Sys Rev Pharm 2020;11(12):2050-2053
A multifaceted review journal in the field of pharmacy

Colloid-Chemical and Antimicrobial Properties of Ribavirin Aqueous Solutions

[1]Sardushkin M.V., [2]Shiryaeva Y.K., [3]Donskaya L., [4]Vifor R., [5]Donina M.V.

[1-5]Mendeleev University of Chemical Technology of Russia
[1]s_makar@mail.ru, [2]yuliashiryaeva988@yandex.ru, [3]donskaya.1863@mail.ru, [4]viforimma@mail.ru, [5]ethersy@yandex.ru

Methods of quantitative determination of ribavirin were developed using capillary electrophoresis and UV spectroscopy. It is shown that aqueous solutions of ribavirin exhibit not only antiviral, but also antibacterial activity against Pseudomonas aeruginosa, Staphylococcus aureus, Carynebacteria stationis and Bacillus subtilis and antimykotic activity against Candida albicans.

Index Terms: antimicrobial activity, capillary electrophoresis, ribavirin, UV spectroscopy.

The work was supported by Mendeleev University of Chemical Technology of Russia. Project Number K-2020-024.

INTRODUCTION

The habituation and resistance of microorganisms to known antimicrobial drugs, in particular to antibiotics, is a major problem today. The synthesis of new biologically active compounds and the development of modern drugs based on them is an urgent task. However, it is necessary to pay attention to the fact that the properties of some substances already known are not sufficiently studied.

From this point of view, an attractive object of research is ribavirin, a widely used antiviral drug as part of a number of complex therapies, in particular, against hepatitis C viruses and certain strains of influenza virus. Despite the fact that the antiviral properties of ribavirin have been known for a long time, the mechanism of its action has not been clearly studied [1-3], moreover, few studies indicate that in addition to antiviral properties, ribavirin also exhibits antibacterial properties [4].

The purpose of this study was to identify the antimicrobial activity of ribavirin in relation to a number of microorganisms, as well as to develop methods for its quantitative determination by modern methods of physical and chemical analysis.

EXPERIMENTAL

Ribavirin (1-β-D-ribofuranosyl-1H-1,2,4-triazole- 3-carboxamide or 1 - [(2R,3R,4S,5R) - 3, 4 - dihydroxy - 5-(hydroxymethyl) oxolan-2-yl] - 1H - 1, 2, 4 – triazole – 3 - carboxamide; Fluka, USA; analytically pure, >98%; CAS 36791-04-5) and Ribavirin Canon tablets and capsules (200 mg, Canonpharma Production, batch 030817; Russia) were used in the work.

Measurements were made on a Shimadzu UV-2600 spectrophotometer (Japan). Capillary electrophoresis was performed using the "Kapel-103RT" device manufactured by Lumex (Russia). Evaluation of antibacterial and antimycotisch activity was carried out by the disco-diffusion method and the method of serial dilutions. *Staphylococcus aureus* FDA 209P, *Carynebacteria stationis* VKPM-B-10645, *Bacillus subtilis* VKPM-B-13183, *Essherichia coli* ATCC 25922, *Pseudomonas aeruginosa* VKPM-B-8243, *Candida albicans* VKPM-Y-3108 and *Aspergillus niger* VKPM-F-428 was used as test-organisms.

RESULTS AND DISCUSSION

Fig. 1 shows the structural formula of ribavirin:

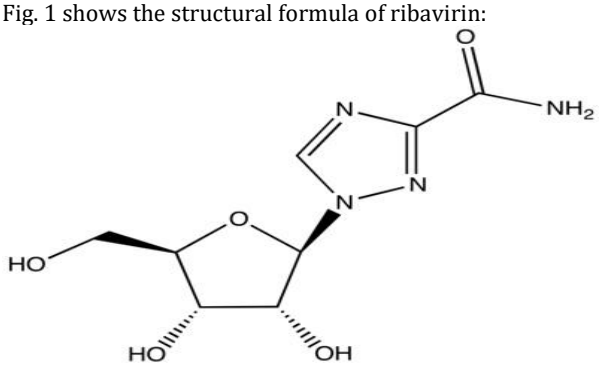


Figure 1. Structural formula of ribavirin.

There are a lot of methods for the quantitative determination of ribavirin in the literature [5-7], however, all of them are quite time-consuming and require specific equipment and reagents. The presence of a triazole ring in the ribavirin molecule suggests that its solutions can be analyzed by UV spectroscopy. Moreover, the development of this analysis method will make possible in the future to use other modern analytical equipment with UV detection. Fig. 2 shows the aqueous ribavirin solution UV spectrum. As you can see, the maximum absorption corresponds to a wavelength of 206 nm. Fig. 3 shows the calibration dependence of the optical density on the ribavirin concentration.

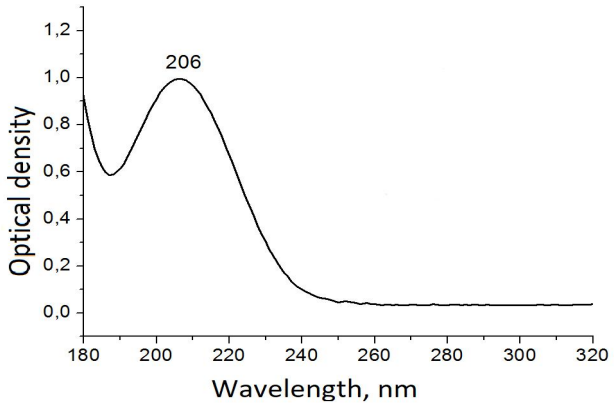


Figure 2. UV absorption spectrum of an aqueous solution of ribavirin (0.002%) in a 1-cm cuvette

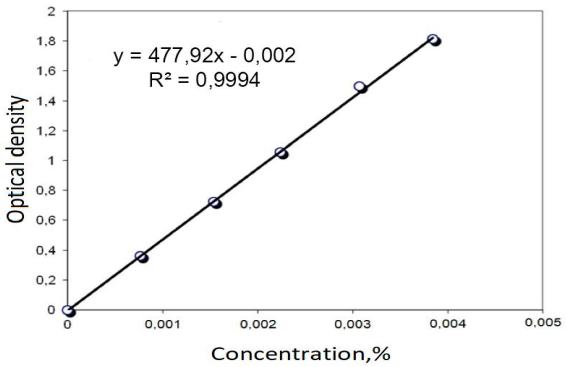


Figure 3. Ribavirin solution concentration dependence of its optical density

It should be noted that the position of the maximum absorption of ribavirin aqueous solutions does not depend on the solution pH, which allows to determine the content of ribavirin in both acidic and alkaline environments.

Table I presents results from obtained data statistical processing using a least-squares method.

Ribavirin tablets and capsules containing 200 mg of the main substance were analyzed according to the established method. For this purpose, a tablet was placed into a 100-mL volumetric flask, treated with distilled H_2O (70 mL), and shaken to disperse it completely. The volume of the resulting suspension was adjusted to the mark with doubly distilled H_2O and stirred. The suspension was filtered through No. 4 filter paper. The first portion of the filtrate (10 mL) was discarded. Next, filtrate (2 mL) was transferred into a 100-mL volumetric flask. The solution volume was adjusted to the mark using doubly distilled H_2O and stirred.

The optical density of the resulting solution was measured relative to doubly distilled H_2O in a 10-mm cuvette at 206 nm. Table II presents the obtained results.

Similar results were obtained for capsule, too. For doing this, the contents of the capsule were also placed in a 100-mL volumetric flask and subjected to the same procedures as in the case of tablets.

Thus, a quantitative method for determining ribavirin by UV spectroscopy was developed. The second method was capillary electrophoresis with UV detection.

Table I. Aggregation dependence of optical density of solutions of ribavirin on it concentration

f	$-x$	$-y$	b	a	$t\ (P; f)$ if P = 95%
4	$2{,}304 \cdot 10^{-3}$	1,0991	477,92	$-2 \cdot 10^{-3}$	2,78
s_0^2	r	s_x if $n_j = 1 \quad y_j = \bar{y}$	ΔX	$\frac{\Delta X \cdot 100}{\bar{x}}$, %	
$3{,}7 \cdot 10^{-4}$	0,9997	$3{,}13 \cdot 10^{-5}$	$8{,}7 \cdot 10^{-5}$	3,78	

Table II. Statistical characteristics of the results of the method for determining the content of ribavirin in tablets Ribavirin Canon 200 mg (ser. 030817)

Dosage of ribavirin tablets was 200 mg	
№ tablets	Ribavirin found, mg
1	195,2
2	202,2
3	207,0
4	195,6
5	200,4
Statistical characteristic	**Value**
Arithmetic mean, C	200,08
Dispersion, D	24,09
Standard (mean-square) deviation, S	4,91
Confidence interval of the average value	4,30

Conducting the experiment in the mode of zone electrophoresis, when the method is based on differences in the electrophoretic mobility of charged particles, the release time of ribavirin was very long due to the neutrality of its molecule. To solve this problem, conditions for micellar electrophoresis were selected, since in this case it is possible to determine neutral molecules due to the introduction of a surfactant into the buffer and the formation of a pseudo-stationary micellar phase. The optimal buffer solution was 20 mmol of tetraborate and 40 mmol of SPS.

Fig. 4 and 5 show a typical electrophoregram and calibration dependence.

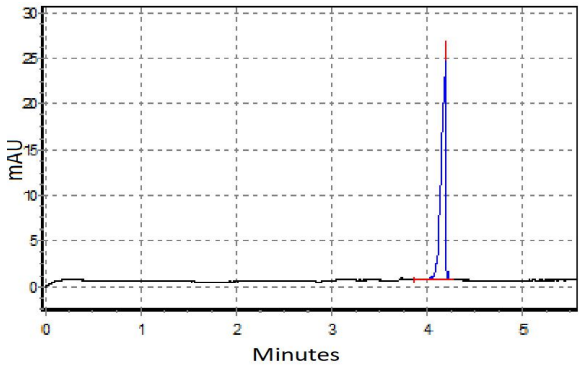


Figure 4. Electrophoregram obtained by micellar electrophoresis, ribavirin concentration 0.968 mg/mL

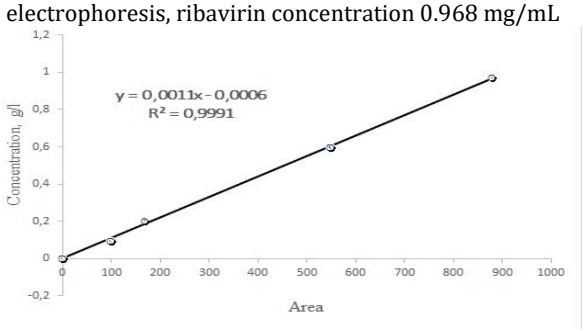


Figure 5. Calibration dependence for micellar electrophoresis

It should be noted that the proven methods of UV spectroscopy and capillary electrophoresis are in a good agreement with each other. The obtained results were used later in the study of the ribavirin aqueous solutions biological activity.

At the first stage of these studies, it was found that in aqueous solutions ribavirin shows a noticeable surface activity at various interfaces (Fig. 6 and 7), probably due to the presence of both hydrophilic and hydrophobic parts in the molecule (Fig. 1).

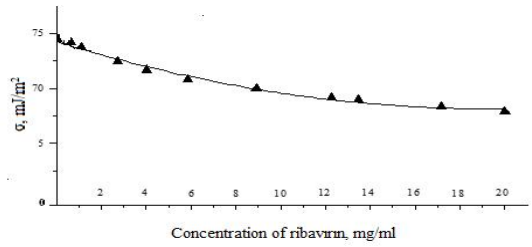


Figure 6. Surface tension σ isotherm of ribavirin aqueous solution

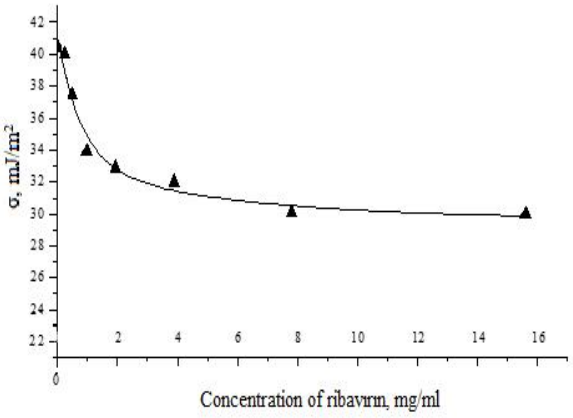


Figure 7. Ribavirin interfacial tension σ isotherm at the water-decane interface

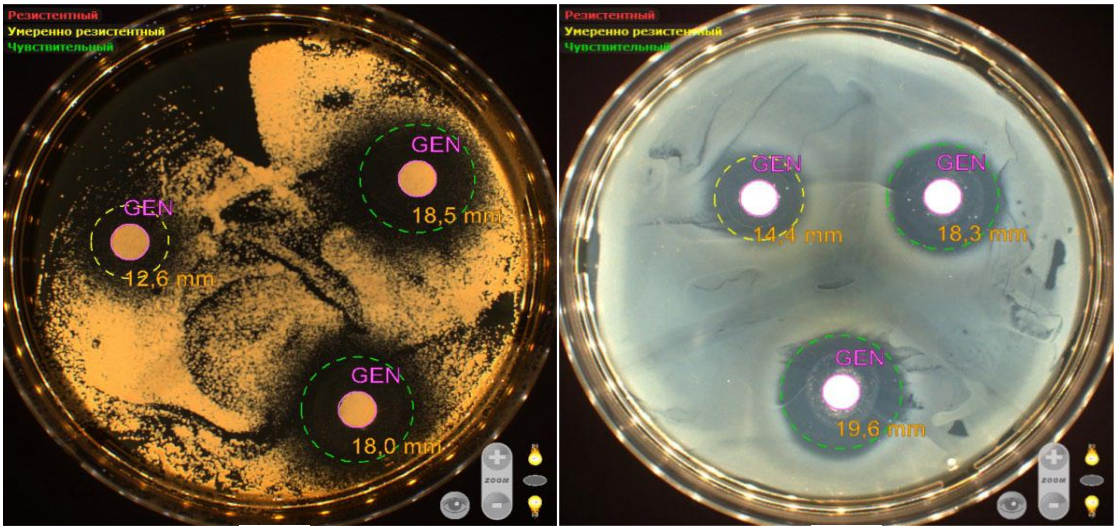
Based on the results presented in Fig. 6 and 7, the parameters of ribavirin adsorption layers were calculated (III), which can be useful in clarifying the mechanism of ribavirin action as an antiviral agent.

The obtained data may also be of interest for identifying the antibacterial mechanism of ribavirin action. The term "non-antibiotics" found in the literature can also be attributed to this compound. In particular, the author of the publication [8] observed the inhibition of *Salmonella typhi* in mice and found that ribavirin inhibits the virulence of *Salmonella typhi in vivo*, as well as *in vitro*.

The authors of this paper initially detected the antimicrobial activity of ribavirin against 2 test microorganisms – *Pseudomonas aeruginosa* and *Candida albicans* (Fig. 8).

Table III. Parameters of ribavirin adsorption layers at different phase interfaces

Surface activity g, mJ·m/mol	Maximum adsorption Γ_{max}, μmol/m²	Landing area S_0, nm²	Thickness of the adsorption layer δ, nm
At the interface between water solution and air			
2,25	1,38	1,20	0,16
At the interface between the water and decan phases			
2,50	1,52	1,15	0,15



(a) (b

Figure 8. Inhibition zones at ribavirin concentrations: 6, 8, 9 mg/mL (a) of *Candida albicans*, (b) of *Pseudomonas aeruginosa*.

It has been shown that increasing the ribavirin concentration above 9 mg/mL to prevent the growth of Pseudomonas aeruginosa and Candida albicans is impractical. It should be noted that this concentration is not at all high and much less than the recommended therapeutic doses used, for example, in the composition of the Devirs cream based on ribavirin, in which the concentration of the active substance is 75 mg/mL.

Later, it was found that ribavirin also shows antibacterial activity against some other microorganisms (IV). The "plus" sign indicates the presence of microbial growth, the "minus" sign indicates the absence of growth.

Table IV. Antibacterial activity of ribavirin at different concentrations of the aqueous solution

Test-organisms	Ribavirin concentration, mg/mL		
	10	5	2,5
St. aureus	—	—	+
C. stationis	—	+	+
E. coli	+	+	+
B. subtilis	—	+	+
As. niger	+	+	+

As can be seen from the table, at comparable concentrations of the previous experiment (no higher than 10 mg/ml), ribavirin is active against *Staphylococcus aureus*, *Carynebacteria stationis* and *Bacillus subtilis* and does not show activity against *E. coli* and *Aspergillus niger*.

CONCLUSION

1. The possibility of quantitative determination of ribavirin in aqueous solutions by UV spectroscopy and capillary electrophoresis was shown.
2. It was found that aqueous solutions of ribavirin have surface-active properties.
3. Ribavirin antimicrobial activity was detected against gram-positive (*Staphylococcus aureus, Carynebacteria stationis* and spore-forming *Bacillus subtilis*), gram-negative (*Pseudomonas aeruginosa*) bacteria, and fungi (*Candida albicans*).

Authors thanks phD, docent Mendeleev University of Chemical Technology of Russia K.I. Kienskaya and phD, docent Mendeleev University of Chemical Technology of Russia I.A. Butorova.

REFERENCES

1. Marukian S. et al., "Hepatitis C virus induces interferon-λ and interferon-stimulated genes in primary liver cultures," Hepatology, vol. 54, no. 6, pp. 1913–1923.
2. Dusheiko G., Nelson D., Reddy K., "Ribavirin considerations in treatment optimization," Antivirial Therapy, vol. 13, no.1, pp. 23-30.
3. Ghany M.G., Nelson D.R., Strader D.B. et al., "An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American Association for the Study of Liver Diseases," Hepatology, vol. 54, no. 4, pp. 1433–1444.
4. Kruszewska H, Zareba T, Tyski S. "Search of antimicrobial activity of selected non-antibiotic drugs," Acta Pol Pharm, vol. 59, no. 6, pp. 436-439.
5. Stryjek-Kaminska D., Ochsendorf F., Rőder C., "Photoallergic skin reaction to ribavirin," The American journal of gastroenterology, vol. 94, no. 6, pp. 1686-1688.
6. El-Brashy A.M., Sheribah Z.A., Sharaf El-Din M.K., El-Gamal R.M., "Kinetic determination of ribavirin in drug formulations," International journal of biomedical science: IJBS, vol. 3, no. 1, pp. 65-71.
7. Paroni R., Sirtori C.R., Borghi C., Kielne M.G., "High-performance liquid chromatographic determination of ribavirin in serum and urine and of its urinary metabolite 1, 2, 4-triazole-3-carboxamide," Journal of Chromatography B: Biomedical Sciences and Applications, vol. 64, pp. 189-196.
8. Mandal, R. S. et al. "Ribavirin suppresses bacterial virulence by targeting LysR-type transcriptional regulators," Scientific reports, vol. 6, p. 39454; doi: 10.1038/srep39454 (2016).