STUDY AND MODELING OF EXTRACTION PROPERTIES OF FLUOROORGANIC SOLVENTS IN REGARD TO LOW-POLAR BIOLOGICALLY ACTIVE SUBSTANCES FROM PLANT RAW MATERIALS

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Abstract
The goal of this work was the studying and modelling of the extraction properties of fluoroorganic solvents in regard to low-polar biologically active substances (BAS) from plant raw materials (PRM).

Keywords: fluoroorganic solvents, extraction properties, mathematical model

Introduction
Currently, separation of low-polar biologically active substances (BAS) from plant raw materials with the use of liquefied gases and supercritical fluids are considered to be the most promising technologies in the world [1-5].

It is conditioned by the fact that these technologies have a number of advantages: high yield of low-polar BAS (90% - 98%), sparing extraction conditions, environmental, fire, and explosion safety, low toxicity to humans.

However, these technologies also have a number of significant drawbacks: the need to use special equipment that has to operate under excessive pressure (2 - 350 bar), the need to use a refrigeration unit for vapour condensation, additional energy costs for the operation of the refrigeration unit, low selectivity of extraction of fatty oils and essential oils between each other, etc.

It should be noted that only in the supercritical state (at elevated temperature and high pressure), carbon dioxide allows selective separation of essential oils from vegetable raw materials in their joint presence with triglycerides [6].

Therefore, it is urgent to search for new solutions in the technology of selective isolation of low-polar BAS from PRM, which allow avoiding the above disadvantages.

One of such innovative solutions can be the use of fluoroorganic solvents owing to their unique physical and chemical properties driven by the presence of fluorine atoms in the carbon skeleton of the molecule, which gives them weak intermolecular forces and
causes these substances to have low boiling points, evaporation heat, heat capacity, surface tension, toxicity, and also gives them hydrophobic and even lipophobic properties [7]. The analysis of the literature revealed that at the moment, fluoroorganic solvents of a new generation have appeared on an industrial scale in Russia; they have the advantages in a number of parameters in comparison to liquefied gases and supercritical fluids. Some fundamental parameters of fluoroorganic solvents and liquefied gases (from the technological point of view) are presented in Table I [8-13].

### Table I

Some fundamental parameters of fluoroorganic solvents and liquefied gases

| Parameter                        | Type of fluoroorganic solvent and liquefied gas |
|----------------------------------|-----------------------------------------------|
| 1. Empirical formula             | Novec 1230, Novec 7100, MR654, R141b, R22, R227ea, RC318, R744 |
| 2. Molecular weight, g/mol       | -                                             |
| 3. Pressure 20 °С, MPa           | -                                             |
| 4. Boiling point, °С             | -                                             |
| 5. Heat of vaporization, kJ/L    | -                                             |
| 6. Density 20 °С, kg/m³          | -                                             |
| 7. Dielectric constant           | -                                             |
| 8. Global warming potential (GWP)| -                                             |
| 9. Toxicity, ppm                 | -                                             |
| 10. Approx. price as to 08/2019, US dollars per kg | - |

As can be seen from the data in Table I, fluoroorganic solvents in many physical, chemical, environmental, and toxicological parameters do not differ very much from freons, but are inferior to carbon dioxide. At the same time, their price is slightly higher than that of freons and much higher than of carbon dioxide, which is one of the limiting factors of their widespread use in industry.

However, owing to their unique properties, these solvents are increasingly being used in various sectors of the economy as solvents, heat-conducting liquids, and fire extinguishing agents [14-15].

As a result of the patent search, we have found only a few patents in the EU and the US, regarding the methods for extraction of fatty and essential oils from vegetable raw materials using fluoroorganic solvents or their mixture with other solvents [16-18].

These results show the availability of limited information on the extraction properties of fluoroorganic solvents for use in the pharmaceutical industry, as well as high prospects for research in this field.

Taking into account the above material, we have conducted systematic studies on the extraction properties of fluoroorganic solvents with respect to various low-polar BAS, and on their basis, an attempt is made to explain theoretically and describe quantitatively the experimental data obtained.

The goal of this work was the studying and modelling of the extraction properties of fluoroorganic solvents in regard to low-polar biologically active substances (BAS) from plant raw materials (PRM).

### Materials and Methods

#### Plant raw materials

Ground plant raw materials with a particle size of 0.1 - 0.5 mm were used for research: *Anethum graveolens* L. fruits, LLC Pharmaceutical shop “Medicinal plants”, Kharkiv, Ukraine, batch no. 981117, bbd 09/2020. *Curcuma longa* L. roots, “Goldiee”, Nayaganj, Kanpur, India, batch no. 827, bbd 01/2020. *Foeniculum vulgare* Mill. fruits, LLC Pharmaceutical shop “Medicinal plants”, Kharkiv, Ukraine, batch no. 135117, bbd 08/2020. *Eucalyptus viminalis* Labill. leaves, “Krasnogorskleskredstva” company, Krasnogorsk, Russia, batch no. 100917, bbd 10/2020. *Hypericum perforatum* L. herbs, LLC Pharmaceutical shop “Medicinal plants”, Kharkiv, Ukraine, batch no. 120717, bbd 07/2019. *Laurus nobilis* L. leaves, LLC “Standart”, Krasnodar, Russia, batch no. 02042019, bbd 04/2020. *Pistacia sativa* L. fruits (Globular cultivar), “Pnsemena” company, Ukraine, batch no. 454124, bbd 12/2021. *Pimpinella anisum* L. fruits, LLC Pharmaceutical shop “Medicinal plants”, Kharkiv, Ukraine, batch no. 135117, bbd 08/2020. *Silybum marianum* L. fruits, “Biocor” company, Penza, Russia, batch no. 040919, bbd 09/2021. *Syzygium aromaticum* L. buds, “Lechev” firm, Kharkiv, Ukraine, batch no. 082018, bbd 08/2020.
Chemicals
Carvone (CAS 6485-4D-1); eugenol (CAS 97-53-0); trans-anethole (CAS 4180-23-8), xanthotoxin (CAS 298-81-7) were from Sigma-Aldrich, Merck, with content \( \geq 98.0\% \); evalcalin standard sample of the All Russian Scientific Research Institute of Medicinal and Aromatic Plants (a sum of macrocarpales); spissum extract of Chlorophyllit of the State Pharmacopoeia of Ukraine (a sum of cupric chlorophylls, euglobals with monoterpenoid structure and macrocarpales), were used as reference substances.

Fluoroorganic solvents
Novec 1230 and Novec 7100 were from the USA, 3M Company; MR6S4 was from Great Britain, Aesica Queenborough Ltd; R141b DGX was from France, Arkema Inc., with content \( \geq 99.0\% \).

Reverse phase high performance liquid chromatography (RP HPLC)
Analysis of biologically active substances was carried out using the method of RP HPLC on Agilent Technologies device, series Agilent 1200 Infinity, USA. The process of analysis was carried out under the following conditions: 1% aqueous solution of formic acid was used as mobile phase (A), and ethanol 96% vol. was used as mobile phase (B), with a linear gradient elution mode; mobile phase velocity was 0.5 mL/min; chromatographic column: Supelco Ascentis express C18, length 100 mm, internal diameter 4.6 mm, particle size 2.7 µm; chromatographic column temperature: 35°C; sample volume: 1 µL. The conditions for RP HPLC analysis were the same as in the work by Zhilyakova et al. [19]. Analytical wavelengths: 240 nm (carvone), 275 nm (euglobal and macrocarpales), 280 nm (eugenol), 284 nm (anethole) and 302 nm (xanthotoxin).

GC-MS method of analysis
Qualitative analysis of extracts was carried out by GC-MS method using equipment GCMS-QP2010 Ultra with a mass analyser, by Shimadzu, Japan. Column: Zebron ZB-5MS, length 30 m, inside diameter 0.25 mm, film thickness 0.25 µm; liquid phase: 5% – polysilarylene and 95% – polydimethylsiloxane; temperature settings: from 70°C to 325/350°C. Gas-carrier: helium with a stable flow of 3.0 mL/min. Detection regime: total ion current (SCAN) in the range m/z from 30 to 500 Da with scanning velocity 1.000 and result time 0.5 sec. The injection volume was 1 µL.

Method of extraction
A sample of ground plant raw material weighing 5.00 g (particle size 0.1 - 0.5 mm) was placed in the Soxhlet extractor; the raw material was poured with 25.0 mL of the extractant and extracted for a specified period. The resulting solution was evaporated until an extract was obtained, and the latter was blown with airflow for 5 - 10 minutes. The extract was dissolved in ethanol 96% vol. The solution was weighed, its density was determined, and it was analysed for the content of biologically active substances using the method of RP HPLC.

Theoretical part
To construct a mathematical model that quantitatively describes the extraction properties of fluoroorganic solvents, the authors used two working hypotheses based on the laws of physical and colloidal chemistry and two molecular descriptors [20, 21].

Working hypothesis 1: the extraction properties of fluoroorganic solvents should be associated with a change of chemical potential in the extraction system, which is represented by two types of Gibbs energies: surface energy (\( \Delta G_1 \)) and interaction energy of BAS molecules with solvent molecules (\( \Delta G_2 \)).

Working hypothesis 2: surface energy (\( \Delta G_1 \)) is directly proportional to the limit value TPSA of BAS molecule for Fluoroorganic solvent (LTPSA), and interaction energy of BAS molecules with solvent molecules (\( \Delta G_2 \)) is directly proportional to the product of surface energy and the exponent of the relative fraction of fluorine atoms in the molecule of fluoroorganic solvent (1-\( \varphi_F \)). These working hypotheses are presented below in equations (1) - (4):

\[
R T \ln \left( \frac{N_0 - N}{N} \right) = \Delta \mu, \quad (1)
\]

\[
\Delta \mu = \Delta G_1 + \Delta G_2, \quad (2)
\]

\[
\Delta G_1 = \sigma \cdot LTPSA, \quad (3)
\]

\[
\Delta G_2 = \sigma \cdot LTPSA \cdot \exp \left[ k \cdot (1 - \varphi_F + b) \right], \quad (4)
\]

where \( \sigma \) is solvent surface tension, N/m²; LTPSA is the experimental value of the limit value of the molecular descriptor TPSA for molecules of low-polar BAS extracted by solvent, Å²; \( \Delta G_2 \) is a change of Gibbs energy (J) due to energy of interaction of BAS molecules with solvent molecules, expressed in terms of surface energy and parameter (1-\( \varphi_F \)), in the form of equation (4):

\[
LTPSA = \frac{A}{1 + \exp \left[ k \cdot (1 - \varphi_F + b) \right]}, \quad (5)
\]

824
Parameter ($\varphi_F$) was calculated by equation (6):

$$
\varphi_F = \sum_i n_i \frac{n_F}{n_i},
$$

(6)

where $n_F$ is the number of fluorine atoms in the solvent molecule, units;

$n_i$ is the number of $i$-atoms in the solvent molecule, units.

A molecular TPSA descriptor for BAS was found in PubChem Database [13].

**Calculation method**

Regression analysis was used to determine the degree of consistency between the theoretically developed mathematical model (5) and the experimentally obtained data. At the same time, the values of constants and their errors were found using Origin 6.1 program.

**Results and Discussion**

In the first part of the research, systematic studies of the extraction properties of fluoroorganic solvents Novec 1230, Novec 7100, MR6S4, and R141b were carried out with respect to various types of low-polar BAS from plant raw materials (mainly from essential oils), the materials of which are partially presented in such works as [22–24]. In this case, the extraction of BAS from plant raw materials was carried out using the circulation method in Soxhlet apparatus for three hours. The results of the studies are presented in Table II.

**Table II**

Results of systematic studies on the extraction properties of fluoroorganic solvents

| Type of PRM | Type of BAS | Yield of BAS, %* | Type of solvent | Note |
|-------------|-------------|------------------|-----------------|------|
| 1. Anethum graveolens L. fruits | Carvone | 97 ± 4 | Novec 1230 | Limonene was detected. Triglycerides were undetected |
| | | 95 ± 4 | Novec 7100 | Limonene and triglycerides were detected |
| 2. Curcuma longa L. roots | Sum of curcuminoids | 17 ± 1 | MR6S4 | Curcumene, zingiberene, Ar-tumerone and curlone were detected |
| | | 16 ± 1 | R141b | |
| 3. Foeniculum vulgare Mill. fruits | Anethole | 94 ± 4 | Novec 1230 | Fenchone was detected. Triglycerides were undetected |
| | | 83 ± 4 | Novec 7100 | Fenchone and triglycerides were detected |
| 4. Eucalyptus viminalis Labill. leaves | Euglobals of monoterpenoid structure | 48 ± 3 | MR6S4 | 1,8-cineole was detected. Chlorophylls and macrocarpales were undetected |
| | Macrocarpales | 44 ± 2 | | 1,8-cineole and chlorophylls were detected |
| | Euglobals monoterpenoid structure | 99 ± 4 | R141b | |
| | Macrocarpales | 51 ± 3 | | |
| 5. Hypericum perforatum L. herbs | Hyperforin | None | Novec 1230 | Adhyperforin was detected. Chlorophylls were undetected |
| | | 99 ± 4 | Novec 7100 | |
| 6. Laurus nobilis L. leaves | Eugenol | 99 ± 3 | Novec 1230 | Costunolide, methyl eugenol, 1,8-cineole were detected |
| | | 99 ± 3 | Novec 7100 | |
| | | 99 ± 4 | R141b | Costunolide, methyl eugenol, 1,8-cineole, chlorophylls were detected |
| 7. Pastinaca sativa L. fruits | Xanthotoxin | None | Novec 1230 | Octyl butyrate was detected. Triglycerides were undetected |
| | | 72 ± 4 | Novec 7100 | Xanthotoxol, bergapten, octyl butyrate and triglycerides were detected |
| | | 75 ± 4 | MR6S4 | |
| | | 66 ± 3 | R141b | |
| 8. Pimpinella anisum L. fruits | Anethole | 99 ± 4 | Novec 1230 | Triglycerides were undetected |
| | | 94 ± 3 | Novec 7100 | Triglycerides were detected |
| 9. Silybum marianum L. fruits | Triglycerides | None | Novec 1230 | |
| | | ≤ 10 | Novec 7100 | |
| | | 99 ± 4 | MR6S4 | |
| | | 99 ± 4 | R141b | |
| 10. Syzygium aromaticum L. buds | Eugenol | 48 ± 3 | Novec 1230 | Caryophyllene, humulene, eugenyl acetate were detected |
| | | 86 ± 5 | Novec 7100 | |

* The mean value and its confidence interval (Mean ± SEM) are calculated with repeat counts $n = 3$ and significance level $p = 0.05$.

Conditions: extraction method: circulating in Soxhlet apparatus, extraction time: three hours, the ratio of PRM to solvent 1:5 (w/w).
As can be seen from the results of Table II, the yield for most of the studied low-polar BAS (anethole, carvone, eugenol, hyperforin, and triglycerides) for three hours of extraction reaches 83 - 100%, 66 - 75% for xanthotoxin, 44 - 53% for euglobals, and 16 - 17% for the sum of curcuminoids. The obtained results were the basis for a number of patents of the Russian Federation for new methods for obtaining essential oils and extracts enriched with target low-polar BAS (furanocoumarins, monoterpene series euglobals, hyperforin, etc.) [25-31]. These results show that the technology of use of fluoroorganic solvents is comparable with those for separation of low-polar BAS from PRM using liquefied gases and supercritical fluids in terms of fire, explosion, and environmental safety, low toxicity and yield of BAS, but surpasses them in its selectivity, simplicity of organization, and conditions of the extraction process [1, 32, 33].

In the second part of the research, the analysis of the obtained data was carried out for the possibility of their mathematical formalization, construction of a mathematical model and prediction of the extraction properties of fluoroorganic solvents.

For this purpose, the authors used the following molecular descriptors: relative fraction of fluorine atoms in the solvent molecule (φF) and topological polar surface area of the low-polar BAS molecule (TPSA). The results obtained are presented in Table III.

### Table III

The extraction properties of fluoroorganic solvents and molecular descriptors of low-polar BAS and solvents

| Type of BAS | TPSA, Å² | Type of fluoroorganic solvent |
|-------------|----------|------------------------------|
|             | Novec 1230 | Novec 7100 | MR6S4 | R12/R22 [1] | R141b |
| 1. Caryophyllene, curcumene, humulene, limonene, zingiberene | 0 | Y* | Y | Y | n/a** | Y |
| 2. Anethole, 1,8-cineole | 9 | Y | Y | n/a | Y | n/a |
| 3. Carvone | 17 | Y | Y | n/a | Y | n/a |
| 4. Ar-tumerone, cunelone | 19 | Y | Y | n/a | n/a | Y |
| 5. Fenchone | 26 | Y | Y | n/a | Y | n/a |
| 6. Methyl Eugenol | 29 | Y | Y | n/a | n/a | n/a |
| 7. Heptyl Butyrate | 36 | P | Y | Y | n/a | Y |
| 8. Costunolide | 49 | N | Y | Y | Y | Y |
| 9. Eugenol | 60 | N | Y | Y | Y | Y |
| 10. Acetyl Eugenol | 71 | N | Y | n/a | n/a | n/a |
| 11. Xanthotoxin, bergapten | 79 | N | P | Y | Y | Y |
| 12. Xanthotoxol | 84 | N | P | Y | n/a | Y |
| 13. Adhyperforin, hyperforin | 95 | N | N | P | n/a | Y |
| 14. Triglycerides | 96 | N | N | P | Y | Y |
| 15. Euglobal IIb | 115 | N | N | N | n/a | Y |
| 16. Macropolar C | 30 ± 5 | 85 ± 5 | 95 ± 5 | 110 ± 5 | 115 ± 5 |
| 17. Chlorophyll a | 0.63 | 0.50 | 0.47 | 0.40 | 0.13 |
| 18. Macropolar A | 0.82 |

* Y means that BAS is extracted by the solvent during 3 h (yield ≥ 50%); N means that BAS is not extracted by the solvent during 3 h (yield ≤ 10%); P means that BAS is poorly extracted by the solvent during 3 h (10% ≤ yield ≤ 50%); ** n/a means that data is not available.

Qualitative data on the possibility/impossibility of extraction of low-polar BAS, fluoroorganic solvent (lines 1 - 18) presented in Table III, allow to formalize and find the limit value of the molecular descriptor LTPSA of low-polar BAS for solvents (line 19) and associate it with the second molecular descriptor, the relative fraction of fluorine atoms in the solvent molecule (φF) (line 20).

In the third part of the studies, the experimentally obtained data were compared as for LTPSA and φF (lines 19, 20 in Table III) and the developed mathematical model (equation (5)) using regression analysis.

Figure 1 presents the experimental points and parameters of the regression line, which is constructed according to equation (5) in Origin 6.1 program.

![Figure 1](Image)
From Figure 1 it is seen that the experimental data are in good agreement with the theoretically developed mathematical model in the form of equation (5) (empirical constants are $A = 116.0 \pm 1.3$, $k = -16.2 \pm 0.6$, $b = 0.44 \pm 0.01$, and the coefficient of determination is $R^2 \geq 0.99$), which gives grounds for adoption of working hypotheses that have been suggested to explain the extraction properties of fluoroorganic solvents. It should be noted that constant ($A$) in equation (5) can be interpreted as the maximum value of $LTPSA$ molecular descriptor for low-polar BAS molecules, above which BAS molecules will not pass into the fluoroorganic solvent even with a minimum content of fluorine atoms. This is explained by the emergence of significant repulsive forces between the polar part of BAS molecules and solvent molecules.

In general, resulting equation (5) allows: (1) theoretical calculation of $LTPSA$ value of BAS molecules that can be extracted with the fluoroorganic solvent with a given relative fraction of fluorine atoms in its molecule; (2) theoretical selection of the fluoroorganic solvent with the desired value of the parameter of relative fraction of fluorine atoms in its molecule for selective extraction of target low-polar BAS from PRM by their TPSA, and this parameter must meet the condition $TPSA < LTPSA$.

Thus, for the first time, the authors systematically studied the extraction properties of various fluoroorganic solvents with respect to low-polar BAS with a circulating extraction method in Soxhlet apparatus. The obtained data showed comparable results in environmental safety, toxicity, extraction time, and yield of BAS with the technology of liquefied gases and supercritical fluids, but at the same time demonstrate advantages in selectivity, simplicity of organization and conditions of the extraction process.

The use of these types of solvents is suitable for selective extraction of low-polar BAS; at the same time, the development of a new direction in the technology of integrated processing of PRM, which meets the basic principles of "green chemistry", is rather promising.

A mathematical model that allows predicting the possibility or impossibility of extraction of low-polar BAS molecules by the fluoroorganic solvent or, on the contrary, helps to choose the necessary type of the fluoroorganic solvent for extraction of a certain type of BAS has been suggested.

**Conclusions**

It has been found that the yield of low-polar BAS from PRM depends on the value of the polar part in the BAS molecule and the relative proportion of fluorine atoms in the fluoroorganic solvent molecule. A mathematical model that quantitatively describes the extraction properties of fluoroorganic solvents using molecular descriptors such as the relative fraction of fluorine atoms in the molecule of the fluoroorganic solvent and $LTPSA$ has been developed. The developed mathematical model allows theoretical prediction and selection of the optimal type of the fluoroorganic solvent for selective extraction of target low-polar BAS from PRM. The obtained practical results showed advantages in selectivity, simplicity of organization, and conditions of the extraction process using fluoroorganic solvents in comparison with the technologies of liquefied gases and supercritical fluids, which is confirmed by a number of patents of the Russian Federation. Conducted systematic studies on extraction properties of various types of fluoroorganic solvents in regards to low-polar BAS from PRM, significantly expand our knowledge in the field of phytotechnology and can be used in the future for the development of new methods for isolation of different types of low-polar BAS on their basis.

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**Conflict of interest**

The authors declare no conflict of interest.

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