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DEVELOPMENT OF A NEW SOLUTION FOR DETERMINING THE SOLUBILITY LIMIT OF QUERCETIN AND OTHER POORLY SOLUBLE SUBSTANCES IN AQUEOUS SOLUTIONS USING THE METHOD FOR DETERMINING TOTAL ORGANIC CARBON

Nataliia Khanina, Victoriya Georgiyants, Vadim Khanin

Aim. *Given the incompleteness of literature data on the solubility of quercetin and the importance of this physicochemical characteristic in the study of its bioavailability, there is a need to develop an alternative method for accurately quantifying the solubility limit of quercetin.*
Materials and methods. *The concentration of quercetin in the samples was determined by directly determining the total organic carbon. For measurements, a total organic carbon analyzer 450 TOC (METTLER TOLEDO) was used with a range of measured values of 0.05–1000 ppbC µgC/l.*
Results. *The exact limit of the solubility of quercetin, as a poorly soluble substance, has been established. Having previously measured the value of total organic carbon in the prepared solutions, we obtained data on the concentration of quercetin in solutions depending on the pH of the solution.*
Having built a graphical dependence of the measured values of the concentration of a substance on the pH values of the studied solutions, we obtain a mathematical equation of the reliance. Using the resulting function equation, one can approximate the concentration value of a substance with a pH value of 7.0. This value will be the solubility limit of the test substance for neutral media.
Conclusions. *As a result of the research, a new method was proposed for the quantitative determination of the solubility limit of a substance, with an accuracy not exceeding 5.0 %. The method is based on measuring the concentration of total carbon in acidic solutions with different pH values and subsequent approximation of the obtained dependence of the pH value equal to 7.0*
Keywords: *quercetin, identification, quantitation, total organic carbon, method development, bioequivalence, biowaiver, solubility, dissolution test*

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1. Introduction

In recent years, more and more people have turned to the using herbal drugs for the treatment and prevention of various diseases. The importance of the research on herbal preparations is that it allows us to use them more effectively in medical practice, reduce the risks of side effects, and improve the quality of life of people. Approximately more than 3000 varieties of flavonoids have been identified, and it has aroused particular interest recently because of their potential beneficial effect on human health. They have reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor, antioxidant, and treatment of neurodegenerative disorders [1]. Flavonoids are categorized into six classes according to their chemical structure flavonols, flavones, flavanones, flavanols, isoflavones, and anthocyanidins [2].

Quercetin, a plant-derived aglycone form of flavonoid glycoside, has been used as a nutritional supplement and may be beneficial against various diseases. Some of the beneficial effects include cardiovascular protection, anticancer, antitumor, anti-ulcer, anti-allergy, anti-viral, anti-inflammatory activity, anti-diabetic, gastroprotective effects, antihypertensive, immunomodulatory, and anti-infective [3, 4].

One of the core most remarkable properties of quercetin is its ability to modulate inflammation. Quercetin inhibits inflammatory enzymes cyclooxygenase (COX) and lipooxygenase, thereby decreasing inflammatory mediators such as prostaglandins and leukotrienes [5, 6].

The neuroprotective effects of quercetin are mainly due to its antioxidant capacity and ability to trap free radicals. Quercetin supplementation may affect mitochondrial biogenesis, energy production and electron transport chain performance, ROS production modification, and mitochondrial defects. It can also pass through the blood-brain barrier [7, 8].

Quercetin, along with ascorbic acid, reduces the incidence of oxidative damage to human lymphocytes and neurovascular structures in the skin and inhibits damage to neurons. It protects brain cells against oxidative stress, which damages tissue, leading to Alzheimer's and other neurological conditions [9]. Flavonoids are believed to be crucial in protecting neuronal injury [10].

Flavonoid exerts neuroprotective actions within the brain, including a potential to protect neurons against damage induced by neurotoxins. It also has the additional ability to suppress neuroinflammation and promote memory, learning, and cognitive functions. Flavonoids are also found to exhibit protective features capable of preventing more serious degenerative diseases and many forms of cerebrovascular disease associated with dementia and stroke, affecting predominantly elderly people [11]. Flavonoid-rich plant or food supplement improves cognition functions and protects vulnerable neurons by enhancing existing neuronal function or by stimulating neuronal regeneration [12–14].

Several studies have been conducted to study the effect of quercetin on the COVID-19 virus, which has presented a particular danger in recent years and caused one of the largest pandemics. One study published in the journal Nutrients in 2020 found that quercetin can reduce the production of cytokines that promote inflammation in the lungs, which may be useful in treating COVID-19 [15, 16].

However, the issue of quercetin bioavailability is still under study and debate. When conducting such research, we are forced to adhere to certain standards. One of these is the Biopharmaceutics Classification System, BCS (Biopharmaceutics Classification System) guidance. It, in particular, defines the criteria and requirements for the characteristics of substances that can be studied by this method [17].

It is a system of classifying drugs based on their physicochemical properties, which helps study and predict their solubility and bioavailability. Solubility is a key parameter that affects the effectiveness and safety of drugs since only dissolved components can be absorbed by the body. It is based on two parameters: solubility coefficient and penetration coefficient. The solubility coefficient characterizes the ability of the medicinal substance to dissolve in water or other solvents, and the penetration coefficient – the ability of the medicinal substance to penetrate through cell membranes [17].

According to the BCS system, medicinal products are classified into four classes. Class 4 drugs have low solubility and low penetration coefficients. These drugs have difficulties with dissolution and absorption by the body. This class includes, for example, almost all-natural plant extracts, as they contain components that are poorly soluble in water or other solvents. Belonging to class 4 and the physicochemical properties of these substances create a big obstacle in the way of studying these substances since research can only be carried out "*in vivo*", which makes research dramatically more expensive. Quercetin also belongs to lipophilic compounds. For this reason, the bioavailability of quercetin is very often low [17].

The solubility limit is a specific characteristic of a substance. Using this feature of measurements, it can be argued that both selective and non-selective analysis methods are suitable for research. Since the object of study is a nonionic organic substance, methods that determine the concentration of charged particles in a solution are unsuitable for solving the problem. However, the method of determining total organic carbon (not a selective analysis method) can be considered in this case. With sufficient sensitivity, at the level of one mg/ml, with a wide linear concentration range from 0.05 mg/ml to 1000 mg/ml, it is the best method for investigating the concentration of quercetin in acidic aqueous solutions. At the same time, this method does not require measurements of the reference substance, which allows you to confirm or refute the results obtained earlier [18].

The method of determining the solubility limit of quercetin in aqueous solutions by the HPLC-MS method successfully allowed us to establish the value of this characteristic. However, working with very low concentrations of quercetin creates a problem with the accuracy of the obtained measurements, and the requirements for measurement error are very strict. A second argument in favour of using a total organic carbon analyzer is the fact that this method does not require the use of standards for analysis. That greatly simplifies and lowers the cost of the research process. It is known from the literature that quercetin has good solubility in acid solutions, at least 0.06 mg/ml [19]. Thus, for quercetin solutions in acidic and weakly acidic environments, it is possible to obtain fairly accurate values of the solubility limit.

Using the above considerations, there was a need to create a methodology for the quantitative determination of the solubility limit of quercetin by the method of determining total organic carbon in acidic and slightly acidic water environments. It was also necessary to develop a method for the quantitative determination of the solubility limit of a substance with a determination error of no more than 5.0 %, which would allow comparison of the determination results with the data of other methods, particularly chromatographic.

2. Planning (methodology) of research

The exact determination of the solubility limit of substances that are poorly soluble in aqueous solutions, such as quercetin, requires the following stages:

a) development of a method for the quantitative determination of quercetin in acid solutions in the range of different pH values by the method of determining total organic carbon;

b) having established the value of total organic carbon in the prepared solutions, we obtain data on the concentration of quercetin in the solutions depending on the pH of the solution;

c) measurement of the quantitative value of the solubility limit of quercetin in solutions with different pH values;

d) the last stage consists of the analysis of the obtained measurement data and the calculation of the quantitative value of the solubility limit of quercetin using models of mathematical extrapolation.

The research consists of theoretical and practical substantiation of the need to establish the exact value of the solubility limit of quercetin by an alternative method. In the process of studying the bioavailability of such poor-

ly soluble substances as quercetin, it is of great importance to establish the exact value of the solubility limit.

3. Materials and methods

3. 1. Materials

Quercetin substance – Sophora Japonica Extract, 98 % HPLC UV Quercetin Powder 117-39-5 (Wuhan Recedar Biotechnology Co., Ltd). The analyzer of total organic carbon 450 TOC (METTLER TOLEDO), range of measured values 0.05–1000 ppbC μgS/l, pH meter 827 pH lab; 230 V, EU with Primatrode (Metrohm), balance XP 205, Analytical Balance (METTLER TOLEDO), Water for injection (>15 mΩ·cm [0.067 μS/cm]), 773185, (MilliporeSigma), Hydrochloric acid solution, volumetric, 0.1 M HCl (0.1N), (100 %), endotoxin-free, 10037 (MilliporeSigma), class A measuring glassware.

3. 2. Sample preparation methodology

Four solutions of quercetin with an initial concentration of 6.0 mg/ml were used as the research object.

Preparation of 4 test solutions: 6.0 mg (exact weight) of carefully ground and dried to constant weight quercetin sample was placed separately in 1000 ml flasks, 900 ml of water was added to each flask and potentiometrically titrated with 1 M hydrochloric acid until pH values of 3.0, 4.0, 4.5, 5.0 were obtained for each solution respectively. After that, the obtained suspensions were dissolved in an ultrasonic bath for 10 min and brought to the mark with the same solvent with subsequent mixing.

The concentration of quercetin in the obtained samples was determined directly by the method of determining the total organic carbon.

The validation of the methodology was carried out following the requirements of the State Pharmacopoeia of Ukraine [20]. Before conducting the main validation tests, the presence of documents confirming the suitability of the used equipment, raw materials, and reagents was monitored.

3. 3. Processing of received data

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

Based on the obtained measurement results, the concentration of quercetin was calculated for each tested solution with different pH values.

Plotting the graphical dependence of the obtained values of the concentration of quercetin on the pH values of the investigated solutions allows for obtaining a mathematical equation of the dependence. Using the constructed equations of functions, by the method of approximation, we obtain the value of the concentration of the substance at a pH value of 7.0. This will be the solubility limit of the tested substance for neutral media.

4. Result

Quercetin is one of the typical representatives of bioflavonoids, the bioavailability of which cannot be studied according to the requirements of the BCS system.

However, using the technique we developed to quantify quercetin, it is possible to study the dissolution kinetics of such substances in aqueous media with a pH close to neutral, as required by BCS.

All lipophilic substances, as a rule, dissolve well in aqueous environments with acidic, slightly acidic, slightly alkaline, and alkaline pH values. In particular, quercetin is highly soluble in acidic media. Thus, it becomes possible to accurately determine the solubility limit for solutions of the substance under investigation in acidic or alkaline media because the high solubility of the substance sharply increases the limit of quantitative detection of the technique.

Thus, we get some solutions where the only carbon source is the quercetin we introduced. Using the method of determining total carbon, based on the technology of continuous measurement, in the flow of the analyzed liquid passing through a quartz tube of a spiral shape, with constant exposure to UV radiation. This leads to the complete oxidation of quercetin with the formation of carbonic acid and the appearance of ionic conductivity. The device calculates the total content of organic carbon by differential electrical conductivity, which is measured by high-precision conductometric sensors.

Having previously measured the value of total organic carbon in the prepared solutions, we obtained data on the concentration of quercetin in the solutions depending on the pH of the solution.

After plotting the graphical dependence of the measured values of the concentration of the substance on the pH values of the investigated solutions, we obtain a mathematical equation of the reliance. Using the resulting equation of the function, it is possible to approximate the value of the concentration of the substance at a pH of 7.0. This value will be the solubility limit of the investigated substance for neutral media.

Below are the results of measuring quercetin solutions in acidic environments with pH values of 3.0, 4.0, 4.5, and 5.0 (Tables 1–4).

Table 1

Measured values of total carbon at pH 3.0

No.	TOC of the solvent, ppbC	Measured value of TOC, ppbC	Corrected value of TOC, ppbC	The concentration of quercetin in the sample, mg/mL
1	0.009	0.150	0.141	0.00236
2	0.009	0.155	0.146	0.00245
3	0.009	0.153	0.144	0.00242
Relative standard deviation, %				1.75

Table 2

Measured values of total carbon at pH 4.0

No.	TOC of the solvent, ppbC	Measured value of TOC, ppbC	Corrected value of TOC, ppbC	The concentration of quercetin in the sample, mg/mL
1	0.009	0.092	0.083	0.00139
2	0.009	0.090	0.081	0.00136
3	0.009	0.093	0.084	0.00141
Relative standard deviation, %				1.84

For the measured values, a statistical analysis of the obtained values was carried out, which included the calculation of average values, standard deviation, and

relative standard deviation. The obtained values are shown below in Table 5.

Table 3

Measured values of total carbon at pH 4.5

No.	TOC of the solvent, ppbC	Measured value of TOC, ppbC	Corrected value of TOC, ppbC	The concentration of quercetin in the sample, mg/mL
1	0.009	0.086	0.077	0.00129
2	0.009	0.085	0.076	0.00127
3	0.009	0.087	0.078	0.00131
Relative standard deviation, %				1.29

Table 4

Measured values of total carbon at pH 5.0

No.	TOC of the solvent, ppbC	Measured value of TOC, ppbC	Corrected value of TOC, ppbC	The concentration of quercetin in the sample, mg/mL
1	0.009	0.075	0.066	0.00110
2	0.009	0.076	0.067	0.00112
3	0.009	0.075	0.066	0.00111
Relative standard deviation, %				1.12

Table 5

Average values of total carbon at pH: 3.0, 4.0, 4.5, 5.0

No.	The pH value	Average values concentration of quercetin in the sample, mg/mL	Average values concentration of quercetin in the sample, mg/mL
1	3.0	0.00241	0.00236
2	4.0	0.00139	0.00139
3	4.5	0.00129	0.00129
4	5.0	0.00111	0.0011
Relative standard deviation, %			0.5657

For the total carbon analyzer used for the measurement, according to the manufacturer's data, the measurement error should not exceed ±0.1 ppbC at a TOC concentration <2.0 ppbC (water quality>15 mΩ·cm [0.067 µS/cm]). Reproducibility of measurements should have an error of no more than ±0.05 ppbC<5 ppbC, ±1.0 %>5 ppbC. The error of sample preparation for the method used in the measurements was 0.2 %.

Thus, taking into account the above data, it can be stated that the obtained results are statistically valid since a significant measurement error does not burden them, no more than 2 %, which allows them to be used to predict the value of the solubility limit for environments with a pH of 7.0 by the method of extrapolation of the obtained dependence of the measured values of the pH value of the investigated solutions.

Using the averaged values of the concentration of quercetin, a graphical dependence of the measured values of the pH value of the investigated solutions was constructed. The resulting dependence graph is shown below (Fig. 1).

The main task of the study was to find the value of the concentration of quercetin at neutral pH, which would satisfy the BCS requirements [17].

The solution to this problem consists of an attempt to approximate the obtained experimental data with a certain mathematical function and the extrapolation method to find the concentration point at pH=7. For this purpose, two variants were considered: a polynomial function of the 2nd degree and a power function. The obtained results are presented in Fig. 2, 3.

The regression analysis of the obtained dependencies made it possible to calculate a number of statistical parameters: standard deviation (SD_{rest}), Fisher's test (F), determination test (R^2), and probability (%) [21]. The values are given in Tables 6, 7.

After conducting a comparative analysis of the obtained values of the specified statistical parameters, the power function was determined as the model that most accurately describes the presented graph.

In order to assess how adequately we established the correlation between the measured parameters, we used the coefficient of determination (R^2) and Fisher's test, which helps to avoid a biased assessment when choosing.

For the equation of the power function, the requirements of the Fisher test are fulfilled. Given this value, as well as the coefficient of determination, residual standard deviation, and probability, it can be used to predict the required value – the dissolution limit. For this, the obtained experimental dependence was approximated by a power function (Fig. 2).

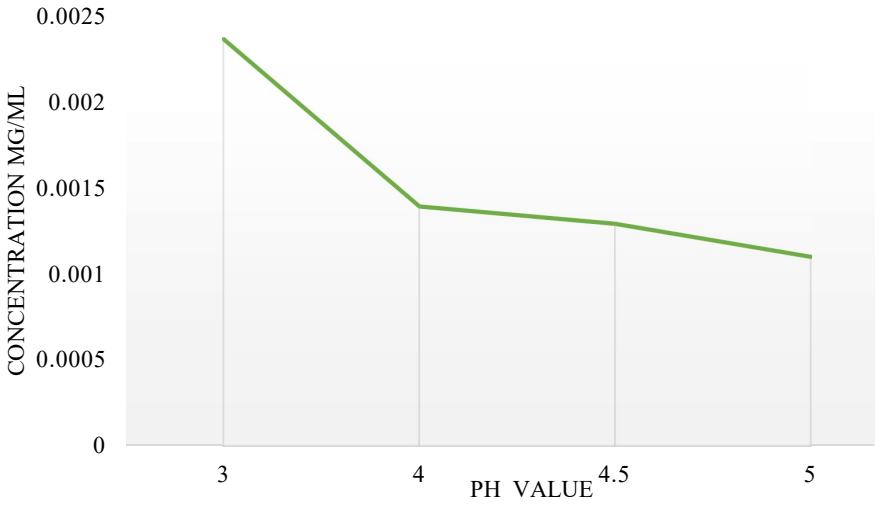

Fig. 1 Graph of the dependence of the measured values on the pH value of the investigated solutions

According to the above tables, the value of R^2 for the power function and for the 2nd-order polynomial was almost the same value (0.97). However, the probability value (%) for the 2nd-order polynomial was 12.9 % compared to the static function, which was 2.6 %. Fisher's criterion (F) for the power function was 37.048, and for the polyno-

mial function – 32.120. The residual standard deviation (SD_{rest}) for the power function was 0.092, and for the 2nd-order polynomial was 0.099. The results evaluate the polynomial dependence of the 2nd degree as less suitable for the interpretation of the research results [22]. In graphs 2 and 3, the power function and polynomial of the 2nd degree, respectively, we can observe the location of the concentration points obtained by theoretical calculation and experimentally. It is clear that the location of the points, both theoretical and practical, is best determined by the power function.

Knowing the pH value at which we should obtain the value we are interested in, we can calculate from the equations of the proposed functions the value of the concentration of the solubility limit. Thus, using the power function and solving the system of equations using the Excel package, we obtained the concentration value corresponding to the pH 7 point. The obtained value corresponds to the solubility limit of quercetin. The obtained value was 0.7 μg/ml as a result of solving the static function equation.

It should be noted that the obtained data are correlated with the data obtained by us in the first experiment, during which the method of tandem chromatography-mass spectrometry was used as a method of analytical analysis. In an experiment using the HPLC-MS method, values of 3.02 μg/ml were obtained for the 3rd-degree polynomial. [18]. It should be noted that the method using HPLC-MS has a higher error than the method proposed in this article. Because it requires a standard substance, and the sample preparation process is more complicated. The second method, the total organic carbon (TOC) method, has a lower margin of error because it is direct and DOES NOT require standards. The process of sample preparation is simpler, which allows us to conclude that with the help of the second method we managed to get a more accurate result.

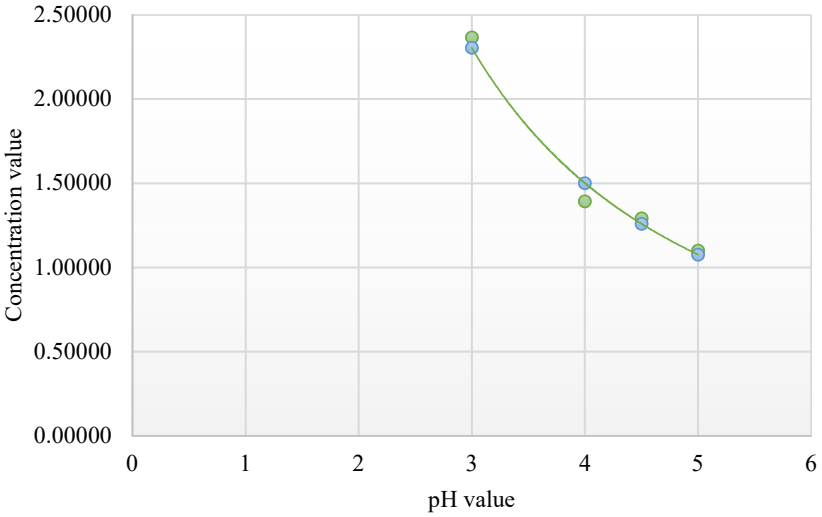

Fig. 2. Graph of a power function

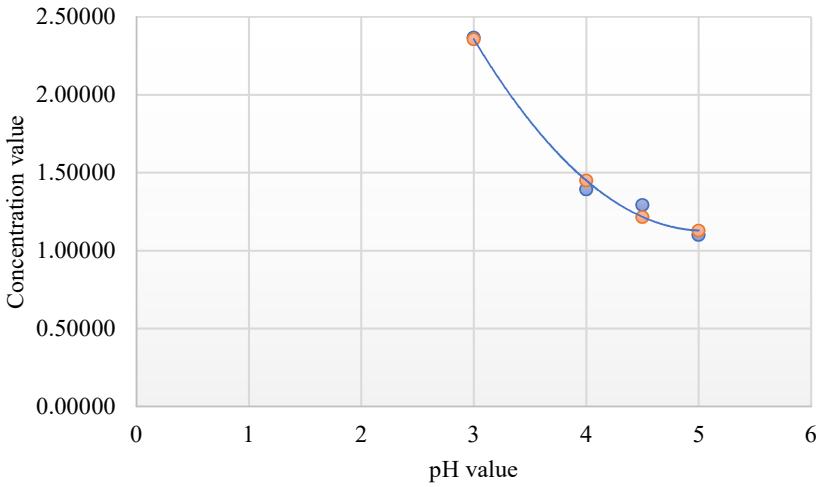

Fig. 3. Graph of a polynomial of the 2nd degree

Table 6

Calculated criteria for the power function

Polynomial equation: $y=A*x^{(-B)}$ $y=11.818x^{(-1.488)}$				
Number of measurements (n)	pH	Output data	Calculated data (Y$calc$)	Squared difference ((Y–$Ycalc$)^2)
1	3	2.3673	2.3045	0.00395
2	4	1.3935	1.5020	0.01176
3	4.5	1.2928	1.2605	0.00104
4	5	1.10057	1.0776	0.00053
Degree of freedom (k)		2	–	–
Degree of freedom (n–k)		2	–	–
Sum (Sum)				0.01728
Residual standard deviation (SD_{rest})				0.09205
Fisher's test (F)				37.048
Test value R^2=1–1/F=				0.9730
Probability % (<5)				2.6

Table 7

Calculated criteria for a polynomial of the 2nd degree

Polynomial equation: $y=A*x\text{^}2+B*x+C$ $y=0.2928x\text{^}2-2.957*x+8.5934$				
Number of measurements (*n*)	pH	Output data	Calculated data (*Ycalc*)	Squared difference ((*Y–Ycalc*)^2)
1	3	2.3674	2.35760	0.00010
2	4	1.3935	1.45020	0.00321
3	4.5	1.2928	1.21610	0.00589
4	5	1.10057	1.12840	0.00077
Degree of freedom (*k*)		3	–	–
Degree of freedom (*n–k*)		1	–	–
Sum (*Sum*)				0.00996
Residual standard deviation (SD_{rest})				0.09982
Fisher's test (*F*)				32.120
Test value $R^2=1-1/F=$				0.9689
Probability % (<5)				12.9

4. 1. Validation of the developed methodology

In the process of developing the TOC method for the quantitative determination of quercetin, its validation was carried out, as part of which it was 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 5 %. Below are the results.

The validation of the TOC method of quantitative determination of the quercetin substance was carried out according to separate validation characteristics: specificity, linearity, convergence, precision, correctness, and intra-laboratory precision [20].

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 $\Delta sp=5\cdot0.32=1.6$ %. At the same time, the real error of sample preparation for the TOC technique used in the measurements was 0.2 %, which is much less than the established criterion. The above calculations showed the insignificance of the influence of the sample preparation error on the result of the quantitative determination.

The solutions for conducting validation measurements were prepared according to the following method: a precise suspension of a sample of the quercetin substance, thoroughly ground and dried to a constant mass, was placed in a 1000 ml flask, and 100 ml of 1M hydrochloric acid was added. After that, the suspension was dissolved in an ultrasonic bath for 10 minutes and brought up to the mark with water, followed by mixing. The blank solution was prepared similarly to the investigated solution.

The linearity assessment was carried out in the range (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 (6 mg/ml), which is in the middle of the range covering the minimum and maximum concentrations of quercetin.

The obtained results were statistically processed by the method of least squares following the requirements of the State Pharmacopoeia of Ukraine [20].

The calculated statistical values *b*, S_b, *a*, S_a, S_r (final standard deviation), and *r* (correlation coefficient) are given in Table 8.

Table 8

Linearity parameters and criteria (for given values)

Parameters	Value
b	63.4165
Sb	0.2846
a	|–0.18143|
Sa	1.7207
RSD_0	0.6614
RSD_0/b	|0.01043|
RSD_y	49.1262
r	|0.9999|

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 (*Si*) were calculated. The obtained results were processed by the method of least squares for the straight-line $Y=b\cdot x+a$ (Table 9).

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

To measure and calculate the metrological assessment of the convergence and correctness of the method, three peak area values were obtained for the comparison solution and 27 peak area values for the model solutions. We calculated the actual values (Xi fact), the ratio of the average values of the peak areas for each of the 27 solutions to the average value of the peak area of the comparison solution, obtaining the values $X_i=(C_i/C_{st})\cdot100$ %, $Y_i=(S_i/S_{st})\cdot100$ %, as well as value $Z_i=(Y_i/X_i)\cdot100$ %, which is the concentration found as a percentage of the input. The results of the calculations are given in Table 10.

Table 9

Linearity parameters and criteria in the normalized coordinates

Parameters	Value	Requirements 1	Requirements 2	Conclusion
b	1.0008	–	–	–
Sb	0.0044	–	–	–
a	|–0.0477|	<=|0.841|	<=|7.9|	are maintained according to the first criterion
Sa	0.4525	–	–	–
RSD_0	0.17398	–	–	–
RSD_0/b	|0.17384|	<=|2.6888|	–	criteria are met
RSD_y	12.9099	–	–	–
r	|0.9999|	>|0.9780|	–	criteria are met

Table 10

Parameters of accuracy and precision

–	Parameters	Value	Requirements 1	Requirements 2	Conclusion
Precision	Δ_Z	0.3029	<=5	–	criteria are met
Accuracy	$\|Z_{cp}–100\|$	|0.03192|	<=0.0958	<=1.58	are maintained according to the first criterion

To assess interlaboratory precision, a relative confidence interval was used for 5 parallel determinations of the quantitative content of substances, which should be smaller than the maximum permissible uncertainty of the analysis results: $\Delta_Z \leq 1.0$ %. Tests were performed using the same batch of quercetin by different analysts on the same carbon analyzer on different days using different measuring vessels. The obtained value of the uncertainty of the analysis results was 0.94 %.

Intra-laboratory precision was confirmed by the fact that the value of the relative confidence interval for five parallel determinations of one series of drugs meets the acceptance criterion.

To prove that the TOC method is specific, it can be considered sufficient that all the requirements for the criteria of linearity, correctness, precision, and intra-laboratory precision are met [21].

In the process of validating the TOC method of quantitative determination of the quercetin substance, results were obtained that meet the criteria of correctness, linearity, precision, and interlaboratory precision, which confirms the specificity of the investigated TOC method.

Thus, the results of the study of the validation parameters of the TOC method of quantitative determination of the quercetin substance were obtained, which fully meet the requirements of the established criteria, which allows us to conclude that the error of the method has a negligible effect on the result of the quantitative determination. This method allows you to correctly measure the concentration of quercetin with a given accuracy (1.6 %) and can be reproduced in laboratory conditions.

4. Discussion

As mentioned earlier, we currently have a variety of literature data regarding the solubility limit of quercetin in aqueous media with a neutral pH. The discrepancy of these data is as much as five orders of magnitude. Therefore, it is practically impossible to use such data, but it is necessary for the production of drugs and the study of their bioavailability. The method for determining the solubility limit of quercetin in aqueous solutions by HPLC-MS, which we initially developed to study this characteristic and establish an accurate value, allowed us to quantify the solubility limit and obtain adequate values [18]. However, working with very low concentrations of quercetin and the inability to compare with literature data created a problem for us in the measurements' reliability.

The solution to this problem required the development of an alternative method by which it would be possible to confirm or refute the results with a probability of 95 %, as required by the pharmacopoeia. Therefore, the quantitative method for determining the solubility limit of quercetin by determining the total organic carbon developed was a response to the above challenge and has several major advantages. First, it eliminates the use of the quercetin standard, significantly reducing the uncertainty of the results. Utilizing of acidic media eliminates the problem of quercetin dissolution in aqueous media and reduces the measurement error at the level of sample preparation with accurate concentration values. Using the above measurements has allowed us to obtain the results of the solubility limit of quercetin by the method of determining total organic carbon method of the same order as the previously obtained results by HPLC-MS, which confirmed the correctness of the results and allowed them to be used for further study of the bioavailability of quercetin in drugs.

Study Limitations. Like any analytical method, determining total organic carbon has several drawbacks in solving our problem. One of these disadvantages is the inability to directly measure the concentration of quercetin at pH 7.0 since we only conduct studies at slightly Another limitation of the method is the inability to measure quercetin concentration below 0.05 ppbC µgC/L when using the specified equipment.

The method for determining total organic carbon is quite simple to implement, does not require many consumables implementation, and does not require any consumables, standards, or special skills. In the pharmaceutical industry, the TOC method is used to monitor the cleanliness of the equipment and the presence of residuals.

Prospects for further research. The quantitative method developed by us can create the prospect of using the method to determine the solubility limit of various classes of organic substances. If further used, this method can be suggested to the pharmacopoeia as a standardized means of determining the solubility limit.

The method for determining total organic carbon is quite simple to implement, however, it has limitations, especially in quantitative analysis. The limitation of the method is the impossibility of measuring the concentration of quercetin below 0.05 ppbC μgC/l. That is, the investigated substances must be well soluble in the solvents chosen for the determination method. Since quercetin is poorly soluble in aqueous media, we are forced to use acidic media. The second limitation of the method is the ability to analyze only organic substances.

In pharmaceutical production, the method of determining total organic carbon is used to control the cleanliness of the equipment and the presence of residual substances. The quantitative technique developed by us can create the prospect of using the method of determining the solubility limit of various classes of organic substances.

5. Conclusions

As a result of the conducted research, a an alternative method of quantitative determination of the solubility limit of a substance was proposed, which does not exceed 5.0 % accuracy. The method is based on measuring the concentration of total carbon in acidic solutions with different pH values and further approximating the obtained dependence of the pH value equal to 7.0.

A comparison of the data obtained by the proposed method (0.7 μg/ml) and the data obtained using the chromatographic method (3.02 μg/ml) allows us to assert that the method for determining total organic carbon allows obtaining a more accurate value of the solubility limit of quercetin in neutral environment This makes it possible to use the developed method as a reference for the solubility limit of lipophilic substances [18].

The verification of the technique showed that the accuracy (no more than 2 %) and reproducibility (0.94 %) of the obtained results fully meet the requirements laid down during its development, the error is no more than 5 % and the reproducibility is no more than 1.0 %.

Conflict of interests

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

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Data availability

The manuscript has no associated data.

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Nataliia Khanina*, PhD Student**,** Department of Pharmaceutical Chemistry**,** National University of Pharmacy**,** Pushkinska str, 53, Kharkiv, Ukraine, 61002

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*