UDC 615.01, 615.03, 615.07, 615.013, 615.011.4
DOI: 10.15587/2519-4852.2023.283293

DEVELOPMENT OF A METHOD FOR THE QUANTITATIVE DETERMINATION OF THE SOLUBILITY LIMITS OF POORLY SOLUBLE IN WATER SUBSTANCES ON THE EXAMPLE OF QUERCETIN

Nataliia Khanina, Victoriya Georgiyants, Vadim Khanin

Aim. *To consider the importance of this physicochemical characteristic as a determining factor in the study of bioequivalence and bioavailability, there is a need to develop a method to quantitatively determine the solubility limit of quercetin.*
Materials and methods. *The quercetin concentration was determined in the obtained samples using chromatographic and external standard methods. The pharmacopeial standard – PS of the SPU was used as a standard. For measurements, an Agilent 1290 liquid chromatograph with an Agilent 6530 TOF mass spectrometric detector was used, using a 50×4.6 mm column filled with a sorbent with a grafted phase of octyl silica gel, particle size – 1.7 μm.*
Results*. The exact limit of the solubility of quercetin, as a poorly soluble substance, has been established. Based on the data obtained, the kinetics of the dissolution of quercetin was studied. In tandem with the QTOF mass spectrometric detector, the HPLC method was utilized in the identification and quantification process. To accurately determine the point that will correspond to the solubility limit of quercetin in water, the obtained experimental dependence was approximated by a polynomial dependence, for which, by solving a system of equations in the Microsoft Excel program, concentration values were found corresponding to the inflection points of the studied dependences.*
Conclusions*. When studying their bioavailability, a new approach has been developed to quantitatively determine the solubility limit of difficult or practically insoluble substances in aqueous media with a neutral pH value. The exact value of the solubility limit for the test sample of quercetin was established, which was 3.02 mcg/ml. The kinetics of the release of quercetin in aqueous solutions was studied*
Keywords*: quercetin, identification, quantitative definition, HPLC, method development, bioequivalence, biowaiver, mass spectrometry, solubility, dissolution testing*

How to cite:
Khanina, N., Georgiyants, V., Khanin, V. (2023). Development of a method for the quantitative determination of the solubility limits of poorly soluble in water substances on the example of quercetin. ScienceRise: Pharmaceutical Science, 3 (43), 58–66. doi: http://doi.org/10.15587/2519-4852.2023.283293

© The Author(s) 2023
This is an open access article under the Creative Commons CC BY license hydrate

1. Introduction

At the current stage of the development of the pharmaceutical industry, medicinal products of plant origin occupy a significant part both in the national market and in the leading markets of the world [1]. According to experts, about 25 % of medicines used in medical practice worldwide are obtained directly from the medicinal plant's raw materials [2]. The popularity of herbal preparations depends on the characteristic feature of most of them: a milder, safer effect on the body and the absence of dangerous side effects compared to preparations of synthetic origin [3, 4]. The group of flavonoids occupies a special place among the most common plant components due to its spectrum of pharmacological action. During the epidemic of COVID-19, drugs based on quercetin have proven themselves as an effective tool in the fight against the virus and a tool for restoring the body after a long period of illness in combination with other known groups of drugs [5, 6]. From the above, it can be concluded that the research of drugs containing components of plant origin is extremely important for further developing pharmaceutical activity in the development of both original and generic drugs [7, 8].

One of the important aspects in the study of medicinal products of any origin is their physicochemical properties, one of which is solubility. Important factors affecting solubility are particle size, moisture, the structure of the crystal lattice [9, 10]. These parameters are rarely considered when conducting studies to determine the solubility of substances. However, these factors can have a significant effect on the thermodynamics of the process, and their parameters must be taken into account [11–13].

To date, in the Pharmacopoeia of Ukraine, there is no way to quantify the solubility of substances, which creates a big problem for sparingly soluble and practically insoluble substances because it is simply impossible to evaluate their solubility quantitatively, using only subjective methods of visual assessment. The European Pharmacopoeia also does not contain similar described methods for determining the exact value of the solubility limit [14, 15].

The biowaiver approach based on the Biopharmaceutical Classification System is intended to reduce the number of in vivo bioequivalence studies, i.e., it can be considered a substitute for in vivo bioequivalence studies [16]. In vivo bioequivalence studies may not be conducted if the assumption of in vivo equivalence can be

justified by satisfactory in vitro data [17, 18]. Given the great value of clinical research, it is very important to be able to carry out the biowaiver procedure based on BCS as a study preceding full-scale clinical trials. This is especially important for developing and producing generic medicines because saving material, time, and human resources will be significant. However, according to the BCS classification, it is impossible to carry out the biowaiver procedure for poorly soluble substances [17, 18]. Thus, for generic drugs containing poorly soluble substances, it is impossible to establish identity with the original, which dramatically increases the risk of obtaining negative results during clinical trials.

The study aimed to develop a method for the quantitative determination of the quercetin solubility limit, given the importance of this physicochemical characteristic as a determining factor in the study of bioequivalence and bioavailability.

To carry out the biowaiver procedure, it is necessary to know the exact solubility limit of the active substance. This is a very important point because we must clearly understand in what volume of the solvent the dose of the active substance can be completely dissolved.

2. Research planning (methodology)

The research consists of theoretical and practical substantiation of the need to establish the exact value of the solubility limit of quercetin. This is the main thing in developing the method of quantitative determination and the study of dissolution kinetics in the process of biowaiver.

The study of the solubility of such poorly soluble substances in aqueous solutions as quercetin requires the following steps:

a) development of a method for quantitatively determining quercetin in aqueous solutions in the range of given concentrations, which has sufficient sensitivity and selectivity;

b) study of the dissolution kinetics of quercetin. Measurement of the dissolution time and concentration of quercetin during the dissolution test in aqueous solutions. Obtaining the value of the optimal dissolution time;

c) measuring the quantitative value of the solubility limit of quercetin in water by gradually diluting the dissolution medium and determining the concentration of quercetin depending on the degree of increase in the volume of the solution and constructing a graphical dependence with a characteristic curve. The concentration at the inflection point corresponds to the solubility limit of quercetin;

d) definition of a mathematical model that should describe the obtained experimental data at a probability level of 5 %. ITS statistical justification for small data samples (less than 10);

e) analysis of the data obtained using the selected mathematical model and calculation of the quantitative value of the solubility limit of quercetin by the method of taking the first derivative for the selected mathematical dependence, which corresponds to the value of the inflection point on the curve.

3. Materials and methods

3. 1. Materials

Quercetin substance – Quercetin HPLC (Wuhan Recedar Biotechnology Co., Ltd). The 1290 Infinity II LC System is an ultra-high performance liquid chromatography instrument (Agilent Technologies), The 6530 Q-TOF LC/MS Quadrupole Time of Flight LC/MS mass spectrometric detector (Agilent Technologies, laser diffraction device (Malvern master-sizer 3000 (USA). Trifluoroacetic acid for HPLC (Sigma-Aldrich, Germany), Acetonitrile P for HPLC (Sigma-Aldrich, Germany).

3. 2. Chromatographic conditions of the HPLC method

For measurements, an Agilent 1290 liquid chromatograph with an Agilent 6530 TOF mass spectrometric detector was used, using a 50×4.6 mm column filled with a sorbent with a grafted phase of octyl silica gel (L1), particle size – 1.7 µm; with thermostatic control (30 °C). Mobile phase A: 0.1 M solution of trifluoroacetic acid, degassed in an ultrasound bath, mobile phase B: acetonitrile P; injection volume – 10.0 µl. The highly selective time-of-flight mass spectrometer had the following settings: ionization type: positive, electrospray (+ESI); measurement mode: scanning in the mass range 50–1500; the voltage on the fragment is 10 V; nitrogen temperature – 350 °C; nitrogen consumption – 10 ml/min; pressure on the nebulizer is 35 PSI; the voltage on the capillary is 4 kV. Elution mode: gradient (Table 1).

Table 1

Gradient program

τ, min	MP A, %	MP B, %
0	100	0
5	100	0
10	50	50
15	50	50
16	100	0
20	100	0

The comparison solution (*c*) was chromatographed, obtaining from 2 to 6 chromatograms. For the peak areas of quercetin, the relative standard deviation (*RSD*) is calculated from the obtained chromatograms.

Obtaining parallel chromatograms (n_0) is stopped when the RSD_{max} requirements for the content tolerance of 15 %, specified in Table 2, are reached [19].

Table 2

Parallel measurement accuracy requirement

n_0	2	3	4	5	6
RSD_{max}	0.76	2.01	2.88	3.56	4.13

20 µl each of the tested solutions and the comparison solution is chromatographed on a liquid chromatograph, and the number of chromatograms is not less than when checking the suitability of the chromatographic system.

3. 3. Processing of received data

Excel software (Microsoft Office 2021) was used to calculate the parameters of the grading equations and construct graphs. Using the obtained measurement results by the external standard method, the concentration of quercetin was calculated for each tested solution, and the dependence of the concentration of quercetin on the volume of the solution was plotted.

The resulting curve will always represent a non-linear relationship with the point of maximum quercetin content – the solubility limit – as a maximum on the curve (Fig. 2).

For a more accurate determination of the maximum position on the curve, the obtained dependence is approximated by a polynomial function of the 2nd or 3rd order with the subsequent taking of the first derivative (Fig. 4, 5). For this, we used a package for mathematical data processing – MicroCal Origin version 3.01 (USA).

4. Result

When formulating the approach, we assumed that the main reasons limiting the solubility of quercetin in aqueous media are thermodynamic and diffusion limitations. The former limits the concentration of quercetin in the solution, and the latter determines the kinetics of the dissolution process.

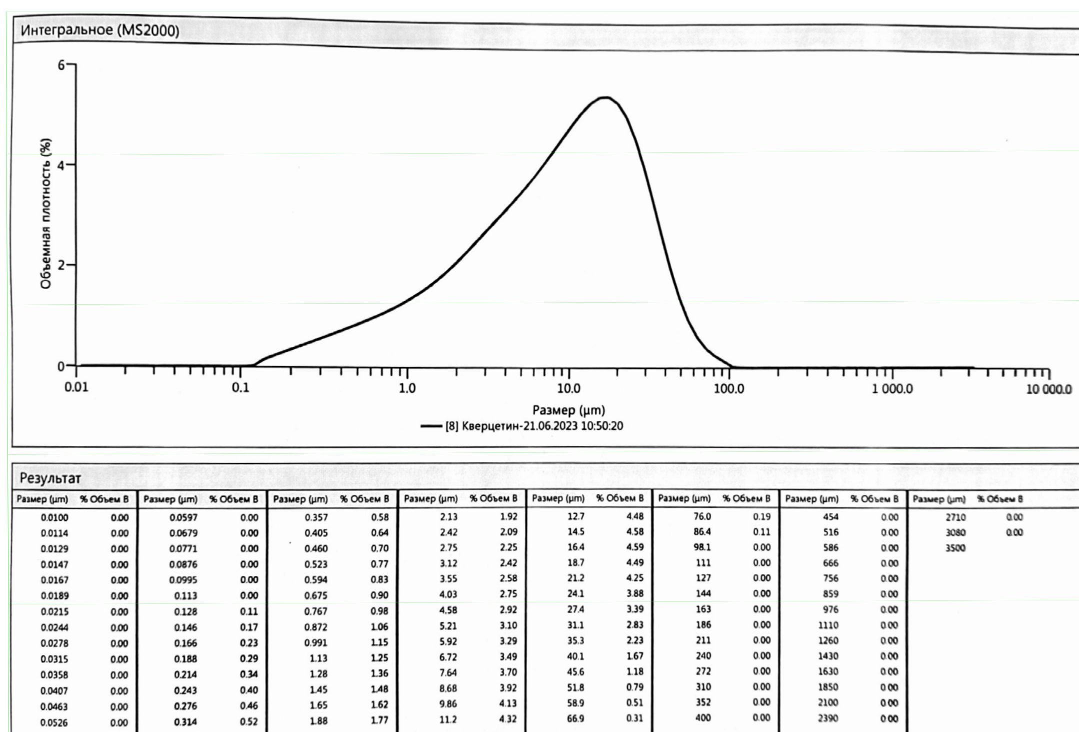
Thus, the experiment was divided into two stages. During the first one, it was necessary to set an hour during which the concentration of quercetin – 6 mg was transferred to the solution in order to guarantee the maximum approximation of the concentration of quercetin in the obtained solution to its equilibrium (pseudo-equilibrium) concentration.

The volume of the solution was determined in such a way (1000 ml) that the dissolution process had no thermodynamic limitations and met the requirements of BCS [17]. According to the conditions of our experiment, for the preparation of the investigated solutions, we took a sample of the quercetin substance for which the moisture content, the content of the substance in the substance, and the degree of pulverization of the substance were characterized by the manufacturer.

The quercetin sample was dried to a constant weight at 500C in a vacuum before use. The content of compatible impurities in quercetin did not exceed 0.2 %.

Since the sample of quercetin was ground before the preparation of the solutions (standard grinding in a mortar was used), the size of the particles obtained after grinding was measured. A laser diffraction device model, MALVERN MASTERSIZER 3000 (USA), was used for measurement. Water was used as a solvent. Fraunhofer scattering model. Below are the results of the measurements.

The results showed that the vast majority of particles are sized from 60 to 90 μm. This size of the particles makes it possible to obtain the kinetics of the dissolution of poorly soluble substances when the equilibrium (pseudo-equilibrium) concentration of quercetin in the solution is reached within a period of 30-40 minutes. These data are in good agreement with the data of the author [19]. Thus, the choice of the dissolution time of the quercetin concentration was justified. Other dissolution conditions met the BCS requirements for the dissolution test conditions during the biowaiver. The dissolution medium is purified water with a pH of 7.0. The volume of the dissolution medium is 1000 ml. Solubility tester according to the USP method 2 (shovel). The rotation speed is 100 rpm. Heating temperature: 37 °C (±0.2 °C). Sampling was carried out from the centre of the vessel after 5, 10, 20, and 30 minutes.



Результат

Размер (μm)	% Объем В	Размер (μm)	% Объем В	Размер (μm)	% Объем В	Размер (μm)	% Объем В	Размер (μm)	% Объем В	Размер (μm)	% Объем В	Размер (μm)	% Объем В	Размер (μm)	% Объем В
0.0100	0.00	0.0597	0.00	0.357	0.58	2.13	1.92	12.7	4.48	76.0	0.19	454	0.00	2710	0.00
0.0114	0.00	0.0679	0.00	0.405	0.64	2.42	2.09	14.5	4.58	86.4	0.11	516	0.00	3080	0.00
0.0129	0.00	0.0771	0.00	0.460	0.70	2.75	2.25	16.4	4.59	98.1	0.00	586	0.00	3500	
0.0147	0.00	0.0876	0.00	0.523	0.77	3.12	2.42	18.7	4.49	111	0.00	666	0.00		
0.0167	0.00	0.0995	0.00	0.594	0.83	3.55	2.58	21.2	4.25	127	0.00	756	0.00		
0.0189	0.00	0.113	0.00	0.675	0.90	4.03	2.75	24.1	3.88	144	0.00	859	0.00		
0.0215	0.00	0.128	0.11	0.767	0.98	4.58	2.92	27.4	3.39	163	0.00	976	0.00		
0.0244	0.00	0.146	0.17	0.872	1.06	5.21	3.10	31.1	2.83	186	0.00	1110	0.00		
0.0278	0.00	0.166	0.23	0.991	1.15	5.92	3.29	35.3	2.23	211	0.00	1260	0.00		
0.0315	0.00	0.188	0.29	1.13	1.25	6.72	3.49	40.1	1.67	240	0.00	1430	0.00		
0.0358	0.00	0.214	0.34	1.28	1.36	7.64	3.70	45.6	1.18	272	0.00	1630	0.00		
0.0407	0.00	0.243	0.40	1.45	1.48	8.68	3.92	51.8	0.79	310	0.00	1850	0.00		
0.0463	0.00	0.276	0.46	1.65	1.62	9.86	4.13	58.9	0.51	352	0.00	2100	0.00		
0.0526	0.00	0.314	0.52	1.88	1.77	11.2	4.32	66.9	0.31	400	0.00	2390	0.00		

Fig. 1. Graph of the distribution of particles in a sample of ground quercetin

The volume of the samples was 1.0 ml. The total volume taken during the analysis was 4.0 ml, which slightly affects the concentration of quercetin in the solutions for which measurements were made.

An aliquot was centrifuged for 10 minutes at 10,000 rpm. 0.5 ml of the upper layer was carefully taken after centrifugation, avoiding hitting the agglomerates of the lower layer, and transferred to vials for chromatography.

Using a mass spectrometric detector made it possible to identify the investigated substance by the molecular ion's mass and sharply increased the selectivity and sensitivity of the developed analytical technique in relation to the spectrophotometric detection of the specified substance.

The results of the first part of the experiment are given in Table 3.

Table 3

The results of determining the quantitative content of quercetin passed into solution depending on the time of analysis

Sampling time, min	The peak area of quercetin was determined, S	Quercetin content, mg/ml	Quercetin content from nominal, %
5	120.157	0.00069	11.44
10	547.437	0.00313	52.14
20	745.922	0.00470	78.33
30	818.272	0.00496	82.71

Based on the obtained results, a dependence characterizing the degree of transition of quercetin into the solution from the time of measurement was constructed. The graph of the concentration of quercetin in the solution versus time is a typical Langmuir isotherm and is shown below (Fig. 2).

The degree of release of quercetin into the solution was calculated as the fraction of quercetin that went into the solution to the initial weight taken for the study and was measured in relative units.

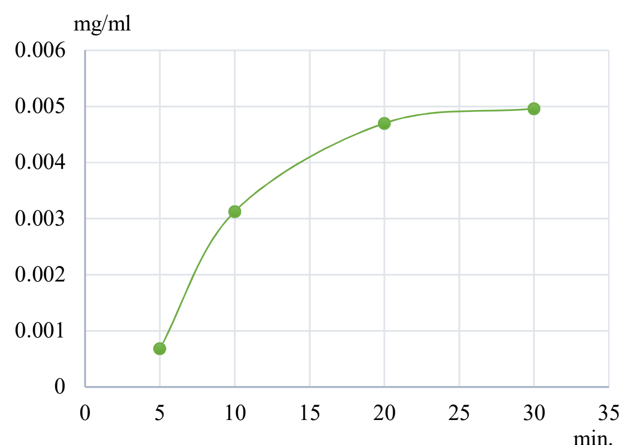


Fig. 2. Graph of the dependence of the concentration of quercetin in the solution on time

Analysis of the resulting dependence allows us to state that the quercetin concentration close to equilibrium (pseudo-equilibrium) is reached within 30 minutes. Following the requirements of BCS, the release of the substance into the solution is satisfactory when 80 % of the initial weight of the substance under investigation is dissolved. It should be noted that the concentration of quercetin in the solution corresponding to these conditions is equal to 0.0047 mg/ml, which is reached after 20 minutes. This indicates that the maximum concentration has been reached, and the variability of all subsequent measurements is due only to the error of the analysis. The obtained dissolution time of the quercetin suspension is in good agreement with the data obtained by the article's authors [19].

The obtained value of the time required to dissolve a selected amount of quercetin in a given volume of solvent allowed us to proceed to establish the actual solubility limit of quercetin by determining its concentration in aqueous solutions.

The second part of the experiment was aimed at quantitatively determining the concentration of quercetin in the solution, corresponding to its solubility limit. For this, five tested solutions of different concentrations and volumes were prepared.

Five investigated solutions were prepared simultaneously:

– test solution 1: 1.0 mg (exact weight) of a thoroughly ground sample of quercetin was placed in a 200 ml flask, 50 ml of water P was added, and the weight was dissolved in an ultrasonic bath for 10 minutes, after which it was brought up to the mark with the same solvent and mixed;

– test solution 2: 1.0 mg (exact weight) of a thoroughly ground sample of quercetin was placed in a 500 ml flask, 300 ml of water P was added, and the weight was dissolved in an ultrasonic bath for 10 minutes, and mixed;

– test solution 3: 1.0 mg (exact weight) of a thoroughly ground sample of quercetin was placed in a 500 ml flask, 350 ml of water P was added, and the weight was dissolved in an ultrasonic bath for 10 minutes, and mixed;

– test solution 4: 1.0 mg (exact weight) of a thoroughly ground sample of quercetin was placed in a 500 ml flask, 400 ml of water P was added, and the weight was dissolved in an ultrasonic bath for 10 minutes, and mixed;

– test solution 5: 1.0 mg (exact weight) of a thoroughly ground sample of quercetin was placed in a 500 ml flask, 400 ml of water P was added, and the weight was dissolved in an ultrasonic bath for 10 minutes, after which it was brought up to the mark with the same solvent and mixed.

Next, the selected samples of the tested samples and the comparison solution were carefully transferred to 2 ml test tubes for centrifugation and centrifuged for 10 minutes at 10,000 rpm. An aliquot of 1 ml of the upper layer was carefully taken after centrifugation, avoiding hitting agglomerates of the lower layer, and transferred to vials for chromatography.

The quercetin concentration was determined in the obtained samples using the chromatographic and external standard methods. The pharmacopeial standard – PS of the SPU was used as a standard.

A comparison solution was prepared according to the following method: 6.0 mg (exact weight) of a thoroughly ground sample of quercetin was placed in a 1000 ml flask, 500 ml of acetonitrile R was added, and the weight was dissolved in an ultrasonic bath for 10 minutes, after which it was brought up to the mark with water R and stirred.

The comparison solution was prepared simultaneously with the preparation of the five tested solutions. All solutions were used immediately after preparation.

Table 4 below shows the concentrations of the tested solutions and the peak areas of quercetin that were measured for these solutions.

Table 4

The quantitative content of quercetin passed into the solution depending on the volume of the test solution

The volume of the solution, l	The theoretical concentration of quercetin, mg/ml	The measured peak area of quercetin, S	Quercetin content, mg/ml	Quercetin content, mcg/ml
0.2	0.00500	170.78	0.0010	1.00
0.3	0.00333	354.58	0.0030	3.00
0.35	0.00286	348.03	0.0029	2.90
0.4	0.00250	289.18	0.0026	2.60
0.5	0.00200	194.58	0.0018	1.80
Output standard deviation (*SD*)			0.00847	0.84

Based on the measurement data, a non-linear dependence was constructed in the "concentration – degree of dilution" coordinates (Fig. 3). The initial part of the curve shows an increase in the concentration of quercetin in the solution with an increase in the volume of the solution. This is probably due to a decrease in the thermodynamic limit of the solubility of quercetin, which leads to an increase in its concentration in the solution with an ever-increasing rate of dissolution of quercetin. This process continues until some equilibrium (pseudo-equilibrium) concentration is reached and is accompanied by a decrease in the dissolution rate of quercetin. A further increase in the volume of the solution, on the contrary, leads to a decrease in concentration since the reached equilibrium (pseudo-equilibrium) concentration of quercetin is subject to increasing dilution. This is expressed on the graph as a descending branch of the curve. The resulting point of inflection of this dependence will correspond to the complete dissolution of the amount of quercetin that was taken to prepare the investigated solution.

In this case, the accuracy of setting the value corresponding to the solubility limit of quercetin is directly proportional to the number of measured values of the concentration of quercetin in the solution and, therefore, the points on the graph of the curve. Carrying out many determinations (at least 20) necessary to achieve the specified accuracy of 5 % when determining the solubility limit makes the proposed method unusually time-consuming and costly. This makes it impossible to use it for widespread use.

Therefore, the authors proposed an approach that allows for a small number of measurements (up to 10) to acquire a more accurate value of this point, which will correspond to the limit of solubility of quercetin in water.

The solution to this problem consists of an attempt to approximate the obtained experimental data with a certain mathematical function. For this, three options were considered: a polynomial of the 2nd degree and a polynomial of the 3rd degree. The obtained results are presented in Fig. 4, 5.

The regression analysis of the obtained dependencies made it possible to calculate a number of statistical parameters: standard deviation (*SD*), Fisher's criterion (*f*), determination criterion (R^2), and probability (%) [20]. The values are given in Tables 5, 6.

By comparing the values of the obtained values of the specified statistical parameters, the variant whose model most accurately describes the presented graph was found – a polynomial of the third degree.

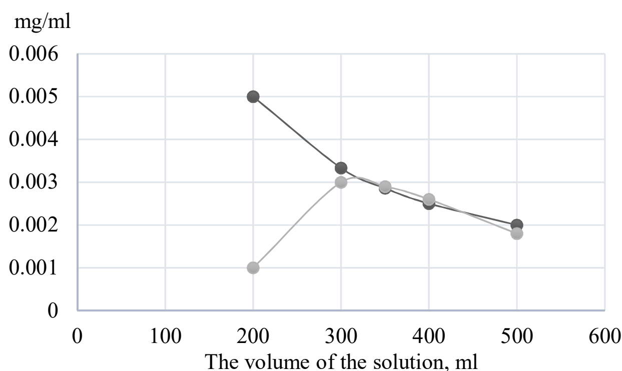


Fig. 3. Graph of the dependence of the concentration of quercetin on the volume of the solvent

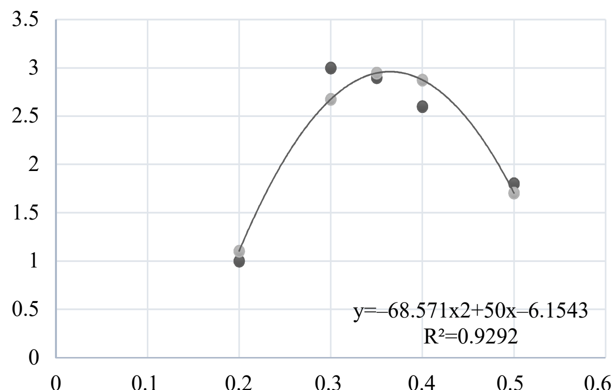


Fig. 4. Graph of a polynomial of the 2nd degree

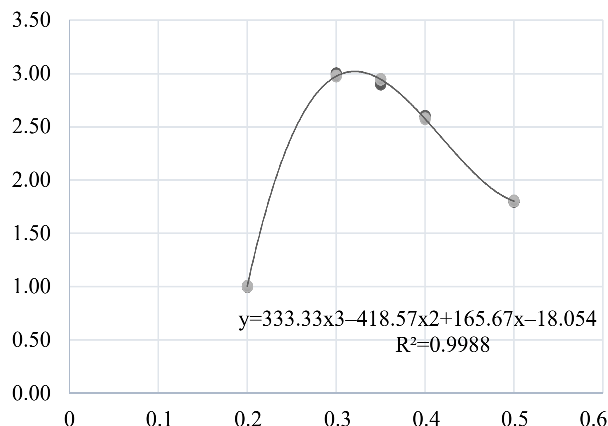


Fig. 5. Graph of a polynomial of the 3rd degree

In order to assess how adequate the correlation between the measured parameters is, we used the value of the coefficient of determination (R^2) and Fisher's test, which helps to avoid a biased assessment when choosing. It is known that with a probability of 0.05, suitable values of the correlation coefficient will be considered to be values greater than 0.99 if we are talking about methods of quantitative determination. A polynomial function of the 3rd order meets this requirement [20].

This variant with the highest coefficient of determination is the most reliable, despite the fact that it has the smallest value of the degree of freedom – 1.

Table 5

Statistical analysis of a polynomial of the 3rd degree

Polynomial equation: $y=A*x^3+B*x^2+C*x+D$; $y=333.33*x^3-418.57*x^2+165.67*x-18.054$ ($R^2=0.9988$)			
Number of measurements (n)	Output data	Calculated data ($Ycalc$)	Squared difference (($Y-Ycalc$) ^2)
1	1.00	1.00384	0.0000147
2	3.00	2.97561	0.0005949
3	2.90	2.94720	0.0022277
4	2.60	2.57592	0.0005798
5	1.80	1.80475	0.0000226
Output Standard Deviation (SD)	0.84	–	–
Degree of freedom (k)	4	–	–
Degree of freedom ($n-k$)		1	–
Sum (Sum)			0.00344
Residual standard deviation (SD_{rest})			0.05864
Fisher's test (F)			208.736
Test value $R^2=1-1/F=$			0.99521
Probability %			0.477
Criterion %			<5

Table 6

Statistical analysis of a polynomial of the 2nd degree

Polynomial equation: $y=A*x^2+B*x+C$; $y=-68.571x^2+50*x-6.1543$ ($R^2=0.9292$)			
Number of measurements (n)	Output data	Calculated data ($Ycalc$)	Squared difference (($Y-Ycalc$) ^2)
1	1.00	1.10286	0.01058
2	3.00	2.67431	0.10607
3	2.90	2.94575	0.00209
4	2.60	2.87434	0.07526
5	1.80	1.70295	0.00942
Output Standard Deviation (SD)	0.84	–	–
Degree of freedom (k)	3	–	–
Degree of freedom ($n-k$)		2	–
Sum (Sum)			0.203428
Residual standard deviation (SD_{rest})			0.318927
Fisher's test (F)			7.059
Test value $R^2=1-1/F=$			0.8583
Probability %			12.8
Criterion %			<5

Estimated values of the Fisher test were calculated as the ratio of the squares of the residual standard deviations obtained for the sample of measured quercetin concentrations and the values calculated for the selected correlation dependences of a given probability of 0.05 [20].

For a polynomial of the 3rd degree, the requirements of the Fisher criterion are fulfilled. Given this and the value of the coefficient of determination, the residual standard deviation, and the probability, it can be used to predict the required value – the dissolution limit. For this, the obtained experimental dependence was approximated by a polynomial dependence of the third degree (Fig. 5). For a polynomial of the 2nd degree (Fig. 4), the requirements of the Fisher criterion are also fulfilled, but the calculated value of R^2 for it is less important than for a polynomial of the third degree. Also, for a polynomial of the 2nd degree, we have a higher probability value of 12.8 % compared to a polynomial dependence of the 3rd degree, which corresponds to a value of 0.477 %. All this makes the polynomial dependence of the second degree less suitable for approximating experimental results [21].

In graphs 4 and 5, the polynomial of the 2nd degree and the polynomial of the 3rd degree, respectively, we can observe the location of the concentration points obtained by theoretical calculation and experimentally. It is visible that the location of points, both theoretical and practical, is best determined by a polynomial function of the 3rd order.

Knowing the type of correlation dependence, it is easy to calculate the value of the solubility limit by taking the first derivative, the value of which will correspond to the value of the inflection point of the analyzed curve. Thus, using the polynomial dependence of the third degree and solving the system of equations using the MicroCal Origin mathematical package, we obtained the concentration value corresponding to the inflection point of the investigated dependence. The obtained value corresponds to the solubility limit of quercetin.

According to the resolution of the equations of polynomial functions given above, the results were obtained after taking the first derivative. For the polynomial of the 2nd degree, the calculated concentration value was 2.96 mcg/ml, and for the polynomial of the 3rd degree – 3.02 mcg/ml. These values are consistent with data confirming the limited solubility of quercetin in aqueous media with a neutral pH. Also, the result obtained confirms the fact that a polynomial function of both the 2nd and 3rd order can be used to find the solubility limit. Thus, in the work of I. Kovalevska, quercetin belongs to practically insoluble substances (0.435 g/100 ml of purified water at pH 7.0) [22].

Validation of the developed methodology.

Validation was carried out for the HPLC mass spectrometric technique for the quantitative determination of quercetin in aqueous solutions. As part of this validation, it is shown that the error of determining the content of quercetin in the sample does not exceed the maximum error for the specified method in the entire concentration range of 95–105 % of the nominal value. Below are the results.

The validation of the methodology for the quantitative determination of the quercetin substance was carried out according to separate validation characteristics:

specificity, linearity, accuracy, precision, and intra-laboratory precision [14].

To estimate the error of sample preparation of model solutions and the standard solution, the theoretical values of the uncertainty of the analytical operation were calculated, which was Δ_{sp}=1.06 %≤5·0.32=1.6 %. Therefore, the calculated uncertainty of sample preparation and analysis as a whole should ensure sufficient measurement accuracy. Chromatography solutions were prepared according to the above analytical method. The blank solution was prepared similarly to the investigated solution.

The linearity assessment was carried out in the range (of 80–120 %) of the application of the technique according to the standard method. The study of the nature of the dependence of the signal as a function of concentration was carried out using 9 model solutions for analysis with exact concentration weights: 80, 85, 90, 95, 100, 105, 110, 115, and 120 %. At the same time, the concentration taken as 100 % was the concentration of quercetin, which is in the middle of the range covering the min and max concentrations of quercetin.

The obtained results were statistically processed by the method of least squares in accordance with the requirements of the Federal State of Ukraine. The construction of the calibration graph was carried out in normalized coordinates. For each of the nine sample solutions, the average values of the peak area (S_i) were calculated. The obtained results were processed by the method of least squares for the line $Y=b*x+a$. The calculated statistical values b, S_b, a, S_a, S_r (final standard deviation), and r (correlation coefficient) are shown in Table 7.

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

To measure and calculate the metrological assessment of the *precision* and *accuracy* of the method, three values of the peak area for the reference solution and 27 values of the peak area for the model solutions were obtained. We calculated the actual values, the ratio of the average values of the peak areas for each of the 27 solutions to the average value of the peak areas of the comparison solution, obtaining the values $Xi=(C_i/C_{st})*100$ %, $Yi=(S_i/S_{st})*100$ %, and also the value $Zi=(Y_i/X_i)*100$ %, which is the concentration found as a percentage of the input. The results of the calculations are given in Tables 8–10.

Table 7

Characteristics of linear regression

Parameters	Value		
b	3.9034		
s_b	0.0059065		
a	0.79		
s_a	0.41272		
s_0	0.71375		
s_0/b	|0.183|		
s_Y	157.23		
r	|0.99999|		
Reference solution	Average S_{St}	C_{St}	RSD_{St}, %
Reference	391.5	100	0.181

Table 8

Values of dispersion

Test solutions	Name	Average S_i	C_i	RSD_i, %
1	5	19.45	5	0.3636
2	10	39.75	10	0.17789
3	20	79.65	20	0.08878
4	30	117.65	30	0.0601
5	60	236.25	60	0.02993
6	70	273.55	70	0.02585
7	80	313.65	80	0.02254
8	90	351.45	90	0.02012
9	100	390.85	100	0.018092
10	120	469.15	120	0.015072

Note: Student (95, 1, 11)=1,7956

Requirements to the maximum permissible RSD_P: 1.67 %.

The calculated value until the convergence of the measurements is maintained.

Table 9

Results of analysis of model solutions and their statistical processing

Test solutions	Name	Average S_i	C_i	Y_i	X_i	RSD_i, %	Z_i, %
1	5	19.45	5	4.97	5.00	0.36	99.36
2	10	39.75	10	10.15	10.00	0.18	101.53
3	20	79.65	20	20.34	20.00	0.09	101.72
4	30	117.65	30	30.05	30.00	0.06	100.17
5	60	236.25	60	60.34	60.00	0.03	100.57
6	70	273.55	70	69.87	70.00	0.03	99.82
7	80	313.65	80	80.11	80.00	0.02	100.14
8	90	351.45	90	89.77	90.00	0.02	99.74
9	100	390.85	100	99.83	100.00	0.02	99.83
10	120	469.15	120	119.83	120.00	0.02	99.86

Table 10

Accuracy and precision parameters

Validation parameter	Parameter	Value	Requirements 1	Requirements 2	Conclusions
Precision	Δ_Z	1.43	≤3	–	Maintained
Accuracy	$\|Z_{cp}-100\|$	|0.28|	≤0.45	≤0.96	Maintained on the first criterion

Note: s_Z (%)=0,78103; Student (95, 1, 9)=1,83310

5. Discussion

The main and outstanding advantage of the proposed method of quantitative determination of the solubility limit is that we were able to quantitatively determine the solubility limit of poorly soluble substances with an accuracy that does not exceed the error of the chromatographic method of determination, which was 1.6 %. Prior to this, no methods would allow for the standardization of the conditions for the quantitative determination of the solubility limit for a given measurement error. The pharmacopoeias of the leading countries do not contain such data. The lack of such data makes it impossible to study the bioavailability of poorly water-soluble substances by in vitro methods.

The main example for comparing the results was the method proposed by Igor Zenkevich and described in work devoted to the study of the solubility limit of quercetin under conditions of pH variability [23, 24]. However, the comparative analysis reveals significant differences. Firstly, in the work of Igor Zenkevich cited above, the study of the solubility limit of quercetin was carried out at pH values corresponding to acidic or alkaline environments, which requires further extrapolation of the data for pH 7.0. We can conduct all studies at pH values corresponding to acidic, slightly acidic, and neutral values of the dissolution medium. This is an advantage and difference since this approach is more similar to the real model for conducting drug bioavailability studies. Such studies assume that the pH value is always neutral [17].

The second significant difference is that in the work of Igor Zenkevich, the determined value of the quercetin solubility limit is outside the range of measured values. Which significantly reduces the accuracy of the obtained value since it was obtained by extrapolation. In our study, the value of the solubility limit is within the experimentally studied interval, which makes the accuracy of our measurements much higher.

Study limitations. Measurements carried out according to the scheme proposed by the authors of the article will have limitations related only to the specificity of the technique used in the research data for the quantitative determination of quercetin. Since this was an HPLC technique with mass spectrometric detection, the main limitations will be the sensitivity of the detector, the linear range of measured concentrations, and its ability to detect the substance under investigation in the sample. Also, to conduct this research, it is necessary to use a standard sample of the substance under investigation with a specified value of the quantitative content of the substance and an error that does not exceed the error of the analysis, which makes this method more expensive to implement [25, 26].

Prospects for further research. There is interest in studying the dissolution profiles for other substances of substances that are limitedly soluble, as well as highly soluble in water. Implementation of the developed method for a deeper study of the kinetics and in vivo experiments in the study of the bioavailability of drugs.

For a more reliable assessment of the obtained values of the solubility limit, it is necessary to obtain these values by another analytical method, preferably by direct determination without using comparative methods with standard samples.

6. Conclusions

The requirements for correctness are met according to the first criterion.

To assess interlaboratory precision, we used a relative confidence interval for 5 parallel determinations of the quantitative content of substances, which should be less than the maximum permissible uncertainty of the analysis results: $\Delta z \leq 1.6$ %. The tests were carried out using the same series of the drug by different analysts on the same chromatograph on different days using different-sized dishes.

The interlaboratory precision is confirmed by the fact that the value of the relative confidence interval for five parallel determinations of one series of the drug satisfies the acceptance criterion (Δz=0.22 %≤1.6 %).

Since the method of high-performance liquid chromatography (HPLC) which was used in the method is specific, it is enough to fulfil all the requirements for the criteria of linearity, accuracy, precision, and interlaboratory precision.

In the process of verification of the method of quantitative determination of the quercetin substance, the validation characteristics of the HPLC method according to the standard method were studied: accuracy, linearity, precision, specificity, and interlaboratory precision.

The validation characteristics of the method do not exceed the critical error value (1.6 %) and are characterized by qualitative analytical indicators. This technique can be correctly reproduced in laboratory conditions.

As a result of the study, a new approach has been developed to quantify the solubility limit of difficult or practically insoluble substances in aqueous media with a neutral pH value when studying their bioavailability.

Quantitative analysis of the resulting solutions was carried out by high-performance liquid chromatography using a mass spectrometric detector. The exact value of the solubility limit for the studied sample of quercetin was established using a polynomial function of the 2nd and 3rd order, which amounted to 2.96 mcg/ml and 3.02 mcg/ml, respectively.

The value of the quercetin solubility limit thus obtained made it possible to study the kinetics of the release of quercetin into aqueous solutions.

Conflict of interests

The authors declare that they have no conflict of interest concerning this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

Funding

The study was performed without financial support.

Data availability

The manuscript has no associated data.

References

1. Mudrak, I. H., Zaliska, O. M. (2011). Analiz dynamiky dokazovykh danykh pro likarski roslynni zasoby u bazi Kokrana. Farmatsevtychnyi chasopys, 3, 75–78.

2. Sahoo, N., Manchikanti, P., Dey, S. (2010). Herbal drugs: Standards and regulation. Fitoterapia, 81 (6), 462–471. doi: https://doi.org/10.1016/j.fitote.2010.02.001

3. Borghetti, G. S., Carini, J. P., Honorato, S. B., Ayala, A. P., Moreira, J. C. F., Bassani, V. L. (2012). Physicochemical properties and thermal stability of quercetin hydrates in the solid state. Thermochimica Acta, 539, 109–114. doi: https://doi.org/10.1016/j.tca.2012.04.015

4. Kaşıkcı, M., Bağdatlıoğlu, N. (2016). Bioavailability of Quercetin. Current Research in Nutrition and Food Science Journal, 4 (2), 146–151. doi: https://doi.org/10.12944/crnfsj.4.special-issue-october.20

5. Parasuraman, S., Anand David, A., Arulmoli, R. (2016). Overviews of biological importance of quercetin: A bioactive flavonoid. Pharmacognosy Reviews, 10 (20), 84–89. doi: https://doi.org/10.4103/0973-7847.194044

6. Zheng, Y.-Y., Ma, Y.-T., Zhang, J.-Y., Xie, X. (2020). COVID-19 and the cardiovascular system. Nature Reviews Cardiology, 17 (5), 259–260. doi: https://doi.org/10.1038/s41569-020-0360-5

7. Althans, D., Schrader, P., Enders, S. (2014). Solubilisation of quercetin: Comparison of hyperbranched polymer and hydrogel. Journal of Molecular Liquids, 196, 86–93. doi: https://doi.org/10.1016/j.molliq.2014.03.028

8. WHO Model List of Essential Medicines, 18th list (2013). World Health Organization. Available at: https://apps.who.int/iris/handle/10665/93142

9. Abraham, M. H., Acree, W. E. (2014). On the solubility of quercetin. Journal of Molecular Liquids, 197, 157–159. doi: https://doi.org/10.1016/j.molliq.2014.05.006

10. Aguda, R., Chen, C.-C. (2016). Solubility of Nutraceutical Compounds in Generally Recognized as Safe Solvents at 298 K. International Journal of Chemical Engineering and Applications, 7 (5), 289–294. doi: https://doi.org/10.18178/ijcea.2016.7.5.591

11. NCBI homepage U.S. National Library of Medicine National Center for Biotechnology Information. Pubchem. Available at: https://pubchem.ncbi.nlm.nih.gov/

12. Chebil, L., Chipot, C., Archambault, F., Humeau, C., Engasser, J. M., Ghoul, M., Dehez, F. (2010). Solubilities Inferred from the Combination of Experiment and Simulation. Case Study of Quercetin in a Variety of Solvents. The Journal of Physical Chemistry B, 114 (38), 12308–12313. doi: https://doi.org/10.1021/jp104569k

13. Srinivas, K., King, J. W., Howard, L. R., Monrad, J. K. (2010). Solubility and solution thermodynamic properties of quercetin and quercetin dihydrate in subcritical water. Journal of Food Engineering, 100 (2), 208–218. doi: https://doi.org/10.1016/j.jfoodeng.2010.04.001

14. State Pharmacopoeia of Ukraine. Vol. 1 (2015). Kharkiv: Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines, 1128.

15. European Pharmacopoeia (2016). Strasbourg: European Department for the Quality of Medicines.

16. Nastanova z klinichnykh doslidzhen. Likarski zasoby. Doslidzhennia biodostupnosti ta bioekvivalentnosti. Nastanova 42–7.1:2005. Kyiv: Ministerstvo okhorony zdorov'ia Ukrainy.

17. M9 Biopharmaceutics Classification System-Based Biowaivers, Guidance for Industry U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER) (2021). Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/m9-biopharmaceutics-classification-system-based-biowaivers

18. Marchyshyn, S. M., Stoiko, L. I., Mosula, L. M. (2018). Vyznachennia flavonoidiv tyrlychu khreshchatoho travy (Gentiana Cruciata l.). Fitoterapiia. Chasopys, 2, 58–61.

19. Grizodub, A. I., Gubarevich, I. G., Nikishina, L. E., Leontev, D. A., Akichev, A. Sh., Glumenko, E. N., Baumer, V. N. (2008). Zavisimost rastvorimosti fensuktcinala ot razmera chastitc. Farmakom, 1, 50–67.

20. Doerfel, K. (1968). Statystyka v analytychnoi khimii. Moscow: Mir.

21. Epshtein, N. A. (2019). Validation of Analytical Procedures: Graphic and Calculated Criteria for Assessment of Methods Linearity in Practice. Drug Development & Registration, 8 (2), 122–130. doi: https://doi.org/10.33380/2305-2066-2019-8-2-122-130

22. Kovalevska, I. V., Ruban, O. A., Hrudko, V. O. (2015). Study of quercetin release of solid dispersions with macromolecular substances. Zbirnyk naukovykh prats spivrobitnykiv NMAPO im. P. L. Shupyka, 24 (5), 318–322. Available at: http://nbuv.gov.ua/UJRN/Znpsnmapo_2015_24 %285 %29__62

23. Zenkevich, I. G., Guschina, S. V. (2010). Determination of dissociation constants of species oxidizable in aqueous solution by air oxygen on an example of quercetin. Journal of Analytical Chemistry, 65 (4), 371–375. doi: https://doi.org/10.1134/s1061934810040064

24. Zenkevich, I. G., Olisov, D. A., Shafigulin, R. V., Bulanova, A. V. (2019). A new approach to the chromatographic determination of quercetin water solubility. Analitika i Kontrol, 23 (3), 386–392. doi: https://doi.org/10.15826/analitika.2019.23.3.013

25. Kovalevska, I. V. (2014). Quercetin physical-chemical characteristics' definition. Current issues in pharmacy and medicine: science and practice, 1 (14): 9–11. Available at: http://nbuv.gov.ua/UJRN/apfimntp_2014_1_5

26. Shubenkova, E. G. (2015). Kolloidnaia khimiia. Poverkhnostnye iavleniia: praktikum. Omsk: Izd-vo OmGTU, 64.

Received date 17.04.2023
Accepted date 22.06.2023
Published date 30.06.2023

Nataliia Khanina*, PhD Student, Department of Pharmaceutical Chemistry, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002; Senior Associate manager of Regulatory Affairs, Meitheal Pharmaceuticals, 8700 W Bryn Mawr Ave #600s, Chicago, IL, USA, 60631

Victoriya Georgiyants, Doctor of Pharmaceutical Sciences, Professor, Head of Department, Department of Pharmaceutical Chemistry, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002

Vadim Khanin, PhD Chemistry, QC Specialist, State Laboratory of Drug Quality Control, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002

***Corresponding author:** *Nataliia Khanina, e-mail: natalykhanina@gmail.com*