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Research of Fluorescent Properties of Telmisartan in Order to Develop an Express Determination in Blood Plasma

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Abstract. Non-peptide selective blockers of AT1-receptors have been developed relatively recently and are actively used in thrombosis therapy. These substances belong to the group of drugs modulating the functioning of the renin-angiotensin-aldosterone system through interaction with angiotensin receptors. However, with a variety of methods for controlling this substance, the problem of quantitative determination of telmisartan in biological fluids remains relevant. Since the substance has a high fluorescent activity, an assumption has been formed on the possibility of developing a method of determining the amount of telmisartan by fluorescent method. The method has high sensitivity and selectivity that allows to determine fluorescence in solutions with concentration from 10^{-12} M. In this work fluorescent properties of telmisartan substance and "Telmista" medicine were obtained, analytical signal registration conditions were defined empirically: solvent – 0.01 M NaOH, excitation wave length – 290 nm, luminescence wave length – 365–490 nm, strobe parameters – signal delay 0.70 μs, signal duration – 4.70 μs. On the basis of the obtained spectra the calibration dependence of the signal intensity on the substance concentration was constructed, and the relative quantum yield was calculated. The influence of solvents in different pH conditions on the intensity of the analytical signal in the model solution was obtained. It was found that the highest intensity of luminescence was observed in 0.01 M NaOH. Quantitative determination of the drug "Telmista" in the model solution was carried out by fluorimetric method. The conditions of signal detection in human blood plasma were determined. This method is characterized by high selectivity, accuracy and sensitivity. The low level of spectral interference allows detection of weak analytical signals. It was found that the excipients meglumine, povidone-K30, lactose monohydrate, sorbitol (E 420), magnesium stearate, included in the drug "Telmista" have no interfering effect on the signal intensity, since the absorption spectra of these excipients are in the region of 500 nm [1: 40]. Fluorimetric analysis of telmisartan often uses a selected combination of chemical reaction products called EDAS (ethylenediamine, diacetylmonoxime, sodium sulfite) reagent. The reaction produces a

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commodity homogeneous mixture that absorbs light of a specific wavelength and emits light in precisely the range that allows the concentration of telmisartan to be accurately measured.
Due to its increased sensitivity, the fluorimetric method of analysis is a very useful tool for measuring telmisartan concentration in human plasma. Moreover, it is able to detect extremely small amounts of the drug substance, which makes it particularly useful for clinical trials and drug analysis. However, the express method of fluorimetric determination of telmisartan will speed up the process of obtaining patient tests many times over. In order to develop such a method, the obtained described in this article were carried out.

Keywords: telmisartan, fluorimetry, luminescence, signal intensity, express method of fluorimetric determination.

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Исследование флуоресцентных свойств телмисартана с целью разработки экспресс-определения в плазме крови

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Аннотация. Непептидные селективные блокаторы AT1-рецепторов разработаны сравнительно недавно и активно используются в терапии тромбозов. Эти вещества относятся к группе препаратов, модулирующих функционирование ренин-ангиотензин-альдостероновой системы посредством взаимодействия с рецепторами ангиотензина. Однако при разнообразии методов контроля этого вещества проблема количественного определения телмисартана в биологических жидкостях остается актуальной. Поскольку вещество обладает высокой флуоресцентной активностью, сформировалось предположение о возможности разработки метода определения количества телмисартана флуоресцентным методом. Метод обладает высокой чувствительностью и селективностью, что позволяет определять флуоресценцию в растворах с концентрацией от 10–12 М. В работе рассмотрены флуоресцентные свойства субстанции телмисартан и препарата «Телмиста», эмпирически определены условия регистрации аналитического сигнала: растворитель – 0,01 М. NaOH, длина волны возбуждения – 290 нм, длина волны люминесценции – 365–490 нм, параметры строба – задержка сигнала 0,70 мкс, длительность сигнала – 4,70 мкс. На основе

полученных спектров была построена калибровочная зависимость интенсивности сигнала от концентрации вещества и рассчитан относительный квантовый выход. Получено влияние растворителей при различных значениях pH на интенсивность аналитического сигнала в модельном растворе. Установлено, что наибольшая интенсивность люминесценции наблюдалась в 0,01 М NaOH. Количественное определение препарата «Телмиста» в модельном растворе проводили флуориметрическим методом. Определены условия детектирования сигнала в плазме крови человека. Этот метод характеризуется высокой селективностью, точностью и чувствительностью. Низкий уровень спектральных помех позволяет обнаруживать слабые аналитические сигналы. Установлено, что входящие в состав препарата «Телмиста» вспомогательные вещества – меглумин, повидон-К30, лактозы моногидрат, сорбит (Е420), магния стеарат – не оказывают мешающего влияния на интенсивность сигнала, поскольку спектры поглощения этих вспомогательных веществ находятся в области 500 нм [1: 40].

Для флуориметрического анализа телмисартана часто используется выбранная комбинация продуктов химической реакции, называемая реагентом ЭДАС (этилендиамин, диацетилмоноксим, сульфит натрия). В результате реакции образуется товарная гомогенная смесь, которая поглощает свет определенной длины волны и излучает свет именно в том диапазоне, который позволяет точно измерить концентрацию телмисартана.

Благодаря своей повышенной чувствительности флуориметрический метод анализа является очень полезным инструментом для измерения концентрации телмисартана в плазме человека. Более того, он способен обнаруживать чрезвычайно малые количества лекарственного вещества, что делает его особенно полезным для клинических испытаний и анализа лекарств. Однако экспресс-метод флуориметрического определения телмисартана многократно ускорит процесс получения анализов пациентов. С целью разработки такого метода были получены результаты, описанные в данной статье.

Ключевые слова: телмисартан, флуориметрия, люминесценция, интенсивность сигнала, экспресс-метод флуориметрического определения.

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Introduction

A relevant group for investigating the fluorescence properties of drugs is the nonpeptide selective AT1-receptor blockers. Drugs of this group refer to antihypertensive agents used to treat high blood pressure.

By chemical structure, nonpeptide AT1-receptor blockers can be divided into 3 main groups:

– Biphenyl tetrazole derivatives: losartan, irbesartan, candesartan, valsartan, and tazosartan;
– biphenyl non-tetrazole compounds: telmisartan;
– non-biphenyl non-tetrazole compounds – eprosartan.

According to their pharmacological activity, AT1-receptor blockers are divided into active dosage forms and prodrugs. For example, valsartan, irbesartan, telmisartan, and eprosartan themselves have pharmacological activity, whereas candesartan cylexetil and azilsartan medoxomil are prodrugs [2: 686]. Telmisartan is of the greatest research interest because of its availability and widespread use in thrombosis therapy. For quality control of drugs containing telmisartan, the most commonly used method is high-performance liquid chromatography [3: 108]. However, the development of new alternative methods for quantitative determination of telmisartan in biological fluids, in order to control the dosage, is quite an urgent task. Telmisartan is a drug that belongs to the group of angiotensin II AT1 receptor antagonists. It is used as a treatment for hypertension (high blood pressure) and to prevent complications associated with hypertension, such as strokes, myocardial infarctions, and other cardiovascular diseases.Telmisartan research is relevant from several perspectives. First, the drug is used in clinical practice, so further research could lead to improvements in the way it is used and the outcomes of hypertension treatment. Secondly, telmisartan can be used in treatment of other diseases related to activation of renin-angiotensin-aldosterone system, such as chronic kidney disease, diabetic nephropathy and others. Telmisartan research could also lead to improved treatments for these diseases.Third, telmisartan is the subject of research to develop new drugs that may be more effective in the treatment of hypertension and other diseases. For example, research into the synthesis of new molecules or biological markers could lead to new drugs that would be more selective and more effective in treating various pathologies.Thus, telmisartan research is important for clinical practice, the development of new drugs and more effective treatment of diseases related to the renin-angiotensin-aldosterone system.

In this work we investigated fluorescent properties of telmisartan in order to develop a method for its quantitative determination in biological fluids by fluorescence method, which has high sensitivity, low cost of analysis and expressiveness.

“Fluorat-02 Panorama” (produced by LLC “Lumex”, St. Petersburg) is a classical research spectrofluorimeter. In fluorimetric obtained, the spectral characteristics of excitation and/or emission of luminescence of the In fluorimetric obtained objects at the moment of exposure to pulses of excitation light are measured. When investigating chemo- or bioluminescence, the intensity of the sample’s own luminescence caused by chemical or biological processes in it is recorded. Installation error of monochromators is not more than 3 nm. The signal-to-noise ratio is 100/1.

The development of a methodology for the quantitative determination of telmisartan in human plasma is of great importance for clinical practice and research in the field of pharmacokinetics and pharmacodynamics. Having an accurate and reliable methodology will make it possible to study telmisartan plasma concentrations in humans in different situations: before and after drug administration, at different points in time, and at different dosages. This will allow more accurate prediction of treatment efficacy and determination of the need for dose adjustments. In addition, the technique of quantifying telmisartan in plasma will allow a more accurate study of the drug’s pharmacokinetic parameters, such as absorption rate, distribution, metabolism and excretion. These data may be useful for a better understanding of the mechanisms of telmisartan action, as well as for the development of more effective drugs based on this drug.

The telmisartan quantification technique may also be useful for monitoring therapy and determining whether the resulting telmisartan concentrations are as expected. This is especially important when

using this drug in the treatment of patients with chronic diseases, such as hypertension, where it is necessary to maintain a certain concentration of the drug in the blood in order to achieve effective blood pressure control. Thus, the development of a methodology for the quantification of telmisartan in human plasma is of great importance for clinical practice and research in the field of pharmacokinetics and pharmacodynamics.

Materials and Methods

Due to the lack of data on the luminescent analysis of telmisartan in blood plasma or other biological fluids, the conditions of detection were chosen empirically. A standard solution of Telmisartan and the investigational drug Telmista produce a signal at the same wavelength.

The object of the analysis is the drug "Telmista", film-coated tablets produced by KPKA, Slovenia, dosage 40 mg. Excipients: sorbitol – 149.84 mg, lactose monohydrate – 60 mg, meglumine – 12 mg, povidone K30–12 mg, sodium hydroxide – 3.38 mg, magnesium stearate – 2.8 mg. A comparison sample was prepared using the pharmaceutical substance Telmisartan manufactured by Sigma-Aldrich, USA. Solutions of 0.01M NaOH, 0.01 M HNO3, and 0.01 M phosphate buffer solution (pH = 7.0) were chosen to investigate the effect of solvent. Sample preparation of test samples: 10 mg of crushed tablets of Thelmist was placed in 100 ml volumetric flasks, solvents were added and samples were diluted 10-fold. A cuvette with the analyzed solution was placed in the cuvette compartment, the excitation wavelength was set to 290 nm, and the luminescence registration spectrum was recorded.

The research was carried out on a spectrofluorimeter "Fluorat-02 Panorama" manufactured by "Lumex" company, St. Petersburg.. The instrument is designed for various spectral-kinetic, spectrofluorimetric, bioluminescent and chemoluminescent research of the analysis of the objects in a wide wavelength range. Spectrofluorimeter has an interdisciplinary range of applications, it is used for environmental research of aquatic environments and petroleum products, scientific research on measurement of spectral characteristics of luminescence and luminescent tags, as well as in medical, technological and geological research. Fig. 1 shows the intensity of the luminescence spectrum of telmisartan in 0.01 M NaOH solution (1), which is about 3 times higher than in 0.01 M HNO3 solution (2) and 5 times higher than in 0.01 M phosphate buffer solution (3). Presumably, this effect is due to the poor solubility of the tested substance in 0.01 M HNO3 and 0.01 M FBR solutions, as well as in water and alcohols.

For further research, therefore, 0.01 M NaOH solution was chosen as the solvent.

The method of external standards was used to construct the calibration curve. The standard solutions of telmisartan in 0.01M NaOH were prepared: 100, 500, 900, 1300, 1700, 2000, 2400 and 2800 μl of telmisartan initial solution with 1 mg/dm^3 concentration was put into separate 10 ml volumetric flasks and the solution was diluted to the mark with a solvent. Luminescence intensity of each solution was measured at λvosb = 290 nm, λlum = 365 nm. Determination of the relative quantum yield of fluorescence of telmisartan was performed according to the method [4: 645] using fluorescein as a standard fluorescent substance in 0.01M NaOH aqueous solution with the known fluorescence quantum yield (φfl = 0.92). For this purpose, diluted solutions (~5–10–6 M) of telmisartan and fluorescein in 0.01M NaOH were prepared. The optical densities of the solutions at absorption maxima of 375 nm (fluorescein) and 365 nm (telmisartan) did not exceed 0.01.

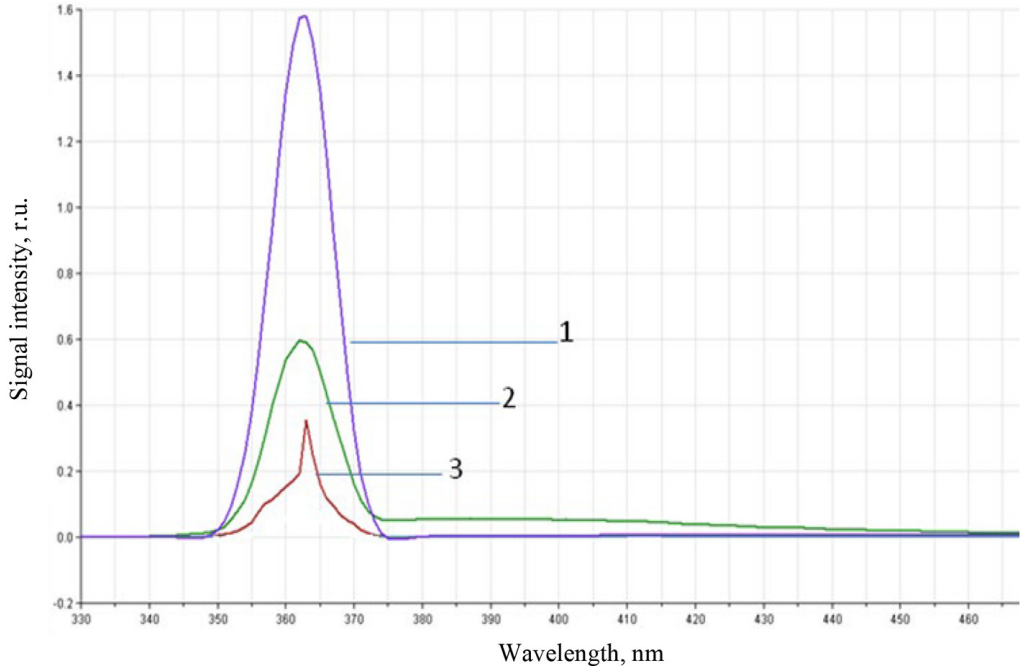

Fig. 1. Spectrum of telmisartan solution in different pH-solvents

Results and discussion

The peak of the main substance was recorded in the wavelength range of 350–370 nm with full fluorimetric correction.

Relative quantum yield of telmisartan fluorescence (φfl) was calculated by formula (1):

$$\varphi_{\text{фл.}} = \frac{S_2 \times A_1}{S_1 \times A_2} \times 0{,}92 \quad (1)$$

where S 1, S 2 are areas under fluorescence spectra, and A1, A2 are optical densities of fluorescein and telmisartan solutions.

The calculated relative quantum yield of telmisartan fluorescence was 0.69. Thus, telmisartan is an intensely fluorescent substance, indicating that direct fluorescence determinations are possible at relatively low concentrations of the substance in solution.

Fig. 2 shows the luminescence spectra of telmisartan substance using a single region overlay. The signal has the highest intensity at a wavelength of 365 nm.

Fig. 3 shows a graduation diagram of the dependence of signal intensity on the telmisartan content in the model solution. The equation of dependence of analytical signal on telmisartan concentration has the form y = 79.996x, and the correlation coefficient is 0.9745.

The linear range of the determined contents was 0.01–0.3 mg/dm^3. The calculated detection limit was 0.0004 mg/dm^3. For investigating the stability of telmisartan in solution, the luminescence spectra of the model solution freshly prepared and after 24 hours were taken. The peaks on the luminescence spectrum have differences in height, i.e. the signal intensity decreases after 24 hours (Fig. 4). However, the peaks have the highest intensity at 365 nm in both measurements. After 24 hours, the telmisartan peak has a double peak. The data obtained indicate a decrease in the

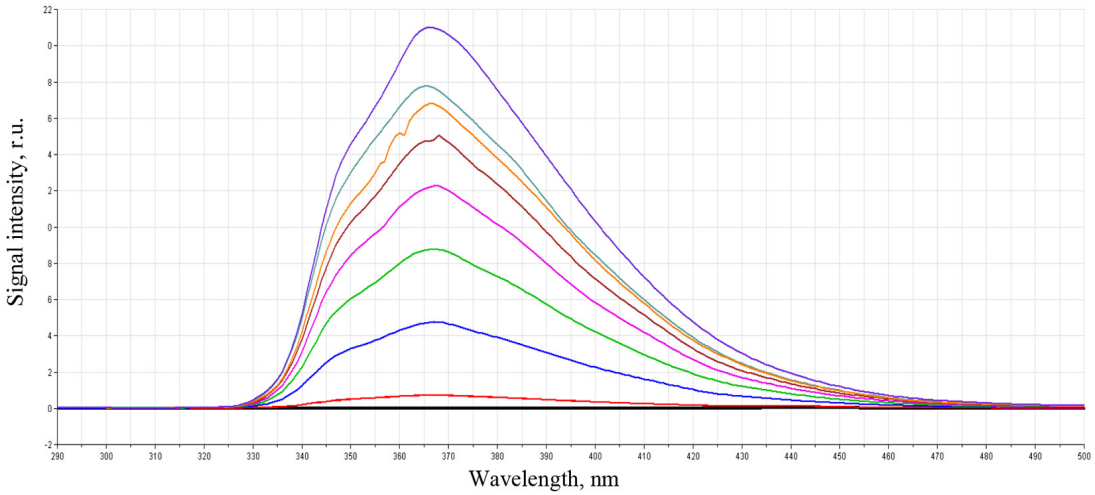

Fig. 2. Luminescence spectrum of telmisartan

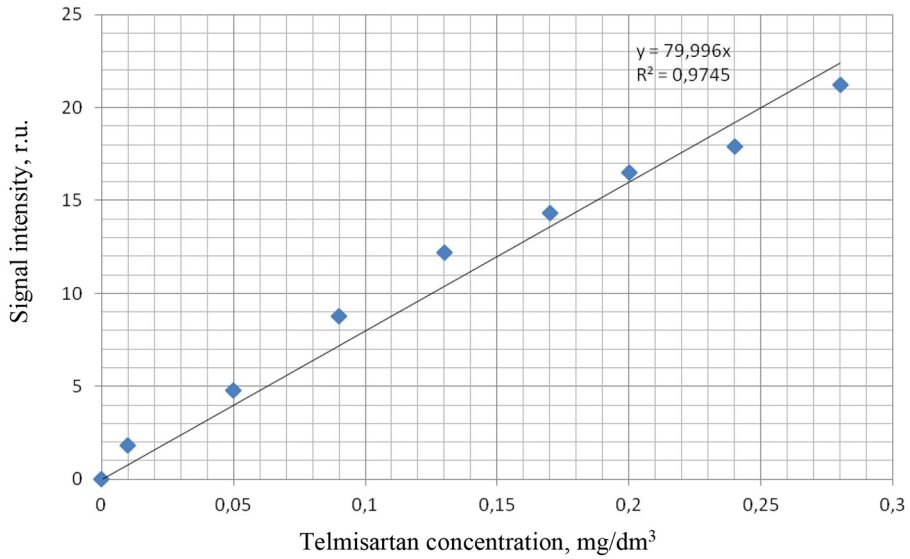

Fig. 3. Graduation chart. Dependence of signal intensity on telmisartan concentration in solution

concentration of the main substance in the solution over a short period of time, which is obviously related to the oxidation process of telmisartan. Only using a fresh solution should be used for further research.

In summary, in the course of research, the conditions were selected and the detection range of the telmisartan peak in the preparation “Telmista” was determined, the relative quantum yield of the substance was calculated and a graded diagram of the dependence of signal intensity on concentration was constructed. substance in solution was constructed. The effect of solvents in different pH-conditions on the intensity of the analytical signal in the model solution was studied. It was found that the greatest intensity of luminescence is observed in 0.01 M NaOH.

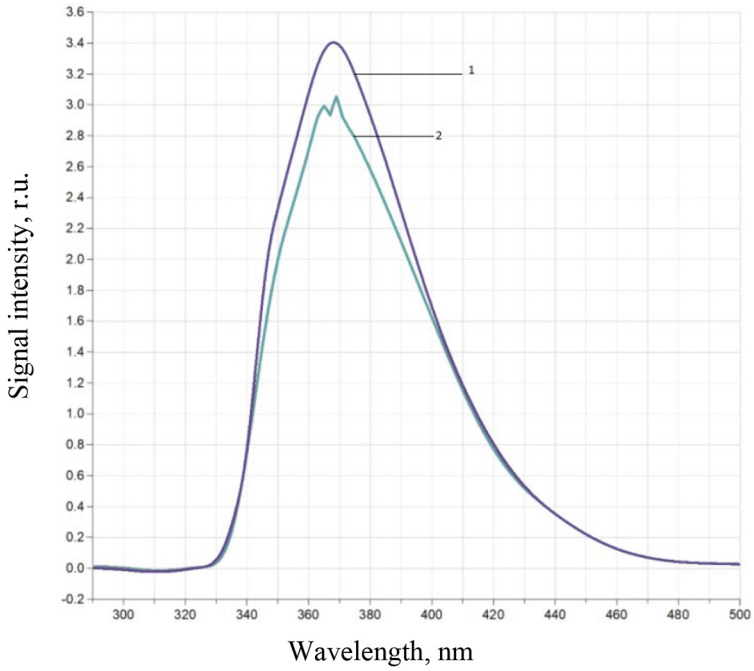

Fig. 4. Luminescence spectrum of the preparation immediately after preparation (1) and after 24 hours (2)

To determine Telmisartan in biological objects, human blood plasma was used, as it is known that this substance binds to blood plasma proteins, mainly to albumin and alpha-1 glycoprotein by 99.5 %.

Using two-dimensional scanning, the maximum signal intensity of the sample was determined at a wavelength of 490 nm (Fig. 5), while in standard conditions of spectral measurements the wavelength is determined at 365 nm (Fig. 6).

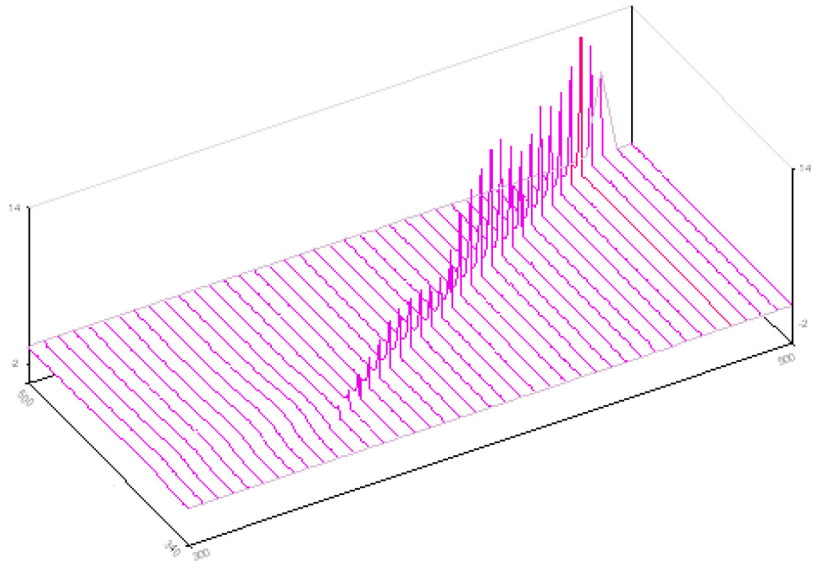

Fig. 5. 2D scan spectrum Telmisartan in blood plasma

Methodology of the experiment:

Human blood plasma, previously thawed and brought to human body temperature. The pharmaceutical substance telmisartan manufactured by Sigma-Aldrich, USA >98 % was used for the experiment. In a flask of 10 dm^3 volume was added a standard solution of the substance telmisartan at a concentration of 0.01–0.1 mg/dm^3, pre-dissolved in isotonic solution of 100, 500, 900, 1300µl, the volume of the working solution was brought to the mark with the plasma solution. Measurements were made in three replays.

The results of telmisartan plasma concentration measurements are summarized in Table 1. The measurements were performed in three repetitions. The concentration of the substance was in the range of 0.01–0.1 mg/dm3, wavelength 365 nm.

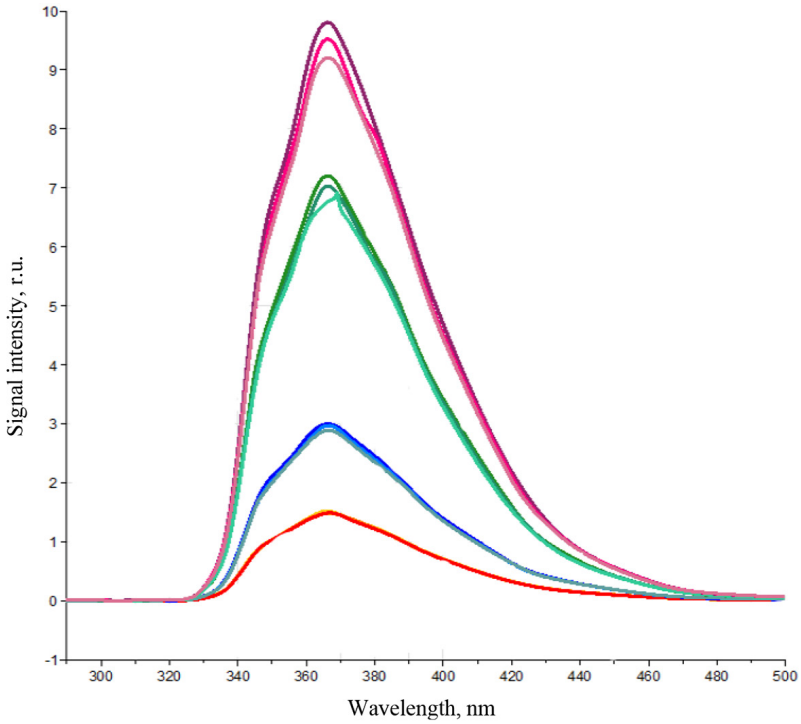

Fig. 6. Telmisartan plasma spectra (standard conditions)

Таблица 1. Результат измерения концентрации телмисартана в модельном растворе плазмы крови

Table 1. Result of telmisartan concentration measurement in model plasma solution

Measurement No.	Measurement results of replays (Signal intensity, r.u.)			Average value (Signal intensity, r.u.)	Concentration mg/dm^3
1	1,72	1,68	1,71	1,71	0,01
2	2,79	2,79	2,79	2,79	0,05
3	6,66	6,79	6,79	6,75	0,09
4	9,24	9,55	9,78	9,52	0,1

Conclusion

The fluorescence properties of telmisartan substance and the drug "Telmista" were considered in the work, the conditions of analytical signal registration were determined empirically, the influence of solvents in different pH – conditions on the analytical signal intensity in a model solution was obtained. It was found that the greatest intensity of luminescence was observed in 0.01 M NaOH. The relative quantum yield was also calculated and the calibration dependence was constructed. Detection conditions for telmisartan in human blood plasma were determined.

As a result of this research it was proved that telmisartan is an intensively fluorescent substance, there is no interfering influence of auxiliary components in the quantitative analysis of the drug. Thus, further studies and development of express method for quantitative determination of telmisartan in biological fluids by fluorimetric analysis have sense and practical importance.

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