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Evaluation of Physicochemical Properties and Biological Activity of Tirzepatide-Based Drugs

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Currently, there is a steady increase in the prevalence of metabolic disorders among the population of developed countries. Among them, obesity and type 2 diabetes mellitus are the most important health problems. Tirzepatide is an innovative drug that is a dual agonist of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptors. The medcine is effective for the treatment of type 2 diabetes mellitus and obesity. The first drug with the active substance tirzepatide in Russia was Tirzetta® (manufacturer LLC «PROMOMED RUS»), which is the first in Russia, but not in the world. The reference drug for it is Munjaro® (INN: tirzepatide, Eli Lilly and Company, USA). To date, the question of the equivalence of these drugs has not been fully studied.

The aim. To conduct a comprehensive comparative evaluation of the reproduced drug Tirzetta® (INN: tirzepatide, manufacturer LLC “PROMOMED RUS”) and the reference drug Munjaro® (INN: tirzepatide, manufacturer Eli Lilly and Company, USA).

Materials and methods. The authenticity and quality of drugs were assessed by physicochemical methods according to the current pharmacopoeia of the EAEU. Spectrophotometry in the UV region, HPLC-MS/OF, and gel filtration chromatography were performed. The analysis of agonism to GIP and GLP-1 receptors was performed *in vitro* using reporter cell lines. The studies were performed in accordance with EMA, FDA, EAEU guidelines and in accordance with the current EAEU pharmacopoeia.

Results. As a result of the evaluation of the physicochemical properties of the studied series of Tirzetta® and the reference drug Munjaro®, it was found out that the absorption spectra in the ultraviolet region, the profile of related impurities and their quantitative content, the profile of high-molecular-weight compounds and their quantitative content, as well as mass spectra in all the studied series were similar. During the evaluation of the biological activity of the Tirzetta® and Munjaro® series, results were obtained that demonstrated the absence of statistically significant differences in the ability to activate GLP-1 and GIP receptors (p <0.0001).

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Conclusion. During the studies, the equivalence of the physicochemical properties and biological activity of the Russian drug Tirzetta® to the comparator drug Munjaro® was confirmed.
Keywords: tirzepatide; peptide; synthetic peptide; glucagon-like peptide-1; glucose-dependent insulinotropic polypeptide; biological activity; safety; physicochemical properties; metabolic syndrome; type 2 diabetes mellitus
Abbreviations: DM — diabetes mellitus; MS — metabolic syndrome; ND — normative documentation; RRT — relative retention time; BMI — body mass index; GLP-1 — glucagon-like peptide type 1; GLP-1-R — glucagon-like peptide type 1 receptor; GLP-1a — glucagon-like peptide type 1 receptor agonist; GIP — glucose-dependent insulinotropic polypeptide; GIP-R — glucose-dependent insulinotropic polypeptide receptor; EMA — European Medicines Agency; LP — medicinal product; INN — international nonproprietary name; EAEU — Eurasian Economic Union; HPLC-MS/OF — high-performance liquid chromatography with mass spectrometry/reversed-phase; HRMS — high-resolution mass spectra; ESI — electrospray ionization; GFC — gel filtration chromatography; cAMP — cyclic adenosine monophosphate.

Оценка физико-химических свойств и биологической активности лекарственных препаратов на основе тирзепатида

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В настоящее время среди населения развитых стран наблюдается устойчивый рост распространенности метаболических нарушений. Из них ожирение и сахарный диабет 2 типа представляют наиболее актуальную проблему для здравоохранения. Тирзепатид является инновационным лекарственным препаратом, представляющим собой двойной агонист рецепторов глюкозозависимого инсулинотропного полипептида (ГИП) и глюкагонподобного пептида типа 1 (ГПП-1). Препарат эффективен для лечения сахарного диабета

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2 типа и ожирения. Первым лекарственным препаратом с действующим веществом тирзепатид в России стал Тирзетта® (производитель ООО «ПРОМОМЕД РУС»), который является первым в России. Референтным лекарственным препаратом для него выступает Мунджаро® (МНН: тирзепатид, Eli Lilly and Company, США). Вопрос об эквивалентности этих лекарственных препаратов является важным и актуальным для уверенности медицинского сообщества в высоком качестве проводимой терапии.

Цель. Провести комплексную сравнительную оценку воспроизведённого лекарственного препарата Тирзетта® (МНН: тирзепатид, производитель ООО «ПРОМОМЕД РУС») и референтного препарата Мунджаро® (МНН: тирзепатид, производитель Eli Lilly and Company, США).

Материалы и методы. Оценка подлинности и качества лекарственных препаратов осуществлялась физико-химическими методами согласно действующей фармакопеи ЕАЭС. Проводили спектрофотометрию в УФ области, ВЭЖХ-МС/ОФ, гель-фильтрационную хроматографию. Анализ агонизма к рецепторам ГИП и ГПП-1 проводили *in vitro* при помощи репортерных клеточных линий. Проведённые исследования выполнены в соответствии с руководствами EMA, FDA, ЕАЭС и согласно действующей фармакопее ЕАЭС.

Результаты. В результате оценки физико-химических свойств исследуемых серий препарата Тирзетта® и референского препарата Мунджаро® установлено, что спектры поглощения в ультрафиолетовой области, профиль родственных примесей и их количественное содержание, профиль высокомолекулярных соединений и их количественное содержание, а также масс-спектры во всех исследуемых сериях были аналогичны. В ходе оценки биологической активности серий препарата Тирзетта® и Мунджаро® были получены результаты, демонстрирующие сопоставимость биологической активности вышеуказанных препаратов и высокую эффективность исследуемого препарата в отношении активации рецепторов ГПП-1 и ГИП (p <0,0001).

Заключение. В ходе проведённых исследований была подтверждена эквивалентность физико-химических свойств и биологической активности российского лекарственного препарата Тирзетта® препарату сравнения Мунджаро®.

Ключевые слова: тирзепатид; пептид; синтетический пептид; глюкагоноподобный пептид-1; глюкозозависимый инсулинотропный полипептид; биологическая активность; безопасность; физико-химические свойства; метаболический синдром; сахарный диабет 2 типа

Список сокращений: СД — сахарный диабет; МС — метаболический синдром; НД — нормативная документация; ОВУ — относительное время удерживания; ИМТ — индекс массы тела; ГПП-1 — глюкагонподобный пептид типа 1; ГПП-1-Р — рецептор глюкагонподобного пептида типа 1; ГПП-1а — агонист рецептора глюкагонподобного пептида типа 1; ГИП — глюкозозависимый инсулинотропный полипептид; ГИП-Р — рецептор глюкозозависимого инсулинотропного полипептида; EMA — Европейское агентство лекарственны средств; ЛП — лекарственный препарат; МНН — международное непатентованное наименование; ЕАЭС — Евразийский экономический союз; ВЭЖХ-МС/ОФ — высокоэффективная жидкостная хроматография с масс-спектрометрией/обращенно-фазовая; HRMS — масс-спектры высокого разрешения; ESI — ионизационное электрораспыление; ГФХ — гель-фильтрационная хроматография; цАМФ — циклический аденозинмонофосфат.

INTRODUCTION

Metabolic diseases, including type 2 diabetes mellitus (DM) and obesity, are among the most accute problems in modern healthcare. According to the Russian register as of January 01, 2023, 4,962,762 people (3.31% of the Russian Federation population) are registered for dispensary observation, of which 92.33% (4.58 million) have type 2 DM. The incidence of type 2 DM among the adult population is 191.4 per 100,000 population [1].

Metabolic syndrome (MS), the main components of which are arterial hypertension (33.5%), hypercholesterolemia (29.0%) and obesity, is becoming an epidemic in economically developed countries. Up to 16–30% of residents of these countries suffer from various forms of MS, which is associated with a multiple increase in the risk of cardiovascular diseases and an increase in mortality [2]. According to the World Obesity Atlas, it is expected that by 2035 more than 1.77 billion people will be overweight (body mass index [BMI] = 25–29.9 kg/m^2), and 1.53 billion will suffer from obesity (BMI > 30 kg/m^2) [3]. Forecasts are disappointing and show a steady increase in these indicators up to 2050 [4].

Methods for preventing and treating various forms of MS are based on a multimodal approach, including lifestyle changes, drug and surgical methods, personalized monitoring technologies, and psychosocial support [5, 6]. The use of new classes of drugs and evidence-based prevention strategies helps to improve metabolic control and reduce the development of complications.

Over the past decade, new effective drug treatments for type 2 DM and obesity have been developed. Thus, glucagon-like peptide 1 receptor agonists (GLP-1a) — semaglutide and liraglutide — have shown high efficacy in achieving weight loss and improving glycemic control [7, 8]. The evolution of the pharmacological approach based on agonism of GLP-1 receptors has manifested in the emergence of multiagonists of the “incretin axis” components, which contributes to a significant increase in glycemic

control and comprehensive correction of metabolic disorders [9]. The results of preclinical and clinical studies have demonstrated the high therapeutic potential of these drugs [9]. To date, the only registered drug (LP) of this class is tirzepatide, a dual agonist of GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) receptors [10]. Tirzepatide was first registered in 2021 in the USA (trade name [TN] — Mounjaro®, manufacturer Eli Lilly and Company) [11]. Tirzepatide preparations have not been registered in the Russian Federation, and therefore, for a long time, the population of the Russian Federation did not have access to the drugs based on this compound. The high efficacy of tirzepatide in reducing weight, treating the main components of obesity and metabolic syndrome, and preventing the risks of associated complications has been demonstrated in clinical studies, which has increased the need to develop domestic LPs to expand the geography of its use [12].

In January 2025, tirzepatide first appeared on the Russian market under the trade name Tirzetta®, manufactured by PROMED RUS LLC. The chemical synthesis method, which was used in the production of peptide molecules of the drug, has a number of potential advantages: a more controlled production process, a reduced risk of contamination with biological impurities, and the possibility of precise control of the structure of the final product [13]. However, for complex peptides such as tirzepatide, chemical synthesis requires careful optimization of reaction conditions and purification methods. In this regard, the own production technology of the substance used for the domestic drug Tirzetta® is of particular importance.

It is worth noting that proving the bioequivalence of reproduced drugs is becoming an integral part of the process of their registration, which is confirmed by the current requirements of both national regulators and international agencies, such as the European Medicines Agency (EMA). In April 2024, the discussion of the EMA guideline "Guideline on the Development and Manufacture of Synthetic Peptides" was completed, which defines the main requirements for methods of characterization, quality control and production processes of drugs based on synthetic peptides[1] [14]. This guideline serves as the foundation for ensuring that reproduced drugs meet established criteria for quality, safety and efficacy. According to the provisions of the guideline, to confirm the equivalence of two peptides in the composition of the drug, it is sufficient, and in many cases even more representative, to present evidence of compliance of their physicochemical properties and biological activity with accurate modern research methods.

THE AIM. To conduct a comprehensive comparative study of the physicochemical properties and biological activity of Tirzetta® (INN: tirzepatide) and the reference drug Mounjaro® (INN: tirzepatide).

MATERIALS AND METHODS

Samples

In order to form a representative quality profile and obtain reliable data on the comparability of the active substance tirzepatide in two equivalent preparations, three series of the reproduced drug were used. Information about the studied series is presented in Table 1.

Comparative studies of the physicochemical properties of drugs

To analyze the spectral characteristics of the drugs, spectrophotometry was performed in the ultraviolet region (200–400 nm) in accordance with the recommendations of the current pharmacopoeia of the EAEU, Article 2.1.2.53[2]. When comparing absorption spectra in the UV region, the solutions of each series of each drug were diluted with water for injection to a tirzepatide concentration in the solution of 0.5 mg/mL. The initial solutions of each series were obtained by mixing the contents of 7 cartridges of syringe pens and taking an averaged sample. The analysis was performed on a Shimadzu UV-1800 spectrophotometer (Shimadzu, Japan).

High-resolution mass spectra (HRMS) were recorded on an LCMS-9030 instrument (Shimadzu, Japan) using electrospray ionization (ESI). Measurements were performed in positive ion mode. Samples were dissolved in deionized water to a concentration of 1.25 mg/mL and introduced in a volume of 0.1 µL into the mass spectrometer dispenser without separation. The following parameters were used: capillary voltage — 4.5 kV; mass scanning range — 100–5000 m/z; external calibration — with a solution of NaI in MeOH/H_2O; drying and heating

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gases (nitrogen) — 10 l/min each; spraying gas (nitrogen) — 3 l/min; interface temperature — 300°C; acetonitrile / water (5 / 95) flow rate — 0.4 ml/min. Data were processed using LabSolutions v.5.114.

Confirmation of authenticity and determination of the quantitative content of tirzepatide were performed by high-performance liquid chromatography (HPLC). The determination was carried out in accordance with the requirements of the EAEU pharmacopoeia (Article 2.1.2.28).

The analysis was performed using a high-pressure liquid chromatograph with a UV detector on a diode matrix on a Kinetex C18, 100 Å column, (4.6 × 150 mm, particle size 2.6 µm), filled with L1 type sorbent (manufacturer Phenomenex, cat. No. 00F-4462-E0). Tirzepatide was identified at a wavelength of 210 nm (optimal for peptide bond). The column temperature was 30°C, the mobile phase flow rate was 0.7 ml/min, the autosampler temperature was 5°C, and the chromatography time was 30 min. Elution was performed at a mobile phase ratio (A/B) of 45:55. Under these conditions, the approximate retention time of the tirzepatide peak was from 14 to 20 min.

Next, a standard sample (SS) of a given concentration was prepared. For this: about 15.0 mg (exact weight) of tirzepatide SS was placed in a 10 mL volumetric flask, 8 mL of solvent was added, mixed until the substance was completely dissolved, the volume of the solution was brought to the mark with the solvent and mixed again (tirzepatide concentration — 1.5 mg/mL).

Authenticity was confirmed by comparing the retention time and UV spectrum of the test peak with the tirzepatide standard. The quantity of the substance was calculated by comparing the peak areas of the analyzed samples and the standard.

Before starting the measurements, the suitability of the chromatographic system was checked according to section 2.1.2.36.

Chromatographic System Suitability Test (SST):

The chromatographic system is suitable if:

- The relative standard deviation of the peak area values of tirzepatide for five consecutive chromatograms of the standard solution is not more than 2.0%;
- The relative standard deviation of the retention time of tirzepatide peaks for five consecutive chromatograms of the standard solution is not more than 2.0%;
- The symmetry factor calculated for the tirzepatide peak on the chromatogram of the standard solution is 0.8–2.0;
- The number of theoretical plates calculated for the tirzepatide peak on the chromatogram of the standard solution is not less than 1000.

The quantitative determination of impurities was also performed by reversed-phase HPLC (RP-HPLC) according to section 2.1.2.28 of the Eurasian Economic Union Pharmacopoeia using a gradient elution mode.

To analyze impurities, a standard solution was prepared: approximately 15.0 mg (exact weight) of the tirzepatide standard was placed in a 10 mL volumetric flask, 8 mL of solvent was added, stirred until the substance was completely dissolved, the volume of the solution was brought to the mark with the solvent, and stirred again. Then, 1 mL of the resulting solution was placed in a 100 mL volumetric flask, the volume of the solution was brought to the mark with the solvent, and mixed (tirzepatide concentration — 0.015 mg/mL).

A 100 mM phosphate buffer solution pH 7.5 was used as a solvent. Mobile phase A was prepared: 9.2 g of ammonium dihydrogen phosphate R was placed in a 1000 mL beaker, dissolved in 800 mL of water for chromatography R, the pH of the resulting solution was adjusted to 3.7 ± 0.1 potentiometrically using phosphoric acid R, 100 mL of acetonitrile for chromatography R was added, and stirred. Quantitatively transferred to a 1000 mL volumetric flask, the volume of the solution was brought to the mark with water for chromatography R and stirred. The mobile phase was filtered through a nylon membrane filter with a pore size of 0.45 µm (Water Laboratory LLC, cat. No. NY045050L or equivalent quality), discarding the first 100 mL of filtrate and degassing under vacuum.

Mobile phase B: 200 mL of water for chromatography R, 600 mL of acetonitrile for chromatography R, and 200 mL of 2-propanol R were mixed. Stir and sonicate for about 15 min, cool to room temperature.

For dosing the test solution, 2.5 mg, the contents of 7 syringes / cartridges were mixed and an average sample was taken. Then, 1.5 mL of the drug was placed in a 5 mL volumetric flask, the volume of the solution was brought to the mark with the solvent and mixed (tirzepatide concentration — 1.5 mg/mL). 2 solutions were prepared. For a dosage of 5 mg, the contents of 4 syringes/cartridges were mixed and an average sample was taken. Then, 0.75 mL of the drug was placed in a 5 mL volumetric flask, the volume of the solution was brought to the mark with the solvent

and mixed (tirzepatide concentration — 1.5 mg/mL). 2 solutions were prepared.

Placebo solution: 3.0 mL (for a dosage of 2.5 mg), 1.5 mL (for a dosage of 5 mg), 1.0 mL (for a dosage of 7.5 mg), 0.75 mL (for a dosage of 10 mg), 0.6 mL (for a dosage of 12.5 mg), 0.5 mL (for a dosage of 15 mg) of a mixture of excipients included in the drug were placed in a 10 mL volumetric flask, the volume of the solution was brought to the mark with the solvent and mixed.

Chromatographic System Suitability Test (SST):

The chromatographic system is suitable if:

- The signal-to-noise ratio for the tirzepatide peak on the chromatogram of the solution for checking sensitivity is not less than 10;
- The relative standard deviation of the peak area values of tirzepatide on five consecutive chromatograms of the standard solution is not more than 5.0%;
- The symmetry factor of the tirzepatide peak on the chromatograms of the standard solution is 0.8–2.0;
- The number of theoretical plates calculated for the tirzepatide peak on the chromatogram of the standard solution is not less than 1000;
- The peak-to-valley ratio (p/v) between the tirzepatide peak and the peak with a relative retention time of about 1.11 on the chromatogram of the solution for checking resolution is not less than 1.2.

Table 2 shows the gradient programming parameters.

Under the conditions described, the approximate retention time of tirzepatide tirzepatide was from 23 to 27 minutes.

The quantitative determination of high molecular weight compounds was performed by gel filtration chromatography (GFC) in accordance with the requirements of the Eurasian Economic Union Pharmacopoeia 2.1.2.28. The study was performed on a high-pressure liquid chromatograph with a UV detector on a column filled with a sorbent (silica gel with chemically modified dihydroxypropane groups (L20) Waters Insulin HMWP, 7.8 mm × 300 mm, particle size 10 µm, (manufacturer Waters)). The detection wavelength was 280 nm, the mobile phase flow rate was 0.5 mL/min, the column thermostat temperature was 50°C, the autosampler temperature was 5°C, the injected sample volume was 40 µL; the chromatography time was 30 min with an isocratic elution mode. According to the validation results, the retention time of the monomer peak was about 16 min. The approximate retention time of high molecular weight compound peaks is about 15 min (immediately before the monomer peak). All studies were performed in three independent replicates.

The solution prepared according to the following procedure was used as the mobile phase: 29.2 g of sodium chloride R and 1.56 g of sodium dihydrogen phosphate R were placed in a 1000 mL volumetric flask, dissolved in 400 mL of water for chromatography R, 0.34 mL of phosphoric acid R and 500 mL of 2-propanol R were added, and stirred. The volume of the solution was brought to the mark with water for chromatography R and stirred. Filtered under vacuum through a nylon membrane filter with a pore diameter of 0.45 µm (Water Laboratory LLC, cat. No. NY045050L or equivalent quality), discarding the first portions of the filtrate.

For a dosage of 2.5 mg, 1.5 mL of the aged solution was placed in a 5 mL volumetric flask, the volume of the solution was brought to the mark with the solvent, mixed and filtered through a regenerated cellulose (RC) membrane filter with a pore size of 0.45 µm (Water Laboratory LLC, cat. No. SFRCL04525 or equivalent quality) (tirzepatide concentration — 1.5 mg/mL).

For a dosage of 5 mg, 0.75 mL of the aged solution was placed in a 5 mL volumetric flask, the volume of the solution was brought to the mark with the solvent, mixed and filtered through a regenerated cellulose (RC) membrane filter with a pore size of 0.45 µm (Water Laboratory LLC, cat. No. SFRCL04525 or equivalent quality) (tirzepatide concentration — 1.5 mg/mL).

The standard solution was prepared similarly to the above method “Confirmation of authenticity and determination of the quantitative content of tirzepatide”. The test solution was prepared according to the method for quantitative determination of impurities.

Chromatographic System Suitability Test (SST):

The chromatographic system is suitable if:

- The relative standard deviation, calculated from the areas of the tirzepatide monomer peaks on the chromatograms of the standard solution with repeated injections, is not more than 5.0%;
- The symmetry factor for the tirzepatide monomer peak on the chromatogram of the standard solution is 0.8–2.0;
- The number of theoretical plates calculated for the tirzepatide monomer peak on the chromatogram of the standard solution is not less than 1000;

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- The signal-to-noise ratio for the tirzepatide monomer peak on the chromatogram of the solution for checking sensitivity is not less than 10.

In vitro biological activity study

A comparative *in vitro* study of the biological activity of the investigated drugs was performed on two model systems, which are cell lines expressing the human GLP-1 receptor (GLP1R/CRE-Luc/HEK293, Cobioer, cat. No. CBP71117, China) or GIP (GIPR/CRE-Luc/HEK293 Cobioer, cat. No. CBP71346, China) with a reporter construct stably integrated into the genome under the control of a Cre-dependent promoter. Treatment of cells of this model system with agonists of the GGP-1 or GIP receptor activates the signaling pathway, which causes the expression of the luciferase gene. In the absence of agonists, the receptor is not activated, and the luminescence signal is low. In the presence of an agonist, luminescence activated by GLP-1R or GIPR (depending on the cell line) can be detected in a dose-dependent manner by detecting bioluminescence [14].

GIPR/CRE-Luc/HEK293 and GLP1R/CRE-Luc/HEK293 cell lines were cultured according to the manufacturer's instructions. To study agonism, cells were seeded into the wells of a 96-well plate at a rate of 40 thousand cells/well in a volume of 100 µL per well. After 24 hours, the comparator drug Mounjaro® or samples of the drug Tirzetta® (INN: Tirzepatide) were added to the cells in a volume of 10 µL per well so that the required drug concentration was achieved upon addition. The range of investigated concentrations was selected according to the manufacturer's instructions and ranged from 10^{-12} to 10^{-6} M. Testing was carried out in a concentration range covering both the minimum and maximum effective doses (according to the manufacturer's instructions). Growth medium, which was added in the same volume, was used as a negative control. After adding the samples, the cells were incubated at 37°C in a CO_2 incubator for the required incubation time (5 hours according to the manufacturer's instructions). Then, the cells were lysed using a buffer containing the luciferase substrate (D-luciferin) using a commercial kit for analyzing firefly luciferase activity, ONE-Step™ Luciferase Assay System (BPS Biosciences, China) in accordance with the manufacturer's protocol. 100 µL of the working substrate solution was added to each well of the plate, and the luminescence signal intensity was determined using the luminometric module of the Perkin-Elmer EnVis system (Perkin Elmer, USA) after 15–20 minutes of incubation after application. The specific activity of the investigated drugs (ED_{50}) was calculated as the dose at which 50% of the maximum effect is achieved, based on the activity values obtained from three independent tests.

Statistical analysis

Molecular ions in the spectra were analyzed in LabSolutions v.5.114. The statistical processing of the results was performed using GraphPad Prizm 10.4.2 software (GraphPad Software, USA). The acceptance criteria for the results were selected according to the requirements of the current pharmacopoeia of the EAEU FS 2.3.12.0, Decision of the EEC No. 85, as well as according to the Guidelines for Preclinical Studies of Medicines edited by Mironov A.N. (2013)[3,4]. An assessment of the parameters of the initial data was carried out (the presence of outliers, data distribution, assessment of the homogeneity of variances) to determine further statistical methods according to the FEAES. The statistical significance of the model was verified using Fisher's F-test at $p < 0.05$ and a coefficient of determination R2 from 0.7 to 1. The choice of using R2 and the F-test together is due to the need to obtain the most objective assessment of the quality of the model. Only when this indicator is achieved is the model considered statistically reliable and suitable for further use[5,6].

Preliminary processing of biological activity data included normalization of the initial luminescence intensity values.

The presence of outliers in the sample was determined using the ROUT method. The final processing included constructing a dose-response curve based on normalized data using a non-linear regression method. The dose-response analysis and comparison of the test samples were carried out taking into account

[3] Guidance for preclinical studies of medicines; Scientific Center for Expertise of Medical Products of the Ministry of Health and Social Development of Russia; Volume 1. Moscow: Grif and K; 2012. 944 p. EDN: SDEWMP. Russian

[4] Decision of Council of the Eurasian Economic Commission of November 3, 2016 No. 85 «About approval of Rules of carrying out researches of bioequivalence of medicines within the Eurasian Economic Union». Available from: https://www.alta.ru/tamd oc/16sr 0085/?ysclid=mfpibknu6g985461506

[5] Guidance for preclinical studies of medicines; Scientific Center for Expertise of Medical Products of the Ministry of Health and Social Development of Russia; Volume 1. Moscow: Grif and K; 2012. 944 p. EDN: SDEWMP. Russian

[6] Yunkerov VI, Grigoriev SG. Mathematical and statistical processing of medical research data; Saint Petersburg: Kirov Military Medical Academy; 2002. 266 p. EDN: XYHSQB. Russian

the application of a mathematical model according to the following parameters: the value of the upper and lower asymptotes, the value of the slope (β); the value of the relative specific activity of the sample.

A 4-parameter logistic model was chosen for statistical analysis — an extended form of the classical logistic function, which includes four parameters for a more accurate description of the dose-effect curve: minimum effect (lower asymptote), maximum effect (upper asymptote), EC_{50} (dose causing 50% effect), β — slope.

The primary data obtained in the study were presented as mean (M) and standard deviation (SD).

RESULTS

UV spectroscopy

To date, the current pharmacopoeias do not contain established optical density values for tirzepatide. The results of our study showed that the UV absorption spectra of the reproduced drug Tirzetta® and the reference Mounjaro® are similar (Fig. 1, Table 3).

Thus, the absorption spectra in the ultraviolet region of Tirzetta® and Mounjaro® are similar, which indicates the identity of the molecule's structure.

High-performance liquid chromatography coupled with mass spectrometry provides high-precision determination of molecular weight by ion peaks in the MS spectrum. Figure 2 shows the mass spectra of the medicinal product Mounjaro® and Tirzetta®.

Thus, the mass spectra in all the studied series of Tirzetta® and Mounjaro® are comparable and correspond to the calculated mass of tirzepatide.

Determination of authenticity and quantitation of tirzepatide by reversed-phase high-performance liquid chromatography

In the chromatograms of all test solutions, there was a peak corresponding to the retention time of the tirzepatide peak under the specified conditions. The chromatograms of the test and standard samples were comparable (permissible deviation ±15% from the retention time of the standard sample) (GPhM.1.2.1.2.0001 Chromatography)[7]. The content of tirzepatide in all samples corresponded to the norms (Fig. 3).

[7] GPhM.1.2.1.2.0001 Chromatography. The State Pharmacopoeia of the Russian Federation XV edition. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-2/1-2-1/1-2-1-2-khromatograficheskie-metody-analiza/khromatografiya/. Russian

Determination of impurities

The results of the quantitative determination of impurities are presented in Table 4.

The profile of related impurities and their quantitative content in the samples of the Mounjaro® and Tirzetta® corresponds to the regulatory documentation for the drugs. However, our own technology for the synthesis and purification of the active pharmaceutical substance made it possible to significantly reduce the number of hydrophilic and hydrophobic impurities.

The quantitative assessment of high-molecular-weight compounds in the composition was performed by gel-filtration HPLC. The evaluation was performed at a wavelength of 280 nm. The retention time of tirzepatide for all drugs ranged from 15 to 18 minutes. The peaks of high-molecular-weight compounds were located directly in front of the tirzepatide peak. The retention time was about 15 minutes (Fig. 4).

The results of the quantitative determination of high-molecular-weight compounds in samples are presented in Table 5.

Thus, the profile of high-molecular-weight compounds and their quantitative content in the Mounjaro® and Tirzetta® are similar.

Comparative *in vitro* bioactivity study

The bioactivity study of drugs was conducted by assessing the sensitivity upon activation of GIP and GLP-1 receptors in cell culture. A comparative *in vitro* study of the bioactivity of the reproduced drug Tirzetta® and the reference drug Mounjaro® on the GLP1R/CRE-Luc/HEK293 and GIPR/CRE-Luc/HEK293 reporter cell line model was performed in three independent biological replicates. The obtained primary data and calculated results met the system suitability requirements and acceptance criteria.

The results of the primary data evaluation on the GLP1R/CRE-Luc/HEK293 model (Table 6) showed that the coefficients of variation do not exceed 30% in each experimental point, and the data spread (i.e., variance) is homogeneous.

The coefficients of variation for each drug concentration value, as well as the results of the assessment of the homogeneity of the sample variance, are presented. In Figure 5, samples of Tirzetta® and the reference drug Mounjaro® demonstrates a positive dose-response relationship in the experimental model.

The results of the statistical analysis of the curves are presented in Table 7 as M ± SD with the given values of the coefficients of covariance CV (%).

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The results of the primary data assessment on the GIPR/CRE-Luc/HEK293 model (Table 8) showed that the CV do not exceed 30% in each experimental point, and the data spread (i.e., variance) is homogeneous.

In the presented graph (Fig. 6), samples of Tirzetta® and Mounjaro® demonstrate a positive dose-response relationship in the experimental model GIPR/CRE Luciferase Reporter HEK293.

A 4-parameter logistic model was used for statistical analysis. The are presented in Table 9 as M ± SD with the values of the CV (%).

Thus, during the assessment of the biological activity of Tirzetta® and Mounjaro®, results were obtained that demonstrate the absence of statistically significant differences in the ability to activate GLP-1 and GIP receptors ($p < 0.0001$).

The obtained data allow us to characterize the developed method, as well as the selected test systems, as having high discriminative ability. This is confirmed by the low values of the variance of the average luminescence indicators, which, in turn, ensures high power of the analysis of variance. The low level of within-group variability contributes to increasing the sensitivity of statistical tests to detect even minimal intergroup differences.

Thus, the conformity of the biological activity profile of the reproduced drug Tirzetta® (INN: tirzepatide; solution for subcutaneous administration, 2.5 mg, manufacturer JSC “Biochemist”, Russia) to the reference drug Mounjaro® (INN: tirzepatide, solution for injection, 5 mg, Eli Lilly and Company, USA) was confirmed within the framework of work using a reproducible and accurate method.

DISCUSSION

In recent years, the concept of multi-agonism in endocrinology has developed significantly. Multi-agonists of the “incretin axis” are a promising tool for controlling metabolic disorders. The evolution of the pharmacological approach based on GLP-1 receptor agonism includes the study of various dual and triple multi-agonists capable of simultaneously activating GLP-1, GIP, and glucagon receptors.

Simultaneous activation of GLP-1 and GIP receptors overcomes the limitations of GLP-1 receptor agonist monotherapy [9]. GLP-1 and GIP are incretin hormones that are released in the intestine in response to nutrient intake and stimulate the activity of β-cells of the pancreas with subsequent insulin secretion. The key insulinotropic effect of GLP-1 is carried out through a receptor associated with the class B G-protein and is manifested through the formation of cyclic adenosine monophosphate (cAMP) [12]. GIP, consisting of 42 amino acid residues and secreted by neuroendocrine k-cells of the duodenum and jejunum in response to nutrient intake, stimulates insulin secretion to a greater extent compared to GLP-1 [9]. The most important feature of GIP is their location directly in adipocytes, which leads to weight loss precisely due to fat, and not muscle tissue, ensuring the formation of a silhouette and, most importantly, metabolically healthy weight loss. It is also worth noting that the activation of GIP neutralizes some undesirable reactions that may occur against the background of the use of GLP-1 agonists in some cases. Thus, GIP activation leads to a decrease in the frequency of nausea and other reactions from the gastrointestinal tract [10].

The successful development of chemically synthesized tirzepatide can stimulate further research in the field of chemical synthesis of other incretin drugs, including GLP-1 receptor agonists and future multi-agonists, which may lead to the creation of new, more effective therapeutic options [9].

The specifics of determining the specific activity of drugs based on complex protein and peptide molecules require an integrated approach, including both physicochemical and biological methods of analysis [15].

Assessment of physicochemical properties

Assessment of protein absorption of UV radiation is a reliable and sensitive method for determining the structure of proteins, which affects their folding and functionality [16]. Spectrophotometry showed identical absorption curves in the UV region: maximum absorption in the region below 230 nm, a shoulder in the range of 225–230 nm and a pronounced peak at 291 nm. The coincidence of the spectra indicates the conformational equivalence of both drugs [17].

Of particular importance is the absence of differences in the profile of related impurities and degradation products, since these factors can affect the safety and efficacy of the drug. Such impurities in a drug containing a peptide can be formed as a result of the destruction of the active substance during production or storage and affect the efficacy and safety of the final product [18]. Such impurities also include peptides with an incorrectly structured structure resulting from errors in the amino acid sequence, removal of individual amino acid residues, as well as oxidation or racemization of amino acids [19].

Table 1 — Study objects

Drug Name	Manufacturer	Series	Expiration Date
Tirzetta®, solution for subcutaneous administration, 2.5 mg (Tirzetta®-1)	JSC "Biochimik", Russia	010124	01/2026
Tirzetta®, solution for subcutaneous administration, 2.5 mg (Tirzetta®-2)	JSC "Biochimik", Russia	020124	01/2026
Tirzetta®, solution for subcutaneous administration, 2.5 mg (Tirzetta®-3)	JSC "Biochimik", Russia	030124	01/2026
Mounjaro®, solution for injection, 5 mg	Eli Lilly and Company, USA	D665365A	04/2025

Table 2 — Gradient elution program for the assessment of impurities in tirzepatide drugs

Time, min	Mobile phase A, %	Mobile phase B, %	Note
0→7	54→(45 ± 4)	46→(55 ± 4)	1 linear gradient.
7→37	(45 ± 4)	(55 ± 4)	1 stage of isocratic elution.
37→49	(45 ± 4)→10	(55 ± 4)→90	2 linear gradient.
49→52	10	90	2 stage of isocratic elution.
52→53	10→54	90→46	3 linear gradient, transition to equilibrium.
53→60	54	46	3 stage of isocratic elution, transition to equilibrium.

Table 3 — Results of spectrophotometry of tirzepatide drugs

Drug Name	Manufacturer	Series	λ_{max}, nm	λ_{min}, nm
Tirzetta®, solution for subcutaneous administration, 2.5 mg	JSC "Biochimik", Russia	010124	281.0 ± 0.7	249.4 ± 1.2
Tirzetta®, solution for subcutaneous administration, 2.5 mg	JSC "Biochimik", Russia	020124	281.2 ± 1.1	249.1 ± 0.6
Tirzetta®, solution for subcutaneous administration, 2.5 mg	JSC "Biochimik", Russia	030124	281.2 ± 0.4	249.3 ± 0.8
Mounjaro®, solution for injection, 5 mg	Eli Lilly and Company, USA	D665365A	281.2 ± 0.5	248.9 ± 0.9

Table 4 — Results of quantitative determination of impurities in samples of the studied drugs

Parameter (norm)	Tirzetta® 010124	Tirzetta® 020124	Tirzetta® 030124	Mounjaro® D665365A
Hydrophilic impurities, %				
RRT 0.81 (not more than 1.5%)	0.05 ± 0.01	0.06 ± 0.05	0.20 ± 0.08	0.34 ± 0.03
RRT 0.90 (not more than 4.0%)	–	–	0.06 ± 0.01	0.28 ± 0.04
RRT 0.94 (not more than 1.5%)	0.07 ± 0.04	0.07 ± 0.02	0.13 ± 0.07	–
Sum of hydrophilic impurities (not more than 7.0%)	0.12 ± 0.041	0.13 ± 0.054	0.39 ± 0.107	0.62 ± 0.05
Hydrophobic impurities 1, %				
RRT 1.11 (not more than 3.0%)	0.05 ± 0.02	–	–	0.49 ± 0.13
RRT 1.18 (not more than 2.0%)	0.11 ± 0.08	0.21 ± 0.06	0.48 ± 0.11	0.96 ± 0.14
Sum of hydrophobic impurities 1 (not more than 5.0%.	0.16 ± 0.082	0.21 ± 0.06	0.48 ± 0.11	1.45 ± 0.191
Hydrophobic impurities 2 (not more than 2.0%)	–	–	–	–
Single unidentified impurity (not more than 1.0%)	–	–	–	–
Sum of all impurities (not more than 10.0%)	0.28 ± 0.092	0.34 ± 0.081	0.87 ± 0.153	2.075 ± 0.197

Note: RRT — relative retention time.

Table 5 —Results of quantitative determination of high molecular weight compounds (HMWC) in the studied drugs

Drug Name	Manufacturer	Series	HMWC, %
Tirzetta®, solution for subcutaneous administration, 2.5 mg	JSC "Biochimik", Russia	010124	0.32%
Tirzetta®, solution for subcutaneous administration, 2.5 mg	JSC "Biochimik", Russia	020124	0.23%
Tirzetta®, solution for subcutaneous administration, 2.5 mg	JSC "Biochimik", Russia	030124	0.20%
Mounjaro®, solution for injection, 5 mg	Eli Lilly and Company, USA	D665365A	0.68%

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Table 6 — Assessment of primary data GLP1R/CRE-Luc/HEK293

log[C], M	The coefficient of variation, %				*p*-value
	Tirzetta®-1	Tirzetta®-2	Tirzetta®-3	Mounjaro®	
-5	7.0	5.6	3.2	7.5	0.5931
-6	5.3	7.7	3.4	4.8	0.5761
-7	6.8	5.9	3.8	10.6	0.0800
-8	4.0	7.1	9.1	9.3	0.0750
-9	9.9	8.3	8.2	15.2	0.0280
-10	11.6	15.9	26.0	25.9	0.4119
-11	25.8	23.6	17.9	11.3	0.1467
-12	28.0	7.6	18.7	9.3	0.0682

Note: * — estimating the spread of data; $p > 0.05$ — the homogeneity of the variances is confirmed.

Table 7 — Results of the analysis of logistic curves GLP1R/CRE-Luc/HEK293

Drug	Coefficient of determination, R^2	Fischer's F-test	Log EC_{50}	Ratio of the angle of inclination β to the referent
Tirzetta®-1	0.9983	F = 6900 (p < 0.0001)	-8.71 ± 0.014 CV = 0.2	0.98
Tirzetta®-2	0.9977	F = 4771 (p < 0.0001)	-8.67 ± 0.016 CV = 0.2	0.93
Tirzetta®-3	0.9985	F = 6112 (p < 0.0001)	-8.71 ± 0.014 CV = 0.2	0.93
Mounjaro®	0.9948	F = 2630 (p < 0.0001)	-8.89 ± 0.020 CV = 0.2	–

Table 8 — Assessment of primary data GIPR/CRE-Luc/HEK293

log[C], M	The coefficient of variation, %				Homogeneity of dispersions*
	Tirzetta®-1	Tirzetta®-2	Tirzetta®-3	Mounjaro®	
-8	14.6	14.6	13.1	7.4	0.5189
-9	12.5	10.3	22.6	7.3	0.2959
-10	16.6	11.8	29.1	23.2	0.4751
-11	11.7	12.5	23.0	12.6	0.0908
-12	19.6	13.9	16.7	24.5	0.7829
-13	20.2	23.6	13.5	19.0	0.4650
-14	6.3	12.8	5.1	28.6	0.6686
-15	15.0	18.4	25.9	14.2	0.7094

Note: * —homogeneity of the variances was determined by evaluating the spread of data, the column shows the values of the *p*-value Brown-Forsythe test.

Table 9 — The results of the analysis of logistic curves GIPR/CRE Luciferase Reporter HEK293

Drug	Coefficient of determination, R^2	Fischer's F-test	Log EC50	Ratio of the angle of inclination β to the referent
Tirzetta®-1	0.9365	F = 180.6 (p < 0.0001)	-12.12 ± 0.097 CV = 0.8	0.8
Tirzetta®-2	0.9489	F = 227.4 (p < 0.0001)	-12.20 ± 0.176 CV = 1.45	0.8
Tirzetta®-3	0.8613	F = 68.3 (p < 0.0001)	-12.18 ± 1.310 CV = 10.8	1.1
Mounjaro®	0.9697	F = 312.0 (p < 0.0001)	-12.20 ± 0.385 CV = 3.2	–

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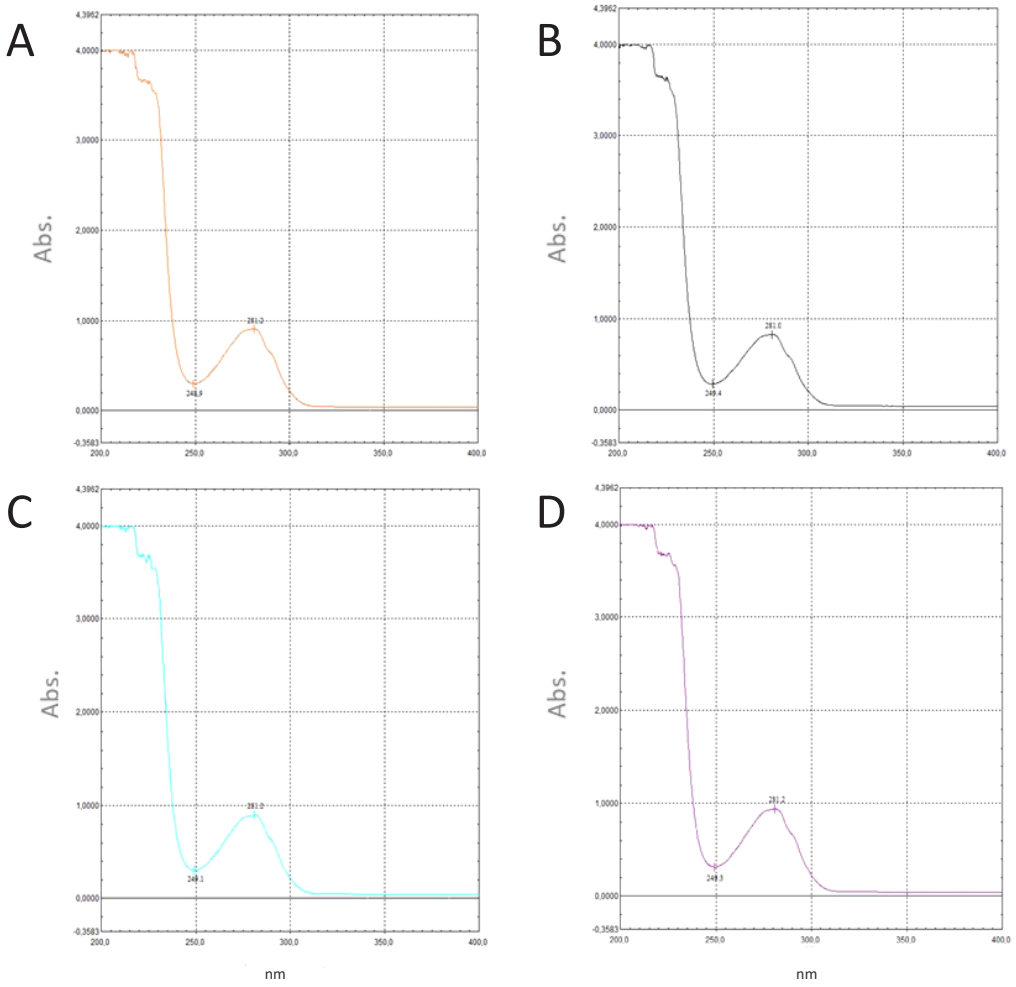

Figure 1 — Absorption spectrum of tirzepatide drug.
Note: A — Mounjaro® (D665365A); B — Tirzetta® (010124); C — Tirzetta® (020124); D — Tirzetta® (030124).

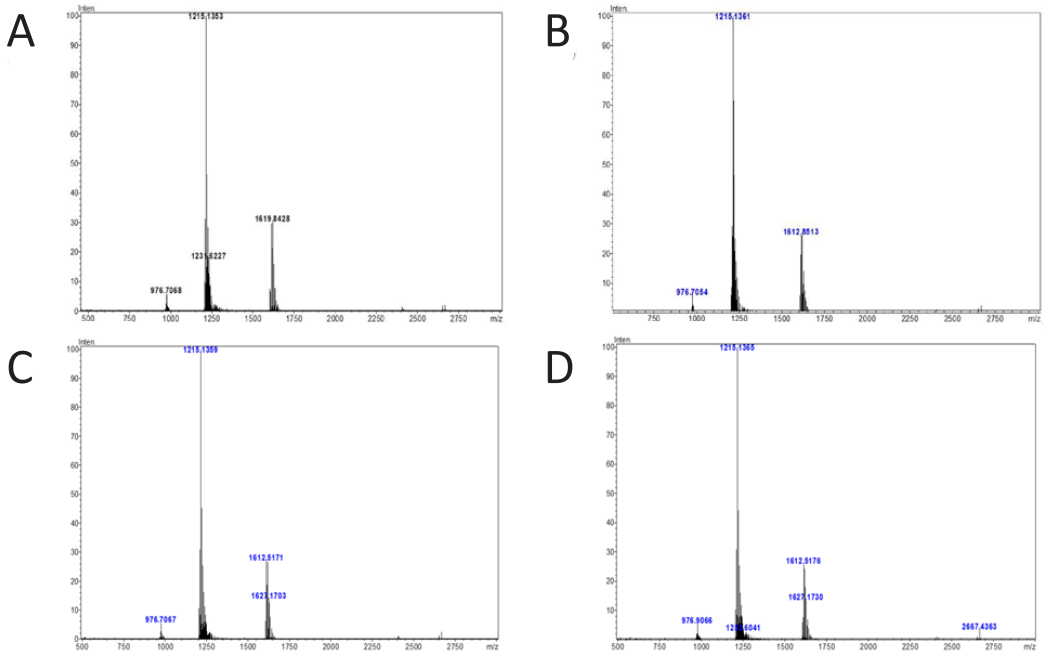

Figure 2 — Mass spectra of solutions of tirzepatide drugs.
Note: A — Mounjaro® (D665365A); B — Tirzetta® (010124); C — Tirzetta® (020124); D — Tirzetta® (030124).

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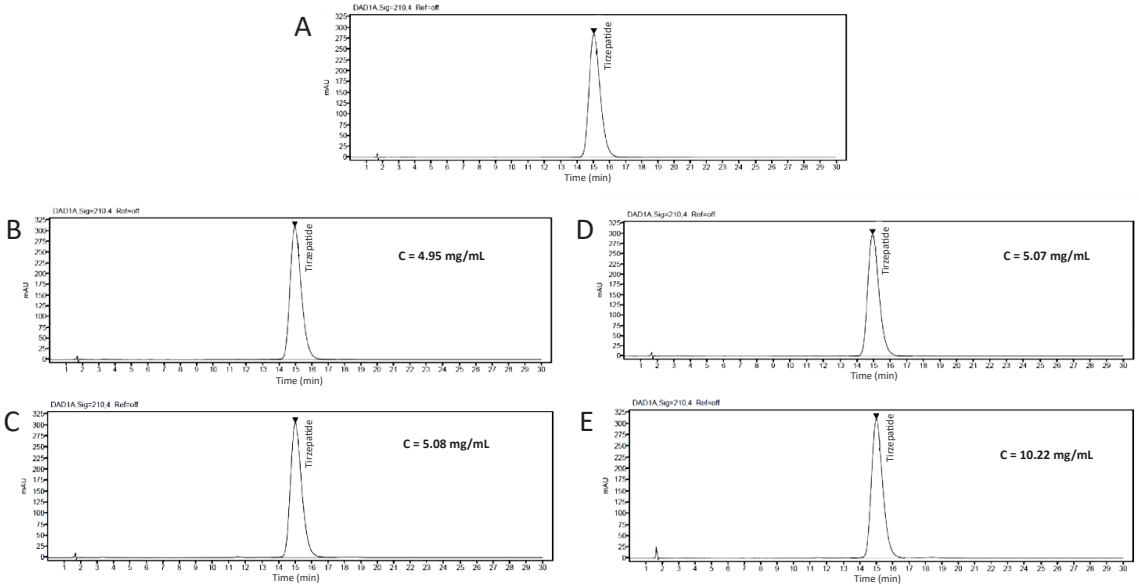

Figure 3 — Chromatograms of the tirzepatide drugs solutions.
Notes: A — Tirzepatide standard; B — Tirzetta® (series 010124); C — Tirzetta® (series 020124); D — Tirzetta® (series 030124); E — Mounjaro® (D665365A).

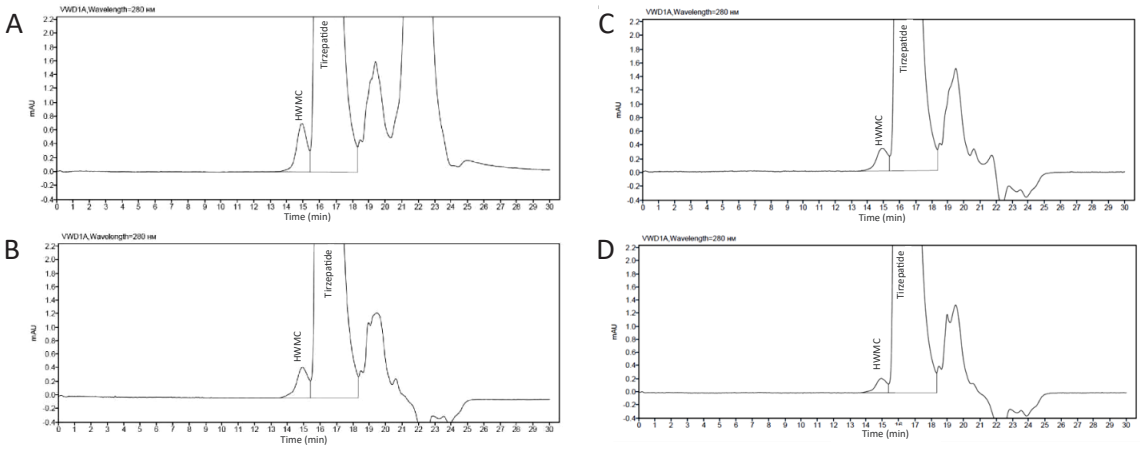

Figure 4 — Chromatograms of tirzepatide drugs and impurities.
Note: A — Mounjaro® (D665365A); B — Tirzetta® (010124); C — Tirzetta® (020124); D — Tirzetta® (030124).

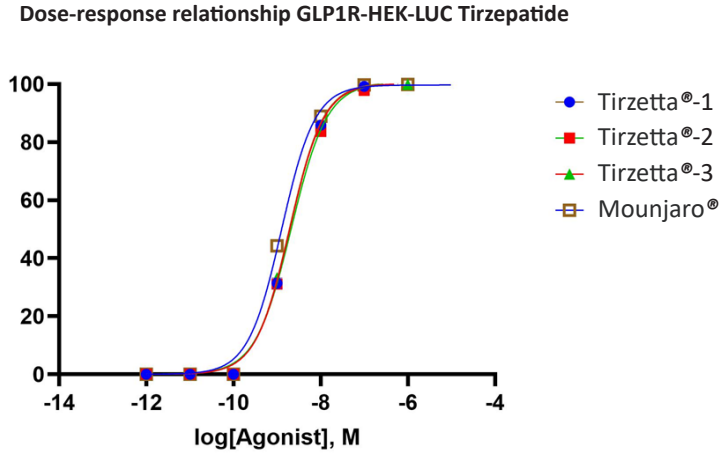

Figure 5 — Dependence of the luminescent signal value on the concentration of the agonist GLP-1R (*n* = 3).
Note: The data is presented in M ± SD, as well as with logistic curves (based on the results of nonlinear regression).

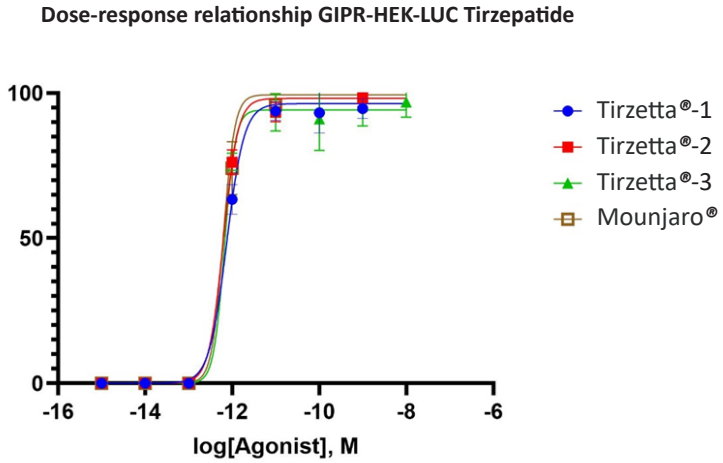

Figure 6 — Dependence of the luminescent signal value on the concentration of the agonist GIPR (*n* = 3).
Note: The data is presented in M ± SD, as well as with logistic curves (based on the results of nonlinear regression).

Chromatographic methods are the "gold standard" for characterizing peptide drugs and allow detecting even minor differences in the structure of molecules. The identity of the chromatographic profiles of the active substance (tirzepatide) in the composition of Tirzetta® and Mounjaro® confirms their structural equivalence [15].

Modern trends in pharmaceutical design determine the need for an integrated approach, in which not only the active pharmaceutical substance is optimized, but also the composition of excipients, in particular, preservatives in parenteral dosage forms. The abandonment of the use of phenol and benzyl alcohol in favor of innovative delivery systems makes it possible to exclude these components from the formulation. This approach is aimed to minimize the risks of developing hypersensitivity reactions and increasing the overall safety profile of pharmacotherapy, as well as toxicity [20].

The drug Tirzett[a]® contains 4.2 times fewer impurities than the reference drug Mounjaro®, which suggests that the former is safer for patients. It should be taken into account that therapy for type 2 diabetes and obesity is lengthy and may subsequently turn into lifelong medication. Impurities such as phenol and benzyl alcohol can accumulate in the body with prolonged use and cause toxic effects. The elimination of impurities from the composition of Tirzetta® provides a number of pharmacotechnological and clinical advantages, which include reducing local toxicity and irritation at the injection site, which improves the tolerability of therapy, especially in sensitized patients, as well as with prolonged use. In addition, the possibility of systemic toxic effects and undesirable interactions with other drugs due to the properties of preservatives is excluded. Therefore, the development of preservative-free forms meets modern regulatory requirements and helps to increase adherence to treatment due to an improved safety and biocompatibility profile.

Thus, the study confirms that today in Russia it has been possible to develop an effective method for producing peptide drugs, which reduces the formation of racemic impurities, simplifies the purification of the target product, increases its purity and yield, and reduces the cost. It is also worth noting that chemical synthesis can provide a number of advantages compared to biotechnological production, including greater reproducibility of the process, a reduced risk of microbiological contamination, and a potentially lower production cost. These factors are especially important for ensuring the availability of innovative drugs for the general population [21]. Given the increasing prevalence of type 2 diabetes and obesity, the availability of effective drugs is becoming critical for public health [1].

In vitro biological activity assessment

The use of reporter cell lines is one of the most effective screening approaches for assessing the biological activity of drugs in vitro [22]. This model is genetically modified cells into which reporter genes have been introduced under the control of promoters that are activated when a ligand binds to a receptor and subsequent activation of the signaling pathway. Such lines allow quantifying the activity of receptor interactions by measuring the expression of the reporter — usually a fluorescent protein. The level of

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bioluminescence is directly proportional to the amount of enzyme and the binding activity of a particular transcription factor [23].

The comparability of the results of GIP and GLP-1 receptor activation between the studied drugs indicates the preservation of the key structural and functional characteristics of the tirzepatide molecule, which determine the properties and biological activity of the peptide. This is critical because it is the dual activity against the two incretin receptors that determines the unique therapeutic profile of tirzepatide [9].

The totality of the results fully confirms the initial hypothesis about the bioequivalence of the reproduced drug Tirzetta® and the reference drug Mounjaro®. This fact indicates that the synthesis and purification conditions are optimized in the production of the drug Tirzetta® in order to minimize the formation of structural variants and impurities [24]

Confirmation of bioequivalence allows extrapolation of previously obtained safety and efficacy data of the reference drug to reproduced drugs without studying the latter in large-scale clinical trials [25].

Clinical efficacy

To date, two main registered tirzepatide drugs are known in global practice — Mounjaro® and Zepbound® (Eli Lilly and Company, USA). Despite the fact that both drugs contain the same active substance — tirzepatide — they have different indications. Thus, Mounjaro® is registered for the treatment of type 2 diabetes mellitus, while Zepbound® is used for the treatment of obesity and related conditions. Tirzetta® is registered for both indications [26].

In addition to tirzepatide, other dual agonist molecules have been studied. Thus, NNC0090-2746 passed two phases of clinical trials (Phase 1 and Phase 2a) in patients with type 2 diabetes mellitus [27]. Studies have shown that the drug has a hypoglycemic effect and reduces body weight, however, its development did not progress beyond Phase 2a — it did not reach Phase 3 clinical trials and was not registered for medical use. In the second phase study, NN00090-2746, characterized by balanced activity against GLP-1 and GIP receptors, was administered daily subcutaneously at a dose of 1.8 mg for 12 weeks. Against the background of NN00090-2746 therapy, compared with placebo, there was a decrease in HbA1c level by 0.96% ($p < 0.001$) and body weight by 1.67% ($p = 0.06$).

Tirzepatide, the activity of which is more pronounced against GIP receptors, was administered weekly subcutaneously in various doses (1, 5, 10 and 15 mg) for 26 weeks. The degree of reduction in HbA1c level was dose-dependent (1.73, 1.89 and 2.07%), while 45–90% of patients on tirzepatide therapy reached the target HbA1c level.

Analyzing the data from these studies, it can be concluded that tirzepatide is more effective than NNC0090-2746 in reducing HbA1c levels and body weight, which was observed after 12 weeks of therapy. This may be due to differences in the chemical structure, affinity and balance of activity of the drugs against GLP-1 and GIP receptors [9].

The results of the Phase II study served as the basis for the start of Phase III clinical programs with tirzepatide in patients with type 2 DM (SURPASS) and in patients with obesity (SURMOUNT) [27]. Clinical studies have demonstrated unprecedented efficacy of tirzepatide. The drug achieve the significant and sustained weight loss, as well as a marked improvement in cardiometabolic parameters in patients with obesity and/or type 2 DM [28–31]. In general, treatment with tirzepatide led to a weight loss of approximately 8–15% of the initial body weight in patients with type 2 diabetes and from 15 to 23% in patients with obesity and overweight, which is comparable in efficacy to some surgical interventions. There is a decrease in waist circumference, on average, by 15 cm, which indicates a decrease in the severity of visceral obesity. A significant proportion of patients achieve weight loss ≥ 5%, and a significant proportion — weight loss of ≥ 15–20%, which significantly exceeds the results of previous drugs and corresponds to or exceeds the best modern analogues. In addition to changes in weight, there was a decrease in systolic blood pressure by 4.9–6.4 mm Hg, improvement in glycemic control (decrease in HbA1c by 1.8–2.4%), as well as positive dynamics of the lipid profile: total cholesterol decreased by 0.25–0.37 mmol/Ll, LDL — by 0.31–0.43 mmol/L, HDL increased by 0.05–0.08 mmol/L. The main advantage of tirzepatide in the treatment of patients with obesity is a 94% reduction in the risk of diabetes mellitus. The introduction of this drug into practice is a transition from managing existing diabetes to active prevention of this disease. This is the most effective way to reduce the global burden of type 2 diabetes mellitus for society as a whole. Side effects are mainly associated with the gastrointestinal tract, but are usually mild and reversible. No serious complications or increased risk of hypoglycemia have been identified. Thus, tirzepatide provides effective, versatile and well-tolerated treatment for long-term weight and metabolic health management.

Study limitations

Despite the fact that the use of multiple methodological approaches ensures a high degree of reliability of the study results, it is necessary to note a number of limitations that should be taken into account when interpreting the results. Thus, reporter cell lines used to assess in vitro activity are simplified models of receptor interaction. The real physiological environment is characterized by a much greater complexity of intercellular interactions and regulatory mechanisms [32].

CONCLUSION

The results of a comparative study of the physicochemical characteristics and biological activity of Tirzetta® (manufacturer PROMED RUS LLC) and Mounjaro® (Eli Lilly and Company, USA) convincingly demonstrated the equivalence of these two drugs.

The totality of the data obtained indicates that Tirzetta® is a high-quality drug that provides comparable therapeutic efficacy with the reference drug, with better safety indicators. From a scientific point of view, the data obtained contribute to the understanding of structure-functional relationships in the tirzepatide molecule and confirm the stability of key pharmacophore groups.

The results of the study create a scientific basis for further development of peptide multiagonists, opening up prospects for expanding the availability of innovative drugs.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

P.I. Makarevich, N.A. Alexandrushkina — investigation, data analysis; P.A. Podlesnaya, Yu.G. Kazaishvili, A.V. Taganov — administration, data analysis, writing—original draft; V.S. Shcherbakova, K.Ya. Zaslavskaya, P.A. Bely — conceptualization, data analysis, writing—review & editing; K.N. Koryanova, E.S. Mishchenko, L.I. Shcherbakova, I.N. Dyakova — data analysis, writing—review & editing.

All the authors confirm their authorship compliance with the ICMJE international criteria (all the authors made a significant contribution to the conceptualization, conduct of the study and preparation of the article, read and approved the final version before publication).

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