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STUDY OF DISTRIBUTION OF BIOLOGICALLY ACTIVE SUBSTANCES FROM FLOWERS OF HELICHRYSUM ARENARIUM BETWEEN PHASES OF THE EXTRACTION SYSTEM

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The aim of this study is to confirm the adequacy of the proposed hypothesis, which explains and quantitatively describes the distribution of biologically active substances (BAS) within the extraction system consisting of Helichrysum arenarium flowers and the solvent using a regressive analysis for the theoretically predicted coordinates.

Materials and methods. For this research, milled officinal flowers of Helichrysum arenarium (Helichrysum arenarium L. flores) were used. The analysis of the extractions was carried out by RP HPLC method. Isosalipurposide, salipurposide, and chlorogenic acid of ≥98.0% purity were used as reference substances. The analytical wavelengths were 370, 290, and 325 nm.

Results. The obtained experimental data are well-approximated by regressive linear equations in the theoretically predicted coordinates $1/C=f(V)$ and $\ln(b/a)=f(1/T)$. Wherein, the coefficient of determination of regressive equations was $R^2 \geq 0.998$, which indicates functional dependence between the studied parameters and confirms the adequacy of the developed mathematical model. The experimental work identified the necessity of implementation of additional constant values into the mathematical model.

Conclusion. A new hypothesis was proposed to explain and quantitatively describe the distribution of BAS in the extraction system of Helichrysum arenarium flowers and 80% ethanol. With this working hypothesis, mathematical models were developed and their adequacy was proved using a regressive analysis in the theoretically predicted coordinates. The results obtained could not deny that a mechanism of BAS distribution between the phases is explained and described by the classic Boltzmann distribution for discrete values of molecular energy (or quantum distribution according to Fermi and Dirac).

Keywords: flowers of Helichrysum arenarium; isosalipurposide; salipurposide; chlorogenic acid; equilibrium; classic Boltzmann distribution for discrete values of molecular energy

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ИЗУЧЕНИЕ РАСПРЕДЕЛЕНИЯ БИОЛОГИЧЕСКИ АКТИВНЫХ ВЕЩЕСТВ ИЗ ЦВЕТКОВ БЕССМЕРТНИКА ПЕСЧАНОГО МЕЖДУ ФАЗАМИ ЭКСТРАКЦИОННОЙ СИСТЕМЫ

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Цель. Проверка адекватности выдвигаемой рабочей гипотезы, которая объясняет и количественно описывает распределение БАВ в экстракционной системе из цветков бессмертника песчаного и растворителя, с помощью регрессионного анализа в предсказанных теорией координатах.

Материалы и методы. Для исследований использовали измельченное фармакопейное растительное сырье «Бессмертника песчаного цветки» (*Helichrysum arenarium* L. flores). Анализ извлечений проводили с помощью ОФ ВЭЖХ метода. В качестве стандартных веществ использовали изосалипурпозид, салипурпозид, хлорогеновую кислоту ФСО ГФУ, содержание ≥98,0 %. Аналитические длины волн 370, 290 и 325 нм.

Результаты. Экспериментальные данные хорошо аппроксимируются регрессионными линейными уравнениями в предсказанных теорией координатах 1/C=f(V) и ln(b/a)=f(1/T). При этом коэффициент детерминации регрессионных уравнений, имеет значение $R^2 \geq 0,998$, что говорит о функциональной зависимости между изучаемыми параметрами и подтверждает адекватность разработанных уравнений. Эксперимент выявил необходимость введения в математическую модель дополнительной константы.

Заключение. Предложена рабочая гипотеза, которая объясняет и количественно описывает распределение БАВ в экстракционной системе из цветков бессмертника песчаного и этанола 80 % об. С помощью рабочей гипотезы разработаны математические модели, адекватность которых доказана с помощью регрессионного анализа в предсказанных теорией координатах. Полученные результаты не отвергают гипотезу, что механизм распределения БАВ между фазами в экстракционной системе объясняется и описывается классическим распределением Больцмана для дискретных значений энергии молекул (или квантовым распределением Ферми-Дирака).

Ключевые слова: цветки бессмертника песчаного; *Helichrysum arenarium*, изосалипурпозид; салипурпозид; хлорогеновая кислота; равновесие; классическое распределение Больцмана для дискретных значений энергии молекул

INTRODUCTION

Flowers of Helichrysum arenarium (*Helichrysum arenarium* L. flores) are the officinal raw material within the territory of the Russian Federation, the Republic of Belarus, Ukraine, the Republic of Kazakhstan, etc. This raw material is used for the production of the drug product Flamin, which is manufactured in the form of tablets, granules and in-bulk substance. It is used in treatment of liver and gallbladder diseases. In addition, biologically active substances (BAS) from that raw material have antioxidant, antibacterial, antiviral, antihyperlipidemic, and cytotoxic effects [1–20].

In our previous research [21], the authors justified the mechanism of dielectric constant impact on equilibrium concentration of isosalipurposide in the extractions. However, the obtained model neither explains nor describes the mechanism of BAS distribution in the extraction system when the equilibrium status is reached within it. That is why the studies aimed at the development of the equilibrium status of the extraction process are relevant.

THE AIM of this study is to confirm the adequacy of the proposed hypothesis, which explains and quantitatively describes the distribution of BAS in the extraction system of *Helichrysum arenarium* flowers and the solvent using a regressive analysis in the theoretically predicted coordinates.

MATERIALS AND METHODS

Raw materials and chemical reagents

For this study, milled plant raw material of *Helichrysum arenarium* was used, and it was bought at pharmacy Medical herbs Ltd., Kharkiv, Ukraine, lot number 530617, expiry date 07.2020 [22].

An aqueous solution of ethanol 80%±1%, was used as an extracting solution. Qualitative and quantitative analyses were carried out using RP HPLC with reference substances.

Isosalipurposide, salipurposide, and chlorogenic acid of ≥98.0% purity were used as reference substances. Analytical wavelengths were 370, 290, and 325 nm.

The main validation parameters of the analytical method and suitability of RP HPLC system for the assay of isosalipurposide, salipurposide, and chlorogenic acid, are shown in Table 1.

Methods of obtaining extracts

A precisely weighed amount of the raw material (1 g) was put into a hermetic flask, the required volume of the solvent was added, which additionally was weighted and put into a refrigerator/thermostat at the temperature of 4, 20, 40 and 60±1°C. The proportions of the raw material and the solvent at each temperature, were 1:5 (1:10), 1:15, 1:20, 1:40 w/v. The extraction mixture was decocted for 24 hours. After that, the extract was removed and its assay was performed using RP HPLC method. The mean value and the standard error of mean were calculated at the repeat count *n*=3 and the significance level *P*=0.95.

Analysis methods via RP HPLC

The analysis of the extractions was carried out using the chromatographic equipment of Agilent Technologies, "Agilent 1200 Infinity" series, manufactured in the USA. The detailed description is available in the following articles [21, 23].

Theoretical part

In order to explain the mechanism and a quantitative description of BAS distribution between a solid phase of the medicinal plant raw material and a liquid phase of the solvent, the authors proposed the following hypothesis: the mechanism of equilibrium molecular distribution of BAS between two phases in the extraction system is explained and described by the classic Boltzmann distribution for discrete values of molecular energy (or quantum distribution according to Fermi and Dirac), equation (1). This hypothesis allows developing a mathematical model, which will describe the experimental data in the theoretically predicted coordinates, as shown by equations (2) and (3):

$$\frac{n}{n_0} = \frac{1}{1 + \exp\left(\frac{\Delta G}{kT}\right)} \qquad (1)$$

where n is quantity of BAS in the solvent with energy equals ΔG, mol;

n_0 is an overall quantity of BAS, mol;

ΔG is the difference of Gibb's energy for BAS molecules in the extraction system, J;

k is Boltzmann constant value, $1.38 \cdot 10^{-23}$ J/K;

T is absolute Kelvin's temperature, K.

$$\frac{1}{C} = \frac{1}{n_0} \cdot V + \exp\left(\frac{\Delta G}{kT}\right) \cdot \frac{1}{n_0} = \frac{M}{m_0} \cdot V + K_H \cdot \frac{M}{m_0} = a \cdot V + b \qquad (2)$$

$$\ln K_H = \ln\left(\frac{b}{a}\right) = \frac{1}{T} \cdot \frac{\Delta G}{k} \qquad (3)$$

where C is concentration of BAS in the extracting solution, g/ml;

m_0 is *an* overall (initial) content of BAS in the raw material, g;

V is the volume of the extracting solution, ml;

M is the weight of the raw material in the extraction system, g;

a is a constant that equals to the reversed value of overall BAS content in the raw material (M/m_0);

b is a constant that equals to multiplicity of Henry's constant and (K_H) and the reversed value of overall BAS content in the raw material (M/m_0), ml/g.

To determine the degree of adequacy of the proposed hypothesis, the authors used the regressive analysis of the experimental data in the theoretically predicted coordinates $1/C=f(V)$ and $\ln(b/a)=f(1/T)$. The obtained data were processed with preset for the data analysis in MS Excel 2010.

Table 1 – The main validation parameters of analytical method and suitability of RP HPLC system for the assay of isosalipurposide, salipurposide, and chlorogenic acid

Parameter	Pharmacopoeia criteria [22]	Isosalipurposide	Salipurposide (sum of isomers)	Chlorogenic acid
1. Retention time, min*	–	20.1±0.2	11.9±0.2 and 12.8±0.2	6.3±0.3
2. Separation coefficient	≥1.5	3.0	3.1 and 3.2	11.0
3. Number of theoretical plates	≥1,000	103,458	39,541 and 29,267	12,282
4. Relative Standard Deviation, RSD, %	≤2.0	1.2	1.2	0.8
5. LOD, g/mL	–	$4.1 \cdot 10^{-5}$	$5.5 \cdot 10^{-6}$	$2.2 \cdot 10^{-5}$
6. LOQ, g/mL	–	$1.3 \cdot 10^{-4}$	$1.7 \cdot 10^{-5}$	$6.5 \cdot 10^{-5}$
7. Determination coefficient, r^2	≥0.98	0.9999	0.9999	0.9999
8. Linear regressive equation, C(g/ml) = f(S(mAU·sec))	-	$C=(2.79 \pm 0.06) \cdot 10^{-7} \cdot S$	$C=(3.94 \pm 0.01) \cdot 10^{-7} \cdot S$	$C=(2.92 \pm 0.04) \cdot 10^{-7} \cdot S$

** Note. The average value and its error (X±ΔX) were calculated on the basis of the repeat count n = 3 and the significance level P = 0.95.*

Table 2 – Constant values for BAS from Helichrysum arenarium flowers

BAS	Constant value		
	ΔG, J/mol	g	m_p, % wt.
1. Isosalipurposide	5,390±380	–1.5±0.2	22.0±3.0
2. Salipurposide	19,930±1,030	–7.2±0.4	0.075±0.004
3. Chlorogenic acid	18,640±2,160	–5.4±0.9	0.45±0.08

Note. The number of experiments: n=4, the significance level: P=0.95.

Table 3 – Values of overall (initial) BAS content in the raw material (m_0) found by the experiment and theoretical calculation

BAS	Theoretically calculated content, m_0/M, % wt.*	Experimental values, m_0/M, % wt.**
1. Isosalipurposide	1.58±0.06	1.46±0.07
2. Salipurposide	0.47±0.02	0.43±0.02
3. Chlorogenic acid	0.19±0.04	0.19±0.01

*Note. * The number of experiments: n=4, the significance level: P=0.95.*
*** The number of experiments: n=3, the significance level: P=0.95.*

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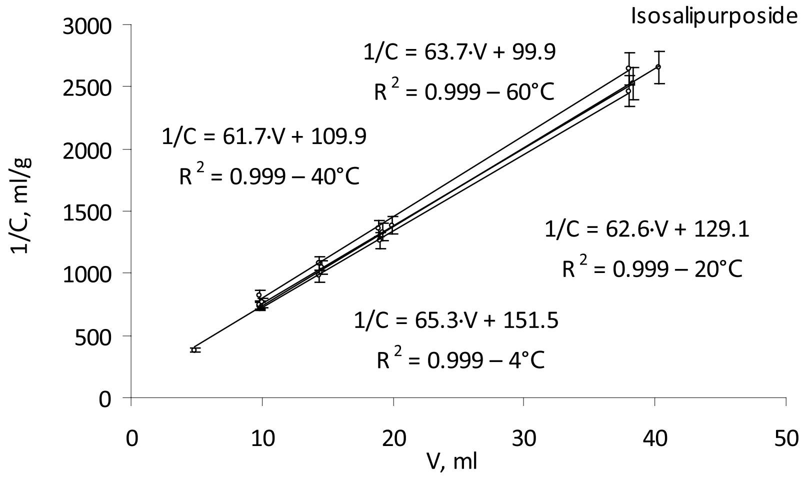

Figure 1 – Regressive equation of isosalipurposide concentration dependence on the volume of the extracting solution in coordinates 1/C=f(V)

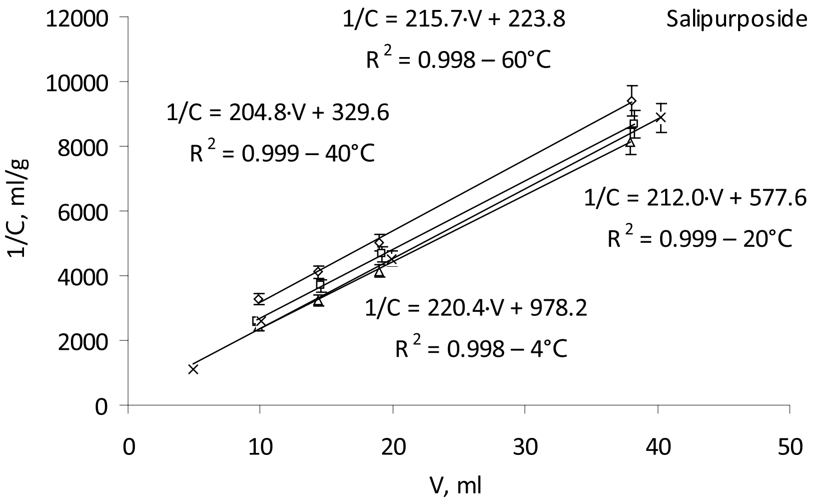

Figure 2 – Regressive equation of salipurposide concentration dependence on the volume of the extracting solution in coordinates 1/C=f(V)

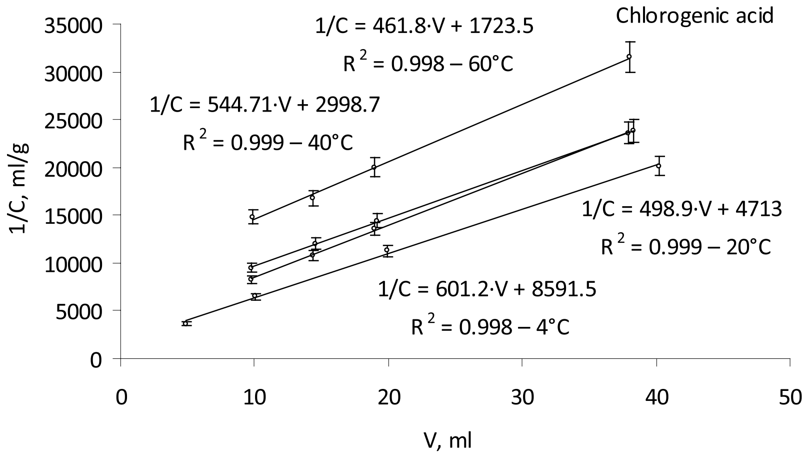

Figure 3 – Regressive equation of chlorogenic acid concentration dependence on the volume of the extracting solution in coordinates 1/C=f(V)

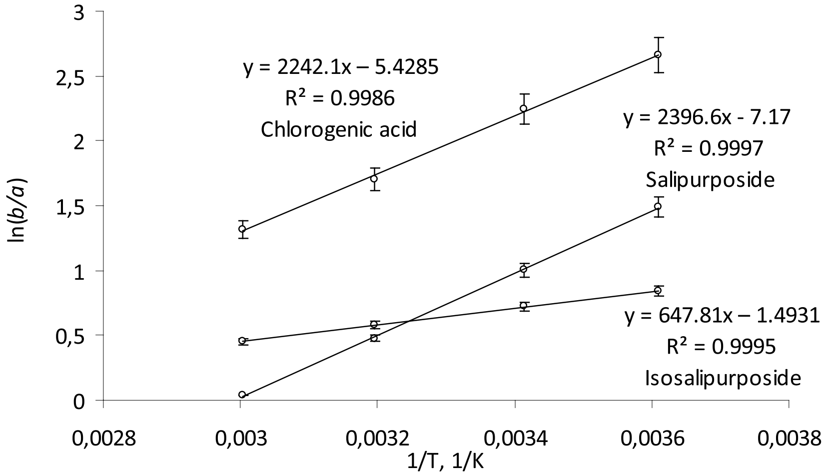

Figure 4 – Regressive equations of empiric constant values' (a, b) dependence on temperature in coordinates ln(b/a)=f(1/T) for BAS from Helichrysum flowers

RESULTS AND DISCUSSIONS

The experimental data and regressive linear equations of isosalipurposide, salipurposide, and chlorogenic acid concentration dependence on the volume of the extracting solution in the theoretically predicted coordinates are shown in Figures 1, 2, and 3.

As Figures 1, 2, and 3 show, the experimental points are well-approximated by regressive linear theoretically predicted coordinates $1/C=f(V)$. Wherein, the coefficient of determination of the regressive equations was $R^2 \geq 0.998$. It determines the functional dependence between the studied parameters and confirms the adequacy of the equation (2).

Thereafter, the obtained results were used for building up the regressive linear equations of dependence of Henry's constant for isosalipurposide, salipurposide and chlorogenic acid in the theoretically predicted coordinates. They are shown in Figure 4.

As Figure 4 shows, the dependence of Henry's constant values on the temperature is well-approximated for isosalipurposide, salipurposide, and chlorogenic acid by regressive linear equations in the theoretically predicted coordinates $\ln(b/a)=f(1/T)$. Wherein, the coefficient of the determination of the regressive equations was $R^2 \geq 0.998$. It proves the functional dependence between the studied parameters and confirms the adequacy of the equation (3). However, the obtained results have identified the additional constant value (g) in the equation (3), which was not predicted by the theory, hence, this requires to add this experimentally found constant value ($g=\ln(m_p/100)$) into the initial equation (1).

The constant values (ΔG, g and m_p) for BAS from *Helichrysum arenarium* flowers, found in accordance with the proposed theoretical equations (2) and (3) and also the experimental outcome, are summarized in Table 2.

As Table 2 shows, constant value ΔG, which expresses the energy of the BAS interphase distribution, is found at 5-20 kJ/mol level. It is well-complied with the values of the physical adsorption of the substances on the adsorbents [24]. Judging by this fact, BASes in the medicinal plant raw material are found to be in a bound or even adsorptive status, as it was discovered by M.S. Tsvet at the beginning of the XXth century [25].

The conclusive test of the proposed hypothesis was performed, comparing the experimentally obtained and theoretically calculated values of the overall (initial) content of BAS in the raw material (m_0/M), which are shown in Table 3.

As Table 3 shows, the experimentally found and theoretically calculated values of the overall (initial) content of BAS in *Helichrysum arenarium* flowers (m_0/M), do not differ from each other. This fact additionally confirms the adequacy of the equation (2).

Hence, the obtained experimental results are well-complied with theoretically developed mathematical models via equations (2) and (3). However, the experiment identified the necessity of adding constant (g) into the mathematical model, wherein equation (1) transforms to the following:

$$\frac{n}{n_0} = \frac{1}{1+\exp\left(\frac{\Delta G}{kT} - g\right)} \quad (4)$$

Hence, the proposed hypothesis concerning the mechanism of the interphase distribution of BAS in the extraction system, is explained and described by the classic Boltzmann distribution for discrete values of the molecular energy (or quantum distribution according to Fermi and Dirac), is not denied.

Thus, the proposed hypothesis and the developed mathematical model based on it, explain the mechanism of the BAS distribution in the extraction system between the phases; make it possible for us to find the constants required; forecast the equilibrium (limiting) concentration of BAS in the extract; and choose/calculate the optimal values of the volume and temperature of the extractant to achieve certain values of the exhaustive degree of the plant raw material for BAS.

CONCLUSION

A new hypothesis was proposed to explain and quantitatively describe the distribution of BAS in the extraction system of *Helichrysum arenarium* flowers and 80% ethanol. With this hypothesis, the mathematical models have been developed, their adequacy has been proved using a regressive analysis in the theoretically predicted coordinates. The constant values have been found, they are present in the mathematical model. The necessity of the addition of a new constant value has been identified experimentally. The obtained results do not deny that the mechanism of BAS distribution between the phases is explained and described by the classic Boltzmann distribution for discrete values of the molecular energy (or quantum distribution according to Fermi and Dirac).

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AUTHORS’ CONTRIBUTION

All authors have equally contributed to the research work.

CONFLICT OF INTEREST

Authors state there’s no conflict of interest between them.

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