Journal of Organic and Pharmaceutical Chemistry

Original Research

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UDC 615.01:615.03:615.07:615.074:615.013:615.011.4

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A New Method for Studying the Kinetics of the Release of Poorly Soluble API from Solid Oral Dosage Forms on the Example of Quertin®

Abstract

In this paper, it is proposed to consider a new method developed for studying the kinetics of release of substances that are poorly soluble in aqueous media on the example of quercetin. The study object was the drug containing plant bioactive components – Quertin® chewable tablets, 40 mg, 3 blisters, 10 pcs – produced by PJSC SIC "Borshchahivskiy CPP". An Agilent 1290 Infinity II LC System liquid chromatograph with an Agilent 6530 mass selective detector (Agilent Technologies) was used for the analysis. Solubility profiles were studied in accordance with the requirements of the Biopharmaceutical Classification System (BCS). The solubility limit of the substance in the media studied has been determined. A method for the quantitative determination of quercetin in test media in the range of specified concentrations with high sensitivity and selectivity has been developed. The dissolution of Quertin® chewable tablets in 3 different aqueous dissolution media with pH 1.2, pH 4.5 and pH 6.8 was studied, the dissolution profiles were compared, and the f_2 factor was calculated. This factor is a criterion for evaluating the study by comparing dissolution kinetics with *in vivo* results. The results obtained indicate that the approach proposed to studying the kinetics of the release of substances that are sparingly soluble in aqueous solutions allows us to correctly assess the release of such substances in accordance with the requirements of the BCS. The method developed has been validated.

Keywords: quercetin; Biopharmaceutical Classification System; quantitative determination; method development; bioequivalence; bioavailability; solubility; dissolution test

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Новий метод дослідження кінетики вивільнення важкорозчинної АФІ з твердих пероральних лікарських форм на прикладі Квертину®

Анотація

У статті запропоновано розглянути новий розроблений метод дослідження кінетики вивільнення речовин, що погано розчиняються у водних середовищах, на прикладі кверцетину. Об'єктом дослідження був препарат з рослинними біоактивними компонентами Квертин® жувальні таблетки, 40 мг, 3 блістери по 10 шт., виробництва ПАТ НВЦ «Борщагівський ХФЗ». Для аналізу використовували рідинний хроматограф Agilent 1290 Infinity II LC System з мас-селективним детектором Agilent 6530 (Agilent Technologies). Профілі розчинності вивчали відповідно до вимог біофармацевтичної системи класифікації. Визначено межу розчинності речовини в досліджуваних середовищах. Розроблено методику кількісного визначення кверцетину в досліджуваних середовищах у діапазоні заданих концентрацій, яка має високу чутливість і селективність. Досліджено розчинення жувальних таблеток Квертин® у 3 різних водних середовищах з pH 1,2, pH 4,5 та pH 6,8, порівняно профілі розчинення та розраховано коефіцієнт f_2, який є критерієм для оцінки дослідження шляхом порівняння кінетики розчинення з *in vivo* результатами. Отримані результати свідчать про те, що запропонований підхід дозволяє правильно оцінити вивільнення важкорозчинних у воді речовин відповідно до вимог біофармацевтичної системи класифікації. Розроблений метод було валідовано.

Ключові слова: кверцетин; біофармацевтична система класифікації; кількісне визначення; розробка методу; біоеквівалентність; біовейвер; розчинність; випробування на розчинення

ISSN 2308-8303 (Print) / 2518-1548 (Online)

Citation: Khanina, N. V.; Georgiyants, V. A.; Khanin, V. A.; Zupanets, I. A. A New Method for studying the Kinetics of the Release of Poorly Soluble API from Solid Oral Dosage Forms on the Example of Quertin. *Journal of Organic and Pharmaceutical Chemistry* **2023**, *21* (3), 50–60.
https://doi.org/10.24959/ophcj.23.290665
Received: *11 September 2023*; **Revised:** *4 November 2023*; **Accepted:** *4 November 2023*

Funding: The work is a part of the research of the National University of Pharmacy on the topic "Organic synthesis and analysis of biologically active compounds, drug development based on synthetic substances" (the state registration No. 01144000943; the research period of 2019–2024).
Conflict of interests: The authors have no conflict of interests to declare.

■ Introduction

In vitro studies can be conducted to confirm the equivalence of medicinal products in solid dosage forms of systemic action for oral administration. The decision to register a generic medicinal product without conducting *in vivo* bioequivalence studies based on *in vitro* studies following the international practice is called the "biowaiver" procedure [1, 2].

The biowaiver procedure methodology based on the Biopharmaceutical Classification System (BCS) is intended to reduce *in vivo* bioequivalence studies, i.e., it can be considered as a surrogate for *in vivo* bioequivalence studies. *In vivo* bioequivalence studies may not be conducted if the assumption of *in vivo* equivalence can be justified by satisfactory *in vitro* data [3, 4].

The bioavailability procedure is based on the BCS, which allows all active substances to be divided into four classes according to their solubility in aqueous solutions and their permeability. When the aforementioned indicators are combined with the drug dissolution, the system takes into account three main factors: drug dissolution, biopharmaceutical solubility, and the degree of permeation of the active substance [1].

The study of quercetin solubility is a matter of particular interest since the available sources do not provide an unambiguous and uniform value for the solubility limit of this substance. As a part of the study of the quercetin bioavailability, we developed methods for determining the exact solubility limit [5, 6]. The exact value of this physicochemical parameter appears to be extremely important as it is the basis for further studies of bioequivalence and bioavailability of the drug.

Studies must guarantee immediate release properties and confirm comparability between the drugs tested, i.e., the drug under research and the reference drug must show the similar *in vitro* dissolution under physiologically relevant experimental pH conditions. However, this does not establish an *in vitro*/*in vivo* correlation. The *in vitro* dissolution should be studied within the pH range of 1–6.8 (at least pH 1.2, 4.5, and 6.8). Additional studies may be necessary at pH values, at which the active substance has minimal solubility. The use of any surfactants is not acceptable [1, 3].

Comparative *in vitro* dissolution studies must comply with the current Pharmacopoeial standards. Thus, a detailed description of the experimental conditions and analytical methods, including validation data, should be provided [3].

The development of a new methodology and implementation of a modified procedure for studying the release kinetics within *in vitro* studies for poorly soluble substances may affect the following aspects of the pharmaceutical development:

- reduction of cases of biological and pharmacodynamic non-bioequivalence of generic and branded drugs;
- ensuring a higher level of quality for new and existing generic drugs;
- ensuring a higher level of safety through the in-depth study of adverse effects of existing and new generic drugs;
- reducing the cost of the development of new drugs containing low-soluble substances by partially or entirely eliminating the *in vivo* stage;
- the possibility of creating new generic drugs.

The drug Quertin® ("Research and Production Center "Borshchahivskiy CPP") is an example of the above problem since it contains quercetin, which is characterized by very low solubility in the aqueous medium, and, considering the fact that it belongs to the 4th class according to the BCS classification, the study of its dissolution and release kinetics is a very complex process. We have studied the dissolution limit of quercetin, which now provides the basis for continuing research and implementing the next stage, which includes the study of the drug itself.

The aim of the work is to develop a new approach to the study of the release kinetics of

substances that are poorly soluble in aqueous media, for instance, quercetin, which is a component of Quertin® chewable tablets, 40 mg, and further confirm the results obtained by *in vivo* data.

■ Materials and methods

Materials

One batch of Quertin®, chewable tablets, 40 mg, 3 blisters of 10 pcs manufactured by the "Research and Production Center "Borshchahivskiy CPP" was used as the study object. The quercetin substance (CAS registration number 117-39-5) was used as a standard sample – lemon-yellow crystals, slightly soluble in water, diethyl ether, ethanol, chloroform, soluble in acetic acid and alkalis. A laboratory electronic balance (ABT 120-5DM), a pH meter (Starter ST2100, Ohaus), a semi-automatic dissolution testing system "Pharma Test" type PT-DT70 (meets the requirements of the State Pharmacopoeia of Ukraine (SPhU) General Article 2.9.3. "Dissolution Test for Solid Dosage Forms"), an Agilent 1290 Infinity II LC System liquid chromatograph with an Agilent 6530 mass-selective detector (Agilent Technologies), measuring glassware of accuracy class A were applied in the study.

Conditions for the assay by HPLC-MS

For the measurements, an Agilent 1290 liquid chromatograph with an Agilent 6530 TOF mass spectrometry detector, a 50×4.6 mm column filled with a sorbent with a grafted octyl silica gel phase (L1), particle size – 1.7 µm; with thermostatic control (30°C) was used. *Mobile phase A*: 0.1 M trifluoroacetic acid solution degassed in an ultrasonic bath; *mobile phase B*: acetonitrile P; the injection volume – 10.0 µL. The highly selective time-of-flight mass spectrometer had the following settings: the ionization type – electrospray ionization in the positive mode (ESI+); measurement mode – scanning in the mass range of 50–1500; the voltage at the fragmenter – 10 V; the nitrogen temperature – 350°C; the nitrogen flow rate – 10 mL min^{-1}; the nebulizer pressure – 35 psi; the capillary voltage – 4 kV; the elution mode – gradient (**Table 1**).

Table 1. The gradient program

Time, *min*	Mobile phase A, *%*	Mobile phase B, *%*
0	100	0
5	100	0
10	50	50
15	50	50
16	100	0
20	100	0

Preparation of the reference solution

0.6 mg (accurate weight) of thoroughly grounded quercetin was placed in a 100 mL flask, 50 mL of acetonitrile *R* was added, and the mass was dissolved in an ultrasonic bath for 10 min, after which it was diluted to the volume with water *R* and stirred.

The preparation of test solutions is described below in the following section.

The reference solution was prepared simultaneously with test solutions. All solutions were used immediately after preparation.

The methodology for studying the release of quercetin according to the requirements of the SPhU dissolution test.

The *in vitro* dissolution of the tablets was studied in the pH range of 1–6.8 (pH 1.2, 4.5, and 6.8). Additional studies that might be required at pH values at which the active substance had minimal solubility were not conducted. No surfactants were used in the experiment.

For statistical evaluation, we used 6 units of the medicinal product in each study.

The number of test samples for these studies was 6 samples, 1 batch in total. The number of measurements was 156 concentration determinations for two aliquots of each of the three media and 13 sampling time points.

The standard conditions of the study were:

- the medium volume – 1000 mL;
- the rotation speed of the blade – 100 rpm;
- the temperature – 37+0.5°C;
- the dissolution media – (1) buffer solution, pH 1.2; (2) buffer solution, pH 4.5; (3) buffer solution, pH 6.8;
- the sampling timetable – 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39 min.

The sampling was performed with the volume compensation for a dissolution medium.

Methods

2 Tablets of Quertin® chewable tablets (40 mg) were placed in two reservoirs of the Pharma Test dissolution test system type PT-DT70, 1000 mL of an appropriate buffer was added, and the dissolution test was performed under the given conditions. When the sample time was reached, 900 mL of the corresponding buffer was replaced, using an aliquot of the selected 900 mL of the sample for further quantification. The replacement of 900 mL was performed 6 times for each reservoir for even and non-even points of the sampling schedule. The sampling was performed from the center of the reservoir. The volume of

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samples taken was 900.0 mL. This volume was selected experimentally to avoid the sample loss during the analysis.

An aliquot was centrifuged for 10 min at 10,000 rpm. After centrifugation, 1.0 mL of the upper layer was carefully removed, avoiding contact with the agglomerates of the lower layer, and transferred to chromatography vials.

The sample preparation methodology

In compliance with Guideline 42-7.4:2022 [1], the similarity of dissolution profiles was studied in dissolution media, which were buffer solutions with pH 1.2, 4.5, and 6.8. As buffer solutions for the test, the solutions recommended by the SPhU for the dissolution test in accordance with Article 2.9.3 were used [3].

Buffer solution – pH 1.2 (SPhU): 250.0 mL of 0.2 M sodium chloride solution *P* (11.69 g in 1000.0 mL of water *R*) was placed in a 1000 mL volumetric flask, 425.0 mL of 0.2 M hydrochloric acid solution was added, and the solution was diluted to the volume with water *R*.

Buffer solution – pH 4.5 (SPhU): 2.99 g of sodium acetate *R* was dissolved in water *R*; 14.0 mL of 2 M acetic acid solution *R* was added, and the solution was diluted to the volume of 1000.0 mL with water *R*.

Buffer solution – pH 6.8 (SPhU): 250 mL of 0.2 M potassium dihydrogen phosphate solution *R* and 112 mL of 0.1 M sodium hydroxide solution *R* were mixed.

Degassing of all dissolution media was carried out in accordance with the degassing procedure described in Section 2.9.3 of the SPhU [3].

For the analysis, the required volume of the solvent was preliminarily calculated by dividing the dose of quercetin in one tablet – 40 mg – by the value of the solubility limit of quercetin. The solubility limit of quercetin was previously found using the methodology developed by us earlier and then published [5, 6]. This value was 0.0031 mg mL^{-1}. Thus, to determine the release of quercetin in each of the media, 13 L of the solvent were required for 13 solvent changes in the dissolution beakers.

Processing of the data obtained

Excel program (Microsoft Office 2021) was used to calculate the parameters of the classification equations and to draw graphs.

Based on the primary data (peak areas) and calculated parameters (concentrations), graphical dependences of the quercetin concentration values obtained on time were plotted and tabulated.

Results and discussion

Selection of chromatography conditions

The substance quercetin is described in the monograph of the European Pharmacopoeia. This monograph does not provide a chromatographic method for the quantitative determination, or a method for determining the content of impurities in the substance. In connection with the above, we have developed a method for the chromatographic quantitative determination of quercetin in the finished dosage form for the dissolution test since the presence of a complex matrix of excipients can affect the result of quantification. This method uses a gradient type of elution to increase the degree of separation of the substance components with excipients contained in the drug. In addition, the method uses mass-spectrometric detection of the substances, which significantly increases the sensitivity and selectivity of the method. Another advantage of the new method was the use of a chromatographic column with a particle size of 1.7 µm, which made it possible to increase the efficiency of the separated peaks along with sensitivity. All of this allowed determining rather low concentrations of the substances of interest (approximately 0.1 ppm – 1 ppm) without the loss of accuracy, precision, and reproducibility.

Under the conditions we chose, we obtained full ion current chromatograms for samples in three media with pH values of 1.2, 4.5, and 6.8, which were planned to use for the dissolution test (**Figures 1–4**). We used the quercetin substance as a standard sample since the uncertainty of the analysis did not exceed 3%.

The effect of the pH on the dissolution test

The pH-dependent solubility profile of the active substance should be determined and discussed. The active substance is considered highly soluble if the highest single dose of the immediate-release formulation is completely soluble in 250 mL of buffer solutions in the pH range of 1–6.8 at 37±1°C. To prove this, at least three buffer solutions within this range (preferably at pH 1.2, 4.5, and 6.8) and an additional pK_a value if it is within the above pH range should be used. Repeated determinations at each pH value may be necessary to achieve a clear classification of the solubility of the active substance (e.g., by flask shaking or other reasonable method). The pH of each buffer solution should be checked before and after the introduction of the active substance into the buffer solution [1, 3].

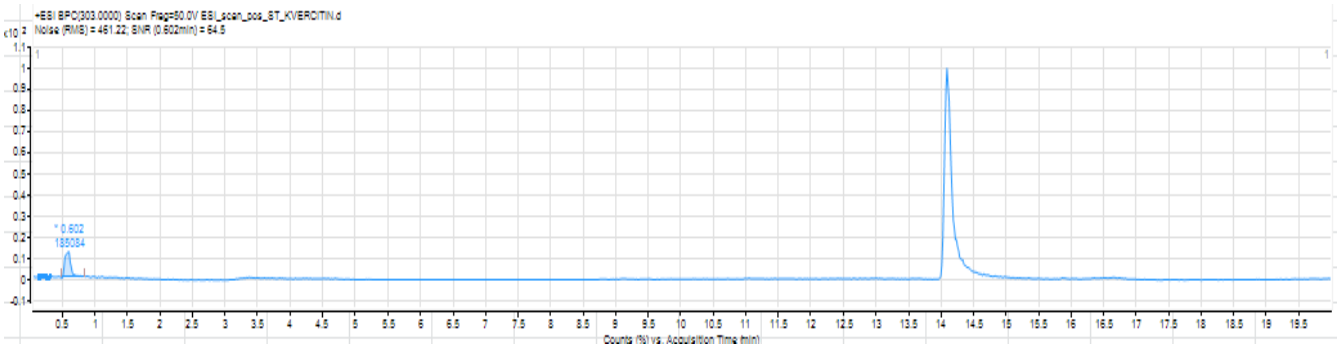

Figure 1. A typical chromatogram of the solution of a standard quercetin sample

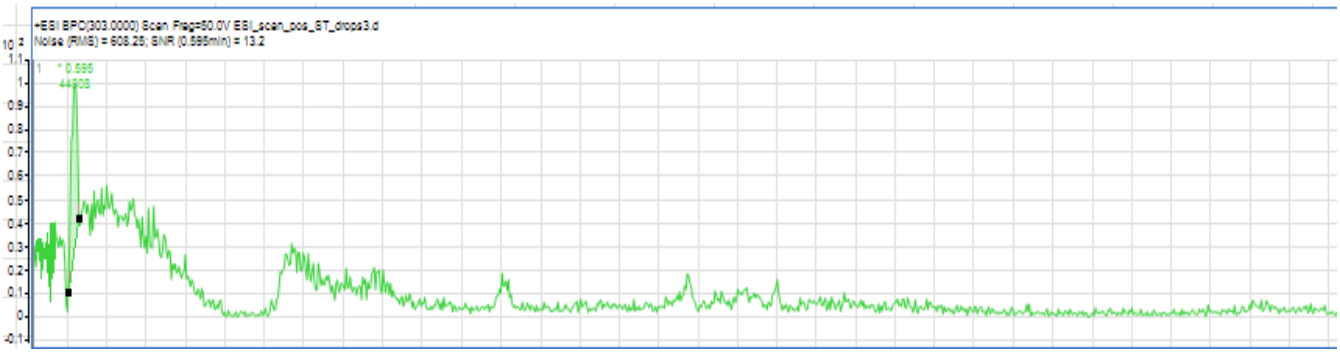

Figure 2. A typical chromatogram of the test solution of Quertin® drug

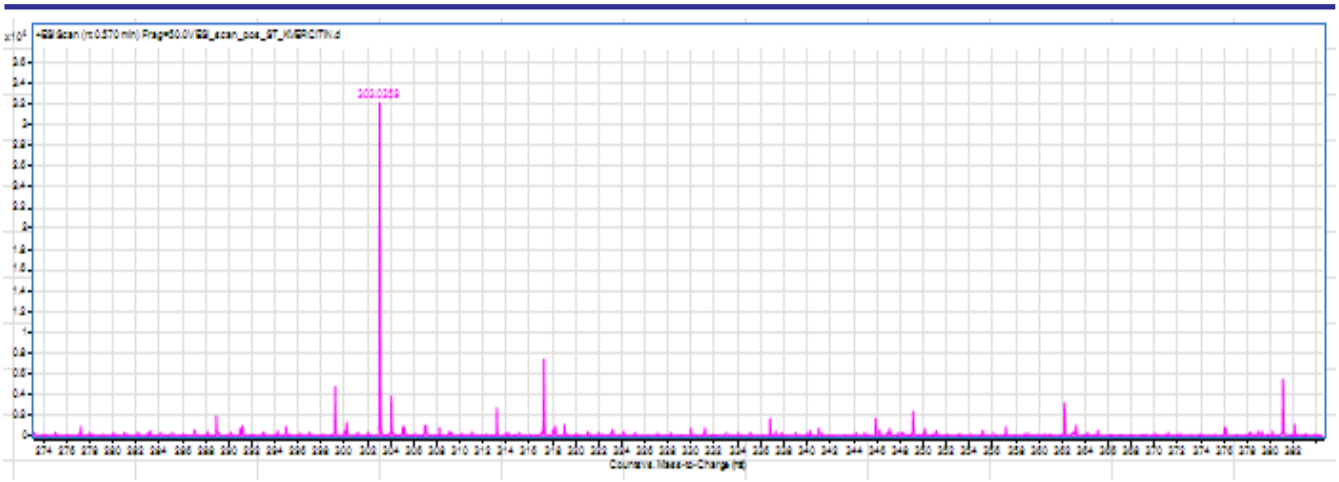

Figure 3. A typical mass-spectrum of the solution of a standard quercetin sample

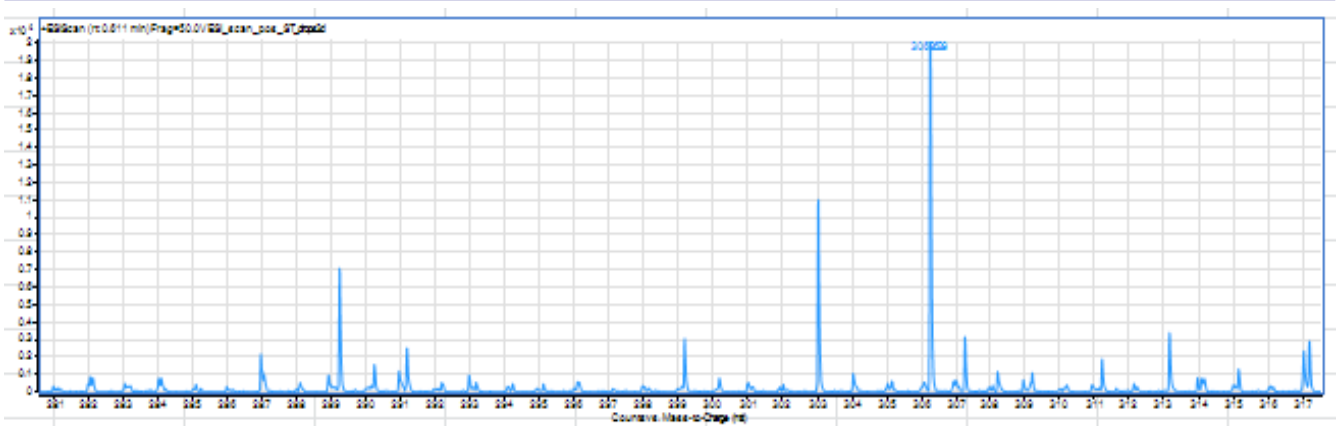

Figure 4. A typical mass spectrum of quercetin in Quertin® drug

ISSN 2308-8303 (Print) / 2518-1548 (Online)

Before studying the dissolution profiles, the pH of the dissolution media to be used for the dissolution test was investigated. The study was conducted for the pH of three buffer solutions under the above conditions for the dissolution test.

The effect of the components of the drugs under study on the pH of the dissolution medium is given below in **Table 2**.

Since the test drug did not significantly affect the pH of the dissolution medium (the deviation of the pH value was less than 0.05), a positive conclusion was made that these dissolution media could be used for the dissolution test.

Results of the dissolution profiles study

After studying the dissolution profiles, the following data were obtained for Quertin® chewable tablets in 3 different aqueous dissolution media with pH 1.2, pH 4.5, and pH 6.8 (**Table 3**).

The degree of the quercetin release into the solution was calculated as the ratio of the amount of quercetin transferred to the solution to the initial sample taken for the study and measured in relative units.

The graphical dependencies (**Figures 5–7**) allow us to conclude that the results obtained meet the BCS requirements for biowaiver results, the relative standard deviation of the first point does not exceed 20%, and the subsequent ones do not exceed 10%. The quercetin content is 85% and is achieved for all media studied in 30 min, which correlates well with the data obtained in the previous studies for the quercetin substance [5, 6] where the content was also achieved in 30 min.

Validation of the methodology developed

To use this methodology for quercetin solubility tests, according to the bioassay scheme for three dissolution media with pH 1.2, 4.5, and 6.8, the method was validated as a quantitative determination method with a maximum uncertainty value of 1.6%. The method was validated by individual validation characteristics: specificity, linearity, convergence, precision, accuracy, and intra-laboratory precision [3].

Table 2. The effect of the components of the drugs under study on the pH of the dissolution medium

Sample No.	pH to dissolution	pH after dissolution	pH to dissolution	pH after dissolution	pH to dissolution	pH after dissolution
1	1.244	1.244	4.540	4.542	6.771	6.782
2		1.246		4.543		6.780
3		1.243		4.542		6.773
4		1.235		4.540		6.779
5		1.245		4.542		6.775
6		1.245		4.540		6.777
7		1.243		4.543		6.780
8		1.244		4.542		6.765
9		1.243		4.544		6.760
10		1.242		4.545		6.760
11		1.243		4.546		6.775
12		1.240		4.546		6.780

Table 3. The concentrations of quercetin obtained in media with pH 1.2, 4.5, and 6.8

Dissolution time, *min*	Dissolution medium – pH 1.2		Dissolution medium – pH 4.5		Dissolution medium – pH 6.8	
	Concentration of quercetin, *%*	RSD, *%*	Concentration of quercetin, *%*	RSD, *%*	Concentration of quercetin, *%*	RSD, *%*
3	7.5	11.4	4.1	17.3	2.7	18.8
6	22.5	6.6	13.8	9.6	10.7	9.8
9	32.5	4.9	22.3	8.2	25	9.4
12	45	7.8	31.2	9.8	33.2	9.2
15	55	9.1	58.0	9.9	51.2	8.9
18	65	6.8	63.9	8.8	63.9	8.9
21	72.5	5	74.7	7.5	71	8.1
24	80	7.1	83.9	7.7	78.5	7.9
27	87.5	4.2	88.6	6.9	82	7.3
30	92.5	5.7	92.2	6.1	88.5	6.9
33	95	4.99	96.1	5.8	95.2	6.1
36	97.5	4.8	98.4	5.5	97.2	6.5
39	100	4.2	100.0	5.1	100	6.1

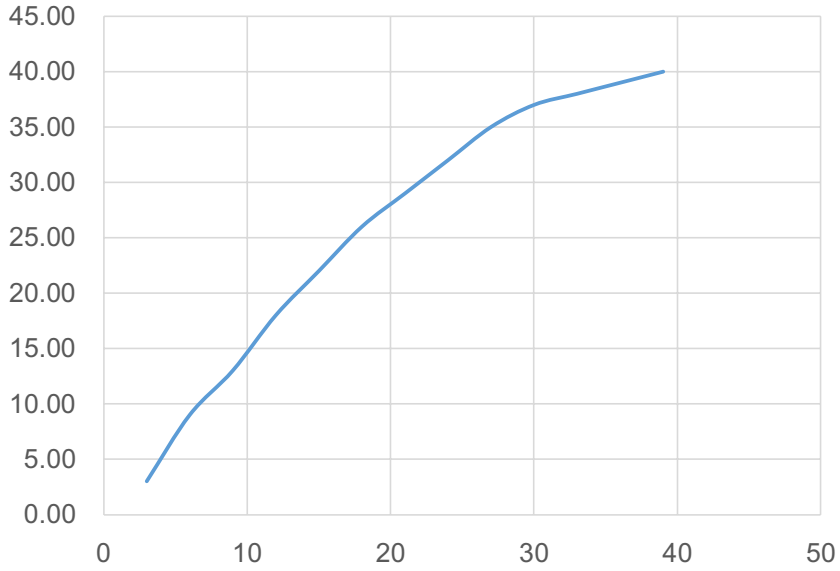

Figure 5. The release graph of quercetin with pH 1.2 for Quertin® chewable tablets

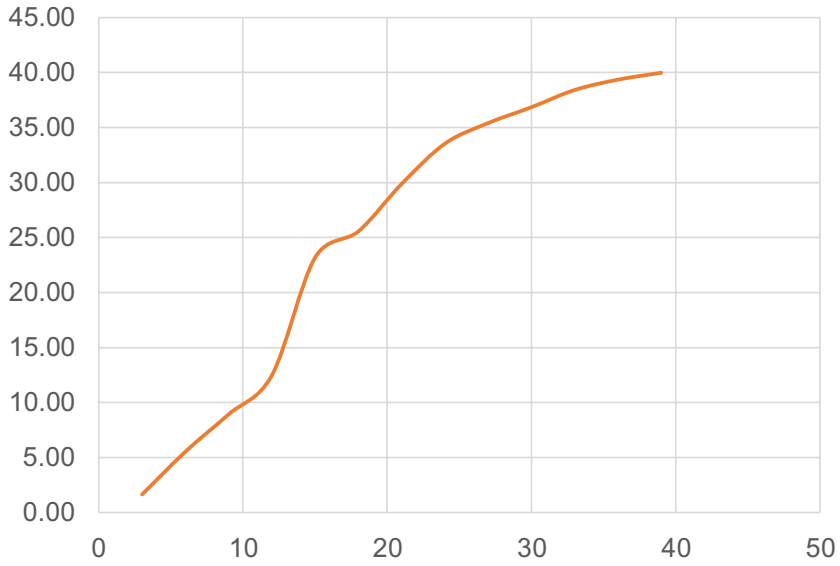

Figure 6. The release graph of quercetin with pH 4.5 for Quertin® chewable tablets

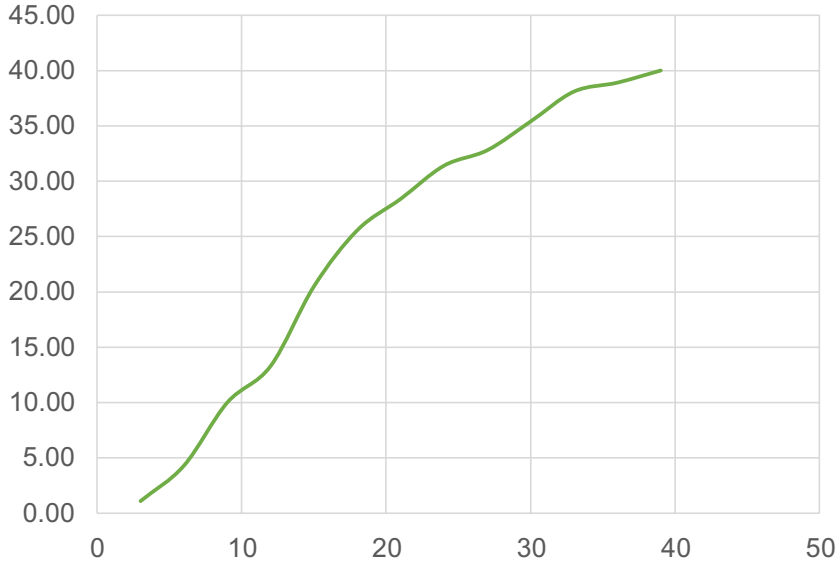

Figure 7. The release graph of quercetin with pH 6.8 for Quertin® chewable tablets

ISSN 2308-8303 (Print) / 2518-1548 (Online)

Since the high-performance liquid chromatography (HPLC) method used in the methodology is specific, it is sufficient to prove that the methodology is specific if all the requirements for the criteria of linearity, accuracy, precision, and intra-laboratory precision are met.

The model solutions for chromatography were prepared according to the analytical procedure described above. The blank solution was prepared similarly to the test solution of the drug.

The linearity was assessed in the range (80–120%) of the method according to the standard method. The nature of the signal dependence on the concentration was studied using 9 model solutions for the analysis with accurate concentration weights: 80, 85, 90, 95, 100, 105, 110, 115 i 120%. At the same time, the concentration taken as 100% was the quercetin concentration, which was in the middle of the range covering the minimum and maximum quercetin concentrations [3].

The results were statistically processed by the least squares method according to the requirements of the SPhU [1]. The calibration graph was constructed in the normalized coordinates (**Figure 8**). The average values of the peak area (S_i) were calculated for each of nine solution samples. The results were processed by the least squares method for the line Y = b*x + a. The calculated statistical values of b, S_b, a, S_a, S_r (final standard deviation) and r (correlation coefficient) are given in **Table 4**.

In our case, the requirements for the linear regression parameters are met over the entire range of the methodology (80–120%).

To measure and calculate the metrological assessment of the accuracy and precision of the method, three peak area values were obtained for the reference solution and 27 peak area values for the model solutions. The actual values, the ratio of the average peak area values for each of 27 solutions to the average peak area value of the reference solution were calculated, obtaining the values $X_i = (S_i/S_{st}) \times 100\%$, $Y_i = (S_i/S_{st}) \times 100\%$, and the value $Z_i = (Y_i/X_i) \times 100\%$ (it is the concentration found as a percentage of the input material). The calculation results are shown in **Tables 5–7**.

Requirements for the maximum allowable RSDP are 1.7%. The calculated value is stored until the measurement results match.

To assess the intra-laboratory precision, the relative confidence interval for 5 parallel determinations of the quantitative content of substances was used, which should be less than the maximum permissible uncertainty of the analysis results: $\Delta z \leq 1.6\%$. Tests were performed using the same batches of the drug by different analysts on the same chromatograph on different days using different measuring dishes.

The intra-laboratory precision was confirmed by the fact that the value of the relative confidence interval for five parallel determinations of one batch of the drug meets the acceptance criterion ($\Delta z = 0.22\% \leq 1.6\%$).

Comparison of the results with the results of *in vivo* studies

To fulfill the task formulated for this study, it was necessary to study the following aspects:

- the first step was to conduct the solubility study and classify the active bioactive substance – quercetin – according to the biopharmaceutical classification system (BCS class); study the solubility limit of a substance in test media;

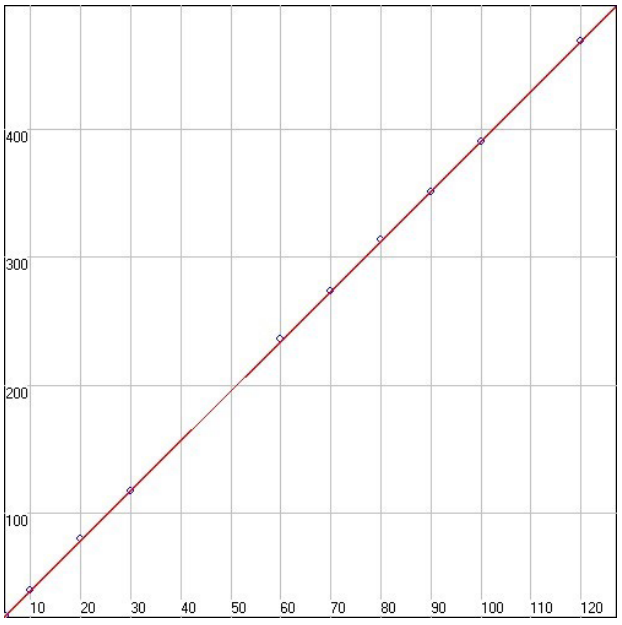

Figure 8. The linear regression of the peak area against the quercetin concentration in the normalized coordinates

Table 4. Linear regression characteristics

Parameter	Value		
b	4		
S_b	0.006		
a	0.8		
S_a	0.4		
S_0	0.7		
S_0/b	| 0.19 |		
S_Y	157.1		
r	| 0.99999 |		
Reference solution	Average S_{St}	C_{St}	RSD_{St}, %
Reference	391.4	100	0.18

Table 5. Variance values

Test solutions	Name	Average S_i	C_i	RSD_i, %
1	5	19.5	5	0.4
2	10	39.8	10	0.2
3	20	79.7	20	0.09
4	30	117.7	30	0.06
5	60	236.3	60	0.03
6	70	273.6	70	0.026
7	80	313.7	80	0.02
8	90	351.5	90	0.02
9	100	390.9	100	0.018
10	120	469.2	120	0.015

Note: Student t-test (95, 1, 11) = 1.7956

Table 6. Results of the analysis of model solutions and statistical processing

Test solutions	Name	Average S_i	C_i	Y_i	X_i	RSD_i, %	Z_i, %
1	5	19.5	5	4.9	5.00	0.4	99.4
2	10	39.8	10	10.2	10.00	0.18	101.5
3	20	79.7	20	20.3	20.00	0.09	101.7
4	30	117.7	30	30.1	30.00	0.06	100.2
5	60	236.3	60	60.3	60.00	0.03	100.6
6	70	273.6	70	69.9	70.00	0.03	99.8
7	80	313.7	80	80.11	80.00	0.02	100.1
8	90	351.5	90	89.8	90.00	0.02	99.8
9	100	390.9	100	99.8	100.00	0.02	99.8
10	120	469.2	120	119.8	120.00	0.02	99.9

Table 7. The accuracy and correctness parameters obtained

Parameter	Name	Value	Requirements 1	Requirements 2	Conclusions
Precision	Δ_Z	1.4	≤ 3		meets
Accuracy	$\lvert Z_{cp} - 100 \rvert$	| 0.29 |	≤ 0.45	≤ 0.96	meet for the 1st criteria

Note: S_z (%) = 0.78103; Student t-test (95, 1, 9) = 1.83310

- as the second step, the development of a method for the quantitative determination of quercetin in test media in a range of specified concentrations with a high sensitivity and selectivity;
- the study of the dissolution of Quertin® chewable tablets in 3 different aqueous dissolution media with pH 1.2, pH 4.5 and pH 6.8, comparison of dissolution profiles and calculation of the f_2 factor, which is the criterion for evaluating the study by comparing dissolution kinetics with *in vivo* results as a finalizing step.

The chemical formula shows that quercetin is an aglycone without a carbohydrate group, which determines its chemical and pharmaceutical properties. A quercetin glycoside is formed by the addition of glucose, rhamnose, or rutinose moieties, which replace one of the hydroxyl groups in its structure, usually in position 3, thus forming a glycosidic bond [7]. This significantly affects the solubility and absorption of quercetin *in vivo* [8]. Hence, the regularity is that the presence of a carbohydrate molecule in the structure of a quercetin glycoside contributes to its better solubility in water compared to a quercetin aglycone [9]. Usually, the term "quercetin" refers only to the aglycone; in medical research, this term is used to define the glycoside molecule of quercetin.

The study of pharmacokinetic properties of quercetin *in vivo* and its complex with pectin (Quertin® chewable tablets, 40 mg) was performed in 8 outbred rabbits of both sexes weighing 2500-3000 g. The rabbits were divided into 2 experimental groups of 4 animals each: *Group 1* – rabbits receiving oral quercetin in the dose of 10.0 mg kg^{-1}; *Group 2* – rabbits receiving an oral combination of quercetin and pectin in the dose of 10.0 mg kg^{-1} for quercetin. As a bioanalytical method for determining the concentration of quercetin and its metabolites (methoxy-, sulfate- and/or glucuronic conjugates) in biological samples, ultra-performance liquid chromatography

ISSN 2308-8303 (Print) / 2518-1548 (Online)

Table 8. Average values of the quercetin (*Q*) and isorhamnetin (*Ir*) content

The study object		The time of blood sampling, *hours*						
		0	0.25	0.5	1.0	2.0	4.0	8.0
Quercetin (substance)	*Q*	<25.0	324.3	279.3	257.8	176.9	121.1	104.3
	Ir	<25.0	122.8	103.4	92.2	85.1	66.8	36.6
	Sum	<25.0	441.7	378.1	345.9	258.3	184.9	139.3

with a mass-selective detector was used. The total concentration of quercetin and its metabolites in the blood plasma was expressed as a pure quercetin (**Table 8**).

The results of the study illustrate that the intragastric administration of quercetin contributed to the appearance and fluctuation of the concentration of the active substance in the blood of experimental animals of both groups.

When analyzing the average values, it was found that the peak concentrations of quercetin and its metabolites were formed in the plasma of animals during the first 25 min and then gradually decreased. Based on the calculations of the mean values of the quercetin concentration, pharmacokinetic curves were constructed, reflecting the dependence of the total content of quercetin and its metabolites (isorhamnetin) in the blood serum of experimental animals on time.

Thus, the results obtained allow us to characterize the degree of the quercetin release within *in vivo* tests by the nature of pharmacokinetic dependence on time.

Using the similarity factor f_2 calculated as described below, the similarity of the dissolution profiles was found from the *in vivo* studies and *in vitro* dissolution profiles obtained for Quertin® chewable tablets, 40 mg, in this study:

$$f_2 = 50 \cdot \log\left[\frac{100}{\sqrt{1+\frac{\sum_{t=1}^{t=n}[R(t)-T(t)]^2}{n}}}\right]$$

Since the calculated value of the similarity factor f_2 amounted to 58, the dissolution profiles were considered similar since the similarity factor $f_2 \geq 50$.

The HPLC method with mass-spectrometric detection will always have limitations related to the design features of the equipment used, such as the sensitivity of the detector, the linear range of concentrations measured, and its ability to detect the substance under research in the sample. Such studies also require the use of a standard sample of the test substance, which can complicate and increase the cost of the study.

First, there is an interest in studying the dissolution profiles, according to the conditions of the method, for other substances that are limitedly soluble. The implementation of the method developed will allow us to study the *in vitro* kinetics when studying the bioavailability of drugs that the BSC classifies as *Class 4*. This will necessarily lead to a reduction in the cost of the process of developing new drugs due to the partial or complete exclusion of the *in vivo* stage.

■ Conclusions

The solubility profiles of quercetin in 3 different aqueous dissolution media with pH 1.2, pH 4.5 and pH 6.8 were obtained for the first time for the drug Quertin® chewable tablets, 40 mg, in compliance with the BCS requirements.

The results indicate that the approach proposed to studying the kinetics of the release of substances that are sparingly soluble in aqueous solutions allows us to correctly assess the release of such substances in accordance with the BCS requirements.

The studies have shown that the *in vitro* dissolution profiles are in good agreement with the results of pharmacokinetic studies of quercetin release *in vivo*. Since the same drug is used for the studies, this confirms the correctness of the *in vitro* results obtained by the methodology developed.

In the process of validation of the method for the quantitative determination of quercetin in Quertin® chewable tablets, 40 mg, the variational characteristics of the method by the standard method were studied: accuracy, linearity, precision, specificity, and intra-laboratory precision. The variational characteristics of the method do not exceed the critical error value (1.6%) and are characterized by qualitative analytical parameters. This method can be correctly reproduced in laboratories and does not depend on excipients.

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ISSN 2308-8303 (Print) / 2518-1548 (Online)