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Performing a physiologically relevant test for cladribine tablets

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Abstract

Introduction. The introduction of devices – analogues of GIS (hereinafter – Gastro-intestinal simulator) is one of the current ways to develop in-vitro assessment of the quality of solid dosage forms. Testing on a physiologically relevant test device (hereinafter referred to as PRT) makes it possible to predict pharmacokinetic profiles due to more relevant conditions, including the use of biorelevant dissolution media, physiological volumes of the gastrointestinal tract, as well as transit between them.

Aim. Conduct a study of cladribine tablets on a physiologically relevant tester in order to predict the behavior of the drug in the human gastrointestinal tract.

Materials and methods. The objects of the study are "Mavenclad®, tablets, 10 mg" (series 2200754, expiration date until 04.2025, NERPHARMA, S.r.L., Italy) and "Cladribine, tablets, 10 mg" of Russia with valid expiration date. During the study, the reagents necessary for the preparation of biorelevant dissolution media and assay by HPLC were used. Physiologically relevant test were carried out using an apparatus of our own production, consisting of a DT-6 dissolution tester (ERWEKA GmbH, Germany), a water bath equipped with a Thermomix WB-4 heating element (B. Braun, Germany), and peristaltic pumps (Kamoer, China). The assay of released cladribine was assessed using a HPLC system "Khromatek-Kristall HPLC 2014" (JSC CDO "Khromatek", Russia) using a validated method at a wavelength of 252 nm, analysis time – 7 min, column – Grace HPLC Column Platinum C18-EPS, 250 × 4.6 mm, 5 mm (Grace, USA), temperature – 35 °C, elution mode – isocratic (A : B 80 : 20), mobile phase A – 0.1 % H_3PO_4 solution, phase B – acetonitrile.

Results and discussion. Profiles were obtained to assess the dynamics and degree of release of the studied drugs in various parts of the human gastrointestinal tract. Despite the expected degradation of cladribine in an acidic environment (pH1.2), under physiologically relevant conditions, the drug reached the third section (small intestine model) without degradation. Complete release of cladribine from the test and reference dosage forms was observed. Also, in the future, based on the data obtained, it is possible to predict pharmacokinetic profiles using physiologically based pharmacokinetic modeling approaches.

Conclusion. A PSF study was conducted for the drugs "Mavenclad®, tablets, 10 mg" and "Cladribine, tablets, 10 mg". Assay was carried out by HPLC-UV method. The test results showed complete release of both drugs and reaching the intestinal tract, indicating the absence of degradation of cladribine in the region simulating the stomach.

Keywords: cladribine, physiologically relevant test, biorelevant media, HPLC-UV, FaSSIF, FaSSGF

Conflict of interest. The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

Contribution of the authors. Andrey M. Poluyanov and Igor E. Shokhin invented and developed the experiment. Polina A. Losenkova, Danila D. Gvozdev, Alexandra V. Suvorova conducted a physiologically relevant test. Polina A. Losenkova quantitatively assessed the release using high-performance liquid chromatography. Polina A. Losenkova and Yuri V. Medvedev participated in data processing. Yuri G. Kazaishvili, Kira Ya. Zaslavskaya, Victoria S. Shcherbakova, Polina A. Losenkova. All authors participated in the discussion of the results.

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Проведение физиологически релевантного теста для таблеток кладрибина

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Резюме

Введение. Внедрение аппаратов – аналогов GIS (далее – Gastro-intestinal simulator) является одним из актуальных путей развития *in-vitro*-оценки качества твердых лекарственных форм. Испытания на приборе для физиологически релевантных тестов (далее – ФРТ) позволяют предсказать фармакокинетические профили за счет более релевантных условий, среди которых использование биорелевантных сред растворения, физиологичные объемы отделов ЖКТ, а также транзит между ними.

Цель. Провести исследование таблеток кладрибина на физиологически релевантном тестере с целью предсказания поведения препарата в ЖКТ человека.

Материалы и методы. Объектами исследования являются «Мавенклад®, таблетки, 10 мг» (серия 2200754, срок годности до 04.2025, NERPHARMA, S.r.L., Италия) и «Кладрибин, таблетки, 10 мг» отечественного производства с действующим сроком годности. Во время исследования использовались реактивы, необходимые для приготовления биорелевантных сред растворения и проведения количественного определения методом ВЭЖХ. Физиологически релевантный тест

проводили на аппарате собственного производства, состоящем из тестера растворения DT-6 (ERWEKA GmbH, Германия), водяной бани, оснащенной нагревательным элементом Thermomix WB-4 (B. Braun, Германия), насосов перистальтических (Kamoer, Китай). Количественное содержание высвободившегося кладрибина оценивали на высокоэффективном жидкостном хроматографе «Хроматэк-Кристалл ВЭЖХ 2014» (ЗАО СКБ «Хроматэк», Россия) по валидированной методике при длине волны 252 нм, время анализа – 7 мин, колонка – Grace HPLC Column Platinum C18-EPS, 250 × 4.6 мм, 5 мм (Grace, США), температура – 35 °C, режим элюирования – изократический (A : B 80 : 20), подвижная фаза A – 0,1%-й раствор H_3PO_4, фаза B – ацетонитрил.

Результаты и обсуждение. Были получены профили, позволяющие оценить динамику и степень высвобождения исследуемых ЛС в различных отделах ЖКТ человека. Несмотря на ожидаемую деградацию кладрибина в кислой среде (pH 1,2), в физиологически релевантных условиях препарат достиг третьего отдела (модель тонкого кишечника) без деградации. Наблюдалось полное высвобождение кладрибина из лекарственной формы для тестового и референтного лекарственных препаратов. Также в дальнейшем, исходя из полученных данных, можно предсказать фармакокинетические профили при помощи подходов физиологически обоснованного фармакокинетического моделирования.

Заключение. Проведено исследование ФРТ для препаратов «Мавенклад®, таблетки, 10 мг» и «Кладрибин, таблетки, 10 мг». Количественное определение проводилось методом ВЭЖХ-УФ. По результатам испытания было отмечено полное высвобождение обоих препаратов и достижение отдела, имитирующего кишечник, что указывает на отсутствие деградации кладрибина в отделе, имитирующем желудок.

Ключевые слова: кладрибин, физиологически релевантный тест, биорелевантные среды, ВЭЖХ-УФ, FaSSIF, FaSSGF

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Вклад авторов. А. М. Полуянов и И. Е. Шохин придумали и разработали эксперимент. П. А. Лосенкова, Д. Д. Гвоздев, А. В. Суворова провели физиологически релевантный тест. П. А. Лосенкова проводила количественную оценку высвобождения методом высокоэффективной жидкостной хроматографии. П. А. Лосенкова и Ю. В. Медведев участвовали в обработке данных. Ю. Г. Казаишвили, К. Я. Заславская, В. С. Щербакова, П. А. Лосенкова участвовали в написании текста статьи. Все авторы участвовали в обсуждении результатов.

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INTRODUCTION

The implementation of devices similar to GIS is one of the relevant and effective ways of development of modern in vitro studies of solid dosage form drugs [1]. Physiologically relevant testing allows for evaluation of drug release with a high level of discrimination. The advantage of this method is the use of biorelevant dissolution media simulating the composition of various sections of the gastrointestinal tract. Such media are represented by Fasted State Simulated Intestinal Fluid (FaSSIF) and Fasted State Simulated Gastric Fluid (FaSSGF) [2, 3].

It is important that this kind of testing be performed during pharmaceutical development of new drugs, especially if subsequent biowaiver study is foreseen, which is allowed for the Biopharmaceutics Classification System (BCS) Class I and Class III drugs.

As a BCS III drug, Cladribine is one of the drugs to which the biowaiver procedure may be applied. It should be noted that, due to higher pH in the model chamber (section) simulating the stomach (pH 2.84), it becomes possible to conduct PRT for this molecule because, to literature data, in acidic media Cladribine is degraded with formation of 2-chloro-6-aminopurin (chemical equation of Cladribine hydrolysis reaction is shown in Figure 1).

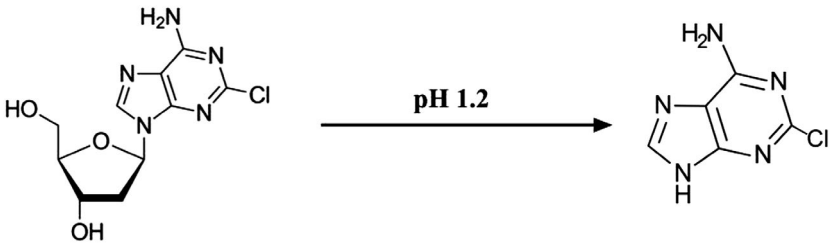

Figure 1. **Scheme of hydrolysis of the substance cladribine**

MATERIALS AND METHODS

Objects of study

The objects of study were as follows: "Mavenclad®, tablets, 10 mg" (series: 2200754, expiration date: 04.2025, NERPHARMA, S.r.L., Italy), and "Cladribine, tablets, 10 mg" (domestic production, expiration date: valid).

Reagents and solutions

The following reagents were used in the study: purified water, type I; concentrated hydrochloric acid (HCl), class "puriss." ("extra pure", LLC "Sigma Tec", Russia); concentrated orthophosphoric acid (H_3PO_4) concentrated (class "for HPLC", Scharlau, Spain); sodium hydroxide (NaOH), class "p.a." ("GR for analysis", LLC "Component-Reaktiv", Russia); sodium phosphate dibasic (Na_2HPO_4), anhydrous, class "puriss." ("extra pure", LLC "Component-Reaktiv", Russia); sodium chloride (NaCl), class "puriss." ("extra pure", LLC "Component-Reaktiv", Russia); 3F Powder media preparation mix (Biorelevant, United Kingdom); acetonitrile (ACN) (class "HPLC gradient grade", Biosolve, France).

We carried out the physiologically relevant test using a test apparatus of our own assembly, consisting of a DT-6 dissolution tester (ERWEKA GmbH, Germany), a water bath equipped with a Thermomix WB-4 heating element (B. Braun, Germany), and peristaltic pumps (Kamoer, China). Chromatographic separation and evaluation of quantitative content of Cladribine were carried out using a high-efficient liquid chromatograph "Khromatek-Kristall HPLC 2014" (JSC CDO "Khromatek", Russia) set at wavelength of 252 nm. Analysis run time reached 7 minutes. The column used was Grace HPLC Column Platinum C18-EPS, 250 × 4.6 mm, 5 mm (Grace, USA) placed in a column thermostat maintaining temperature of 35 °C throughout the analytical cycle. Separation was performed in isocratic elution mode (A : B 80 : 20); mobile phase A was presented by 0.1 % H_3PO_4 solution, phase B – by acetonitrile. Before the analysis, the procedure was validated by the following parameters: "specificity", "calibration curve (linearity)", "accuracy and repeatability", "accuracy and precision on different days", "stability". Analytical range of the method was from 0.0025 mg/ml to 2.0000 mg/ml.

RESULTS AND DISCUSSION

In the course of the study, samples obtained by PRT of Cladribine preparations were analyzed; assay was carried out by HPLC method.

PRT Procedure

In the experiment, the release of the drug was evaluated taking into account the physiological transit within the human GIT. Schematic view of the test apparatus is shown in Figure 2.

For the first chamber (section) of the test system we used FaSSGF solution – a biorelevant dissolution medium simulating the composition of gastric fluid in fasted state with a pH value of 2.0. To 50 ml of solution 250 ml of purified water was added (the volume corresponds to the amount of water that is usually washed down with a tablet). Total starting volume was 300 ml, and pH value of the solution was 2.84,

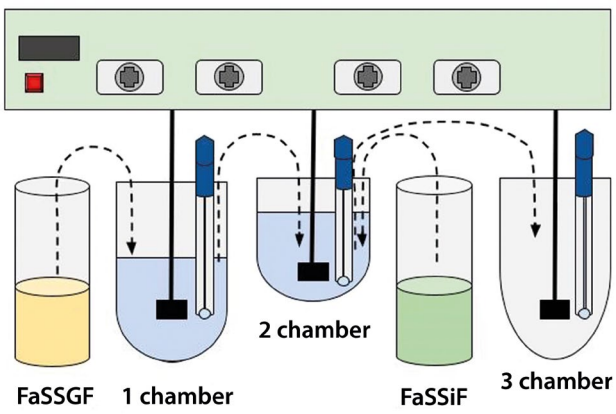

Figure 2. **Scheme of apparatus**

which corresponds to literature data on pH values of the stomach of healthy people [4–6]. The chamber simulating the stomach was drained according to the equation of first order kinetics, gastric half-emptying time was 18 minutes, while complete emptying time (to residual volume of 50 ml) was 38.6 minutes [7]. It should be noted that the stomach emptying rate affects the time that takes for an oral drug form to reach the small intestine, which, in its turn, limits absorption of the active substance by intestinal enterocytes.

For simulation of physiological conditions of duodenum, biorelevant solution medium FaSSIF with a pH value of 6.5 was selected. The starting volume remained constant throughout the test at 75 ml, which corresponds to physiological capacity of the duodenum [7, 8].

The third chamber, simulating the intestine, serves as a receiver of the content of the second chamber, so the starting volume was zero, but by the end of the test it reached 390 ml.

During the test, permanent secretion of FaSSGF and FaSSIF solutions into the first and second chambers was carried out with a rate of 1 ml/min. Figure 3 shows the curves representing the actual amounts of the fluids in different chambers of the apparatus at different time points.

Within each chamber a pH meter electrode was placed to measure and record pH values at each sampling time point (Figure 4).

Mixing of the fluids was carried out by type II (paddle) apparatus with a modification in the form of two round holes (Figure 5). The rotation speed of the paddle was 25 rpm; in order to simulate the actual gastrointestinal motility, "burst" mode of rotation rate acceleration was activated, so that every 5 minutes the speed of paddle rotation increased to reach 180 rpm, then remained constant at that rate for 15 sec, and then the conditions returned to their initial state [7, 8].

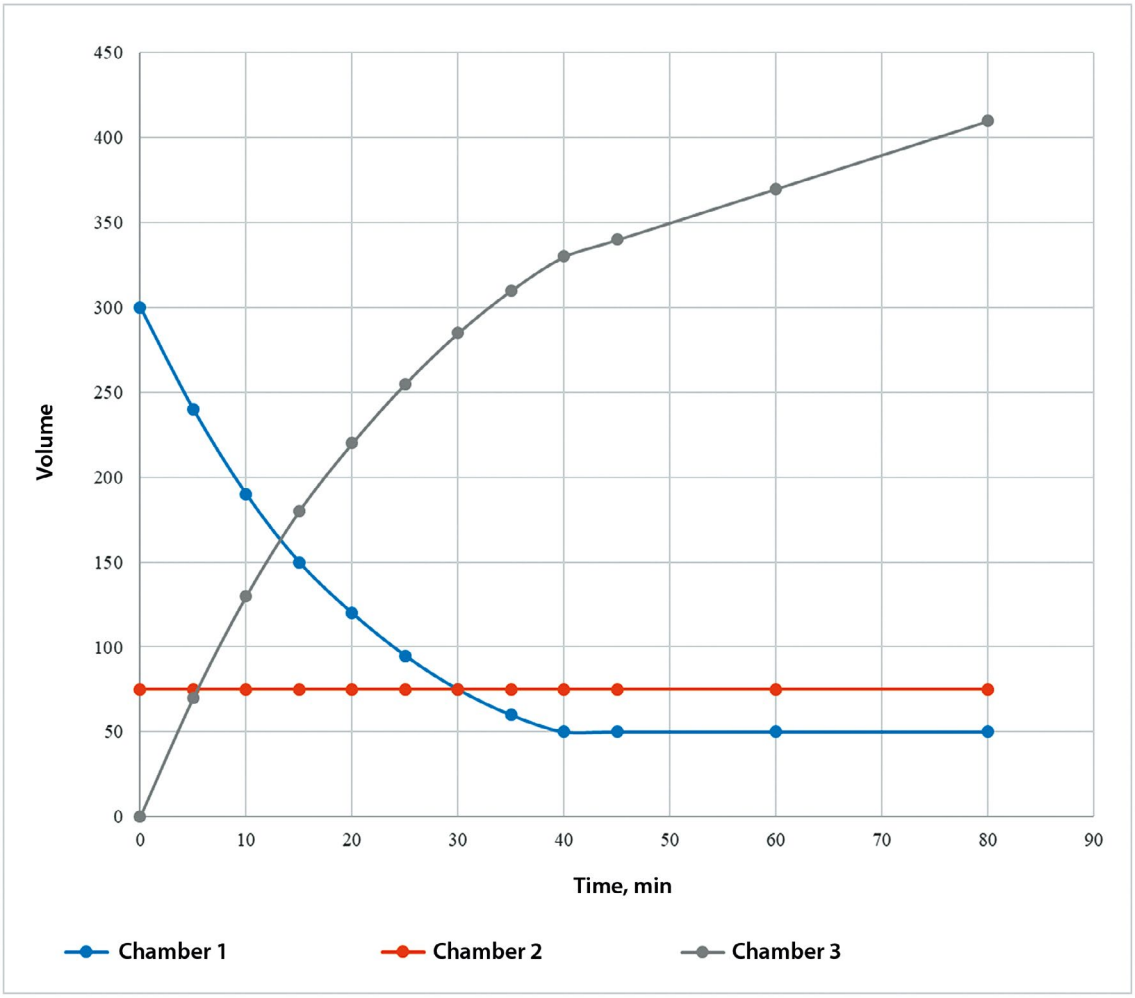

Figure 3. **Diagram of volumes**

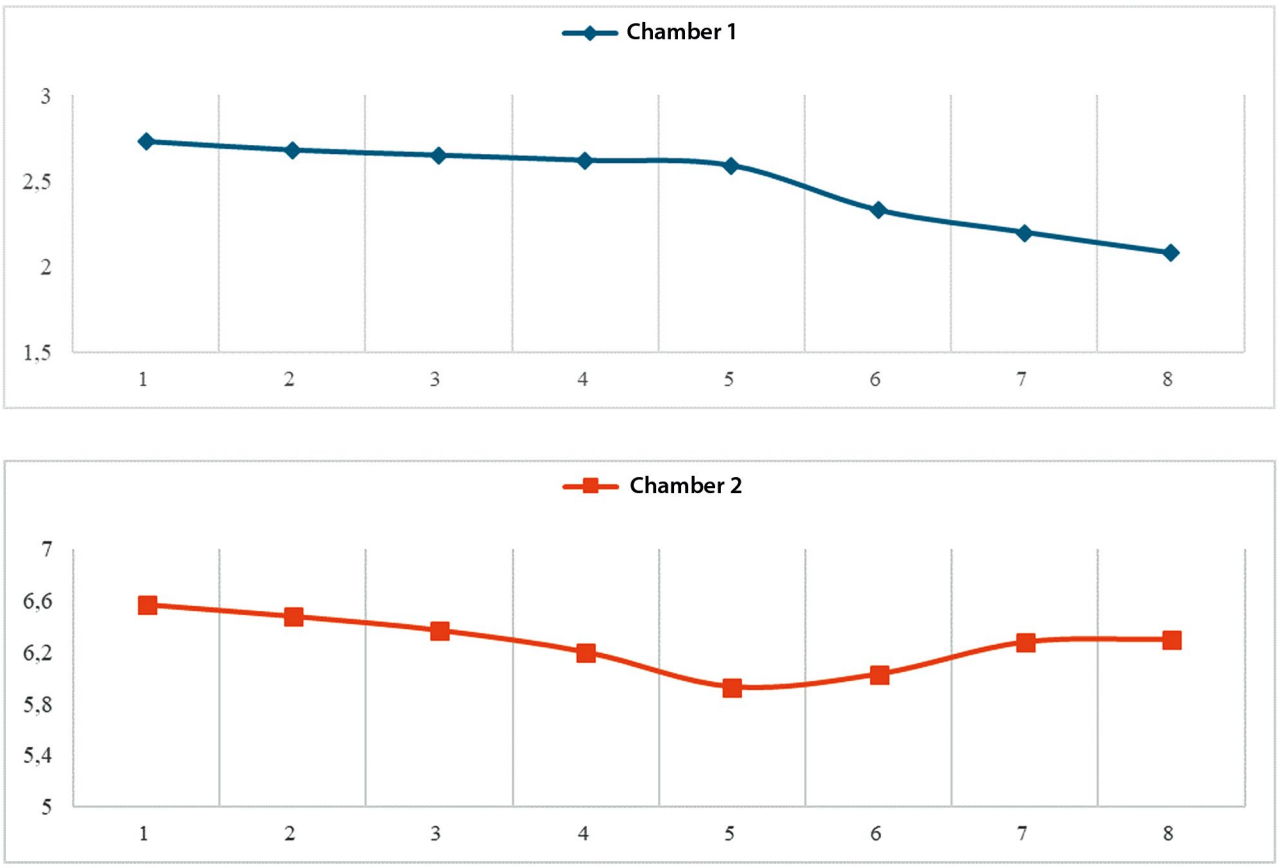

Figure 4. **The pH value in each of the chambers**

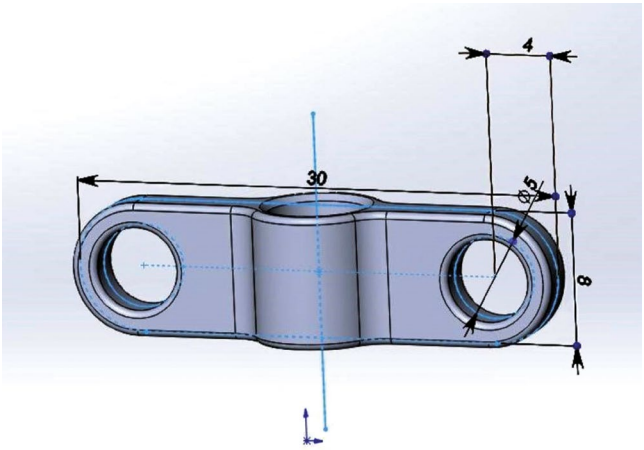

Figure 5. **Shape of the PRT paddle**

The sampling time points – 10, 15, 20, 25, 30, 45, 60, and 80 minutes – were selected in such a way as to ensure a complete dissolution profile of the studied drugs in each section of the system, with due account for the time of transit through the human gastrointestinal tract.

Assay was carried out in accordance with a developed and validated HPLC-UV procedure. In all studied samples, a single peak was observed with a retention time of about 4.25 min; an example of the chromatogram is shown in Figure 6. Each sample was evaluated for concentration, which was then used for plotting the release profiles (represented in Figures 7 and 8).

It is worth noting that in preliminary tests of Cladribine tablets this substance was found to degrade in a compendial dissolution medium with pH 1.2. In this PRT study, we used a pH 2.84 medium to simulate the human fasting stomach after taking a tablet with water, which is more consistent with the real conditions of the human organism. We used a medium having pH of 2.84, which is more relevant to the actual conditions of the human organism. The time the drugs spent in the stomach simulating chamber was 40 minutes with gastric half-emptying time equal to 18 minutes, and it was found that in these conditions the substance did not disintegrate and passed to the third section of the apparatus in an unchanged state.

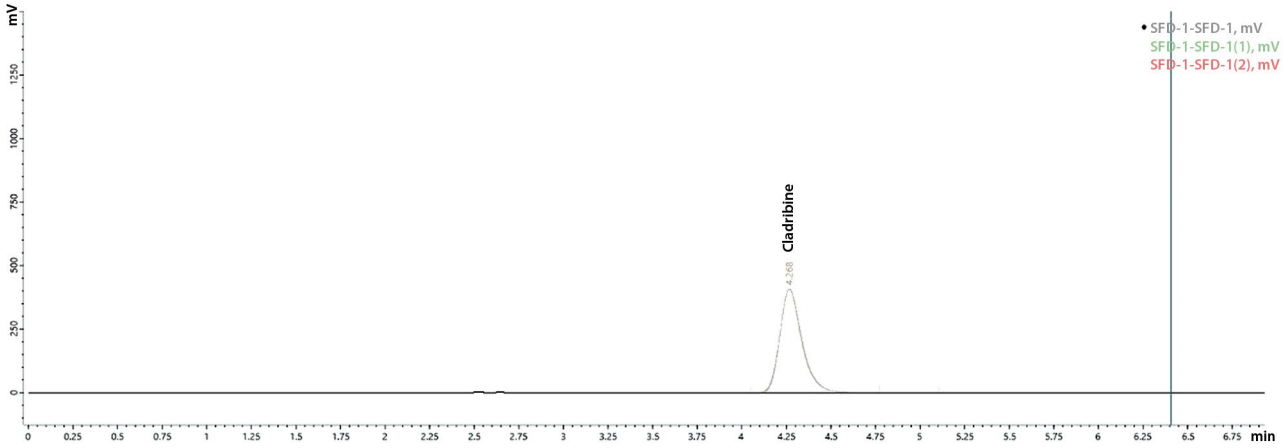

Figure 6. **Sample of chromatogram**

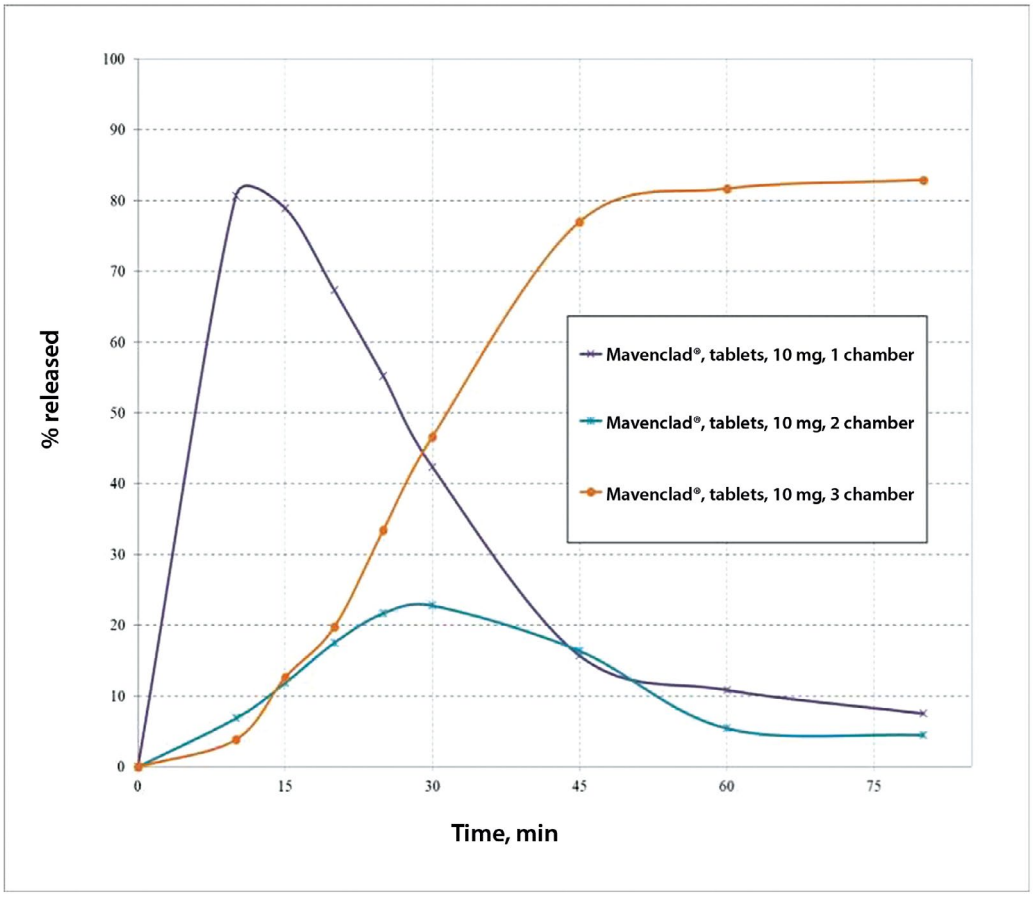

Figure 7. **Averaged profiles of the dissolution of cladribine in the drug "Mavenclad®, tablets, 10 mg" in three chambers of the apparatus**

Since the classical methods of assessing the equivalence of dissolution profiles are not applicable to PRT study (for example, f2, due to parabolic shape of this profile), comparability of the drug under test and reference drug was proven and evaluated by the rate of substance transfer and completeness of release in the third section of the test apparatus simulating the intestine, where the substance is absorbed. The data

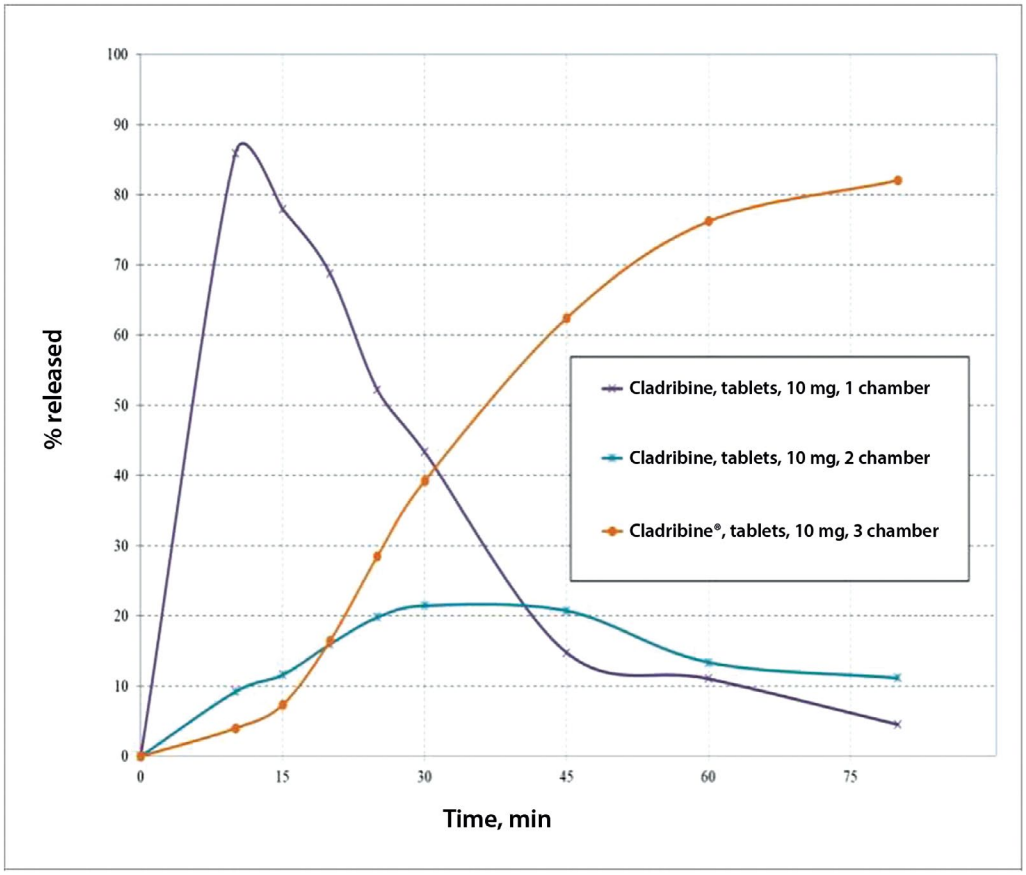

Figure 8. Averaged profiles of the dissolution of cladribine in the drug "Cladribine, tablets, 10 mg" in Three chambers of the apparatus

obtained allows predicting pharmacokinetic profiles through physiologically based pharmacokinetic modeling approaches and with the help of such programs as PK Quest, Gastro Plus, PK-sim, and SimCyp.

CONCLUSION

A PRT study of the drug products "Mavenclad®, tablets, 10 mg" and "Cladribine, tablets, 10 mg" was conducted. Assay was carried out in accordance with HPLC-UV validated procedure. Following the test results, it was recorded that Cladribine reached the third section of the test apparatus and was released completely from the dosage form for both the drug being tested and reference drug under physiologically relevant conditions, which allows to conclude their comparability in physiologically relevant conditions.

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