TLC – DENSITOMETRY INVESTIGATION OF STABILITY OF GALANTAMINE PEPTIDE ESTERS AT PHYSIOLOGICAL TEMPERATURE AND DIFFERENT PH VALUES

D. Tsvetkova[1], D. Obreshkova[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University – Sofia

Abstract. In current work were summarized the experimental results from the study of chemical stability of Galantamine peptide esters in different aqueous buffer solutions with pH = 2, pH = 7.4, pH = 9 at physiological temperature for 6 h. The applied conditions were: stationary phase: Silicagel $G_{60}F_{254}$; mobile phase: n – butanol : water = 30 : 10 v/v, reflectance – absorbance mode at λ = 282 nm.
Linear regression analysis was employed. The obtained regression equations show the proportional accordance between spot area and concentration.
The experimental results show that the examined Galantamine esters are resistant at physiological temperature to chemical hydrolysis in aqueous buffer solutions with pH = 2, pH = 7.4, pH = 9, which is proved by the fact that during 6 h of experiment it is not observed the statistical significant difference between the values of area of spots.

Key words: Galantamine, peptide esters, stability, pH

Introduction

Alzheimer's disease is a progressive neurodegenerative brain disorder associated with loss of neurons and destroying of memory and thinking and is characterized with presence of amyloid plaques and neurofibrillary tangles [1]. For pharmacological treatment of Alzheimer's disease [2, 3] are applied inhibitors of acethylcholinesterase: Galantamine [1, 4], Donepezil and Rivastigmine [1]. The most promising new therapeutic strategy for therapy is the application of γ – secretase inhibitors [5].

Very important factor for the pharmacological activity of every compound is its stability at different physiological conditions. The degradation of drugs can occur through either reversible or irreversible processes. The most common factors that affect this stability include temperature, light, pH, oxidation and enzymatic degradation [6]. HPLC is very often used method for stability analysis and for analysis of related substances in tablet dosage forms [7]. TLC densitometric methods are developed and applied for the identification, determination and investigation of stability of different drugs, using as stationary phase silicagel $G_{60}F_{254}$: 1) Betaxolol: chloroform : methanol : ammonia 25 % = 18 : 4 : 0.2 and λ = 280 nm [8]; 2) Carteolol in pharmaceutical dosage forms: chloroform : methanol = 5 : 1 and λ = 254 nm [9]; 3) Tramadol: chloroform : toluene : ethanol = 9 : 8 : 1 and λ = 270 nm [10]; 4) Aceclofenac: chloroform : methanol : ammonia = 48 : 11.5 : 0.5 and λ = 274 [11]; 5) Prednisolone acetate (243 nm) and Chloramphenicol (278 nm): chloroform : methanol = 14 : 1 [12]; 6) Clopidogrel: carbon tetrachloride : chloroform : acetone = 6 : 4 : 0.15 and λ = 230 nm [13]. The aim of current study is to investigate the chemical stability in different aqueous buffer solutions with pH = 2, pH = 7.4, pH = 9 of new synthetized Galantamine peptide esters, which possesses both acethylcholinesterase and γ – secretase inhibitory activity [14].

Materials and methods

Materials

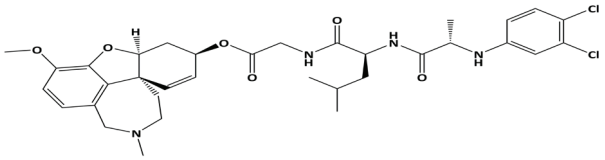
I. 6-O-N[N(3,4-dichlorophenyl)-D,L-Alanil-L-Leucil-L-Glycil]-Galantamine (GAL – LEU) and 6-O-N[(N-3,4-dichlorophenyl)-D,L-Alanil-L-Valil-L-Glycil]-Galantamine (GAL – VAL) (syntetized from prof. Vesenkov from Department of Organic Chemistry, University of Chemical

Technology and Metallurgy) (Fig. 1.) [14].

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).

6-O-N[N(3,4-dichlorophenyl)-D,L-Alanil-L-Leucil-L-Glycil]-Galantamine



6-O-N[(N-3,4-dichlorophenyl)-D,L-Alanil-L-Valil-L-Glycil]-Galantamine

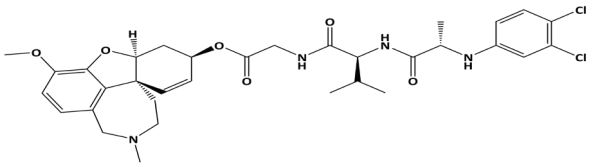


Fig. 1. *Structures of Galantamine peptide esters.*

Method: TLC – densitometry

I. Instrumentation

TLC densitometric scanner TR 541a, performed in the reflectance – absorbance mode at λ = 282 nm and 10 μl sample syringe (Hamilton, Bonaduz, Switzerland) were used for the chromatographic procedure**.**

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 R 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.

IV. Preparation of solutions of GAL – LEU and GAL – VAL for linearity.

An accurately weighed quantity of GAL – LEU and GAL – VAL: 100 mg, 150 mg, 200 mg, 225 mg, 250 mg, 275 mg, 300 mg was dissolved in Buffer solution pH 2.0 to 1.0 ml to obtain solutions with concentration correspondingly: 1.10^{-1} g/ml; $1.5.10^{-1}$ g/ml; 2.10^{-1} g/ml, $2.25.10^{-1}$ g/ml, $2.5.10^{-1}$ g/ml, $2.75.10^{-1}$ g/ml, 3.10^{-1} g/ml.

V. 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 GAL – LEU and GAL – VAL was dissolved in buffer solution with pH = 2.0, pH = 7.4, pH = 9.0 to obtain concentration of 1 mg/1 ml. From every solutions were taken 10 μl at every 30 min. during interval of 6 hours. Chromatograms were prepared on TLC plates Silicagel $G_{60}F_{254}$ using the mobile phase n – butanol : water = 30 : 10 v/v. 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 increasing concentration of respective compound and were analyzed separately by the written TLC densitometric method. The obtained results for spot area (A) and concentration (C) are shown on Table 1.

Linear regression analysis was employed to calculate the regression equations and the correlation coefficients. The obtained regression equations show the proportional accordance between spot area and concentration. Calibration curves for linearity are illustrated on Fig. 2. (pH = 2), Fig. 3. (pH = 7.4) and Fig. 4. (pH = 9).

Very important factor for the pharmacological activity of every compound is its stability at different physiological conditions. For the assessment of stability of acethylcholinesterase inhibitor Rivastigmine is described TLC method by using stationary phase: Silicagel $G_{60}F_{254}$, mobile phase: methanol : butanol : water : ammonia = 5 : 4 : 1 : 0.01 and densitometry in absorbance mode at λ = 263 nm [15].

In previous our experiments was confirmed that

Table 1. *Results for spot area for GAL – LEU and GAL – VAL at pH = 2, pH = 9*

N:	Compound	GAL – LEU			GAL – VAL		
	C [g/ml]	Area at pH = 2	Area at pH = 7.4	Area at pH = 9	Area at pH = 2	Area at pH = 7.4	Area at pH = 9
1.	1.10^{-1}	59200	61200	65000	76800	61000	50000
2.	$1.5.10^{-1}$	88300	90200	97000	112700	97800	59800
3.	2.10^{-1}	123400	123800	135800	142000	125600	93600
4	2.25^{-1}	136900	136500	147500	166500	131600	102000
5.	$2.5.10^{-1}$	145200	152400	163500	190400	148300	114700
6.	$2.75.10^{-1}$	167500	166500	179600	207400	172000	126900
7.	3.10^{-1}	177600	183300	197400	224400	190600	135300

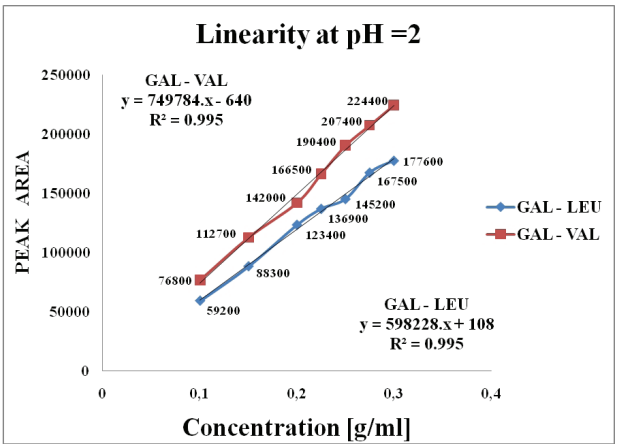


Fig. 2. *Calibration curve for linearity of GAL – LEU and GAL – VAL at pH = 2.*

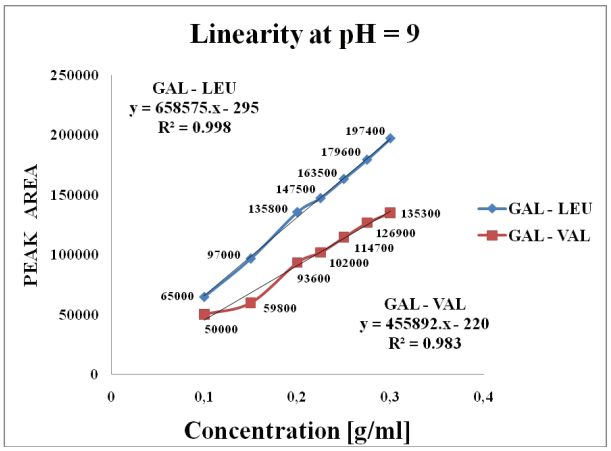


Fig. 4. *Calibration curve for linearity of GAL – LEU and GAL – VAL at pH = 9.*

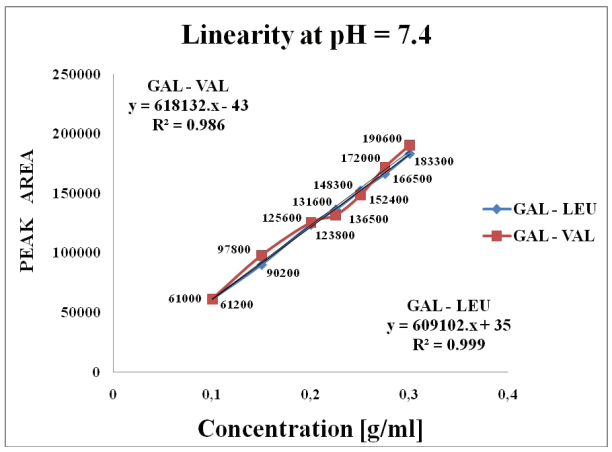


Fig. 3. *Calibration curve for linearity of GAL – LEU and GAL – VAL at pH = 7.4.*

the examined Galantamine peptide esters are resistant in period of 6 h at room temperature and at different aqueous buffer solutions with pH values corresponded to the pH values in stomach (pH = 2), blood (pH = 7.4) and intestine (pH = 9) [16]. The aim of current study is to apply the TLC densitometric method for the investigation of chemical stability of esters for 6 h at 37 oC, corresponded to the body temperature and in aqueous buffer solutions with pH = 2, pH = 7.4 and pH = 9.

The content of every peptide ester is determined by method of calibration curve using the respective regression equation at different pH. For all of the obtained results for content of peptide esters 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 relation UC < 2.03 shows, that all experimental results for UC are lower, than standard requirement: Umax = 1.96 (n = 11) and it isn't necessary to

Table 3. *Chemical stability of GAL – LEU for 6 h at t = 37°C and pH = 2, pH = 7.4, pH = 9.*

Compound		GAL – LEU								
N:	Time t [min.]	pH = 2			pH = 7.4			pH = 9		
		Area	C mg/ml	UC	Area	C mg/ml	UC	Area	C mg/ml	UC
1.	0	6100	10.02	0.13	7200	11.76	1.12	5900	9.41	0.92
2.	30	6900	11.35	1.76	7200	11.76	1.12	6200	9.86	0.02
3.	60	6800	11.19	1.56	6800	11.11	0.61	6500	10.32	0.98
4.	90	6400	10.52	0.74	6400	10.45	0.09	6500	10.32	0.98
5.	120	6100	10.02	0.13	5500	8.97	1.06	6400	10.17	0.67
6.	150	6000	9.85	0.07	5700	9.30	0.80	6700	10.62	1.60
7.	180	5500	9.01	1.10	5600	9.14	0.93	5700	9.10	1.56
8.	210	5800	9.51	0.49	5700	9.30	0.80	6200	9.86	0.02
9.	240	5500	9.01	1.10	5700	9.30	0.80	6100	9.71	0.29
10.	270	5600	9.18	0.89	6100	9.96	0.29	6100	9.71	0.29
11.	360	5700	9.35	0.68	7700	12.58	1.76	5800	9.25	1.25
$\overline{X}$			9.91			10.33			9.85	
SD			0.82			1.28			0.48	

Table 4. *Chemical stability of GAL – VAL for 6 h at t = 37°C and pH = 2, pH = 7.4, pH = 9*

Compound		GAL – VAL								
N:	Time t [min.]	pH = 2			pH = 7.4			pH = 9		
		Area	C mg/ml	UC	Area	C mg/ml	UC	Area	C mg/ml	UC
1.	0	7700	11.12	0.89	5500	8.97	0.97	4600	10.57	0.51
2.	30	7300	10.59	1.00	5800	9.45	0.23	4600	10.57	0.51
3.	60	7700	11.12	0.89	6300	10.26	1.02	4700	10.79	0.05
4.	90	7900	11.39	1.86	5400	8.81	1.22	4800	11.01	0.62
5.	120	7600	10.99	0.43	5700	9.29	0.48	4600	10.57	0.51
6.	150	7300	10.59	1.00	6500	10.59	1.52	4500	10.35	1.08
7.	180	7400	10.72	0.54	5500	8.97	0.97	4700	10.79	0.05
8.	210	7400	10.72	0.54	5600	9.13	0.72	4800	11.01	0.62
9.	240	7200	10.46	1.46	6500	10.59	1.52	5000	11.45	1.74
10.	270	7500	10.86	0.04	6100	9.94	0.52	4900	11.23	1.18
11.	360	7600	10.99	0.43	5900	9.61	0.02	4400	10.13	1.64
X			10.87			9.60			10.77	
SD			0.28			0.65			0.39	

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 $(SD)^2$.

The experimental data show that between the value of spot area of zero time sample and spot areas of all samples stored under 37°C no statistical significant difference is observed. This results confirm the chemical stability of esters at 37°C and different pH.

On Table 3. (GAL – LEU) and Table 4. (GAL – VAL) are presented the results for: 1) spot area; 2) quantity of compounds: C [mg/ml]; 3) Chauvenet's criterion for content of compounds (UC).

Conclusion

The results during 6 h of the experiment show that the examined Galantamine esters are resistant at 37°C 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 fact that during 6 hour of experiment it is not observed the statistical significant difference between the values of spot area.

Acknowledgements

This article was prepared with the financial support from Grant project N:14/2012 (Contract N:26/17.07.2012) – Medical University – Sofia, Bulgaria.

The authors would like to thank to prof. Ljubomir Vesenkov from the Department of Organic Chemistry, University of Chemical Technology and Metallurgy – Sofia, for the providing of substances of Galantamine peptide esters.

References

1. De A, Bala NN, DasGupta P. Alzheimer's Disease and its Management. Int J Res Pharm Biomed Sci 2011; 2(4): 1439-1443.
2. Massoud F, Léger GC. Pharmacological treatment of Alzheimer disease. Can J Psychiatry 2011; 56(10): 579-588.
3. Winslow, BT, Onysko MK, Stob CM, Hazlewood KA. Treatment of Alzheimer disease. Am Fam Physician 2011; 83(12): 1403-1412.
4. Aronson S, Van - Baelen B, Kavanagh S, Schwalen S. Optimal dosing of galantamine in patients with mild or moderate Alzheimer's disease: post Hoc analysis of a randomized, double - blind, placebo - controlled trial. Drugs Aging 2009; 26(3): 231-239.
5. Salomone S, Caraci F, Leggio GM, Fedotova J, Drago F. New pharmacological strategies for treatment of Alzheimer's disease: focus on disease - modifying drugs. Br J Clin Pharmacol 2012; 73(4): 504-517.
6. Briscoe CJ, Hage DS. Factors affecting the stability of drugs and drug metabolites in biological matrices. Bioanalysis 2009; 1(1): 205-220.
7. Tsvetkova B, Pencheva I, Zlatkov A, Peikov P. High-performance liquid chromatographic assay of indomethacin and its related substances in tablet dosage forms. Int J Pharm Pharm Sci 2012; 4(Suppl. 3): 549-552.
8. Kwiecie, A, Krzek J, Walczak M, Mazur M. Development and validation of stability - indicating TLC - densitometric method for determination of Betaxolol with LC-ESI/MS analysis of degradation product. Acta Poloniae Pharm Drug Res 2013; 70 (4): 643-652.
9. Ameeduzzafar JA, Asgar A. Stability - indicating HPTLC method of carteolol in bulk drug and in pharmaceutical dosage forms. J Planar Chromatogr - Modern TLC 2013; 26(1): 86-92.
10. Krzek J, Starek M. Quality assessment for tramadol in pharmaceutical preparations with thin layer chromatography and densitometry. Biomed Chromatogr 2004; 18(8): 589-599.
11. El-Saharty YS, Refaat M, El-Khateeb SZ Stability - indicating spectrophotometric and densitometric methods for determination of aceclofenac. Drug Dev Ind Pharm. 2002; 28(5): 571-582.
12. Musharraf SG, Fatima U, Sultana R. Stress degradation studies and development of stability-indicating TLC-densitometry method for determination of prednisolone acetate and chloramphenicol in their individual and combined pharmaceutical formulations Chem Cent J 2012; (6): 7.
13. Agrawal H, Kaul N, Paradkar AR, Mahadik KR. Stability indicating HPTLC determination of clopidogrel bisulphate as bulk drug and in pharmaceutical dosage form. Talanta 2003; (61): 581-589.
14. Vezenkov L, Georgieva M, Danalev D, Ivanov Tch, Ivanova G. Synthesis and characterization of new Galanthamine derivatives comprising peptide moiety. Protein

and Peptide Letters 2009; 16(9): 1024-1028.

15. Salem MY, El - Kosasy AM, El - Bardicy MG, Abd El - Rahman MK. Spectrophotometric and spectrodensitometric methods for the determination of rivastigmine hydrogen tartrate in presence of its degradation product. Drug Test Anal 2010; 2(5): 225-233.

16. Tsvetkova D, Obreshkova D, Danchev N. Study of stability of Galantamine peptide esters at room temperature and different pH values. Int J Pharm Pharm Sci 2013; 5(1): 41-45.

✉ **Corresponding author**
Dobrina Tsvetkova
Faculty of Pharmacy, Medical University
2 Dunav Str. 1000 Sofia
Tel.: +35929236566
Fax.: +35929879874
e-mail: dobrinka30@abv.bg