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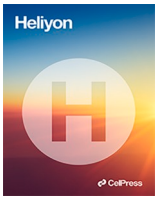

Research article

Development of a sustainable procedure for smartphone-based colorimetric determination of benzalkonium chloride in pharmaceutical preparations

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ABSTRACT

A sustainable procedure offering green, simple, and rapid analysis was developed to determine benzalkonium chloride (BKC) in pharmaceutical preparations. The determination using smartphones was based on the ion pair colorimetric reaction with bromothymol blue (BTB), which produces a yellow color. The intensity of the product color, which is proportional to the concentration of BKC, was detected and evaluated using a smartphone camera and an image processing application. The procedure was performed in a microliter and was rapidly detected within 1 min after incubation. This offered high throughput at 28 samples per well plate in duplicate. Linear calibration, which was a plot of BKC concentrations and relative red intensities, was in the range of 2.0–24.0 μg/mL with an R^2 of 0.997. The limits of detection (LOD) and quantitation (LOQ) were 1.0 and 3.2 μg/mL, respectively. This work was successful in applying it to pharmaceutical materials, disinfectant products, and pharmaceutical products containing BKC. It was discovered that the concentrations of BKC as an active ingredient in pharmaceutical materials were 82% w/v, whereas those in disinfectant products ranged from 0.4 to 2.1% w/v. In pharmaceutical products, ophthalmic drops and nasal sprays contain BKC as preservatives in the 0.01–0.02, and the 0.02% w/v, respectively. The results obtained by the proposed procedure compared with a reference titration method showed no significant differences at a 95% confidence level with 1.2–3.4% RSDs. This promotes the efficiency of pharmaceutical preparations regarding infection prevention and control by ensuring that available disinfectants contain a sufficient concentration of BKC. Additionally, this improves the efficiency of pharmaceutical preparations for quality control of pharmaceutical products by ensuring that the available preservatives maintain a sufficient concentration throughout the lifespan of the products.

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1. Introduction

Disinfectants are crucial to prevent the spread of diseases used by healthcare facilities and various industries. Numerous disinfectants containing quaternary ammonium compounds (QACs) are available because of their low toxicity and being environmentally friendly. The antimicrobial activity of QACs is achieved when the cationic charge forms an electrostatic interaction with negatively charged phospholipids in the membrane, and the hydrophobic alkyl tail integrates in the lipid core, reducing membrane fluidity and impairing osmoregulation. Therefore, QACs can enter cells and engage with intracellular targets. As a result, membranes are disrupted, activities on the membrane are blocked, low molecular weight molecules leak out, proteins and nucleic acids are degraded, and the cell wall is lysed [1]. These compounds are used in a wide range of situations and in a variety of ways. However, critical aspects of chemical disinfection in healthcare settings have to be realized and guidelines followed [2]. One of the defined practices is regarding the concentration of disinfectant. Although the concentration of disinfectant is an important factor affecting the efficiency of pharmaceutical preparation regarding infection prevention and control (IPC), using excessive amounts may cause future biocidal resistance, toxicity and irritation. Therefore, the correct dosage should be controlled to complete the efficacy of disinfection and to prevent future biocidal resistance, toxicity and irritation. One of the most frequently overused QACs is benzalkonium chloride (BKC), which is structured as a benzyl-dimethyl-ammonium chloride linked to alkyl chains (C12 and C14) [3]. BKC, as an active ingredient, was extensively used in disinfectants and antiseptics [4]; for example, BKC was used for the preparation of hand sanitizer for alcohol-based hand gels [5]. To prevent the spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in hospitals and the wider public, only 0.2% of BKC is sufficient [6,7], and a 0.1% BKC concentration is recommended for use for cleansing and sterilizing medical equipment [7]. In addition, 0.25% of BKC was used to inhibit *Streptococcus mutans* and *S. sobrinus* in dental equipment [8]. Commercial BKC products are available in two different uses: a concentrate that needs to be diluted with water onsite and a ready-to-use product [9]. These BKC products have been used in various ways, such as diluting before use, soaking the medical device, or reusing. To ensure complete efficiency of disinfection and to avoid any excess that could lead to biocidal resistance and toxicity in the future, the concentration of the active form in any product must be evaluated. Especially products prepared onsite because they contaminate oxidizing agents, and negatively charged ions in water can convert BKC to an inactive form, causing antimicrobial activity eventually to decrease as a result [10]. In addition, benzalkonium chloride is also used as a preservative in the preparation of pharmaceutical products, such as ophthalmic drops and nasal sprays [11,12].

Various techniques have been proposed to determine BKC concentration, including high-performance liquid chromatography [13–15], electrochemistry [16] and spectrophotometry, using chromogenic agents such as eosin, eosin-Y, methyl orange, bromophenol blue (BPB), bromothymol blue (BTB), and bromocresol green [17–25]. However, all require expensive equipment, experience, time consumption, and cannot be analyzed onsite. For onsite analysis, commercial colorimetric test strips are frequently used, but they are expensive and offer a range of concentrations that are determined using naked-eye estimation [26,27]. Recently, developing an alternative approach to be adapted for a local practice to follow the guidelines related to Infection Prevention and Control (IPC) is recommended [28,29]. Unfortunately, discovering the expected analytical technology that could be accessed would be rare at primary medical care levels, particularly in developing nations. Therefore, developing simple and cost-effective analytical techniques as alternative methods to control the quality and stability of BKC-containing preparations would be beneficial because of their easy accessibility and user-friendly green analysis. These are to ensure IPC management and promote well-being according to the Sustainable Development Goals (SDGs). Additionally, quality control of onsite pharmaceutical preparations containing BKC must be achieved as often as needed and maintained even in community memorials or health-promoting hospitals in primary healthcare settings.

The cost-effective, eco-friendly approach has received a lot of attention in any field [30]. A cost-effective alternative procedure has been developed that is often designed based on a colorimetric reaction for pharmaceutical analysis to provide miniaturized analysis [31]. This reduces the use of chemicals and the generation of waste. Based on image processing technology, the portable platform and a smartphone as a detector were used in the pharmaceutical field, for example, to determine fluoroquinolones [32,33]. Regarding colorimetric smartphone-based detection of QACs, simple platforms, including a microcentrifuge tube and a paper-based platform, were used. Although the related report used a microcentrifuge tube in a micro-scale analysis, it required liquid-liquid microextraction by chemical solvent [34]. Another, using a paper-based platform and polydiacetylene liposome as a chromogenic agent, detected the gradient product color to evaluate the concentration of QACs through the color bar lengths. However, the platform also requires the fabrication and immobilization of a chromogenic agent on a paper-based surface [35]. Increasing the high-throughput sample and preventing errors from collating with standards that are not in the same frame would be possible in the future.

This research aimed to develop a simple, green, and sustainable downscaled smartphone-based colorimetric procedure to determine BKC in pharmaceutical preparations. This research produced an ideal approach that does not require advanced equipment or analytical expertise, avoids toxic reagents, uses an available reagent, and ensures low-cost and rapid analysis in order to overcome the drawbacks of the procedures reported in the literature, namely the requirements of expensive equipment and experience, time consumption, and toxic solvents. The available micro-well plate platform was used to operate the colorimetric reaction. The color change due to the yellow product of the formation of ion pairs between BKC and BTB was monitored with a smartphone to detect the intensity of color and then convert it to BKC concentration. The obtained great analytical characteristics, including effectiveness, sensitivity, accuracy, and precision, represent a cost-effective green chemical analysis, implying a sustainable pharmaceutical analysis.

2. Materials and methods

2.1. Chemicals, materials, and equipment

BKC (≥95%) was purchased from Sigma-Aldrich Company (Darmstadt, Germany). BTB (95%), a chromogenic agent, was obtained from Sigma-Aldrich Company (Buchs, Switzerland). Ethanol was acquired from ACI Labscan Limited (Bangkok, Thailand). Potassium phosphate dibasic and potassium phosphate monobasic, purchased from Ajax Chemicals (Auckland, New Zealand), were used for phosphate buffer preparation. The commercial disinfectant products, including iodine (Meiyume Manufacturing, Pathumthani, Thailand), sodium hypochlorite (Loba Chemie, Maharashtra, India), peracetic acid (Thai Peroxide, Samutprakran, Thailand), ethyl alcohol-containing product (Pose Health Care, Bangkok, Thailand), and chloroxylenol (Reckitt Benckiser Healthcare, Cileungsi, Indonesia), were used for the selectivity study. Benzyldimethyl-n-dodecylammonium chloride (Alfa Aesar, Ward Hill, USA), Sucrose (ACI Labscan Limited, Bangkok, Thailand), di-sodium Tetraborate (Loba Chemie, Mumbai, India) and 2′-7′-dichlorofluorescein (Acros Organics, New Jersey, USA) were used for the titration method. Deionized Water was produced using an Elgastat-Maxima purification system (18.2 MΩ cm, ELGA, UK).

The 96-well microplate (flat bottom) was made from polystyrene (Corning, AZ, USA). The spectrophotometric measurements were carried out using a microplate reader (SPECTROstar Nano, BMG LABTECH, Ortenberg, Germany). PH700 Benchtop pH Meter was used for buffer preparation (Apera Instruments, Wuppertal, Germany). A smartphone was the iPhone 11.0 Pro, Apple, China.

2.2. Procedure

The downscaled color ion-pair analysis was performed using a 96-well plate with flat sterilization bottle and a smartphone as a detector following the related publication [36]. Briefly, 200 μL of the sample or standard and 50 μL of reagent solution were added to each well. Then the reactions were mixed and incubated to produce a color ion pair. The color BKC-BTB ion pair was then detected using a microplate reader for absorption spectra investigation. For the proposed procedure, the colored BKC-BTB ion-pair was detected using a smartphone. The 96-well plate containing the colored ion pair was placed into a light-controlled box with the LED light source at the bottom, and a single-shot photograph covering the 96 wells was taken using a smartphone with flash-off through a hole at the top of the light-controlled box. The image (JPEG format) of color products was imported to the color analysis application, namely, ColorMeter® (Version 2.2.0), for processing red, green, and blue (RGB) values in a square area covering the BKC-BTB color product. Then the intensity of the R was converted to BKC concentration. The proposed alternative green chemical analysis procedure is shown in Fig. 1.

2.3. Optimization of the proposed alternative green analysis for the BKC determination

The optimum condition for BKC determination was investigated using the range of standard BKC concentration at 0–24.0 μg/mL (2.0, 4.0, 8.0, 12.0, 16.0, 20.0, and 24.0 μg/mL). The investigation procedure was performed following section 2.1. The parameters include the pH of the phosphate buffer, concentration of phosphate buffer, concentration of BTB, and incubation time. Table 1 presents details regarding the fixed and variable parameters in each condition.

2.4. reference method

Precipitation titration was selected as a reference method [37]. Briefly, 50 mL of the sample was titrated with 0.07 M sodium tetraphenylboron. Before titration, 0.5 g of powdered sucrose was added to the sample to prevent coagulation of the precipitate, and

Fig. 1. Proposed alternative green procedure for determination of benzalkonium chloride. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Parameters for the optimum conditions to determine BKC.

Condition	Parameter	Concentration/Values							
1	pH of phosphate buffer	7.0	7.5	8.0					
	Concentration of phosphate buffer (M)	0.50							
	Concentration of BTB (μg/mL)	40							
	Incubation time (min)	1							
2	pH of phosphate buffer	7.5							
	Concentration of phosphate buffer (M)	0.05	0.10	0.25	0.50	1.00			
	Concentration of BTB(μg/mL)	40							
	Incubation time (min)	1							
3	pH of phosphate buffer	7.5							
	Concentration of phosphate buffer (M)	0.50							
	Concentration of BTB(μg/mL)	10	20	30	40	50	60		
	Incubation time (min)	1							
4	pH of phosphate buffer	7.5							
	Concentration of phosphate buffer (M)	0.50							
	Concentration of BTB(μg/mL)	40							
	Incubation time (min)	1	2	3	4	5	7	10	15

0.2% dichlorofluorescein in ethanol was used as an indicator. The indicator typically turns a deep pink right before the endpoint is reached, and the color transition from red to yellow is fairly rapid, indicating the endpoint.

3. Results and discussion

3.1. Investigation of the BKC-BTB ion pairing

In this work, the visible absorption of the ion pair of BKC-BTB was investigated at wavelengths 340–800 nm. The BKC-BTB ion pairs of 2.0–24.0 μg/mL of BKC and 40 μg/mL of BTB were prepared by mixing 2.5–30 μg/mL BKC and 200 μg/mL BTB in 0.5 M phosphate buffer pH 7.5. The absorption spectrum showed two absorption peaks, as shown in Fig. 2A. BTB showed maximum absorbance of acidic and basic form at 400 nm and 620 nm, respectively [37]. The absorption spectrum changed after BTB binding with BKC following equation below.

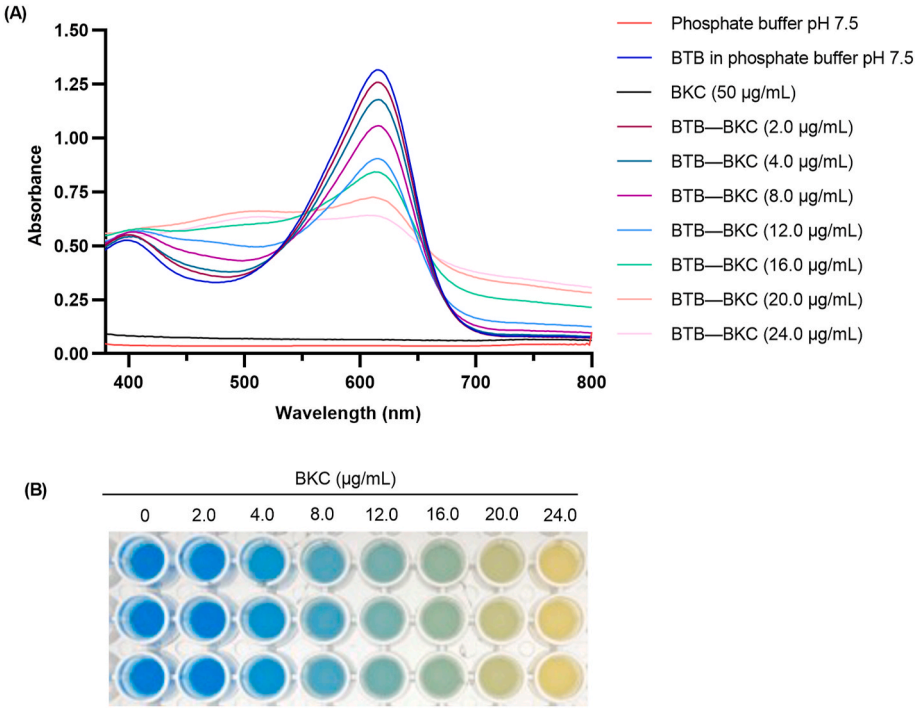

Fig. 2. (A) visible spectrum of BKC (2.0 − 24.0 μg/mL) and BTB (40 μg/mL) color complex in 0.50 M phosphate buffer pH 7.5 and (B) color of the BKC-BTB ion pair at different concentrations of BKC in triplicate. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

$$QA^{\oplus}Cl^{\ominus} + BTB^{\ominus}Na^{\oplus} \leftrightharpoons QA{-}BTB + NaCl$$

Where $QA^{\oplus}Cl^{\ominus}$ is quaternary ammonium salt as benzalkonium chloride, $BTB^{\ominus}Na^{\oplus}$is sodium salt of bromothymol blue, and QA−BTB is the ion pair between benzalkonium chloride and bromothymol blue.

The equation demonstrates the ion-pairing between BTB and BKC involving stoichiometric replacement of the cation of BTB with quaternary ammonium ion [38]. The absorption spectrum of the mixture showed a bathochromic shift from 400 nm. This shift was brought about by the ion association of BKC^{+} interacting with BTB^{-} to form an ion pair. This interaction influences electron delocalization in BTB^{-}, causing it to rearrange into the yellow form [39]. The BKC-BTB ion pair structure was proposed in Fig. S1. It was not only a wavelength of maximum absorbance shift, but absorbance also increased. The cause could be the interaction producing a yellow color that becomes darker as the concentration of BKC, which is a limiting substrate, increases. The maximum absorbance decreased at 620 nm, which was inversely related to the concentration of BKC. Such a decrease in maximum absorbance at 620 nm involved the BTB in basic form, which was used for ion pairing with BCK [24].

The absorption spectra were congruent with the color appearance observed in this work, as shown in Fig. 2B. The BTB solution was observed as blue because it contained the basic form, dominating the acidic form by a significant amount. The mixture solution containing BKC-BTB ion pairs exhibited varying shades of green and yellow, corresponding to the increasing concentration of BKC. The observed color solution was agreeable with the absorbance ratio at 620 nm and 400 nm. Using image processing, the color change of the mixture was analyzed. The red (R) intensity is observed to be the highest response among red (R), green (G), and blue (B) when concentrations of BKC were varied (see Fig. S2).

3.2. Optimization of the proposed alternative green analysis for the BKC determination

3.2.1. The pH of the phosphate buffer

The optimum pH of the phosphate buffer to determine BKC was investigated at pH 7.0, 7.5, and 8.0, which covered the range of the acid dissociation constant (pK_a) of BTB [38, 41]. In this range of pH, BTB was in the basic form that was ready to pair an anion ion with a cation of BKC. The results were explained in terms of linear regression of the calibration plot, considering linearity and sensitivity. The calibrations were plotted between the concentration of BKC and the relative color intensity of red (Δintensity of red), which was the intensity of red when contrasted with a blank background, as shown in Fig. 3. The results under the condition of 0.50 M phosphate buffer at pH 7.5 gave the wide linear range (0–24.0 μg/mL), the highest sensitivity with the slope of linear calibration as 5.839 and offered good linearity at the coefficient of determination (R^2) as 0.9952. The obtained linear range using 0.50 M phosphate buffer at pH 7.0 provided a lower sensitivity than at pH 7.5 despite the same wide linearity. At pH 8.0, the linear range was narrower (4.0–24.0 μg/mL), and the sensitivity, which was considered from the limit of detection, was worse among the three conditions. This is because bromothymol blue demonstrates a range of colors at different pH levels; the blank solution displays distinct color intensities at pH 7.0, 7.5, and 8.0. At a pH of 8.0, the blank appeared more intense than at pH 7.0 and 7.5, making it challenging to distinguish the difference in color intensity between the blank and the color product produced using a low amount of BCK. This phenomenon is illustrated in Fig. S3. As a result, it was decided that pH 7.5 in the 0.50 M phosphate buffer was appropriate because it demonstrated the highest sensitivity and a wide range of linear calibration.

3.2.2. Concentration of the phosphate buffer

The concentration of phosphate buffer at pH 7.5 was varied from 0.10 to 1.00 M. The results were interpreted in terms of linear regression of the calibration plot considering linear range, sensitivity, and R^2. Calibration plots between Δ intensity of red and the concentration of BKC were obtained under different concentrations of phosphate buffer conditions, as shown in Fig. S4. The results reveal the effect of the concentration of phosphate buffer on the ion-pair formation by considering the intensity of red. The highest red intensity was observed at 0.10 M phosphate buffer, and it decreased as the phosphate buffer concentration increased. Obviously, at above 12 μg/mL of BKC under 1.00 M phosphate buffer, the ion-pair formation appeared to be constrained. This involves ionic strength affecting the BTB dissociation. The ionic strength increased by increasing the concentration of phosphate buffer, causing reduced BTB dissociation [40]. The reduced ion dissociation of BTB resulted in decreased ion-pair formation. Practically, the linear calibration, sensitivity (slope of linear calibration), and R^2 were considered. The highest sensitivity was produced under 0.10 M phosphate buffer

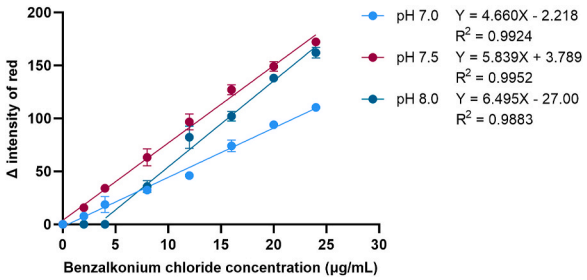

Fig. 3. Calibration plots between Δ intensity of red and concentration of BKC under different pH levels of 0.5 M phosphate buffer condition in triplicate. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

condition, but the linear range was narrow (0–12.0 μg/mL). Under 0.25 and 0.5 M phosphate buffer conditions, the linear ranges were wider (0–24.0 μg/mL), and the sensitivity (7.7593 and 7.7882) and R^2 (0.9918 and 0.9981) were highest. It indicated that the range of 0.25–0.50 M of concentration of phosphate buffer was suitable. In this work, 0.5 M phosphate was used to ensure sufficient buffer capacity.

3.2.3. BTB concentration

The optimum concentration of BTB was investigated to produce the proposed procedure with the highest sensitivity. Calibration plots between Δ intensity of red and the concentration of BKC were obtained under different concentrations of BTB (10, 20, 30, 40, 50, and 60 μg/mL), as shown in Fig. S5. At BTB concentrations of 10, 20, and 30 μg/mL, the linear ranges were 0–8.0, 0–12.0, and 0–20.0 μg/mL BCK, respectively. The linear range was expanded to 0–24.0 μg/mL BCK at 40 μg/mL BTB. In addition, the linear ranges were narrower and shifted to 2.0–24.0 and 4.0–16.0 μg/mL BKC at 50 and 60 μg/mL BTB, respectively. This could be explained by the higher concentration of BTB expressing a dark color, making it difficult to indicate the difference in signal between low and absent BCK.

Considering the wide range, statistical metrics in a linear regression of the calibration identified sensitivity (slope of linear calibration) and R^2 using different concentrations of BTB in the range of 0–60.0 μg/mL as shown in Fig. 4. The metrics in a linear regression obtained in the condition of 40 μg/mL of BTB, provided the high sensitivity (the linear calibration slope of 7.6288) and the best linearity with $R^2 = 0.9974$, indicating the sufficient ability to determine BKC.

3.2.4. Incubation time

The formation of the BKC-BTB ion pair was monitored at various incubation times (from 1 to 15 min). The results showed that the BKC-BTB ion pair formed rapidly. The observed intensity of R values confirmed that all reactions were rapid and that incubation time had not impacted the formation of colored ion pairs. The rapid completion and spontaneity of the reaction between BKC and BTB can be described by the negative ΔG° (the free energy change of the ion-pair) values that were studied and calculated using the Benesi-Hildebrand method by Issa et al. [23]. The complete reaction and stable products significantly proved that incubation time at 1 min exhibited no significant difference with other incubation times ($p < 0.05$, two-way ANOVA test), providing a high throughput sample as 28 samples per a 96-microwell plate in duplicate.

3.3. Analytical characteristics

3.3.1. Calibration linearity, LOD, and LOQ

Analytical characteristics of the proposed procedure compared with the spectrophotometric methods demonstrated in Table 2. The range of linear calibration was 2.0–24.0 μg/mL with R^2 at 0.997. The LOD (3.3σ) and LOQ (10σ) were 1.0 and 3.2 μg/mL, where σ served as the standard deviation of the Y-intercept, divided by the slope of the linear calibration graph. respectively. The LOD and LOQ from this work were as good as the related publications using similar chromogenic agents and other agents and measured by a spectrophotometer [20]. Although slightly poorer than that of the related publication using Eosin Y as a chromogenic agent and tetradecyldimethyl (ethylbenzyl)ammonium chloride (TDBAC) as standard, the working range was wider [19]. Compared with related publications regarding digital image processing, which was rarely reported, the sensitivity of this work was higher than that of that using a paper-based platform [35]. Although the sensitivity was not as excellent as the others reported by Amelin et al., this work could avoid the solvent extraction step to give a simple procedure [34]. This promotes the use of fewer chemicals and the generation of less waste. Moreover, the steps of analysis were also reduced, providing greater ease of use and rapid analysis.

3.3.2. Accuracy and precision

Percent recovery was investigated to evaluate the accuracy, and repeatability and intermediate precision were evaluated for precision with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) validation of analytical procedures [41,42]. These were studied by spiking standard BKC concentrations (2.0, 4.00, 8.0, and 12.0

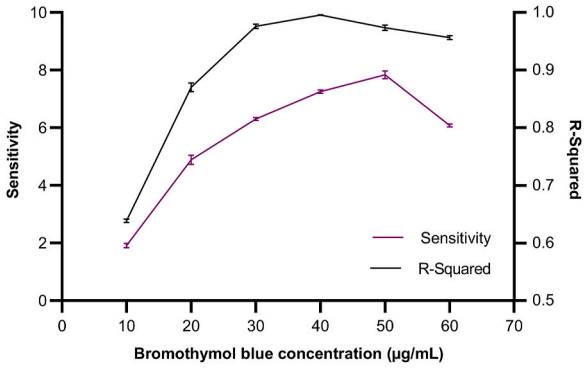

Fig. 4. Sensitivity and R^2 of linear calibrations with concentrations of BKC in the range of 0–24.0 μg/mL obtained using different concentrations of BTB in 0.50 M phosphate buffer at pH 7.5.

Table 2
Analytical characteristics of the proposed procedure compared with the conventional spectrophotometric methods.

Technique	Chromogenic agent	Linear range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)	Reference
Conventional Spectrophotometric method	Eosin Y and Triton X-100	0.5–10 of TDBAC	0.53	1.77	[14]
	Bromothymol blue	2.79–130 of BKC	2.79	8.45	[18]
	Bromocresol green	2.38–145 of BKC	2.38	7.20	
	Bromophenol blue	7.12–95 of BKC	7.12	21.56	
	Xylenol orange	2.41–115 of BKC	2.41	7.30	
Digital image processing	10,12-pentacosadiynoic acid and dimyristoyl phosphatidylcholine	90.52–434.52 of DDAC	14.48	47.07	[25]
			8.09	28.30	
		101.08–808.6 of BAC	27.20	85.00	
		119.0–679.98 of CPC			
	Eosin	0.4–10 of BKC	0.1	0.4	[24]
	Bromothymol blue	2.0–24.0 of BKC	1.0	3.2	Present work

*BKC: Benzalkonium chloride.
TDBAC: Tetradecyldimethyl(ethylbenzyl) ammonium chloride
DDAC: Didecyl dimethyl ammonium chloride
BAC: Benzyldimethyltetradecyl ammonium chloride
CPC: Cetylpyridinium chloride

μg/mL) in the sample with ten replications three times daily for three days. Percent recovery and RSDs for repeatability and intermediate precision are summarized in Table S1. Recoveries were calculated as follows: %recovery $= (C_f - C_u) \times 100/C_A$, where C_f is the concentration of BKC found for the fortified sample, C_u is the BKC concentration present originally for the unfortified sample, and C_A is the added concentration of standard BKC. The results showed that the percentage of recovery was 98–109%. The RSDs showed excellent repeatability and intermediate precision over three days ranging from 2–4, and 2–5%, respectively.

3.4. Interference

Following ICH Q2(R2) regarding interference described that the presence of other substances (such as impurities, degradation products, related substances, matrix, or other components present in the operating environment) must not impact the identification and/or quantification of an analyte in order to demonstrate specificity or selectivity [42]. In this work, these substances, which may be interfering compounds, were investigated. Investigations were made into the active substance, which was used in disinfectants functioning with BKC, like ethanol. The ethanol concentration of 70% w/v, which existed in the common commercially available disinfectants, was mixed with 0.5% BKC for the investigation. Other active compounds and components in commercial disinfectants, including sodium hypochlorite, peracetic acid, iodine, and chloroxylenol, which may contaminate in the operating environment, were also investigated. The concentrations of sodium hypochlorite, peracetic acid, iodine, and chloroxylenol found in the ready-to-use commercially available disinfectants were investigated at 0.21, 0.16, 1, and 0.12%, respectively. A spiking experiment of species into a matrix was selected for investigation and demonstrated sufficient non-interference and verification of accuracy/recovery following recommendations in ICH Q2(R1) [41]. The concentration ratios of the interfering compounds to BKC, found in real applications, were used to investigate by adding an interfering compound into 10.0 μg/mL BKC. The recovery percentage for each interfering substance that covered a specification acceptance criterion following ICH Q2(R2) is shown in Table S2. The results showed that the proposed procedure could accurately detect the concentration of BKC adulterated with 70% ethanol. This implies its potential

Table 3
Determining BKC concentration with the proposed procedure and titration method in samples.

Sample	Active pharmaceutical ingredients Concentration of BKC (%w/v)		Sample	Preservative Concentration of BKC (%w/v)	
	Proposed procedure[a]	Titration[b]		Proposed procedure[a]	Titration[b]
S1	82 ± 2	81 ± 1	S7	0.02 ± 0.0004	0.02 ± 0.0003
S2	82 ± 2	82 ± 2	S8	0.01 ± 0.0003	0.01 ± 0.0001
S3	82 ± 3	82 ± 1	S9	0.02 ± 0.0003	0.02 ± 0.0002
S4	0.4 ± 0.01	0.42 ± 0.01	S10	0.02 ± 0.0002	0.02 ± 0.0001
S5	2.1 ± 0.1	2.08 ± 0.04	S11	0.02 ± 0.0005	0.02 ± 0.0003
S6*	0.4 ± 0.01	0.41 ± 0.01			

*The sample is the BKC-based formulation with alcohol as an additional active substance.
[a] The dilution factors of S1–S3 were 80000, the dilution factors of S4 and S6 were 410, the dilution factor of S5 was 2000, and the dilution factor of S7–S11 were 10.
[b] The dilution factors of S1 – S3 was 10000, the dilution factors of S4 and S6 were 50, the dilution factor of S5 was 250, the dilution factors of S7–S9, and S11 were 25, and the dilution factor of S10 was 33.

applicability to disinfectant formulations that include a combination of ethanol and BKC. Furthermore, the precision of BKC evaluation remained unaltered even in the presence of contamination from substances such as sodium hypochlorite, peracetic acid, iodine, or chloroxylenol. This suggests that these compounds did not cause any interference in actual practical usage.

3.5. Application to medical disinfectant formulations

The proposed procedure was applied to detect BKC in three commercial pharmaceutical materials and three commercial finished products. The results showed no significant differences ($p < 0.05$) between the proposed procedure and the reference titration method, as summarized in Table 3. It succeeded in quantitatively controlling the concentration of BKC in pharmaceutical materials and disinfectant products as perfectly as the reference method and those of the related publications [34,35]. Comparing related colorimetric smartphone-based strategies for QACs, the proposed procedure reduced the need for liquid-liquid solvent extraction and the fabrication and immobilization of a chromogenic agent on a paper-based surface. In addition, this increases the high-throughput sample and prevents errors from collating with standards that are not in the same frame. The proposed procedure was advantageous according to the guidelines of green chemical analysis, including using less analytical volume (250 μL), excluding solvent extraction, and using a smartphone as a commonly available detector. Furthermore, it demonstrated competitive benefits in rapid operation, providing high sample throughput with a simple procedure. The proposed procedure operated within 60 of the 96 wells on a plate in a single-shot photograph. Four wells were allocated for the standard, while the remaining 56 were used for 28 samples in duplicate. For the operation, an eight-head micropipette was used for solution handling, leading to the analytical operation being completed in 2 min and the evaluation taking 10 min. Therefore, the proposed procedure took only 12 min for the analysis of 28 samples in duplicate. The spectrophotometric method [24] required 4 min for each sample: 1 min for ion-pair formation, 2 min for extraction, and 1 min for measurement. As a result, the analytical process for 28 duplicate samples took 4 h without evaluation. In comparison to the methodologies for detecting BKC in pharmaceuticals (conventional spectrophotometric method and HPLC), the proposed procedure could reduce the cost of chemicals, equipment, and overhead, as well as the time it takes to clean the equipment and issues related to waste. In addition, the proposed procedure can be carried out at the site. The comparison regarding limitations, costs, and environmental impact was summarized in Table S3.

3.6. Greenness and sustainability study

The greenness of the proposed procedure was assessed via the Green Analytical Procedure Index (GAPI) [43] and Analytical GREEnness (AGREE) [44] compared with the spectrophotometric method [24] and the reference titration method [45]. The evaluation criteria of GAPI, involving sample preparation using chemicals, solvents, and instruments, were divided into three levels: red, yellow, and green. The overall GAPI of the proposed procedure was more directional toward green than the spectrophotometric method and the reference method. The proposed procedure met the criteria of GAPI: no preservation, transport, and storage of samples; use of less volume of reagent and waste; use of a slightly toxic and high-safety reagent; and less energy consumption. The GAPI picograms obtained from the assessment of the proposed procedure, the spectrophotometric method, and the reference method are shown in Fig. S6. The AGREE, based on the assessment criteria from the 12 principles of green analytical chemistry, was also used to evaluate the greenness of the proposed procedure. The proposed procedure met the criteria of GREEnness regarding avoiding sample treatment, saving energy, reducing the use of samples and reagents, avoiding the generation of a large volume of waste, eliminating, or replacing toxic reagents, and increasing the safety of the operator. The results showed that the degree of greenness of the proposed procedure was higher than that of the spectrophotometric method and the reference method, as shown in Fig. S6. Furthermore, the sustainability of the proposed procedure was assessed using the Need, Quality, and Sustainability (NQS) index [46] in comparison with the spectrophotometric method and the reference method. The results exhibited a similar trend to the greenness, as shown in Fig. S7, where the proposed procedure provided a higher NQS index than the spectrophotometric and the reference methods, respectively. It demonstrated that the proposed procedure could be used as an alternative technique with a greener and more sustainable approach for qualitative control of BKC-containing products in community memorials or health-promoting hospitals in primary healthcare settings, especially in developing countries.

4. Conclusion

A sustainable procedure offering rapid and environmentally friendly determination of benzalkonium chloride (BKC) is available by utilizing a microwell plate platform with smart detection. This is based on the hydrophobic ion pair of BKC and a commonly commercially available bromothymol blue indicator. The proposed procedure shows excellent potential for cost-effective, high-throughput applications in remote areas with limited resources, infrastructure, and services for determining BKC in pharmaceutical products required by hospitals at all levels in Thailand and developing countries. The results from 11 samples, which included disinfectant materials, ophthalmic drops, and nasal sprays containing 0.01–82% w/v BKC, showed no significant differences when compared to titration as a reference method. The evaluation of greenness and sustainability through the Green Analytical Procedure Index, Analytical GREEnness, and the Need, Quality, and Sustainability Index demonstrates an edge over conventional and reference methods. Therefore, it would be appropriate to regulate the concentration of active compounds in pharmaceutical products and supplies in places providing primary medical care, including community memorials and health-promoting hospitals. This constitutes the development of sustainable pharmacologic analysis for infection prevention and control and to promote the SDGs, specifically Goal 3: Good health and well-being, involving strengthening the capacity of all countries, in particular developing countries, for early

warning, risk reduction, and management of national and global health risks.

Data availability

The original contributions generated for this study are included in the article; the data presented in this study are available on request from the corresponding author.

CRediT authorship contribution statement

Suphakorn Katib: Data curation, Formal analysis, Methodology, Writing – original draft. **Sutasinee Apichai:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **Thanawat Pattananandecha:** Formal analysis, Investigation. **Jutamas Jiaranaikulwanitch:** Writing – review & editing. **Busaban Sirithunyalug:** Writing – review & editing. **Kate Grudpan:** Writing – review & editing. **Chalermpong Saenjum:** Conceptualization, Funding acquisition, Project administration, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28965.

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