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The study of dissolution kinetics of drugs with riboxinum (inosine)

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ABSTRACT

The study of dissolution kinetics of drugs in the form of tablets with riboxinum (inosine) has been carried out in accordance with the requirements of the "biowaiver" procedure, the recommendations of the SPhU and the WHO requirements in order to assess the possibility of replacing the pharmacokinetic studies *in vivo* by tests *in vitro*. The possibility to use the recommendations of the "biowaiver" procedure for the registration of generics with riboxinum has been found.

Key words: standardization, riboxinum, dissolution, tablets.

INTRODUCTION

Inosine is a derivative of purine and is considered as a precursor of ATP. It has the anabolic action, activates metabolism of the myocardium, increases the activity of some enzymes of the Krebs cycle, stimulates the synthesis of nucleotides, inhibits the process of destruction of sarcolemma of ischemic cardiac myocytes and provides intracellular transport of energy. By improving microcirculation the drug reduces the size of the necrosis area and myocardial ischemia [1]. Traditionally in the countries of the former USSR inosine is used as a drug under the name of Riboxin.

When using riboxinum the increased glycolysis, increase in the activity of lactate dehydrogenase, activation of the aerobic pathways of glucose: enzymes of the Krebs cycle, the pentose phosphate cycle, aerobic decarboxylation of pyruvic acid, the respiratory chain of electron transport providing the normal process of the tissue respiration are observed [2].

It is known from literature that riboxinum (inosine) is successfully used in treating chronic ischemic heart disease, in the post-infarction period, dystrophy of the myocardium, arrhythmias, circulatory inefficiency caused by chronic respiratory diseases, tonsillogenic heart disease, changes of central hemodynamics caused by cerebral palsy, etc. [3-6].

Inosine is a natural metabolite of a living organism, has low toxicity and almost no side effects; it makes possible to use it intravenously (up to 0.4 g per day) and in the form of tablets in rather high doses (2.4 g per day). The injectable form of riboxinum is used in severe cardiac pathologies; tablets of riboxinum have a mild action and are widely used by the population, including the preventive purposes. It is noted that under the effect of drugs the health of patients improves already on the second day, vivacity and increased efficiency appear [7].

In Ukraine tablets with riboxinum are produced by different manufacturers. The aim of our research was to study dissolution kinetics of drugs in the solid dosage form with riboxinum in order to assess their equivalence under conditions *in vitro* according to the "biowaiver" procedure [8,9].

MATERIALS AND METHODS

The study object are a generic drug “Riboxin” in the form of film-coated tablets (manufactured by “Technolog” PJSC, Ukraine); the reference drugs in the form of tablets “Riboxin-BCPP” (manufactured by PJSC SIC “Borshchahivskiy CPP”, Ukraine) and “Riboxin” (film-coated tablets manufactured by “Borisovskiy zavod meditsinskikh preparatov” JSC BAT, Republic of Belarus). These drugs contain the same amount of the active pharmaceutical ingredient – riboxinum and similar excipients, i.e. they are pharmaceutically equivalent drugs.

The riboxinum substance by “Starlake Bioscience Co. Inc. Znaoqing Guangdong Starlake Biochemical Pharmaceutical Factory” company (China) and the reference standard of riboxinum (inosine) – 9-β-D-ribofuranosyl hypoxanthine, batch 1 dated 20.04.2012 (RS SPhU) were used in the work for preparation of the reference solution. Analytical studies were carried out on a Specord 205 spectrophotometer of “Analytik Jena AG” firm (Germany), and an “Erweka” device (Germany) for dissolving solid dosage forms.

The study of dissolution kinetics was conducted in accordance with the monograph of the SPhU, Supplement 2 “5.N.2. Studies on bioavailability and bioequivalence of generic medicines” [10], Guidance on bioavailability and bioequivalence research [11], methodological recommendations [12], as well as the WHO Guide [13,14] in three media with different pH values: hydrochloric acid solution with pH 1.2, acetate buffer solution with pH 4.5 and phosphate buffer solution with pH 6.8. All buffer solutions were prepared according to the SPhU [10]. Degassing of the dissolution media was carried out by heating to a temperature of (40+2)°C followed by filtration under vacuum through a membrane filter with the pore size of 45 μm and vigorous stirring under vacuum for 5 min.

The conditions for performing the "Dissolution" test: “Erweka” apparatus, the device with basket was used; the volume of the dissolution medium – 1000 ml; the temperature of the dissolution medium – (37.0+0.5)°C; the rotation speed of the basket – 100 rpm. Sampling was carried out in 15, 30 and 45 min manually with a 10.0 ml pipette from the plot midway between the surface of the dissolution medium and the basket at the distance of 2 cm from the wall of the dissolution vessel. The samples obtained were filtered through a filter paper with the pore size of from 2 to 3 μm. 5.0 ml of the filtrate obtained was diluted to the volume of 100.0 ml with the corresponding dissolution medium. The volume selected was compensated by the corresponding dissolution medium. To obtain statistically reliable results the test was carried out on 12 samples of each of the study objects.

The equivalence of dissolution kinetics of drugs in the form of tablets with riboxinum was assessed by the value of the similarity factor (f_2), which should be from 50 to 100, in order to make a conclusion about conformity of the kinetic curves:

$$f_2 = 50 \times \log f(x) = \left\{ \left[1 + \left(\frac{1}{n} \right)_{t=1}^n (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}$$

where: n – is the number of control points;

R(t) – is the mean value of the quantitative determination of the active substance passed into the solution at each specified sampling point when studying the reference drug (%);

T(t) – is the mean value of the quantitative determination of the active substance passed into the solution at each specified sampling point when studying the generic drug (%).

For each time interval the standard deviation of the mean value (SD) was calculated. It must keep the following requirements: should be less than 10% starting from the second to the last point of control; less than 20% for the first time point.

RESULTS AND DISCUSSION

Riboxinum is well absorbed in the digestive tract, rapidly distributed in tissues, metabolized in the liver where it is completely utilized in the biochemical reactions of the body. It is eliminated mainly by the kidneys as metabolites [1].

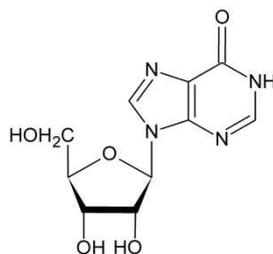


Fig. 1. Riboxinum (inosine)

Riboxinum (inosine) (Fig. 1) – 9-β-D-ribofuranosyl hypoxanthine – is sparingly soluble in water, practically insoluble in 96 % alcohol and chloroform.

Determination of solubility was carried out for the riboxinum substance, and it was found that the highest single dose (2400 mg of riboxinum) was soluble in 250 ml of the hydrochloric acid medium with pH 1.2, acetate buffer solution with pH 4.5 and phosphate buffer solution with pH 6.8.

The assay of riboxinum when conducting the research *in vitro* was performed by spectrophotometry. The optical density of test solutions was measured at the absorption maximum at the wavelength of 249 nm in buffer solutions with pH 1.2, 4.5 and 6.8, in a cell with the layer thickness of 10 mm. In parallel, the optical density of the reference solution (riboxinum RS) was measured. The typical absorption spectra of riboxinum RS when studying dissolution kinetics are presented in Fig. 2.

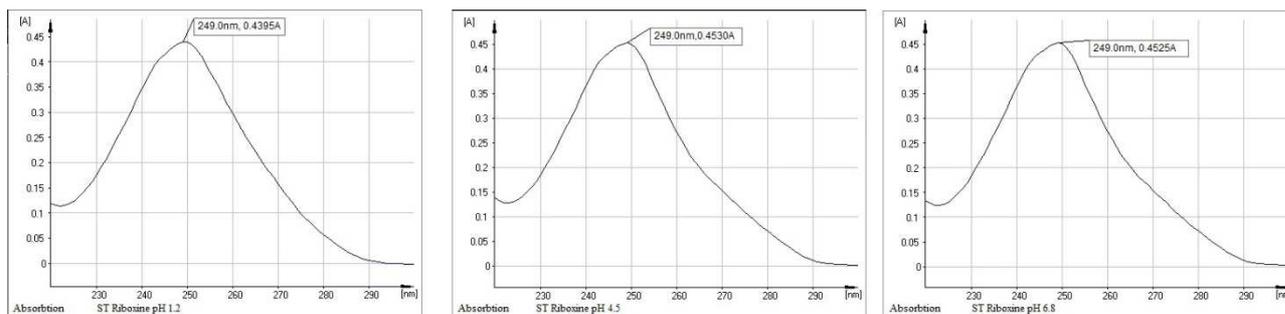


Fig. 2. The UV absorption spectra of riboxinum RS in buffer solutions

Kinetic curves of dissolution of riboxinum for “Riboxin”, “Riboxin-BCPP”, and the reference drug “Riboxin” RB in three dissolution media are presented in Fig. 3–5.

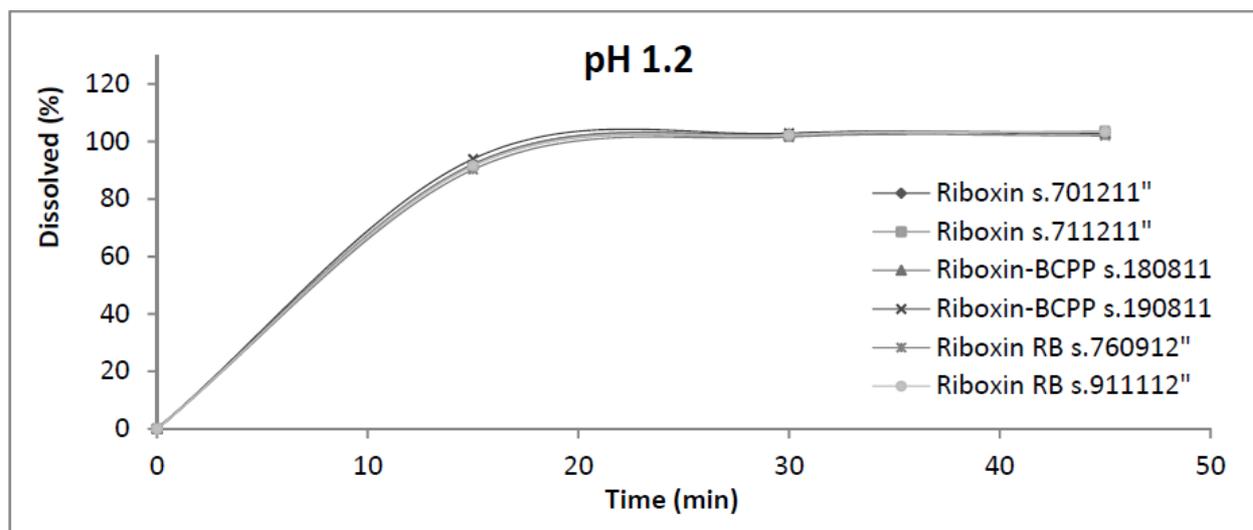


Fig. 3. Kinetic curves of dissolution of riboxinum in a buffer solution with pH 1.2

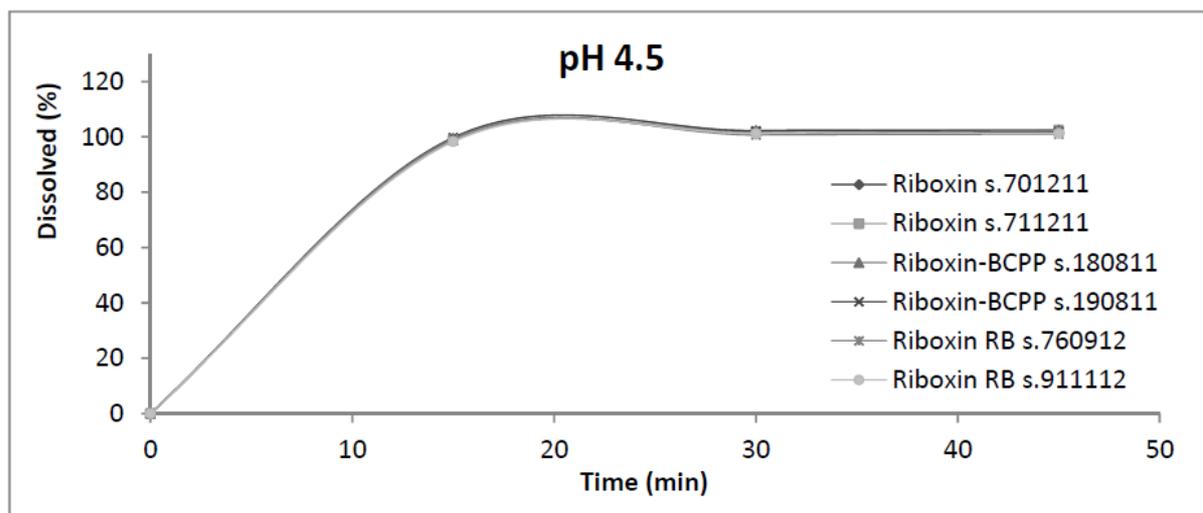


Fig. 4. Kinetic curves of dissolution of riboxinum in a buffer solution with pH 4.5

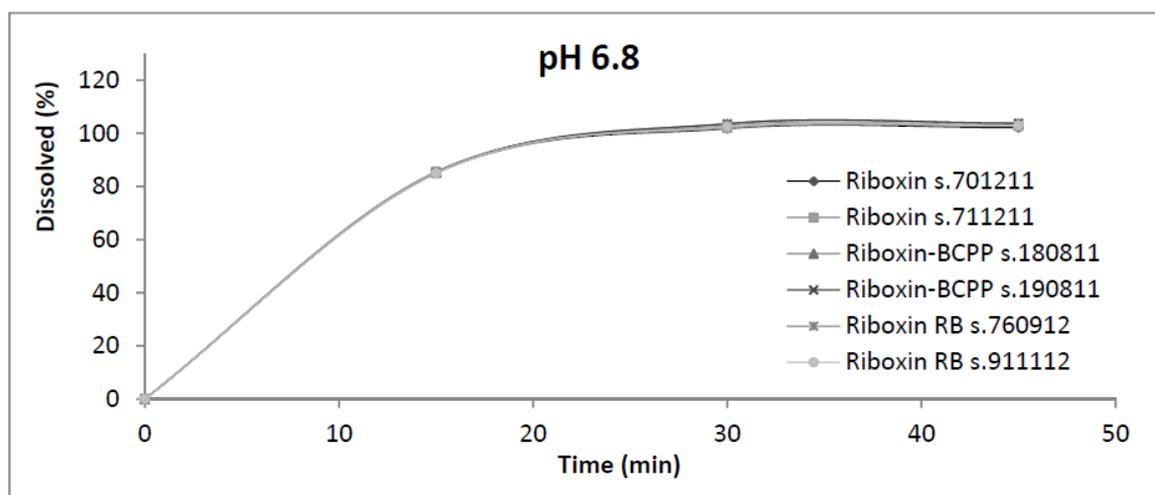


Fig. 5. Kinetic curves of dissolution of riboxinum in a buffer solution with pH 6.8

As shown in Fig. 3–5, the dissolution profiles (kinetic curves of dissolution) of drugs “Riboxin”, “Riboxin-BCPP” and “Riboxin” RB in each of the three dissolution media recommended are similar.

To confirm the equivalence of drugs *in vitro* the values of riboxinum dissolution at each time point were determined and the value of the similarity factor was calculated. The results obtained are given in Table 1.

Table 1 The results of *in vitro* studies to confirm the equivalence of drugs “Riboxin”, “Riboxin-BCPP” and “Riboxin” RB

No.	Time, min	Dissolution of riboxinum, %					
		“Riboxin” tablets		“Riboxin-BCPP” tablets		“Riboxin” RB tablets	
		s.701211	s.711211	s.180811	s.190811	s.760912	s.911112
The medium with hydrochloric acid and pH 1.2							
1	15	91.95	91.61	92.14	93.95	90.25	91.53
2	30	102.65	101.77	102.59	102.89	101.54	102.31
3	45	102.95	103.59	102.63	102.92	102.01	103.33
The similarity factor f_2 is not calculated		More than 85% of substances are released for 15 min					
Acetate buffer solution with pH 4.5							
1	15	98.60	98.72	99.59	99.78	99.23	98.28
2	30	101.18	101.79	102.32	102.14	100.69	101.25
3	45	101.63	102.46	102.49	101.90	100.97	101.37
The similarity factor f_2 is not calculated		More than 85% of substances are released for 15 min					
Phosphate buffer solution with pH 6.8							
1	15	85.02	85.57	85.12	85.19	84.99	85.03
2	30	101.76	102.06	103.60	103.65	102.92	102.56
3	45	102.19	102.72	103.74	103.88	103.08	102.98
The similarity factor f_2 is not calculated		More than 85% of substances are released for 15 min					

On the basis of the data obtained it has been found that the equivalence of dissolution profiles for all recommended dissolution media is observed (pH 1.2, 4.5 and 6.8) for the drugs studied. In all three dissolution media the release of riboxinum is more than 85% in 15 min (Table 1), i.e. the drugs under research can be classified as "highly soluble", and their equivalence can be determined by the method *in vitro*.

The studies conducted have shown that riboxinum can be referred to class I of the biopharmaceutical classification system, i.e. substances with a high biopharmaceutical solubility and a high penetration rate. It will allow conducting comparative studies *in vitro* to confirm the equivalence of drugs.

CONCLUSION

According to the results of the comparative study of dissolution kinetics the equivalence of dissolution profiles for the drugs under research – “Riboxin”, “Riboxin-BCPP” and “Riboxin” RB at pH 1.2, 4.5 and 6.8 has been determined without calculating the similarity factor (the release of riboxinum is more than 85% in 15 min in three media). Using the "biowaiver" procedure it has been found that the drugs with riboxinum studied are equivalent.

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