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STUDY OF BAICALIN HYDROLYSIS KINETICS IN THE PROCESS OF ITS EXTRACTION FROM SCUTELLARIA BAICALENSIS GEORGI ROOTS

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The aim of this study was to investigate the kinetics of baicalin hydrolysis in the process of its extraction from *Scutellaria baicalensis* Georgi roots.

Materials and methods. For the studies, *Scutellaria baicalensis* Georgi roots with a particle range of 0.1–0.5 mm were used. The method of extraction was a simple maceration during a specified period of time, the ratio of plant raw material : extractant was 1:10 w/v at the temperature of 24±1°C. Baicalin and baicalein contents were analyzed by reverse phase high performance liquid chromatography (RP HPLC) at the analytical wavelength of 275 nm. The extractant was a water solution of ethanol 26, 43, 59, 72, 81, 97±1% v/v. The time of the extraction was from 1 to 24 hours.

Results. The experimental points of dependency of baicalin concentration in the extract on the time of extraction for ethanol solutions with a concentration of 43 and 72% v/v are closely approximated by a linear equation in coordinates ln$C$=f(t). The value of determination coefficient is more than R^2>0,99. Half lifetime for baicalin has been calculated: for ethanol with the concentration of 43% v/v it is 4.3±0.7 hours, and for ethanol with the concentration of 72% v/v it is 42.3±1.8 hours.

Conclusion. Baicalin hydrolysis kinetics in the process of its extraction from *Scutellaria baicalensis* Georgi roots with 43 and 72% v/v ethanol concentration. has been studied. It has been established that the process of baicalin hydrolysis is well described by the first order kinetic equation. The constants of baicalin hydrolysis during its extraction from *Scutelaria baicalensis* roots with ethanol having different concentrations have been calculated. Recommendations on technology optimization for baicalin or baicalein extraction from *Scutellaria baicalensis* Georgi roots have been given.

Keywords: *Scutellaria baicalensis* Georgi roots, baicalin, baicalein, hydrolysis, first order reaction, half lifetime

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ИЗУЧЕНИЕ КИНЕТИКИ ГИДРОЛИЗА БАЙКАЛИНА ПРИ ЕГО ЭКСТРАКЦИИ ИЗ КОРНЕЙ ШЛЕМНИКА БАЙКАЛЬСКОГО

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Цель. Изучить кинетику гидролиза байкалина при его экстракции из корней шлемника байкальского.
Материалы и методы. Корни шлемника байкальского с размером частиц 0,1-0,5 мм. Используемый метод экстракции – простая мацерация в течение заданного промежутка времени, при соотношении сырье: экстрагент 1:10 м/о и температуре 24±1°С. Содержание байкалина и байкалеина анализировали с помощью обратно-фазовой высокоэффективной жидкостной хроматографии (ОФ ВЭЖХ) при длине волны 275 нм. Экстрагент: водные растворы этанола 26, 43, 59, 72, 81, 97±1% об. Время настаивания от 1 до 24 часов.
Результаты. Экспериментальные точки зависимости концентрации байкалина в извлечении от времени настаивания для этанола с концентрацией 43 и 72% об., хорошо аппроксимируются линейным уравнением в координатах lnC=f(t). Коэффициент детерминации боле R^2>0,99. Рассчитано время полураспада байкалина в этаноле с концентрацией 43% об., которое составило 4,6±0,5 часа, в этаноле с концентрацией 72% об., данный показатель равен 42,3±1,8 часа.
Заключение. Изучена кинетика гидролиза байкалина при его экстракции из корней шлемника байкальского с помощью этанола с концентрацией 43 и 72% об. Установлено, что процесс гидролиза байкалина хорошо описывается кинетическим уравнением первого порядка. Найдены константы процесса гидролиза байкалина во время его экстракции из корней шлемника байкальского с помощью этанола различной концентрации. Даны рекомендации по оптимизации технологии выделения байкалина или байкалеина из корней шлемника байкальского.
Ключевые слова: корень шлемника байкальского, байкалин, байкалеин, гидролиз, реакция первого порядка, время полураспада

INTRODUCTION

Scutellaria baicalensis Georgi is a plant of *Lamiaceae* family that grows in the Russian Federation in Transbaikal, Amur River and Primorye regions, as well as in Mongolia and China. The plant raw material used for medicinal purposes is the root.

The root contains flavonoids (baicalin – more than 9%, baicalein – up to 5%, wogonoside – up to 4%, wogonin – up to 0.7%, scutelarein, etc.), steroids (beta-sitosterol, stigmasterol, etc.), coumarins and some other compounds [1, 2].

Biologically active compounds (BACs) from *Scutellaria baicalensis* Georgi roots have different useful pharmacological effects. They have effects on the central nervous system (sedative, hypotension, and anticonvulsant). BAC from *Scutellaria baicalensis* Georgi roots are useful for liver as they demonstrate hepatoprotective and antioxidant activities; besides, they decrease inflammation processes, inhibit the growth of pathogenic microorganisms (bacteria and viruses), have a cytotoxic effect on different cancer cell lines, etc. [3–18].

Therefore, BACs from this type of plant raw material (PRM) have useful pharmacological properties, and all kinds of research in the field of technology of their extraction is important.

According to the known scientific literature data, the type of extractant, the temperature and time of the extraction have a great influence on the qualitative and quantitative composition of the extract obtained [19, 20].

These kinds of influence are determined by the presence of the active form of beta-glucuronidase enzyme in the cells of *Scutellaria baicalensis* Georgi roots. After wetting the raw material with the extractant containing water, this enzyme starts hydrolyzing baicalin actively to its aglicone (baicalein) and glucuronic acid [21].

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This fact should be taken into account during the development of quality control methods and extraction technology of BAC from this PRM.

Therefore, the decision to study the process of baicalin hydrolysis during its extraction from *Scutellaria baicalensis* Georgi roots was made up. Moreover, this information may be used as a starting point for optimization of BAC extraction technology from this type of PRM in further researches.

The aim of this study was to investigate the kinetic process of baicalin hydrolysis during its extraction from *Scutellaria baicalensis* Georgi roots.

MATERIALS AND METHODS

Object of investigation

Scutellaria baicalensis Georgi roots were purchased from LLC Pharmaceutical shop "Medicinal plants", Kharkiv, Ukraine, lot No. 921217, best before IX/2020. For the studies, the roots were ground to the particle fraction of 0.1 to 0.5 mm using high-speed multifunction grinder HC-500Y, China.

Extraction method

For the studies, a simple maceration method for a certain period of time was used. Hereby, the PRM:extractant ratio was 1:10 w/v at the temperature of 24±1°C. For that, a precisely weighed amount of 1.0 g of ground PRM was put into an airtight flask, 10.0 ml of the extractant was added, then the flask was sealed and left for a specified period of time. After that the extract was decanted, centrifuged at 3,000 rpm for 5 min. and delivered to the analysis for the contents of baicalin and baicalein.

Before the analysis, the extract was additionally centrifuged at 13,000 rpm for 5 min. Hydroethanolic solutions 26, 43, 59, 72, 81, 97±1% v/v were used as extractants.

Sample preparation

The analysis of the initial content of baicalin and baicalein in the plant raw material was carried out by a simple maceration method under the following conditions: ethanol 43% v/v was used as an extractant, the ratio of plant raw material : extractant was approximately 1:50 m/v, the extraction time was 30 min at the temperature of 95±5°C (water bath). A precisely weighed amount of 1.0 g of ground PRM was put into a flask, 50.0 ml of extractant (ethanol 43% vol.) was added; the flask was connected to a backflow condenser and the process of extraction took place in water bath for 30 min.

Then the flask was cooled, the extract was decanted and the plant raw material was rinsed out with an additional portion of the extractant (5.0 ml). The obtained extract was added to the great bulk of the extract and weighed. The ultimate extract was analyzed by the method of reverse phase high performance liquid chromatography (RP HPLC). The density of the extract was determined by method 1 according to general pharmacopoeia monograph 1.2.1.0014.15 [22].

The contents of baicalin and baicalein in the plant raw material ($X_{1,2}$, %) was calculated by the following equation (1):

$$X_{1,2} = \frac{C \cdot M \cdot 100}{m \cdot \rho} \quad (1)$$

where

C – baicalin or baicalein concentration, g/ml;

M – mass of the extract, g;

m – plant raw material, g;

ρ – density of the extract, g/ml.

Method of analysis

Qualitative contents of baicalin and baicalein in the extracts were analyzed by reverse phase high performance liquid chromatography (RP HPLC). The analyses were carried out with Agilent Technologies equipment, Agilent 1200 Infinity series, the USA. More details on RP HPLC analysis conditions are described in this work [23].

As standards, baicalin and baicalein of the State Pharmacopoeia of Ukraine with the content ≥95.0 were used. The analytical wavelength was 275 nm.

The main parameters for the validation method of the analysis and suitability of RP HPLC system for the determination of baicalin and baicalein are presented in Table 1.

Table 1 – Main parameters for validation method of the analysis and suitability of RP HPLC system for baicalin and baicalein determination

Parameter	Pharmacopoeia limitation [22]	Baicalin	Baicalein
Retention time (t_R), min.*	–	22,6±0,5	29,4±0,5
Asymmetry coefficient (T)	0,8-1,5	1,35	0,94
Separation coefficient (R_s)	≥1,5	1,58	1,62
RSD of peak's area, %	≤2,0	1,6	1,5
LOD, g/ml	–	$2,9 \cdot 10^{-5}$	$3,9 \cdot 10^{-6}$
LOQ, g/ml	–	$8,8 \cdot 10^{-5}$	$1,2 \cdot 10^{-5}$
Determination coefficient, r^2	≥0,98	0,9992	0,9999
Calibration linear equation, C(g/ml)=f(S(mAU·s))	–	$C=(2,52±0,10) \cdot 10^{-7} \cdot S$	$C=(1,78±0,01) \cdot 10^{-7} \cdot S$

* Note. The mean value and its confidence interval (Mean±SEM) are calculated with repeat counts n=3 and significance level P=0.95.

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RESULTS AND DISCUSSION

Fig.1 presents a chromatogram of the extract during the determination of baicalin and baicalein contents in the plant raw material according to the subsection "Sample preparation" in the section "Materials and methods".

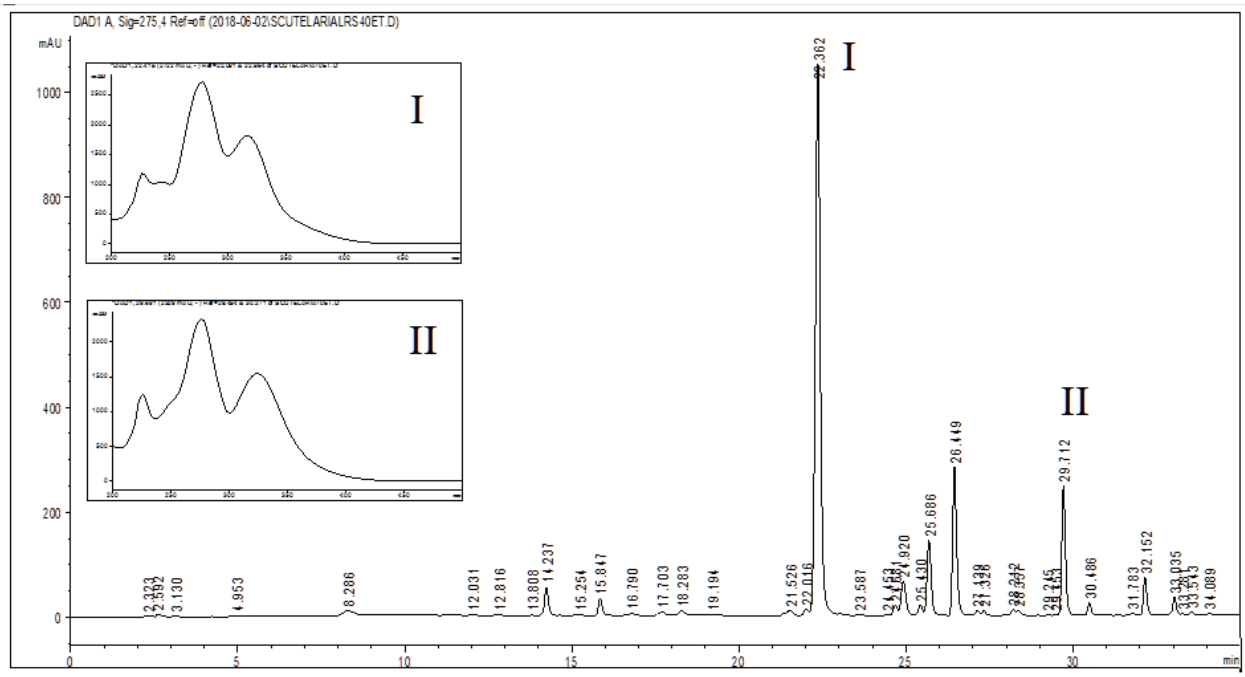

Figure 1 – Chromatogram of the extract obtained during determination of baicalin and baicalein contents in the plant raw material

Note: the analytical wavelength was 275 nm; I – baicalin; II – baicalein.

As Fig.1 shows, baicalin (I) dominates in the extract obtained (the retention time was 22.4 min). After the substitution of the experimental values of baicalin/baicalein peak area into the regression equation (see Table 1), the concentration of these substances in the ultimate extract was calculated and then the calculation of their contents in the plant raw material was carried out using the equation (1). The initial content of baicalin in the plant raw material was 14.8% m/m, and the one for baicalein was 1.89% m/m.

Fig. 2 presents a chromatogram of the extract at the analytical wavelength of 275 nm obtained under the following conditions: ethanol 72% v/v was used as an extractant, the time of maceration was 13.3±0.2 h, the temperature was 24±1°C, and the ratio of the plant raw material to the extractant was 1:10 m/v.

As Fig. 2 shows, the two substances, baicalin (I) and baicalein (II), dominate in the extract, while in the PRM it was only baicalin that was dominating. It means that for 13.3 ± 0.2 hours of infusion, hydrolysis of baikalin occurred with the formation of a significant amount of baikalein.

The results of RP HPLC analysis of baicalin and baicalein yield into the extracts at different concentrations of ethanol under the above-mentioned conditions (the time of maceration – 13.3±0.2 h, the temperature – 24±1°C, and the ratio of the plant raw material to the extractant – 1:10 m/v) are presented in Fig. 3.

The yield of baicalin was calculated compared to its initial value in PRM. The yield of baicalein (X_3), was calculated equivalent to its hypothetical content in PRM given that the total amount of baicalin (X_1), transforms into it ($X_3=X_2+X_1 \cdot Mr_2/Mr_1=1,89+14,8 \cdot 270,2/446,4=10,9\%$ mass.). The repeat count is n=3, and the significance level is P=0.95.

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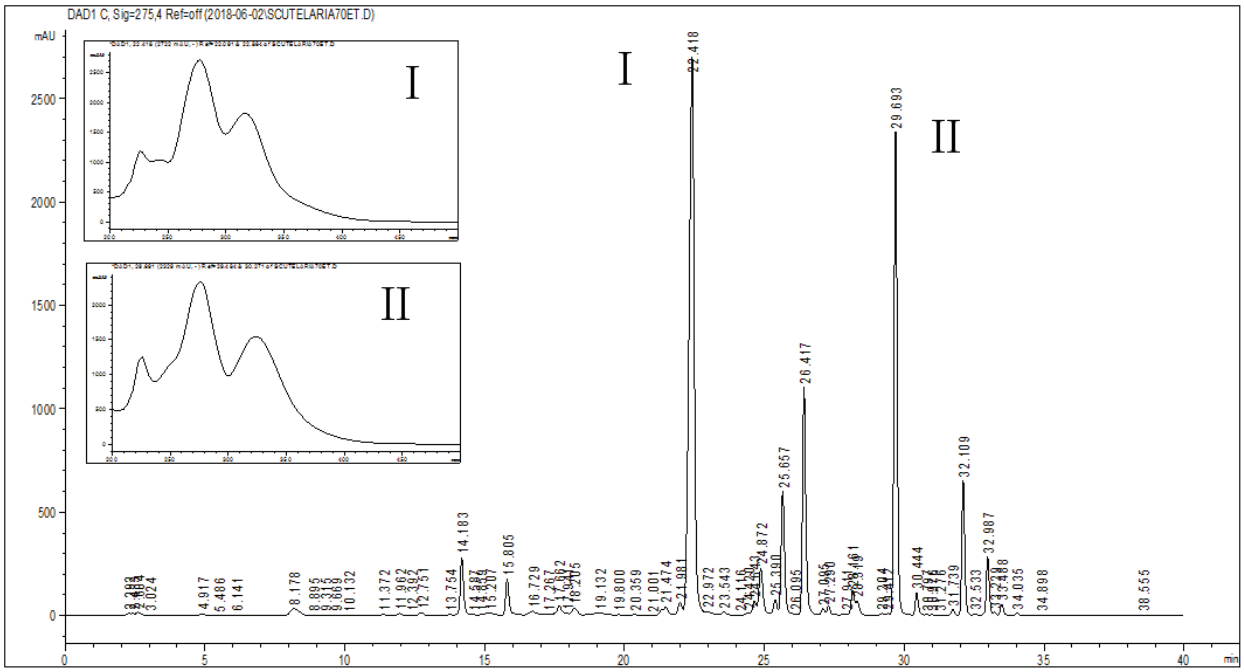

Figure 2 – Chromatogram of the extract from Scutellaria baicalensis Georgi roots
Note: analytical wavelength was 275 nm; I – baicalin; II – baicalein

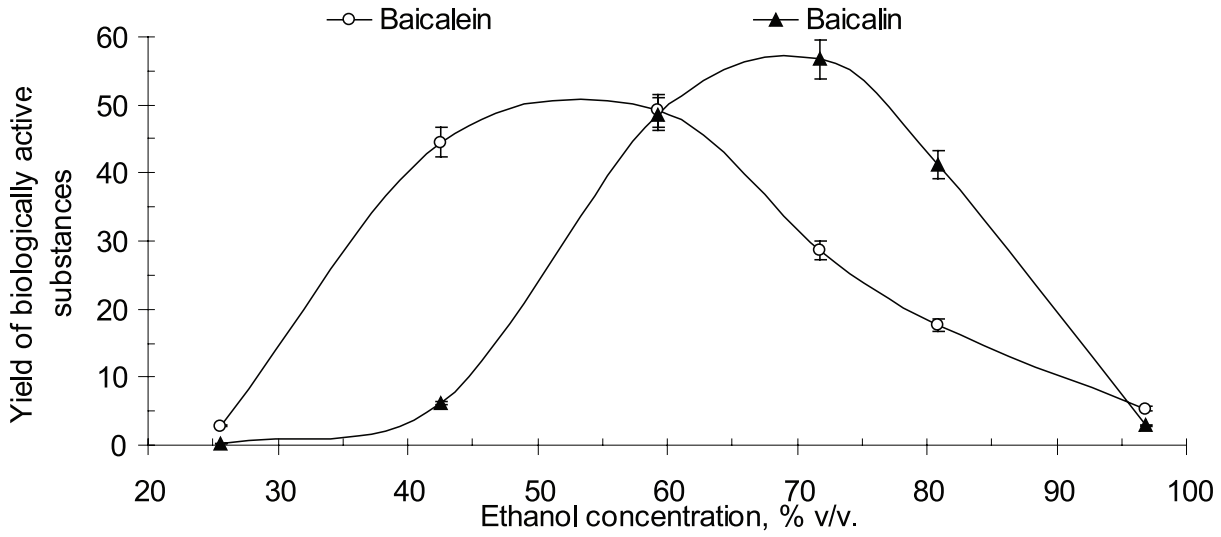

Figure 3 – Dependency of baicalin and baicalein yield on ethanol concentration

Empirical graphs presented in Fig. 3 show that under the experimental conditions (the time of maceration of 13.3±0.2 h, the temperature of 24±1°C, the ethanol concentration of 43% v/v, the PRM / extractant ratio of 1:10 m/v), a considerable part of baicalin disintegrates up to baicalein. Herewith, the yield of baicalin into this extractant was 6.2% of its initial content in the PRM, and the yield of baicalein was 44.5% of its hypothetic content in the PRM. These values make it possible to calculate the percentage of converted baicalin, which was 44.8%={100·[(10.9-1.89)·44.5/100]·446.4/(270.2·14.8)}, i.e. almost a half of baicalin of its initial content in the plant raw material disintegrated. Its residual part (49%=100–44.8–6.2) did not possibly dissolve in the ethanol of this concentration and remained in the PRM. That fact requires additional studies.

On the empirical curves obtained, the maximum for baicalin yield in ethanol with the concentration range of 70±5% v/v is clearly seen, and for baicalein it takes place in ethanol with the concentration range of 53±10% v/v. It is interesting to notify the existence of the interception (isobestic) point of empirical curves for ethanol with

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the concentration of 62±3% v/v, at which 50% value of each component yield is observed.

Moreover, the curves also show that baicalin and baicalein are practically not extracted by ethanol with the concentration of less than 30% v/v and more than 90% v/v.

In ethanol with the concentration from 40 to 60% v/v, the maximum yield of baicalein up to 45–50% from its hypothetic content in PRM is seen. It can probably be explained by its partial solubility in ethanol at this concentration, as it has already been mentioned above.

From the abovementioned data we can suppose that the activity of a beta-glucuronidase enzyme in *Scutellaria baicalensis* Georgi roots is not inhibited by ethanol and has a high level of activity in ethanol with the concentration from 30 to 90% v/v.

The next stage of the work was connected with the study of baicalin hydrolysis kinetics in *Scutellaria baicalensis* Georgi roots under the same conditions of the extraction process.

For this purpose, ethanol with the concentration of 43 and 72% v/v was used. Hereby, an assumption that hydrolysis of baicalin should occur in the first order reaction was made, thus the experimental data in coordinates ln*C*=*f*(*t*) should be closely approximated by the linear regression equation.

The results of processing of the obtained experimental data are presented in Fig. 4.

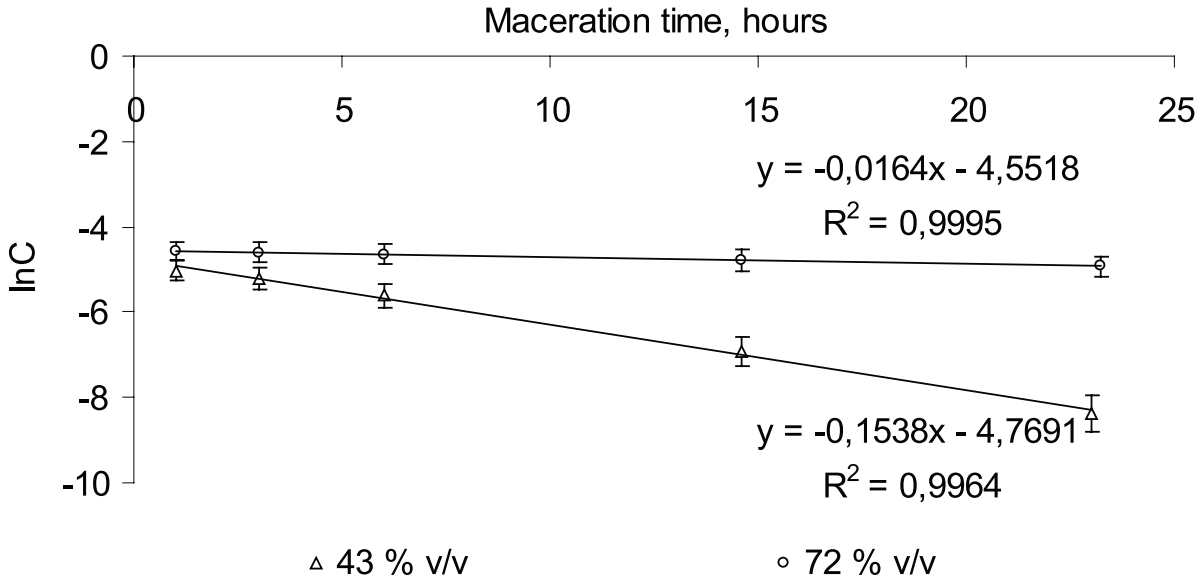

Figure 4 – Dependency of change in baicalin concentration in the extract on the maceration time with ethanol 43 and 72% v/v in coordinates lnC=f(t)

As Fig. 4 shows, the experimental points for the dependency of baicalin concentration in the extract on the maceration time are closely approximated in the predicted coordinates by the linear equation (the determination coefficient is more than R^2>0.99, which indicates the functional dependency between the parameters).

Therefore, this experiment confirms our assumption about the mechanism of baicalin hydrolysis in *Scutellaria baicalensis* Georgi roots. It follows the first order kinetic equation. It should be pointed out that hereby, ethanol affects the energy of the enzymatic hydrolysis process and slows it down with an increase in the concentration of ethanol in the extraction mixture.

The constants obtained, as well as some other derivative parameters that can be calculated from them in the view of chemical kinetic laws for the first order reactions, are presented in Table 2.

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Table 2 – Values of experimentally obtained constants and some other derivative parameters

Constant/parameter	Ethanol 43% v/v	Ethanol 72% v/v
Slope of regressive curve, 1/h (k)	0.15±0.02	0.0164±0.0007
Intercept, b	–4.8±0.2	–4.6±0.1
Initial concentration of baicalin, g/ml (C_0=exp[b])	0.0082±0.0004	0.0101±0.0002
Half lifetime of baicalin, h ($t_{½}$=ln2/k)	4.6±0.5	42.3±1.8

As Table 2 shows, the values of the derivative parameter of the initial baicalin concentration in ethanol, 43 and 72% v/v, are close but statistically different (0.0082±0.0004<0.0101±0.0002, g/ml). Moreover, the values calculated are less than the ones determined experimentally for baicalin in PRM by 1.8 and 1.4 times, respectively (0.0148±0.0007 g/ml). These inconsistencies require additional experiments and theoretical interpretation.

It should be pointed out that such a derivative parameter as half lifetime of baicalin in ethanol with the concentration of 43% v/v is by about one order lower than the one in ethanol with the concentration of 72% v/v (4.6±0.5<<42.3±1.8, h).

The half lifetime baicalin values calculated show that to obtain the extract with a maximum baicalin content and a minimum baicalein content, it is reasonable to use the technology of rapid extraction (for 1–2 h) and ethanol with the concentration of 70–80% v/v.

And if baicalein extraction is necessary, it is recommended to use maceration for not less than 12 h and ethanol with the concentration of 30–60% v/v.

In general, the results obtained give the possibility to describe baicalin kinetic hydrolysis within a framework of the laws of chemical kinetics and catalysis.

Moreover, to study the influence of the type and composition of the extractant on the baicalin yield and its hydrolysis kinetics, we should also use laws of physical chemistry and additional studies that provide means for development of an advanced type of the mathematical model with the introduction of energy activation of the hydrolysis process.

These results can be used for further development of the extraction technology of baicalin and baicalein from *Scutellaria baicalensis* Georgi roots.

CONCLUSION

Baicalin hydrolysis kinetics at its extraction from *Scutellaria baicalensis* Georgi roots with ethanol concentration of 43 and 72% v/v has been studied. It has been found out that the process of baicalin hydrolysis is well described by the first order kinetic equation. The constants of baicalin hydrolysis process during its extraction from *Scutellaria baicalensis* roots with ethanol having different concentrations have also been found out.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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