PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

AVALIAÇÃO DA BIOEQUIVALÊNCIA EM VOLUNTÁRIOS SAUDÁVEIS DE TABLETS ORODISPERSÍVEIS COM OLANZAPINA

ASSESSMENT OF OLANZAPINE ORODISPERSIBLE TABLETS BIOEQUIVALENCE IN HEALTHY VOLUNTEERS

ОЦЕНКА БИОЭКВИВАЛЕНТНОСТИ ДИСПЕРГИРУЕМЫХ ТАБЛЕТИРОВАННЫХ ФОРМ ОЛАНЗАПИНА У ЗДОРОВЫХ ДОБРОВОЛЬЦЕВ

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RESUMO

A forma dispersa de comprimidos (ODT) dos medicamentos é conveniente para os pacientes; a eficácia de seu uso aumenta significativamente com o tratamento de não conformidade de transtornos mentais. Após o vencimento da patente do medicamento original, a produção de genéricos com o mesmo ingrediente farmacologicamente ativo geralmente começa. Devido a diferenças nas cargas e nas técnicas de fabricação, mesmo cópias de alta qualidade do produto original podem não ser equivalentes. Portanto, o objetivo deste estudo foi avaliar a bioequivalência da forma ODT do medicamento original e genérico com 10 mg de olanzapina. Depois de consumir o medicamento original e o genérico, em dois estágios, com o estômago vazio de voluntários saudáveis, foram coletados amostras de sangue venoso em tubos a vácuo com heparina. O período entre as doses dos medicamentos foi de 21 dias. A extração (LLE) da olanzapina foi realizada com uma mistura de n-hexano:isopropanol (3:1) com pH 8,0, e analisada por cromatografia líquida-espectrometria de massa em tandem (armadilha de íons quadrupolo-linear). O grau de extração da olanzapina do sangue (amostras modelo, 20 ng/ml) foi de 75,2% (CV 4,6%). O limite quantitativo para a olanzapina por HPLC-MS/MS (LLOQ) foi de 0,50 ng/ml (CV 2,0%, precisão 17,6%). Os estudos conduzidos em sistema aberto randomizado mostraram um perfil de biodisponibilidade e bioequivalência satisfatório do medicamento original e olanzapina genérica. Os parâmetros farmacocinéticos calculados para os valores médios convertidos logaritmicamente, verificados com um intervalo de confiança de 90%, foram: C_{max} - 95,93-99,80% (valor médio 97,64%); AUC_{0-t} - 95,43-100,35% (valor médio 97,75%); C_{max} / AUC_{0-t} - 94,44-101,73% (valor médio 97,87%).

Palavras-chave: *olanzapina, comprimido orodispersível, bioequivalência genérica, LC-MS / MS.*

ABSTRACT

Dispersible tablet form (ODT) of drugs is convenient for patients to take; the effectiveness of its use increases significantly with non-compliance treatment of mental disorders. Upon the expiration of the patent for the original drug, the production of generics with the same pharmacologically active ingredient often starts. Due to differences in fillers and manufacturing techniques, even high-quality copies of the original product may not be equivalent to it. Therefore, the aim of this study was to evaluate the bioequivalence of the ODT form of the original drug and generic with 10 mg olanzapine. After taking the original drug and generic in two stages, on an empty stomach from healthy volunteers, from the vein were sampled bloods into vacuum heparin tubes. The washout period between doses of the drugs was 21 days. The extraction (LLE) of olanzapine was carried with a mixture of n-hexane:isopropanol (3:1) at pH 8.0, and analyzed by liquid chromatography-tandem mass spectrometry (quadrupole-linear ion trap). The degree of extraction olanzapine from bloods (model samples, 20 ng/ml) was 75.2% (CV 4.6%). The quantitative limit for olanzapine by HPLC-MS/MS (LLOQ) was 0.50 ng/ml (CV 2.0%, accuracy 17.6%). Conducted on an open randomized cross scheme studies showed a satisfactory bioavailability and bioequivalence profile of the original drug and generic containing olanzapine. The calculated pharmacokinetic parameters for the logarithmically converted mean values verified with a 90% confidence interval were: C_{max} - 95.93-99.80% (average value 97.64%); AUC_{0-t} - 95.43-100.35% (average value 97.75%); C_{max} / AUC_{0-t} - 94.44-101.73% (average value 97.87%).

Keywords: *olanzapine, orodispersible tablet, bioequivalence, generic, LC-MS/MS*.

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АННОТАЦИЯ

Диспергируемая таблетированная форма (ДТ) лекарственных препаратов удобна для приема пациентами; эффективность ее применения существенно возрастает при нонкомплаенсе лечения психических расстройств. По окончании срока действия патента на оригинальное лекарство часто запускается производство дженериков с тем же фармакологически активным ингредиентом. Из-за различий в наполнителях и технологиях изготовления даже высококачественные копии оригинального препарата могут быть не эквивалентны ему. Поэтому целью данного исследования было оценить биоэквивалентность ДТ формы оригинального препарата и дженерика с содержанием оланзапина 10 мг. После приема оригинального препарата и дженерика в два этапа, натощак у здоровых добровольцев из вены отбирались пробы крови в вакуумные пробирки с гепарином. Период вымывания между дозами препаратов составлял 21 день. Извлечение оланзапина проводили методом жидкостно-жидкостной экстракции смесью н-гексан: изопропанол (3:1) при pH 8,0, анализировали методом жидкостной хроматографии с тандемной масс-спектрометрией (квадруполь-линейная ионная ловушка). Степень извлечения оланзапина из крови (модельные образцы 20 нг/мл) составила 75,2% (CV 4,6%). Предел количественного определения оланзапина методом ВЭЖХ-МС/ МС составил 0,50 нг/мл (CV 2,0%, точность 17,6%). Проведенные по открытой рандомизированной перекрёстной схеме исследования показали удовлетворительный профиль биодоступности и биоэквивалентности оригинального препарата и дженерика, содержащих оланзапин. Рассчитанные фармакокинетические параметры для логарифмически преобразованных средних значений верифицированные с 90%-ным доверительным интервалом составили: C_{max} - 95,93-99,80% (среднее значение 97,64%); AUC_{0-t} - 95,43—100,35% (среднее значение 97,75 %); C_{max}/AUC_{0-t} – 94,44-101,73% (среднее значение 97,87%).

Ключевые слова: *оланзапин, диспергируемая таблетка (ДТ), биоэквивалентность, дженерик, ВЭЖХ-МС/МС.*

1. INTRODUCTION:

Development of second generation of atypical antipsychotics, such as olanzapine (a thienobenzodiazepine derivative), provided a possibility for new, safer standards of psychiatric disorders treatment (San, Casileas, Ciudad, *and* Gilaberte, 2011).

Olanzapine binds to serotonin (5-$HT_{2A/2C}$, 5-HT_3, 5-HT_6), dopamine (D_1, D_2, D_3, D_4, D_5), and muscarinic ($M_{1–5}$) receptors, as well as to α_1-adrenoreceptors and histamine H_1-receptors, which predetermines its pharmacological activity. Olanzapine C_{max} in serum is reached within 5-8 hours; the concentrations (1-20 mg) are changing linearly, proportional to the dose of the drug. The degree of plasma protein binding is high (FB 0.93) (Tolmacheva, 2019).

Orodispersible tablets (ODT) of olanzapine, manufactured using Zydis® technology, provide several advantages over traditional tablets as they increase patient compliance and more comfortable for use in patients who have difficulty swallowing (San, Casillas, *and* Gilaberte, 2008; Seager, 1998; Sreenivas, Dandagi, Gadad, *and* Godblou, 2005). It should be noted that olanzapine absorption depends heavily on the manufacturing technology and dosage form composition: particle size and excipients affect both rate and degree of API absorption, considering that olanzapine dissolves faster in the acidic environment of the stomach than in the neutral environment of saliva (Chauhan, Kadliya, Patel, Patel, *and* Patel, 2014; Hobbs, Karagianis, Treuer, *and* Raskin, 2013; Kozlova, Zabolotnaya, *and* Maslova, 2015; Maher, Ali, Salem, *and* Abdelrahman, 2016; Sun, McDonnell, *and* von Moltke, 2018).

Taking into account increasing trends for wider use of generic (reproduced) drugs in the Russian Federation and worldwide, including drugs released in form of generic ODTs, obligatory tests must be performed in order to confirm their bioequivalence to the original drugs (Russian Federation. Federal Law, 2010). Robust and inexpensive, generic drugs are often preferred due to optimal combination of clinical efficacy, safety and lower costs of treatment (Shabelsky, 2015). However, the conclusion about equivalence of the generic and original drug should be based on the results of comprehensive studies (Russian federation. Ministry of Health and Social Development, 2008; Yin *et al*., 2016; Yu, Lou, Ruon, Jiang, *and* Chen, 2012).

Now increasingly a highly sensitive and reproducible method liquid chromatography-tandem mass spectrometry has for the quantification olanzapine in whole blood (plasma, serum) (Cavalcanti Bedor *et al*., 2015; Domingues *et al*., 2016; Steuer *et al*., 2015; Uřinovská *et al*.,

2012).

Olanzapine is a weak base (pKa1 5.0; pKa2 7.4, pKa314, 7), has a log P 3.0 and strongly binds to blood proteins. Various extraction variants are used to extract olanzapine from blood samples (Nielsen *and* Johansen, 2009; Poothong, Lundanes, Thomsen, *and* Haug, 2017), but the liquid-liquid extraction (LLE) method is simpler to perform and economicals. Therefore, in a carried study, a used optimized variant of liquid-liquid extraction a blood sample for analysis olanzapine developed using the special program "ASD/Percepta".

Olanzapine is administered in doses of 5-40 mg / day, the level and time to reach its maximum concentration in the blood varies depending on the dosage form used, and its pharmacokinetic profile is affected by the genotype of the subject (Chen *et al*., 2012; Citrome *et al*., 2009; Fan *et al*., 2020; Markowitz *et al*., 2006; Polasek *et al*. 2018; Sathirakul *et al*., 2003).

A critical analysis of the publications showed that patients prefer the dispersible form of the drug and studies are being conducted in different countries to study the bioequivalence of generics from various manufacturers, especially it actual for the often administered doses of olanzapine 10-20 mg/day (Cánovas *et al*., 2011; Chatsiricharoenkul *et al*., 2011; Chatsiricharoenkul, 2009; Du *et al*., 2019; Elshafeey, Elsherbiny, *and* Fathallah, 2009; Sun *et al*., 2016; Waykar *and* Kulkarni, 2012; Zakeri-Milani, Islambulchilar, Ghanbarzadeh, *and* Valizadeh, 2013).

Therefore, the aim of the study was to compare bioavailability and bioequivalence of a single, orodispersible dose of olanzapine (10 mg), manufactured by Teva Operations Poland Sp.z.o.o. (Olanzapine-DT-Teva, generic drug (T)) and by Catalent U.K. (Zyprexa® Zydis®, original drug, (R)).

2. MATERIALS AND METHODS:

Reference standards and reagents: Olanzapine and clozapine reference standards (RS) (≥ 98% purity, HPLC) were purchased from Sigma-Aldrich (USA). Ascorbic acid (pharmacopoeial quality) was purchased from FARM, ФС-42-2668-95. Methanol (HPLC-MS grade), isopropanol, *n*-hexane, formic acid, and ammonium acetate (HPLC grade) were obtained from Merck KGaA (Germany). Deionized water for HPLC analysis was obtained using Barnstead Easypure II, Model 7134 (Thermo Fisher Scientific, USA) water purification system.

Instrumentation: Atom 80 (Biotron, Spain) automatic shaker, Eppendorf 5415C (Eppendorf, Germany) centrifuge, Eppendorf Concentrator 5301 (Eppendorf, Germany). HPLC-ESI–MS/MS was conducted on a Shimadzu (Columbia, MD) HPLC system composed of two LC-20AD pumps with a CTO-20A column oven, DGU-20A3 degasser and CBM 20A controller connected to a SIL20A autosampler all interfaced with an API Sciex 3200 triple quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA). Analyst 1.6.3 software (AB Sciex, Foster City, CA) was utilized for instrument control, data acquisition, and analyte mass spectrometric parameter optimization.

Volunteers: A total of 18 healthy, non-smoking male volunteers (Caucasian, age 18-45) were included in the study after signing informed consent agreement and undergoing medical examination.

Study design: An open randomized crossover study was carried out in two stages: a course of original drug was followed by a 21-day washout period, then a course of generic drug. The drugs were administered on an empty stomach. Both drug products during study satisfied manufacturers' quality requirements and were within their shelf-life limits. Blood samples were withdrawn prior to drug administration and 1.0, 2.0, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, 8.0, 9.0, 10.0, 12.0, 16.0, 24.0, 36.0, 48.0, 72.0, 120.0, and 168.0 hours after olanzapine administration. Blood samples from cubital vein were collected into vacuum test tubes containing heparin and allowed to stand for 5-10 min before centrifugation. After centrifugation for 10 min at 2500 RPM, separated plasma was transferred into labeled plastic vials. All samples were stored at -40°C in low-temperature freezer (Sanyo, Japan) until analysis.

Analytical procedure: The method of liquid-liquid extraction was developed based on calculations of the physicochemical parameters of olanzapine by the specialized ACD/Percepta program (pKa, LogP, LogD). Also, using this program “ACD/Percepta”, an internal standard of clozapine (LogP 3,37) was selected, which has physico-chemical parameters similar to olanzapine (Figure 1 and 2). The selection of the internal standard is very important for the sample preparation and analysis stage, as it allows the sample preparation to rectify the effect of error on the measurement results by correcting it

mathematically (using the ratio).

The LogP distribution coefficient (octanol/water) determines the lipophilicity of the compound and its distribution between two immiscible phases, with different polarity properties. Indices of acidity (pKa) of olanzapine allow to determine optimal solution pH for its dissolution, and LogD characterizing dependence between LogP and pKa was used to achieve maximum extraction of olanzapine (Sanjivanjit, 2019). As can be seen from the figures, the non-ionized form of substances already prevails at a pH above 8, and the change Log D is insignificant. On the basis of the obtained data, a protocol of operational procedures for analysis (SOP) was developed.

In order to extract olanzapine from plasma samples, 2 mcl of clozapine methanol solution (internal standard, 225 ng/ml) were added to 100 mcl of plasma in 2 mL Eppendorf centrifuge test tubes, followed by 25 mcl of 5% ascorbic acid solution, 400 mcl of carbonate buffer (pH 8), 1 ml of n-hexane-isopropanol mixture (3:1), and 150 mg of sodium chloride. The mixture was shaken for 10 minutes using automatic shaker. After that, all samples were centrifuged for 10 min at 12500 RMP. Seven hundred microliters of organic phase were transferred into vial using automatic pipette and were evaporated until dry. Dry residue was reconstituted in 100 mcl of methanol and transferred into glass inserts, which were, in turn, placed into 1,5 ml chromatographic vials. Solutions were analyzed LC-MS/MS. Separation was carried out in gradient mode (mobile phase – 0.10% ammonium acetate (A) and methanol (B), starting with 10% A, flow rate – 0,4 ml/min) on ProntoSIL 120-5-C18 AQ (2.0 x 75 mm) chromatographic column at 40°C. Injection volume – 5 mcl. Olanzapine retention time was 5.37 min (Figure 3).

Detection was performed using M+H values and MRM transition, using main and daughter ions: 313.2/256.2 m/z for olanzapine (Figure 4) and 327.3/270.2 m/z – for clozapine (internal standard, Figure 5). Olanzapine content was determined using internal standard method with automatic integration by standard AB Sciex software (Analyst 1.6.3).

The quantification procedure was validated using several parameters presented in table 1 to prove its suitability for bioequivalence studies.

Thus, the results obtained comply with the requirements of GOST, EMA, FDA and EURASES Validation Guides (Europian Medicines Agency, 2011; USA. Food and drug Administration, 2018; GOST R 56431-2015; Collegion of the eurasian economic commission, 2018) and the technique was found to be suitable for quantitative determination of olanzapine in the blood. Then concentration of olanzapine in blood samples of volunteers was determined validated method as described SOP.

Pharmacokinetic parameters calculation and statistical data processing: All calculations were performed using SigmaPlot 12.0 (SYSTAT Software, USA) and Microsoft Excel 2007 (Microsoft, USA).

The following pharmacokinetic parameters were calculated for each participant, using olanzapine concentrations determined after administration of original drug and generic: C_{max} (maximum measured concentration), T_{Cmax} (time until C_{max}), k_{el} (elimination rate constant, which was derived using slope of the final (monoexponential) part of the pharmacokinetic curve, described using non-linear regression analysis), $T_{1/2}$ (elimination half-life, calculated as natural logarithm (ln) (2)/k_{el}), AUC_{0-t} (area under pharmacokinetic curve from time of administration (0 hours) until final blood sample withdrawal (*t* hours); calculated using trapezium rule), $AUC_{0-\infty}$ (from time of administration to infinity; $AUC_{0-\infty}$= AUC_{0-t} + C_t/k_{el}, where C_t is the last measured drug concentration in plasma), $AUC_{0-t}/AUC_{0-\infty}$ (this ratio, expressed as per cent, is a measure of adequacy of blood sampling time points), C_{max}/AUC_{0-t} (relative absorption rate). Consequent analysis of pharmacokinetic data included calculation of relative olanzapine bioavailability after administration of generic drug versus administration of original product ($f' = AUC_{0-t}(T)/AUC_{0-t}(R)$ and calculation of $f'' = C_{max}(T)/C_{max}(R)$ ratio for each drug. The following descriptive statistics parameters were calculated for all pharmacokinetic values: mean ($\acute{X}$), standard deviation (SD), geometric mean (G), coefficient of variation (CV), median, and maximum (Max) and minimum (Min) values. Values of main pharmacokinetic parameters (AUC_{0-t}, C_{max}, and C_{max}/AUC_{0-t}), after logarithmic transformation, were analyzed using ANOVA with $p < 0,05$. For common randomized crossover study, statistical model of dispersion analysis included the following factors, which influence observed data variability: drug administration sequence, stages of study, volunteers (within sequence). Dispersion analysis was used to test hypotheses about statistical significance of each of the mentioned factors to the overall variability. Obtained residual variance was used in calculation of 90% confidence interval (CI) for the ratio of mean values of corresponding

parameter.

The conclusion about bioequivalence of compared olanzapine products was based on the assessment of 90% CIs for the ratio of logarithmically transformed mean values of main pharmacokinetic parameters (AUC_{0-t}, C_{max}, C_{max}/AUC_{0-t}). The drugs were considered bioequivalent if 90% CI limits for AUC_{0-t} and $AUC_{0-\infty}$ were within 80—125% range. For C_{max}, C_{max}/AUC_{0-t}, and $C_{max}/AUC_{0-\infty}$, values, which have higher variability, the range was 75—133% (Russian federation. Ministry of Health and Social Development, 2008; Pisarev, Ulyashova, Vdovin, *and* Tiseyko, 2013).

The study was carried out in accordance with ethical norms, principles of good laboratory practices and good clinical practices, following SOP and study protocol, which were developed in consistence with relevant local regulatory framework (Russian Federation. Federal Law, 2010; GOST 33044-2014; GOST R 52379-2005).

3. RESULTS AND DISCUSSION:

Obtained averaged pharmacokinetic curves of olanzapine concentrations in plasma of health volunteers (Figure 6) suggest that the type of the dependence for both studies products is essentially the same.

Using obtained pharmacokinetic curves, individual olanzapine pharmacokinetic parameters (C_{max}, AUC_{0-t} and C_{max}/AUC_{0-t}.) required for bioequivalence assessment were calculated for each participant. Values of AUC_{0-t}, T_{Cmax}, k_{el}, and $T_{1/2}$ were also calculated in order to obtain additional information. Mean values of calculated pharmacokinetic parameters and other descriptive statistics parameters are presented in Table 2.

Results of dispersion analysis (Table 3) allowed to accept null hypothesis that the difference in mean values of main pharmacokinetic parameters is not caused by difference in composition or manufacturing technology of the drugs under comparison: the 'Drug' factor (5% level of significance) contributed insignificantly into overall variability of olanzapine pharmacokinetic parameters (AUC_{0-t}, C_{max}, C_{max}/AUC_{0-t}). The critical value for assessing the contribution of drug type factors is value $F_{0,05;1;17}$ 4,45.

The effects due to 'administration period' and 'administration sequence' were also insignificant ($p < 0.05$). Factor 'Volunteer (intra-sequence)' had significant effect on all pharmacokinetic parameters ($p > 0.05$), but it indicates only intraindividual variability with the volunteer group. Comparison of the obtained data showed that studied olanzapine products do not significantly differ from each other in terms of their pharmacokinetics, and individual values dispersion is identical for both drugs. Mean value (±SD) of olanzapine relative bioavailabilities (f') after administration of generic drug versus original products was 0,936 ± 0.272 and the ratio of maximum concentrations (f'') was 0,950± 0,099.

Calculated 90% CI for the ratios of mean log values of olanzapine AUC_{0-t}, C_{max}, C_{max}/AUC_{0-t}, and intraindividual variability coefficients of these parameters are presented in Table 4.

Confidence interval for the ratio of olanzapine log AUC_{0-t} values was 96,23 - 100,45% (mean = 98.21%); thus, calculated 90% CI is within bioequivalence criteria. Confidence intervals for logarithmically transformed C_{max} and C_{max}/AUC_{0-t} values were 95,97 - 99,61% (mean = 97,70%) and 95,83 - 101,87% (mean = 98.60%), also satisfying bioequivalence criteria (Russian federation. Ministry of Health and Social Development, 2008; Romodanovsky, Goryachev, Solovieva, *and* Eremenko, 2018).

4. CONCLUSION:

It was shown that the values of 90% CIs for the ratio of log transformed olanzapine AUC_{0-t}, C_{max}, and C_{max}/AUC_{0-t} values were 96,23-100,45%; 95,97-99,61%, and 95,83-101,87%, respectively. These results satisfy the currently accepted criteria of bioequivalence, stated in the relevant regulatory documents of the Russian Federation. Therefore, in can be concluded that two studied olanzapine orodispersible dosage forms (10 mg, manufactured by Teva Operations Poland Sp.z.o.o. and by Catalent U.K.) are bioequivalent.

5. REFERENCES:

1. Cánovas, M., Torres, F., Domenech, G., Cebrecos, J., Pelagio, P., Manríquez, M., … Cabre, F. (2011). Bioequivalence evaluation of two dosage forms of olanzapine 10 mg formulations in healthy volunteers. *Arzneimittelforschung, 61*(2), 75–79.
2. Cavalcanti Bedor, N. C., Galindo Bedor, D. C., De Sousa, C. E. M., Bonifacio, F. N., Branco, D. D. M. C., Leal, L. B., *and* De Santana, D. P. (2015). The development and validation of a method for quantifying

olanzapine in human plasma by liquid chromatography tandem mass spectrometry and its application in a pharmacokinetic study. *Clinical and Experimental Pharmacology and Physiology, 42*(3), 305–313.

3. Chatsiricharoenkul, S. . (2009). In vivo bioequivalence study of 10 mg Olanzapine tablets in healthy Thai volunteers. *Basic and Clinical Pharmacology and Toxicology, 105*, 102.
4. Chatsiricharoenkul, S., Thangboonjit, W., Pongnarin, P., Konhan, K., Sathirakul, K., *and* Kongpatanakul, S. (2011). Bioequivalence Study of 10 mg Olanzapine Tablets in Healthy Thai Volunteers. *J Bioequiv. Availab, 3*(5), 82-85.
5. Chauhan, K. V., Kadliya, P. N., Patel, B. A., Patel, K. N., *and* Patel, P. A. (2014). Formulation Optimization and Evaluation of Orodispersible Tablets of an Antipsychotic Drug Using Solubility Enhancement Technique. *Journal Club for Pharmaceutical Sciences, 1*(1), 33-57.
6. Chen, Q., Zhang, M.-Q., Lui, Y.-M., Li, S.-J., Lu, Ch., Liu, G.–Y., … Jia, J.-Y. (2012). Pharmacokinetics and bioequivalence of 2 tablet formulations of olanzapine in healthy Chinese volunteers: a randomized, open-label, single-dose study. *Arzneimittelforschung, 62*(11), 508-512.
7. Citrome, L., Stauffer, V. L., Chen, L., Kinon, B. J., Kurtz, D. L., Jacobson, J. G., *and* Bergstrom, R. F. (2009). Olanzapine plasma concentrations after treatment with 10, 20, and 40 mg/d in patients with schizophrenia: an analysis of correlations with efficacy, weight gain, and prolactin concentration. *Journal of Clinical Psychopharmacology, 29*(3), 278–283.
8. Collegion of the eurasian economic commission. (2018). Guidance on the validation of analytical methods for testing drugs.
9. Domingues, D., Pinto, M., De Sousa, I., Hallak, J., Crippa, J., *and* Queiroz, M. (2016). Determination of drugs in plasma samples by high-performance liquid chromatography-tandem mass spectrometry for therapeutic drug monitoring of schizophrenic patients. *Journal of Analytical Toxicology, 40*(1), 28-36.
10. Du, P., Li, P., Liu, H., Zhao, R., Zhao, Zh., Yu, W., … Liu, L. (2019). Open-Label, Randomized, Single-Dose, 2-Period, 2-Sequence Crossover, Comparative Pharmacokinetic Study to Evaluate Bioequivalence of 2 Oral Formulations of Olanzapine Under Fasting and Fed Conditions. *Clinical Pharmacology in Drug Development, Oct. 8*. https://doi.org/10.1002/cpdd.743
11. Elshafeey, A. H., Elsherbiny, M. A., *and* Fathallah, M. M. (2009). A single-dose, randomized, two-way crossover study comparing two olanzapine tablet products in healthy adult male volunteers under fasting conditions. *Clinical Therapeutics, 31*(3), 600-608.
12. Europian Medicines Agency. (2011). Guideline on bioanalytical method validation.
13. Fan, L., Zhang, L., Zheng, H., Cheng, J., Hu, Y., Liu, J., *and* Fan, H. (2020). Pharmacokinetics and Bioequivalence of 2 Olanzapine Orally Disintegrating Tablet Products in Healthy Chinese Subjects Under Fed and Fasting Conditions. *Clinical Pharmacology in Drug Development, May 15*. https://doi.org/10.1002/cpdd.765
14. GOST 33044-2014 “Principles of Good Laboratory Practice”.
15. GOST R 52379-2005 “Good Clinical Practice”.
16. GOST R 56431-2015 “Quality Management System. Medical Products. Process Validation Guide”.
17. Hobbs, D, Karagianis, J., Treuer, T., *and* Raskin, J. (2013). An In Vitro Analysis of Disintegration Times of Different Formulations of Olanzapine Orodispersible Tablet: A Preliminary Report. *Drugs in RandD, 13*, 281–288.
18. Kozlova, Zh. M., Zabolotnaya, P. G., *and* Maslova, M. N. (2015). Bystrodispergiruemye oral'nye sistemy dostavki. *Novaya nauka: ot idei k rezul'tatu, 2*, 75–78.
19. Maher, E. M., Ali, A. M. A., Salem, H. F., *and* Abdelrahman, A. A. (2016). In vitro/in vivo evaluation of an optimized fast dissolving oral film containing olanzapine coamorphous dispersion with selected carboxylic acids. *Drug Delivery, 23*(8), 3088–3100.

20. Markowitz, J. S., DeVane, S. L., Malcolm, R. J., Gefroh, H. A., Wang, J.-Sh., Zhu, H.-J., *and* Donovan, J. L. (2006). Pharmacokinetics of olanzapine after single-dose oral administration of standard tablet versus normal and sublingual administration of an orally disintegrating tablet in normal volunteers. *J Clin Pharmacol., 46*(2), 164–171.

21. Nielsen, M. K., *and* Johansen, S. S. (2009). Determination of olanzapine in whole blood using simple protein precipitation and liquid chromatography-tandem mass spectrometry. *Journal of Analytical Toxicology, 33*(4), 212-217.

22. Pisarev, V. V., Ulyashova, M. M., Vdovin, K. A., *and* Tiseyko, N. I. (2013). Bioequivalence study of tablet forms of risperidone, olanzapine and quetiapine in healthy volunteers. *Pharmacokinetics and Pharmacodynamics, 1*, 14–22.

23. Polasek, T. M., Tucker, G. T., Sorich, M. J., Wiese, M. D., Mohan, T., Rostami-Hodjegan, A., … Rowland, A. (2018). Prediction of olanzapine exposure in individual patients using physiologically based pharmacokinetic modelling and simulation. *British Journal of Clinical Pharmacology, 84*(3), 462-476

24. Poothong, S., Lundanes, E., Thomsen, C., *and* Haug, L. (2017). High throughput online solid phase extraction-ultra high performance liquid chromatography-tandem mass spectrometry method for polyfluoroalkyl phosphate esters, perfluoroalkyl phosphonates, and other perfluoroalkyl substances in human serum, plasma, and whole blood. *Analytica Chimica Acta, 957*, 10-19.

25. Romodanovsky, D. P., Goryachev, D. V., Solovieva, A. P., *and* Eremenko, N. N. (2018). Principles of Statistical Evaluation of Bioequivalence Studies in the Context of Current Regulatory Requirements and Legal Acts. *The Bulletin of the Scientific Centre for Expert Evaluation of Medicinal Products, 8*(2), 92-98.

26. Russian Federation. Federal Law “On circulation of medicines” No 61-FZ dated April 12, 2010 (as amended by Federal Law of December 27, 2018 No 511-FZ). Retrieved from https://legalacts.ru/doc/federalnyi-zakon-ot-12042010-n-61-fz-ob/

27. Russian Federation. Ministry of Health and Social Development. (May 12, 2008). Guidelines of the Ministry of Health and Social Development of the Russian Federation “Bioequivalence assessment of medicinal products”.

28. San, L., Casileas, M., Ciudad, A., *and* Gilaberte, I. (2011). Dispergiruemye tabletki olanzapina: obzor effektivnosti i komplaensa (referat). *Psihiatriya i psihofarmakoterapiya imeni P.B. Gannushkina, 4*, 51–58.

29. San, L., Casillas, M., *and* Gilaberte, I. (2008). Olanzapine orally disintegrating tablet: a review of efficacy and compliance. *CNS Neuroscience and Therapeutics, 14*(3), 203-214.

30. Sanjivanjit, K. (2019). *Bhal Lipophilicity Descriptors: Understanding When to Use LogP and LogD*. Toronto, Canada: Advanced Chemistry Development, Inc.

31. Sathirakul, K., Chan, C., Teng, L., Bergstrom, R. F., Yeo, K. P., *and* Wise, S. D. (2003). Olanzapine pharmacokinetics are similar in Chinese and Caucasian subjects. *Br J Clin Pharmacol, 56*(2), 184–187.

32. Seager, H. (1998). Drug-delivery Products and the Zydis Fast-dissolving Dosage Form. *Journal of Pharmacy and Pharmacology, 50*(4), 375–382.

33. Shabelsky, V. S. (2015). Original drugs and generics: is there an optimal balance on the pharmaceutical market? *Young Scientist, 8*, 684 - 689.

34. Sreenivas, S. A., Dandagi, P. M., Gadad, A. P., *and* Godblou, A. M. (2005). Orodispersible tablets: New-fangled drug delivery systems – A review. *Indian Journal of Pharmaceutical Education and Research, 39*(4), 177–181.

35. Steuer, A., Poetzsch, M., Koenig, M., Tingelhoff, E., Staeheli, S., Roemmelt, A., *and* Kraemer, T. (2015). Comparison of conventional liquid chromatography-tandem mass spectrometry versus microflow liquid chromatography-tandem mass spectrometry within the framework of full method validation for simultaneous quantification of 40 antidepressants and neuroleptics in whole blood. *Journal of Chromatography A, 1381*, 87-100.

36. Sun, L., McDonnell, D., Liu, W., Carter, D., Simmons, A., *and* von Moltke, L. (2016). Bioequivalence evaluation of three olanzapine-containing tablet formulations in healthy volunteers. *Clinical Pharmacology in Drug Development, 5*, 19.

37. Sun, L., McDonnell, D., *and* von Moltke, L. (2018). Bioequivalence of Olanzapine Given in Combination With Samidorphan as a Bilayer Tablet (ALKS 3831) Compared With Olanzapine-Alone Tablets: Results From a Randomized, Crossover Relative Bioavailability Study. *Clinical Pharmacology in Drug Development, 8*(4), 459-466.

38. Tolmacheva, E. A. (Ed.). (2019). *Vidal Reference Book "Medicinal Products in Russia"*. Moscow, Russia: Vidal Rus.

39. Uřinovská, R., Brozmanová, H., Šištík, P., Šilhán, P., Kacířová, I., Lemr, K., *and* Grundmann, M. (2012). Liquid chromatography-tandem mass spectrometry method for determination of five antidepressants and four atypical antipsychotics and their main metabolites in human serum. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *907*, 101-107.

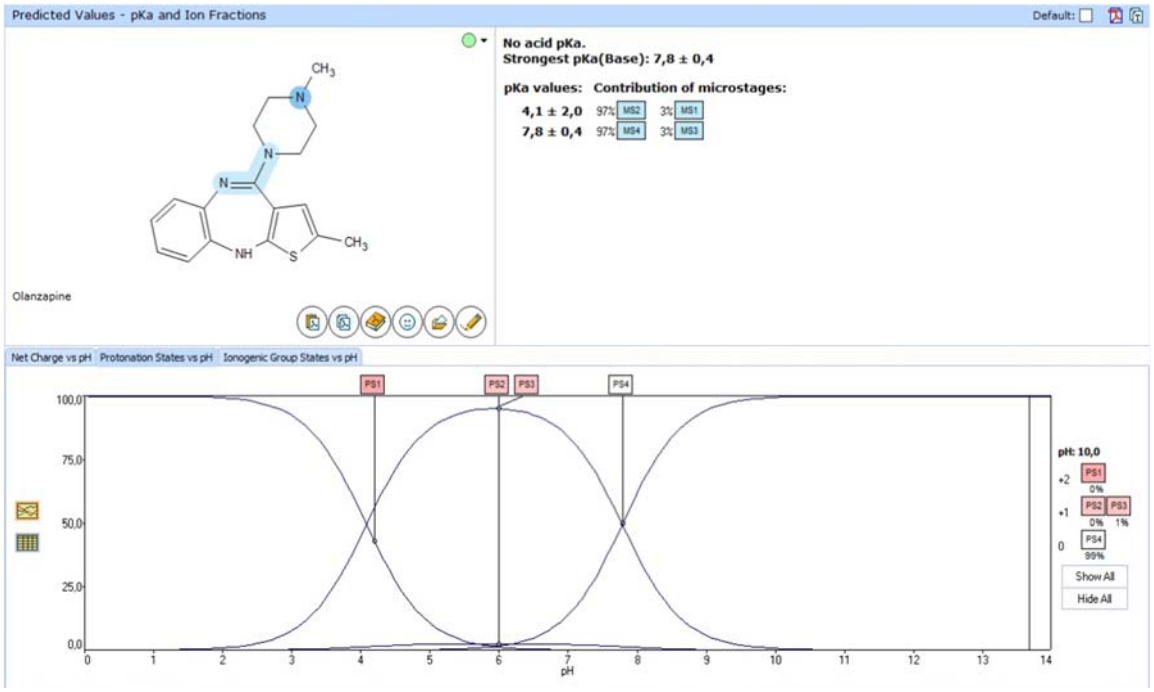
40. USA. Food and drug Administration. (May, 2018). Bioanalytical Method Validation Guidance for Industry.

41. Waykar, R., *and* Kulkarni, Y. (2012). Development and bioequivalence study of olanzapine 10mg tablets. *International Journal of Pharmaceutical Sciences and Research*, *3*(9), 3370-3375.

42. Yin, A., Shang, D., Wen, Y., Li, L., Zhou, T., *and* Lu, W. (2016). Population pharmacokinetics analysis of olanzapine for Chinese psychotic patients based on clinical therapeutic drug monitoring data with assistance of meta-analysis. *Eur J Clin Pharmacol., 72***,** 933–944.

43. Yu, L.-Y., Lou, H.-G., Ruon, Z.-R., Jiang, B., *and* Chen, J.-L. (2012). Pharmacokinetics and bioequivalence of domestic olanzapine tablets in healthy Chinese volunteers. *Chinese Pharmaceutical Journal, 47*(23), 1934-1936.

44. Zakeri-Milani, P., Islambulchilar, Z., Ghanbarzadeh, S., *and* Valizadeh, H. (2013). Single dose bioequivalence study of two brands of olanzapine 10 mg tablets in Iranian healthy volunteers. *Drug Research, 63*(7), 346–350.



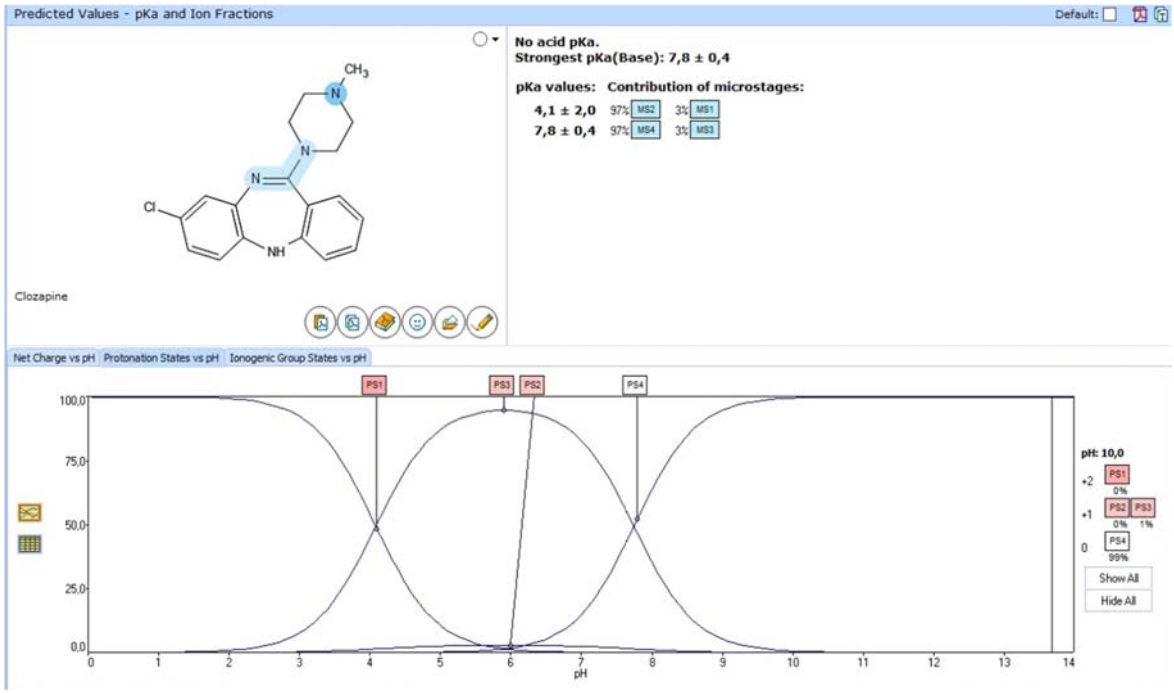


Figure 1. Acidity and isoelectric curve of substances

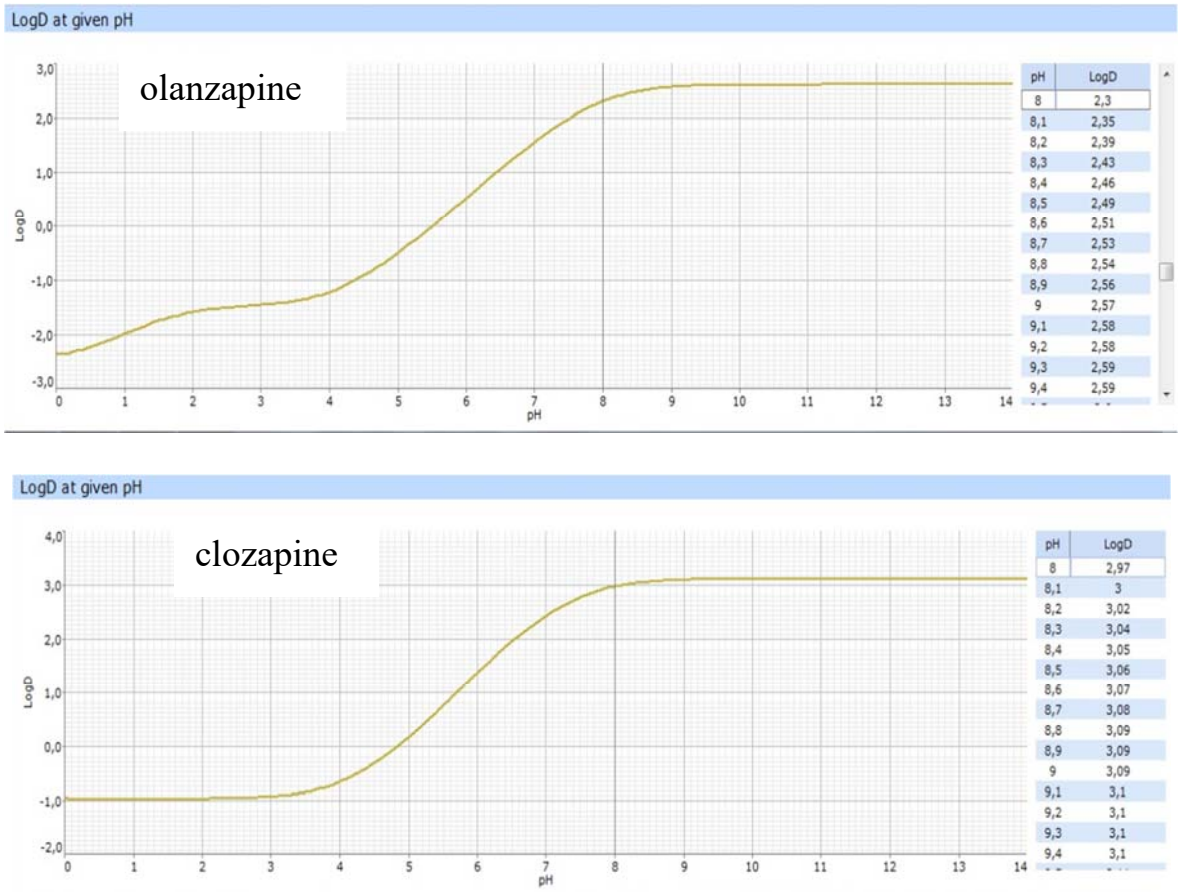


Figure 2. Dependence Log D from pH

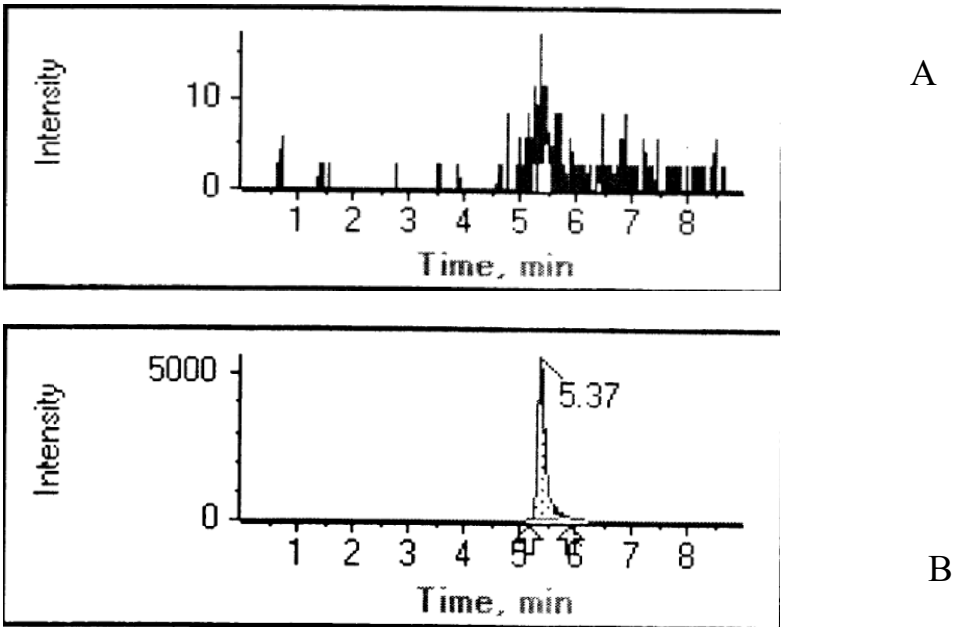


Figure 3. Typical blood extraction chromatogram: A-blank, B-containing olanzapine

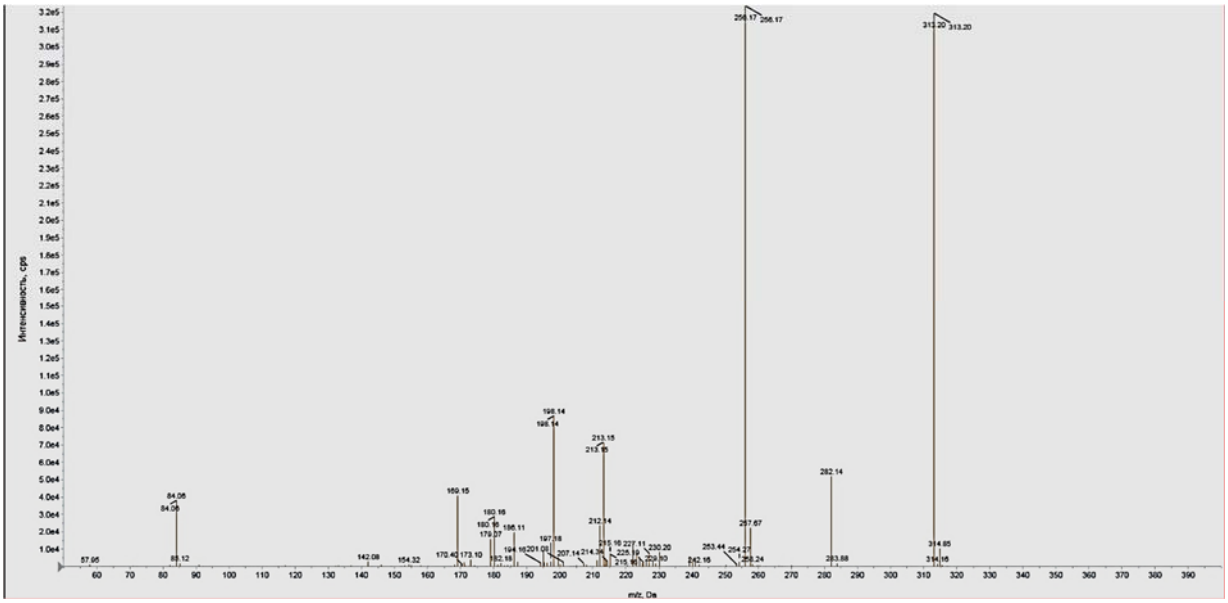


***Figure 4*.** Mass spectrum of olanzapine (direct input to the MS detector in EPI mode (TIC)

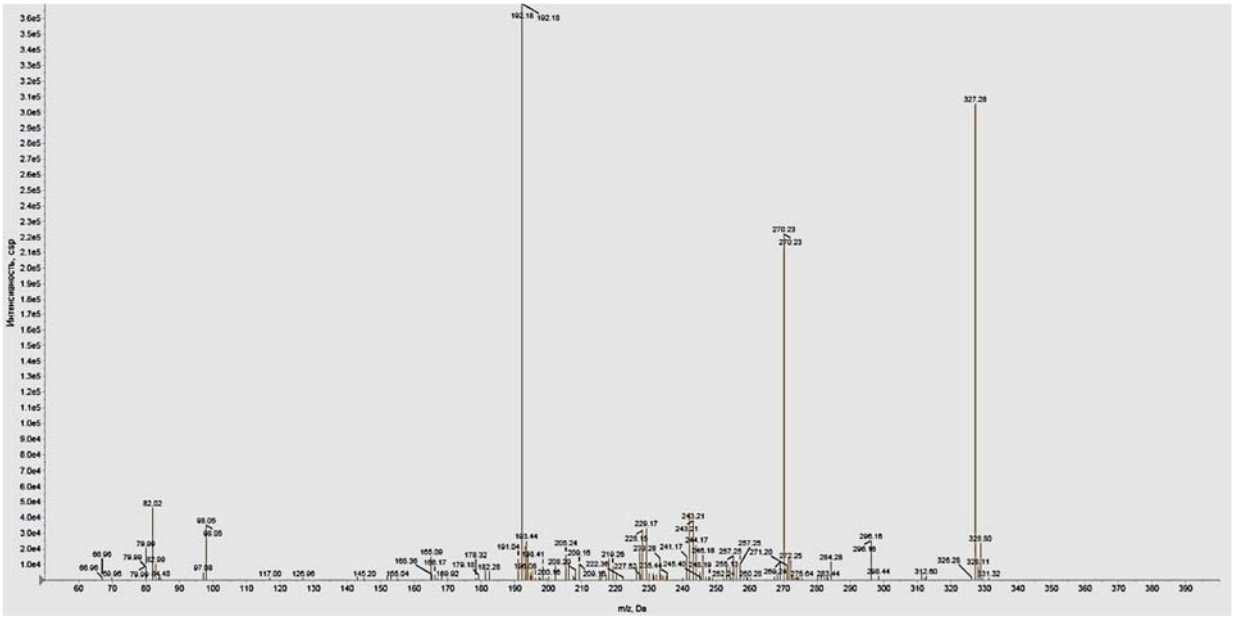


***Figure 5*.** Mass spectrum of clozapine (direct input to the MS detector in EPI mode (TIC)

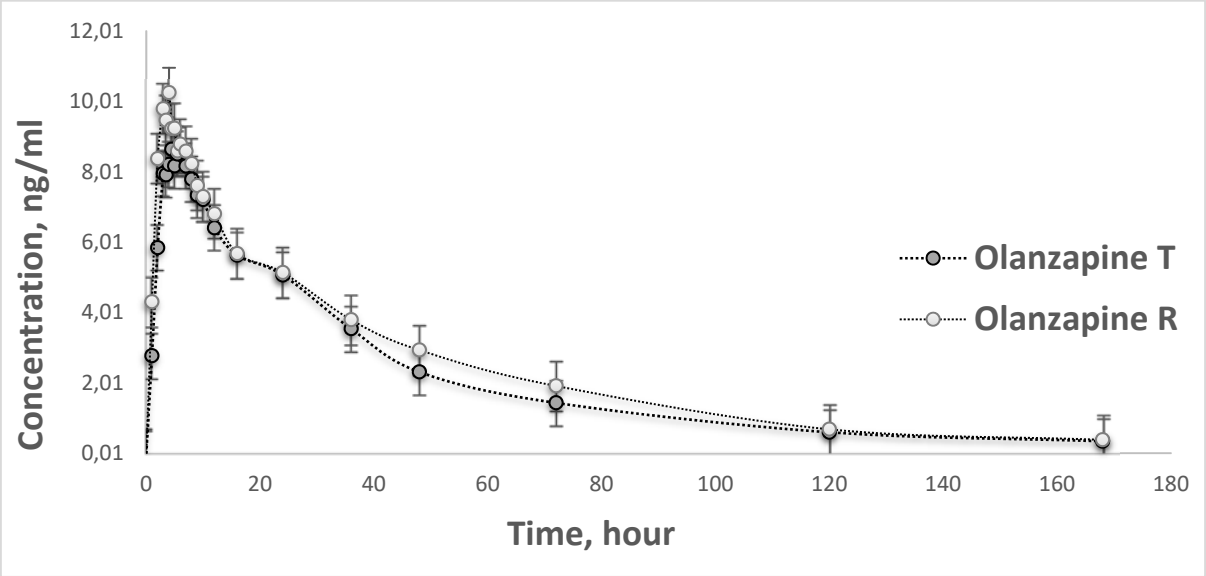


Figure 6. Averaged pharmacokinetic curves of olanzapine concentration in plasma of 20 volunteers after single administration of studied drugs (10 mg)

Table 1. *Method validation*

Validation parameter	Testing sample	Results		Criteria	
Calibration curve N=3	Expended Concentration, ng/ml	CV,%	Accuracy, %	CV,%	Accuracy, %
	0.25	-	-		
	0.5	2,0	117.6		<20
	1.0	2.8	98.2		<15
	5.0	1.6	93.3	<20%	<15
	10.0	1.0	91.9		<15
	15.0	0.6	100.1		<15
	25.0	1.3	93.7		<15
	50.0	1.0	105.3		<15
Detection Limit (LOD) 0,25 ng/ml Limit of quantification (LLOQ) 0,5 ng/ml N=9		Linear regression with weighting 1/x; y = 0.92594x + - 0.04408 (0.5 ng/ml–50 ng/ml)			
Linearity		r=0,99810		r ≥0.9900	
Recovery,%	2.0 ng/ml	92,3%			
	20.0 ng/ml	75,2%			
	40.0 ng/ml	75,0%			
Accuracy, % N=6	2.0 ng/ml	99.96%		100±15%	
	20.0 ng/ml	102.22%			
	40.0 ng/ml	104.29%			
Precision N=6 (CV%)	2.0 ng/ml	2.35%		<10%	
	20.0 ng/ml	4.61%			
	40.0 ng/ml	6.45%			
Inter day precision, % N=6	2.0 ng/ml	6.4%		<10%	
	20.0 ng/ml	7.12%			
	40.0 ng/ml	6.60%			

Validation parameter	Testing sample	Results	Criteria
Selectivity N=6	Blank,	No interferention	
Calibration standard: olanzapine			<20%
IS: closapine	0.5 ng/ml		<5%
Carry-over (N=6), %	Blank after		
Calibration standard: olanzapine	50 ng/ml	1,60	<20%
IS: closapine		0,01	<5%
Stability, % N=3 (freeze and thaw -40°C - +20°C)	0.5 ng/ml	93,93%	100±15%
	50 ng/ml	111,78%	

Table 2. *Pharmacokinetic parameters after single administration of the studied olanzapine products*

Parameter		$\bar{X}$	Min	Max	Med	SD	G	CV, %	C.I. 90%
C_{max}, ng/ml	T	10,62	8,26	12,40	10,58	1,06	10,56	10,02	0,44
	R	11,26	7,72	13,40	11,36	1,28	11,18	11,40	0,53
T_{Cmax}, h	T	5,28	3,00	10,00	4,50	1,94	4,98	36,79	0,80
	R	4,33	2,00	9,00	4,00	1,76	4,01	40,70	0,72
AUC_{0-t}, ng•h/ml	T	354,84	199,08	444,96	367,29	71,99	347,19	20,29	29,52
	R	400,17	217,25	556,86	422,25	101,37	386,98	25,33	41,56
$AUC_{0-\infty}$, ng•h/ml	T	379,57	205,91	521,04	380,36	85,27	369,77	22,48	34,96
	R	400,18	217,26	556,87	422,26	101,37	386,98	25,33	41,56
C_{max}/ AUC_{0-t}, h^{-1}	T	0,03	0,02	0,05	0,03	0,01	0,03	26,40	0,003
	R	0,03	0,02	0,06	0,03	0,01	0,03	27,79	0,003
C_{max}/ $AUC_{0-\infty}$,h^{-1}	T	0,03	0,02	0,05	0,03	0,01	0,03	21,48	0,003
	R	0,03	0,02	0,06	0,03	0,01	0,03	26,40	0,003
k_{el}, h^{-1}	T	0,017	0,01	0,028	0,016	0,01	0,017	26,71	0,003
	R	0,017	0,01	0,028	0,016	0,01	0,017	27,58	0,003
$T_{1/2}$, h	T	42,64	24,93	64,18	43,19	10,64	41,36	24,96	4,36
	R	42,88	24,93	64,18	43,59	10,92	41,53	25,46	4,62

Table 3. *The results of dispersion analysis of pharmacokinetic parameters lnAUC_{0-t}, lnC_{max}, ln C_{max} /ln AUC_{0-t} determining the bioavailability of olanzapine from dosage form*

Source of Variation	ln AUC_{0-t}			
	SS	DF	MF	F
Between Treatments/Preparat	0,106	1	0,106	1,699
Between Subjects	1,332	17	0,0784	2,287
Residual	0,788	17	0,0463	-
Total	2,226	35	-	-
Source of Variation	ln C_{max}			
	SS	DF	MF	F
Between Treatments/Preparat	0,0289	1	0,0289	2,180
Between Subjects	0,353	17	0,0208	5,029
Residual	0,0978	17	0,0057	-
Total	0,480	35	-	-
Source of Variation	ln C_{max} /ln AUC_{0-t}			
	SS	DF	MF	F
Between Treatments/Preparat	0,0242	1	0,0242	0,542
Between Subjects	0,995	17	0,0585	0,789
Residual	0,521	17	0,0306	-
Total	1,540	35	-	-

Notes: SS is the sum of squared deviations; DF - the number of degrees freedom; MS is the root mean square error; F - calculated value Fisher's F-test (at a significance level of α = 5%)

Table 4. *Values of pharmacokinetic parameters after logarithmic data transformation*

Parameter		GMean	SD	CV, %	ratio T/R, %	CV_{intra}, %	90% CI Lower limit Upper limit, %
C_{max}	T	2,4109	0,1029	4,37	97,70	14,50	95,97-99,61
	R	2,3554	0,1262	5,23			
AUC_{0-t}	T	5,8458	0,2224	3,80	98,21	28,55	96,23-100,45
	R	5,9523	0,2743	4,60			
$AUC_{0-\infty}$	T	5,8458	0,2224	3,80	98,21	28,55	96,23-100,45
	R	5,9523	0,2743	4,60			
C_{max}/AUC_{0-t}	T	3,4873	0,1917	5,49	98,60	24,54	95,83-101,87
	R	3,5368	0,2289	6,45			