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Research Article

# STUDY OF STABILITY OF GALANTAMINE PEPTIDE ESTERS AT ROOM TEMPERATURE AND DIFFERENT PH VALUES

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**ABSTRACT**

In current work are summarized the experimental results from the study of chemical stability of Galantamine peptide esters 3,4 – dichlorophenyl – Alanil – Leucil – Glycil – Galantamine (LEU – GAL) and 3,4 – dichlorophenyl – Alanil – Valil – Glycil – Galantamine (VAL – GAL) in different aqueous buffer solutions with pH = 2, pH = 7.4, pH = 9 at room temperature in period of 6 hours. The applied chromatographic TLC conditions were: stationary phase: TLC aluminum plates (Merck) precoated with silica gel $G_{60}F_{254}$; mobile phase: n – butanol: water = 30: 10 v/v; length run – 120 mm. Densitometric analysis was carried out in reflectance – absorbance mode at λ = 282 nm.

Linear regression analysis was employed to calculate the regression equations and the correlation coefficients. The obtained regression equations, showing the proportional accordance A = f (C) at pH = 2 were: y = 8388.x – 3.704, $R^2$= 0.998 (LEU – GAL); y = 3021.x – 0.391, $R^2$= 0.999 (VAL – GAL).

The examined Galantamine esters are resistant at room temperature to chemical hydrolysis in aqueous buffer solutions with pH = 2, pH = 7.4, pH = 9, which is proved by the following facts: 1) on chromatograms didn't exist spots with $t_R$, corresponded to $t_R$ of the respective derivative: $t_R$ = 0.23 (LEU – GAL); $t_R$ = 0.33 (VAL – GAL); 2) the area of spots remains similar during 6 hour of experiment. At pH = 2 the content of LEU – GAL is in interval 10.22 mg – 10.58 mg and the quantity of VAL – GAL is in interval 9.07 mg – 11.05 mg.

**Keywords**: Galantamine, Peptide esters, Stability, pH.

## INTRODUCTION

Alzheimer's disease is a progressive neurodegenerative disorder associated with loss of neurons in brain [1] and presence of excessive amounts of neurotic plaques containing amyloid β protein [2]. For therapy of neurodegenerative Alzheimer's disease are applied inhibitors of acethylcholinesterase [3]: Donepezil [4], Galantamine [4, 5], Rivastigmine [4, 6] and Tacrine [7]. Alzheimer's disease is pathologically characterized by amyloid plaques and neurofibrillary tangles [8]. In the brain of patients a major pathological alteration are senile plaques, composed of amyloid ß – peptide (Aß) in length from Aß38 to Aß42 [9]. Aß is generated by the consecutive cuts of proteases ß – and γ – secretase, which liberate the amyloidogenic peptide from its precursor, the ß – amyloid precursor protein (APP) by endoproteolysis 10. ß – secretase (BACE) mediates the N – terminal cleavage, producing a membrane – associated C – terminal fragment (CTFß) of ßAPP 11, 12. The latter is further processed to Aß by γ – secretases, which cleave within the single transmembrane region 13. Subsequent aggregation is thought to result in the formation of neurotoxic protofibrils and the deposition of amyloid plaques 14.

One of the most promising therapeutic strategies to slow progression of Alzheimer's disease pathology is the application of γ – secretase inhibitors, which reduce the amyloid Aß40/42 peptide (Aß) production [15, 16] by blocking γ – secretase activity [17]. The first in vivo test of γ – secretase inhibitor is of dipeptide DAPT, which reduces ß – amyloid peptide levels in brain 18.

For stability analysis are applied the following methods: HPLC (Bendamustine hydrochloride) [19], HPTLC (Ezetimibe) [20].

The aim of current study is to investigate the chemical stability of new synthetized esters of Galantamine in different aqueous buffer solutions with pH = 2, pH = 7.4, pH = 9.

## MATERIALS AND METHODS

### MATERIALS

I) 3,4 – dichlorophenyl – Alanil – Leucil – Glycil – Galantamine (LEU – GAL) and 3,4 – dichlorophenyl – Alanil – Valil – Glycil – Galantamine (VAL – GAL) (syntetized from prof. Vesenkov from Department of Organic Chemistry, University of Chemical Technology and Metallurgy) (Fig. 1.) [21].

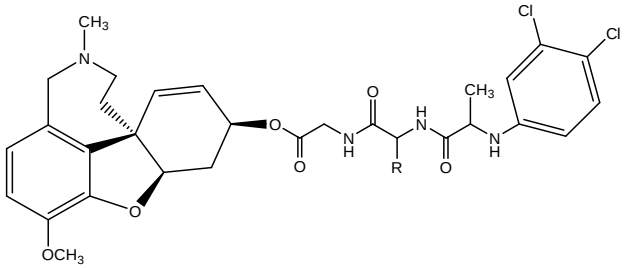

**Fig. 1: Structures of Galantamine peptide esters.**

R = – $CH(CH_3)_2$ 3,4 – dichloro – Alanil – Leucil – Glycil – Galantamine

R = – $CH_2CH(CH_3)_2$ 3,4 – dichloro – Alanil – Valin – Glycil – Galantamine

II) Reagents with analytical grade quality: n – butanol, water, boric acid, 0.1 mol/l HCl, 0.1 M sodium hydroxide, 1 M sodium hydroxide, disodium hydrogen phosphate, sodium chloride, potassium chloride, potassium dihydrogen phosphate.

III) TLC plates: Silicagel $G_{60}F_{254}$, 20 cm x 20 cm (Merck).

### METHOD: TLC – densitometry.

### I. Instrumentation

The chromatographic procedure was carried out using 10 µl sample syringe (Hamilton, Bonaduz, Switzerland) and TLC densitometric scanner TR 541a, performed in the reflectance – absorbance mode at λ = 282 nm.

**II. Chromatographic TLC conditions:** stationary phase: precoated with Silicagel $G_{60}F_{254}$ TLC plates; mobile phase n – butanol: water = 30: 10 v/v; detection at λ = 282 nm; length run – 120 mm.

### III. Buffer preparation:

Buffer reagents were of reagent grade. Buffer solutions were prepared according to European Pharmacopoeia 5.0: 01/2005:40103 as follows:

**1) Buffer solution pH 2.0: 4000200**:

6.57 g of potassium chloride were dissolved in water R and to the obtained solution 119.0 ml 0.1 mol/l HCl were added. The mixture was diluted in volumetric flask to 1000.0 ml with water R.

**2) Phosphate buffered saline pH 7.4: 4005000.**

2.38 g of disodium hydrogen phosphate R, 0.19 g of potassium dihydrogen phosphate R and 8.0 g of sodium chloride R were dissolved in water R. The obtained solution was diluted in volumetric flask to 1000.0 ml with the same solvemt.

**3) Buffer solution pH 9.0: 4007000.**

Solution I. 6.18 g of boric acid R were dissolved in 0.1 M potassium chloride R and solution was diluted in volumetric flask to 1000.0 ml with the same solvent.

Solution II. 0.1 M sodium hydroxide.

Buffer solution pH 9.0 was prepared by mixing of 1000.0 ml Solution I and 420.0 ml Solution II.

**4) Solutions with NaOH.**

a) to 5 ml of 1 mol/l solution of 3,4 – dichlorophenyl – Alanil – Leucil – Glycil – Galanthamine (Mr = 672.2) were added 5 ml 1 mol/l NaOH

b) to 5 ml of 1 mol/l solution of 3,4 – dichlorophenyl – Alanil – Valil – Glycil – Galanthamine (Mr = 658.2) were added 5 ml 1 mol/l NaOH

**IV. Preparation of solutions for linearity of 3,4 – dichlorophenyl – Alanil – Leucil – Glycil – Galantamine (LEU – GAL).**

An accurately weighed quantity of LEU – GAL: 10 mg, 30 mg, 40 mg, 45 mg, 80 mg, 100 mg, 110 mg, 120 mg, 125 mg, 130 mg, 135 mg, 140 mg, 160 mg, 180 mg was dissolved in Buffer solution pH 2.0 to 1.0 ml to obtain solutions with concentration correspondingly: $1.10^{-2}$g/ml; $3.10^{-2}$g/ml; $4.10^{-2}$g/ml, $4.5.10^{-2}$g/ml, $8.10^{-2}$g/ml, $1.10^{-1}$g/ml, $1.1.10^{-1}$g/ml, $1.2.10^{-1}$g/ml, $1.25.10^{-1}$g/ml, $1.3.10^{-1}$g/ml, $1.4.10^{-1}$g/ml, $1.6.10^{-1}$g/ml, $1.8.10^{-1}$g/ml.

**V. Preparation of solutions for linearity of 3,4 – dichlorophenyl – Alanil – Valil – Glycil – Galantamine (VAL – GAL).**

An accurately weighed quantity of VAL – GAL: 10 mg, 25 mg, 90 mg, 125 mg, 130 mg, 135 mg, 350 mg, 410 mg, 430 mg, 470 mg, 500 mg was dissolved in Buffer solution pH 2.0 to 1.0 ml to obtain solutions with concentration correspondingly: $1.10^{-2}$g/ml; $2.5.10^{-2}$g/ml; $9.10^{-2}$g/ml, $1.25.10^{-1}$g/ml, $1.3.10^{-1}$g/ml, $1.35.10^{-1}$g/ml, $3.5.10^{-1}$g/ml, $4.1.10^{-1}$g/ml, $4.3.10^{-1}$g/ml, $4.7.10^{-1}$g/ml, $5.10^{-1}$g/ml.

**VI. Preparation of solutions of Galantamine esters in buffers with pH = 2, pH = 7.4, pH = 9.**

An accurately weighed quantity of 1 mg respectively of LEU – GAL and VAL – GAL was dissolved separately in buffer solution with pH = 2.0, pH = 7.4, pH = 9.0 to obtain concentration of the examined compounds: 1 mg/1ml. Samples of 10 µl from the investigated solutions were taken at every 30 min. during interval of 6 hour and chromatograms were recorded on TLC plates Silicagel $G_{60}F_{254}$ and mobile phases: n – butanol: water = 30: 10 v/v. The chromatograms were scanned in reflectance – absorbance mode at λ = 282 nm.

**RESULTS AND DISCUSSION**

**I. Linearity.**

For the investigation of analytical parameter linearity were prepared solutions with inecreasing concentration of respective compound and were analyzed separately by the written TLC densitometric method. The proportional accordance between the spot area (A) and concentration (C) in g/ml is found and the results are shown on Table 1.

**Table 1: Results for analytical patameter linearity for LEU – GAL and VAL – GAL.**

| N: | 3,4 – dichlorophenyl – Alanil – Leucil – Glycil – Galantamine | | 3,4 – dichlorophenyl – Alanil – Valil – Glycil – Galantamine | |
|---|---|---|---|---|
| 1. | [g/ml] | Spot area (A) | [g/ml] | Spot area (A) |
| 2. | $1.10^{-2}$ | 83 | $1.10^{-2}$ | 30 |
| 3. | $3.10^{-2}$ | 265 | $2.5.10^{-2}$ | 74 |
| 4. | $4.10^{-2}$ | 326 | $9.10^{-2}$ | 270 |
| 5 | $4.5.10^{-2}$ | 365 | $1.25.10^{-1}$ | 379 |
| 6. | $8.10^{-2}$ | 670 | $1.3.10^{-1}$ | 391 |
| 7. | $1.10^{-1}$ | 822 | $1.35.10^{-1}$ | 404 |
| 8. | $1.1.10^{-1}$ | 908 | $3.5.10^{-1}$ | 1078 |
| 9. | $1.2.10^{-1}$ | 993 | $4.1.10^{-1}$ | 1235 |
| 10. | $1.25.10^{-1}$ | 1034 | $4.3.10^{-1}$ | 1292 |
| 11. | $1.3.10^{-2}$ | 1089 | $4.7.10^{-1}$ | 1420 |
| 12. | $1.35.10^{-2}$ | 1124 | $5.10^{-1}$ | 1506 |
| 13. | $1.4.10^{-2}$ | 1220 | | |
| 14. | $1.6.10^{-2}$ | 1331 | | |
| 15. | $1.8.10^{-2}$ | 1504 | | |

Linear regression analysis was employed to calculate the regression equations and the correlation coefficients. The obtained regression equations, showing the proportional accordance A = f (C) are: y = 8388.x – 3.704, $R^2$= 0.998 (LEU – GAL); y = 3021.x – 0.391, $R^2$= 0.999 (VAL – GAL).

On Fig.1. (LEU – GAL) and Fig. 2. (VAL – GAL) are illustrated the calibration curves for linearity.

On Table 2. (LEU – GAL) and Table 3. (VAL – GAL) are presented the results from 6 h, t = 20 °C at pH = 2 for: 1) spot area (A): $A_{LEU - GAL}$, $A_{VAL - GAL}$; 2) quantity of compounds [mg/ml]: [LEU – GAL], [VAL – GAL]; 3) Chauvenet's criterion for spot area (UA): U $A_{LEU - GAL}$, U $A_{VAL - GAL}$; 4) Chauvenet's criterion for content of compounds (UC): U[LEU – GAL], U[VAL – GAL].

The results during 6 h of the experiment show that the examined Galantamine esters are resistant at room temperature to chemical hydrolysis in aqueous buffer solutions with pH = 2, pH = 7.4, pH = 9, selected as model of pH of the stomach, blood and intestine. The chemical stability of derivatives is proved by the following facts: 1) on chromatograms didn't exist spots with $t_R$, corresponded to $t_R$ of the respective derivative: $t_R$ = 0.23 [LEU – GAL]; $t_R$ = 0.33[LEU – GAL]; 2) the area of spots remains similar during 6 hour of experiment.

For all of the obtained results for spot area and for content of LEU – GAL and VAL – GAL is necessary to estimate the Chauvenet's criterion (U), because when U for one value is higher than the relevant standard criterion (USt), the result must be removed as unexpected. The relations: U $A_{LEU - GAL}$ < 2.03, U [LEU – GAL] < 2.03 (Table 2.) and U $A_{VAL - GAL}$ < 2.03, U [VAL – GAL] < 2.03 (Table 3.) show, that all experimental results for UA and UC are lower, than standard requirement: Umax = 2.03 (n = 13), and it isn't necessary to remove data for A and C. For the calculation of sample standard deviation (SD) is applied the Bessel's correction, in which the denominator N – 1 (degrees of freedom) is used instead of N and in this case $(S)^2$is an unbiased estimator for $(SD)^2$.

On Table 4. (LEU – GAL) and Table 5. (VAL – GAL) are presented the results from 6 h, t = 20 °C at pH = 7.4, pH = 9 and solutions mol/mol with NaOH for: 1) spot area (A): $A_{LEU - GAL}$, $A_{VAL - GAL}$; 2) Chauvenet's criterion for spot area (UA): U $A_{LEU - GAL}$, U $A_{VAL - GAL}$. All values of UA are lower, than standard requirement: Umax = 2.03 (n = 13).

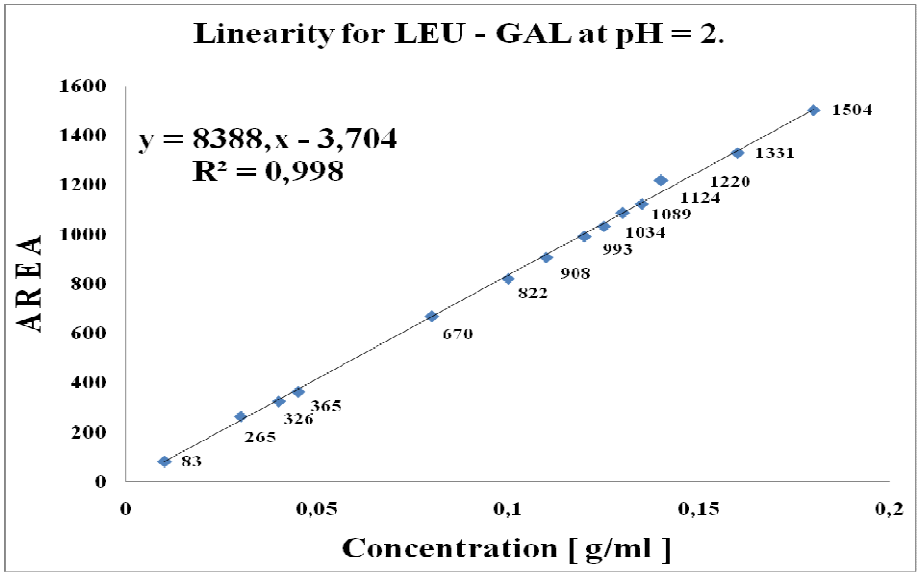


**Fig. 1: Calibration curve for linearity of LEU – GAL at pH = 2.**

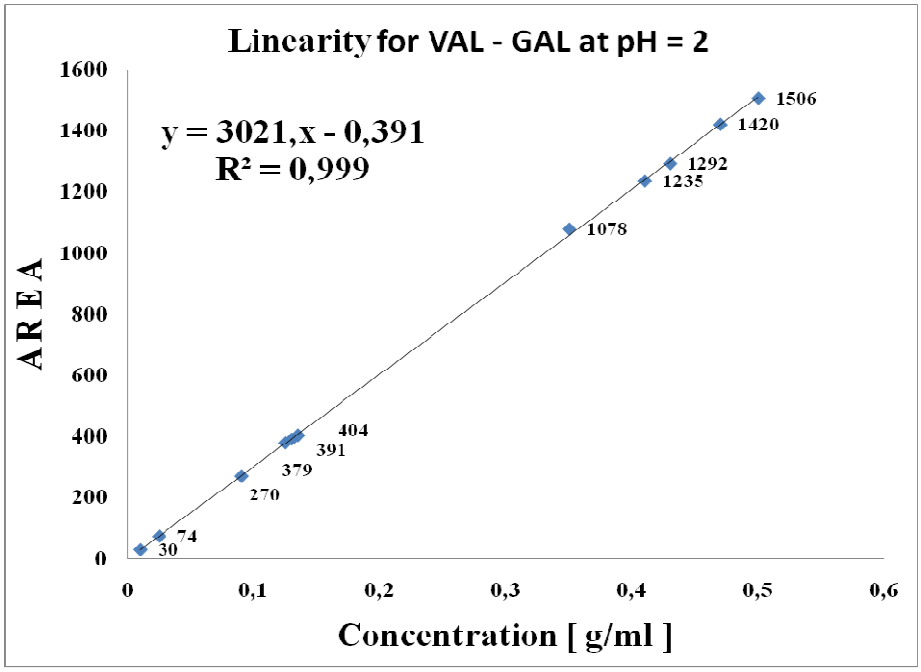


**Fig. 2: Calibration curve for linearity of VAL – GAL at pH =2.**

**Table 2: Spot area and content [mg/ml] of LEU – GAL for 6 h, t = 20 °C at pH = 2.**

| T [min.] | Spot area (A) | | Content (C) [mg/ml] | |
|---|---|---|---|---|
| | $A_{LEU-GAL}$ | U $A_{LEU-GAL}$ | [LEU – GAL] | U [LEU – GAL] |
| 0 | 84 | 0.79 | 10.46 | 0.77 |
| 30 | 83 | 0.14 | 10.34 | 0.15 |
| 60 | 84 | 0.79 | 10.46 | 0.77 |
| 90 | 84 | 0.79 | 10.46 | 0.77 |
| 120 | 82 | 1.07 | 10.22 | 1.08 |
| 150 | 83 | 0.14 | 10.34 | 0.15 |
| 180 | 82 | 1.07 | 10.22 | 1.08 |
| 210 | 82 | 1.07 | 10.22 | 1.08 |
| 240 | 84 | 0.79 | 10.46 | 0.77 |
| 270 | 82 | 1.07 | 10.22 | 1.08 |
| 300 | 84 | 0.79 | 10.46 | 0.77 |
| 330 | 82 | 1.07 | 10.22 | 1.08 |
| 360 | 85 | 1.73 | 10.58 | 1.69 |
| $\overline{X}$ | 83.15 | | 10.36 | |
| SD | 1.07 | | 0.13 | |

**Table 3: Spot area and content [mg/ml] of VAL – GAL for 6 h, t = 20 °C at pH = 2.**

| N: | T [min.] | pH = 2 Spot area | | pH = 2 Content [mg/ml] | |
|---|---|---|---|---|---|
| | | $A_{VAL-GAL}$ | U $A_{VAL-GAL}$ | [VAL – GAL] | U [VAL – GAL] |
| 1. | 0 | 30 | 0.36 | 10.06 | 0.36 |
| 2. | 30 | 27 | 1.20 | 9.07 | 1.19 |
| 3. | 60 | 29 | 0.16 | 9.73 | 0.16 |
| 4. | 90 | 28 | 0.68 | 9.40 | 0.67 |
| 5. | 120 | 28 | 0.68 | 9.40 | 0.67 |
| 6. | 150 | 28 | 0.68 | 9.40 | 0.67 |
| 7. | 180 | 31 | 0.88 | 10.39 | 0.88 |
| 8. | 210 | 29 | 0.16 | 9.73 | 0.16 |
| 9. | 240 | 27 | 1.20 | 9.07 | 1.19 |
| 10. | 270 | 28 | 0.68 | 9.40 | 0.67 |
| 11. | 300 | 32 | 1.39 | 10.72 | 1.39 |
| 12. | 330 | 33 | 1.91 | 11.05 | 1.91 |
| 13. | 360 | 31 | 0.88 | 10.39 | 0.88 |
| 14. | $\overline{X}$ | 29.31 | | 9.83 | |
| 15. | SD | 1.93 | | 0.64 | |

**Table 4: Spot area for LEU – GAL for 6 h, t = 20 °C at different pH values.**

| T [min.] | pH = 7.4 Spot area | | pH = 9 Spot area | | mol/mol with NaOH Spot area | |
|---|---|---|---|---|---|---|
| | $A_{LEU-GAL}$ | U $A_{LEU-GAL}$ | $A_{LEU-GAL}$ | U $A_{LEU-GAL}$ | $A_{LEU-GAL}$ | U $A_{LEU-GAL}$ |
| 0 | 23 | 0.17 | 31 | 0.5 | 29 | 0.57 |
| 30 | 27 | 1.35 | 31 | 0.5 | 32 | 1.74 |
| 60 | 26 | 0.97 | 34 | 0.04 | 27 | 0.21 |
| 90 | 27 | 1.35 | 41 | 1.32 | 31 | 1.35 |
| 120 | 21 | 0.94 | 25 | 1.6 | 26 | 0.6 |
| 150 | 22 | 0.56 | 36 | 0.41 | 26 | 0.6 |
| 180 | 22 | 0.56 | 29 | 0.87 | 25 | 0.99 |
| 210 | 27 | 1.35 | 35 | 0.22 | 25 | 0.99 |
| 240 | 20 | 1.32 | 44 | 1.86 | 24 | 1.38 |
| 270 | 20 | 1.32 | 26 | 1.42 | 28 | 0.18 |
| 300 | 22 | 0.56 | 36 | 0.41 | 31 | 1.35 |
| 330 | 23 | 0.17 | 38 | 0.77 | 26 | 0.6 |
| 360 | 25 | 0.59 | 33 | 0.14 | 28 | 0.18 |
| $\overline{X}$ | 23.46 | | 33.77 | | 27.54 | |
| SD | 2.63 | | 5.49 | | 2.57 | |

**Table 5: Spot area for VAL – GAL for 6 h, t = 20 °C at different pH values.**

| T [min.] | pH = 7.4 Spot area | | pH = 9 Spot area | | mol/mol with NaOH Spot area | |
|---|---|---|---|---|---|---|
| | $A_{VAL-GAL}$ | U $A_{VAL-GAL}$ | $A_{VAL-GAL}$ | U $A_{VAL-GAL}$ | $A_{VAL-GAL}$ | U $A_{VAL-GAL}$ |
| 0 | 46 | 1.85 | 45 | 0.08 | 132 | 1.20 |
| 30 | 55 | 0.05 | 37 | 1.86 | 129 | 0.55 |
| 60 | 53 | 0.37 | 44 | 0.31 | 127 | 0.12 |
| 90 | 55 | 0.05 | 49 | 0.8 | 130 | 0.76 |
| 120 | 58 | 0.68 | 47 | 0.36 | 127 | 0.12 |
| 150 | 52 | 0.58 | 40 | 1.20 | 124 | 0.53 |
| 180 | 62 | 1.52 | 51 | 1.25 | 120 | 1.40 |
| 210 | 52 | 0.58 | 46 | 0.14 | 130 | 0.76 |
| 240 | 50 | 1.0 | 53 | 1.69 | 121 | 1.18 |
| 270 | 54 | 0.16 | 47 | 0.36 | 121 | 1.18 |
| 300 | 53 | 0.37 | 47 | 0.36 | 135 | 1.84 |
| 330 | 63 | 1.73 | 44 | 0.31 | 125 | 0.32 |
| 360 | 59 | 0.89 | 40 | 1.20 | 123 | 0.75 |
| $\overline{X}$ | 54.77 | | 45.38 | | 126.46 | |
| SD | 4.75 | | 4.5 | | 4.63 | |

## CONCLUSION

The examined Galantamine peptide esters 3,4 – dichlorophenyl – Alanil – Leucil – Glycil – Galantamine and 3,4 – dichlorophenyl – Alanil – Valil – Glycil – Galantamine are resistant at different aqueous buffer solutions with pH = 2, pH = 7.4, pH = 9 at room temperature in period of 6 hours. At pH = 2 content of LEU – GAL is in interval 10.22 – 10.58 mg and quantity of VAL – GAL is in interval 9.07 mg – 11.05 mg.

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