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Application Enzymatic Method for Analysis Vaginal Tabletas “Fluomizin”

Aplicación método enzimático para análisis comprimidos vaginales “fluomizin”

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Abstract

Introducción: Desarrollo y validación de un nuevo método cinético-fotométrico enzimático para la determinación cuantitativa de cloruro de decualinio en tabletas vaginales “Fluomizin”, basado en la inhibición de la reacción de hidrólisis enzimática de acetilcolina.

Método: El contenido de cloruro de dequalinio en las tabletas “Fluomizin” № 6 se determinó por el grado de inhibición de la reacción enzimática, que se evaluó por la cantidad residual de acetilcolina en la reacción bioquímica. Como indicador, se usó la reacción de oxidación de p-fenetidina con ácido peracético, que se forma durante la reacción auxiliar de perhidrólisis con exceso de peróxido de hidrógeno durante un cierto período de tiempo en la mezcla de reacción.

Resultados: Se determinaron las condiciones óptimas para la determinación: pH seleccionado 8,35, concentración de acetilcolina (0,05 mg / ml), colinesterasa (0,4 mg / ml), peróxido de hidrógeno (10%) y *p*-fenetidina (1%)tiempo de reacción (20 min).

Conclusiones: Un método enzimático cinético-fotométrico para determinar el contenido de cloruro de dequalinium en píldoras vaginales “Fluomizin” № 6. La validación del método desarrollado para parámetros tales como linealidad, precisión, precisión y límite de cuantificación. Se determinaron los parámetros cinéticos Km (constante de Michaelis-Menten) y Vmax (velocidad máxima).

Palabras clave: cloruro de dequalinio; fluomizina; método cinético-fotométrico enzimático.

Abstract

Introduction: Development of a new enzymatic kinetic-photometric method for analysis of Dequalinium chloride, based on the use of enzymatic hydrolysis of acetylcholine, for determine the amount of Dequalinium chloride in the vaginal tablets “Fluomizin” No 6.

Method: The amount of Dequalinium chloride (DCh) 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.

Results: Optimal conditions were determined: pH 8.35 was selected, influence of nature of buffer solution, concentration of acetylcholine (0.05 mg / ml), cholinesterase (0.4 mg / ml), hydrogen peroxide (10,0%) and *p*-phenethedine (1 %), the incubation time (10 min).

Conclusions: The procedure for determination of Dequalinium chloride content in the vaginal tablets “Fluomizin” No 6 was developed. The kinetic parameters Km (Michaelis–Menten constant) and Vmax (maximal velocity) were determined.

Keywords: Dequalinium chloride; Fluomizin; enzymatic-photometric method.

Introduction

Dequalinium chloride (DCh) is a chemically 1-[10-(4-amino-2-methylquinolin-1-ium-1-yl)decyl]-2-methylquinolin-1-ium-4-amine;dichloride. The molecular formula is $C_{30}H_{26}Cl_2N_4$ and molecular weight is 527.6 g / mol. Dequalinium chloride (DCh) is a quaternary ammonium cation that contains two quaternary quinolinium units linked by an N-decylene chain. It is an antiseptic and disinfectant agent with a broad bactericidal and fungicidal activity. It is most commonly available as a dichloride salt but is available as other various salts as well. It is used in wound dressings and mouth infections and may also have antifungal action, but may associate with skin ulceration. Dequalinium chloride is used as an active ingredient in tablets as Fluomizin for vaginal bacterial infections and in topical bacteriostatic formulation as Dequadin[1-6].

It is a white crystalline power, soluble in water, free soluble in methanol and acetonitrile. Thera are several methods for the determination Dequalinium chloride. Literature review reveals that there are few methods described for the estimation of DCh in biological fluid and even impurity of DCh and its tablet formulation are reported with respect to HPLC method which includes normal-phase HPLC and Reverse phase-HPLC, gas-liquid chromatograph. The literature search for spectrophotometric methods of dequalinium reports the need of chromophoric reagent and indication agent for its determination[7-12]. Thus, the development of simple, less time consuming and photometric method for the assay of Dequalinium chloride in tablets of "Fluomisin" is a very actual problem.

So, the attempt was made to develop a simple, accurate, precise specific enzyme photometric method for the quantitative estimation of Dequalinium chloride in vaginal tablets "Fluomizin"[13].

The content of DCh in the dosage form in vaginal tablets "Fluomizine" 10 mg (X, *mg*) was calculated by the formula:

$$X = \frac{50.00 \times w \times U_W \times \bar{m}}{U_{RS} \times 100\% \times m}$$

50.00 is the weight of the sample DCh reference, mg to one table, mg;

w – the content of the basic substance in reference sample of DCh, %; degree of ChE inhibition in working experience, %; U_{RS} - is degree of ChE inhibition in the reference sample experiment, %; - average weight of the tablet, g; m - weight of a sample of powdered tablets, g.

U_{RS} - the degree of inhibition of the enzymatic hydrolysis of Acetylcholine *U*, %, in the presence of DCh was calculated using the formula: U = (tg αc - tg αmin) / (tg αmax - tg αmin)^x 100%, where,

- $tg\alpha_c$ – 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});
- $tg\alpha_{max}$ – slope of the kinetic curve A vs time for a procedure [(ACh + H_2O_2) + *p*-Ph], (min^{-1}).

The calibration curve was linear in the concentration range of 0.2 µg / mL – 0.8 µg/mL of DCh with a correlation coefficient of 0.999. The limit of determination was calculated as 20% degree of ChE inhibition and was 0.35 µg / mL.

The kinetic methods of analysis are increasingly used in various fields of analytical practice, especially in the analysis of clinical trials by enzymatic reactions. These methods are based on the principle - if the detected particles can react with any other substance - the initial velocity is approximately proportional to the initial concentration of the determined particles. Therefore, measuring the initial reaction rate allows to determine the initial concentrations of the reactants. There is no need to wait until the reactants reach equilibrium, so this method of analysis can be performed very quickly. This is especially important for slow reactions.

There are two ways for determine the initial enzyme concentration [E_0]. One is the “saturation” of the enzyme with the substrate, the initial concentration of the substrate [S] is very high compared to Km (Mechaelis constant, see equation d [S] / dt = k_2 [S] [E_0] / [S] + Km). In this case, the equation takes a simplified form: $d[S]/dt = k_2 [E_0]$.

Therefore, the reaction rate haves been remains constant for a significant period of time, until [S] decreases to the level of Km. In practice, it is necessary to prepare two reaction mixtures for the determination: the first contains an unknown concentration of enzyme, the second - a known concentration of enzyme. Comparing the reaction rates of the two systems, have calculate the unknown concentration of the enzyme (comparison method). However, there is no need for [S] to be greater than Km.

Therefore, according to another method, the initial reaction rate is measured when the substrate concentration does not have time to change significantly. Therefore, it is only necessary to determine that both reaction mixtures have the same substrate concentration (regardless of its value), because the initial reaction rate will be directly proportional to the enzyme concentration. When determining the reagent concentration, it is most convenient to measure the initial velocity to interpret the results of slow reactions. This reduces the effects of temperature changes, side effects and other uncontrolled factors. When spectrophotometric control of the reaction course is used by changing the light absorption of the product, it is possible to determine the initial reaction rate by the initial slope of the graphical dependence of absorption on time. For convenience, facilitation of automation and reliability of results, many kinetic techniques use advanced devices that allow you to directly record the initial reaction rate, and if they are properly calibrated, consequently, the concentration of the substance being determined.

An example of the application of kinetic methods is the determination of the enzyme cholinesterase by measuring the rate of selective hydrolytic cleavage of acetylcholine at the presence of the enzyme cholinesterase. New kinetic photometric method for DCh determination based on an inhibition of the enzymatic (cholinesterase) reaction was proposed. The reaction rate was detected at unhydrolised acetylcholine residue, which is determined by the amount of peracetic acid, wich producted during the impact of H_2O_2 on it. Indicator reaction is a reaction of peracetic acid with 4-ethoxyaniline interaction that leads to the formation of azoxyphenetole with λ_{max} = 350 nm (lgε =4.18). The measurement velocity of changing of light absorption vs. time (ΔA/Δt, min^{-1}) give a chance to quantitatively determination of DCh.

The initial reaction rate is determined by measuring the change in light absorption over a period of time (ΔA / Δt, min^{-1}). Since the limiting stage of the whole process is the formation of peracetic acid in the perhydrolysis reaction, the reaction mixture (acetylcholine + hydrogen peroxide) is pre-incubated for 10 min before adding *p*-phenetidine (*p*-Ph). This time was established experimentally in separate experiments. Under the selected conditions, the observed change in light absorption of the reaction product as a function of time is linear over the investigated period of time (for the next 15 min) and does not reveal the curvature characteristic of the pseudo-first-order process.

Méthodes

Measurements were performed at 37 ^{0}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 laboratory ionometer “I-130” NPO “Analitpribor”. The utensils were used from the set of the AL-4M military radiometric and chemical laboratory.

Pharmaceutical preparation vaginal tablets No 6 “Fluomizin”. It contains: Dequalinium chloride 10 mg. Rottendorf Pharma GmbH, Medinova AG.

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

Pharmaceutical preparation “Fluomizin” No 6.

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 pharmacopoeia drug 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 2.0 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 15 min on photoelectric colorimeter, optical filter No 2 and 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 α, 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 2.0 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 photoelectric colorimeter CPC-2, using optical filter No 2 and 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}).

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 DCh (WSS or *model solution* or *a 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 2.0 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 photoelectric colorimeter CPC-2, optical filter No. 2 and 1.0 cm cuvette were used. Every time before the experiment the test tube content was shaked 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 + *p*-Ph], $tg\alpha_{c_i}$(min^{-1}).

Preparation of DCh solutions:

• *working standard solution* (WSS): 0.05000 g (accurately weighed) of the working standard sample (WSS) of DCh 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 20 °C, 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 20°C, plug the flask and mix thoroughly.

• *a reference solution* (RS): 0.05000 g (accurately weighed) of the reference standard sample of DCh 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 20 °C, mix thoroughly (concentration 20 µg / ml);

In order to estimate the accuracy and precision of the proposed method, standard solutions of 0.2 µg / ml, 0.3 µg /ml, 0.4 µg /ml, 0.5 µg /ml, 0.6 µg /ml, 0.7 µg /ml, 0.8 µg /ml 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 DCh was constructed by the calibration dependence in absolute and normalized coordinates (normalization was performed by comparison solution) of the species and respectively. The least squares method calculated the parameters of the obtained calibration dependences (angular coefficient and its standard deviation, free term and its standard deviation, correlation coefficient and residual standard deviation), which are given in Table. 1.

Table data shows that the parameters constructed in the normalized coordinates of the calibration linear dependence of the DCh determination are characterized by satisfactory linearity for a given range and satisfy the requirements of the maximum permissible uncertainty.

Correctness and precision. To verify the accuracy and precision of the method, an experiment was conducted with a series of model solutions DCh 1 - 7, for which the content of DCh was calculated by the calibration graph and the parameter “found / entered”, which was used to calculate the systematic and random errors of the technique (Tab 2).

Table 1: Linearity parameters of the developed method for DCh determination

Parameter	$U = b_1 \times c + a_1$	$Y = b_2 \times X + a_2$	Creteria	Conclusion
b	97.1	1.45	–	–
s_b	0.9	0.01	–	–
a	14.1	-42.00	–	–
s_a	0.5	1.50	–	–
R_c	0.9997	0.9997	> 0.9994	responds
RSD_0	0.49	1.47	< 1.51%	responds
LOD	0.05 μg/mL	10%	< 32%	responds

Data RSD_{RR} = 1,43%, Δ_{RR} = $RSD_{RR} \cdot t$ *(95%, n-1)* = 2,76 ($\Delta_{RR,}$ % ≤ max Δ_x =3,05%) testify to the satisfactory correctness and precision of the developed method for determining DCh.

General recommended procedure

For determine the quantitative content of DCh in the vaginal tablets “Fluomizin” enzymatic kinetic-photometric method was developed. It is based on the ability of DCh to suppress the catalytic activity of cholinesterase to decompose acetylcholine in biochemical reaction[16]. As a result of the inhibition of activity, acetylcholine is created in the tube, unreacted with cholinesterase. The content of DCh is determined by the degree of inhibition of the enzyme reaction. The latter is evaluated for the residual acetylcholine that has not reacted with cholinesterase. Determination of excess acetylcholine in the reaction mixture is performed by kinetic-photometric method. Indicative reaction is the oxidation of *p*-phenetidine by acetic acid, which is formed during the auxiliary reaction of perhydrolysis. An auxiliary reaction takes place when excess hydrogen peroxide is added to the reaction mixture. The result is a colored product - azoxyphenetol (λ_{max} = 358 nm) over a period of time.

The paper[16] describes the process of selecting the optimal experimental conditions.

The pH of 7.5 - 8.5 and the temperature of 37 - 39 °C are found to be optimal for the reaction of enzymatic hydrolysis of ACh in the presence of ChE. The use of phosphate buffer with a pH of 8.2 - 8.5 provides the highest speed of three analytical reactions: enzymatic, auxiliary reaction of perhydrolysis and indicator reaction of oxidation of *p*-Ph.

Calibration graph procedure

Kinetic curves (Fig.1) of analytic indication reaction of *p*-phenetidine oxidation by hydrogen peroxide in the presence of the system: [(ChE + Inh) + Ach] +H_2O_2 + *p*-Ph, [(ChE + ACh) + H_2O_2 + *p*-Ph], ACh+(ChE+DC), [(ACh + H_2O_2) + *p*-Ph] were linear, for the first 15 minutes. This enables the use for assessing of the reaction rate the slope angle tangent (angular coefficient of slope) of the derived kinetic lines, built in the coordinates optical density (A) - time (t, min) min^{-1} as the value of the analytical signal, corresponding to a certain content of an inhibitor in a sample.

The calibration graph was constructed using the values obtained from seven replicate samples of the same DCh content (Fig. 2). The linear regression equation was as follows: U (%) =97.5 c – 14.1 (where «c» is the DCh concentration, μg/mL;

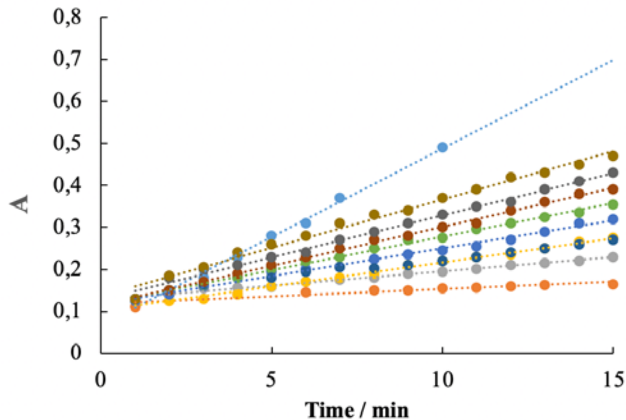

Figure 1. Kinetic curves of the coupled oxidation reaction of p-phenetidine with excess hydrogen peroxide: 1 - ChE + ACh, 2-7 - ACh + (ChE + DCh); c(ACh) = 0.05 mg/mL; [ChE] = 0.25 U; c(DCh), μg/ml: 2 – 0.1, 3 – 0.2, 4 – 0.3, 5 – 0.4, 6 – 0.5, 7 – 0.6, 8 – 0.7, 9 – Ach.

The degree of inhibition of the enzymatic hydrolysis of Acetylcholine *U*, %, in the presence of DCh was calculated using the formula: $U = ((tg\alpha_c - tg\alpha_{min}) / (tg\alpha_{max} - tg\alpha_{min}))\cdot$ 100% where,

- $tg\alpha_c$ – 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});
- $tg\alpha_{max}$ – slope of the kinetic curve A vs time for a procedure [(ACh + H_2O_2) + *p*-Ph], (min^{-1}).

The calibration curve was linear in the concentration range of 0.2 μg / mL – 0.8 μg/mL of DCh with a correlation coefficient of 0.999. The limit of determination was calculated as 20% degree of ChE inhibition and was 0.35 μg / mL.

Results

The results of the experiments allowed to develop a new method for the quantitative determination of ChE inhibitors 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.

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 · 10^{-4}M - 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.

Have showed dependence of the rate of the enzymatic reaction vs the concentration of ChE to.

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). Therefore, the experiments were performed at a concentration of ChE 0.24 mg / ml.

The results of the study of the effect of hydrogen peroxide concentration are shown, that at concentration of hydrogen peroxide ≥ 1%, the rate of the indicator reaction becomes maximum and does not change in the future. The optimal concentration of hydrogen peroxide is 1%.

Excess *p*-Ph (1 %) was used to ensure the conditions of the pseudo-first order course of the *p*-Ph oxidation reaction.

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 $1/v = 1/V_{max} + K_м/(V_{max} \cdot 1/[S])$, which describes a rectilinear dependence, y=a+b*x, 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].

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. 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 = 0.0995). According to dependence, the values of the maximum enzyme hydrolysis rate were Vmax $1.64 \cdot 10^{-5}$ mol · L^{-1} · min^{-1} and Michaelis constant Km were $6.49 \cdot 10^{-4}$.

Quantitative determination of DCh in vaginal tablets “Fluomizine” 10mg No 6.

5 tablets of “Fluomizine” 10 mg were crashed and 1.00580 g (exect weight) tablets powered dissolve in double distilled water in 100.0 ml (Solution 1). Then, 5 ml of the filtered solution was taken into a 500 ml flask and adjusted to 500 ml with double distilled water. The analysis was carried out according to the *general procedure*.

2.00 ml of the resulting solution was introduced into a 20 ml tube and determined according to the procedure described. Similarly perform the determination with the reference solution (RS).

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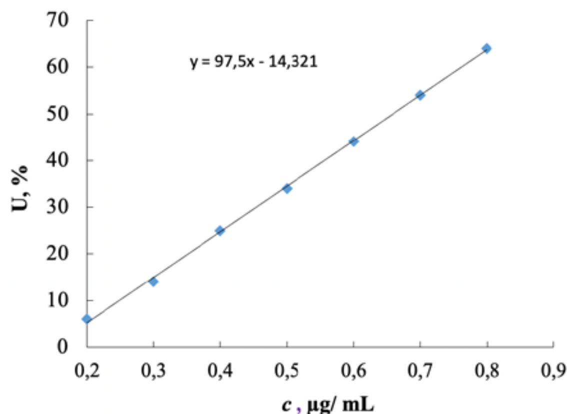

Figure 2. Curve of the graduated dependence inhibition degree (ChE U, %) vs DCh concentration.

Discussion

According to dependence 1/u = 39,556 · $[S]^{-1}$ + 0,6091·10^5 , the values of the maximum enzyme hydrolysis rate Vmax and Michaelis constant Km were calculated, equal to 1.64 · 10^{-5} mol · L^{-1} · min^{-1} and 6.49 · 10^{-4}.

An enzymatic kinetic-photometric method for the determination content of Dequalinium chloride in vaginal tablets "Fluomizin" No 6 was developed.

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