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Research Article

A New Colorimetric kinetic method for determination of Scopolamine butylbromide based on the cholinesterase activity inhibition

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ABSTRACT

A new method for enzyme-kinetic determination of scopolamine butylbromide (SBB), based on the SBB ability to inhibit the reaction of hydrolytic acetylcholine decay over cholinesterase enzyme is elaborated. Scopolamine butylbromide content is determined by the degree of enzyme reaction inhibition, which is measured using unreacted acetylcholine in enzyme reaction: determination of acetylcholine is carried out by kinetic tangent method with *p*-phenetidine oxidizing reaction, formed in the previous perhydrolysis reaction (with excess hydrogen peroxide) by peracetic acid. Indicator reaction rate is determined by the photometric method by the increase of absorbance formed azoxyphenetole (λmax=350 nm). The proposed method was applied successfully to analyze scopolamine butylbromide in tablets dosage form. No interference was observed from common pharmaceutical excipients. In the optimum conditions, the calibration curve is linier over the range 1 ... 6 μmol / L (r=0,996). The relative standard deviation was ± 7.4% (*n*=5) for 1.15 μmol / L and ± 1.95% (*n*=5) for 5.75 μmol / L (δ =-1.22 % and 0.03% respectively). The limit of quantitation (for the reaction inhibition grade 20%) $2 \cdot 10^{-6}$ mol / L (*n*=5). For seven determinations of active substance content in scopolamine buthylbromide substance drug was found to be 99.62%, RSD=1.72% (δ = + 0.41%; compared with HPLC method). A recovery of scopolamine buthylbromide in *"Spasmobru"* tablets 10 mg is 100.51%, RSD=1.65% (δ = + 0.5%).

Key words: *scopolamine butylbromide, enzyme-kinetic method, cholinesterase, electrophotocolorimetry, perhydrolysis.*

INTRODUCTION

Hyoscine *N* – butylbromide – is semisynthetic derivative alkaloid hyosciamine, of Nitrogen butylated, which is in death cherries, henbane, stramony, scopolia. Its chemical name is [7(S)-(1-α, 2-β, 4-β, 5-α,7-β)]-9-butyl-7-(3-hydroxy-1-oxo-2-phenyl-propoxy)-9-methyl-3-oxa-9-azoniatricyclonon bromide (SBB) (*syn*. Butyl scopolamine, hyoscine butylbromide), and it belongs to peripheral neurotropic spasmolytins, M-cholinolytic according to the formulary (Fig. 1).

It is used against spastic state of nonstriated muscle, as well as against irritable bowel syndrome, for active gastric ulcer treating, GIT and biliary dyskinesia, new-borns pylorospasm, sinus bradycardia, bradyarrhythmia in a case of atrio-ventricular block, parkinsonism, dysmenorhoea. Hyperexcitation, central origin sickliness, hypersalivation, diarrhoea, spastic constipation, nocturnal enuresis, premedication before surgical operations and diagnostic procedures, sea and air sickness are also indications for this medicine prescription [1].

Such a broad spectrum of SBB pharmacological action explains availability of great many both of SBB-based individual drug products and combination drugs on a pharmaceutical market[2-3].

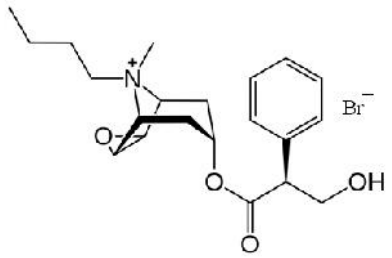

Fig. 1
Structure of Scopolamine butylbromide.

Nowadays, there are no monographs for substance and preparation of scopolamine butylbromide in State Pharmacopoeia of Ukraine [4].

The European Pharmacopoeia recommends using argentometric titration for quantitative determination of hyoscine butylbromide. Titration final point is determined by potentiometry, using a silver indicator electrode paired with argentums chloride reference electrode [5].

For the quantitative determination of SBB Japanese Pharmacopoeia suggests non-aqueous titration 0.1 mol / L with perchloric acid in acetate acid and acetic anhydride conditions[6].

For SBB determination in injection dosage form, which contains a lidocaine hydrochloride and paracetamol and some pharmaceutical formulation and in human plasma HPLC method with solid phase extraction and UV detection are used [7-10].

The objective sensitive HPLC–DAD method for simultaneous determination of three most commonly prescribed drugs: hyoscine, ketoprofen, and ibuprofen was proposed [11]

A simple procedure was described for the determination of scopolamine by square-wave voltammetry using a cathodically pretreated boron-doped diamond electrode. Cyclic voltammetry studies indicate that the oxidation of scopolamine is irreversible at a peak potential of 1.59 V (*vs.* Ag/AgCl (3.0 mol L^{-1} KCl)) in a 0.50 mol L^{-1} sulfuric acid solution. The method was successfully applied to the determination of scopolamine in pharmaceutical formulations with minimum sample preparation [12].

The described methods require availability of number of chemical solvents, expensive equipment that limits its everyday use in the chemist analyst work.

Our processed methods are based on the obvious scopolamine butyl bromide ability to inhibit the reaction of acetylcholine hydrolysis (ACh) over the cholinesterase enzyme (ChE). The reaction rate is assessed at unhydrolised acetylcholine residue, which is determined by the amount of acetate acid, made during the impact of H_2O_2 on it. Indicator reaction is a reaction of acetate acid with *p*-phenetidine (Ph) interaction that leads to the formation of azoxyphenetole from λmax = 358 nM (ε = 15 10^3 L mol^{-1} ·cm^{-1}) [15, 16].

MATERIALS AND METHODS

For light absorbance of solutions "photoelectric concentration colorimeter («CPC-2»)"was used (Zagorsky Optical & Mechanical Plant, Russia). Using the filter №2 (λ_{ef} = 364 nm) and quartz cell of 1.0 cm.

pH value was measured at Ionomer I - 160M laboratory (Belarus) by using EGL 43-07 pH glass electrode together with auxiliary chloride silver electrode of EAL-1M3.1 type, saturated with potassium chloride.

For research reagents were used: *p*- phenetidine (4 - ethoxyaniline - 98%) (SIGMA - ALDRICH); A0281408 series, New Jersey, USA; *p* - phenetidine hydrochloride (Ph), extracted from the base by hydrogen chloride precipitation in the chloroform solution.

Pharmacopoeial acetylcholine chloride medicine - 0.2 g per amp/5 ml (manufactured by "VECTOR" – State Science Center of virology and biotechnology in Russian Federation" (Russia).

Dry protein drug of cholinesterase from horse serum - 80 mg / fL (VI class), 22 AE / mg (manufactured by SMU "Biomed", Russia).

"Stabilized Hydrogen Peroxide 30-40%" (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% [17].

Scopolamine butylbromide substance, $C_{21}H_{30}BrNO_4$ (Sigma), w(SBB) = 99.21% (according to HPLC).

"Spasmobru" pills, 10 mg, №20, STE 301 series, BRUPHARMEXPORT, Belgium (additive agents: Lactose monohydrate, microcrystalline cellulose, sodium starch glycolate (type A), silica dioxide, magnesium stearate).

The enzyme reaction substrate of acetylcholine chloride solution (ACh) preparation.

The ampoule's content of pharmacopoeia drug acetylcholine chloride 0.2 g is in 200 ml of double-distilled water dissolved. For that end, open an ampoule, add 4.0 ml of water with pipette, and shake until acetylcholine is completely dissolved. Then pour the acetylcholine solution into 200 ml capacity measuring bottle and dilute double-distilled water to the volume.

***Cholinesterase solution preparation* (*ChE*).**
Add 10.0 ml double - distilled water in a flask, containing 80 mg of dry cholinesterase drug, shake up and thermostat for 10 minutes at 38 ° C above Zero.

***Phosphate buffer solution preparation* (pH 8.35).**
Pour 35.75 g of disodium hydrogen phosphate in 500 ml flask, add 300 ml double-distilled water, dissolve it, add 19 ml of 0.1 mol/L solution of hydrochloric acid, stir and dilute double distilled water to 500.0 ml. The ready solution pH is potentiometrically controlled.

Hydrogen peroxide solution 10%. It is prepared by the appropriate high-test hydrogen peroxide dilution with double-distilled water. The exact hydrogen peroxide content in ready 10% solution is determined permanganatometrically [17].

p-phenetidine hydrochloride solution preparation 1%.
Dissolve 1.00 g *p*-phenetidine hydrochloride in 80 ml of double-distilled water into 100 ml capacity measuring bottle and dilute it to volume.

Scopolamine butylbromide, reference standard solution, 10 µg / ml (RS). Dissolve 0.05040 g (precisely weighed quantity) of scopolamine butylbromide powder in 40 ml double-distilled water in 50 ml capacity measuring bottle and dilute to volume. 1.0 ml ready solution is pipetted and transferred into 100 ml capacity measuring bottle. After these actions, dilute double distilled water to the volume at 20ºC, cork the bottle and mix thoroughly.

Calibration. In a graduated test tubes with ground plug gradually add phosphate buffer (pH = 8.4) 10.0 ml of 0.2 mol / L in each one, respectively, from 1.00 ml to 5.00 ml of scopolamine butylbromide RS solution and add 2.0 ml of cholinesterase while stirring, switch a timer, shake up each solution thoroughly and thermostate for 20 min, then quickly add 1.0 mL of 1% acetylcholine solution, switch on timer, shake thoroughly and thermostate 10 min again, then add 2.0 ml of 10% hydrogen peroxide solution, keep for 10 min in thermostat and add 1.0 ml of 1% *p*-phenetidine solution (Ph), dilute distilled water to volume at 20 ml. Switch on timer and every other minute scan photometrically each solution for 20 min on photoelectric colorimeter CPC-2, use colour filter №2 and 1.0 cm cuvette. Every time before test tube contents shaking, plug it thoroughly. Buffered solution with double - distilled water as reference solution is used.
According to the optical - time relations the kinetic curves are plotted and the slope of the first 10 minutes is found ($tg\alpha_{c_i}$). According to data received a slope - finite analyte concentration calibrated relation is obtained, *c*, µmol / L. A calibration curve equation is solved by the least squares method (Linear regression): tgα =bc+a, where a,b are Y-axis intercept and slope, (tgα, min^{-1}) respectively.

Standard technique for scopolamine butylbromide in standardized test solutions determining. Dissolve 0.05 g (precisely weighed quantity) of scopolamine butyl bromide powder in 40 ml of double-distilled water in 50 ml capacity measuring flask and dilute with double - distilled water to volume. 1.0 ml of the ready solution is transferred with pipette in 100 ml capacity measuring flask. After this, dilute with double - distilled water to volume at 20ºC, cork the flask and mix thoroughly.
Add 10.0 ml 0.2 mol / L of phosphate buffer solution (pH = 8.4) in 20 ml capacity graduated test tube with ground plug consistently, a definite volume of ready inhibitor solution (from 1.0 to 5.0 ml) and continue determination as first one at graph plotting. All experiments are carried out five times. SBB concentration in standardized test solution is calculated by the formula:

$$C_x = \frac{tg\alpha - a}{b}$$

tgα - is a slope, available from operational experiment, min^{-1};
a, b - Y-axis intercept and slope of calibration curve equation (tgα = bc + a), respectively.

RESULTS AND DISCUSSION

Figure 2 shows the kinetic curves of couple oxidation of *p*-phenetidine by hydrogen peroxide in presence of different concentrations of SBB with a linear character at the initial stage. This enables the use for assessing the reaction rate of 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.
We have also calculated a degree of inhibition of hydrolysis enzyme reaction of acetylcholine, U, in presence of SBB. For this purpose, two additional control experiments are conducted.

1) Transfer successively into a 20 ml graduated test tube 10.0 mL of 0.2 mol / L phosphate buffer solution (pH = 8.4), 1.0 ml of acetylcholine, 2.0 ml of 10% of hydrogen peroxide solution, mix thoroughly the mixture and keeping to thermostat for 10 min at +38°C. Then add 1.0 ml of 1% solution of *p*-phenetidine and dilute twice with distilled water to a volume of 20 ml, mix again, start a stopwatch and every minute measure the solution optical density value on photocolorimeter CPC-2, using a light filter No.2 and a 1.0 cm cuvette. Every time before shaking the content in the test tube, plug it thoroughly. As a comparison solution, use the buffer solution with twice distilled water (20.0 mL). As per dependence of optical density on time, build the kinetic curves and find the slope angle tangent of linear section in first 10 min ($tg\alpha_{V_{max}}$).

2) Into another graduated test tube with a ground glass stopper, consistently transfer 10.0 mL of 0.2 mol / L phosphate buffer solution (pH = 8.4), 1.0 ml of acetylcholine solution, 2.0 ml of cholinesterase solution and incubate for 20 min at + 38 ° C, then add 2.0 ml of 10% solution of hydrogen peroxide, mix the mixture and incubate again for 10 min at 38 ° C. Then add 1.0 ml of 1% solution of *p*-phenetidine, mix, dilute twice with distilled water to 20.0 ml and mix the solution again, start a stopwatch and measure every minute the solution optical density on the photoelectric colorimeter CPC-2, using a light filter No 2 and a 1.0 cm cuvette. Every time before shaking the content in the test tube, plug it thoroughly. As the comparison solution, use the buffer solution with twice distilled water. per dependence of optical density on time, build the kinetic curves and find the slope angle tangent of linear site in first 10 min ($tg\alpha_{min}$).

The degree of inhibition of the enzymatic hydrolysis of acetylcholine *U*, %, in presence of SBB is calculated using the formula:

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

where $tg\alpha_{c_i}$ – rate of oxidation reactions of Ph with peroxyacetic acid, which is formed in reaction of perhydrolyses, not used in reaction of enzyme hydrolysis of acetylcholine (ACh) in presence of C_i inhibitor concentration (SBB) min^{-1};

$tg\alpha_{V_{max}}$ – rate of Ph oxidation reaction with peroxyacetic acid, which is formed in reaction of acetylcholine perhydrolyses (in absence of cholinesterase enzyme and inhibitor) min^{-1};

$tg\alpha_{min}$ – rate of oxidation reactions of Ph with peroxyacetic acid, which is formed in reaction of perhydrolyses, not used in reaction of hydrolysis of acetylcholine (ACh) in presence of cholinesterase (ChE) in absence of inhibitor (SBB) with hydrogen peroxide, min^{-1}.

The equation of dependence of the inhibition degree of the enzymatic hydrolysis reaction of acetylcholine on concentration of scopolamine buthylbromide had a linear character: $U,\% = 3.847 \cdot 10^6 c + 12.103$, (r = 0,986%), where c (SBB) in mol / L (Figure 3).

Limit of quantitation (LOQ) was calculated as the value of a final concentration of inhibitor in solution at 20% degree of inhibition of the reaction, which was $2 \cdot 10^{-6}$ mol/L.

Method of determining the active substance content in scopolamine buthylbromide substance. Dissolve 0.05 g (*m*, exact weight) of the test substance powder in 40 ml of twice distilled water in a 50 ml volumetric flask and dilute with water to the mark. Withdraw with a pipette 1.00 ml of the obtained solution and transfer into a 100 ml volumetric flask. Then adjust the volume with twice distilled water to the mark at 20ºC, plug the flask and mix thoroughly.

Similarly, the standard sample solution of scopolamine buthylbromide preparation, which contains 1.00 mg in 1.00 ml of the solution.

Transfer consequently into a 20 ml graduated test tube with a ground stopper 9.0 ml of 0.2 mol / L phosphate buffer solution (pH = 8.35), 5.00 ml of the inhibitor test solution (or RS SBB with concentration 0.010 mg / ml) and add 2.0 ml of cholinesterase solution, start the stopwatch and then the solution is vigorously shaken and kept in a thermostat for 20 minutes, then quickly add 1.0 mL of 1% solution of acetylcholine, start a stopwatch and shake vigorously again and keep in a thermostat for additional 10 min, then add 2.0 ml of 10% hydrogen peroxide solution, keep in an incubator for 10 minutes and add 1.0 ml of 1% solution of *p*-phenetidine. Start the stopwatch and for 20 min every minute the solution is processed on the «CPC-2» (light filter №2). Thickness of the cuvette is 1.0 cm.

According to optical density value depending on time (in minutes), build the kinetic curve and determine the angle tangent during the first 10 minutes. Additionally perform further two control experiments, as before, in determining the degree of inhibition of the enzymatic reaction of acetylcholine hydrolysis.

According to the obtained data, calculate the degree of inhibition U, %.

Content of scopolamine buthylbromide in substance w (SBB), in %, is calculated using the formula:

$$w_{st}\,(SBB) = \frac{U_x \cdot m_{st} \cdot 100}{U_{st} \cdot m \cdot \omega_{st}} \cdot 100\%$$

where U_x – inhibition rate in working experiment with a sample of solution of the test substance, %;
U_{st} – inhibition rate in experiment with a sample of RS solution of SBB, %;
m – weight of SBB test substance, g;
m_{st} - weight of SBB standard substance drug, g;
w_{st} – weight content of SBB in RS, %.
The results of the quantitative determination *of the active substance content in scopolamine buthylbromide* are given in Table. 2.

Methods of quantitative determination of scopolamine buthylbromide in tablets "Spasmobru" 10 mg. Dissolve 0.1 g (exact weight) of powder of crashed tablets in 40 ml of twice distilled water in a 50 ml volumetric flask and dilute with water to the mark. With a pipette withdraw 1.00 ml of the obtained solution and transfer into a 100 ml volumetric flask of. Then dilute the volume with twice distilled water to the mark at 20ºC, plug the flask and mix thoroughly.
Similarly, the standard sample solution of scopolamine buthylbromide preparation, which contains 1.00 mg in 1.00 ml of the solution.
Transfer consequently into a 20 ml graduated test tube with a ground stopper 9.0 ml of 0.2 mol / L phosphate buffer solution (pH = 8.35), 5.00 ml of the inhibitor test solution (or RS of SBB with concentration 0.010 mg / ml) and add 2.0 ml of cholinesterase solution, start the stopwatch and then the solution is vigorously shaken and kept in a thermostat for 20 minutes, then quickly add 1.0 mL of 1% solution of acetylcholine, start a stopwatch and shake vigorously again and keep in a thermostate for additional 10 minutes, then add 2.0 ml of 10% hydrogen peroxide solution, keep in an incubator for 10 minutes and add 1.0 ml of 1% solution of *p*-phenetidine. Start the stopwatch and for 20 minutes every minute the solution is processed on the «CPC-2» (light filter №2). Thickness of the cuvette is 1.0 cm. Use buffer solution as reference solution.
According to optical density value depending on time (in minutes), build the kinetic curve and determine the angle tangent during the first 10 minutes. Additionally perform further two control experiments.
1) Transfer consequently into a 20 ml test tube 9.0 ml of 0.2 mol / L of phosphate buffer solution (pH = 8.35), 1.0 ml of acetylcholine solution, 2.0 ml of 10% hydrogen peroxide solution, the mixture is vigorously shaken and kept in a thermostat for 10 minutes at 38°C. Then add 1.0 mL of 1% *p*-phenetidine solution and dilute with twice distilled water to 20 ml, shake again, start a stopwatch and after every minute measure the value of optical density of the solution on the «CPC-2», using the light filter №2 and 1.0 cm cuvette. Use buffer solution as reference solution. Build the kinetic curve of dependence of optical density on time and find the angle slope tangle.
2) Transfer consequently into another test tube 9.0 ml of 0.2 mol / L of phosphate buffer solution (pH = 8.35), 1.0 ml of acetylcholine solution, 2.0 ml of 10% cholinesterase solution and keep in a thermostate for 20 minutes at 38°C, then add 2.0 mL of 10 % of hydrogen peroxide solution, shake the mixture and keep in a thermostat for additional 10 minutes at 38°C. Then add 1.0 ml of 0.1% *p*-phenetidine solution, mix, dilute with twice distilled water to 20 ml, shake again, start a stopwatch and after every minute measure the value of optical density of the solution on the «CPC-2», using the light filter №2 and 1.0 cm cuvette. Use buffer solution as reference solution. Build the kinetic curve of dependence of optical density on time and find the angle slope tangle. Calculate the inhibition grade *U*, %.

Content of scopolamine buthylbromide in "Spasmobru" tablets 10 mg No.10, in mg, is calculated using the formula:

$$m\,(SBB) = \frac{U_x \cdot m_{st} \cdot 100 \cdot \overline{m}}{U_{st} \cdot \omega_{st} \cdot m}$$

where U_x – inhibition rate in working experiment with a sample of solution of the test substance, %;
U_{st} – inhibition rate in experiment with a sample of solution of SBB working standard sample,%;
m – weight of tablets powder SBB, mg;
m_{st} - weight of SBB standard sample, mg;
w_{st} – weight content of SBB in RS, %.
$\overline{m}$ - table average weight, in mg.
Results of assay of SBB in "Spasmobru" tablets 10mg are presented in Table 3.

CONCLUSION

In this study, the simple and sensitive enzyme-kinetic method was developed for the assay of scopolamine buthylbromide in bulk and tablet dosage form. It is based on the SBB ability to inhibit the reaction of hydrolytic decay of acetylcholine over cholinesterase enzyme. Scopolamine butylbromide content is determined by the degree of enzyme reaction inhibition, which is measured using unreacted acetylcholine in enzyme reaction: determination of acetylcholine is carried out by kinetic tangent method with *p*-phenetidine oxidizing reaction, formed in the previous perhydrolysis reaction (with excess hydrogen peroxide) by peracetic acid. Indicator reaction rate is determined by the photometric method by the increase of absorbance formed azoxyphenetole (λmax=350 nm). In the optimum conditions, the calibration curve is linier over the range 1 ... 6 μmol / L (r=0,996). The relative standard

deviation was ± 7.4% (n=5) for 1.15 µmol / L and ± 1,95% (n=5) for 5.75 µmol / L (δ =-1.22% and -0.03% respectively).The limit of quantitation (for the reaction inhibition grade 20%) $2 \cdot 10^{-6}$ mol / L (n=5). For seven determinations of the active ingredient content in scopolamine buthylbromide substance drug was found to be 99.62%, RSD=1.72% (δ = + 0.41%; compared with HPLC method). A recovery of scopolamine buthylbromide in *"Spasmobru"* tablets 10 mg is 100.51%, RSD=1.65% (δ = + 0.5%).

Table 1

Metrological characteristics of results of scopolamine buthylbromide kinetic determination in model solutions

S.No	**SBB taken, mol /L**	**SBB found, $\overline{X}$ ±ΔX, mol /L**	**Metrological characteristics (n=5; P=0,95)**
1.	$1.15 \cdot 10^{-6}$	$(1.14\pm0.11)\cdot10^{-6}$	*RSD*=7.43% δ= -1.22%
2.	$2.30\cdot10^{-6}$	$(2.32\pm0.14)\cdot10^{-6}$	*RSD*=4.90% δ=0.96%
3.	$3.45\cdot10^{-6}$	$(3.46\pm0.17)\cdot10^{-6}$	*RSD*=3.83% δ=0.28%
4.	$4{,}60\ 10^{-6}$	$(4.60\pm0{,}14)\cdot10^{-6}$	*RSD*=2.38% δ = 0 %
5.	$5{,}75\cdot10^{-6}$	$(5.75\pm0.14)\cdot10^{-6}$	*RSD*=1.95% δ =- 0.03%

***Note* : SBB taken, *a*; $\delta=(\overline{X}-a)\cdot100\%/a$**

Table 2

The results of the quantitative determination of the active substance content in scopolamine buthylbromide

The active substance contenet in SBB, %	Found, $\overline{X}$ ±ΔX, %	Metrological characteristics (n=5; P=0.95)
99.21*	99.62±1.59	*RSD*=1.72% (δ=0.41%)

Note. * Determined by HPLC method (*a*); $\delta=(\overline{X}-a)\cdot100\%/a$

Table 3

Metrological characteristics of results of the quantitative determination of scopolamine buthyl bromide in "Spasmobru" 10 mg tablets

SBB taken, mg	SBB found, $\overline{X}$ ±ΔX		Metrological characteristics (n=5; P=0.95)
	mg	%	
9.80*	9.85±0.15	100.51±1.53	*RSD*=1.65% (δ=0.5%)

Note. *as per HPLC method date from the certificate, *a*; $\delta=(\overline{X}-a)\cdot100\%/a$

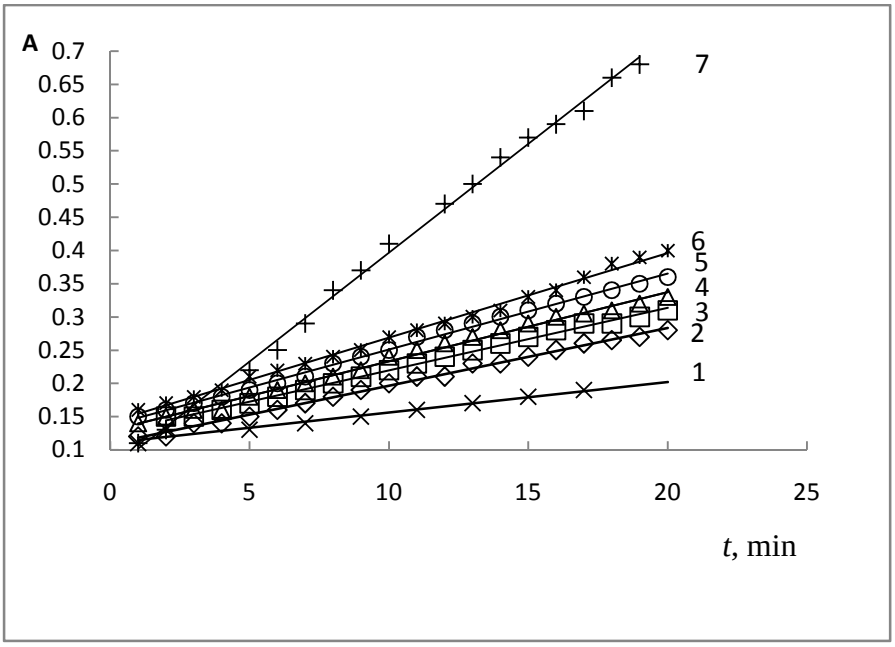

Fig.2
Kinetic curves of couple oxidation *p*- phenetidine by hydrogen peroxide in presence of the system: *1* – ACh+ChE, *2- 6* –ACh+(ChE+SBB) , *7* – ACh. *C*(ACh) = 0.1%; ChE = 0.25 U;*C* (SBB, 10^{-6} mol/l: *2*–1.0, *3*– 2.0, *4*–3.0, *5* –4.0 , *6* – 5.0.

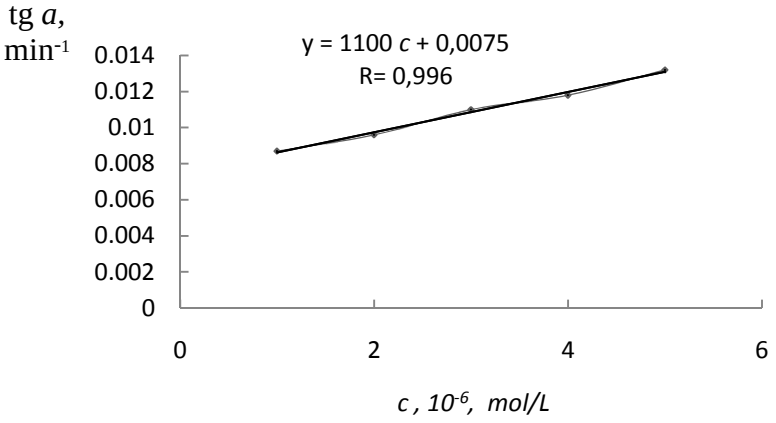

Fig.3
Curve of dependence of the inhibition degree of the enzymatic hydrolysis reaction of acetylcholine vs. concentration of scopolamine buthylbromide .

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