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STUDY OF THE STABILITY OF THE SUBSTANCE 3-[2-(4-PHENYL-1-PIPERAZINO)-2-OXOETHYL]QUINAZOLINE-4(3*H*)-ONE UNDER STRESSFUL CONDITIONS

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The aim of the research was to study the stability of a new pharmaceutical substance 3-[2-(4-phenyl-1-piperazino)-2-oxoethyl]quinazoline-4(3*H*)-one under stress conditions.

Materials and methods. The study was conducted in accordance with the recommendations of the ICH guidelines. The object of the study was a previously unknown derivative of quinazoline-4(3*H*)-one: 3-[2-(4-phenyl-1-piperazino)-2-oxoethyl] quinazoline-4(3*H*)-one synthesized in Volgograd state medical university. The following laboratory equipment was used: HPLC chromatograph, HPLC-MS, centrifuge, electronic scales, pH meter, thermostat, laboratory filters.

The computational experiment was conducted on a computer with an Intel Xeon E3-1230 processor using the programs ORCA 4.1. and GROMACS 2019.

Results. The influence of the most unfavorable environmental factors, such as high temperature, light, oxidants, hydrolysis in acidic and alkaline environments, affect the stability of the test substance. The results of the computer-based stability prediction were confirmed by HPLC and HPLC-MS, and the degradation products of the substance under stressful conditions were determined. The conducted studies showed that the test substance is stable to UV radiation at the wavelength of 365 nm, at the elevated temperature (80°C), to the action of oxidants. But it is unstable to hydrolysis: in an alkaline medium of sodium hydroxide 1M, a break in the amide group occurs with the formation of 2-(4-oxoquinazoline-3-yl)acetic acid and 1-phenylpiperazine. And in an acidic environment, hydrochloric acid 1M is also destroyed, but it is significantly reduced, presumably due to the protonation and stabilization of tertiary nitrogen atoms in the molecule.

Conclusion. The conducted research makes it possible to conclude that the test substance 3-[2-(4-phenyl-1-of piperazino)-2-oxoethyl]quinazoline-4(3*H*)-one is stable to aggressive environmental factors, with the exception of hydrolysis in an alkaline environment that will be further considered in the preparation of regulatory documents for this pharmaceutical substance.

Keywords: quinazoline-4(3*H*)-one derivative, pharmaceutical substance, stress testing, stress test modeling, thermolysis, photolysis, hydrolysis, high-performance liquid chromatography, photostability, thermalstability, GROMACS, ORCA

Abbreviations: State Pharmacopoeia (SF), high performance liquid chromatography (HPLC), high performance liquid chromatography with mass spectrometry (HPLC-MS), ultraviolet radiation (UV radiation).

ИЗУЧЕНИЕ СТАБИЛЬНОСТИ СУБСТАНЦИИ 3-[2-(4-ФЕНИЛ-1-ПИПЕРАЗИНО)-2-ОКСОЭТИЛ]ХИНАЗОЛИН-4(3*H*)-ОНА В СТРЕССОВЫХ УСЛОВИЯХ

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Цель работы – изучение стабильности новой фармацевтической субстанции 3-[2-(4-фенил-1-пиперазино)-2-оксоэтил]хиназолин-4(3*H*)-она под воздействием стресс-условий.
Материалы и методы. Исследование выполнено в соответствии с рекомендациями руководства ICH. Объектом исследования было ранее не изученное производное хиназолин-4(3*H*)-она: 3-[2-(4-фенил-1-пиперазино)-2-оксоэтил] хиназолин-4(3*H*)-он, синтезированное в Волгоградском государственном медицинском университете. Было использовано лабораторное оборудование: ВЭЖХ-хроматограф, ВЭЖХ-МС, центрифуга, электронные весы, pH-метр, термостат, лабораторные фильтры. Вычислительный эксперимент проводился на компьютере с процессором Intel Xeon E3-1230 с использованием программ ORCA 4.1. и GROMACS 2019.
Результаты. Изучено и определено влияние неблагоприятных факторов внешней среды, таких как: высокая температура, свет, действие окислителей, гидролиза в кислой и щелочной среде на стабильность исследуемого вещества. Результаты компьютерного прогнозирования стабильности были подтверждены с помощью ВЭЖХ и ВЭЖХ-МС, а также определены продукты деструкции субстанции в стрессовых условиях. Проведенные исследования показали, что исследуемое вещество стабильно к воздействию УФ-облучения при длине волны 365 нм, повышенной температуры (80°С), действию окислителей и нестабильно к гидролизу: в щелочной среде натрия гидроксида 1М происходит разрыв по амидной группе с образованием 2-(4-оксохиназолин-3-ил)уксусной кислоты и 1-фенилпиперазина; а в кислой среде кислоты хлористоводородной 1М также происходит деструкция, но она значительно снижается, предположительно, за счет протонирования и стабилизации третичных атомов азота в молекуле.
Заключение. Проведенные исследования позволяют сделать вывод о том, что исследуемая субстанция 3-[2-(4-фенил-1-пиперазино)-2-оксоэтил]хиназолин-4(3*H*)-он стабильна к агрессивным факторам внешней среды, за исключением гидролиза в щелочной среде, что в дальнейшем будет учитываться при составлении нормативной документации этой фармацевтической субстанции.
Ключевые слова: производное хиназолин-4(3*H*)-она, фармацевтическая субстанция, стресс-тестирование, моделирование стресс-тестов, термолиз, фотолиз, гидролиз, высокоэффективная жидкостная хроматография, фотостабильность, термостабильность, GROMACS, ORCA
Список сокращений: Государственная фармакопея (ГФ), высокоэффективная жидкостная хроматография (ВЭЖХ), высокоэффективная жидкостная хроматография с масс-спектрометрией (ВЭЖХ-МС), ультрафиолетовое излучение (УФ-излучение).

INTRODUCTION

Stress tests of drugs are artificially recreated unfavorable environmental conditions in order to establish degradation products of medicinal substances.

Various factors that accelerate the rate of chemical reactions are used: high temperatures, light (in the ultraviolet and visible regions of the spectrum), high humidity, changes in the acidity/alkalinity of the medium, exposure to various oxidizing agents and other air components. The study and analysis of the degradation products and half-life of drugs during stress testing should be taken into account when developing methods for determining foreign impurities, quantitative determination, production, storage, transportation, and other regulatory documents being developed for a particular drug [1].

The results of stress tests also allow us to assess the impact of short-term deviations from the declared storage conditions and various ways of destruction of the studied substances, to determine the most unfavorable environmental factor, to which the test substance is more sensitive. Stability data for the resistance of drug substances to air components, also allow a more rational approach to the choice of its primary packaging [2–4].

In particular, the GPM.1.1.0009.18 "Stability and shelf lives of medicinal products" of the State Pharmacopoeia of the Russian Federation (XIVth edition) indicates that the study of drug stability should include stress, accelerated and long-term trials; special attention should be also paid to the development of a program for studying the stability of new drugs. For new pharmaceutical substances, the SP of the XIVth edition recommends conducting stress tests on a compulsory basis [1].

THE AIM of the research was to study the stability of a new pharmaceutical substance 3-[2-(4-phenyl-1-piperazino)-2-oxoethyl]quinazoline-4(3H)-one under stress conditions.

MATERIALS AND METHODS

The work was performed in accordance with the recommendations of the ICH manual and the SP (XIVth edition) [1, 5].

The object of the study was one series of the substance 3-[2-(4-phenyl-1-piperazino)-2-oxoethyl]quinazoline-4(3*H*)-one (laboratory code: VA-10-21) synthesized in Volgograd State Medical University. The structural formula is shown in Fig. 1.

HPLC studies were performed using the UltiMate 3000 system ("Dionex", CCA) with a special spectrophotometric detector with an operating wavelength range from 190 to 900 nm. The data collection and processing were performed using a chromatographic data collection and processing system Chromeleon, version 7 ("Dionex", USA).

It is shown that when using acetonitrile and 0.05 M phosphoric acid as the mobile phase, pH=3.5 in the ratio of 25:750, it is possible to provide optimal chromatography conditions. The experimental parameters were as follows: a stainless steel chromatographic column 150×4.6 mm size LunaC 18 with a particle size of 5 microns, a flow rate of 1 ml/min, a column temperature of 25°C, detection at 226 nm, a sample volume of 20 µl, and an analysis time of 40 minutes. HPLC-MS analysis was performed on a Bruker mass spectrometer (Germany) by electrospray ionization in the "Positive" mode. The temperature control of the samples was carried out in the TS-1/20 SPU thermostat (Russia).

As a mobile phase, acetonitrile and a 0.05 M phosphoric acid solution in the ratio of 25:75, with a pH value of 3.5±0.05, were selected. An aqueous solution of phosphoric acid was adjusted to the specified pH value by adding a triethylamine solution. The pH was controlled potentiometrically. The samples were centrifuged before the HPLC analysis using a laboratory centrifuge with Sigma 2–16P accessories (Germany). All the sample solutions had been centrifuged at 8000 min^{-1} for 3 minutes before being placed in the device. The tested solutions had been pre-filtered through Nylon Membrane, 0.2 µm 25 mm Syringe Filters (USA). The samples were weighed on laboratory electronic scales LV 210-A (Russia). The pH value of the solutions was measured on the C axis and using the pH meter pH-150MI (Russia). All the solvents and reagents used in the study, met the requirements of the GPM.1.3.0001.15 GP (XIVth ed.)

Computer modeling of stress tests, was performed on a workstation with an Intel Xeon E3-1230 processor and 16 GB of RAM. The optimization of the VMA-10-21 substance geometry was performed in the ORCA 4.1 program by the density functional theory (UB3LYP) method using the 3–21G* basis. To study the structure of the solid aggregate state of the substance under study, molecular dynamics was modeled by the method of molecular mechanics in the CHARMM36 force field using the GROMACS 2019 program [6, 7]. The Internet service SwissParam was used for parameterization of the molecule and the studied substance [8]. 10 randomized molecules of the test substance were included in the simulated system. Next, the geometry was optimized using the gradient method.

To study the effect of temperature on the stability of the VMA-10-21 substance, a precisely weighed quantity (about 50 mg) was placed in a conical flask with a capacity of 100 ml, 50 ml of 95% ethyl alcohol was added, and placed on an ultrasonic bath for 15 minutes until the substance was completely dissolved. The resulting solution was boiled in the flask with a return refrigerator in a thermostatically controlled water bath (the temperature of 80^0C) for 3 hours, taking the samples every 45 minutes. In order to search for the solid state structure, molecular dynamics was simulated using simulated annealing with a temperature decrease from 1000 K to 273 K for 200 ns with the use of a thermostat at Nose-Hoover [9]. Further on, in order to study the stability of the studied substance to thermolysis, the molecular dynamics of a system of four molecules was modeled using the unlimited Hartree-Fock method with a base set of 3–21G* for 5000 fs with 1 fs step in the ORCA 4.1 program [10]. The temperature control was performed by scaling velocities with a temperature of 400 K and spherical boundary conditions of constant volume.

The effect of UV light on the stability of the VMA-10-21 substance was studied by the following method: a precisely weighed quantity (about 50 mg) was placed in a conical quartz glass flask with a capacity of 100 ml, 50 ml of 95% ethyl alcohol was added, and placed on the ultrasound bath for 15 minutes until the substance was completely dissolved. The resulting solution was exposed to UV light at a wavelength of 365 nm, and the radiation source fully met the ICH requirements. The sampling was performed every 3 hours. Computer modeling of the effect of light was performed using the unlimited Hartree-Fock method, a set of basic functions 3–21G*, a temperature of 400 K, a simulation step of 1 fs, a simulation duration of 5 ps, and a molecule multiplicity of 3.

To study the effect of acids on the stability of the substance, a precisely weighed quantity (about 50 mg) was placed in a conical flask with a capacity of 100 ml, 40 ml of 95% ethyl alcohol was added, and placed on an ultrasonic bath for 15 minutes until the substance was completely dissolved. After that, 5 ml of a 1 M hydrochloric acid solution was added. The resulting solution was boiled in the flask with a return refrigerator in a thermostatically controlled water bath (the temperature of 80°C) within 45 minutes. On completion the time, the solution was cooled down and 5 ml of a 1 M sodium hydroxide solution was added.

The influence of alkalies on the stability of the substance was studied by the following method: a precisely weighed quantity (about 50 mg) was placed in a conical flask with a capacity of 100 ml, 40 ml of 95% ethyl alcohol was added, and placed on an ultrasonic bath for 15 minutes until the substance was completely dissolved. After that, 5 ml of a 1 M sodium hydroxide solution was added. The resulting solution was boiled in the flask with a return refrigerator in a thermostatically controlled water bath (the temperature of 80°C) within 45 minutes. On completion the time, the solution was cooled down and 5 ml of a 1 M hydrochloric acid solution was added.

In order to calculate the most probable hydrolysis products in acid and alkaline media, vibration analysis was performed, and thermodynamic characteristics using the density functional theory (UB3LYP) method and the 6-311G** basis set in the ORCA 4.1 program, were calculated. During acid hydrolysis, the reaction products calculated with protonated tertiary nitrogens. Since the equivalence factor of 0.5 by titrimetric methods had been determined before, for the molecule VMA-10-21, the Gibbs energies of two simultaneously protonated nitrogen atoms of the VMA-10-21 molecule were calculated for the VMA-10-21 molecule in all possible combinations. During alkaline hydrolysis, the reaction products were presented in the form of carboxylic acid salts – COONa. The Gibbs energy of hydrolysis reactions (ΔG_r) was calculated from the difference between the sum of the Gibbs energies of the reaction products (ΔG_{prod}) and the initial compounds (ΔG_{reac}) in accordance with the Hess' law: $\Delta G_r = \sum \Delta G_{prod} - \sum \Delta G_{reac.}$

The thermodynamic characteristics of water, OH^- ions and the analyzed substance VMA-10-21 without protonation, were also determined. The calculation was performed at a temperature of 310K.

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To study the effect of the oxidation process on the stability of the substance, a precisely weighed quantity (about 50 mg) was placed in a conical flask with a capacity of 100 ml, 45 ml of 95% ethyl alcohol was added, and placed on an ultrasonic bath for 15 minutes until the substance was completely dissolved. Then 5 ml of 3% hydrogen peroxide solution was added. The resulting solution was boiled in the flask with a return refrigerator in a thermostatically controlled water bath (the temperature of 80°C) within 45 minutes. On completion the time, the solution was cooled down and analyzed.

RESULTS AND DISCUSSION

The chemical stability of pharmaceutical molecules is of a serious concern, as it affects the safety and effectiveness of the drug. The FDA and ICH guidelines establish requirements to these tests to determine the effect of various environmental factors on the quality of a pharmaceutical substance due to the passage of time. Knowing the stability of the molecule, helps in choosing the right composition and packaging, as well as ensuring proper storage conditions and shelf life, which is important for regulatory documentation. Artificial degradation involves the destruction of drugs and their semi products under more stringent conditions than accelerated tests, which makes it possible to study the stability of the molecule more fully, and determine the most probable degradation ways [11].

Information on the stability of molecules, helps in the manufacture of dosage forms and determination of storage conditions, so it is rational to start degradation studies at an early stage of drug development [12].

The question of whether the degree of degradation of a substance is sufficient, has been a topic of a lot of discussions among pharmaceutical scientists. Decomposition of a drug substance between 5% and 20%, was considered reasonable for chromatographic analyses [13, 14]. Some pharmaceutical scientists believe that 10% decomposition is optimal for the substances with a low molecular weight [15]. It is not necessary that a forced decomposition should lead to a complete decomposition of a substance. The study can be discontinued if a pharmaceutical substance or a drug form is not decomposed after the exposure to stressful conditions [16]. This indicates the stability of the tested molecule. An excessive exposure to stress tests on the sample, may lead to the formation of secondary decomposition products that will not be identified in storage stability studies, and insufficient exposure may not produce decomposition products [17].

Stability study of VMA-10-21 when exposed to high temperatures (thermolysis)

When modeling molecular dynamics in order to search for the structure of the solid state of the substance under study, the VMA-10-21 substance molecules were arranged systematically, forming elements of the crystal lattice (Table 1).

These data were used as the initial location of the molecules for further calculations by the molecular dynamics method for a system of 4 molecules of the substance under study.

Table 2 shows the state of the system in the process of molecular dynamics simulation by the Hartree-Fock method with a basis set of 3-21G**.

As Table 2 shows, according to the results of computer modeling, the chemical structure of the VMA-10-21 molecule did not change, which makes it possible to assume the stability of the molecule when exposed to elevated temperatures.

ICH recommends performing thermal treatment at temperatures of 60–80°C, i.e. at higher temperatures than in the accelerated tests [18]. The maximum recommended temperature was selected.

Fig. 2 shows a chromatogram of the VMA-10-21 alcohol solution before stress testing.

Fig. 3 shows a chromatogram of the VMA-10-21 alcohol solution after stress testing.

The data on the stability of VMA-10-21 for a time of 0–180 minutes, are presented in Table 3.

As follows from the presented data, the substance VMA-10-21 is almost completely resistant to high temperatures. The results of computer modeling coincided with the practical data.

Study of the VMA-10-21 stability under UV light (photolysis)

Photolytic degradation is believed to be caused by wavelengths in the range of 300–800 nm [19]. Photostability studies are an important aspect of drug studies on substance stability, since photolysis can cause photooxidation by a free-radical mechanism, so we chose the most “hard” UV at a wavelength of 365 nm. Fig. 4 shows the obtained chromatogram after 24 hours of UV exposure.

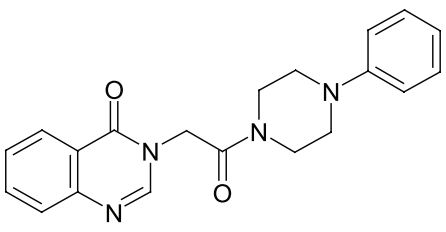

Figure 1 – Structural formula of substance VMA-10-21

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Table 1 – State of the system in the process of molecular dynamics simulation of 10 molecules by the method of molecular mechanics

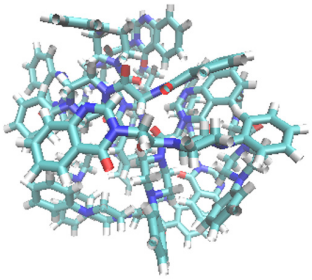
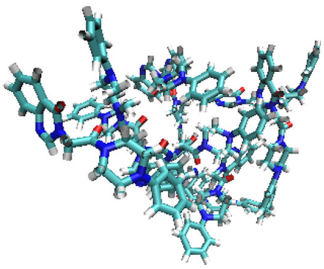
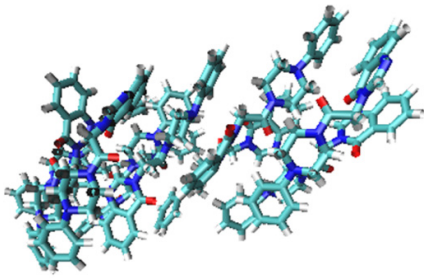
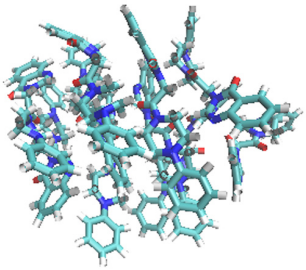
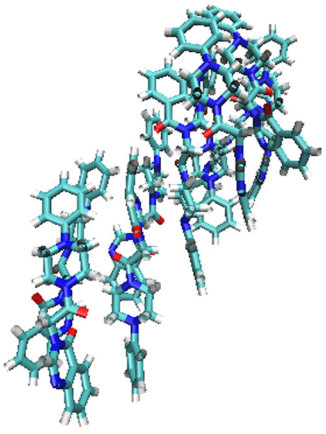
Time, ns	System image
0	
50	
100	
150	
200	

Table 2 – State of the system in the process of molecular dynamics simulation 0–5000 fs

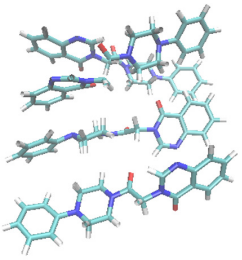
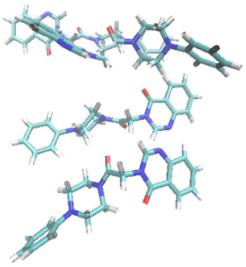
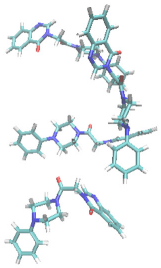
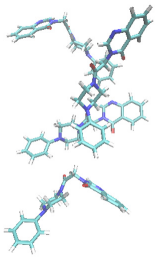
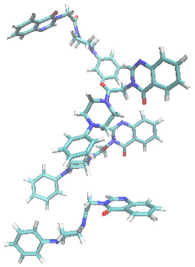
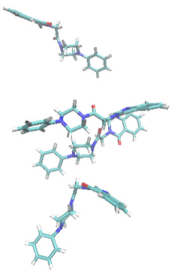
Time, ns	System image
0	
1000	
2000	
3000	
4000	
5000	

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Table 3 – Results of the influence of the stress test (elevated temperature) on the VMA-10-21 stability

Stress test (heating at 80°C)	Contents of VMA-10-21	Degradation percentage
VMA-10-21 without heating	99.68%	–
VMA-10-21 after 45 minutes	99.60%	0.08%
VMA-10-21 after 90 minutes	99.58%	0.1%
VMA-10-21 after 135 minutes	99.54%	0.14%
VMA-10-21 after 180 minutes	99.50%	0.18%

Table 4 – Results of the impact of the stress test (UV light) on the VMA-10-21 stability

Stress test (UV light 365 nm)	Contents of VMA-10-21	Percentage of degradation
VMA-10-21 without UV irradiation	99.68%	–
VMA-10-21 after 3 hours	99.66%	–
VMA-10-21 after 6 hours	99.65%	–
VMA-10-21 after 9 hours	99.61%	–
VMA-10-21 after 12 hours	99.60%	–
VMA-10-21 after 15 hours	99.60%	–
VMA-10-21 after 18 hours	99.59%	–
VMA-10-21 after 21 hours	99.58%	–
VMA-10-21 after 24 hours	99.56%	–

Table 5 – Results of vibrational analysis of all possible combinations for the doubly protonated VMA-10-21 molecule

Molecule	Enthalpy, a.u.	Entropy, cal/mol*K	Enthalpy, kJ/mol	Entropy, kJ/mol*K	Gibbs energy, kJ/mol
VMA-10-21	–1143.540907	35.914	–3002366.237	0.150364735	–3002366.237
I + III	–1144.153274	35.731	–3003974.007	0.149598551	–3003974.007
I + IV	–1144.189237	35.721	–3004068.428	0.149556683	–3004068.428
II+ III	–1144.061293	35.952	–3003300.432	0.150272626	–3003300.432
II + IV	–1144.12641	35.742	–3003732.511	0.150523834	–3003732.511
III + IV	–1144.119529	35.663	–3003903.475	0.149644606	–3003903.475

Table 6 – Results of the conducted vibrational analysis and calculated thermodynamic characteristics and hydrolysis products

The molecule	Enthalpy, a.u.	Entropy, cal/mol*K	Enthalpy, kJ/mol	Entropy, kJ/mol*K	Gibbs energy, kJ/mol
Product 1	–687.469271	106.648	–1805019.32	0.44651	–1805157.74
Product 1 (– COONa)	–686.943269	114.886	–1803638.25	0.48100	–1803787.36
Product 2	–532.492603	93.828	–1398112.58	0.39284	–1398234.36
Product 3	–498.863275	92.872	–1309815.41	0.38884	–1309935.95
Product 4	–721.096936	99.055	–1893312.12	0.41472	–1893440.68
Product 4 (– COONa)	–720.566026	96.379	–1891918.16	0.40352	–1892043.25
OH^-	–75.751778	41.417	–198893.87	0.1734	–198947.62
Water	–76.422293	46.469	–200654.37	0.19456	–200714.68

The data on the stability of the VMA-10-21 when exposed to UV for 0–24 hours, are presented in Table 4.

As follows from the presented data, the substance VMA-10-21 is resistant to UV light, which corresponds to the results of modeling.

Study of VMA-10-21 stability under the influence of acids and bases (hydrolysis)

Hydrolysis is one of the most common chemical decomposition reactions over a wide pH range. It is assumed that high humidity, as one of the parameters of the water content in the ambient air, is a potential threat to the implementation of hydrolytic cleavage reactions. In the study of hydrolysis reactions, the influence of acidic and basic conditions on it is considered as a stress factor for the primary destruction of drugs.

For acid hydrolysis, hydrochloric or sulfuric acids with concentrations of 0.1–1M are used, and for alkaline hydrolysis – sodium or potassium hydroxides with concentrations of 0.1–1M are used [17, 20]. In our study, we selected hydrochloric acid and sodium hydroxide in the maximum allowable concentrations of 1M.

Theoretically, several hydrolysis ways are possible for the VMA-10-21 substance under study. They are shown in Fig. 5.

By the first hydrolysis way, 2-(4-oxoquinazoline-3-yl) acetic acid (product 1) and 1-phenylpiperazine (product 2) are produced, and by the second way, these are 3-methylquinazoline-4-one (product 3) and 4-phenylpiperazine-1-carboxylic acid (product 4).

The results of the vibrational analysis on the search for the most possible tertiary nitrogen atoms involved in protonation, are presented in Table 5.

The protonated nitrogen atom in position 1 of the quinazoline nucleus was denoted as I, in position 3 of the quinazoline nucleus – as II, 1-piperazino – as III, 4-phenyl – as IV. As the results of Table 5 show, protonation of nitrogen atoms in the VMA-10-21 molecule is most probable in positions I and IV. This Gibbs energy was used by the authors in the vibrational analysis for VMA-10-21 in acid hydrolysis reactions.

Table 6 shows the obtained vibrational analysis data for hydrolysis products.

The calculated Gibbs energies of the reactions are presented in Table 7.

As the results obtained show, hydrolysis in an alkaline environment is the most probable for the first hydrolysis way, since the Gibbs energy value is lower for this reaction. It can also be seen that in the acidic environment, due to the higher Gibbs energy of the protonated I + II molecule, the Gibbs energy of hydrolysis in the acidic reaction has increased, so hydrolysis in the acidic environment can be assumed to be less pronounced.

The chromatogram obtained as a result of the interaction of VMA-10-21 solution with hydrochloric acid, is shown in Fig. 6.

The data on the VMA-10-21 stability under the influence of acids are presented in Table 8.

As the presented data show, under the action of a 1 M solution of hydrochloric acid, a partial decomposition of the VMA-10-21 molecule occurs.

The data obtained are shown in Fig. 7.

VMA-10-21 stability data are presented in Table 9.

As the presented data show, under the action of 1 M sodium hydroxide solution, the VMA-10-21 molecule decomposes with a degradation percentage of 92.61%.

Under the action of an alkali solution, the VMA-10-21 molecule decomposes to form two predominant products with retention times of 2.12 min. (about 18%) and 2.98 min. (about 67%). HPLC-MS was used to determine the structural fragments formed as a result of hydrolysis of the products. Fig. 8 and 9 show the obtained mass spectra.

The peak, with a retention time of 2.12 min., corresponds to the molecular ion with a molar mass of 162 g/mol, and the peak with a retention time of 2.98 min., corresponds to the molecular ion with a mass of 186.9 g/mol. The most probable hydrolysis ways calculated as a result of a computational experiment, confirmed the mass detector data.

Based on the obtained data, it can be concluded that the decomposition of the VMA-10-21 molecule occurs at the amide group (way 1 in Fig. 5) with the formation of two main degradation products, which are separated from each other under the selected chromatographic conditions.

Hydrolysis of the VMA-10-21 molecule in the presence of 1M hydrochloric acid occurs with a significantly less decomposition of the molecule. This is probably due to the stabilization of the VMA-10-21 molecule in an acidic environment due to the formation of salts with hydrochloric acid. The main decomposition product in an acidic medium is a molecular ion with a mass of 186.9 g/mol, which also coincides in the retention time (2.98 min.)

Study of VMA-10-21 stability under the influence of oxidizing agents

Hydrogen peroxide, metal ions, oxygen, initiators of radical reactions (azocompounds, N-nitrozoanilides, triazenes, dibenzyls, etc.) are widely used for the forced oxidation of drug substances. It was established that exposure to solutions of 0.1–3% hydrogen peroxide at pH=7 and a temperature of 20°C for seven days, can potentially lead to the appearance of corresponding degradation products [21]. In this study, the maximum allowable hydrogen peroxide solution of 3% was used, but with a shorter time interval.

First, a 3% hydrogen peroxide solution was analyzed without adding the analyzed substance. The resulting chromatogram is shown in Fig. 10.

The data on the stability of VMA-10-21 to oxidizing agents are presented in Table 10.

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Table 7 – Calculated Gibbs energies of hydrolysis reactions.

Way of reaction	Hydrolysis product	Calculated Gibbs energies of reactions, kJ/mol
First	No.1 + No.2 (acidic environment)	1391.01
	No.1 + No.2 (alkaline environment)	–707,86
Second	No.3 + No.4 (acidic environment)	1406.48
	No.3 + No.4 (alkaline environment)	–665.343

Table 8 – Results of the stress test influence (hydrolysis, acid) on the VMA-10-21 stability

Stress test (1M hydrochloric acid)	The contents of the VMA-10-21	Percentage of degradation
VMA-10-21 without hydrolysis	99.68%	–
VMA-10-21 after 45 minutes	98.14%	1.54

Table 9 – Results of the influence of stress test (hydrolysis, alkaline) on the VMA-10-21stability

Stress test (1M sodium hydroxide)	Contents of the VMA-10-21	Percentage of degradation
VMA-10-21 without hydrolysis	99.68%	–
VMA-10-21 after 45 minutes	7.07%	92.61%

Table 10 – Results of the stress test (oxidation) effect on the VMA-10-21stability

Stress test (3% hydrogen peroxide solution)	**Contents of VMA-10-21**	**Percentage of degradation**
VMA-10-21 without addition of hydrogen peroxide	99.68%	–
VMA-10-21 after 45 minutes	98.56%	1.12%

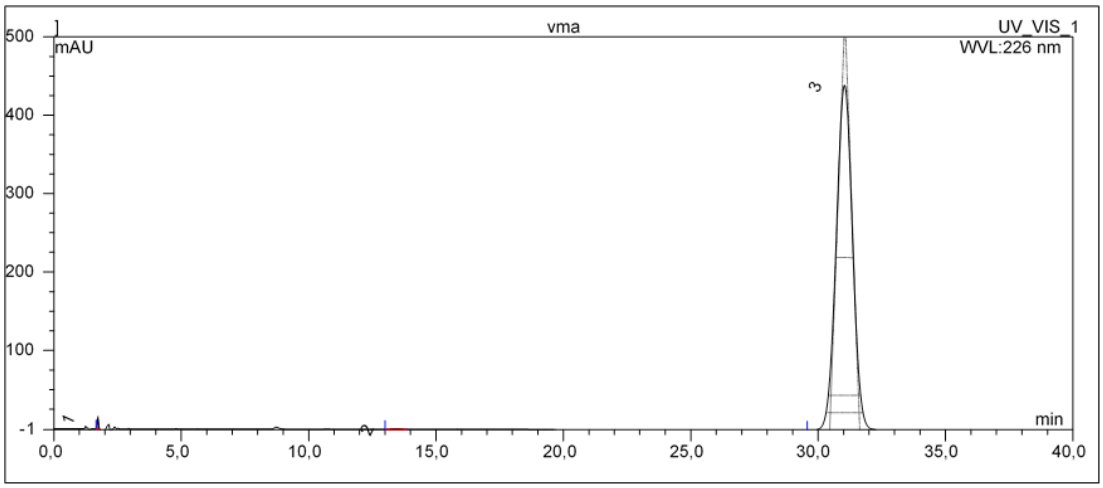

Figure 2 – Chromatogram of VMA-10-21 alcohol solution before stress testing

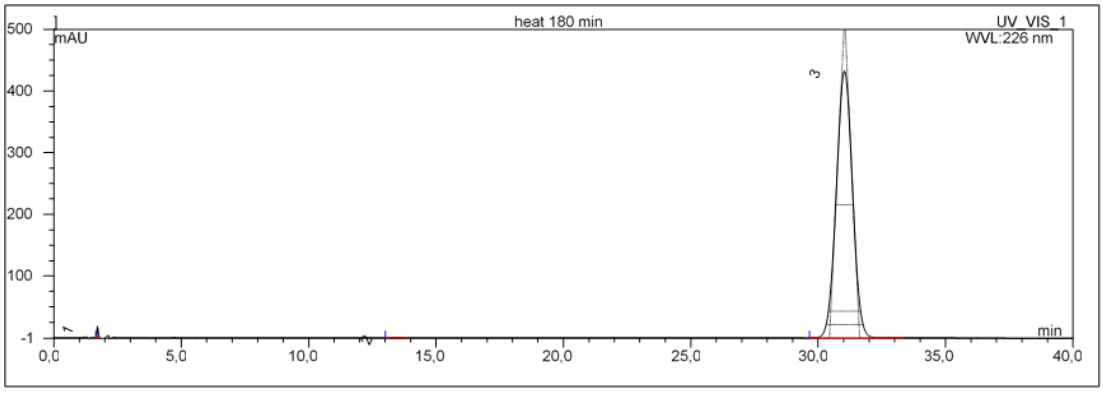

Figure 3 – Chromatogram of VMA-10-21 alcohol solution after heating at 80°C for 180 minutes

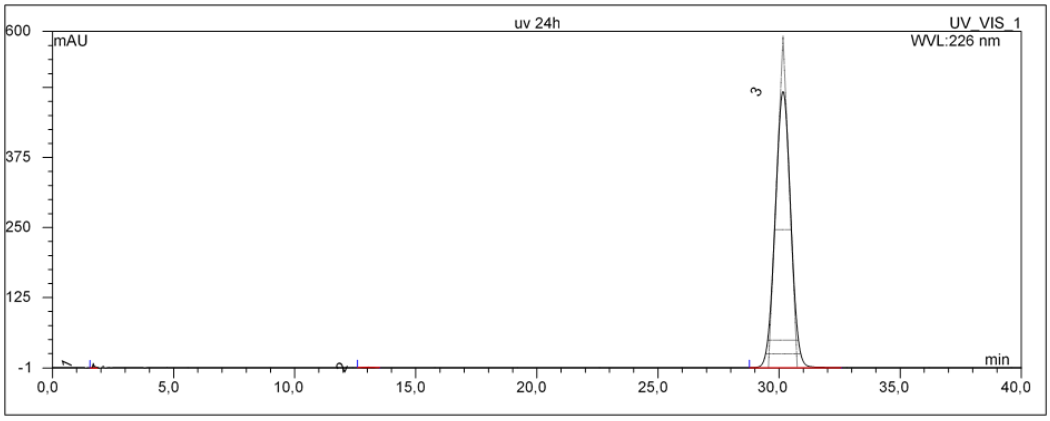

Figure 4 – Alcohol solution VMA-10-21 exposed to UV light after 24 hours

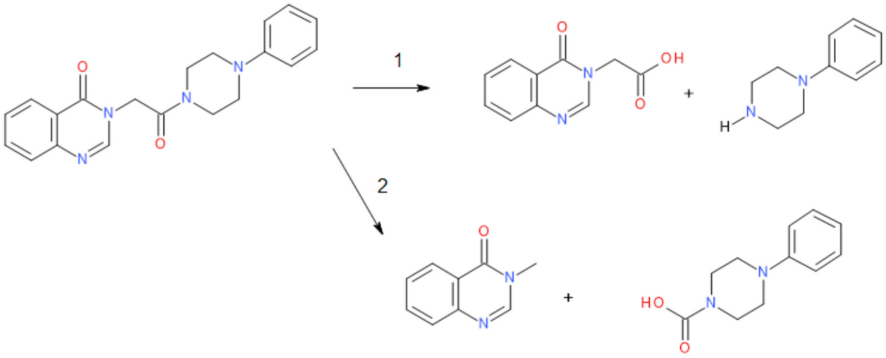

Figure 5 – Suggested ways of hydrolysis of VMA-10-21 substance

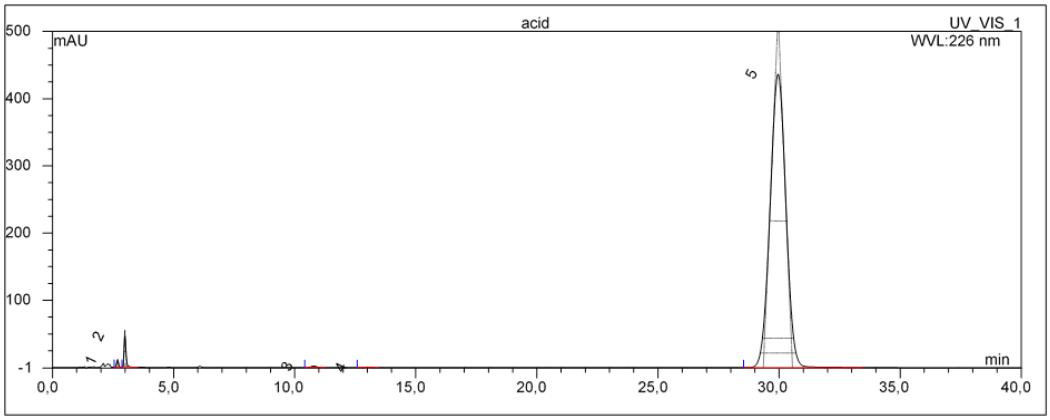

Figure 6 – Chromatogram of VMA-10-21 alcohol solution during hydrolysis with 1M solution of hydrochloric acid after 45 minutes

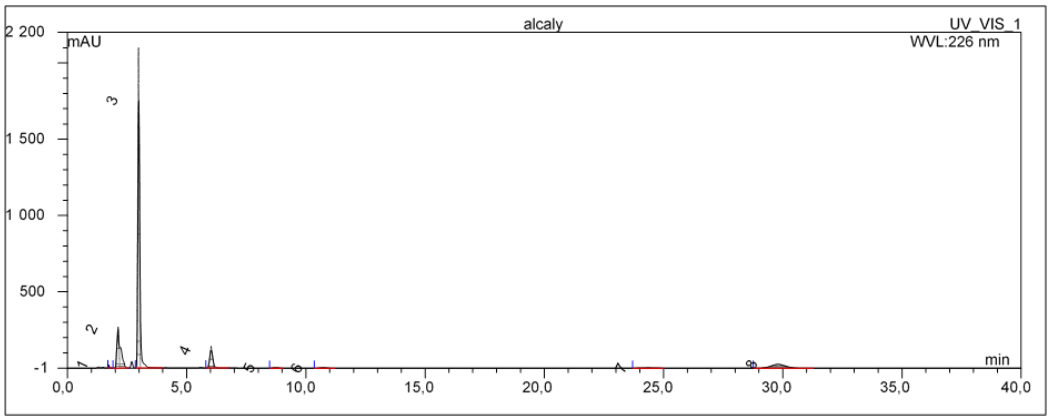

Figure 7 – Alcohol solution of VMA-10-21 during hydrolysis with 1M sodium hydroxide solution after 45 minutes

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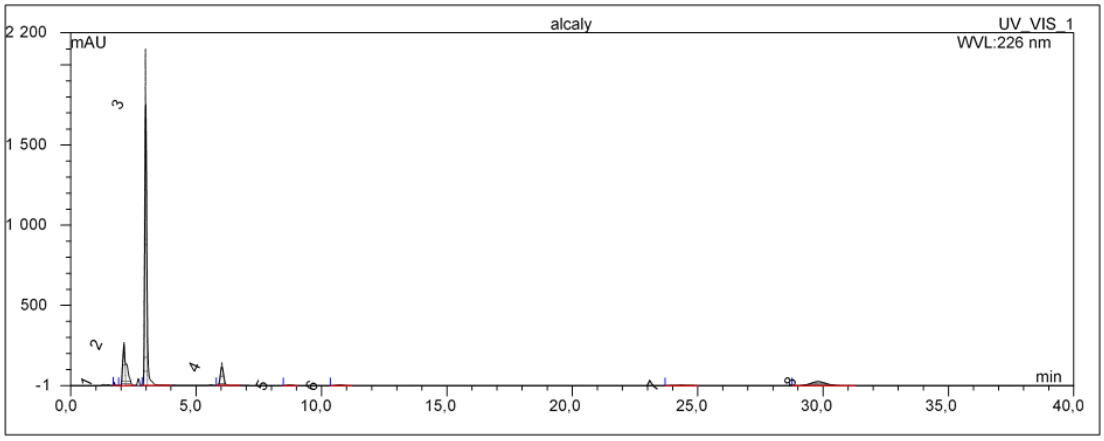

Figure 8 – Mass spectra of a structural fragment with a molecular weight of 162 g/mol

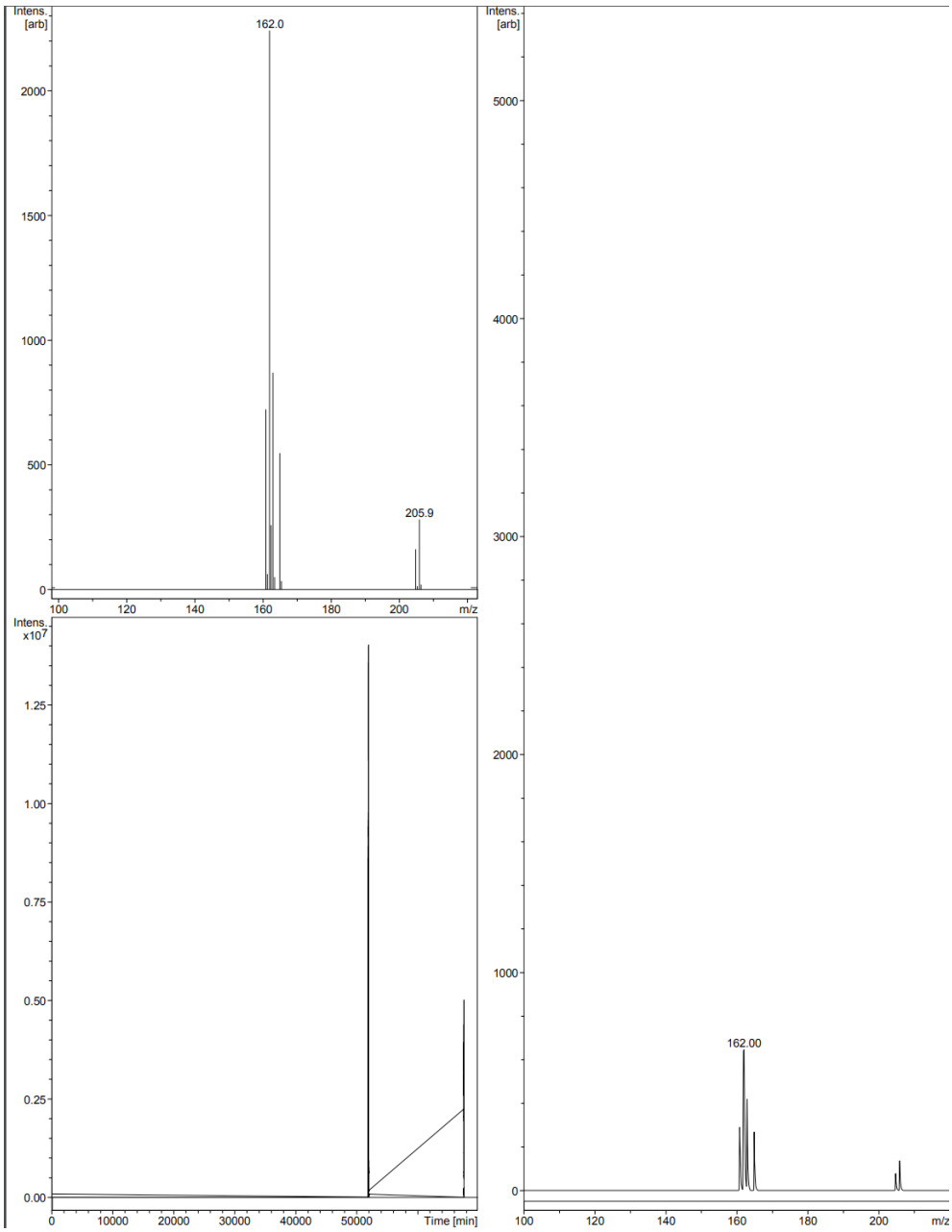

Figure 9 – Mass spectra of a structural fragment with a molecular weight of 186.9 g/mol

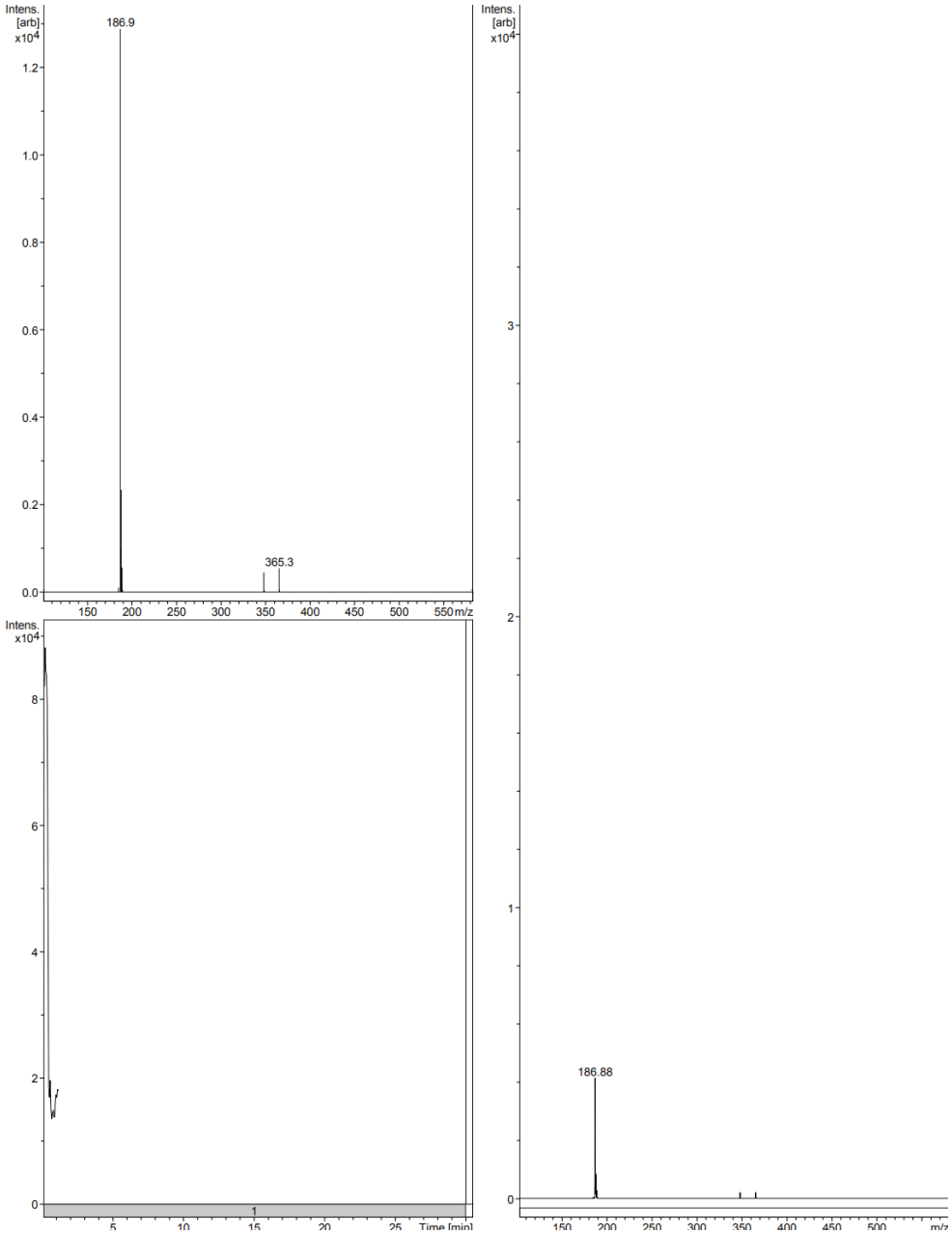

Figure 10 – 3% hydrogen peroxide solution without VMA-10-21

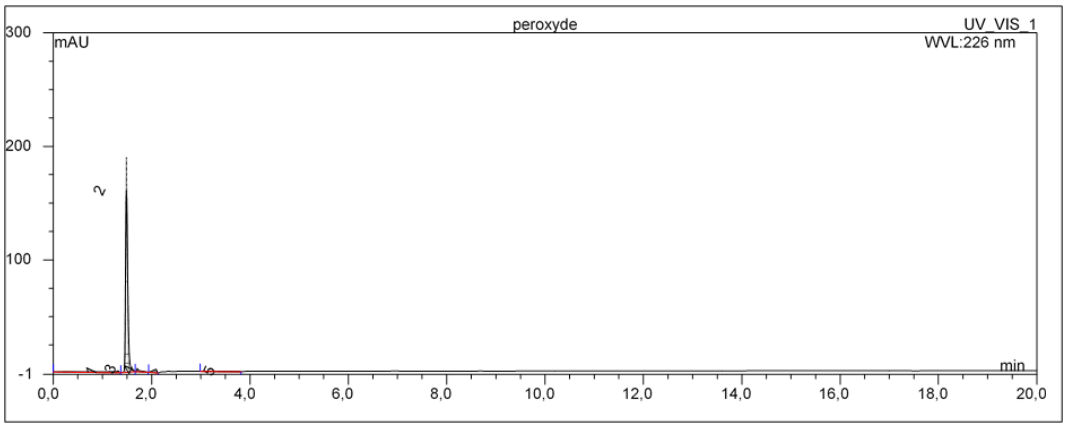

Figure 11 – Alcohol solution of VMA-10-21 with the addition of 3% hydrogen peroxide solution after 45 minutes

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As follows from the presented data, under the action of a 3% hydrogen peroxide solution, the VMA-10-21 molecule is partially decomposed with a degradation percentage of 1.12%.

CONCLUSION

The stability of the new pharmaceutical substance VMA-10-21 was studied in the course of stress tests. As a result of the experiment it was established, that the substance is stable under the action of high temperatures and UV radiation. When conducting the hydrolysis, the investigated substance hydrolyses in alkaline environment at the amide group with the formation of 2 main products, the structural fragments of which were established using the mass detector. In the acid medium, decomposition of the product is greatly reduced, which is likely associated with the increased stability of the molecule due to the formation of salts with hydrochloric acid and the protonation of the two tertiary nitrogen atoms. When the substance is exposed to oxidizing agents (a 3% hydrogen peroxide solution), there is a slight destruction of the molecule (about 1%), which shows the relative stability of the molecule under the action of oxidizing agents. The presented computer calculations have also made it possible to predict the stability and most likely hydrolysis ways of the studied substance, which correspond to practical results. These results will be taken into account in the future when developing regulatory documentation for the substance 3-[2-(4-phenyl-1-piperazino)-2-oxoethyl]quinazoline-4(3*H*)-one.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

CONTRIBUTION OF AUTHORS

T.A. Gendugov – computer analysis and processing of the results obtained, carrying out the practical part of the work; A.A. Glushko – computer analysis and processing of the results; A.A. Ozerov – research conception and strategy, text editing, synthesis and purification of the VMA-10-21substance; L.I. Shcherbakova – research conception and strategy, text editing.

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