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STUDYING THE BIOPHARMACEUTICAL PROPERTIES OF THE DRUG "BISKOR" BY METHODS IN VITRO AND IN VIVO

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The results of the study of the biopharmaceutical properties of the recommended tablets "Biskor" by methods in vitro and in vivo. The first step in a bioavailability study is to determine the solubility or release time of the drug from the dosage form. It has been established that the solubility test in the first approximation characterizes the bioavailability of the drug, since in practice there is a very frequent correlation between the rate of dissolution and absorption. The solubility, foreign impurities and the quantitative content of active substances were studied.

Key words: biopharmacy, in vitro, in vivo, solubility, quantitation, impurities, HPLC

Relevance. The creation of a new generation of medicines is relevant to meet the needs of domestic healthcare in strategically important medicines in order not only to import substitution, but also to ensure national security in the field of drug policy. The introduction of promising scientific developments will ensure the sustainable production of effective drugs and initiate the creation of innovative world-class pharmaceutical products that can compete with foreign manufacturers both in the domestic and foreign markets [1, 4, 6].

30 names are currently known. The need to include beta-blockers in the program for the treatment of cardiovascular diseases (CVD) is obvious: over the past 50 years of cardiac clinical practice, beta-blockers have taken a strong position in the prevention of complications and in the pharmacotherapy of arterial hypertension (AH), coronary heart disease (CHD), chronic heart failure (CHF),

metabolic syndrome (MS), as well as in some forms of tachyarrhythmias. Traditionally, in uncomplicated cases, drug treatment of hypertension begins with beta-blockers and diuretics, which reduce the risk of myocardial infarction (MI), cerebrovascular accident and sudden cardiogenic death.

Bisoprolol is a highly selective beta-adrenergic receptor antagonist that reduces the rate and strength of heart contractions. Drugs of this group protect the heart from excessive excitability [4, 20].

The bioavailability of a medicinal substance is an objective characteristic of therapeutic efficacy [18, 19].

The study of the bioavailability of drugs, preparations or their dosage forms usually begins with experiments in vitro , and ends with experiments in vivo with further clinical study [2, 5, 11].

Study of dissolution in experiments by the in vitro are relatively easy to use and represent an important tool for characterizing the biopharmaceutical quality of drugs. The study of dissolution in experiments in vitro is an important instrumental method for characterizing the biopharmaceutical properties of drugs [11, 12, 14].

Biopharmacy, a branch of pharmaceutical science that studies the relationship between the physicochemical properties of drugs in a particular dosage form and their pharmacological action, appeared after the establishment of the facts of the therapeutic non-equivalence of drugs, i.e. a drug of the same composition, but manufactured by different companies, differed in effectiveness. This was due to a number of reasons: the grinding of medicinal substances; selection of auxiliary components and the difference in technological processes - the so-called. pharmaceutical factors. Each of these factors in itself is decisive in the pharmacological action of drugs. Biopharmacy is the theoretical basis for the technology of dosage forms. One of the biopharmaceutical criteria that determine the therapeutic efficacy of a medicinal substance is its bioavailability. The latter is provided by the dosage form, which must be pharmacokinetically justified and rational in terms of the qualitative and quantitative selection of auxiliary components [11,13, 17].

The first step in a bioavailability study is to determine the solubility or release time of the drug from the dosage form. It has been established that the solubility test in the first approximation characterizes the bioavailability of the drug, since in practice there is a very frequent correlation between the rate of dissolution and absorption [3, 11, 12].

Thus, when developing the composition and technology of any drug, special attention should be paid to the release of the active substance.

The purpose of this work was to study the release time of the active substance from the dosage form – "Biskor" tablets in experiments in vitro and in vivo.

The tablets obtained according to the recommended composition and technology are round, biconvex, film-coated tablets of white or almost white color. In appearance, they comply with the requirements of GF XIII.

The main way to assess the biopharmaceutical properties of drugs in experiments in vitro is the "Rotating Basket" method included in SP XIII. Therefore, to determine the release rate of bisoprolol fumarate from the recommended tablets "Biskor" experiments were carried out by this method.

The Dissolution test was carried out on the Rotating Basket, Paddle Stirrer, and Flow Cell devices; the number of rotations of the paddle or basket, as well as the flow rate, were selected during the experiment. It is known from the literature data that many factors influence the release rate of the active substance: the excipients used, the volume and pH of the dissolving medium, and the basket rotation speed [3, 5, 13, 15, 16].

As a medium for dissolution, solutions were used, the pH values of which correspond to the pH values in different parts of the gastrointestinal tract.

To select the optimal pH value of the dissolving medium, we used dissolving media with different pH values in our studies. As neutral - purified water, acidic - 0.1 n solution of hydrochloric acid and alkaline - 0.1 n sodium hydroxide solution. In the experiments, the volume of the dissolving medium was 900 ml. This volume

was established taking into account the sensitivity of the method developed by us for the quantitative determination of active substances in “Biskor” tablets.

The study was carried out as follows: 1 tablet was placed in a vessel for dissolution.

Every 15 minutes, about 50 ml was taken from the center of the dissolution vessel and filtered through a Blue Ribbon or Millipore paper filter, discarding the first portions of the filtrate.

We measured the optical density of a solution of RSO of bisoprolol fumarate and test solution on a UV spectrophotometer at a wavelength of 240 nm ±1 nm, in a cuvette with a layer thickness of 10 mm, using the dissolution medium as a reference solution.

Amount of RSO of bisoprolol fumarate, which passed into solution from the tablet, in percent (*X*), was calculated by the formula:

$$X = \frac{D_1 \cdot m_0 \cdot 900 \cdot 1 \cdot P \cdot 100}{D_0 \cdot L \cdot 100 \cdot 100 \cdot 100} = \frac{D_1 \cdot m_0 \cdot 0{,}09 \cdot 1 \cdot P}{D_0 \cdot L},$$

Where:

D_1 - optical density of the test solution;

D_0 - optical density of the RSO solution RSO bisoprolol fumarate ;

t_0 - mass of sample RSO RSO bisoprolol fumarate , in mg;

L - the declared amount of RSO of bisoprolol fumarate in one tablet, indicated in the section “Composition per tablet”, in mg;

P - content of RSO of bisoprolol fumarate in RSO RSO bisoprolol fumarate, in %.

Amount of RSO of bisoprolol fumarate , which passed into solution after 45 minutes, should pass at least 75%.

The first stage of the study was to study the effect of the pH of the dissolving medium on the dissolution rate of “Biskor” tablets .

The results of studying the influence of the pH of the dissolving medium on the dissolution rate of the “Biskor” tablets are shown in Fig.1.

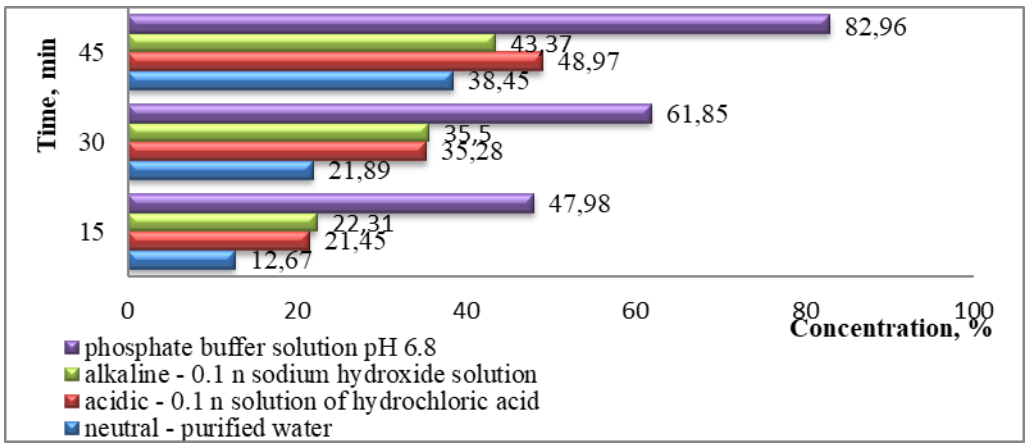

Fig.1. The results of studying the influence of the pH of the dissolving medium on the rate of dissolution of the "Biskor" tablets

As can be seen from the data in Figure 1, the pH of the dissolving medium affects the release rate of the active substance (bisoprolol fumarate) from the studied tablets.

Based on the results of studying the effect of medium pH on the rate of dissolution of "Biskor" tablets, we recommended the use of a phosphate buffer solution pH 6.8 for further research.

When developing the "Dissolution Test", the intensity of the release of biologically active substances, studies were carried out to establish the optimal speed of rotation of the basket.

The experiments were carried out at basket rotation speeds of 50, 100, 150, and 200 rpm.

Figure 2 shows the results of the experiment.

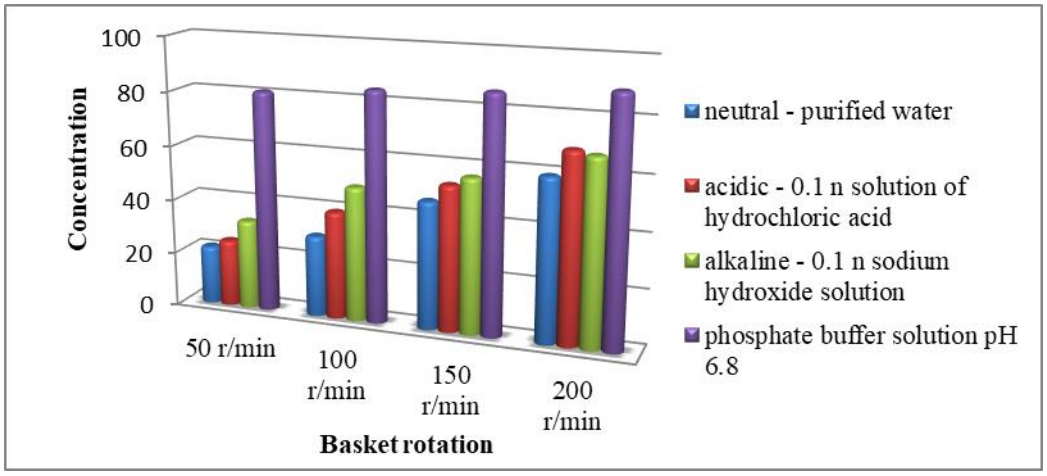

Fig.2. The results of studying the effect of basket rotation speed on the intensity of bisoprolol release fumarate

The figure shows that the release of the active substance from the tablets “Biskor” at different speeds of rotation of the basket occurs intensively.

From the results obtained, it can be seen that the result obtained from the investigated media with solvents in all media, except for the medium with phosphate buffer, does not correspond to the required level (75% or more) after 45 minutes. Also, in experiments with phosphate buffer solution of circulating basket, the rotation speed of basket is 50, 100, 150 and 200 rpm. Almost the same results were obtained with (81.04 - 89.32%).

Thus, for the release of active substances from “Biskor” tablets, the following conditions are determined: the dissolution medium is a phosphate buffer solution pH 6.8. The volume of the dissolution medium is 900 ml. Basket rotation speed -50 rpm. Temperature (37±0.5) °C.

The next stage of the study was carried out by the in vivo. Study by the method “in vivo” began with the study of the bioequivalence of the recommended drug with its analogue. In order to study bioequivalence, studies were carried out to study specific activity on a model of adrenaline hypertension in rabbits.

The specific activity of the compared preparations “Biskor” - tablets and “Bisoprol ®” - tablets, produced by PJSC Farmak, Ukraine was studied on a model of adrenaline hypertension in rabbits [6, 7, 8, 11].

For the experiment, 6 rabbits were used, weighing 2100 - 2400 g, which were divided into 2 groups of 3 individuals. The animals were euthanized under urethane anesthesia, the cervical region was opened, and the carotid artery was found. To record blood pressure, a special glass cannula was installed in the carotid artery, to which it was connected through a mercury manometer to a kymograph.

Rabbits were injected intravenously (through the ear vein) with a 0.001% solution of adrenaline at a dose of 5 μg/kg. With intravenous injection of adrenaline, blood pressure rises for 5 seconds due to irritation of alpha receptors, called the alpha phase, after which, within 1-2 minutes, blood pressure decreases

due to irritation of beta receptors below the level of normal blood pressure, this phase is called the beta phase. This period lasts 2-3 minutes, after which the blood pressure returns to normal.

To study the hypotensive effect, the compared drugs in the form of 1% aqueous solutions were administered to rabbits per os through a pre-installed gastric tube at a dose of 5 mg/kg, blood pressure was measured after 3 hours.

After 5 hours, the rabbits were re-intravenously (through the ear vein) injected with a 0.001% solution of adrenaline at a dose of 5 μg/kg.

Statistical calculations were carried out by the method of variation statistics with the calculation of the Student's criterion using the STATISTICA program for Windows 95 [9, 10, 19].

When studying the antihypertensive activity of the compared drugs, the kymogram reflected an increase in blood pressure, similar to the alpha phase. After that, there was a decrease in blood pressure to normal without the beta phase. This is due to the fact that bisoprolol is a beta blocker and eliminates the beta phase (table 1).

Table 1

Antihypertensive effect of drugs "Biskor" and "Bisoprol ®" PJSC Farmak, Ukraine

A drug	Weight, grams	BP is normal	HELL-Beta phase	Difference HELL	BP-after introductions drugs	AD-beta phase introductions drugs	difference
Recommended tablets "Biskor"	1400	120	100	20	95	95	0
	1200	125	100	25	95	90	5
	1300	130	110	20	100	100	0
	1300±100.0	125±5.0	103.3±5.7	21.6±2.8	96.6 ± 2.8	95±5	1.6 ± 1.8
Tablets "Bisoprol ®" PAR Farmak	1300	125	105	20	95	90	5
	1100	115	100	15	90	90	0
	1300	120	100	20	95	95	0

Ukraine	1233±115.5	120±5	101.7±2.8	18.3±2.8	93.3±2.8	91.6±2.8	1.6 ± 1.8
		P>0.5	P>0.5	P>0.5	P>0.5	P>0.5	P >0 5

The table shows that intravenous administration of adrenaline reduced blood pressure in the ß-phase by 17-3% of the norm. The introduction of adrenaline 5 hours after the drug “Biskor” the level of the ß-phase decreased by only 1.6%, this is due to the fact that the test drug had a blocking effect.

Thus, the study of the acute toxicity of the drug “Biskor” - tablets 5 mg in comparison with the analogue drug “Bisoprol®” - tablets 5 mg, produced by PJSC Farmak Ukraine, has an antihypertensive effect, i.e., the drugs were biologically equivalent in terms of specific action.

The study of the toxicity of the recommended tablets “Biskor” by the in vivo showed that the recommended drug is not toxic (Table 2).

Table 2

The results of determining the acute toxicity (LD_{50}) of the recommended drug “Biskor” with the analogue “Bisoprol ®” PJSC Farmak

Study drugs						
No.	**Recommended tablets “Biskor”**			**Tablets “Bisoprol ®” PJSC Farmak, Ukraine**		
	Route of administration- per os					
animal groups	**animal weight, mg/kg**	**Dose, ml**	**Result**	**animal weight, mg/kg**	**Dose, ml**	**Result**
1	100	0.4	0/6	100	0.4	0/6
2	150	0.6	0/6	150	0.6	0/6
3	200	0.8	0/6	200	0.8	0/6
LD_{50} >200 mg/kg						

Conclusions:

1. Based on the results obtained on the study of the influence of the pH of the medium on the dissolution rate of the “Biskor” tablets, the use of a phosphate buffer solution of pH 6.8 is recommended for further research.

2. In the experiments, the volume of the dissolving medium was set at 900 ml, which was chosen taking into account the sensitivity of the method we developed for the quantitative determination of active substances.

3. Based on the obtained data, for further study of the quality of the finished product from a biopharmaceutical point of view, a basket rotation speed of 50 rpm is recommended, which is included in the ND.

4. Recommended tablets “Biskor” with an analogue drug have an antihypertensive effect, i.e. According to the specific action, the preparations were biologically equivalent and non-toxic.

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