Application of Enzymatic Photometric Kinetic Method for Determination of Benzalkonium Chloride in Various Dosage Forms

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A kinetic-photometric method for accurate and sensitive determination of benzalconium chloride has been described. The method is based on inhibition of enzymatic hydrolysis of acetylcholine by enzyme acethylcholinestherase reaction. The amount of benzalconium chloride was determined by the degree of inhibition of the enzymatic reaction, which was evaluated by the residual unreacted substrate - acetylcholine. Determination of the residual amount of acetylcholine in the reaction mixture was performed by a kinetic-photometric method using an indicator oxidation reaction of p-phenetidine with peracetic acid, which is formed during the auxiliration reaction of perhydrolysis with addition of excess hydrogen peroxide in the reaction mixture over a period of time. The inhibition degree –concentration plot is linear over the range of $1.4 \cdot 10^{-6}$ – $7.0 \cdot 10^{-6}$ mol/ L with correlation coefficient of 0.998. The LOQ was $1.9 \cdot 10^{-6}$ mol/L. The different experimental parameters pH, buffer solution was carefully studied and optimized. The proposed method has been successfully applied to pharmaceutical formulations. Statistical comparison of the results with a good established reported method showed excellent agreement and proved that there is no significant difference in the accuracy and precision. For "Virotec-intim" preparation RSD was 3.2% (δ=- 0.3%).*

Keywords: benzalkonium chloride (BAC), cholinesterase (ChE), "Virotec-intim"

Benzalkonium chloride (BAC) is a quaternary ammonium compounds with disinfection surfactants and antiseptic properties. This active agent has a number of advantages: it can be used in the presence of patients (personnel) in therapeutic-prophylactic institutions and similar, do not cause upper respiratory tract irritation, they, do not have strong odors, and they do not decolorize materials. Same disinfectants based on surfactants (SA) are very convenient for routine and prophylactic disinfection [1]. At the same time, it is often used as a preservative in various dosage forms BAC. The BAC estimation as cationic surfactant antiseptic, disinfectant, and preservative component in medicinal formulations is of important practical interest. The complexity and laboriousness of existing chemical and microbiological methods prevents operational monitoring of their concentrations at local utilization sites [2].

BAC is a mixture of alkylbenzyldimethyl ammonium chlorides with the general formula $[C_6H_5CH_2N(CH_3)_2R]^+Cl^-$, where $R = C_nH_{2n}+1$, n = 8, 10, 14, 18 [3].

Several techniques have been proposed for the quantification of BAC in pure, pharmaceutical dosage forms and in biological fluids, voltammetry [4], chromatography [5], capillary electrophoresis [6-7], atomic absorption spectrometry [8], fluorimetry, [9]; and spectrophotometry [10, 11]. The voltammetry and chromatographic, electrophoretic, atomic absorption spectrometric and chemiluminometric methods utilized dedicated and/or expensive instruments that are not available in most quality control laboratories' analytical technique. Photometry is considered the most convenient analytical technique, because of its inherent simplicity, low cost, and wide availability in most quality control laboratories. However, few spectrophotometric methods were reported for the determination of BAC in its pharmaceutical dosage forms [12, 13]. These methods were associated with some major drawbacks such as decreased selectivity due to measurement in ultraviolet region, and/or decreased simplicity of the assay procedure e.g. tedious precipitation, or liquid–liquid extraction steps are based on the formation of ion-pair complex. The kinetic spectrophotometric method offers an easy, less time consuming, sensitive analysis, by using simple and available reagents, which are able to be used for routine determinations of drug substances. The kinetic spectrophotometric analysis is one of the major interests of analytical pharmacy. This work summarizes the results of much before researches and shows the possibility of determining BAC in various medicinal forms (aerosols, solution for external use, eye drops); it has been proven that auxiliary substances present in the dosage form do not affect the results of determination of BAC in the preparation. It is also shown the possibility of using the developed method to determine BAC as an active pharmaceutical ingredient ("Cutasept F", "Virotec-intime") and as a preservative - "Patanol", 10ml, "Optrex", 10 ml, "Aqua-rinosol", "Apisal".

The amount of BAC was determined by the degree of inhibition of the enzymatic reaction, which was evaluated by the residual unreacted substrate -

acetylcholine. Determination of the residual amount of acetylcholine in the reaction mixture was performed by a kinetic-photometric method using an indicator oxidation reaction of *p*-phenetidine with peracetic acid, which is formed during the auxiliary reaction of perhydrolysis with addition of excess hydrogen peroxide in the reaction mixture over a period of time. The reaction is followed up on spectrophotometer using quartz cells of 1-cm path length. The tangency methods were adopted after full investigation and understanding of the kinetics of the reaction. The proposed method is simple, accurate and sensitive.

Materials and methods

Apparatus: Unicam SP-800 instrument, Beckman DB spectrophotometer. Air thermostat TS-80m. Measurements were performed at 37°C, the temperature of the reaction mixture was provided by water thermostating, the pH of the solutions was monitored by a glass electrode ESL-43-07 on a I-nometer "I-130" NPO "Analitpribor".

Pharmaceutical preparation antiseptic solution "Virotec intim". It contains: BAC 20 mg in 100 g of solution. Manufacturer pharmaceutical factory "Luganskpharmacy", "Valartin Pharma" s.10314.

Acetylcholine Chloride (Pharm Grade) - 0.02 g per amp/5 mL, produced by "VECTOR" – State Scientific Centre of virology and biotechnology in Russian Federation" (Russia).

Sodium Phosphate dibasic, $Na_2HPO_4 \cdot 12H_2O$ (puriss.), CAS 7558-79-4, produced by «ReaChem», Kharkiv, Ukraine.

Dry protein drug of cholinesterase from horse serum - 80 mg/fL (VI class), 27 AU/mg, produced by SMU "Biomed", Russia.

Remark: The catalytic activity of 1 unit (U) has such amount of the given enzyme preparation which coverts 1 μmole of the given substrate in 1 min at the given reaction conditions.

"Stabilized Hydrogen Peroxide 30-40%", (puriss.), (LLC "Inter - Synthes", Boryslav, Ukraine); the content of hydrogen peroxide was determined by SPU according to the monograph "High-test hydrogen peroxide solution 27.5-31.0%

High purity double distilled water was used throughout.

Preparation of 0.2 M Phosphate buffer solution (pH 8.35) 35.75 g Disodium Hydrogen Phosphate dodecahydrate (grade «p.a.»), crystallized ($Na_2HPO_4 \cdot 12H_2O$) was dissolved in 500 mL flask using double-distilled water. 19 mL of 0.1 M solution of Hydrochloric acid solution was added. pH of the final solution was controlled potentiometrically.

Preparation of 10% (wt) Hydrogen peroxide solution. The solution was prepared by the appropriate high-test hydrogen peroxide dilution with double-distilled water. The content of hydrogen peroxide in the working 10% solution was determined by permanganatometric method.

Preparation of 1% (wt) p-Phenetidine hydrochloride solution. *p*-Phenetidine hydrochloride (*p*-Ph or Ph), was extracted from the base by Hydrogen Chloride precipitation in the chloroform solution. 1.00 g of p-phenetidine hydrochloride was dissolved in 80 mL of double-distilled water in 100 mL volumetric flask and after the dissolution brought to the mark.

Preparation of Acetylcholine chloride solution (ACh). The ampoule's content 0.02 g of the Acetylcholine Chloride was dissolved in 20 mL of double-distilled water. For that end, an ampoule was opened, 4.0 mL of water was pipetted, and shake until Acetylcholine was completely dissolved. Then the Acetylcholine solution was transferred into 20 mL volumetric flask and the volume was brought to the mark with double-distilled water.

General recommended procedure

The first part: 10.0 mL portion of 0.2 M phosphate buffer solution (pH = 8.3) was transferred into 20 mL graduated test tube with ground plug, 1.0 mL of 1 mg/mL acetylcholine solution was added and then 3.0 mL of 10% hydrogen peroxide solution was added and the stopwatch was switched on. After that the solution was shake thoroughly and was thermostated for 10 min. Then 1.0 mL of 1% *p*-phenetidine solution was added and brought to the mark with double distilled water in a 20 mL volumetric flask. The stopwatch was switched on and every minute each solution was scanned photometrically for 5 min on spectrophotometer 1.0 cm cuvette were used. The rate of reaction was determined as a slope of the kinetic curve A *vs* time [(ACh + H_2O_2) + *p*-Ph] ($tg\alpha$, min^{-1}).

The second part: 10.0 mL portion of 0.2 M phosphate buffer solution (pH = 8.3) was transferred into 20 mL graduated test tube with ground plug. After that accurate 2.0 mL portion of Cholinesterase was added, then 3.0 mL of 10% hydrogen peroxide solution was added while stirring, shaked up thoroughly and kept for 10 min in a thermostat. Then 1.0 mL of 1% *p*-phenetidine solution was added and brought to the mark with double distilled water. The stopwatch was switched on and every minute the solution was scanned photometrically for 15 min on spectrophotometer, using 1.0 cm cuvette were used. Buffered solution with double distilled water as reference solution was used. The rate of reaction was determined as a slope of the kinetic curve A *vs* time, (ChE) + ACh)t + H_2O_2 + *p*-Ph, (min^{-1}) switched on a stopwatch, and thermostated for 10 min, [(ChE) + ACh) + H_2O_2 + *p*-Ph] $tg\alpha_{min}$ (min^{-1}).- (This phase of the study provided a baseline. By further subtracting the value of this value, we subtracted the influence of the conditions on the conduct of the experiment).

The third part: 10.0 mL portion of 0.2 M of phosphate buffer solution (pH = 8.3) was transferred into 20 mL graduated test tube with ground plug. The accurate volumes (from 0.40 to 3.20 ml) of test solution of BAC (WSS or model solution or a

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reference solution) were added into a standard flask. 2.0 mL of Cholinesterase was added while stirring, the stopwatch was switched on, every solution was shaked up thoroughly and thermostated for 10 min, then quickly 1.0 mL of 1 mg/mL Acetylcholine solution was added and the stopwatch was switched on, shaked thoroughly and thermostated for 10 min again, then 3.0 mL of 10% Hydrogen peroxide solution was added, kept for 10 min in thermostat and after 1.0 mL of 1% *p*-phenetidine solution was added and brought to the mark with double distilled water. The stopwatch was switched on and every minute the solution was scanned photometrically for 15 min on spectrophotometer, using 1.0 cm cuvette were used. Every time before the experiment the test tube content was mixed and plugged thoroughly. Buffered solution with double distilled water as reference solution was used. The rate of the reaction was determined as a slope of the kinetic curve A *vs* time) [(ChE + Inh) + Ach] +H_2O_2 + *n*-Ph], $tg\alpha_{ef}$ (min^{-1}).

Preparation of BAC solutions: working standard solution (WSS): 0.05000 g (accurately weighed) of the working standard sample (WSS) of BAC is dissolved in double distilled water in a volumetric flask with a capacity of 100.0 mL and the volume of the solution is there by reduced to the mark (concentration of 500 μg/mL). Pipette 4.00 mL of the resulting solution, transfer to a volumetric flask of 100.0 mL capacity and bring the volume of the solution with double distilled water to the mark at a temperature of 20ºC, mix thoroughly (concentration 20 μg/mL);

model solution: 1.0058 g (accurately weighed) of the test substance powder dissolved in 40 ml of double distilled water in a 100.0 ml volumetric flask and dilute with water to the mark. Withdraw with a pipette 4.00 ml of the obtained solution and transfer into a 100.0 ml volumetric flask. Then adjust the volume with double distilled water to the mark at 20ºC, plug the flask and mix thoroughly.

In order to estimate the accuracy and precision of the proposed method, standard solutions of 2.8 μmol/L, 4.2 μmol/L, 5.6 μmol/L, 7.0 μmol/L, were analysed according to the recommended procedure. For this purpose, five replicate determinations of each concentration were prepared.

According to the obtained data, the dependence of the degree of inhibition on the concentration of BAC in absolute and normalized coordinates (normalization was performed by comparison solution). The least squares method calculated the parameters of the obtained calibration dependences, which are given in Table 1.

Table data shows that the parameters constructed in the coordinates of the calibration linear dependence of the BAC determination are characterized by satisfactory linearity for a given range and satisfy the requirements of the maximum permissible uncertainty. RSD ≤2.84 (δ <RSD).

Result and discussion

Optimization of parameters

The results of the experiments allowed to develop a new method for the quantitative determination of ChE inhibitors – BAC in aqueous solutions on the principle: "Inhibitor concentration – the rate of formation of a colored product". The optimal conditions for the course of the enzyme reaction were studied: the order of mixing, concentration, time of incubation, the influence of the pH of the medium and the nature of the buffer solution.

In the course of work have been showed the dependence of the rate of the enzymatic reaction on the concentration of the substrate of acetylcholine. It is according to the obtained data in the range of concentrations ACh (0–3.3)×10^{-4} M there is a linear relationship between the rate of the enzymatic reaction and the concentration of the substrate, within the concentration ACh (3.3–8)×10^{-4} M there is a decrease in the reaction rate, probably due to the saturation of the substrate of the active centers of the enzyme, which agrees well with the literature [14]. In subsequent studies, the optimal concentration of ACh was taken 3.3×10^{-4} M.

Table 1. The results of BAC assay in standard solution.

Taken	Found*	RSD, %	Recovery, %	δ**
C(BAC)·10^6, mol L^{-1}				
2.80	2.83±0.10	2.84	101.1	+1.07
4.20	4.22±0.08	1.53	100.5	+0.48
5.60	5.65±0.08	1.14	100.9	+0.89
7.00	7.05±0.08	0.91	100.7	+0.71

* n = 5; P =0. 95 %. RSD = Relative standard deviation; ** δ = ($\overline{x}$ – μ) 100%/ μ

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Dependence of the rate of formation of the colored product of the indicator reaction of oxi-dation of p-Ph on the holding time

Figure 1 showed the dependence of the formation rate of the colored product of the oxidation reactions indicator p-Ph on the exposure time. According to the results, the incubation time was 15 min.

Fig. 1. Dependence of the rate of formation of the colored product of the indicator reaction of oxidation of p-Ph on the holding time of ChE + ACh before the addition of p-Ph, min: 1 - 15; 2 - 10; 3 - 5; 4 - 0; c (p-Ph) = 0.05%, $w(H_2O_2)$ = 1.5 %;T = 310 K, pH 8.4.

Effect of ACh concentration

According to the obtained data in the range of concentrations ACh (0–3.3)×10^{-4} M there is a linear relationship between the rate of the enzymatic reaction and the concentration of the substrate, within the concentration ACh (3.3–8)×10^{-4} M there is a decrease in the reaction rate, probably due to the saturation of the substrate of the active centers of the enzyme, which agrees well with the literature [14]. In subsequent studies, the optimal concentration of ACh was taken 3.3·10^{-4}M.

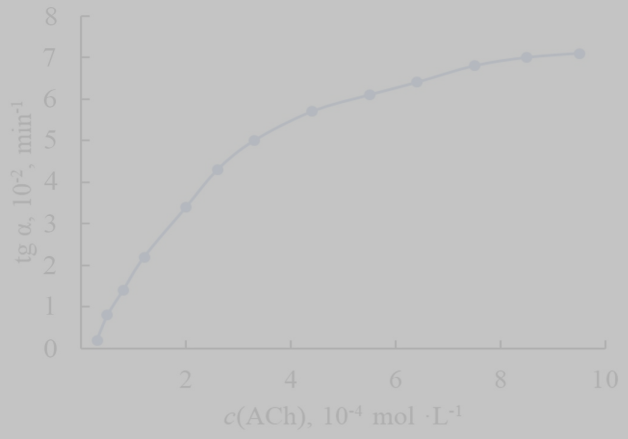

Fig. 2. Dependence of the conditional rate of the enzymatic reaction on the concentration of acetylcholine. C(ChE) = 0.24 mg / ml, w(p-Ph) = 0.03%, $w(H_2O_2)$ = 1.5%; T = 310 K, pH 8.4

Effect of AChE concentration

Fig. 3 have showed dependence of the rate of the enzymatic reaction vs the concentration of ChE.

The linear dependence of the reaction rate on the enzyme concentration is observed in the concentration range of 0.12–0.36 mg/ml (This is 0.25–0.75 ml 4 mg / ml ChE). The experiments were performed at the concentration of ChE 0.24 mg / ml. This concentration corresponds to the midpoint of the line segment.

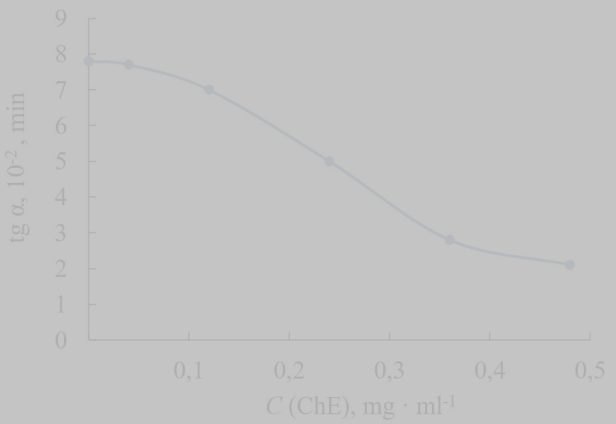

Fig. 3. The influence of ChE concentration on the conditional rate of the enzymatic reaction. c(Ach) = 3.3×10^{-4} M, w(p-Ph) = 0.05%, $w(H_2O_2)$ = 1.5%; T = 310 K, pH 8.4.

Effect of hydrogen peroxide concentration

The results of the study of the effect of hydrogen peroxide concentration are shown in Fig. 4.

The results of the study of the effect of hydrogen peroxide concentration are shown, that at concentration of hydrogen peroxide ≥1.5 %, the rate of the indicator reaction becomes maximum and does not change in the future. The optimal concentration of hydrogen peroxide is 1.5 %.

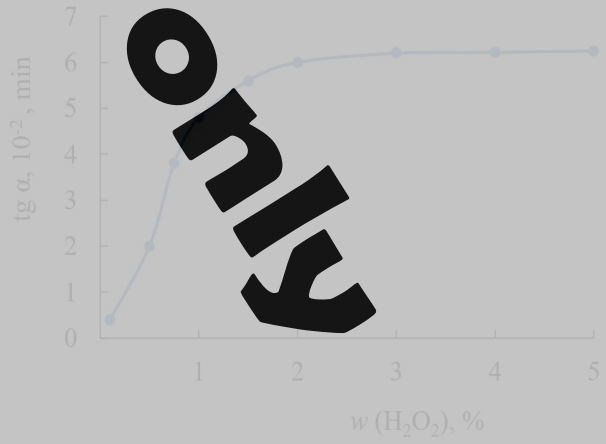

Fig. 4. Influence of hydrogen peroxide concentration on the conditional rate of indicator reaction. c (ChE) = 0.24 mg/ml, c (Ach) = 3.3×10^{-4} M, c (p – Ph) = 0.05 %, T = 37 °C, pH 8.4.

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Effect of p-Ph. Excess *p*-Ph (0.05%) was used to ensure the conditions of the pseudo-first order course of the *p*-Ph oxidation reaction.

Evaluation of the biochemical methods

The optimal conditions for the biochemical reaction were determined: concentrations of the enzyme cholinesterase and the substrate – acetyl-choline. Analysis of the literature [15-17] shows that the optimal conditions for the biochemical reaction of enzymatic hydrolysis of acetylcholine by cholinesterase are: pH 8.2–8.5; substrate concentration $3 \cdot 10^{-4}$ M. Although for cholinesterase, in contrast to acetylcholinesterase, inhibition by excess substrate is not the main interfering factor in the reaction, we set out to find out at what maximum substrate concentration it is still possible to apply the linearization of the Michaelis-Menten equation [19]:

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \frac{1}{[S]}$$

which describes a rectilinear dependence,

$$y = a + bx,$$

where $y = 1/v$; $a = 1/V_{max}$ (a segment intersecting a line on the y-axis); $b = K_м / V_{max}$ (the slope of the line); $x = 1/[S]$ (Fig. 5).

The need for a biochemical reaction with the maximum possible concentration of the substrate follows from our proposed principle of determining the activity of the enzyme – the concentration of undigested substrate: the smaller the substrate will be subjected to enzymatic hydrolytic cleavage, the greater its amount will remain unhydrolyzed – will lead to the formation of a more intense color of the oxidation product of the indicator reaction for a certain predetermined period of time.

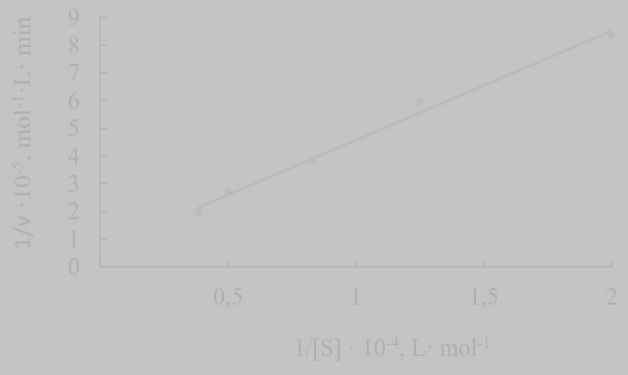

Fig. 5. Linearization of the Michaelis-Menten equation.

Dependence of the inverse rate of the enzyme hydrolysis reaction on the inverse concentration of the acetylcholine substrate were $1/v = 39.556 \cdot [S]^{-1} + 0.6091 \cdot 10^{-5}$ (R = 0.0991). According to dependence, the values of the maximum enzyme hydrolysis rate V_{max} and Michaelis constant K_m were calculated, equal to $1.64 \cdot 10^{-5}$ mol·L^{-1}·min^{-1} and $6.49 \cdot 10^{-4}$. The obtained data confirm the laws of kinetics of enzymatic hydrolysis.

Quantitative determination of BAC in antiseptic solution "Virotec intim".

Kinetic curves, the dependence of light adsorption on at different concentrations of BAC, under optimized conditions, were processed (Fig. 6). Initially, the degree of inhibition of the enzymatic hydrolysis of Acetylcholine *U*, %, in the presence of BAC was calculated using the formula:

$$U = \frac{tg\alpha_{C_i} - tg\alpha_{min}}{tg\alpha_{V_{max}} - tg\alpha_{min}} \cdot 100\%$$

where $tg\alpha_{C_i}$ – slope of the kinetic curve A *vs* time for a procedure [(ChE + Inh) + Ach] +H_2O_2 + *p*-Ph, (min^{-1}); $tg\alpha_{min}$ – slope of the kinetic curve A *vs* time for a procedure [(ChE + ACh) + H_2O_2 + *p*-Ph], (min^{-1}); $tg\alpha_{V_{max}}$ – slope of the kinetic curve A *vs* time for a procedure [(ACh + H_2O_2) + *p* -Ph], (min^{-1})·

According to the obtained data, the dependence of the degree of inhibition of ChE on the concentration of BAC was constructed. The method of the smallest squares was calculated metrological parameters of obtained graduated dependence (angular coefficient *b* and its standard deviation S_b, free member *a* and its standard deviation RSD. (Regression equations calculated from calibration graphs along with standard deviation of the slope (Sb) and intercept (Sa) on the coordinate and the standard deviation of residuals (Sy/x). The high values of the correlation coefficients of regression equations indicate good linearity.

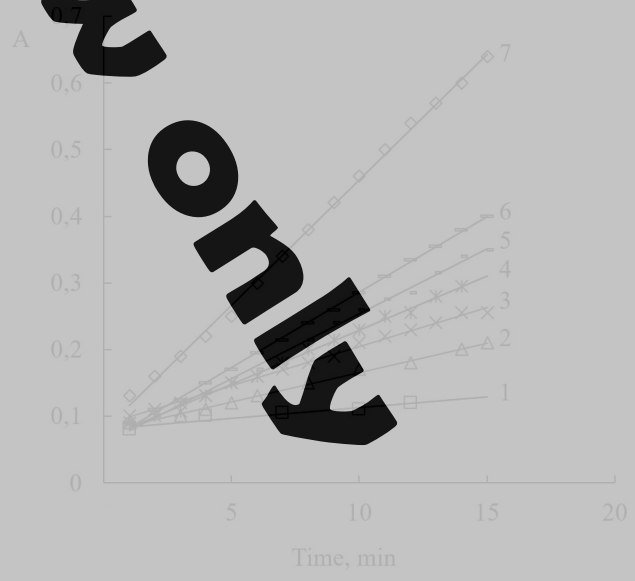

Fig. 6. Kinetic curves of couple oxidation of 0.05% *p*-phenetidine by hydrogen peroxide in the system: *1* – ACh+ChE, *2- 6* –ACh+(ChE+BAC), *7* – ACh. *w*(ACh) = 0.1%; [ChE] = 0.25 U; w(H_2O_2) = 1.5%; c(BAC), 10^{-5} M: 2–1.4; 3– 2.8; 4–3.4; 5 –5.6; 6 – 7.0.

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The calibration curve was linear in the concentration range of (1.4–7.0)×10^{-6} M of BAC with a correlation coefficient of 0.998. LOQ was calculated as 20% degree of ChE inhibition and was 1.9·10^{-6} M.

The performances of the proposed method were verified on samples containing from 1.00 mL to 5.00 mL of BAC solution (WSS) according to the General procedure.

Fig. 7. Curve of the graduated dependence of inhibition degree *vs* BAC concentrations.

The calibration graph was constructed using the values obtained from five replicate samples of the same BAC content. The linear regression equation was as follows: $U(\%) = 0.74c \cdot 10^7 + 5.54$ (R^2=0.998) (where «*c*» is the BAC concentration expressed in mol·L^{-1}; $b = (0.74 \pm 0.04) \cdot 10^7$; $a = 5.5 \pm 2.0$). The calibration curve was linear in the concentration range of (1.4–7.0)×10^{-6} M of BAC.

Recommended procedure for the analyses of antiseptic solution "Virotec -intime"

1.00 mL of the solution "Virotec-intime" was transferred into 5 mL volumetric flask and brought to the mark with double distilled water.

Known volumes of the sample solution (1.00 mL) were analyzed by the proposed kinetic-photometric method (according to general procedure) (Table 2).

1.00 mL of the solution "Virotec" was transferred into 5 mL volumetric flask and brought to the mark with double distilled water.

Known volumes of the sample solution (1.00 mL) were analyzed by the proposed kinetic-photometric method (according to general procedure).

Accuracy and reliability of the proposed method were further ascertained by performing recovery experiments. To a fixed amount of the drug in formulation (pre-analyzed), pure BAC at three different levels of concentration was added, and the total content of BAC was determined by the proposed method.

Table 2. The results of analysis of antiseptic solution "Virotec -intime".

The active ingredient content in "Virotec-intime" BAC, %	Found, %	Metrological characteristics (n=5; P=0.95)
0.02*	0.0198 0.0195 0.0194 0.0210 0.0200	$\bar{x}$=*0.0199%* *S*=0.00064 $\bar{x}$=*0.0003* $\Delta\bar{x}$=0.0008 *RSD=3.2* *(δ*=- 0.3%)*

10 mL of phosphate buffer 0.2 mol L^{-1} solution were transferred into each graduated test tube with ground plug gradually, 0.50 mL of solution "Virotec" sample solution and 1.00 mL, 1.50 mL, 2.00 mL of Benzalkonium chloride solution (WSS) were added respectively. After seeing General procedure. Each test was repeated five times (Fig. 8.). The results compiled in Table 3 show that recoveries were in the range 100.1-101.2% indicating that commonly active pharmaceutical ingredient (API) did not interfere the kinetic photometric determination of the BAC. Thus, the proposed method shows the accuracy and reliability results: RSD < 2.8% and (δ < RSD).

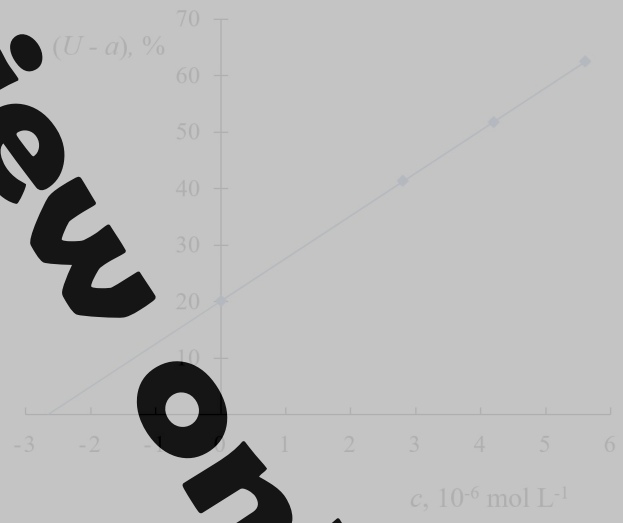

Fig. 8. Results of Recovery Study Using Standard-Addition method.

The universality of the method has been proposed in this article of quantitative determination of BAC is confirmed by the results of the experiments shown in Table 4.

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Table 3. Precision and Accuracy of the proposed of analysis BAC method.

Taken	Added	Found	Found BAC*	RSD, %	Recovery BAC, %	δ, %
C(BAC)·10⁶, mol·L⁻¹			(±△)·10⁶, mol·L⁻¹			
2.80**	-	2.82	-	2.80	100.1	
2.80	2.80	5.65	(2.85±0.10)	2.82	100.8	1.79
2.80	4.20	7.05	(4.25±0.07)	1.33	101.2	1.19
2.80	5.60	8.45	(5.65±0.08)	1.14	100.9	0.89

Notes. *Mean of five measurements (P=0.95). **Certificate Information.

Table 4. The BAC determination in different drugs forms.

Medicine	Medicine forms	Manufacturer pharmaceutical factory	The active ingredient content in BAC, %	Linear range, 10^{-6}, mol/ L	Found, BAC, (X ± ΔX), %	RSD, %	Ref.
"Apisal", 15 mL	nasal spray	Amman Pharmaceutical Industries, Jordan, Lot MC07	0.01	1.1-7.0	(0.0097 ± 0.001)	4.1	[20]
"Aqua-rinosol", 20 mL	nasal spray	"VECTOR" – State Science Center of virology and biotechnology in Russian Federation» (Russia), lot 061015	0.15	1.1-7.0	(0.150 ± 0.004)	1.9%	[21]
"Cutasept F", 50 mL	antiseptic solution	BODE Chemie GmbH, Humburg, lot 404580	0.020 – 0.05	0.5-7.0	0.0282 ± 0.001	2.85	[22]
"Virotec-intime", 50 mL	antiseptic solution	«Lugansk pharmacy», "Valartin Pharma", Ukraine lot.10314.	0.02	1.4-7.5	(0.0199 ± 0.008)	3.2	current article
"Patanol", 10mL	eye drops	15C6FB ALCON-COUVEREUR, B-2870, Puurs, Belgium	0.01	1.4-8.0	(0.0097 ± 0.005)	4.1	current article
"Optrex", 10mL	eye drops	Reckitt Benckiser Healthcare International,lot AS383	0.005	1.4-8.0	(0.004 ± 0.0003)	4.5	[23]

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Conclusion

The proposed method can be easily applied to the determination of BAC in pure and different dosage forms (antiseptic solution, eye drops, nasal spray) which do not require elaborate treatment of the analyte and tedious extraction of the chromospheres produced. The proposed method is sensitive enough to enable determination of a lower amount of the drugs. These advantages encourage the application of the proposed method in routine quality control of the investigated drugs in industrial laboratories. No interference has been observed with excipients found in drug formulations.

Reference

1. Percival, S.; Mayer, D.; Malone, M.; Swanson, T.; Gibson, D.; Schultz, G. Surfactants and their role in wound cleansing and biofilm management. *J. Wound Care*. 2017, 26 (11), 680–690. DOI: 10.12968/jowc.2017.26.11.680.

2. Mikhaleva, N.M.; Kulapina, E.G.; Mikhaleva, O.V. Estimation of cationic surfactants in medicinal formulations. *Pharm. Chem. J.* 2008, 42 (4), 224–227. DOI: 10.1007/s11094-008-0089-7.

3. Gilbert, E.A.; Guastavino, J.F.; Nicollier, R.A.; et al. Synthesis and Properties of Cleavable Quaternary Ammonium Compounds. *J. Oleo Sci.* 2021, 70(1), 59–65. DOI: 10.5650/jos.ess20216

4. Muthuraman, G.; Chandrasekara Pillai, K; Il-Shik Moon. Electrochemical Analysis of Aqueous Benzalkonium Chloride Micellar Solution and Its Mediated Electrocatalytic De-Chlorination Application. Catalysts. 2019, 9(1), 99. DOI: 10.3390/catal9010099

5. Shen, Y.; Xu, S.J.; Wang, S.C.; Tu, J.S. 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.B0920209

6. Altria, K.D.; Elgey, J.; Howells, J.S. Validated capillary electrophoretic method for the quantitative analysis of histamine acid phosphate and of benzalkonium chloride. *J. Chromatogr. B: Biomed. Sci. Appl.*, 1996, 686 (1), 111–117. DOI: 10.1016/S0378-4347(96)00036-9.

7. Hou, Y.H.; Wu, C.Y.; Ding, W.H. Development and validation of a capillary zone electrophoresis method for the determination of benzalkonium chlorides in ophthalmic solutions. *J. Chromatogr. A* 2002, 976 (1-2), 207–213. DOI: 10.1016/s0021-9673(02)00943-3

8. Brycki, B.; Małecka, I.; Koziróg, A.; Otlewska, A. Synthesis, Structure and Antimicrobial Properties of Novel Benzalkonium Chloride Analogues with Pyridine Rings. *Molecules* 2017, 22 (1), 130. DOI: 10.3390/molecules22010130

9. Kümmerera, K.; Eitela, A.; Brauna, U.; et al. Analysis of benzalkonium chloride in the effluent from European hospitals by solid-phase extraction and high-performance liquid chromatography with post-column ion-pairing and fluorescence detection. J. Chromatogr. A 1997, 774, (1–2), 281–286. DOI: 10.1016/S0021-9673(97)00242-2

10. Kovács-Hadady, K.; Fábián, 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.

11. Meshram, D.; Pater, P.; Varshney, P.; et al. Method development and validation of benzalkonium chloride in marketed formulation by UV-visible spectrophotometry using silver nitrate and eosin solution. *World J. Pharm. Pharm. Sci.* 2014, 3(2), 1481–1487.

12. Massouna, H.; Abdelrahman, M.; Mohamed, M. Determination of Azelastine Hydrochloride and Benzalkonium Chloride in Their Ophthalmic Solution by Different Spectrophotometric Methods. *World J. Appl. Chem*. 2017, 2 (2), 48–56.

13. Ma Weixing, Ma Ou Xiaodong, Liu Sha Yinghong. Two Spectrophotometric Methods for the Assay of Benzalkonium Chloride in Bandage Samples. *J. Surfactants Deterg.* 2013, 17 (1), 177–181. DOI: 10.1007/s11743-013-1446-4

14. Guilbault, G.G.; Sadar, M.H. Use of enzymes in Analytical Chemistry. Analytical use of enzymes. *Proceedings of the Analytical Division of the Chemical Society,* 1977, 14(10), 302–306. DOI: 10.1039/ad9771400302

15. Malevany, S.V.; Vodolazkaya, N.A.; Mchedlov-Petrosyan, N.O.; Orlov, V.D. Diacetyl fluoresceine as a fluorogenic substrate cholinesterase. *Kharkiv University Bulletin. Chemical Series*, 2000, 495, 6(29). (in Ukr.).

16. *Musil,* Y.; *Novakova, O.*; *Kunts, K. Modern biochemistry* in schemes. 2nd ed. Moskow, Mir 1984, 216 p. (in Russ.)

17. Masoom, R.S.; Zeid, A.A.; Nafisur, R. Analytical techniques in pharmaceutical analysis: A review. *Arabian J. Chem*. 2017, 10 (1), 1409–1421. https://doi.org/10.1016/j.arabjc.2013.04.016

18. Saroj, D.; Garg, N. Biochemical Methods of Analysis: Theory and Applications. Oxford, U.K.: Alpha Science International, 2010.

19. Guilbault, G.G.; Sadar, M.H. Use of enzymes in Analytical Chemistry. Analytical use of enzymes. *Proceedings of the Analytical Division of the Chemical Society*, 1977, 14 (10), 302–306. DOI: 10.1039/ad9771400302

20. Blazheyevsky, M.E.; Kovalska, O.V. Application of kinetic enzyme method for determination of benzalkonium chloride in aerosol preparation “Apisal®”. Modern aspects of drug development: theses add. International scientific-practical distance conference dedicated to the 100th anniversary of the Department of Analytical Chemistry of NUPh (April 16, 2021). Kharkiv, NFaU, 2021, 64. (in Ukrainian)

21. Koval'ska, O.V.; Blazheyevskiy, M.Ye. Application of the kinetic enzymatic method for benzalkonium chloride determination in aerosol preparation. Peer-reviewed materials digest (collective monograph) published following the results of the CXXXIV International Research and Practice Conference «Modern methods of ensuring health and quality of human life through the prism of development of medicine and biological sciences». November 2016, London, IASHE, 55–57.

22. Blazheyevskiy, M.Ye.; Koval'ska O.V. The enzymatic method for the quantitative determination of benzalkonium chloride in the antiseptic solution “CUTASEPT® F”. *J. Org. Pharm. Chem*. 2021, 19 (4), 33–39. DOI: 10.24959/ophcj.21.244359

23. Blazheyevskiy, M.Ye., Koval'ska, O. The determination of benzalkonium chloride in eye drops. *Ars Pharm*. 2019, 60(2), 79–84.

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