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Comparative analysis of physicochemical properties, bioequivalence, safety and tolerability of the first domestic semaglutide

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Semaglutide is a representative of analogues of the incretin hormone human glucagon-like peptide-1 (GLP-1) and is currently used in Russia for the treatment of type 2 diabetes mellitus (T2DM; in monotherapy and in combination therapy), including patients with obesity and overweight.

The aim of the work was to conduct a comparative assessment of the physicochemical properties, a biological activity, bioequivalence and safety, including tolerability and immunogenicity, of the drug Quincent® (semaglutide, 1.34 mg/ml, a solution for a subcutaneous administration, Promomed Rus LLC, Russia) and the drug Ozempic® (semaglutide, 1.34 mg/ml, a solution for a subcutaneous administration, Novo Nordisk A/S, Denmark) when administered to healthy volunteers.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

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Materials and methods. To assess the degree of similarity of the study drug Quincenta® (semaglutide, 1.34 mg/ml, a solution for a subcutaneous administration, Promomed Rus LLC, Russia) with a chemically synthesized active substance to the original (reference) drug Ozempic® (semaglutide, 1.34 mg/ml, a solution for a subcutaneous administration, Novo Nordisk A/S, Denmark), a comparative study of physicochemical properties and a biological activity was carried out. To assess the bioequivalence of the study drug and the reference drug, an open randomized parallel comparative study with the participation of healthy volunteers (*n*=54), 54 participants of which had been included in the population, was conducted. The volunteers were randomized into 2 groups in a 1:1 ratio, and received a single dose subcutaneously either of the study drug (domestic semaglutide at a dose of 0.5 mg) or the reference drug (foreign semaglutide at a dose of 0.5 mg). The mode of administration was in the morning on an empty stomach. A semaglutide concentration was determined in serum samples using a previously validated enzyme-linked immunosorbent assay (ELISA) method. A quantitative determination of antibodies to semaglutide in the human serum by ELISA was carried out with a microplate photometer using ready-made kits pre-validated by the manufacturer. The conclusion about the bioequivalence of the compared drugs was made using an approach based on the assessment of 90% confidence intervals for the ratios of the geometric mean values of the parameters C_{max}, $AUC_{(0-t)}$ of semaglutide in the measurement original units.

Results. The results of the comparative analysis of the study drug and the reference drug demonstrate the comparability of their physicochemical properties and biological activity. The results of the clinical study demonstrated the bioequivalence of the test drug and the reference drug. Thus, the pharmacokinetic parameters of the drugs were comparable to each other: the C_{max} value for the study drug was 42.088±8.827 ng/ml, for the reference drug Ozempic® it was 42.2556±7.84. Herewith, the half-life for the study drug and the reference drug was 168.39±39.47 and 157.99±28.57 hours, respectively. The resulting 90% confidence intervals for the ratio of the C_{max} and AUC_{0-t} values of the study drug and the reference drug were 90.89–109.15 and 91.66–111.27%, respectively. The tolerability of the drugs in the volunteers was notified as good. No adverse events were recorded during the study. No serious adverse events were reported throughout the study. According to the results of the immunogenicity analysis, no antibodies to Russian-made semaglutide were detected in the blood serum of the volunteers, which indicated the lack of Results. The results of a comparative analysis of the study drug and the reference drug demonstrate the comparability of physicochemical properties and biological activity. The results of the clinical study demonstrated the bioequivalence of the study drug and the reference drug. Thus, the pharmacokinetic parameters of the drugs were comparable to each other: the C_{max} value for the study drug was 42.088±8.827 ng/ml, for the reference drug Ozempic® this figure was 42.2556±7.84. At the same time, the half-life for the study drug and the reference drug was 168.39±39.47 and 157.99±28.57 hours, respectively. The resulting 90% confidence intervals for the ratio of the C_{max} and AUC_{0-t} values of the study drug and the reference drug were 90.89–109.15 and 91.66–111.27%, respectively. Tolerability of the drugs in volunteers was noted as good. No adverse events were recorded during the study. No serious adverse events were reported throughout the study. According to the results of the immunogenicity analysis, no antibodies to Russian-made semaglutide were detected in the blood serum of the volunteers, which indicated the lack of the drug immunogenicity.

Conclusion. In the course of the study, the comparability of the physicochemical properties and biological activity of the studied Russian drug with the chemically synthesized active substance Quincenta® to the reference drug Ozempic® was confirmed: the activity range of the studied drugs was within 80–120% in relation to the standard sample of semaglutide. The bioequivalence and a similar safety profile, including the immunogenicity and tolerability of the Russian drug Quincenta® (semaglutide 1.34 mg/ml, Promomed Rus LLC, Russia) were shown in comparison with the foreign drug Ozempic® (semaglutide 1.34 mg/ml, Novo Nordisk A/C, Denmark).

Keywords: glucagon-like peptide-1; GLP-1; bioequivalence; pharmacokinetics; semaglutide; type 2 diabetes mellitus; physicochemical properties; safety profile; biological activity

Abbreviations: T2DM – Type 2 diabetes mellitus; CVDs – cardiovascular diseases; GK – glycemic control; ASCVDs – atherosclerotic cardiovascular diseases; HbAlc – glycated hemoglobin; GLP-1 – glucagon-like peptide-1; CHF – chronic heart failure; iNGLT-2 – inhibitors of sodium-glucose cotransporter-2; CKD – chronic kidney disease; BMI – body mass index; ARVI – acute respiratory viral infection; AP – arterial pressure; HR – heart rate; RR – respiratory rate; ECG – electrocardiography; AE – adverse event; SAE – serious adverse event; CI – confidence interval.

Сравнительный анализ физико-химических свойств, биоэквивалентности, безопасности и переносимости отечественного семаглутида

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Семаглутид является представителем аналогов инкретинового гормона человеческого глюкагоноподобного пептида-1 (ГПП-1) и в настоящее время в России используется для лечения сахарного диабета 2 типа (СД 2; в монотерапии и в комбинированной терапии), в том числе, у пациентов с ожирением и избыточной массой тела.

Цель. Провести сравнительную оценку физико-химических свойств, биологической активности, биоэквивалентности и безопасности, включая переносимость и иммуногенность, лекарственного препарата Квинсента® (семаглутид, 1,34 мг/мл, раствор для подкожного введения, ООО «Промомед», Россия) и препарата Оземпик® (семаглутид, 1,34 мг/мл, раствор для подкожного введения, Ново Нордиск А/С, Дания) при введении здоровыми добровольцами.

Материалы и методы. Для оценки степени подобия исследуемого препарата Квинсента® (семаглутид, 1,34 мг/мл, раствор для подкожного введения, ООО «Промомед», Россия) с химически синтезированным активным веществом оригинальному (референтному) препарату Оземпик® (семаглутид, 1,34 мг/мл, раствор для подкожного введения, Ново Нордиск А/С, Дания) было проведено сравнительное изучение физико-химических свойств и биологической активности. Для оценки биоэквивалентности исследуемого и референтного препарата было проведено открытое рандомизированное параллельное сравнительное исследование с участием здоровых добровольцев (*n*=54), из них в популяцию для оценки биоэквивалентности вошли 54 участника. Добровольцы были рандомизированы в 2 группы в соотношении 1:1 и получали однократно подкожно утром натощак, либо исследуемый препарат (отечественный семаглутид в дозе 0,5 мг), либо референтный препарат (зарубежный семаглутид в дозе 0,5 мг). Концентрацию семаглутида определяли в образцах сыворотки крови с помощью предварительно валидированного метода иммуноферментного анализа (ИФА). Количественное определение антител к семаглутиду в сыворотке крови человека методом ИФА было проведено с помощью фотометра для микропланшетов с использованием готовых предварительно валидированных производителем наборов. Вывод о биоэквивалентности сравниваемых препаратов делали с использованием подхода, основанного на оценке 90% доверительных интервалов для отношений средних геометрических значений параметров C_{max}, $AUC_{(0-t)}$ семаглутида в исходных единицах измерения.

ОРИГИНАЛЬНАЯ СТАТЬЯ
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Результаты. Результаты сравнительного анализа исследуемого и референтного препарата демонстрируют сопоставимость физико-химических свойств и биологической активности. Результаты клинического исследования продемонстрировали биоэквивалентность исследуемого препарата и препарата сравнения. Так, фармакокинетические параметры препаратов были сопоставимы между собой: величина C_{max} для исследуемого препарата составила 42,088±8,827 нг/мл, для препарата сравнения Оземпик® данный показатель составил 42,2556±7,84. При этом период полувыведения для исследуемого препарата и препарата сравнения составил 168,39±39,47 и 157,99±28,57 ч, соответственно. Полученные 90%-ные доверительные интервалы для отношения значений C_{max} и AUC_{0-t} исследуемого препарата и референтного препарата составили 90,89–109,15 и 91,66–111,27%, соответственно. Переносимость препаратов у добровольцев была отмечена как хорошая. При проведении исследования не было зафиксировано нежелательных явлений. В течение всего исследования не было зарегистрировано ни одного серьёзного нежелательного явления. По результатам анализа иммуногенности у добровольцев не были выявлены антитела к семаглутиду российского производства в сыворотке крови, что свидетельствовало об отсутствии иммуногенности препарата.

Заключение. В ходе проведенного исследования была подтверждена сопоставимость физико-химических свойств и биологической активности исследуемого российского препарата с химически синтезированным активным веществом Квинсента® препарату сравнения Оземпик®: диапазон активности исследуемых препаратов находился в пределах 80–120% по отношению к стандартному образцу семаглутида. Показана биоэквивалентность и сходный профиль безопасности, включая иммуногенность и переносимость российского препарата Квинсента® (семаглутид 1,34 мг/мл, ООО «Промомед», Россия) в сравнении с зарубежным препаратом Оземпик® (семаглутид 1,34 мг/мл, Ново Нордиск А/С, Дания).

Ключевые слова: глюкагоноподобный пептид-1; ГПП-1; биоэквивалентность; фармакокинетика; семаглутид; сахарный диабет 2-го типа, физико-химические свойства, профиль безопасности, биологическая активность

Список сокращений: СД 2 – сахарный диабет 2 типа; ССЗ – сердечно-сосудистые заболевания; ГК – гликемический контроль; АССЗ – атеросклеротические сердечно-сосудистые заболевания; HbAlc – гликированный гемоглобин; ГПП-1 – глюкагоноподобный пептид-1; ХСН – хроническая сердечная недостаточность; иНГЛТ-2 – ингибиторы натрий-глюкозного котранспортера-2; ХБП – хроническая болезнь почек; ИМТ – индекс массы тела; ОРВИ – острая респираторная вирусная инфекция; АД – артериальное давление; ЧСС – частота сердечных сокращений; ЧДД – частота дыхательных движений; ЭКГ – электрокардиография; НЯ – нежелательное явление; СНЯ – серьёзное нежелательное явление; ДИ – доверительный интервал.

INTRODUCTION

Given the increasing prevalence of type 2 diabetes mellitus (T2DM) and its associated complications[1] [1], the need to develop highly effective treatment strategies for this serious disease has never been greater. The focus of treatment for type 2 diabetes has traditionally been a glycemic control[2], but in recent years, the standards of care have emphasized the importance of a multifactorial approach that includes the correction of cardiovascular disease (CVD) risk factors such as hyperglycemia, as well as overweight/obesity, hypertension, and dyslipidemia[3] [1, 2].

T2DM people are known to have a 2- to 4-fold higher risk of developing CVDs than people without diabetes, and cardiovascular events are more likely to occur at an earlier age. CVDs are the main cause of death in T2DM[4] patients [3, 4]. It has been proven that influencing cardiovascular risk factors as a part of the T2DM treatment can reduce mortality from the disease itself and its complications. Thus, the Steno-2 study [5] showed that a comprehensive control of diabetes risk factors can reduce the incidence of cardiovascular events by more than 50%.

But although a harder glycemic control (GC) has been shown to be effective against microvascular complications [6] and recent observational studies [7, 8] have demonstrated that increased HbA1c levels are associated with a greater risk of cardiovascular events, the precise role of the GC in reducing CVDs risk remains to be detected [9].

In the treatment of T2DM patients, it is necessary to personalize the choice of glucose-lowering therapy, taking into account the individual characteristics of the patient (especially indications of a high risk of atherosclerotic CVDs (ASCVDs) or existing ASCVDs, a chronic heart failure (CHF), chronic kidney diseases (CKD), obesity, high-risk hypoglycemia) and a dominant clinical problem[5]. Thus, in patients with T2DM and existing cardiovascular diseases (CVDs), the preference should be given to glucose-lowering drugs[6] with a proven cardiovascular safety[7] [1, 2, 4, 10].

[1] International Diabetes Federation. IDF Diabetes Atlas (2021). Available from: https https://www.diabetesatlas.org/data/en/region/3/eur.html
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[3] Ibid.
[4] International Diabetes Federation. IDF Diabetes Atlas, 2021.
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The key element regulating an insulin production in the body is glucagon-like peptide-1 (GLP-1). Glucagon-like peptide-1 receptor (GLP-1) agonists act similarly to the incretin hormone GLP-1 and mediate their effects through its receptors, which are expressed in the pancreas, gastrointestinal tract, heart, lungs, kidneys and brain. GLP-1 receptors in the pancreas and brain have been shown to be responsible for the corresponding improvements in GC and weight loss. Functional effects in the pancreas include a glucose-dependent insulin release as well as an up-regulation of insulin biosynthesis and glucokinase and glucose transporters. The impact on GLP-1 receptors also causes a glucose-dependent decrease in the glucagon secretion, which, in turn, reduces a hepatic glucose output. In the pancreas, GLP-1 receptors are predominantly localized to insulin-producing beta cells, with a markedly weaker expression on the acinar cells of the exocrine pancreas. Most of GLP-1 is produced in the gastrointestinal tract. In the brain, GLP-1 is produced in neurons and is likely a neuropeptide with physiologically and pharmacologically significant effects on the food intake and body weight, a potential neuromodulatory role, and possible effects in a number of other neuropathological conditions, including neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease), brain injuries and strokes, as well as depression, anxiety and addiction [11].

The main advantage of a number of GLP-1 drugs, including semaglutide, is the ability to reduce the risks of cardiovascular events and improve renal outcomes in T2DM patients [12].

The first drug of this kind from the arGLP-1 class was exenatide, which had been approved for use by the U.S. Food and Drug Administration (FDA). Since 2005, it has been successfully used in clinical practice in the USA, European countries, and since 2007 – in Russia. Currently, six arGLP-1 drugs, each with a unique drug delivery strategy, have been approved by the FDA, and several more are being developed. Considering a rapid elimination as the main problem for the clinical use of arGLP-1, researchers have successfully developed and implemented various strategies to increase the half-life of these drugs, including a sequential modification and an increase in the duration of their action [13].

However, in general, arGLP-1, including semaglutide, is the ability to reduce overall mortality and the risk of major cardiovascular events (Major Adverse Cardiovascular Event, MACE), such as non-fatal heart attack, non-fatal stroke or death from CVD and improve renal outcomes in patients with T2DM [14].

Semaglutide is one of the latest drugs from the arGLP-1 group approved both in Russia[8] and in many foreign countries. This drug, semaglutide, was developed established on a large body of research based on liraglutide [15].

The semaglutide molecule has a 94% homology with human GLP-1 and 3 main structural modifications compared to human GLP-1: 1) a substitution of an amino acid at position C_8 (alanine to α-aminoisobutyric acid), which prevents the peptide destruction by the dipeptidyl peptidase-4 (DPP-4) enzyme; 2) acylation of lysine in the main part of the peptide and the attachment of a C_{18} fatty acid at position C_{26} to ensure strong and specific binding to albumin; 3) the substitution of an amino acid at position C_{34} (lysine to arginine) – this prevents the addition of a C_{18}-dibasic fatty acid at the wrong site of the semaglutide molecule [16].

In preclinical and pharmacokinetic studies in T2DM adults, it was shown that, compared with liraglutide, which is administered once a day, semaglutide has an even longer half-life (from 7 days), which allows its use once a week [16–19]. The effect of semaglutide did not change in patients with an impaired renal or hepatic function, with the exception of a terminal renal and hepatic failure [20, 21]. Semaglutide is excreted mainly in urine (approximately 3% unchanged) and also in feces [22].

At the doses of 0.5 and 1.0 mg, semaglutide was approved for use in type 2 diabetes in the USA in 2017[9] and by the European Medicines Agency in 2018[10].

Semaglutide is registered in the Russian Federation and is currently included in the clinical recommendations of the Ministry of Health of the Russian Federation[11] for the treatment of T2DM patients, as well as in the Algorithms for a specialized care for T2DM patients [2]. According to these documents, semaglutide is recommended for use in T2DM patients with an indication of a high risk of ASCVDs, as well as with an already established diagnosis of ASCVDs as priority

[8] State register of medicines of the Russian Federation. Quincenta®. Available from: https://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=bf3309b5-3cd1-491a-bef6-ac1db65daa4c

[9] US Food and Drug Administration. OZEMPIC (semaglutide) injection prescribing information, 2017. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/209637lbl.pdf

[10] Novo Nordisk Company Announcement. Ozempic® (semaglutide) approved in the EU for the treatment of type 2 diabetes. February 2, 2018. Available from: https://www.novonordisk.com/bin/getPDF.2167679.pdf

[11] Clinical guidelines Type 2 diabetes mellitus in adults, 2022.

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therapy, as well as in patients with CKD in stages 1–4 for nephroprotection in the presence of contraindications or intolerance to sodium-glucose cotransporter inhibitors-2 (iNGLT-2) in the presence of a concomitant diagnosis of obesity, which determines its relevance for Russian patients. Herewith, semaglutide was presented on the Russian pharmaceutical market only in the form of a foreign drug, which is currently unavailable. In this regard, it seems relevant to develop and localize the production of a full cycle from the substance to the finished dosage form and the subsequent study of the bioequivalence of the domestic analogue of semaglutide to ensure the country's medicinal independence and increase an access for our fellow citizens to a modern high-quality drug.

According to the FDA guidelines[12], alpha-amino acid polymers, such as glucagon, semaglutide, etc., containing up to 40 amino acid residues, are considered not protein molecules, but peptides. According to the FDA, to confirm the equivalence of a synthetic peptide and a biotechnologically derived semaglutide contained in a precursor drug, it is sufficient to prove the structural identity of the active pharmaceutical substance (APS) using modern analytical methods.

The Promomed Rus LLC company has developed its own technology for the production of API using methods of chemical synthesis and isolation of semaglutide into a finished dosage form for the treatment of type 2 diabetes. The Quincenta® (solution for subcutaneous administration, 0.25/0.5 mg/dose, 1 mg/dose) has passed the entire cycle of necessary studies and registered in the Russian Federation (LP-008828 dated 17 October 2023). The full production cycle from the substance to the finished dosage form on the territory of the Russian Federation allows, on the one hand, to ensure maximum control over the quality of the product, and on the other side to guarantee the uninterrupted supply of the population with a vital drug.

To further assess the quality and safety of the developed drugs, their registration in the Russian Federation in accordance with Russian regulatory requirements, in addition to physicochemical methods of analysis and preclinical studies, it is necessary to conduct a study of the pharmacokinetics, safety and immunogenicity of the drug Quicenta® (semaglutide 1.34 mg/ml, Promomed Rus LLC, Russia) in comparison with the foreign predecessor drug Ozempic® (semaglutide 1.34 mg/ml, Novo Nordisk A/S, Denmark).

THE AIM of the work was to conduct a comparative assessment of the physicochemical properties, a biological activity, bioequivalence and safety, including tolerability and immunogenicity, of the drug Quincenta® (semaglutide, 1.34 mg/ml, a solution for a subcutaneous administration, Promomed Rus LLC, Russia) and the drug Ozempic® (semaglutide, 1.34 mg/ml, a solution for a subcutaneous administration, Novo Nordisk A/S, Denmark) when administered to healthy volunteers.

MATERIALS AND METHODS

Physicochemical properties and biological activity

In order to assess the degree of comparability (similarity) of the test drug with the chemically synthesized active substance Quicenta® (semaglutide 1.34 mg/ml, Promomed RUS LLC, Russia) to the original (reference) drug Ozempic® (semaglutide 1.34 mg/ml, Novo Nordisk A/S, Denmark), a comparative study of their physicochemical properties and biological activity was carried out. In order to form a representative quality profile and obtain reliable data on comparability in accordance with Decision No. 89 "On approval of the Rules for conducting research on biological medicinal products of the Eurasian Economic Union", three series (samples) of the original (reference) drug and three series (samples) of its synthetic analogue (test drug) were used in the research.

Spectrophotometry in the ultraviolet light (200–400 nm)

The absorption spectra in the ultraviolet region for domestic / test and foreign / reference drugs were obtained using a Shimadzu UV-1800 spectrophotometer (Shimadzu, Japan) in the spectral wave range of 190–1100 nm. To carry out the analysis, working solutions of each of the drugs (3 series) were prepared by diluting with water for the injection to a concentration of semaglutide in a solution of 0.025 mg/ml.

Mass spectrophotometry (MALDI-TOF MS)

The confirmation of the semaglutide peptide presence in the domestic drug under study was carried out using a mass spectrophotometry. The mass spectra were recorded on an Axima Confidence time-

[12] U.S. Food and Drug Administration. ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin Guidance for Industry, 2021. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/andas-certain-highly-purified-synthetic-peptide-drug-products-refer-listed-drugs-rdna-origin

of-flight spectrometer (Shimadzu Biotech, Japan) in a high-resolution reflectron mode with a nitrogen laser (λ=337 nm). M/z scanning was carried out in the range from 500 to 5000 Da, using 2.5-dihydroxybenzoic acid (DHB) and sinapic acid (SA) as the matrix.

To prepare the target, the drug was adjusted to the concentration of 1 mg/ml with deionized water, then 20 µl of the resulting solution was mixed with 20 µl of the matrix solution (10 mg/ml in 50% acetonitrile/0.1% aqueous trifluoroacetic acid). The resulting mixtures were applied to a stainless-steel target and dried in air.

Relative molar ratio of amino acids

The relative molecular ratio of amino acids in the drugs was determined by HPLC. The analysis was carried out on a high-pressure liquid chromatograph equipped with an Agilent 1260 Infinity LC UV detector (Agilent Technologies, USA), using a stainless steel AccQ Tag Amino Acid Analysis Column (150×3.9 mm, 4 µm) at the wavelength of 254 nm.

Solutions of the drugs were hydrolyzed by adding a 6 M solution of hydrochloric acid containing 0.1% phenol and 1% thioglycolic acid. The hydrolysis process was carried out at the temperature of 110°C for 24 h. The derivatization of hydrolysates of the test solutions and a solution of standard amino acid samples was carried out using a derivatizing reagent by heating the solutions to 55°C for 10 min.

Size exclusion-high-performance liquid chromatography

The size exclusion chromatography method was used to determine the quantitative content of high molecular weight impurities in the preparations. The analysis was carried out on a Tosoh TSK-gel G 2000 SWXL column (7.8×300 mm, 5 µm) using an Agilent 1260 Infinity LC liquid chromatograph with UV detection (Agilent Technologies, USA).

Reversed-phase high-performance liquid chromatography

The reverse phase chromatography method was used to determine the quantitative content of semaglutide and related impurities in the test and reference drugs. The analysis was carried out using a liquid chromatograph with UV detection Prominence (Shimadzu, Japan), using a Jupiter 4u Proteo 90A column (4.6×250 mm, 4 µm; Phenomenex, USA).

Comparative studies of drugs biological activity *in vitro*

A comparative biological activity of the test and reference drugs was assessed *in vitro* on the CHO-K1/GLP-1R cell culture (GenScript, USA). This cell line has GLP-1 receptors, to which the active substance, semaglutide, binds. The cultivation of the cell line was carried out using the RPMI culture medium (PanEco, Russia) with the addition of a penicillin / streptomycin solution (1%) and fetal bovine serum (10%), under standard conditions (the temperature – 37±1°C, the CO_2 content – 5±1%), for 2 days. The results were assessed using the cAMP-GloTM Assay kit (Promega, USA) in accordance with the instructions.

Bioequivalence and comparability of safety, tolerability profile and immunogenicity

An open randomized parallel comparative study of the domestic drug Quincenta® (semaglutide 1.34 mg/ml, Promomed Rus LLC, Russia), produced on the basis of synthetic semaglutide (hereinafter referred to as the test drug), and the foreign drug Ozempic® (semaglutide 1, 34 mg/ml, Novo Nordisk A/S, Denmark), which contains a molecule obtained by biotechnological means (hereinafter referred to as the reference drug), was conducted. The test and reference drugs were comparable in composition.

Study design

This research was a single-center, open-label, randomized, parallel bioequivalence study of a single subcutaneous fasting dose of the test / reference drugs in healthy volunteers.

A flowchart of the study design is presented in Fig. 1.

Objects of study and eligibility criteria

A total of 54 healthy volunteers, male and female, aged 18 to 45 years (34.20±6.25 years) were included in the study. All participants signed an informed consent form and expressed their ability and willingness to comply with all requirements of the Study Protocol.

The main inclusion criteria were: a body weight > 50 kg; BMI 18.5–26 kg/m^2 inclusive; a verified diagnosis "healthy" according to standard clinical, laboratory and instrumental examination methods; negative results of tests for the use of alcohol, psychotropic and narcotic substances and willingness to stop drinking alcohol during the participation in the study. The participants had

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been warned to use reliable methods of contraception and to abstain from a sperm donation throughout the study and for 2 months after the end of the study.

The main criteria for non-inclusion included were: the presence of known allergies, drug intolerance, chronic diseases of various organ systems; mental illness; hypersensitivity to study drugs; history of semaglutide or other analogues of human GLP-1 use (for less than 6 months before screening), taking medications that have a pronounced effect on hemodynamics and/or a liver function for less than 2 months before screening; taking other medications, including herbal and homeopathic medications, vitamins and/or dietary supplements (biologically active supplements), for less than 4 weeks before screening; inability to perform subcutaneous injections; any history of difficulty with blood collection or any vasovagal seizures during blood collection; history of surgical interventions on the gastrointestinal tract (except appendectomy). The participants were not considered for inclusion in the study if they had the following diseases and conditions either: a history of medullary thyroid cancer, including a family history; a history of type 2 multiple endocrine neoplasia; a severe depression; suicidal thoughts or behavior, including a history; acute infectious diseases or ARVI symptoms for less than 4 weeks before screening.

The volunteers were excluded from the study if they had refused to participate in the clinical trial, if they were taking drugs for prohibited therapy and if they had been tested positive for the use of alcohol, psychotropic and/or narcotic substances, if there were gross violations of the requirements and procedures of the Study Protocol, if adverse events occurred, as well as if during the study, the volunteer had any diseases or conditions that made his further participation in the study impossible. The study physician may have decided to exclude a volunteer in the best interests of the volunteer.

Concomitant medications and exclusion criteria were assessed throughout the volunteer's participation in the study. The total duration of the study for each volunteer was no more than 35 days (including the screening period).

Randomization procedure

Each volunteer who had met all the inclusion criteria and had not met any of the non-inclusion criteria was assigned a randomization number in accordance with the randomization plan prepared for this study in the WinPepi 11.65 program (ETCETERA 3.26 module) using the random number generation method. The randomization number of the volunteer was entered by the research physician into the Register of Clinical Study Participants in Screening/Randomization. If a volunteer had left the study prematurely, their randomization number was not reused and the volunteer could not subsequently return to the study.

Study conditions and duration

The study was conducted from July 10 to October 2, 2023, at the research center of the Yaroslavl Region Clinical Hospital No. 3 (Yaroslavl, Russia).

Description of manipulations and methodology

Administration of drugs

The volunteers who had met the inclusion criteria and those who had not met the non-inclusion criteria were randomized into 2 groups in a 1:1 ratio. Group I (*n*=27) received Russian semaglutide (studied drug), group II (*n*=27) received reference drug. The reference / test drug was administered by the medical personnel in the morning on an empty stomach at a single subcutaneous dose of 0.5 mg in the abdomen.

The choice of doses for this study was based on the information provided in the current instructions for medical use of the reference drug and the articles devoted to the study of semaglutide preparations. In contrast to the starting dose of 0.25 mg, the dose of 0.5 mg was minimally therapeutic. The selection of this dose was based on the safety of use in healthy volunteers, since when using semaglutide in a dose of more than 0.5 mg, there was a risk of developing side effects from the gastrointestinal tract. In addition, there was experience with the use of semaglutide at a dose of 0.5 mg in healthy volunteers, which showed its good tolerability[13] [23–26]. The mode of administration was consistent with the method of use of the reference drug[14] and the planned method of use of the test drug in clinical practice[15].

To administer the test / reference drugs, the volunteers were admitted to the hospital the

[13] NCT02060266 Trial Investigating the Absorption, Metabolism and Excretion After a Single Subcutaneous Dose of [3H]-Semaglutide in Healthy Male Subjects. Available from: https://clinicaltrials.gov/study/NCT02060266

[14] State register of medicines of the Russian Federation. Ozempic®. Available from: https://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=9859f6af-8ad6-4704-9d20-1bcc87b7dafc

[15] Ibid.

evening before and at least 10 hours before the drug administration. During the period of their stay in the hospital, the volunteers complied with the rules of their stay. The duration of hospitalization was no more than 3 days. Throughout the study, from the start of the screening examination until the completion of the final examination, the volunteers abstained from eating for at least 10 h before the administration of the test / reference drug.

Preparation and sampling

After randomization and before the baseline blood samples had been collected to assess pharmacokinetic and immunogenicity parameters, the volunteers were placed with a heparinized cubital catheter, which was removed after blood sampling at 12 h (day 1). After the catheter removal, the blood was collected from volunteers by venipuncture.

The blood samples were taken to determine pharmacokinetic parameters at the following time points: 1, 0.5, 0 h (day 1) before the administration of the test / reference drug and then after 2, 8, 12 (day 1), 24 (day 2) , 36 (day 2), 48 (day 3), 72 (day 4), 96 (day 5), 144 (day 7), 192 (day 9), 240 (day 11), 360 (day 16) and 480 h (day 21) after the administration of the test / reference drug.

The blood samples were taken to study immunogenicity no more than 15 min before the administration of the test / reference drug (the initial (0) sample) and 480 h (day 21) after their administration. The blood samples for the immunogenicity parameters analysis were collected separately from the blood samples for the evaluation of pharmacokinetic parameters.

Therefore, the study collected 16 blood samples per volunteer (6 ml each) for pharmacokinetic studies and 2 blood samples per volunteer (6 ml each) for immunogenicity studies.

At screening, at the stage of hospitalization (morning before randomization), upon discharge from the hospital and on Days 11 and 21 of the study, the blood samples were taken for clinical, biochemical tests and/or determination of blood glucose levels using a glucometer, the total volume of which in each specified per day was no more than 15 ml.

The blood samples were collected in test tubes to obtain serum with a coagulation activator. After the clot formation, the tubes were centrifuged, the resulting serum was carefully transferred into pre-labeled cryovials, dividing the serum into three 500 µl aliquots: two for the main analysis (aliquots A and B), the third for repeat analyzes (aliquot C). The serum samples were frozen immediately after the receipt, transferred into cryovials and stored at the temperature not exceeding –70°C.

Analytical method

Pharmacokinetics was assessed by the concentration of semaglutide in the blood plasma, and antibodies to it – in the blood serum of each volunteer after a subcutaneous administration of the test / reference drug. In order to comprehensively characterize the pharmacokinetic properties of the test / reference drug, the study included blood sampling to cover the entire pharmacokinetic profile, including the elimination phase. The quantitative determination of semaglutide in the serum samples by ELISA was carried out using a microplate photometer, HiPo MPP-96 (Biosan, Latvia). Semaglutide concentrations were calculated using GraphPad Prism 8.4.3 software. The determination of semaglutide in the serum samples was carried out using a previously validated enzyme-linked immunosorbent assay (ELISA) method using commercially available “KRIBIOLISA™ Semaglutide (Ozempic™) ELISA kits”; antibodies to semaglutide using the KRIBIOLISATM Anti-Liraglutide ELISA kit. The analytical range was 50–4000 pg/ml. The preparation of calibration samples from the kit was carried out by diluting the standard sample. The analytical range was selected in accordance with the instructions for the kit.

Safety and tolerability assessment

The conclusion about the bioequivalence of the compared drugs was made using an approach based on the assessment of 90% confidence intervals (CI) for the ratios of the geometric mean values of the parameters C_{max}, $AUC_{(0–t)}$ of semaglutide in the original units of measurement. The drugs were considered bioequivalent if the boundaries of the estimated CI for C_{max}, $AUC_{(0–t)}$ of semaglutide were within the range of 80.00–125.00%.

In the course of the study, a clinical observation of volunteers was carried out with the assessment of physical examination data, including a survey about the volunteers’ complaints, basic vital signs (blood pressure, heart rate, respiratory rate, body temperature), 12-lead ECG, laboratory parameters of clinical, biochemical blood tests, a general urine analysis, determining glucose levels using a glucometer.

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The safety and tolerability criteria included the frequency and severity of adverse events (AEs) as measured by abnormal laboratory tests, physical examination, vital signs, and ECG; the number of cases of early termination of participation in the study due to the development of AEs and/or serious adverse events (SAEs), including those related to the test / reference drug; the frequency of volunteers with detected antibodies to semaglutide; the assessment of the overall tolerability of the test / reference drug on a Likert scale. The safety and tolerability of liraglutide were assessed for all the volunteers. The identification of AEs occurred from the moment of administration of the study drugs until the end of the volunteers' participation in the study.

The pharmacokinetic parameters[16] were as follows: a maximum concentration of the substance in the blood serum (C_{max}); the time to reach C_{max} (T_{max}); area under the concentration-time curve from the moment of drug administration to the last detectable concentration at time point t ($AUC_{(0–t)}$); the area under the pharmacokinetic curve from the zero time to infinity ($AUC_{(0–\infty)}$); the ratio of the area under the concentration-time curve from the moment of the drug administration to the last detectable concentration at the time point to the area under the pharmacokinetic curve, starting from the zero time value to infinity ($AUC_{(0–t)}$ / $AUC_{(0–\infty)}$); the terminal elimination rate constant (K_{el}); half-life ($T_{1/2}$); the volume of distribution (Vd); the residual (extrapolated) area under the curve, determined by the formula: $V_d = AUC_{(0–\infty)} – AUC_{(0–t)}$ / $AUC_{(0–\infty)}$ ($AUC_{(t–\infty)}$).

Ethical approval

The study complied with the ethical principles set forth in the most recent revision of the Declaration of Helsinki, the rules of Good Clinical Practice of the Eurasian Economic Union, the Rules of Good Clinical Practice of the International Council for Harmonization (ICH E6 GCP R2), as well as other legislation applicable to this study. The clinical trial protocol was approved by the Ministry of Health of Russia (Extract from Protocol No. 347 dated July 5, 2023) and the Ethics Council of the Ministry of Health of Russia (Extract from Protocol No. 337 of the meeting dated June 27, 2023), as well as the Local Independent Ethics Committee at the research center of the state budgetary healthcare institution Yaroslavl region "Clinical Hospital No. 3" (Extract from Protocol No. 186 dated July 7, 2023).

[16] If necessary, additional pharmacokinetic calculations could be performed.

Statistical analysis

To calculate the number of participants, the data on the coefficients of intra-individual variability (CV_{inter}) of the parameters C_{max}, AUC_{0-t} of semaglutide, presented in the Assessment Report Ozempic[17], were used. According to the results of the study, after a single subcutaneous administration to the healthy volunteers, CV_{inter} semaglutide for the main pharmacokinetic parameters C_{max} and AUC did not exceed 24%[18]. The calculation of the required number was carried out using the PASS 11.4.12 program.

For the standard two-parallel group design conditiosn, assuming a 90% CI of 80.00–125.00%, CV_{inter}=24%, α=0.05, the power of 80%, the group ratio of 0.95, it was necessary to include at least 50 healthy volunteers who would have completed the study and would be included in the statistical analysis. Taking into account the possible dropout during the study, the randomization of 54 healthy volunteers (27 volunteers in each study group) was planned.

For pharmacokinetic calculations, the actual time of blood sampling was used. The calculation of pharmacokinetic parameters, statistical analysis of safety indicators and presentation of results were carried out using statistical packages (StatSoft Statistica version 13.3 and the R Project program (version 3.5.1, GPL-2/GPL-3 license) with the *bear* extension, version 2.8.3-2. No interim analysis was performed.

For all pharmacokinetic parameters, the following statistical parameters were calculated: arithmetic mean, geometric mean, standard deviation of the mean, coefficient of variation, median, minimum and maximum values, and variability.

A statistical analysis was carried out based on the assumption of a log-normal distribution of $AUC_{(0–t)}$, $AUC_{(0–\infty)}$, C_{max} and a normal distribution of other pharmacokinetic parameters, with the exception of T_{max}. After the log transformation, these scores were analyzed using the analysis of variance (ANOVA). The statistical analysis of the study was carried out at a standard significance level of α=0.05.

[17] Assessment report Ozempic EMA/21773/2022. 11 November 2021. Committee for Medicinal Products for Human Use (CHMP). Available from: https://www.ema.europa.eu/en/documents/variation-report/ozempic-h-c-004174-x-0021-epar-assessment-report-variation_en.pdf

[18] Assessment report Ozempic, semaglutide, Procedure No. EMEA/H/C/004174/0000, 14 December 2017, Committee for Medicinal Products for Human Use (CHMP). Available from: https://www.ema.europa.eu/en/documents/assessment-report/ozempic-epar-public-assessment-report_en.pdf

For the randomized parallel groups comparative study, the ANOVA statistical model included the following factor contributing to the observed variation in the data: a drug. The analysis of variance was used to test a hypothesis about the statistical significance of the contribution of the specified factor to the observed variability.

The conclusion about the bioequivalence of the compared drugs was made using an approach based on the assessment of 90% confidence intervals for the ratios of the geometric mean values of the semaglutide parameters C_{max}, $AUC_{(0-t)}$ in the original units of measurement.

The descriptive statistics is presented for all safety and tolerability indicators collected during the study. To analyze frequencies, the proportions were compared using a two-sided version of the Fisher's exact test or the χ^2 test. To compare quantitative continuous indicators, the Student's *t*-test (in the case of a normal distribution) or the Mann-Whitney *U*-test (in the case of a non-normal distribution) were used. The differences were considered statistically significant at $p < 0.05$.

RESULTS

Physicochemical properties and biological activity.

Spectrophotometry in the ultraviolet area (200–400 nm)

The results of the spectrophotometric determination of the test / reference drugs are presented in Table 1 and Fig. 1.

Based on the data obtained, it can be concluded that the absorption spectra of the foreign reference drug Ozempic® and the domestic test drug Quincenta® corresponded to each other in terms of the positions of absorption maxima and minima in the region of 200–400 nm, which, in turn, indicates the identity of the studied drugs.

Mass spectrophotometry (MALDI-TOF MS)

The results of the MALDI-TOF MS analysis confirm the presence of kDa[19], in the study domestic peptide drug with a mass of 4.1, corresponding to the mass of the reference drug semaglutide (Fig. 3).

Relative molar ratio of amino acids

In the course of the study, comparable molar ratios of amino acids were obtained in the samples of the study drugs Ozempic® and Quincenta® (Table 2).

[19] Assessment report Ozempic EMA/21773/2022, 2021.

Size exclusion-high-performance liquid chromatography

The results obtained in the study demonstrated the comparable retention time of high molecular weight protein impurities. Moreover, at the time of the analysis, the domestic drug of semaglutide contained on average 1.6 times less high-molecular impurities than the foreign drug.

Typical chromatograms obtained when determining the content of high-molecular impurities in the test and reference preparations are presented in Fig. 4 and 5. The content analysis results of the of high-molecular impurities are presented in Fig. 6.

Reversed-phase high-performance liquid chromatography

The results of determining the quantitative content of semaglutide and related impurities in the test and the reference drugs are presented in Table 3.

The data obtained demonstrate that the quantitative content of related impurities and the active substance semaglutide in the samples of the studied drugs are comparable. It should be also noted that at the time of the analysis, the content of impurities in the domestic drug was 2.5 times lower compared to the foreign drug. At the same time, the foreign drug contained on average 5.6 times more hydrophilic impurities, and almost twice as many hydrophobic impurities in comparison with the domestic drug.

Comparative studies of biological activity

The obtained data from a comparative analysis of the biological activity of the test and reference drugs *in vitro* are presented in Table 4.

The results demonstrate the presence of a comparable biological activity of the study domestic drug with a chemically synthesized active substance, to a foreign drug: the range of activity of the studied drugs was in the range of 80–120% in relation to the standard sample of semaglutide.

Bioequivalence and comparability of safety and tolerability profile

Population

All volunteers were included in the population to assess safety, tolerability and immunogenicity, for the pharmacokinetic analysis and bioequivalence assessment. The average age of the volunteers in the population was 34.20±6.25 years, the average body weight was 70.41±9.42 kg, the average height was 172.61±7.38 cm, the average BMI was 23.51±1.

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39 kg/m^2. The demographic and baseline anthropometric characteristics of the volunteers did not differ between the groups (Tables 5 and 6).

Pharmacokinetics and bioequivalence

After the use of the Russian and reference drugs, their average values of the main and additional pharmacokinetic parameters are presented in Table 7.

As follows from the presented data, the average values of both main and additional pharmacokinetic parameters obtained after the use of the test and reference drugs were comparable to each other. It can be concluded that the pharmacokinetic profiles of the test and reference drugs are similar.

The results of assessing the ratio of geometric mean pharmacokinetic parameters AUC_{0-t}, C_{max} of the studied semaglutide drugs and 90% CIs for these ratios demonstrate the equivalence of the main pharmacokinetic parameters (AUC_{0-t}, C_{max}) (Table 8). According to the results of the statistical analysis, the obtained 90% CI for the ratio of C_{max}, AUC_{0-t} values of the studied Russian and foreign drugs were 90.89–109.15 and 91.66–111.27%, respectively. The coefficients of the intra-individual variation, calculated on the basis of the analysis of variance, were 20.29% for the C_{max} value and 21.50% for the AUC_{0-t} value.

Thus, the intervals obtained during the study were fully consistent with the equivalence limit of 80.00–125.00% for C_{max} and AUC_{0-t}, clearly demonstrating the bioequivalence of the study drug.

Safety

All the volunteers completed the study entirely in accordance with the approved study protocol. During the study, no AEs were recorded. In 100% (54) cases, the volunteers' tolerability was rated as "good." No SAEs were identified in the volunteers during the study or after its completion. No deaths were observed. There were no cases of pregnancy of the sexual partner of a study participant during the study or after its completion. No abnormalities were found in the results of clinical and biochemical blood tests, in the determination of blood glucose levels, general urinalysis, vital signs, physical examination and ECG.

Immunogenicity assessment

According to the results of the immunogenicity parameters analysis, no antibodies to semaglutide were detected in the blood serum of the volunteers, which indicated the absence of the drug's immunogenicity. No unexpected results were notified during the study.

Thus, the study drug semaglutide and the reference drug had a similar safety profile. At the same time, no cases of immunogenicity were observed for the Russian drug, which confirms a high safety profile and reduced the risk of ineffective therapy.

DISCUSSION

The efficacy and safety of semaglutide compared with placebo or active reference drugs (sitagliptin, exenatide, insulin glargine, dulaglutide, liraglutide) in adult patients with T2DM were consistently studied in a series of phase III clinical trials combined by a large SUSTAIN research program, which included a total of more than 10 thousand patients [27–33]. Semaglutide therapy was significantly better than placebo and comparator and reduced HbA1c by 1.2–1.5% when using a dose of 0.5 mg and by 1.5–1.8% when using a dose of 1.0 mg, compared with baseline values, and resulted in achieving target values of HbA1c <7.0% in 78.7% of patients, and HbA1c <6.5% in 66.7% of patients [27–32]. It is worth noting that 74.3% of the subjects receiving semaglutide achieved the composite endpoint of HbA1c <7.0% without severe or symptomatic hypoglycemia or weight gain. In contrast, 65.7% of patients receiving semaglutide achieved weight loss of 5% or more, and 26.7% – 10% or more, which was significantly better compared to the placebo group and the comparison group [27, 31, 32].

A retrospective observational study also showed that at a dose of 0.5–1.0 mg after 32 weeks of treatment, semaglutide reduced HbA1c by an average of 1.38%, weight by 6.03 kg, and significantly improved BP and lipid levels, reduced the number of glucose-lowering and lipid-lowering drugs taken, leading to better a patient satisfaction with the results of diabetes control and eating behavior [34].

Additionally, semaglutide, compared with lixisenatide, exenatide, liraglutide, albiglutide, and dulaglutide, demonstrated the highest rates of BG improvement and weight loss [35]. A similar benefit was also shown in two meta-analyses comparing the results of semaglutide studies with the study results of sodium-glucose cotransporter-2 inhibitors (SGLT-2) empagliflozin, canagliflozin and dapagliflozin in people with inadequate control of type 2 diabetes with one or two oral antidiabetic agents [36] or metformin monotherapy [37] found out that semaglutide was superior to iSGLT in reducing HbA1c levels and weight loss.

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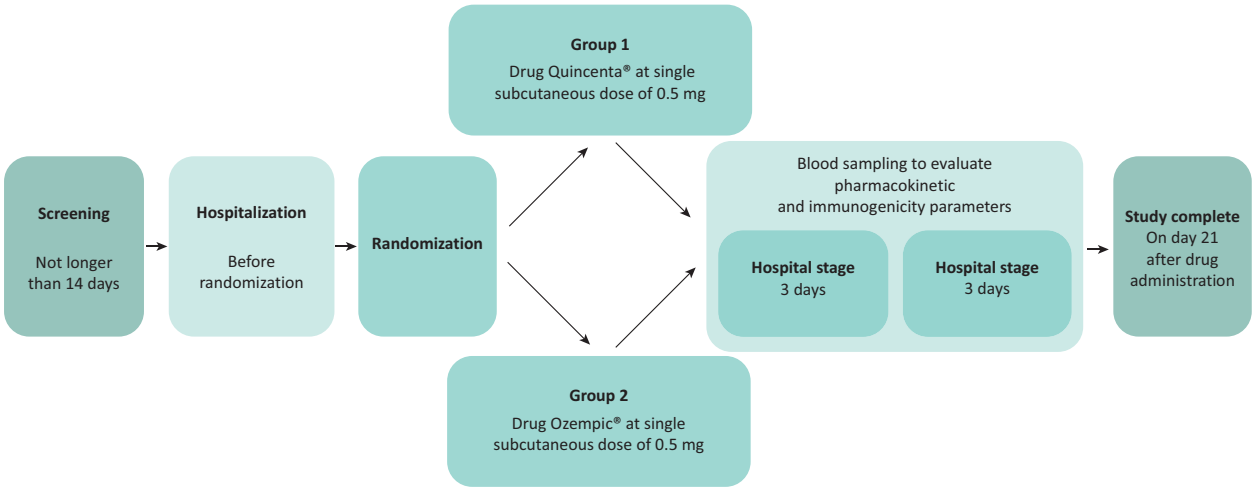

Figure 1 – Study design

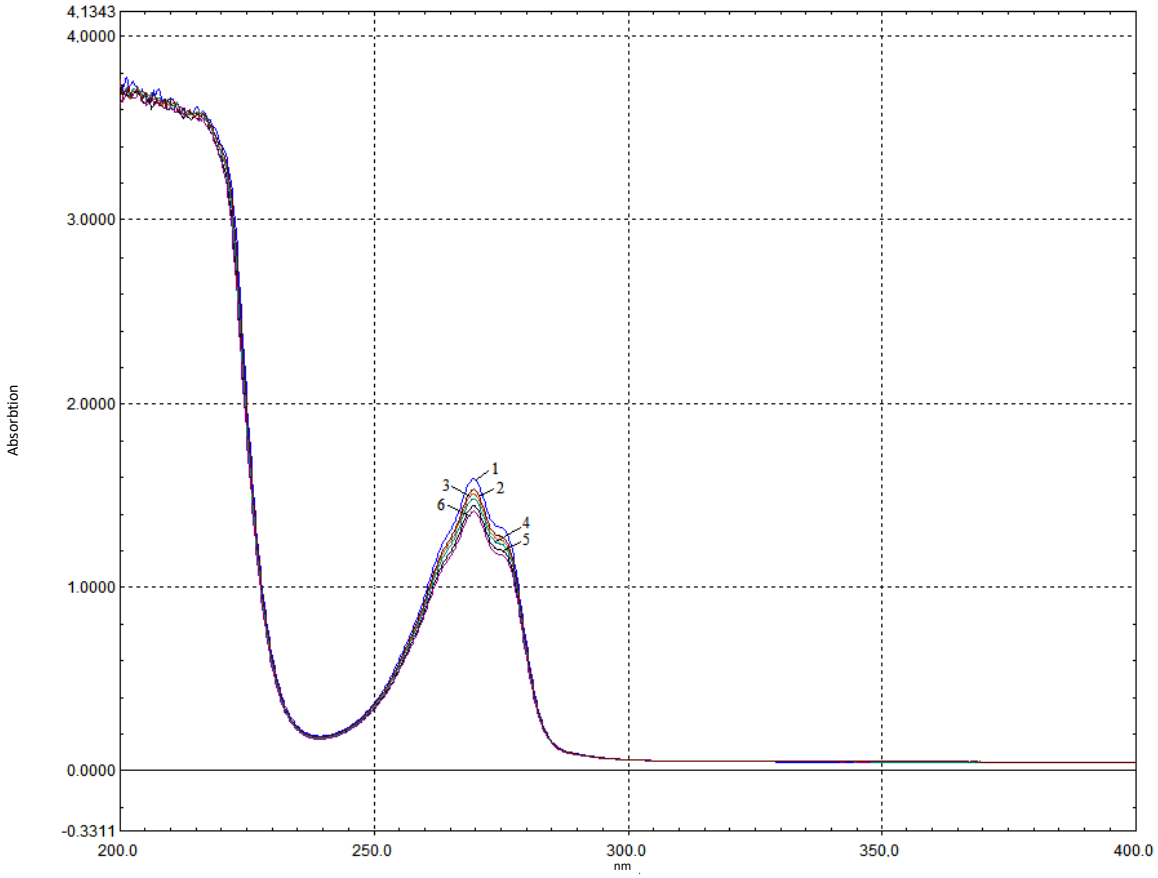

Figure 2 – Absorption spectra overlay of test and reference drugs

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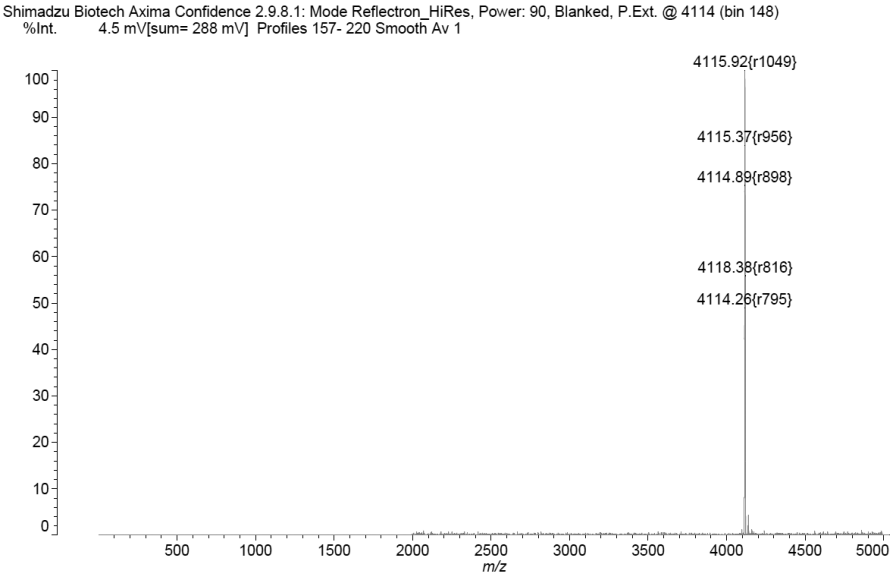
Series 1



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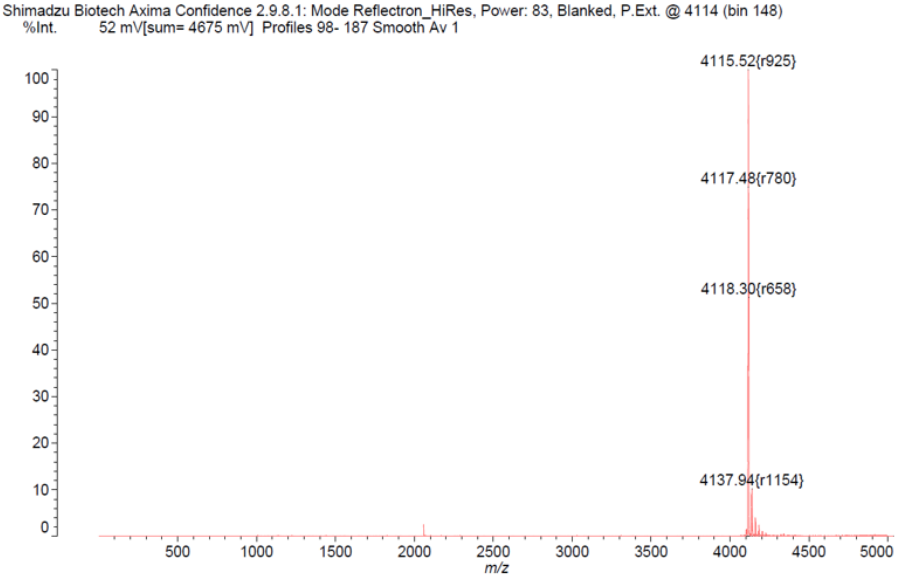

Series 3

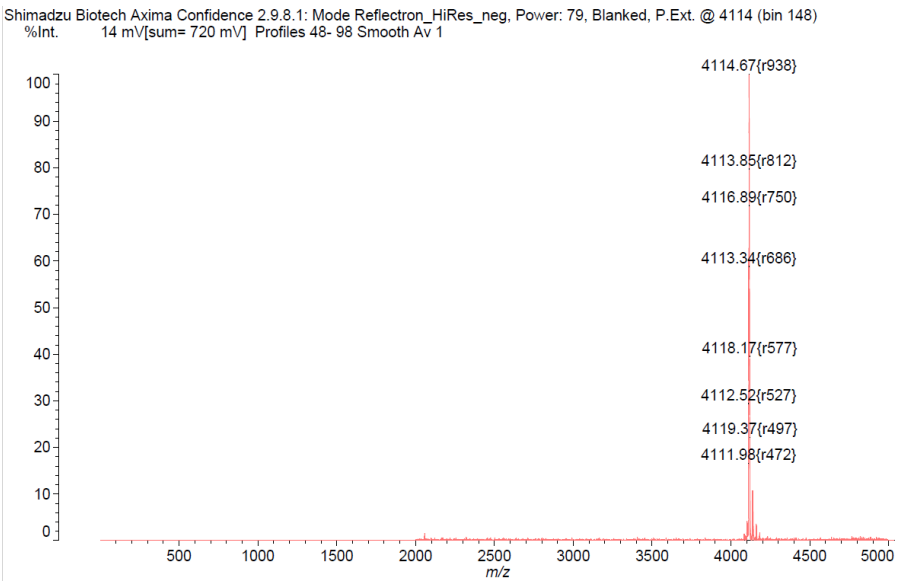

Figure 3 – MALDI-TOF MS mass spectra obtained from study of three series of test drug Quincenta®

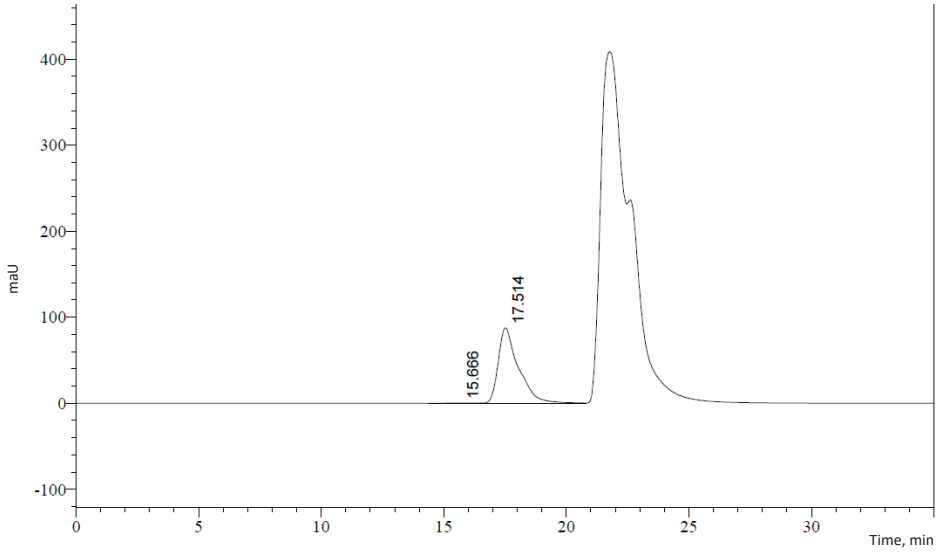

Figure 4 – Chromatogram obtained by determining content of high molecular weight proteins in reference drug

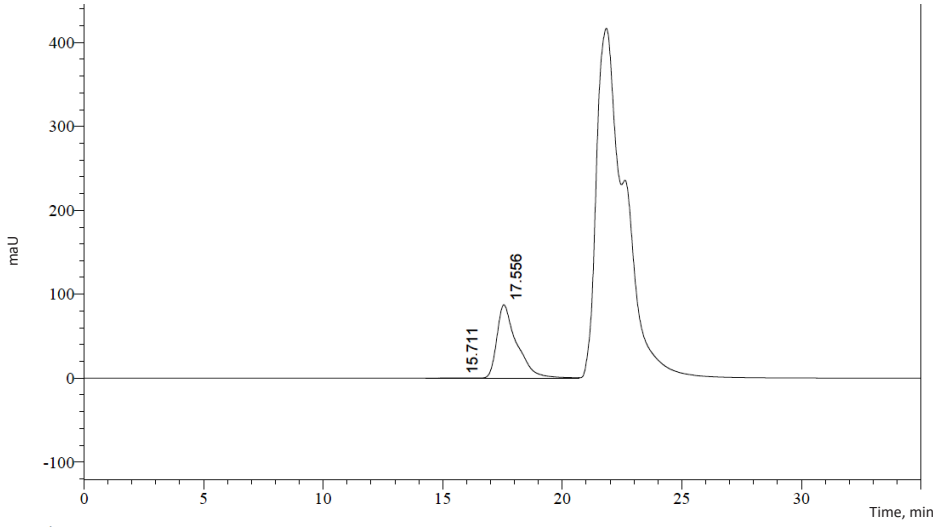

Figure 5 – Chromatogram obtained when determining the content of high molecular weight proteins in test drug

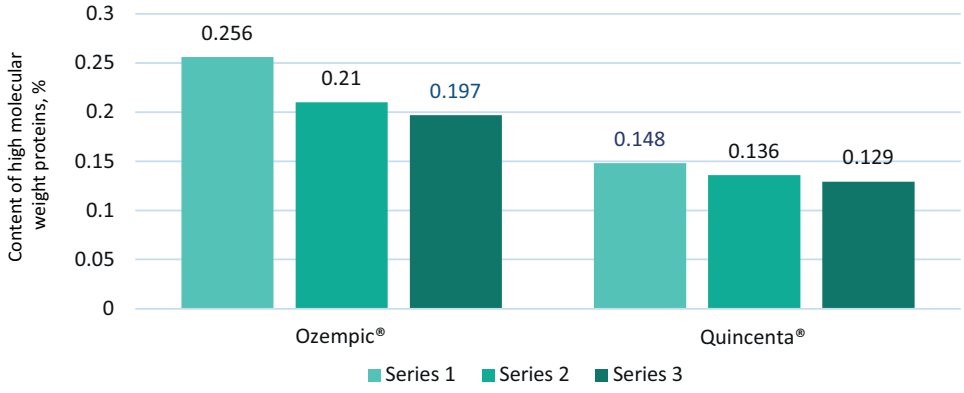

Figure 6 – Results of content analysis of high-molecular impurities in test and reference drugs

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Table 1 – Spectrophotometric analysis results of test / reference drugs in UV area

No	Object of analysis	Series	λ_{max}	λ_{min}
1	Reference drug	1	269.6	239.4
2		2	269.7	239.2
3		3	269.4	239.5
4	Test drug	1	269.6	239.7
5		2	269.3	239.2
6		3	269.1	239.6

Table 2 – Results of determining relative molar ratio of amino acids in samples of test and reference drugs

Amino acid name	Relative molar ratio of amino acids					
	Ozempic®			Quincenta®		
	Series No. 1	Series No. 2	Series No. 3	Series No. 1	Series No. 2	Series No. 3
Asp	1.1	0.9	1.2	0.9	1.0	1.1
Glu	5.0	5.3	5.4	5.2	5.0	4.9
His	1.1	0.9	1.2	1.0	1.1	0.9
Thr	1,9	2.0	2.3	2.2	1.8	2.1
Aeea	1,9	1.7	1,9	2.0	1.8	1.9
Tyr	1.0	0.9	1.1	1.1	0.8	1
Lys	1.0	1.1	1.0	1.2	0.9	1.1
Leu	2.1	1.8	2.1	1.9	2.2	2.2
Ser	2.7	2.5	2.9	3.1	2.8	2.6
Gly	4.1	4.4	3.9	4.0	4.2	3.8
Arg	2.0	1.7	2.2	1.8	2.1	1.9
Ala	3.0	2.7	2.9	2.8	2.9	3.3
Aib	1.0	0.8	1.0	0.9	1.1	1.1
Val	2.0	2.3	1.8	1.9	2.1	2
Ile	1.0	1.2	0.9	0.9	1.0	1.2
Phe	2.0	1.7	2.1	2.2	1.9	2
Trp	1.0	1.0	0.7	1.0	0.8	1.1

Table 3 – Results of quantitative determination of related impurities using reverse-phase high-performance liquid chromatography in the test and reference drugs

Index	Ozempic®			Quincenta®		
Series	1	2	3	1	2	3
Quantitative determination of semaglutide, mg/ml	1.35	1.34	1.33	1.37	1.39	1.35
Amount of impurities, %	2.683	2.312	2.751	0.944	1.124	1.036
Hydrophilic impurities, %	0.821	0.933	1.06	0.135	0.215	0.148
Hydrophobic impurities 1, %	1.737	1.308	1.62	0.716	0.834	0.825
Hydrophobic impurities 2, %	0.125	0.071	0.049	0.093	0.075	0.063

Table 4 – Comparative study results of biological activity of domestic and foreign drugs

Drug	Series No	EC_{50}	Activity % of RS
Ozempic®	1	1.379	93.62
	2	1.725	117.11
	3	1.232	83.64
Quincenta®	1	1.317	89.41
	2	1,731	117.52
	3	1.559	105.84
Semaglutide RS	–	1.473	–

Note: RS – reference standard.

Table 5 – Descriptive characteristics of volunteers' demographic and anthropometric data

Index	*n*	*p*	Quincenta®			Ozempic®		
			Mean value	CI –95.000%	CI 95.000%	Mean	CI –95.000%	CI 95.000%
Age, completed years	27	0.085940	35.6667	33.1337	38.1997	32.7404	30.4273	35.0541
Body weight, kg	27	0.119176	72.2222	68.3608	76.0837	68.6037	65.0962	72.1112
Height, cm	27	0.310119	173.7407	170.8119	176.6695	171.4815	168.5841	174.3788
BMI, kg^2/m	27	0.161126	23.7952	23.2588	24.3316	23.2274	22.6794	23.7754

Note: CI – confidence interval; BMI – body mass index.

Table 6 – Analysis of gender distribution

Gender	Groups comparison Pearson X^2 test	Quincenta®		Ozempic®	
		Frequency	Percentage	Frequency	Percentage
Male	0.5859	14	51.85185	12	44.44444
Female	0.5859	13	48.14815	15	55.55556

Table 7 – Value of calculated confidence intervals for calculated pharmacokinetic parameters

Parameter	Average ratio	90% CI		Valid range, %
		Low level	High level	
AUC_{0-t}	1.00	91.66	111.27	80–125
C_{max}	0.99	90.89	109.15	80–125

Another important advantage of semaglutide is a low risk of hypoglycemia, but the risk increases when it is combined with sulfonylureas and/or insulin [38].

But perhaps the most valuable feature came from the SUSTAIN 6 trial, which demonstrated that subcutaneous semaglutide, compared with placebo, was associated with a significant reduction in the incidence of CVD-related deaths, nonfatal myocardial infarctions, or nonfatal cerebrovascular accidents (p <0.001) (combined RR=0.74; 95% CI 0.58 to 0.95) and also with fewer cases of nephropathy [39]. A focused study (FLOW; NCT03819153) is currently investigating the effect of subcutaneous semaglutide on renal outcomes in people with T2DM and CKD.

A retrospective analysis also showed [40] that when taking semaglutide, there was a reduction in the relative and absolute risk of serious adverse cardiovascular events compared with comparators. Although the absolute risk reduction was small, there was a trend (p=0.06) for the greatest relative risk reduction in those with the lowest cardiovascular risk. This phenomenon may be explained by the fact that more advanced stages of diabetes may be more resistant to the beneficial effects of arGLP-1 on CVD outcomes.

Another pool of studies examined the effectiveness and safety of semaglutide in weight loss in overweight and obese patients without diabetes during the STEP clinical trial program. Thus, in the STEP 1,3,4, and 8 studies, semaglutide at a dose of 2.4 mg once a week led to an average weight loss of 14.9–17.4% from baseline by week 68, with 69–79% of participants achieved ≥10% weight loss, and 51–64% achieved ≥15% weight loss [41–44]. In the STEP 5 study, the mean weight loss with semaglutide 2.4 mg at week 104 was 15.2% of baseline compared with 2.6% with placebo [45]. An improvement in the main cardiometabolic risk factors was also found: a decrease in waist circumference, a decrease in blood pressure, normalization of lipid levels and C-reactive protein, as well as an improvement in physical functions and quality of life with a good safety profile of semaglutide [41–43, 45, 46].

Bandyopadhyay S. et al. [47] conducted a systematic review and meta-analysis to evaluate the effectiveness and safety of semaglutide therapy in patients with non-alcoholic fatty liver disease and non-alcoholic steatohepatitis and found a significant decrease in the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), a significant decrease in liver lipid content, improvement in its elasticity, HbA1c and lipid profile indicators.

The identification of structurally related peptide impurities and their characterization is a critical challenge in the pharmaceutical development. These impurities may result from during the production degradation or storage and may affect the efficacy and safety of

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the finished product [48]. Semaglutide impurities can include peptides of imperfect structure, resulting from the insertion of an undesired amino acid or deletion (the absence of one or more amino acid residues), oxidation or racemization of amino acids [49].

There are methods for obtaining semaglutide by solid-phase synthesis that can reduce the formation of racemic impurities, simplify the purification of the target product, its purity and yield, and also reduce costs [50, 51].

The biological activity of peptide molecules directly correlates with their atomic size arrangement, while a configuration inversion at a particular peptide chiral center can cause a local spatial redistribution of critical functional groups [52]. That is why, when developing a method for producing semaglutide by chemical synthesis, a special attention is paid to controlling the racemization of amino acids.

During the analysis of literature data, it was revealed that in the foreign drug Ozempic® (Novo Nordisk A/S, Denmark), containing semaglutide of biotechnological origin, the formation of three isomers containing in its structure amino acids in the D-conformation D-His1, D-Ser8, D-Asp9, is possible. As it is known, the isomerization of even one amino acid in a certain peptide chain can have a significant impact on the overall conformation of the peptide molecule and affect its biological activity [53, 54]. Based on this fact, when developing technologies for obtaining the substance semaglutide, the main task for the specialists of Promomed Rus LLC was to minimize the amount of potential impurities.

At the study time of domestic and foreign drugs, it was found that Quincenta® (Promomed Rus LLC, Russia), which contains semaglutide of a synthetic origin, contains 2.5 times less impurities compared to the foreign drug.

Based on the results obtained in the course of comparative studies, it can be concluded that today it has been possible to achieve not only the production of a substance of the appropriate pharmacopoeial quality, but also to minimize the formation of by-products, thereby reducing the total amount of impurities in the finished medicinal product. It is important to note that peptide preparations obtained by microbiological synthesis consist of amino acids in the L-conformation, which corresponds to the life processes of natural microorganisms, while in the process of chemical synthesis D-isomers can be formed, which, as stated above, can critically change the drug quality and lead to unexpected pharmacological effects. Thus, control of spontaneous isomerization processes and obtaining a product of the required stereochemical purity are critical to the proper quality of the drug. In this regard, it is worth noting that at the moment, the Russian drug Quincenta® is the only domestic drug based on semaglutide, which uses its own technology for the production and purification of API, which ensures a high level of quality control of the resulting substance, the exclusion of undesirable impurities and isomerization and, consequently, contributes to achieving high efficiency and safety of the therapy.

Injection therapy for diabetes is the most effective treatment method. However, it is known that, despite its effectiveness, this method of therapy is characterized by a low level of adherence, and one of the ways to overcome this problem is the use of simple and convenient syringe pens [55]. That is why, for Russian drugs based on arGLP-1, in particular, the drug Quicenta®, a partner of the Promomed Group of Companies, the Medsintez Plant LLC, developed a special syringe pen that ensures a dosing accuracy, the administration ease of the drug, and also the consumption of the drug without unnecessary losses at the stage of selecting an individual dose during a long-term use. This multi-dose syringe pen has a unique advantage, as it allows a patient to fully use the active substance without leaving any residue from the cartridge.

According to the instructions for use of the drug, if after the injection the dose selector stops before the zero mark aligns with the pointer, it means that the patient, due to the characteristics of titration, did not receive the required dose of the drug. In this situation, the dose selector indicates the number of units that must be administered before the full dose of the drug is delivered from the new multi-dose pen. A similar design is not provided for in any current arGLP-1 dosage form registered on the domestic market. It is also worth noting that the above syringe pen is compatible with needles from any manufacturer, which is important from the point of view of a possible reduction in the availability of foreign-made needles. Thus, we can say that the design of the developed syringe pen for Russian drugs based on arGLP-1 from Promomed Rus LLC, in particular, the drug Quincenta®, not only helps to increase patient adherence to treatment, but also provides pharmacoeconomic advantages therapy, excluding an irrational disposal of unused medicine.

CONCLUSION

As a result of the studies, a sufficient amount of data was collected confirming the similarity of the physicochemical and biological properties of the drug with the chemically synthesized active substance semaglutide Quincenta® (a solution for a subcutaneous administration 1.34 mg/ml, Promomed Rus LLC, Russia) with the reference drug Ozempic® (a solution for a subcutaneous administration 1.34 mg/ml, Novo Nordisk A/S, Denmark). Based on this, it can be concluded that the quality, safety and effectiveness of the Russian drug with a synthetic analogue of the active substance are similar, and in some respects even exceed the reference drug.

Reducing the socioeconomic burden of diabetes and obesity is one of the most important tasks of the Russian healthcare system. Semaglutide may be the drug of choice for patients with diabetes and obesity, in particular, in combination with CVD, due to its high effectiveness in controlling glycemic levels and weight loss, restoring metabolic health parameters, and a proven protective effect in reducing the risks of cardiovascular events. The entry of Russian drugs – arGLP-1 into the market is a major step towards providing patients with the necessary therapy, especially in the face of a shortage of foreign predecessors. An open-label, randomized cross-over comparative study of bioequivalence, safety, tolerability and immunogenicity in healthy volunteers confirmed the equivalence of the test drug Quincenta® and the reference drug Ozempic®, and also demonstrated its high safety profile, tolerability and lack of immunogenicity. Based on the data obtained, the drug Quincenta® was registered in the Russian Federation.

The use of our own technology of chemical synthesis and purification of the resulting substance in the production of the drug determines the required stereochemical purity of the product, a reduction in the level of impurities, predictable properties and a low risk of adverse immune reactions. It is advisable to conduct further clinical studies to assess the effectiveness and safety of therapy for patients with diabetes mellitus and obesity or overweight, as well as to identify potential new possibilities for arGLP-1 therapy, including the Russian analogue of semaglutide.

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The sponsor had no influence on the selection of material for publication, analysis or interpretation of data.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

A.S. Ametov, L.A. Balykova – development of the clinical trial concept, analysis and description of the results, text correction; I.E. Shokhin – organization and conduct of physical and chemical research, interpretation of results; E.A. Rogozhina – organization and conduct of studies of physicochemical and biological properties, discussion of the design and results of the study; T.G. Bodrova – analysis and selection of literary sources, writing the text of the article, organizing and conducting physical and chemical studies, interpreting the results; M.E. Nevretdinova – analysis and selection of literary sources, interpretation of results, writing the text of the article; P.A. Bely – implementation of the research design, processing of research data; K.Ya. Zaslavskaya, V.S. Scherbakova – development of the design and concept of the study, writing the text of the article; D.V. Kurkin – analysis and description of the results; K.N. Koryanova – analysis and description of results, search and analysis of literary sources; E.S. Mishchenko – analysis and description of the results; E.Yu. Kesova, E.D. Kozlov, E.S. Samoshkina, D.N. Andreev, Yu.G. Kazaishvili, S.M. Noskov – development of the design and concept of a clinical trial. All authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work.

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