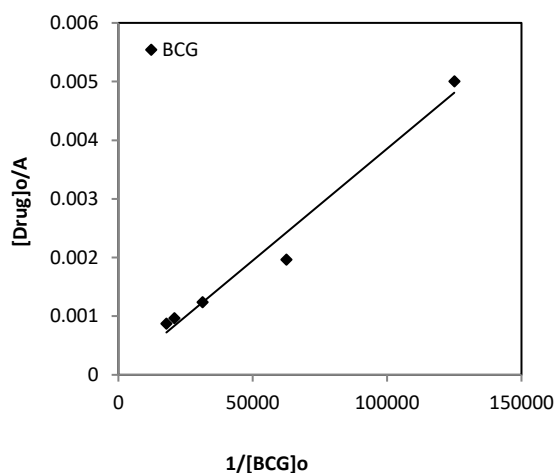
$$[A_o] / [A_{\lambda}^{AD}] = 4.08 \times 10^{-5} + 1/ [D_o] (3.81 \times 10^{-8}) \quad (\text{BKC-BCG})$$

$$[A_o] / [A_{\lambda}^{AD}] = 5.19 \times 10^{-4} + 1/ [D_o] (2.81 \times 10^{-8}) \quad (\text{BKC-BPB})$$

$$[A_o] / [A_{\lambda}^{AD}] = 3.49 \times 10^{-4} + 1/ [D_o] (3.82 \times 10^{-8}) \quad (\text{BKC-BTB})$$

$$[A_o] / [A_{\lambda}^{AD}] = 7.54 \times 10^{-4} + 1/ [D_o] (2.77 \times 10^{-8}) \quad (\text{BKC-XO})$$

The intercept of this line with the ordinate is  $1/\epsilon_{\lambda}^{AD}$  and the slope equals  $(1/K_c^{AD} \cdot \epsilon_{\lambda}^{AD})$ . The values of association constant ( $K_c^{AD}$ ) and the molar absorptivity ( $\epsilon_{\lambda}^{AD}$ ) listed in table (2) confirm the stability of the ion-pairs formed.



**Figure 8:** Benesi-Hildebrand plots (a) BKC-BCG, (b) BKC-BPB, (c) BKC-BTB, (d) BKC-XO complexes

Other methods can be applied for the determination of the apparent formation constant of BKC complexes. Generally, Job’s method.<sup>31</sup> which has been used to study the equilibria in a solution of the complex compounds is more convenient. However, the equation derived for calculating the formation constant could be applied to the results of other spectrophotometric methods especially the molar ratio method.<sup>32</sup>

The following equation<sup>35</sup> was used:

$$K_f = (A/A_m) / [1 - (A/A_m)]^{n+1} C^n n^n \quad (7)$$

Where:  $A_m$  is the maximum absorbance obtained from Job’s method for continuous variation curve,  $A$  is the absorbance corresponding to the intersection of the two tangents of the continuous variation curve,  $C$  is the concentration corresponding to the maximum absorbance and  $n$  is the amount of the drug in the reaction product.

Furthermore, the standard free energies of complexation  $\Delta G^\circ$  were calculated using the following equation:

$$\Delta G^\circ = -2.303 RT \log K \quad (8)$$

Where:

$\Delta G^\circ$  is the free energy change of the ion-pair

R is the gas constant 1.987 cal /mol kelvin

T is the temperature in Kelvin

K is the association or formation constant of the ion-associate (L/mol)

Table 2 shows the calculated parameter for BKC ion pairs under study.

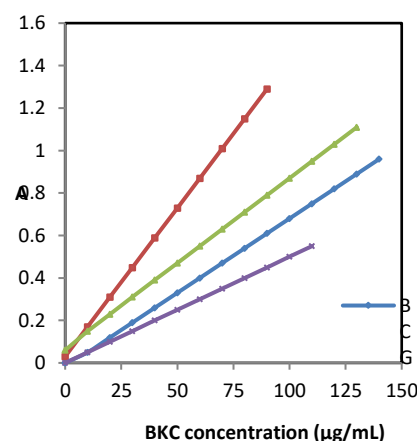
**Table 2:** Association, formation constants, molar absorptivity, and free energy change for BKC ion pairs.

Parameter	Reagent			
	BCG	BPB	BTB	XO
$\epsilon_{\max}$ (L mol <sup>-1</sup> cm <sup>-1</sup> )	2.45x10 <sup>4</sup>	1.92x10 <sup>3</sup>	2.86x10 <sup>3</sup>	1.32x10 <sup>3</sup>
$K_c^{AD}$	1.07x10 <sup>3</sup>	1.84x10 <sup>4</sup>	9.13x10 <sup>3</sup>	2.71x10 <sup>4</sup>
$K_f$ (L mol <sup>-1</sup> ) (n=1)	3.96x10 <sup>3</sup>	3.56x10 <sup>4</sup>	1.04x10 <sup>5</sup>	7.98x10 <sup>3</sup>
$\Delta G^\circ$ (kcal mol <sup>-1</sup> )	4.9x10 <sup>-3</sup>	9.33x10 <sup>-3</sup>	1.37x10 <sup>-4</sup>	1.33x10 <sup>-4</sup>

The results obtained, Table 2, showed that the formation and association constants of the formed complexes indicate the stability of the complexes. On the other hand, the negative values of  $\Delta G^\circ$  indicate the spontaneity of the reaction.

#### Calibration Curves

From the resulting calibration curves, it is obvious that Beer's law is obeyed in wide concentration ranges 2.37-145, 7.1-95, 2.79-130, 2.41-115  $\mu\text{g/mL}$  for BCG, BPB, BTB, and XO methods respectively, Fig. 9. The molar absorptivity and Sandell sensitivity values were calculated and tabulated in Table 3. The methods have high molar absorptivity and low Sandell sensitivity values and hence they are very sensitive. The correlation coefficients ( $r^2$ ) calculated from the least-squares equation amount to 0.999, 0.998, 0.999, and 0.999 for BCG, BPB, BTB, and XO methods respectively, indicating good linearity of the calibration curves.



**Figure 9:** Calibration curves for the determination of BKC using BCG, BPB, BTB, and XO.

**Table 3:** Validation parameters for the evaluation of BKC using BCG, BPB, BTB, and XO.

Parameter	Reagent			
	BCG	BPB	BTB	XO
$\lambda_{\max}$ (nm)	632	591	614	590
Beer's law range ( $\mu\text{g/mL}$ )	2.38-145	7.12-95	2.79-130	2.41-115
Ringbom range ( $\mu\text{g/mL}$ )	16.35-67.36	11.42-58.13	8.54-61.77	9.63-71.24
Molar absorptivity (L/mol cm)	2.45x10 <sup>4</sup>	1.92x10 <sup>3</sup>	2.86x10 <sup>3</sup>	1.32x10 <sup>3</sup>
Sandell sensitivity ( $\mu\text{g/cm}^2$ )	12.71x10 <sup>-2</sup>	12.5x10 <sup>-2</sup>	13.04x10 <sup>-2</sup>	12.88x10 <sup>-2</sup>
LOD ( $\mu\text{g/mL}$ )	2.38	7.12	2.79	2.41
LOQ ( $\mu\text{g/mL}$ )	7.20	21.56	8.45	7.3
Slope (a) (mL/ $\mu\text{g cm}$ )	7.9x10 <sup>-3</sup>	8.0x10 <sup>-3</sup>	7.7x10 <sup>-3</sup>	7.8x10 <sup>-3</sup>
Intercept (b)	0.139	0.138	0.142	0.141
Correlation coefficient ( $r^2$ )	0.9998	0.998	0.999	0.999
RSD %	1.33	0.89	1.24	1.51
$K_c^{AD}$ (L/mol)	1.07x10 <sup>3</sup>	1.84x10 <sup>4</sup>	9.13x10 <sup>3</sup>	2.71x10 <sup>4</sup>
$\Delta G^\circ$ (kcal/mol)	4.9x10 <sup>-3</sup>	9.33x10 <sup>-3</sup>	1.37x10 <sup>-4</sup>	1.33x10 <sup>-4</sup>
$K_f$ (L/mol)	3.96x10 <sup>3</sup>	3.56x10 <sup>4</sup>	1.04x10 <sup>5</sup>	7.98x10 <sup>3</sup>

## Validation of the methods

Experimental methods aim to prove their validity for applicability for its intended purpose. The purpose of the analytical procedure should be clearly understood, as this will regulate the validation characteristics to be assessed. Typical validation attributes, which should be highlighted, are accuracy, precision, detection limit, quantitation limit, linearity, and range.<sup>36</sup>

## Accuracy and Precision

The accuracy of an analytical procedure reflects the closeness of similarity between the value which is accepted either as a traditional true value or an accepted reference value and the observed value. This is sometimes called the truth. In order to achieve higher accuracy levels in photometry, it is more essential to pick an acceptable concentration range and test the accuracy analysis than to demonstrate compliance with Beer's law. To characterize a reasonable concentration range, Ringbom plots<sup>28</sup> for the ideal concentration range can be acquired by plotting the photometric data of (%T) as ordinates against the logarithm of concentration as abscissas, Fig. 9. The precision of an analytical technique represents the near agreement (degree of dispersion) between a set of measurements obtained from various sampling of the same homogeneous sample under the specified conditions. The low values of %RSD recorded in Table 3 are clear evidence on the precession of the proposed methods.

## Limit of detection (LOD) and Limit of quantification (LOQ)

Limit of detection (LOD) and limit of quantification (LOQ) were estimated for validation of the analytical procedures<sup>36</sup> according to the following equations:

$$\text{LOD} = 3s/m \quad (9)$$

$$\text{LOQ} = 10s/m \quad (10)$$

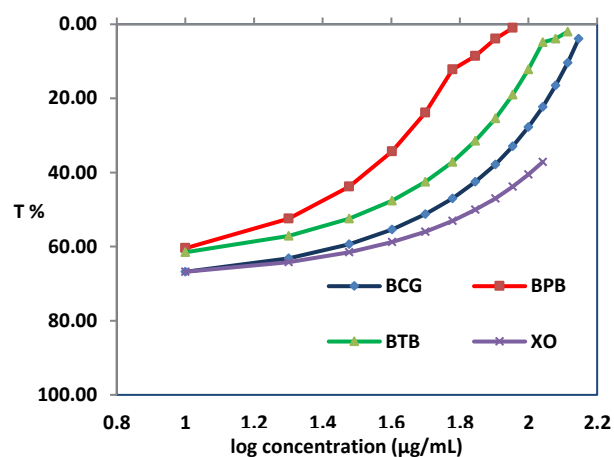
Where (s) is the standard deviation of recreating determination values under the same conditions as for the sample analysis without the analyte, and (m) is the slope of the calibration curve. Values tabulated in Table 3 proved the sensitivity of the proposed methods.

**Table 4:** Evaluation of the precision of the proposed methods on BKC pure samples and their Pharmaceutical formulation: Zora C Lozenges® (1.0 mg / lozenges)

Reagent	Method	Taken (µg/mL)	Found (µg/mL)	%Recovery ±SD	RSD %	t-Test	f-Test
BCG	Pure	3.5	3.62	103.30 ± 0.07	1.97	1.33	8.43
		10.5	10.20	97.11 ± 0.19	1.87		
		17.5	17.70	101.11 ± 0.37	2.13		
	Zora C®	17.5	17.52	100.13 ± 0.20	1.16	1.54	1.43
		35	34.96	99.89 ± 0.38	1.11		
		52.5	51.85	98.75 ± 0.47	0.92		
BPB	Pure	3.5	3.39	99.73 ± 0.03	0.95	0.56	14.02
		10.5	10.45	99.54 ± 0.15	1.52		
		17.5	17.17	98.10 ± 0.07	0.44		

## Linearity and Range

Based on the results of the calibration curves; it is clear that the analytical methods proposed are linear across very broad concentration ranges up to (145, 95, 130, and 115 µg/mL) for BCG, BPB, BTB, and XO methods respectively). The values of correlation coefficients: 0.999, 0.998, 0.999, and 0.999 also demonstrate the linearity of the calibration curves for BCG, BPB, BTB, and XO methods respectively.



**Figure 10:** Ringbom plots for BKC complexes with BCG, BPB, BTB, and XO acid dyes.

## Application to Pharmaceutical formulation

The methods suggested were extended to the estimation of BKC in various disinfectant and antibacterial, namely Zora C Lozenges®. In the experimental section, the specific procedures for determining the drug being investigated in pharmaceutical disinfectant were clearly discussed. The findings were statistically correlated with those recorded from the standard method by the student's T- and F-test.<sup>37</sup> For the four cited acid dyes BCG, BPB, BTB, and XO and using the calibration curve technique, recovery studies were carried. Table 4 shows the recovery studies for the four methods, while Table 5 shows the statistical analysis of the data.



	Zora C <sup>®</sup>	17.5	16.94	96.78 ± 0.58	3.47	0.46	28.41
		35	36.13	103.21 ± 0.55	1.53		
		52.5	51.9	98.92 ± 0.95	1.83		
BTB	Pure	3.5	3.62	103.48 ± 0.04	1.12	1.11	13.59
		10.5	10.06	95.83 ± 0.19	1.95		
		17.5	17.75	101.44 ± 0.38	2.17		
	Zora C <sup>®</sup>	17.5	17.76	101.50 ± 0.33	1.86	0.98	27.44
		35	33.96	97.02 ± 0.30	0.89		
		52.5	54.22	103.27 ± 0.94	1.74		
XO	Pure	3.5	3.37	96.29 ± 0.09	2.73	0.50	11.57
		10.5	10.13	95.50 ± 0.19	1.95		
		17.5	17.73	101.34 ± 0.38	2.15		
	Zora C <sup>®</sup>	17.5	17.71	101.20 ± 0.29	1.68	2.37	8.52
		35	34.87	99.61 ± 0.93	2.66		
		52.5	54.18	103.20 ± 0.82	1.52		

Another way to validate the proposed methods is to compare them with previously approved methods, Table 5

**Table 5:** Comparison between the proposed methods and published spectrophotometric methods

Reagent/Method	Linear range µg/mL	LOD µg/mL	LOQ µg/mL	Slope	Recovery* ± SD%	r <sup>2</sup>	RSD%	ε	λ max
Reported <sup>13</sup>	1 -5			0.062	98.74 ± 0.49	0.999	0.44 ± 0.71	6.2x10 <sup>4</sup>	517
Ion-pair formation (Proposed)									
BCG	2.38 – 145	2.38	7.20	0.0079	100.18±0.38	0.9998	1.33	7.8x10 <sup>3</sup>	632
BPB	7.12 – 95	7.12	21.56	0.008	99.52±0.30	0.9981	0.89	7.9x10 <sup>3</sup>	591
BTB	2.79 – 130	2.79	8.45	0.0077	100.25±0.40	0.9997	1.24	7.6x10 <sup>3</sup>	614
XO	2.41 – 115	2.41	7.3	0.0078	99.37±0.48	0.9994	1.57	7.7x10 <sup>3</sup>	590

\*: Average of three replicate measurements for the reported method and the proposed one.

## CONCLUSION

It is obvious from the literature survey of BKC that the most popular technique used for its detection is chromatography, which is expensive, time consuming and requires highly sophisticated instruments.

Two spectrophotometric methods were proposed for the determination of BKC in its pure form and in pharmaceutical formulations depending on the formation of ion-pairs or ion-associate complexes with BCG, BPB, BTB, and XO, which in turn can be extracted in water-immiscible organic solvents or measured directly at their corresponding absorption maxima. The data obtained from Job's method revealed that the stoichiometry of the reaction was 1:2 molar ratios in all cases, plus 1:1 for BPB.

The high values of association indicate the stability of the ion-pair. Negative values of  $\Delta G^\circ$  indicate spontaneity of the reaction. Beer's law was evident in the concentrations up to 145, 95, 130, and 115 µg/mL, respectively, reflecting the good linearity of the calibration curves. Low relative (RSD %) values indicate the precision of the proposed method.

β-correction method of calculations was applied to BPB method to enhance its selectivity. On the other hand,

extraction in chloroform was carried out in case of BCG and BTB methods to eliminate the blank absorbance and increase the sensitivity of the methods.

## REFERENCES

- Lackner M, Guggenbichler JP, Antimicrobial Surfaces, Ullmann's Encyclopedia of Industrial Chemistry. Wiley-VCH, 2013. DOI: 10.1002/14356007.q03\_q01.
- Mohamed, SH, Issa YM, Elfeky SA, Extraction-free spectrophotometric assay of the antitussive drug pentoxyverine citrate using sulfonephthalein dyes, Spectrochimica Acta A, Mol Biomol Spectrosc, 2019; 222: 117186. DOI: 10.1016/j.saa.2019.117186
- Sherif OE-S, Issa YM, Abo-Dena AS, β-Correction and extraction to overcome spectral overlap in spectrophotometric determination of levocetirizine dihydrochloride, IJRPC, 2014; 4(1): 181-191.
- Mohamed SH, Issa YM, Salim AI, Simultaneous quantification of simeprevir sodium: A hepatitis C protease inhibitor in binary and ternary mixtures with sofosbuvir and/or ledipasvir utilizing direct and H-point standard addition strategies, Spectrochim Acta Part A: Mol Biomol Spectrosc, 2019; 210: 290-297. DOI: 10.1016/j.saa.2018.07.017.
- Dung NT, Dat ND, Bau LH, Thao LQ, Extractive spectrophotometric methods for determination of ciprofloxacin in pharmaceutical formulations using sulfonephthalein acid dyes, Vietnam J Chem, 2017; 55(6): 767-774. DOI: 10.15625/2525-2321.2017-00542.
- Rozsypal T, Halamek E, Kobliha Z, Spectrophotometric determination of tris(2-chloroethyl)amine using acid dyes, J. Appl



- Spectrosc., 2018; 84(6): 1139-1144. DOI:10.1007/s10812-018-0599-3.
7. Kovács-Hadady K, Fábíán I, The Determination of Benzalkonium Chloride in Eye-Drops by Difference Spectrophotometry, *J Pharm Biomed Anal*, 1998; 16(5): 733-740. DOI: 10.1016/S0731-7085(97)00085-X
  8. Sakai T, Ohno N, Co-extraction-spectrophotometric Determination of Berberine with Quinine and Bromophenol blue, *Chem Lett.*, 1982; 11 (1): 107-110. DOI: 10.1246/cl.1982.107
  9. Ma W, Ma X, Sha O, Liu Y, Two Spectrophotometric Methods for the Assay of Benzalkonium Chloride in Bandage Samples, *J Surfactants Deterg.*, *J Surfact Deterg*, 2014; 17(1): 177-181. DOI 10.1007/s11743-013-1446-4
  10. Sharma A, Tapadia K, Silver Nanoparticles-Based Nano-drop Spectrophotometric Determination of Cationic Surfactants Coupled with Hydrophobic Interaction; An Application to Pharmaceuticals and Environmental Samples, *Orient J of Chem*, 2016; 32(5): 2641-2651. DOI: 10.13005/ojc/320536
  11. Hassouna MEM, Abdelrahman MM, Mohamed MA, Simultaneous Determination of Azelastine hydrochloride and Benzalkonium chloride by RP-HPLC Method in their Ophthalmic Solution, *J Forensic Sci Crim Inves*, 2017; 1(3): 555565-555575. DOI:10.19080/JFSCI.2017.01.555565
  12. Arai K, Kimura M, Kusu F, Takamura K, Extraction/spectrophotometric determination of drugs of quaternary ammonium salts based on ion association between an anionic dye and quaternary ammonium ions, *BUNSEKI KAGAKU*, 1996; 45(8): 783-787. DOI: 10.2116/bunsekikagaku.45.783
  13. Patel P, Varshney P, Patel D, Meshram D, Method Development and Validation of Benzalkonium chloride in Marketed Formulation by UV-visible Spectrophotometry using Silver Nitrate and Eosin solution, *World Journal of Pharmacy and Pharmaceutical Sciences*, 2014; 3(2): 1481-1487.
  14. Gaber M, Shawish HMA, Khedr AM, Abed-Almonem KI, Determination of Benzalkonium chloride preservative in pharmaceutical formulation of eye and ear drops using new potentiometric sensors, *Mater Sci Eng C*, 2012; 32(8): 2299-2305. DOI: 10.1016/j.msec.2012.06.018
  15. Shawish HMA, Khedr AM, Abed-Almonem KI, Gaber M, A comparative study of solid and liquid inner contact Benzalkonium chloride ion-selective electrode membranes, *Talanta*, 2012; 101: 211-219. DOI: 10.1016/j.talanta.2012.09.005.
  16. Burger K, Pethö G, A new coated-wire cobalt(II)-selective electrode based on the Benzalkonium tetrathiocyanatocobaltate(II) ion pair, *Anal Chim Acta*, 1979; 107: 113-119. DOI: 10.1016/S0003-2670(01)93202-7.
  17. Billow JA, Baker HW, Direct Titrimetric Determination of Aromatic Quaternary Ammonium Salts and Long Chain Aliphatic Sulfates and Sulfonates, *J Pharm Sci*, 1966; 55(12): 1446-1447. DOI: 10.1002/jps.2600551225.
  18. Japanese pharmacopoeia codex 15, Quantitative determination of Benzalkonium chloride.
  19. Kostić DA, Mitić SS, Nasković DČ, Zarubica AR, Mitic MN, Determination of Benzalkonium Chloride in Nasal Drops by High-Performance Liquid Chromatography, *E-Journal Chem*, 2012; 9(3): 1599-1604. DOI: 10.1155/2012/172070.
  20. AlAani H, AlNukkary Y, Determination of Benzalkonium Chloride in Ophthalmic Solutions by Stability-Indicating HPLC Method: Application to a Stability Study, *J Appl Pharm Sci*, 2016; 6(05): 080-089. DOI: 10.7324/JAPS.2016.60513.
  21. Dudkiewicz-Wilczyńska J, Tautt J, Roman I, Application of the HPLC method for benzalkonium chloride determination in aerosol preparations, *Journal of Pharmaceutical and Biomedical Analysis*, 2004; 34(5): 909-920. DOI: 10.1016/j.jpba.2003.09.001.
  22. Parhizkari G, Delker G, Miller RB, Chen C, A Stability-Indicating HPLC Method for the Determination of Benzalkonium Chloride in 0.5 % Tramadol Ophthalmic Solution, *Chromatographia*, 1995; 40(3-4): 155-158. DOI: 0009-5893/95/02.
  23. Shen Y, Xu S, Wang S, Tu J, Determination of Benzalkonium chloride in viscous ophthalmic drops of azithromycin by high-performance liquid chromatography, *J Zhejiang Univ Sci B*, 2009; 10(12): 877-882. DOI:10.1631/jzus.B0920229
  24. Edress LAH, Elhag DE, Development and Validation of Stability Indicating HPLC Method for Benzalkonium Chloride in Betaxolol (0.5%) Ophthalmic Solution, *J Pharm Pharmacol Res*, 2019; 3(2): 019-027 DOI: 10.26502/jppr.0017.
  25. Prince SJ, McLaury H-J, Allen L V, McLaury P, Analysis of Benzalkonium Chloride and Its Homologs: HPLC Versus HPCE, *J Pharm Biomed Anal*, 1999; 19(6): 877-882. DOI: 10.1016/S0731-7085(98)00187-3.
  26. Cybulski ZR, Determination of Benzalkonium chloride by gas chromatography, *J Pharm Sci*, 1984; 73(12): 1700-1702. DOI: 10.1002/jps.2600731209.
  27. Rojsitthisak P, Wichitnithad W, Pipitharome O, Sanphanya K, Thanawattanawanich P, Simple HPLC Determination of Benzalkonium Chloride in Ophthalmic Formulations Containing Antazoline and Tetrahydrozoline, *PDA J Pharm Sci Technol*, 2005; 59(5): 332-337.
  28. Ringbom AZ, *Anal Chem*, 1939; 115: 332-338.
  29. Benesi HA, Hildebrand JH, *J Amer Chem Soc*, 1947;63:437.
  30. Martin AN, Swarbrick J, Cammarata A, *Physical Pharmacy, Physical chemical principles in the pharmaceutical sciences*, 3<sup>rd</sup> ed., 1983; 74(3): 363-364. DOI: 10.1002/jps.2600740338.
  31. Job R, *Advanced physicochemical experiments*, 1<sup>st</sup> ed., *J Chem Educ*, 1965; 42(3): A230. DOI: 10.1021/ed042pA230.
  32. Ewing GW, *Instrumental Methods of Chemical Analysis*, 5<sup>th</sup> ed., McGraw-Hill Inc.,US, Spore, New York, 1985.
  33. Issa YM, Shoukry AF, El-Nashar RM, Conductimetric determination of reproterol HCl and pipazethate HCl and salbutamol sulphate in their pharmaceutical formulations, *J Pharm Biomed Anal*, 2001; 26(3): 379-386. DOI: 10.1016/S0731-7085(01)00415-0.
  34. Sakai T, Extraction—spectrophotometric determination of benzethonium and benzalkonium salts with bomocresol green and quinine, *Anal Chim Acta*, 1983; 147: 331-337. DOI: 10.1016/0003-2670(83)80100-7.
  35. Harvey AE, Manning DL, *Spectrophotometric Methods of Establishing Empirical Formulas of Colored Complexes in Solution*, *J Am Chem Soc*, 1950; 72(10): 4488-4493. DOI: 10.1021/ja01166a044.
  36. Miller JC, Miller JN, Horword E, *Statistics for analytical chemistry*, 3<sup>rd</sup> ed., *Talanta*, 1994; 41(8): 1408. DOI: 10.1016/0039-9140(94)80026-X

**Source of Support:** None declared.

**Conflict of Interest:** None declared.

For any question relates to this article, please reach us at: [editor@globalresearchonline.net](mailto:editor@globalresearchonline.net)

New manuscripts for publication can be submitted at: [submit@globalresearchonline.net](mailto:submit@globalresearchonline.net) and [submit\\_ijpsrr@rediffmail.com](mailto:submit_ijpsrr@rediffmail.com)

