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У журналі розглянуто проблеми синтезу й аналізу органічних та елементоорганічних сполук, аналогів природних сполук і лікарських субстанцій, наведено результати фізико-хімічних досліджень у вищезазначених напрямах. Також з погляду (біо)органічної, фармацевтичної, аналітичної та фізичної хімії проаналізовано питання з різних аспектів рослинництва, ґрунтознавства й дослідження навколишнього середовища.

Для працівників науково-дослідних установ, вищих навчальних закладів та фахівців хімічного, фармацевтичного, біологічного, медичного і сільськогосподарського профілів.

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N-Difluoromethylindazoles

Abstract

Aim. To study the possibility of the N-difluoromethylation and separation of 1-difluoromethyl and 2-difluoromethyl isomers of C-substituted indazoles, as well as some possibilities of functionalization of such molecules.

Results and discussion. The N-difluoromethylation of indazole derivatives containing bromine, iodine, and nitro groups in various positions of the heterocyclic ring was studied. In all cases, the conditions for the separation of isomers – N-difluoromethylation products – in positions 1 and 2 were found. The corresponding amines, esters of carboxylic and boric acids were obtained as a result of further functionalization of 1- and 2-difluoromethylindazole derivatives.

Experimental part. The structure of the compounds synthesized was proven by ^{1}H and ^{19}F NMR spectroscopy methods, as well as by the elemental analysis. The structure of isomeric difluoromethylindazoles was finally confirmed by the SELNOESY and ^{1}H-^{13}C HMBC experiments.

Conclusions. A convenient method for the difluoromethylation of substituted indazoles has been found; difluoromethyl derivatives in positions 1 and 2 of the indazole ring have been obtained in high yields. The method for the efficient separation of isomeric difluoromethylindazoles has been found; some possibilities of their further functionalization have been described.

Keywords: indazoles; N-difluoromethylation; isomer separation

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N-Дифлуорометиліндазоли

Анотація

Мета. Вивчити можливість N-дифлуорометилювання та розділення ізомерних 1-дифлуорометил- і 2-дифлуорометил С-заміщених індазолів, а також деякі можливості функціоналізації таких молекул.

Результати та їх обговорення. Досліджено N-дифлуорометилювання похідних індазолу, що містять атоми брому, йоду та нітрогрупу в різних положеннях гетероциклічного циклу. У всіх випадках визначено умови розділення ізомерів – продуктів N-дифлуорометилювання за положеннями 1 і 2. У результаті подальшої функціоналізації похідних 1- та 2-дифлуорометиліндазолу одержано відповідні аміни, естери карбонової та борної кислот.

Експериментальна частина. Структуру синтезованих сполук підтверджено методами ^{1}H і ^{19}F ЯМР-спектроскопії, а також елементним аналізом. Остаточне підтвердження структури ізомерних дифлуорометиліндазолів здійснено в експериментах SELNOESY та ^{1}H-^{13}C HMBC.

Висновки. Знайдено зручний метод дифлуорометилювання заміщених індазолів та одержано дифлуорометильні похідні за положеннями 1 та 2 індазольного ядра за високими виходами. Знайдено метод ефективного розділення ізомерних дифлуорометиліндазолів та досліджено деякі можливості їх подальшої функціоналізації.

Ключові слова: індазоли; N-дифлуорометилювання; розділення ізомерів

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Introduction

Indazoles are widely known heterocyclic systems that have found application in the synthesis of many practically useful drugs [1]. Alkylation or other reactions of substitution of the hydrogen atom near the nitrogen atoms of indazoles occur at positions 1 or 2 and always lead to a mixture of isomers. The separation of these mixtures is often a difficult task, which can be solved on an industrial scale by fractional distillation; however, it often requires a high efficiency of the fractional separation. For example, the difference in boiling points of 1-methyl and 2-methyl indazoles is 30 °C [2], but in some cases, isomeric N-alkylindazoles can be separated by chromatography only.

The difluoromethylation of nitrogen-containing heterocycles has already become a well-studied chemical process. The known herbicides Sulfentrazon and Carfentrazone were synthesized based on of N-difluoromethylated triazoles. The features of the N-difluoromethylation of nitrogen-containing heterocycles have been studied in our laboratory. Hence, for the first time, we obtained products of the N-difluoromethylation of all simplest (parent) azoles – pyrazole [3], pyrrole, 1,2,3- and 1,2,4-triazoles, and tetrazole [4]. In addition, there were significant studies on the difluoromethylation of ambident azoles: mercaptobenzimidazoles [5], mercaptotetrazoles [6] and cyanoazoles [7].

The difluoromethylation of indazole derivatives has not previously been systematically studied. There are limited reports in the literature that describe the N-difluoromethylation of functionalized indazoles, and two methods have emerged as the most common. The first method generally involves the treatment of indazole derivatives with NaH and an excess of $CHClF_2$ (Freon 22, a cheap difluorocarbene source) at high temperature in DMF [8, 9], which presents a safety concern associated with the thermal instability hazards due to the use of NaH/DMF. Alternatively, the reactions can be performed in THF as a solvent with two moles of NaH and an excess of Freon-22 at elevated temperatures in a closed pressure vessel for an extended period of time to give N-difluoromethylated indazole products in moderate yields [10], or even at atmospheric pressure for indazoles with a methoxycarbonyl group [11]. The isomers (1 and 2-difluoromethylindazole derivatives) were separated chromatographically in most of the above cases. While this approach may be adequate for a small-scale synthesis, it is impractical for large-scale preparative procedures due to the need for a large dilution of the reaction mixture. The second method widely used consists in heating of indazole derivatives with $ClCF_2COONa$ as a difluorocarbene precursor in the presence of a base [12, 13]. However, the yields are generally below average, and the isomers were often not separated. In addition, CHF_3 has been reported as difluorocarbene sources for the N-difluoromethylation of 3-chloroindazole [14]. The difluoromethylation of the unsubstituted (parent) indazole was carried out quite recently using a very expensive reagent – $BrCF_2P(O)(OEt)_2$ [15]. N-difluoromethylindazoles with amino groups are unknown so far.

In this work, we carried out the N-difluoromethylation of indazole derivatives by the action of Freon-22 and alkali using a water-dioxane medium. This method does not require anhydrous solvents and high dilutions of the reaction medium, thus it is convenient for a large-scale synthesis. We chose indazoles containing halogen atoms or nitro groups in different positions as model compounds. We also carried out the reactions of the reduction of the nitro group to the amino group and the substitution of the bromine atom.

Results and discussion

Indazole derivatives containing a bromine atom in different positions of the indazole ring (**1.1**–**1.5**), 3-iodoindazole (**1.6**) and indazole derivatives containing nitro group in positions 5 and 6 (**1.7**, **1.8**) were used as starting compounds. The difluoromethylation was carried out by the action of Freon-22 excess in an aqueous dioxane medium using 5 mol of potassium hydroxide in 40% water solution. In all cases, the reaction proceeded with an exothermic effect was not practically accompanied by side processes, and led to high yields of a mixture of the isomers (Scheme 1).

Compound **2.3** was mentioned earlier, however, the work [16] indicated that the only one isomer was obtained during the N-difluoromethylation of indazole **1.3**, and it was described as a liquid. But, in fact, both isomeric products of the N-difluoromethylation of indazole **1.3** are solid crystalline substances with melting points of 64–65 °C for **2.3** and 68–69 °C for **3.3**. Nevertheless, the mixture of isomers may be liquid.

In all N-difluorometylation experiments performed in this work, we obtained a mixture of isomers. A ratio of the latter depended on the position of the substituent, and was determined by the steric hindrances created by it. The presence

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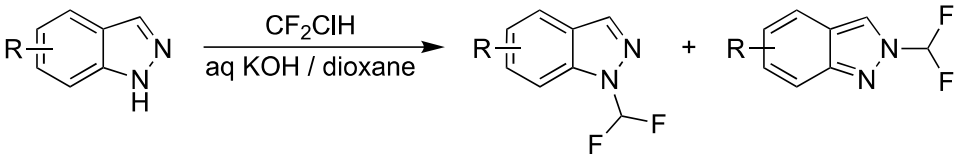


1.1-1.8 **2.1-2.8** **3.1-3.8**

1.1: R = 3-Br; **1.2**: R = 4-Br; **1.3**: R = 5-Br; **1.4**: R = 6-Br; **1.5**: R = 7-Br;
1.6: R = 3-I; **1.7**: R = 5-NO_2; **1.8**: R = 6-NO_2

Scheme 1. The N-difluoromethylation of substituted indazoles

of a substituent in positions 4, 5, or 6 did not create steric hindrances to the N-CHF_2 group, therefore the isomer ratios were close to 1:1 and ranged from 10:9 to 5:4 (Table 1). On the contrary, in the case of 3-bromoindazole, the ratio of isomers was 3:1, and in the case of a larger iodine atom as a substituent in position 3, the ratio was 4:1. The difluoromethylation of 7-bromomindazole (**1.5**) led to 2-difluoromethyl derivative **3.5** as a main product due to steric effects of a bromine atom. The isomer ratio was 1:3 (Table 1). Interestingly, the signal of the difluoromethyl group in the ^{1}H NMR spectra of the minor product **2.5** was strongly shifted downfield to 8.2 ppm compared to 7.45–7.50 ppm in other cases.

The separation of isomers in most cases was carried out chromatographically, on a MN-Kieselgel-60 silica gel using methylene chloride as an eluent. The products of the difluoromethylation in position 1 (compounds **2,** $R_f \approx 0.6–0.8$) were quite well separated from the products of the difluoromethylation in position 2 (compounds **3**, $R_f \approx 0.4–0.6$), except for the cases of 4-bromo- and 6-bromoindazoles (**2.2**, **3.2** and **2.4**, **3.4** when isomers had nearly equal R_f. In the latter cases the isomers were separated by two steps: the initial fractional distillation with an efficient reflux column under oil pump vacuum, giving two enriched fractions (b. p. 70–72 °C / 0.5 Torr for 1-difluoromethyl products, and 83–85 °C / 0.5 Torr for 2-difluoromethyl products) and the subsequent crystallization of each fraction from hexane. The combined mother liquors after evaporation were subjected to the repeated fractional distillation followed by crystallization.

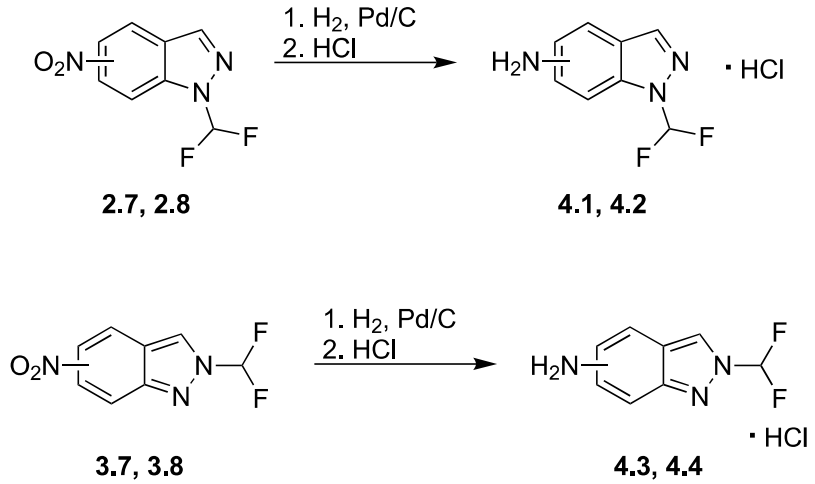
In the ^{1}H NMR spectra of compounds **3.2–3.5** and **3.7**, **3.8**, the singlet signal of the proton in position C-3 was downfield compared to compounds **2.2–2.5** and **2.7**, **2.8** due to the effect of the electron-withdrawing difluoromethyl group. The structure of compounds **3.2–3.5** and **3.7**, **3.8** was finally confirmed by the SELNOESY experiment. When the proton signal at the C^3 atom (the lowest-field singlet) was saturated, the Overhauser effect appeared on the signals of the CHF_2 group, while in the case of compounds **2.2**–**2.5**, **2.7**, and **2.8**, the effect did not appear. The SELNOESY experiment was also carried out with compound **2.4** containing a bromine atom in position 6. When the proton signal was saturated at C-7 (singlet signal of the aromatic ring), the Overhauser effect was also observed on the signals of the CHF_2 group, which unambiguously confirmed the structure of the molecule. The structure of compounds **3.1** and **3.6** was confirmed by ^{1}H-^{13}C HMBC experiments. The correlation between the CHF_2-group proton signal and the highest field carbon signal bonded to iodine or bromine atom was found.

Table 1. The overall yields and ratio of isomers of N-difluorometylindazoles (**2** and **3**)

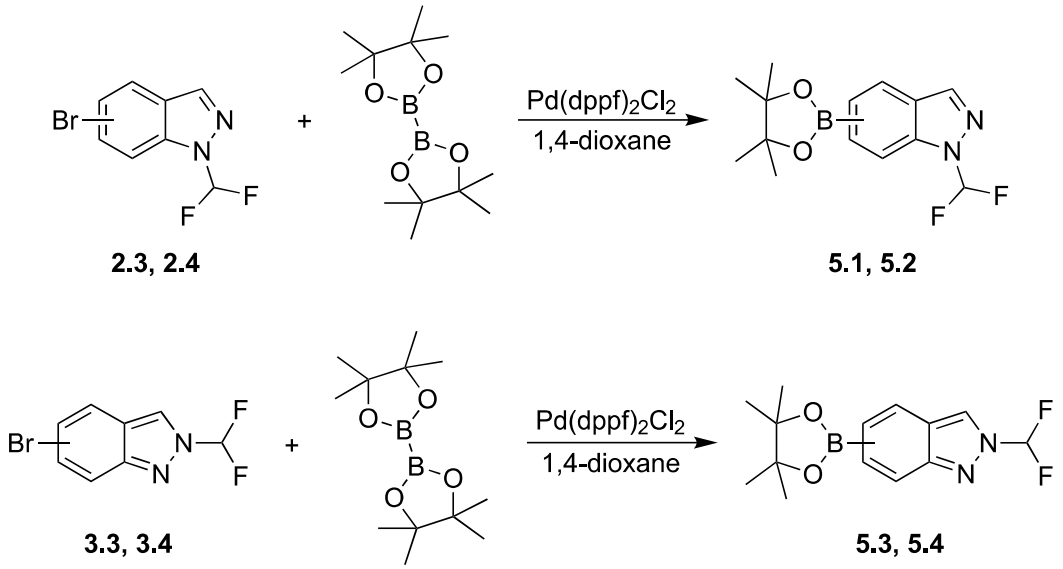
Starting indazole	Substituent	Overall yield **2** + **3**, %	Ratio **2**/**3**
1.1	3-Br	83	3:1
1.2	4-Br	67	7:6
1.3	5-Br	79	5:4
1.4	6-Br	76	10:9
1.5	7-Br	64	1:3
1.6	3-I	81	4:1
1.7	5-NO_2	75	8:7
1.8	6-NO_2	76	10:9

The nitro compounds (**2.7**, **2.8**, **3.7**, and **3.8**) were reduced to the corresponding amino compounds by the hydrogenation on palladium with hydrogen at atmospheric pressure in methanol. However, the final amines were not stable as free bases and darkened quickly after the solvent was evaporated. The crystallization or distillation of these free amines led to their decomposition. Therefore, the amines were converted to the corresponding hydrochlorides (**4.1–4.4**), which were stable while storing and could be recrystallized from alcohol (Scheme 2).

N-Difluoromethylindazoles containing a bromine atom in position 4 and 5 (**2.3**, **2.4**, **3.3** and **3.4**)



Scheme 2. The synthesis of N-difluoromethylindazoles with amino groups in the ring



Scheme 3. The synthesis of N-difluoromethylindazole-(5 or 6)-boropinacolates

were converted into the corresponding pinacolyl esters of boric acid (**5.1**–**5.4**) under the palladium catalysis (Scheme 3). Our attempts to carry out such a modification of compound **3.1**, as well as **3.6**, containing a bromine or iodine atom in position 3 failed. After water-alkali workup of the reaction mixture the only product separated from the dark resins was 2,3-dimethylbutanediol-2.3.

The bromine atom in compounds **2.3** and **3.3** can be replaced by an ester group by the carbonylation in an alcoholic medium in an autoclave in the presence of a palladium catalyst to give methyl N-difluoromethylindazole-(5 or 6)-carboxylates (**6.1**, **6.2**). This method for obtaining of such substances undoubtedly has advantages over the described one for methyl N-difluoromethylindazole-3-carboxylates [11] since it is more suitable for the large-scale syntheses. The resulting esters can easily be hydrolyzed to the corresponding carboxylic acids **7.1**, **7.2** (Scheme 4).

■ Conclusions

A convenient method for the difluoromethylation of substituted indazoles has been found; difluoromethyl derivatives in positions 1 and 2 of the indazole ring have been obtained in high yields. The method for efficient separation of isomeric difluoromethylindazoles has been found and some possibilities of their further functionalization have been described.

■ Experimental part

Melting points were measured in open capillary tubes and were given uncorrected. ^{1}H NMR (400 MHz, $CDCl_3$ or DMSO-d_6) and ^{19}F NMR (376 MHz, $CDCl_3$ or DMSO-d_6) spectra were recorded on a Varian-Mercury-400 spectrometer using TMS and CCl_3F as internal standards. Two-dimensional ^{1}H NMR (400 MHz, $CDCl_3$) and

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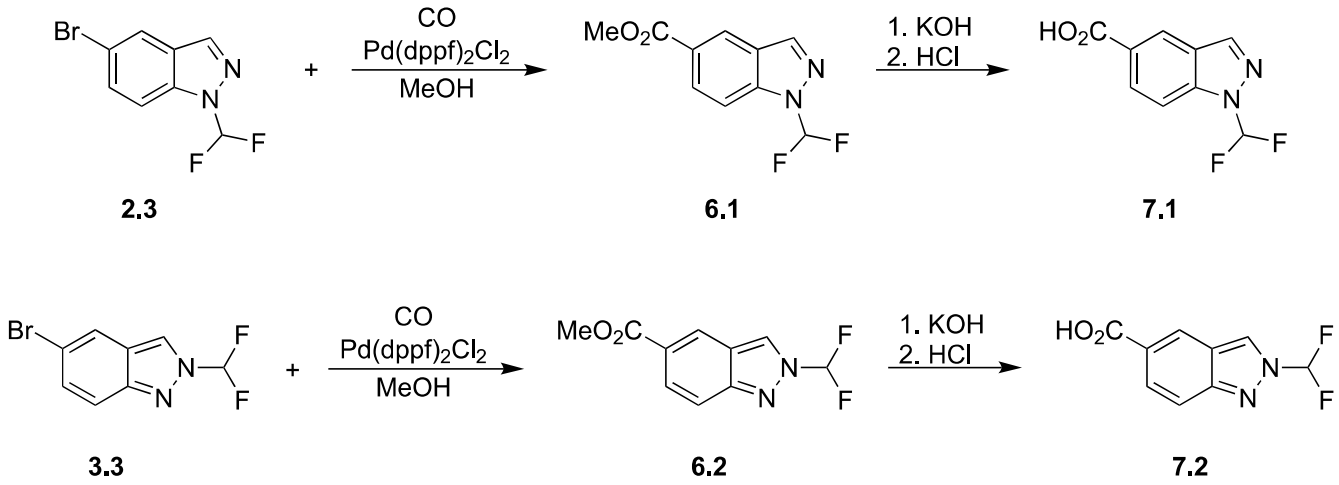

Scheme 4. The synthesis of N-difluoromethylindazole-(5 or 6)-carboxylic acids

^{13}C NMR (125 MHz, $CDCl_3$) were recorded on a Bruker Avance DXR-500 spectrometer. The elemental analysis was performed in the Analytical Chemistry Laboratory of the Institute of Organic Chemistry, National Academy of Sciences of Ukraine. The reaction progress was controlled by TLC on Silufol 254 plates.

The general procedure for the synthesis of N-difluoromethylindazoles (2.1–2.8 and 3.1–3.8)

A solution of a starting indazole **1.1–1.8** (0.3 mol) in dioxane (200 mL) was stirred and treated by adding a solution of KOH (90 g, 1.5 mol) in H_2O (120 mL). In the case of nitroindazoles, a voluminous precipitate of potassium nitroindazolide was formed and then gradually dissolved during the reaction. Freon 22 was bubbled through the vigorously stirred reaction mixture at 40–45 °C until the absorption of gas ceased (the exothermic effect was observed). If, according to the TLC control, the starting indazole remained, the additional KOH (30 g) was added, and Freon 22 was bubbled until the absorption of gas ceased. The overall time of the reaction was about 4–5 h. Water (300 mL) was added, the product was extracted by shaking with MTBE (2×300 mL), the organic layer was separated and washed with water (3×300 mL), dried over anhydrous K_2CO_3, the solvent was evaporated at reduced pressure. The mixture of isomers was separated by chromatography except for the cases **1.2** and **1.4** when the separation was performed by the initial fractional distillation followed by crystallization.

1-Difluoromethyl-3-bromoindazole (**2.1**)

A white solid. Yield – 62%. M. p. 63–64 °C. Anal. Calcd for $C_8H_5BrF_2N_2$, %: C 38.90; H 2.04; N 11.34. Found, %: C 38.75; H 1.98; N 11.27. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 7.38–7.41 (1H, m, ArH); 7.45 (1H, t, J = 60.0 Hz, N-CHF_2); 7.52–7.60 (2H, m, ArH); 7.88–7.91 (1H, m, ArH). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -94.2 (d, J = 60.0 Hz, N-CHF_2).

2-Difluoromethyl-3-bromoindazole (**3.1**)

A white solid. Yield – 21%. M. p. 69–70 °C. Anal. Calcd for $C_8H_5BrF_2N_2$, %: C 38.90; H 2.04; N 11.34. Found, %: C 38.71; H 1.89; N 11.33. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 7.19–7.21 (1H, m, ArH); 7.40–7.48 (2H, m, ArH); 7.70–7.72 (1H, m, ArH); 7.81 (1H, t, J = 60.0 Hz, N-CHF_2). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -95.6 (d, J = 60.0 Hz, N-CHF_2).

1-Difluoromethyl-4-bromoindazole (**2.2**)

A white solid. Yield – 36%. M. p. 55–57 °C. Anal. Calcd for $C_8H_5BrF_2N_2$, %: C 38.90; H 2.04; N 11.34. Found, %: C 38.65; H 2.08; N 11.42. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 7.25–7.29 (1H, m, ArH); 7.32–7.38 (1H, m, ArH); 7.41 (1H, t, J = 60.0 Hz, N-CHF_2); 7.82–7.85 (1H, m, ArH); 8.04 (1H, s, C^3-H). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -94.5 (d, J = 60.0 Hz, N-CHF_2).

2-Difluoromethyl-4-bromoindazole (**3.2**)

A white solid. Yield – 31%. M. p. 62–63 °C. Anal. Calcd for $C_8H_5BrF_2N_2$, %: C 38.90; H 2.04; N 11.34. Found, %: C 38.83; H 2.04; N 11.44. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 7.21–7.23 (1H, m, ArH); 7.32–7.36 (1H, m, ArH); 7.46 (1H, t, J = 60.0 Hz, N-CHF_2); 7.68–7.70 (1H, m, ArH); 8.39 (1H, s, C^3-H). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -95.9 (d, J = 60.0 Hz, N-CHF_2).

1-Difluoromethyl-5-bromoindazole (**2.3**)

A white solid. Yield – 44%. M. p. 64–65 °C. Anal. Calcd for $C_8H_5BrF_2N_2$, %: C 38.90; H 2.04; N 11.34. Found, %: C 38.85; H 2.03; N 11.32. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 7.45 (1H, t, J = 60.0 Hz, N-CHF_2); 7.59–7.61 (1H, m, ArH); 7.66–7.68 (1H, m, ArH); 7.92–7.94 (1H, m, ArH); 8.05 (1H, s, C^3-H). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -94.7 (d, J = 60.0 Hz, N-CHF_2).

2-Difluoromethyl-5-bromoindazole (**3.3**)

A white solid. Yield – 35%. M. p. 68–70 °C. Anal. Calcd for $C_8H_5BrF_2N_2$, %: C 38.90; H 2.04; N 11.34. Found, %: C 38.78; H 2.06; N 11.51. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 7.34–7.41 (1H, m, ArH); 7.46 (1H, t, J = 60.0 Hz, N-CHF_2); 7.60–7.63 (1H, m, ArH); 7.81–7.83 (1H, m, ArH); 8.29 (1H, s, C^3-H). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -95.6 (d, J = 60.0 Hz, N-CHF_2).

1-Difluoromethyl-6-bromoindazole (**2.4**)

A white solid. Yield – 42%. M. p. 75–76 °C. Anal. Calcd for $C_8H_5BrF_2N_2$, %: C 38.90; H 2.04; N 11.34. Found, %: C 38.76; H 2.02; N 11.50. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 7.40–7.42 (1H, m, ArH); 7.45 (1H, t, J = 60.0 Hz, N-CHF_2); 7.60–7.65 (1H, m, ArH); 7.98 (1H, s, C7-H); 8.07 (1H, s, C^3-H). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -94.7 (d, J = 60.0 Hz, N-CHF_2).

2-Difluoromethyl-6-bromoindazole (**3.4**)

A white solid. Yield – 36%. M. p. 50–51 °C. Anal. Calcd for $C_8H_5BrF_2N_2$, %: C 38.90; H 2.04; N 11.34. Found, %: C 38.71; H 2.06; N 11.31. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 7.20–7.22 (1H, m, ArH); 7.45 (1H, t, J = 60.0 Hz, N-CHF_2); 7.57–7.62 (1H, m, ArH); 7.93 (1H, s, C^7-H); 8.34 (1H, s, C^3-H). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -95.1 (d, J = 60.0 Hz, N-CHF_2).

1-Difluoromethyl-7-bromoindazole (**2.5**)

A white solid. Yield – 16%. M. p. 76–77 °C. Anal. Calcd for $C_8H_5BrF_2N_2$, %: C 38.90; H 2.04; N 11.34. Found, %: C 38.96; H 2.11; N 11.12. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 7.16–7.19 (1H, m, ArH); 7.70–7.76 (2H, m, ArH); 8.23 (1H, t, J = 60.0 Hz, N-CHF_2); 8.07 (1H, s, C^3-H). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -90.8 (d, J = 60.0 Hz, N-CHF_2).

2-Difluoromethyl-7-bromoindazole (**3.5**)

A white solid. Yield – 48%. M. p. 103–105 °C. Anal. Calcd for $C_8H_5BrF_2N_2$, %: C 38.90; H 2.04; N 11.34. Found, %: C 38.97; H 2.07; N 11.23. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 7.00–7.04 (1H, m, ArH); 7.55 (1H, t, J = 60.0 Hz, N-CHF_2); 7.56–7.58 (1H, m, ArH); 7.67–7.71 (1H, m, ArH); 8.47 (1H, s, C^3-H). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -94.7 (d, J = 60.0 Hz, N-CHF_2).

1-Difluoromethyl-3-iodoindazole (**2.6**)

A white solid. Yield – 65%. M. p. 72–74 °C. Anal. Calcd for $C_8H_5IF_2N_2$, %: C 32.68; H 1.71; N 9.53. Found, %: C 32.75; H 1.98; N 9.77. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 7.33–7.37 (1H, m, ArH); 7.47 (1H, t, J = 60.0 Hz, N-CHF_2); 7.53–7.61 (2H, m, ArH); 7.72–7.74 (1H, m, ArH). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -94.6 (d, J = 60.0 Hz, N-CHF_2).

2-Difluoromethyl-3-iodoindazole (**3.6**)

A white solid. Yield – 16%. M. p. 92–94 °C. Anal. Calcd for $C_8H_5IF_2N_2$, %: C 32.68; H 1.71; N 9.53. Found, %: C 32.71; H 1.89; N 9.37. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 7.18–7.22 (1H, m, ArH); 7.38–7.40 (1H, m, ArH); 7.45–7.47 (1H, m, ArH); 7.59 (1H, t, J = 60.0 Hz, N-CHF_2); 7.71–7.73 (1H, m, ArH). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -95.6 (d, J = 60.0 Hz, N-CHF_2).

1-Difluoromethyl-5-nitroindazole (**2.7**)

A yellow solid. Yield – 40%. M. p. 157–158 °C. Anal. Calcd for $C_8H_5F_2N_3O_2$, %: C 45.08; H 2.36; N 19.71. Found, %: C 44.97; H 2.47; N 19.63. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 7.55 (1H, t, J = 60.0 Hz, N-CHF_2); 7.85–7.88 (1H, m, ArH); 8.31 (1H, s, C^3-H); 8.38–8.41 (1H, m, ArH); 8.74–8.75 (1H, m, ArH). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -94.1 (d, J = 60.0 Hz, N-CHF_2).

2-Difluoromethyl-5-nitroindazole (**3.7**)

A yellow solid. Yield – 35%. M. p. 117–118 °C. Anal. Calcd for $C_8H_5F_2N_3O_2$, %: C 45.08; H 2.36; N 19.71. Found, %: C 45.12; H 2.41; N 19.89. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 7.52 (1H, t, J = 60.0 Hz, N-CHF_2); 7.82–7.85 (1H, m, ArH); 8.14–8.17 (1H, m, ArH); 8.69 (1H, s, C^3-H); 8.70–8.72 (1H, m, ArH). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -95.3 (d, J = 60.0 Hz, N-CHF_2).

1-Difluoromethyl-6-nitroindazole (**2.8**)

A yellow solid. Yield – 40%. M. p. 135–136 °C. Anal. Calcd for $C_8H_5F_2N_3O_2$, %: C 45.08; H 2.36; N 19.71. Found, %: C 45.22; H 2.42; N 19.91. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 7.55 (1H, t, J = 60.0 Hz, N-CHF_2); 7.95–7.98 (1H, m, ArH); 8.18–8.20 (1H, m, ArH); 8.25 (1H, s, C^3-H); 8.70–8.72 (1H, m, ArH). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -94.8 (d, J = 60.0 Hz, N-CHF_2).

2-Difluoromethyl-6-nitroindazole (**3.8**)

A yellow solid. Yield – 36%. M. p. 117–118 °C. Anal. Calcd for $C_8H_5F_2N_3O_2$, %: C 45.08; H 2.36; N 19.71. Found, %: C 45.10; H 2.33; N 19.55. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 7.54 (1H, t, J = 60.0 Hz, N-CHF_2); 7.82–7.85 (1H, m, ArH); 7.98–8.00 (1H, m, ArH); 8.52 (1H, s, C^3-H); 8.74–8.75 (1H, m, ArH). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -95.7 (d, J = 60.0 Hz, N-CHF_2).

The general procedure for the synthesis of N-difluoromethyl-(5 or 6)-aminoindazole hydrochlorides (4.1–4.4)

To a stirred solution of compound **2.7**, **2.8**, **3.7**, or **3.8** (0.1 mol) in a peroxide-free dioxane (100 mL), 10% Pd/C (1 g) was added. The flask was connected to a vacuum and then filled with hydrogen. The reaction mixture was stirred under H_2 atmosphere until the absorption of gas ceased (about 4 h), and then the catalyst was removed by filtration. The procedure was completed by passing gaseous HCl through the solution of the corresponding amine until the absorption

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of gas was complete; the hydrochloride precipitate obtained was filtered off, washed with MTBE, and dried at 80 °C under the air atmosphere.

1-Difluorometyl-5-aminoindazole hydrochloride (**4.1**)

A white solid. Yield – 92%. M. p. 207–208 °C (dec.). Anal. Calcd for $C_8H_8ClF_2N_3$, %: C 43.75; H 3.67; N 19.13. Found, %: C 43.60; H 3.53; N 19.25. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 7.82–7.85 (1H, m, ArH); 7.92–7.94 (1H, m, ArH); 7.99 (1H, s, C^4-H); 8.24 (1H, t, J = 60.0 Hz, N-CHF$_2$); 8.51 (1H, s, C^3-H); 10.72 (3H, br. s, NH_3^+). ^{19}F NMR (376 MHz, DMSO-d_6), δ, ppm: -95.5 (d, J = 60.0 Hz, N-CHF$_2$).

1-Difluorometyl-6-aminoindazole hydrochloride (**4.2**)

A white solid. Yield – 94%. M. p. 213–214 °C (dec.). Anal. Calcd for $C_8H_8ClF_2N_3$, %: C 43.75; H 3.67; N 19.13. Found, %: C 43.78 H 3.90; N 19.28. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 7.37–7.39 (1H, m, ArH); 7.77 (1H, s, C^7-H); 7.84–7.86 (1H, m, ArH); 8.19 (1H, t, J = 60.0 Hz, N-CHF$_2$); 8.45 (1H, s, C^3-H); 9.50 (3H, br. s, NH_3^+). ^{19}F NMR (376 MHz, DMSO-d_6), δ, ppm: -96.1 (d, J = 60.0 Hz, N-CHF$_2$).

2-Difluorometyl-5-aminoindazole hydrochloride (**4.3**)

A white solid. Yield – 91%. M. p. 203–205 °C (dec.). Anal. Calcd for $C_8H_8ClF_2N_3$, %: C 43.75; H 3.67; N 19.13. Found, %: C 43.56 H 3.62; N 19.11. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 7.42–7.45 (1H, m, ArH); 7.82–7.84 (1H, m, ArH); 7.86 (1H, s, C^4-H); 8.21 (1H, t, J = 60.0 Hz, N-CHF$_2$); 8.99 (1H, s, C^3-H); 10.50 (3H, br. s, NH_3^+). ^{19}F NMR (376 MHz, DMSO-d_6), δ, ppm: -95.9 (d, J = 60.0 Hz, N-CHF$_2$).

2-Difluorometyl-6-aminoindazole hydrochloride (**4.4**)

A white solid. Yield – 94%. M. p. 209–211 °C (dec.). Anal. Calcd for $C_8H_8ClF_2N_3$, %: C 43.75; H 3.67; N 19.13. Found, %: C 43.87 H 3.72; N 19.22. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 7.13–7.16 (1H, m, ArH); 7.74 (1H, s, C^7-H); 7.91–7.93 (1H, m, ArH); 8.22 (1H, t, J = 60.0 Hz, N-CHF$_2$); 9.00 (1H, s, C^3-H); 9.75 (3H, br. s, NH_3^+). ^{19}F NMR (376 MHz, DMSO-d_6), δ, ppm: -95.7 (d, J = 60.0 Hz, N-CHF$_2$).

The general procedure for the synthesis of N-difluoromethylindazole-(5 or 6)-boropinacolates (5.1–5.4)

To a mixture of compound **2.3**, **2.4, 3.3** or **3.4** (0.1 mol), boron-dipinacolate (30 g, 0.12 mol), potassium acetate (20 g) and DMSO (10 mL) in a peroxide-free dioxane (200 mL), Pd(dppf)$_2$Cl$_2$ (2 g) was added under the Ar atmosphere. The mixture was stirred under the inert atmosphere for 24 h at 90 °C. The solid was filtered off, the solvent was evaporated in a vacuum. The residue was treated with 2 M aq KOH (400 mL), and after stirring for 1 h, filtered through a thick filter paper or a filter cloth. The filtration process is often difficult due to the plasticine-like nature of the separated material. However, most of the impurities and gum products are separated in this way. The filtrate was acidified with hydrochloric acid to pH 2–3, the precipitated product was extracted by hot hexane (≈ 60 °C, 200 mL); the hexane solution was washed with hot water (≈ 60 °C, 5×300 mL), filtered through a layer of silica gel (Kieselgel-MN-60, 4–5 cm), after that washed with hexane. After evaporation of the solvent, a pure colorless target product was obtained. If the product was oil, it was distilled in vacuo.

1-Difluoromethylindazole-5-boropinacolate (**5.1**)

A white solid. Yield – 77%. M. p. 118–119 °C. Anal. Calcd for $C_{14}H_{17}BF_2N_2O_2$, %: C 57.17; H 5.83; N 9.52. Found, %: C 57.36 H 5.62; N 9.71. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 1.38 (12H, s, CH_3); 7.48 (1H, t, J = 60.0 Hz, N-CHF$_2$); 7.63–7.65 (1H, m, ArH); 7.75–7.77 (1H, m, ArH); 8.11 (1H, s, C^4-H); 8.30 (1H, s, C^3-H). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -94.8 (d, J = 60.0 Hz, N-CHF$_2$).

1-Difluoromethylindazole-6-boropinacolate (**5.2**)

A white solid. Yield – 72%. M. p. 78–79 °C. Anal. Calcd for $C_{14}H_{17}BF_2N_2O_2$, %: C 57.17; H 5.83; N 9.52. Found, %: C 57.21 H 5.77; N 9.81. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 1.39 (12H, s, CH_3); 7.52 (1H, t, J = 60.0 Hz, N-CHF$_2$); 7.71–7.78 (2H, m, ArH); 8.10 (1H, s, C^4-H); 8.27 (1H, s, C^3-H). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -94.4 (d, J = 60.0 Hz, N-CHF$_2$).

2-Difluoromethylindazole-5-boropinacolate (**5.3**)

A white oil. Yield – 73%. B. p. 128–130 °C / 0.5 Torr. Anal. Calcd for $C_{14}H_{17}BF_2N_2O_2$, %: C 57.17; H 5.83; N 9.52. Found, %: C 57.27 H 5.90; N 9.55. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 1.37 (12H, s, CH_3); 7.50 (1H, t, J = 60.0 Hz, N-CHF$_2$); 7.63–7.78 (2H, m, ArH); 8.21 (1H, s, C^4-H); 8.32 (1H, s, C^3-H). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -94.0 (d, J = 60.0 Hz, N-CHF$_2$).

2-Difluoromethylindazole-6-boropinacolate (**5.4**)

A white oil. Yield – 75%. B. p. 129–131 °C / 0.5 Torr. Anal. Calcd for $C_{14}H_{17}BF_2N_2O_2$, %: C 57.17; H 5.83; N 9.52. Found, %: C 57.33 H 5.80;

N 9.64. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 1.36 (12H, s, CH_3); 7.52 (1H, t, J = 60.0 Hz, N-CHF_2); 7.65–7.80 (2H, m, ArH); 8.20 (1H, s, C^4-H); 8.33 (1H, s, C^3-H). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -94.5 (d, J = 60.0 Hz, N-CHF_2).

The general method for the synthesis of methyl N-difluoromethylindazole-5-carboxylates (6.1, 6.2)

Compound **2.3** or **3.3** (12.4 g, 0.05 mol) was dissolved in anhydrous MeOH (250 mL), then Et_3N (7 g, 0.07 mol) was added, followed by Pd(dppf)Cl_2 (1 g) and the reaction mixture was sealed in a high-pressure autoclave. The carbon monoxide gas was introduced at 40 bar pressure, and the reaction mixture was stirred at 100°C for 16 h. After cooling to room temperature, the residual pressure was vented; the reaction mixture was evaporated to dryness at reduced pressure, quenched with water (250 mL), and then extracted with EtOAc (2×150 mL). The extract was dried over anhydrous Na_2SO_4 and evaporated at reduced pressure, dissolved in CH_2Cl_2 (200 mL) filtered through a layer of silica gel (4–5 cm), washing off the remaining product with CH_2Cl_2 to form pure **6.1**, **6.2**.

Methyl 1-difluoromethylindazole-5-carboxylate (**6.1**)

A white solid. Yield – 85%. M. p. 125–127 °C. Anal. Calcd for $C_{10}H_8F_2N_2O_2$, %: C 53.10; H 3.57; N 12.39. Found, %: C 57.21 H 5.77; N 9.81. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 3.97 (3H, s, CH_3); 7.49 (1H, t, J = 60.0 Hz, N-CHF_2); 7.78–7.81 (1H, m, ArH); 8.17–8.20 (2H, m, ArH); 8.52 (1H, s, C^3-H). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -94.8 (d, J = 60.0 Hz, N-CHF_2).

Methyl 2-difluoromethylindazole-5-carboxylate (**6.2**)

A white solid. Yield – 84%. M. p. 85–86 °C. Anal. Calcd for $C_{10}H_8F_2N_2O_2$, %: C 53.10; H 3.57; N 12.39. Found, %: C 53.21 H 2.77; N 12.09. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 3.92 (3H, s, CH_3); 7.49 (1H, t, J = 60.0 Hz, N-CHF_2); 7.69–7.71 (1H, m, ArH); 7.91–7.93 (1H, m, ArH); 8.46 (1H, s, C^4-H); 8.49 (1H, s, C^3-H). ^{19}F NMR (376 MHz, $CDCl_3$), δ, ppm: -93.7 (d, J = 60.0 Hz, N-CHF_2).

The general method for the synthesis of N-difluoromethylindazole-5-carboxylic acids (7.1, 7.2)

Compound **6.1** or **6.2** (4.5 g, 0.02 mol) was suspended in a mixture of water (50 mL) and THF (10 mL), then NaOH (5 g) was added, and the reaction mixture was heated at 50 °C until complete dissolution. After cooling the solution, it was acidified with 10% HCl to pH 1–2. The precipitate obtained was filtered off, washed with H_2O (2×20 mL), and dried at 80°C under the air atmosphere to obtain pure **7.1** or **7.2**.

1-Difluoromethylindazole-5-carboxylic acid (**7.1**)

A white solid. Yield – 90%. M. p. 215–217 °C. Anal. Calcd for $C_9H_6F_2N_2O_2$, %: C 50.95; H 2.85; N 13.20. Found, %: C 50.99 H 2.71; N 13.11. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 7.91–7.93 (1H, m, ArH); 8.11–8.13 (1H, m, ArH); 8.23 (1H, t, J = 60.0 Hz, N-CHF_2); 8.54 (2H, s, C^3-H and C^4-H); 11.0–12.0 (1H, br. s, COOH). ^{19}F NMR (376 MHz, DMSO-d_6), δ, ppm: -95.5 (d, J = 60.0 Hz, N-CHF_2).

2-Difluoromethylindazole-5-carboxylic acid (**7.2**)

A white solid. Yield – 92%. M. p. 189–191 °C. Anal. Calcd for $C_9H_6F_2N_2O_2$, %: C 50.95; H 2.85; N 13.20. Found, %: C 51.21 H 2.77; N 12.91. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 7.88–7.90 (1H, m, ArH); 7.93–7.95 (1H, m, ArH); 8.20 (1H, t, J = 60.0 Hz, N-CHF_2); 8.56 (1H, s, C^4-H); 9.10 (1H, s, C^3-H); 11.0–12.0 (1H, br. s, COOH). ^{19}F NMR (376 MHz, DMSO-d_6), δ, ppm: -95.4 (d, J = 60.0 Hz, N-CHF_2).

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Titrimetric Methods for Determining Cationic Surfactants

Abstract

Aim. To generalize and systematize information on titrimetric methods for determining quaternary ammonium compounds (QACs).

Results and discussion. The review summarizes and systematizes information on the properties of surfactants, provides their classification, shows the main ways of use in the national economy, and their role in pharmacy and medicine. Currently known titrimetric methods for determining cationic surfactants, in particular quaternary ammonium compounds, which are widely used in medicine and pharmacy, are described and summarized.

Conclusions. As a result of the study, the main directions of developing methods for determining QACs by titrimetry methods have been summarized; the disadvantages and advantages of each of the methods described have been shown. In the future, it can be the basis for developing new and more effective methods of analysis.

Keywords: surfactants; quaternary ammonium compounds; titrimetric methods of analysis

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Титриметричні методи визначення катіонних поверхнево-активних речовин

Анотація

Мета. Узагальнити та систематизувати інформацію про титриметричні методи визначення четвертинних амонійних сполук (ЧАС).

Результати та їх обговорення. В огляді узагальнено й систематизовано інформацію про властивості поверхнево-активних речовин, наведено їх класифікацію, окреслено основні шляхи використання в народному господарстві та їх роль у фармації і медицині. Описано й узагальнено відомі нині титриметричні методи визначення катіонних поверхнево-активних речовин, зокрема ЧАС, що їх широко застосовують у медицині та фармації.

Висновки. У результаті проведеного дослідження окреслено основні напрями опрацювання методик визначення четвертинних амонійних сполук методами титриметрії, розглянуто недоліки й переваги кожного з описаних методів, що в подальшому може стати основою для опрацювання нових, більш ефективних способів проведення аналізу.

Ключові слова: поверхнево-активні речовини; четвертинні амонієві сполуки; титриметричні методи аналізу

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Introduction

Surfactants (a contraction of surface-active agent, devised in 1950) have found application in almost every branch of chemical industry. They play an important role as cleaning, wetting, dispersing, emulsifying, foaming and anti-foaming agents in many products, including detergents, fabric softeners, motor oils, emulsions, soaps, paints, adhesives, inks, anti-fogs, ski waxes, snowboard wax, deinking of recycled papers, in flotation, washing and enzymatic processes, and as laxatives [1]. Personal care products, such as cosmetics, shampoos, shower gels, hair conditioners, and toothpastes, also contain them. Alkali surfactant polymers act to displace dirt and debris using detergents when washing wounds [2] and *via* the application of medicinal lotions and sprays to the surface of the skin and mucous membranes [3]. Therefore, many efforts are being made to understand the physical and analytical chemistry of surface-active agents.

The main property of surfactants that determines how they are used is to reduce the surface tension (or interfacial tension) between two liquids, between a gas and a liquid, or between a liquid and a solid [4]. From the structural point of view surfactants are amphiphilic molecules consisting of a non-polar hydrophobic "water-avoiding" group (the tail), usually a straight or branched hydrocarbon or fluorocarbon chain containing 8–18 carbon atoms attached to a polar or ionic hydrophilic "water-seeking" group (the head) [5]. The water-insoluble hydrophobic group may extend out of the bulk water phase, into the air or into the oil phase, while the water-soluble head group remains in the water phase.

Most commonly, surfactants are classified according to the polar head group into four classes, i.e., non-ionic, cationic, anionic, and zwitterionic/amphoteric surfactants [6]. A non-ionic surfactant has no charged groups in its head. The head of an ionic surfactant carries a full positive, or a full negative charge. If the charge is negative, the surfactant is called anionic; if the charge is positive, it is called cationic. In case a surfactant contains the head with two oppositely charged groups, it is termed zwitterionic (Figure 1).

We would like to note right away that this review will focus specifically on the analytical aspects of cationic surfactants (CS), and the main reason is their high impact on the chemical industry and involvement in almost all spheres of human life. CS first became important more than 70 years ago when their unique bactericidal properties were reported [7, 8]. To quote Anna Gillis: "In the industry they are considered the ultimate workhorses. They decolorize sugar, kill bacteria growing in waterbeds, and help to pull the last driblets of oil from drilling wells. They serve as ingredients in products ranging from underarm deodorants to fiberglass for sail boats. Mostly, they're used to keep the laundry soft. These jacks-of-all-trades are quaternary ammonium compounds, or quats." [9]. CS have the capacity of self-assembling [8], being widely used in biotechnology [11, 12]. They play an important role in biotechnological applications, determining the huge potential of cationic amphiphilic agents as drug carriers in pharmacy and biomedicine [13]. Occurrence of positively charged fragments in an amphiphilic scaffold is responsible for their attractiveness in nanotechnological applications as antimicrobial and bioimaging agents [14, 15],

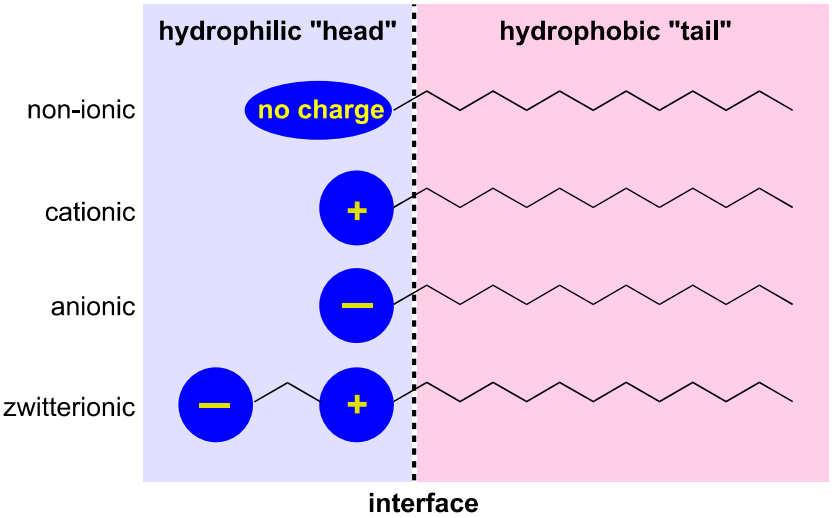

Figure 1. Classes of surfactants

corrosion inhibitors [16], supramolecular catalysts [17], stabilizers of nanoparticles and nanocarriers [18, 19], and especially as drug and gene nanocarriers [20]. Cationic head groups of surfactants provide their high affnity toward biopolyanions, such as DNA [21, 22], cell membranes, and intracellular organelles, such as mitochondrion, thereby initiating the development of a promising area of therapy, the so-called mitochondrion medicine [23, 24]. A series of works focusing on the application of CS in drug delivery have been recently reviewed [25, 26], with the emphasis given to their use for gene therapy [27, 28], as a template for the synthesis of mesoporous materials [29], as well as to their biocidal action against bacteria and fungi [30, 31].

The positive charge within CS is almost invariably centered around one or more nitrogen atoms. Although corresponding analogs containing sulfur, phosphorus, or arsenic have been found, they are considerably more expensive than their nitrogenous counterparts. Hence, they have not found a practical application. Some the most common substances of this class are given in Figure 2.

The charge of CS may be either permanent or only exist in certain pH value ranges. Quaternary ammonium compounds (QACs) retain their cationic character at any pH. Primary, secondary, and tertiary amines can be positively charged, depending on the ambient pH value. A typical fatty primary amine has a pK_b value of approximately 3.4. Although the simple equilibrium between the free base (RNH_2) and its conjugate acid (RNH_3^+) is complicated by adsorption of both species from the solution and by the micelle formation, as a rough guide the concentration of the cationic form is equal to that of the free base at a pH value of 10.6 and greater than it at lower values. Fatty amines are therefore justifiably included as CS in their own right in addition to being intermediates in the synthesis of QACs [32].

The most important property of CS from an environmental perspective is that they are

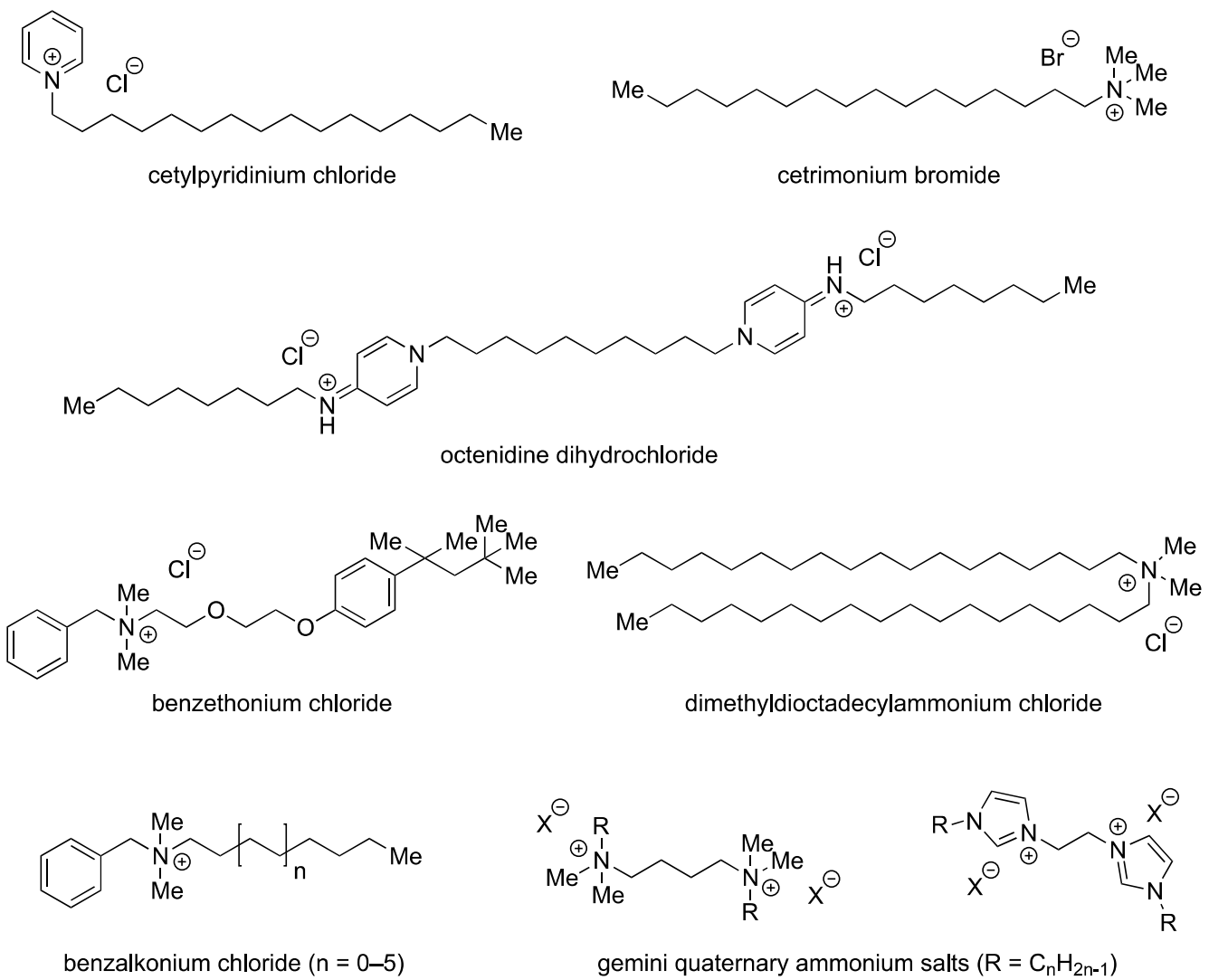

Figure 2. Examples of cationic surfactants

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Table 1. The surfactant market in personal care by type in US$, Bn

	US$ billion					CAGR[a], %
	2015	2016	2018	2021	2024	
Anionics	0.48	0.49	0.52	0.57	0.61	2.7
Nonionics	1.99	2.11	2.31	2.62	2.97	4.7
Cationics	1.76	1.90	2.19	2.68	3.28	7.4
Amphoterics	1.46	1.58	1.82	2.25	2.77	7.7
Others	0.44	0.42	0.44	0.54	0.64	4.7
Total	6.13	6.49	7.29	8.65	10.27	5.9

Note: [a] CAGR (compound annual growth rate) is a business and investing specific term for the geometric progression ratio that provides a constant rate of return over the time period

strongly sorbed by a wide variety of materials, such as natural sediments and soils. Numerous studies indicate that CS are also rapidly and strongly sorbed by many other materials of environmental relevance [33, 34]. A related property of CS is that they form 1:1 complexes with anionic materials, especially anionic surfactants. The complexes are relatively hydrophobic, but largely ionic in character [35]. This leads to the fact that CS may not be detected by some analytical methods, causing a failure to demonstrate their presence in environmental samples or confusion of complexation with degradation in fate studies.

Consumption of CS was estimated to be 190,000 and 150,000 tons in 1987 in the United States and Western Europe, respectively [36] and since then it has been increasing. It is likely that most or at least a large portion of this was sewered. The available data suggest that CS are ubiquitous environmental contaminants, at least in populated areas. Table 1 demonstrates utilization of various groups of surfactants in personal care. It reveals that CS are among the most used ones, and it is confirmed by growth rates and estimated value for 2024.

Considering the huge spread of CS, their toxicity remains a serious problem that hinders their widespread practical use and raises many environmental safety concerns. Thus, the toxicity of QACs, which are antibacterial and antifungal, varies. Dialkyldimethylammonium chlorides used as fabric softeners have a low LD_{50} (5 g kg^{-1}) and are essentially non-toxic, while the disinfectant alkylbenzyldimethylammonium chloride has LD_{50} of 0.35 g kg^{-1}. Prolonged exposure to CS can irritate and damage the skin since they disrupt the lipid membrane that protects the skin and other cells. Many CS are not only potent germicides, but also acutely toxic in the milligram per liter range and lower to aquatic organisms, including algae, fish, mollusks, barnacles, rotifers, starfish, shrimp, and others [37–40]. CS have been shown to elicit acute toxic effects in aquatic organisms by disrupting gill membranes, thus interfering with O_2 exchange [41]. Benzalkonium bromide acts as an inhibitory uncoupler in mitochondria. CS are possibly toxic to higher plants [42].

Taking into account the abovementioned issues concerning enormous spread and toxicity of CS, numerous analytical methods have been developed aiming at controlling their content in various environmental objects and products of different purposes. Many of the older methods are colorimetric, in which CS react with anionic dyes; the surfactant-dye complexes are extracted into an organic solvent, and the absorbance of the solution is measured spectrophotometrically [43]. However, these methods are generally unsuitable for monitoring levels of cationics in sewage or environmental samples or for laboratory studies, in which anionic surfactants are also present, since the affinity of CS for anionic surfactants is often greater than their affinity for the dyes. Here we are going to give a brief overview of classical and new titrimetric analytical methods designed to quantify CS.

■ Results and discussion

Currently, various methods are used for the quantitative and qualitative analysis of CS in analytical chemistry, including pharmaceutical chemistry. They are titrimetry, spectrophotometry, capillary electrophoresis, chromatography, and electrochemical methods.

Direct, reverse and two-phase titration of CS

In particular, to control the composition of a CS substance and the quality of the final products at cosmetic enterprises, the following methods are used: two-phase titration [44], spectrophotometry [45] and chromatography [46]. These methods are fast, simple and allow determining the class of CS. The main disadvantages of these methods, with the exception of chromatography, are as follows: the accuracy of CS determination

depends on the presence of impurities, these methods are not selective in relation to individual representatives of CS, do not allow homologous identification, i.e. with the help of these methods it is impossible to determine the content of an individual CS in the mixture. At the same time, most often only individual identification allows to assess the scope of application, toxicity and biodegradation of CS if they are released into the environment [47].

Titrimetric methods were the first ones widely used for the CS analysis, and they still remain quite common today [48, 49]. Titration is an effective and economical method of measuring the surfactant concentration. High speed and low cost make this method particularly advantageous and suitable for use, even in field conditions.

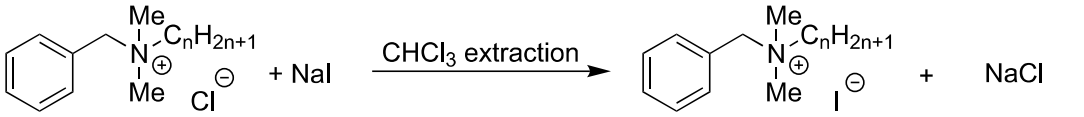
The pharmacopoeial method of analysis of QACs using an example of benzalkonium chloride as a QAC is based on the exchange reaction. A known excess of iodide is added to the sample solution, and the quaternary ammonium iodide is removed when shaking with chloroform. The excess of iodide is titrated by the iodate method. When the chloroform extraction is made from a slightly alkaline solution only quaternary ammonium compounds are measured; if it is made from a slightly acid solution, non-quaternary cationic amine impurities are also included. The difference between assay results obtained from acid and alkaline extractions represents the non-quaternary amine content (Figure 3) [50, 51].

However, the titration of QACs can be performed simpler in an aqueous solution with application of sodium dodecyl sulphate as a titrant and methyl orange or bromophenol blue as an indicator. Cetylpyridinium tetrachlorozincate is recommended as a standard to determine the titrant concentration [52].

Halogen-containing salts of QACs can be titrated in the medium of a non-aqueous solvent – glacial acetic acid, and halogen anions bound to a quaternary nitrogen atom are determined. Such methods are suitable for the analysis of pure samples that do not contain other salts, and other anions. At the same time, the choice of the titrant depends on the halide ion present in the salt (Figure 4) [53].

A more specific analysis of CS is the two-phase titration with an anionic surfactant. A number of indicator dyes, in particular bromophenol blue, methylene blue, which are often used during the analysis of anionic surfactants AS, are also suitable for the titration of cationic ones. [54]. According to Hartley and Runicle [55, 56], bromophenol blue should be used as an indicator for CS. Under these titration conditions, after adding an excess drop of the titrant, the aqueous phase of the solution becomes blue.

The method of the analysis according to *Epton* [57] (with methylene blue as an indicator) is still widely used (Figure 5). In this titration, CS is precipitated by an anionic surfactant in a biphasic mixture of water and chloroform. The ion pair formed must be extracted into chloroform with vigorous shaking. The endpoint is the point at which a purple color of the aqueous phase of the solution becomes colorless. The disadvantages



$$5I^-_{excess} + IO_3^- + 6H^+ \rightleftharpoons 3I_2 + 3H_2O$$

$$3I_2 + 2S_2O_3^{2-} \rightleftharpoons 2I^- + 2S_4O_6^{2-}$$

Figure 3. The scheme of the pharmacopoeial method of analysis of quaternary ammonium compounds

$$2\,B^+Cl^- + Hg(CH_3COO)_2 \rightleftharpoons HgCl_2 + 2\,B*CH_3COOH$$

$$B*CH_3COOH \rightleftharpoons BH^+ + CH_3COO^-$$

$$CH_3COO^- + HClO_4 \rightleftharpoons CH_3COOH + ClO_4^-$$

Note: 'B' represents the quaternary ammonium part

Figure 4. The determination of QACs

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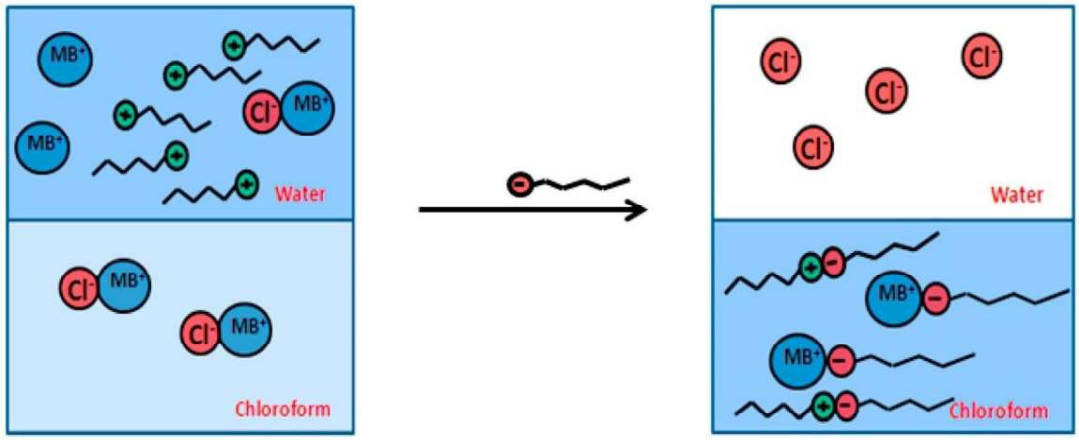

Note: MB^+ – methylene blue

Figure 5. The titration of cationic quaternary ammonium compounds by *Epton*

of this complex method are: the use of chloroform which is a relatively toxic solvent; a long waiting period for the phase distribution; inaccurate visual recognition of the endpoint.

Simultaneously, *Preston* [58] used the change in the surface tension as an indicator to record the endpoint of the titration. However, none of the methods for determining the endpoint of the titration is completely satisfactory. In the first case, the color change is not clear (sharp), and in the other one, the change in the surface tension is affected by the presence of inorganic salts.

Since the scope of application of methods for the quantitative determination of QACs is quite wide, the interest is quite high. Therefore, the method of the two-phase titration with methylene blue for determining the concentration of various cationic surface-active substances in water with a high salt content (22% of dissolved solids) is also presented in the literature. The endpoint of the titration was determined by decolorization, while the blue color completely changed from the aqueous phase to the chloroform phase. Light absorption at the characteristic wavelength of methylene blue was measured using a spectrophotometer. When the absorbance fell below a threshold value of 0.04, the aqueous phase was considered colorless, indicating that the endpoint of the titration had been reached. Using this improved technique, the total error of the titration of CS, such as dodecyltrimethylammonium bromide, in deionized water and high salinity water was 1.27% and 1.32% with detection limits (LOD and LOQ) of 0.149 and 0.215 mM, respectively [59].

J. Cross gave the general rules for the titration of CS by an anionic one. It is valid if:

- one surfactant's alkyl chain should be at least C_{12};
- the titration standards are sufficiently pure and belong to the same class of compounds as the compound being determined;
- nonionic surfactants are absent in the sample analyzed.

The accuracy of the method of non-aqueous titration of CS by an anionic titrant depends on the choice of the titrant and the indicator [60, 61]. When developing titration methods the requirements specified above must be met. It is also necessary to take into account that titration is based on the preservation of surfactants of their ionic character, and therefore, the pH is crucial. All common CS can be titrated at pH 3 using methyl orange as an indicator. At pH 10, only CS belonging to quaternary ammonium salts (QACs) can be determined, at the same time, impurities of non-quaternary amines are insufficiently ionized and do not interfere with titration. At pH 13, only some of the compounds can be identified, including the most common products, such as alkyltrimethyl- and dialkyldimethylammonium and benzyltrialkylammonium salts. Bromophenol blue is a suitable indicator for titrations at pH 10 and 13.

A stepwise titrimetric method for the simultaneous determination of QACs (R_4N^+) and aromatic amines (R_3N) has been developed. The technique is based on the extraction of the ionic associates of R_4N^+ and R_3NH^+ formed with a titrant. Sodium tetrakis(4-fluorophenyl)borate or sodium tetraphenylborate was used as a titrant, and potassium tetrabromophenolphthalein ethyl ether (TBPhE) was used as an indicator. The ionic associate formed between R_4N^+ and TBPhE had a blue color in 1,2-dichloroethane, while the ionic associate formed between R_3NH^+ and TBPhE had a red-violet color. Sample solutions, containing quaternary ammonium and/or amine compounds

were titrated with sodium tetrakis(4-fluorophenyl)borate or sodium tetraphenylborate. When one excessive drop of the titrant was added, the color of the organic phase changed from blue or red-violet to yellow at the endpoint of the titration. On the other hand, in the mixture of R_4N^+ and R_3N the color changed from blue to red-violet in the first endpoint. Therefore, quaternary ammonium compounds and aromatic amines in pharmaceutical preparations can be simultaneously successfully determined by the titration method proposed [62].

Titrimetric methods with the potentiometric determination of the endpoint

Today, a number of titrimetric methods with the potentiometric determination of the endpoint are quite common in the literature. At the same time, to control the endpoint of the titration commercially available perchlorate, nitrate and calcium electrodes can be used. This eliminates the need to manufacture a special liquid membrane or other electrodes, which were most often used until now for the potentiometric titration of surface-active substances. The reverse titration of CS with the standard solution of sodium dodecyl sulfate is usually performed [63]. It is important to note that when the concentration of CS is very low, its adsorption by glassware is significant. Therefore, one should use plastic dishes or glass dishes pre-washed with the sample.

In his work *J. T. Bentglini* compared the methods of determining the quantitative content of QAC derivatives with the visual control of the endpoint and the potentiometric one. To determine the endpoint, standard sodium lauryl sulfate was chosen as a titrant, and a nitrate-ion-selective or surface-active electrode was used. A comparison of the results of these two methods showed that the automatic potentiometric method is more accurate, simpler, faster and, in general, more suitable for use in production laboratories than the biphasic titration method [64].

The potentiometric two-phase titration is also used by the German Institute for Standardization, particularly in the DIN EN 14480 standard. The procedure is as follows: a surfactant solution is introduced into a flask for titration, a two-phase mixture of water and methyl isobutyl ketone/ethanol (1:1) and an emulsifier are added. The resulting emulsion is titrated with intensive stirring. The endpoint of the titration is determined potentiometrically using electrodes sensitive to cationic surface-active surfactants. The method described in this standard has the following advantages compared to the classical *Epton's* titration: the use of safe solvents, expressivity, increased accuracy due to the computerized determination of the endpoint of the titration. The advantage of the method described in the German standard is also its wide range of applications due to the possibility of determining the content of surface-active substances in cosmetic and household products (hair conditioner, bath oil, washing powder), as well as in technical products (industrial cleaning products).

The International Organization for Standardization (ISO) offers separate procedures for the determination of cations with high and low molecular masses. Compounds with a low molecular mass (less than 500), such as alkyltrimethylammonium salts, are dissolved and titrated using 0.005 M sodium dodecyl sulfate (SDS) solution as a titrant (pH adjusted to 2.5 using 1.0 M hydrochloric acid solution). In this method, surfactrode Refill electrodes, the reference electrode Ag/AgCl/3M KCl are used [65]. Compounds with a high molecular mass (more than 500), such as dialkyldimethylammonium fabric softeners, have poor solubility in water and are therefore first extracted from the test sample with isopropanol, then diluted with water, and then titrated with an anionic surfactant. The ISO procedure recommends sodium dodecyl sulfate of high purity for titration. It should be noted that the generally accepted titrant according to GOST standards for the two-phase titration of cations is a tetraphenylborate ion [66]. The titration endpoint can be monitored using fluoroborate ion-selective indicator electrodes and a double jump titration reference electrode [67].

Guang-yu Yuan and co-authors prepared a cationic surface-active ion-selective electrode and described its performance. The electrode had a lower detection limit of about 10^{-6} mol L^{-1}. The concentrations of three CS – cetyltrimetrylammonium bromide (CTAB), dodecyldimethylbenzylammonium chloride (DDMBAC), octadecyltrimethylammonium chloride (OTMAC) were determined by the method of the potentiometric titration using the solution of sodium tetraphenylborate as a titrant. The titration results were satisfactory. Relative errors in potentiometric titration were 2.12%, 3.45% and 4.21%, respectively [68].

Therefore, in view of the above, the biphasic and potentiometric titration are the two main techniques widely used and described in the literature. The biphasic titration is a relatively simple process that requires only minor equipment preparation, but it has a number of disadvantages: it is difficult to determine the titration

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endpoint, laborious, health hazard due to the use of harmful organic solvents, etc. The potentiometric titration has been proven to overcome most of these problems.

Compared to the classical (*Epton*) titration the direct potentiometric titration of CS using surfactants resistant to organic solvents can be easily automated. Even complex matrices, such as fats and oils in bath oils and hair conditioners or strong oxidants in laundry detergents and industrial cleaners, do not interfere with the titration of ionic surfactants, experiments were carried out with CTAB, CPC, benzalkonium chloride (BAC) and didecyldimethylammonium chloride (DDAC). The results obtained show excellent agreement with the *Epton* titration results. Regardless of the substance, the relative standard deviation (RSD) of the triplicate determination was less than 2.1% [69].

Ion-selective electrodes for the determination of quaternary ammonium compounds

A new sensitive potentiometric electrode of a surface-active substance based on highly lipophilic 1,3-didodecyl-2-methylimidazolium and the antagonist ion – tetraphenylborate was developed. This sensor was used as a sensitive material and incorporated into a plasticized PVC membrane**.** The electrode gave a fast Nernst response for the CS studied: cetylpyridinium chloride (CPC), hexadecyltrimethylammonium bromide, and (diisobutylphenoxyethoxyethyl)dimethylbenzylammonium chloride (hiamine) with slopes of 59.8, 58.6, and 56.8 mV decade^{-1}, t = 25 °C, respectively. The electrode served as an endpoint detector during the potentiometric titration of the ion pair of a surfactant using sodium tetraphenylborate as a titrant. Several technical grade CS and several commercial disinfectants were also titrated, and the results were compared to those obtained using the standard two-phase titration method. The electrode showed satisfactory analytical performance in the pH range of 2–11 and excellent selectivity towards CPC compared to all organic and inorganic cations tested [70].

Potentiometric electrodes with plasticized polymer membranes based on organic ion exchangers for the study of cationic and ethoxylated nonionic surface-active substances (CS and EONS) have been proposed. The titration was performed in one step. The anionic titrant (TPB) reacted first with CS:

$$CS^+ + TPB^- \rightarrow CS^+TPB^- \quad (1)$$

$$Ba^{2+} + xEONS \rightarrow [Ba(EONS)x]^{2+} \quad (2)$$

More simply, the above equation can be written as follows:

$$Ba^{2+} + xL \rightarrow Ba(L^{2+})_x \quad (3)$$

where L = EONS.

$$Ba(L^{2+})_x + 2TPB - BaL_x(TPB)_2 \quad (4)$$

The difference of 3–4 pK_s units between the solubility product values of both of the TPB ion associates (Eqs. (1) and (4)) caused the appearance of two distinct inflexions at the titration curve. The first inflexion related to the surfactant that formed with less soluble TPB ion-pair complex (with a lower solubility product value). It was CS. It was shown that ion associates (Eqs. (2) and (4)) were stable up to 60–70 °C, K_S varied in the range from 2×10^{-8} to 5×10^{-10} M. The main electrochemical parameters were also determined. They were the linearity ranges of the electrode function (5×10^{-5} (5×10^{-6})–1×10^{-2} (1×10^{-3}) M) and the slopes of the electrode functions (47–59 mV decade^{-1}), response time (60–90 s), drift potential (2–3 mV in a day), shelf life (3–4 months), limits of detection of tetramethylammonium salts (1×10^{-5}–4×10^{-7} M) [71].

M. Gerhard presented a new type of an ion-selective electrode for determining the content of cetylpyridinium chloride. This new electrode includes screen-printed modified and unmodified ion-selective electrodes for determining cetylpyridinium chloride. Electrodes with screen printing (SPE) show a stable response close to Nernst in the range of concentrations of 1×10^{-2}–1×10^{-6} M cetylpyridinium chloride, at 25 °C in the interval of pH 2–8 with a slope of 60.66 ± 1.10 mV decade^{-1}. The lower detection limit is 8×10^{-7} M, the response time 3 s and the satisfactory shelf life – 6 months. The produced electrodes can also be successfully used in the potentiometric titration of cetylpyridinium chloride using NaTPB. Analytical characteristics of SPE were compared with those for carbon paste electrodes and polyvinyl chloride electrodes (PVC). It was shown that the method could be applied for pharmaceutical preparations with the reproducibility of 99.60% and RSD – 0.53%. The studies used analytical and technical grade cetylpyridinium chloride, as well as various water samples that were successfully titrated, and the results were consistent with those obtained with a commercial electrode and the standard two-phase titration method. The sensitivity of the method proposed was compared

to the official method, and the possibility of field measurements was proven [72].

M. Gerhard with colleagues also investigated the effectiveness of printed carbon ink in the production of simple screen-printed carbon paste electrodes (SPCPE). Such electrodes are used for the potentiometric determination of cetyltrimethylammonium bromidein various pharmaceutical preparations and water samples. Their efficiency is compared to the indicators of electrodes with a carbon paste, an electrode with coating, a graphite and polyvinyl chloride electrode. SPCPE were successfully used for the potentiometric titration of CTAB in model solutions, the potential jump was 1050 mV. The successful application of the method for the analysis of pharmaceutical preparations with a percentage of reproducibility was proven to be 99.20 % and RSD = 0.45%. The electrodes had an almost the Nernst cationic slope – 58.70 ± 1.3 and 56.32 ± 2.4 mV, the method allowed to reach the lower detection limit – 6.8×10^{-7} and 5.80×10^{-7} M, reproducibility – 0.14 and 3.25%, and the reaction time – 3 s; it demonstrated a sufficient shelf life – 6 and 2 month for SPCPE [73, 74].

In order to characterize micellar aggregates of imidazolium-based ionic liquids, a new potentiometric method was developed: a PVC-sensor based on neutral ion-pair complexes of dodecylmethylimidazolium bromide – sodium dodecyl sulfate (C_{12}MeIm(+)DS(-)). The electrode had a linear response in the concentration range of 7.9×10^{-5}–9.8×10^{-3} M with the Nernst inclination of 92.94 mM decade^{-1}, the response time – 5 s and the critical concentration of micelle formation (CCM) – 10.09 mM for C_{12}MeImBr. The performance of the electrode in studying the critical micellar concentration of C_{12}MeImBr in the presence of promazine and promethazine hydrochlorides and three triblock copolymers (P123, L64 and F68) was proven to be satisfactory compared to conductivity measurements. Thus, the electrode makes it possible to implement a simple, clear and relatively fast method for the characterization of micellar aggregates C_{12}MeImBr, supplementing existing conventional techniques [75].

The ISE-electrode was made for determining 1-ethoxycarbonyl) pentadecyltrimethylammonium bromide (Seponex). It was based on using septonex-tetraphenylborate as an electroactive agent and *o*-nitrophenyloctyl ether (o-NPOE) as a plasticizer. The electrode exhibited the response similar to the Nernst one – 59.33 ± 0.85 mV, in the interval of pH from 2 to 9 with a lower detection limit of 9×10^{-7} M, the response time of approximately 5 s, and the storage period of 6 months. The method was used to determine Septonex in pharmaceutical preparations. The percentage of reproducibility of the results was 99.88% with RSD = 1.24%. The electrode was successfully applied in determining Septonex in laboratory-

Table 2. Examples of ion-selective electrodes for potentiometric titration of cationic surfactants

	Composition of an electrode	Titrant	Nernst response, mV decade^{-1}	Cationic surfactants	pH	Ref	LOD or range of linearity
1.	Cationic surfactants	sodium tetraphenylborate	–	DDMBAC, CTAB, OTMAC	3	[68]	10^{-6} mol L^{-1}
2.	1,3-didodecyl-2-methylimidazolium + sodium tetraphenylborate	sodium tetraphenylborate	59.8, 58.6, and 56.8	CPC, CTAB, diisobutyl-phenoxyethoxyethyl) dimethylbenzylammonium chloride (hiamine)	2–11	[70]	–
3.	1,3-didecyl-2-methylimidazolium + tetraphenylborate ion	sodium tetraphenylborate	2–3 mV day^{-1}	CS and EONS	3–10	[71]	1×10^{-5} – 4×10^{-7} M
4.	SPE modified ion-selective electrodes	–	60.66 ± 1.10	CPC	2–8	[72]	LOD = 8×10^{-7} M, 1×10^{-2} to 1×10^{-6} M
5.	SPCPE	–	58.70 ± 1.3	CTAB		[73]	LOD 6.8×10^{-7} M
6.	Dodecyltrimethylammonium + tetraphenylborate + dioctylsebacate DTA+TPB + DOS	–	55.95 ± 0.58	DTAB	3	[74]	LOD = 6.8×10^{-6} mol L^{-1}
7.	C_{12}MeIm $^{+}$-ISE	–	92.94	C_{12}MeImBr	–	[75]	7.9×10^{-5} – 9.8×10^{-3} M
8.	Septonex–tetraphenylborate + -nitrophenyloctylether (*o*-NPOE)	–	59.33 ± 0.85	(Ethoxycarbonyl)penta-decyltrimethylammonium bromide (septonex)	2–9	[76]	LOD = 9×10^{-7} M

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prepared samples by the direct potentiometric method using the calibration curve or the standard application method. The potentiometric titration of Septonex with sodium tetraphenylborate and phosphotungstic acid as a titrant was monitored with the modified screen-printed electrode as an endpoint indicator electrode. Selectivity coefficients for Septonex relative to a number of potential interfering substances were determined. The sensor was highly selective for Septonex over a large number of compounds. Selectivity coefficient data for some common ions showed negligible interference; however, cetyltrimethylammonium bromide and iodide ions interfered significantly. The analytical usefulness of the electrode proposed was evaluated by its application when determining Septonex in laboratory-prepared pharmaceutical samples with satisfactory results. The results obtained with the fabricated sensor are comparable with those obtained by the British Pharmacopeia [76].

As can be seen from the material described, the issue of developing ion-selective electrodes for determining QACs occupies a favorable position in modern analytical chemistry (Table 2).

■ Conclusions

Thus, based on the generalized and systematized information of literary sources, one can conclude that titrimetry is the generally accepted standard method for determining the content of CS. At the same time, it is necessary to note that researchers pay their attention to the prospect of developing new instrumental methods for determining the endpoint of titration since these methods are more accurate, simpler, and faster than visual ones. The combination of titrimetry with instrumental methods expands the possibilities of applying the methods, providing expressivity and increased accuracy in the computerized determination of the endpoint of titration.

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The Synthesis of N-Substituted 4-Fluoro-1,8-naphthalimides

Abstract

Aim. To synthesize 4-fluoro-1,8-naphthalic acid imide and its derivatives substituted in the imide ring.

Results and discussion. 4-Fluoro-1,8-naphthalimide was obtained using acenaphthene as the starting material. N-alkyl-4-fluoro-1,8-naphthalimides were synthesized *via* the phase transfer catalytic alkylation of 4-fluoro-1,8-naphthalimide with haloalkanes. Imidation of 4-fluoro-1,8-naphthalic anhydride with aminoacids resulted in the formation of N-carboxyalkyl-1,8-naphthalimides. These substances can be considered as potential fluorescent labels capable of binding to amino groups of various biological molecules as they contain carboxylic functionality in their structure.

Experimental part. The structure of the compounds synthesized was confirmed by FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy, and mass-spectrometry.

Conclusions. It has been shown that 4-fluoro-1,8-naphthalinedicarboxylic acid imide can be obtained following the synthetic route "acenaphthene – 5-fluoroacenaphthene – 4-fluoro-1,8-naphthalic anhydride – 4-fluoro-1,8-naphthalimide". 4-Fluoro-1,8-naphthalimide can be alkylated by butyl iodide and octyl bromide using tetraalkylammonium salts as a phase transfer catalyst resulted in N-butyl-4-fluoro-1,8-naphthalimide and N-octyl-4-fluoro-1,8-naphthalimide. As a result, N-carboxyalkyl-4-fluoro-1,8-naphthalimides have been obtained for the first time by aminolysis of 4-fluoro-1,8-naphthalic anhydride with glycine, β-alanine and 6-aminocaproic acid.

Keywords: acenaphthene; 1,8-naphthalimide; 1,8-naphthalic anhydride; alkylation; imidation

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Синтез N-заміщених 4-флуоро-1,8-нафталімідів

Анотація

Мета. Синтезувати імід 4-флуоро-1,8-нафталевої кислоти та його заміщені в імідному кільці похідні.

Результати та їх обговорення. Синтезовано 4-флуоронафталімід з використанням аценафтену як вихідної речовини. N-алкіл-4-флуоронафталіміди одержано міжфазно-каталітичним алкілуванням 4-флуоронафталіміду. Імідуванням 4-флуоронафталевого ангідриду амінокислотами отримано N-карбоксиалкіл-4-флуоронафталіміди, які є потенційними флуоресцентними мітками завдяки наявності в їхніх молекулах карбоксильної групи.

Експериментальна частина. Будову синтезованих сполук доведено методами ІЧ-, ^{1}Н ЯМР-, ^{13}С ЯМР-спектроскопії та мас-спектрометрії.

Висновки. З'ясовано, що 4-фторонафталімід може бути отриманий у результаті реалізації синтетичної схеми «аценафтен – 5-флуороаценафтен – 4-флуоронафталевий ангідрид – 4-флуоронафталімід». Доведено, що імід 4-флуоро-1,8-нафталендикарбонової кислоти може бути алкілований в умовах міжфазного каталізу солями тетраалкіламонію, у результаті чого вперше було отримано N-бутил-4-флуоронафталімід і N-октил-4-флуоронафталімід. Уперше синтезовано амінолізом 4-флуоронафталевого ангідриду гліцином, β-аланіном і 6-амінокапроновою кислотою та схарактеризовано N-карбоксиалкілзаміщені іміди 4-флуоро-1,8-нафталендикарбонової кислоти.

Ключові слова: аценафтен; нафталімід; нафталевий ангідрид; алкілування; імідування

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■ Introduction

Derivatives of 1,8-naphthalene dicarboxylic acid imide have attracted much attention in various fields of industry as dyes [1–3], fluorescent brightening agents [4], organic light emitting diode materials [5], fluorescent probes and labels [6, 7]. Moreover, a number of 1,8-naphthalimide derivatives have a high cytotoxic activity against cells of various tumors and viruses and are applied as anti-cancer and antiviral substances [8–10].

Among 4-halo-1,8-naphthalimides, which can be used to obtain fluorophores and are potential biologically active compounds, 4-fluoro-1,8-naphthalimide and its derivatives substituted in the imide ring are not described in the literature. Therefore, the synthesis of these compounds has become the aim of the present work.

■ Results and Discussion

The most common synthetic routes reported for the preparation of 4-chloro- and 4-bromosubstituted 1,8-naphthalimides involve electrophilic halogenation of acenaphthene followed by oxidation of the corresponding 5-haloacenaphthene and the subsequent imidation of 4-halosubstituted 1,8-naphthalic anhydride. The synthesis of 5-fluoroacenaphthene *via* the Balz-Schiemann reaction is described by authors of the manuscript [11] and is applied by us with some changes.

Nitration of acenaphthene (**1**) was carried out by the concentrated nitric acid in acetic acid at 10–15 °C giving 5-nitroacenaphthene (**2**) with the yield of 87%. Further, compound **2** was reduced with sodium dithionite using ethanol and water as the mixed solvent afforded 5-aminoacenaphthene (**3**) with the yield of 78%. The use of the above reducing system allowed us to increase the yield of amine **3** compared to the procedure given in the manuscript [11] where $SnCl_2$ with HCl were used as a reducing agent. Diazotization of amine **3** and the subsequent thermal decomposition of the derived tetrafluoroborate **4** gave desired 5-fluoroacenaphthene (**5**) with the yield of 37% (Scheme 1).

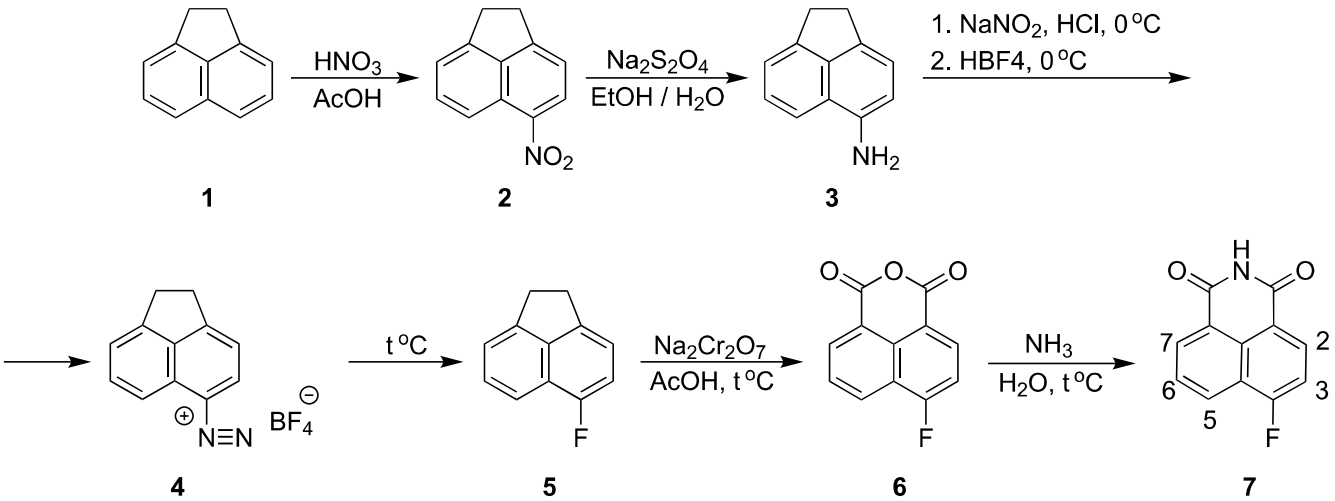
4-Fluoro-1,8-naphthalic anhydride (**6**) was obtained by oxidation of compound **5** with sodium dichromate in acetic acid with the yield of 47%. Initially formed during oxidation 4-fluoro-1,8-naphthalic acid was completely converted to the anhydride by heating at 100–110 °C. Finally, the reaction of anhydride **6** with aqueous ammonia gave the target 4-fluoro-1,8-naphthalimide (**7**) with the yield of 91%.

4-Fluoro-1,8-naphthalimide is a crystalline substance of a light-yellow color with m. p. > 350 °C, which has blue fluorescence.

N-Butyl-4-fluoro-1,8-naphthalimide (**8a**) and N-octyl-4-fluoro-1,8-naphthalimide (**8b**) were synthesized by alkylation of sodium salt of 4-fluoronaphthalimide **7** generated *in situ* with butyl iodide and octyl bromide using tetrabutylammonium bromide (TBAB) as a phase transfer catalyst. The synthesis was carried out in a two-phase system "benzene – 10% aqueous NaOH" resulted in 66% and 62% yields of compounds **8a** and **8b**, respectively (Scheme 2).

The N-alkyl-4-fluoro-1,8-naphthalimides **8a,b** obtained are light yellow crystalline substances with m. p. 102–103 °C for compound **8a** and 82–84 °C for compound **8b**, which have strong blue fluorescence. These compounds have much higher solubility in organic solvents compared to N-unsubstituted imide **7**.

Additionally, the novel N-carboxyalkyl-4-fluoro-1,8-naphthalimides **9a–c** were obtained by



Scheme 1. The synthesis of 4-fluoro-1,8-naphthalimide

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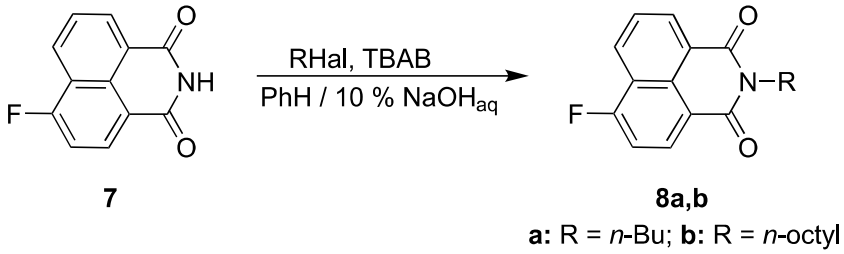


7 **8a,b**

a: R = *n*-Bu; **b:** R = *n*-octyl

Scheme 2. The synthesis of N-alkyl-4-fluoro-1,8-naphthalimides

acylation of glycine, ß-alanine and 6-aminocaproic acid with 4-fluoro-1,8-naphthalic anhydride (**6**). The reactions were carried out using acetic acid as a solvent in order to avoid the possible nucleophilic substitution of the fluorine atom with the amino group (Scheme 3).

Compounds **9a–c** obtained are light yellow crystalline substances with m. p. 247–249 °C (**9a**), 236–238 °C (**9b**) and 176–178 °C (**9c**), which have strong blue fluorescence. These substances can be considered as potential fluorescent labels capable of binding to amino groups of various biological molecules as they contain carboxylic functionality in their structure.

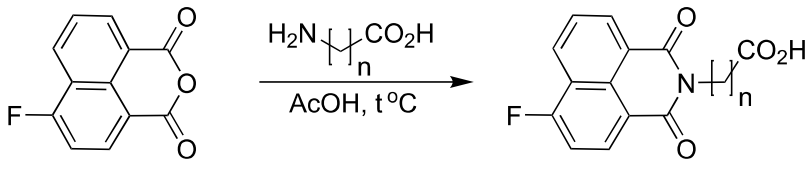
^{1}H and ^{13}C NMR, FT-IR, mass-spectra and data of the elemental analysis fully confirm the structure of compounds **7**, **8a,b**, **9a–c** synthesized.

The FT-IR spectra of compounds **7–9** have two intense absorption bands at 1667–1679 cm^{-1} and 1628–1635 cm^{-1}, corresponding to the characteristic stretching vibrations of the imide carbonyl groups. The absorption band at 1097–1105 cm^{-1} corresponds to the stretching vibrations of the C-F bond. The presence of N-H bond in 4-fluoro-1,8-naphthalimide **7** is confirmed by the absorption at 3170 cm^{-1}. The absorption bands of stretching vibrations of carbonyl and hydroxyl moieties in COOH groups of compounds **9a–c** are observed at 1702–1703 cm^{-1} and 3058–3060 cm^{-1}, respectively.

In the ^{1}H NMR spectra of compounds **7–9** the signals of aromatic protons are observed in the range of 7.6–8.7 ppm (the numeration of aromatic protons is presented in Scheme 1 for imide **7**). Proton H^{3}, interacting with the fluorine nuclei in position 4 and with proton H^{2}, gives the signal in the form of a doublet of doublets with the coupling constant J_{HF} = 10 Hz, while proton H^{2} has the signal in the form of a doublet. The signal of proton H^{6} is observed as a doublet of doublets, interacting with the magnetically inequivalent H^{5} and H^{7}. Protons H^{5} and H^{7} appear in the form of doublets. Signal of the imide proton in compound **7** appears as a singlet at 11.62 ppm. The coupling constants of aromatic protons have the values of 7.6–8.4 Hz, which are common for 1,8-naphthalimide derivatives. The signals of protons of alkyl groups attached to the nitrogen of the imide ring in compounds **8a,b** and **9a–c** are observed in the range of 0.8–4 ppm. The presence of carboxylic proton in the structures of acids **9a–c** is confirmed by the broad singlet in the range of 12.2–12.4 ppm.

Conclusions

It has been shown that 4-fluoro-1,8-naphthalinedicarboxylic acid imide can be obtained following the synthetic route "acenaphthene – 5-fluoroacenaphthene – 4-fluoro-1,8-naphthalic anhydride – 4-fluoro-1,8-naphthalimide". 4-Fluoro-1,8-naphthalimide can be alkylated by butyl iodide and octyl bromide using tetraalkylammonium salts as a phase transfer catalyst resulted in N-butyl-4-fluoro-1,8-naphthalimide and N-octyl-4-fluoro-1,8-naphthalimide. As a result, N-carboxyalkyl-4-fluoro-1,8-naphthalimides have been obtained for the first time by aminolysis of 4-fluoro-1,8-naphthalic anhydride with glycine, ß-alanine and 6-aminocaproic acid.



6 **9a-c**

a: n = 1; **b:** n = 2; **c:** n = 5

Scheme 3. The synthesis of N-carboxyalkyl-4-fluoro-1,8-naphthalimides

■ Experimental part

All reagents were purchased from commercial suppliers without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded at ambient temperature on a BRUKER WM 400 instrument using DMSO-d_6 as a solvent and TMS as an internal standard at 400 MHz and 100 MHz, respectively. IR spectra were obtained on a Perkin Elmer Frontier FT-IR spectrometer using KBr pellets. FAB mass spectra were obtained on a VG 70-70EQ mass spectrometer equipped with a Xe ion gun (8kV). The samples were mixed with a *m*-nitrobenzyl alcohol matrix. Elemental analyses were conducted using an elemental analyzer Vario MICRO cube (determination of C, H, N), and a 9000F Fluoride Analyzer (determination of F), their results were found to be in good agreement (not more than ±0.4%) with the calculated values. The control of the reaction progress and purity of the compounds synthesized were monitored by thin layer chromatography on Silicagel 60 F254 plates (Merck), followed by visualization in UV light, using chloroform as an eluent. Melting points were determined with an electrothermal capillary melting point apparatus.

The synthesis of 5-nitroacenaphthene (2)

Acenapthene (25 g, 0.16 mol) and acetic acid (100 mL) were placed in a 500 mL two-necked round-bottomed flask equipped with a magnetic stirrer, a reflux condenser, a thermometer, and a dropping funnel. The mixture was cooled to 10 °C, and nitric acid (20 mL, d = 1.36 g mL^{-1}) was added dropwise over 20 min while maintaining the temperature at 10–15 °C. The reaction mixture was vigorously stirred for 1 h. The resulting precipitate was filtered off, the filter cake was washed with distilled water and dried at 50 °C to give 28.7 g (87%) of the target product **2** as a yellow solid with m. p. 101.5–102.5 °C (Ref. [1] 101–102 °C).

The synthesis of 5-aminoacenaphthene (3)

5-Nitroacenaphthene (12 g, 0.06 mol) was dissolved in ethanol (120 mL), and hot water (60 mL) was poured into the solution. A solution of sodium dithionite (36 g, 0.21 mol) in water (60 mL) was added portionwise, and the reaction mixture was refluxed for 2 h. The solvents were evaporated, and the concentrated hydrochloric acid (45 mL) was added to the residue. The reaction mixture was heated on a water bath for 15 min, water (500 mL) was added; then the mixture was refluxed for 30 min and filtered hot. The filtrate was neutralized with aqueous ammonia to precipitate a crude product. The procedure of precipitation was repeated to give 8.9 g (78%) of the target product **3** as an off-white solid with m. p. 107–108 °C (Ref. [11] 107.5–108 °C).

The synthesis of 5-fluoroacenaphthene (5)

A mixture of 5-aminoacenaphthene (11.3 g, 0.07 mol), hydrochloric acid (21 mL) and water (100 mL) was refluxed for 30 min, then it was cooled to 0 °C and a solution of sodium nitrite (4.75 g, 0.07 mol) in water (12 mL) was added dropwise with vigorous stirring. The color of the solution changed to dark green. The reaction mixture was stirred for 30 min and filtered. The filtrate was cooled to -5 °C, and tetrafluoroboric acid (12 mL) was added, then the mixture was stirred for 30 min at -5 °C, the green precipitate was filtered and washed with cold methanol. The resulting acenaphthene-5-diazonium tetrafluroborate (**4**) was air-dried, followed by drying in a desiccator over the concentrated sulfuric acid. After that compound **4** was thermally decomposed, and the residue was crystallized from acetic acid to give 4.3 g (37%) of 5-fluoroacenaphthene (**5**) as a white solid with m. p. 94–95 °C (Ref. [12] 93–94 °C).

The synthesis of 4-fluoro-1,8-naphthalic anhydride (6)

Anhydrous sodium dichromate (16 g, 0.06 mol) was added portionwise by stirring to the solution of 5-fluoroacenaphthene (2.6 g, 0.015 mol) heated to 60 °C in acetic acid (50 mL), and the reaction mixture was refluxed by stirring for 6 h. After completion of the reaction the mixture was poured into cold water (100 mL), the precipitate was filtered off, washed with water and air-dried. The crude product was dissolved in 10% aqueous sodium hydroxide (100 mL). The solution was filtered hot, and the pH of the filtrate was adjusted to 2 with the concentrated hydrochloric acid. The resulting precipitate was filtered off, washed with water, and dried at 110 °C to give 1.52 g (47%) of the target product **6** as a light-brown solid with m. p. 220.5–221 °C (Ref. [12] 220–221 °C).

Preparation of 4-fluoro-1,8-naphthalimide (7)

A mixture of 4-fluoro-1,8-naphthalic anhydride (1.1 g, 0.005 mol) and 16% aqueous ammonia was heated on a water bath for 3 h. The reaction mixture was diluted with 10 mL of water. The resulting precipitate was filtered, washed with water, and dried at 110 °C. The crude product was purified by crystallization from acetic acid to obtain 4-fluoro-1,8-naphthalimide (**7**) as a light-yellow solid.

Yield – 1 g (93%). M. p. >350 °C (AcOH). Anal. Calcd for $C_{12}H_6FNO_2$, %: C 66.98; H 2.81; F 8.83;

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N 6.51. Found, %: C 66.95; H 2.82; F 8.80; N 6.49. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 7.64 (1H, dd, J = 8.5, 10.0 Hz, H^3); 7.91 (1H, dd, J = 7.8, 7.6 Hz, H^6); 8.39 (1H, d, J = 7.8 Hz, H^5); 8.43 (1H, d, J = 8.5 Hz, H^2); 8.47 (1H, d, J = 7.6 Hz, H^7); 10.97 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 112.1; 120.9; 125.4; 125.9; 129.8; 130.2; 130.7; 137.6; 138.9; 158.4; 159.1; 168.8 (d, $^1J_{CF}$ = 248 Hz, C^4). FT-IR (KBr), ν, cm^{-1}: 3170 (N-H); 3053; 1698 (C=O); 1672 (C=O); 1583; 1505; 1435; 1356; 1241; 1158; 1105 (C-F); 760. MS (FAB), m/z: 216 [M+H]$^+$.

Preparation of N-butyl-4-fluoro-1,8-naphthalimide (8a)

A mixture of finely ground 4-fluoro-1,8-naphthalimide (0.11 g, 0.5 mmol) and 10% aqueous sodium hydroxide (20 mL) was added to a solution of 1-iodobutane (0.19 g, 1 mmol) and 0.02 g of TBAB in benzene. The reaction mixture was vigorously stirred at 80 °C for 9 h, then it was cooled to the room temperature; the organic layer was separated, dried over calcium chloride and filtered through aluminium oxide to remove the phase transfer catalyst. The solvent was evaporated to give the target product **8a** as a light-yellow solid.

Yield – 0.08 g (66%). M. p. 102–103 °C. Anal. Calcd for $C_{16}H_{14}FNO_2$, %: C 70.84; H 5.20; F 7.00; N 5.16. Found, %: C 70.81; H 5.19; F 7.02; N 5.15. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 0.88 (3H, t, J = 6.4 Hz, CH_3); 1.22–1.24 (2H, m, CH_2); 1.62–1.64 (2H, m, CH_2); 4.04 (2H, t, J = 7.2 Hz, CH_2N); 7.60 (1H, dd, J = 8.8, 9.8 Hz, H^3); 8.40 (1H, d, J = 8.0 Hz, H^5); 8.50 (1H, d, J = 7.0 Hz, H^7); 7.88 (1H, dd, J = 8.0, 7.4 Hz, H^6); 8.42 (1H, d, J = 8.8 Hz, H^2). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 13.8; 19.8; 29.4; 40.1; 111.6; 120.9; 121.8; 125.7; 125.9; 130.2; 130.7; 137.5; 138.9; 158.5; 159.3; 168.7 (d, $^1J_{CF}$ = 250 Hz, C^4). FT-IR (KBr), ν, cm^{-1}: 2926; 2852; 1673 (C=O); 1631 (C=O); 1598; 1450; 1341; 1234; 1104 (C-F); 752. MS (FAB), m/z: 272 [M+H]$^+$.

Preparation of N-octyl-4-fluoro-1,8-naphthalimide (8b)

Compound **8b** was prepared similarly to compound **8a** using 4-fluoro-1,8-naphthalimide (0.11 g, 0.5 mmol), 10% aqueous sodium hydroxide (20 mL), 1-bromooctane (0.2 g, 1 mmol), benzene (20 mL) and TBAB as a phase transfer catalyst. The title compound was obtained as a light-yellow solid.

Yield – 0.1 g (62%). M. p. 82–84 °C. Anal. Calcd for $C_{20}H_{22}FNO_2$, %: C 73.37; H 6.77; F 5.80; N 4.28. Found, %: C 73.35; H 6.78; F 5.78; N 4.29. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 0.83 (3H, t, J = 6.4 Hz, CH_3); 1.22–1.29 (10H, m, $(CH_2)_5$); 1.61–1.63 (2H, m, CH_2); 3.98 (2H, t, J = 6.8 Hz, CH_2N); 7.65 (1H, dd, J = 8.9, 9.4 Hz, H^3); 7.91 (1H, dd, J = 7.8, 7.1 Hz, H^6); 8.45 (1H, d, J = 8.9 Hz, H^2); 8.49 (1H, d, J = 7.8 Hz, H^5); 8.51 (1H, d, J = 7.1 Hz, H^7). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 14.2; 22.7; 26.7; 29.1; 29.5; 30.3; 31.9; 40.4; 111.7; 120.8; 121.9; 125.7; 125.9; 130.1; 130.7; 137.6; 138.9; 158.7; 159.5; 168.8 (d, $^1J_{CF}$ = 250 Hz, C^4). FT-IR (KBr), ν, cm^{-1}: 2924; 2851; 1679 (C=O); 1635 (C=O); 1586; 1438; 1373; 1354; 1232; 1097 (C-F); 781. MS (FAB), m/z: 328 [M+H]$^+$.

The procedure for the synthesis of N-carboxymethyl-4-fluoro-1,8-naphthalimide (9a)

A mixture of 4-fluoro-1,8-naphthalic anhydride (0.1 g, 0.46 mmol) and glycine (0.17 g, 2.3 mmol) in acetic acid (20 mL) was refluxed for 36 h, cooled to room temperature, and poured in cold water (100 mL). The resulting precipitate was filtered, air-dried, and crystallized from ethanol to give the target product **9a** as a white solid.

Yield – 0.08 g (64%). M. p. 247–249 °C. Anal. Calcd for $C_{14}H_8FNO_4$, %: C 61.54; H 2.95; F 6.95; N 5.13. Found, %: C 61.58; H 2.94; F 6.97; N 5.14. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 4.20 (2H, s, CH_2); 8.00 (1H, dd, J = 7.8, 8.4 Hz, H^6); 8.04 (1H, dd, J = 8.0, 10 Hz, H^3); 8.45 (1H, d, J = 7.8 Hz, H^5); 8.61 (1H, d, J = 8.4 Hz, H^7); 8.63 (1H, d, J = 8.0 Hz, H^2); 12.22 (1H, s, COOH). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 42.2; 112.1; 120.9; 125.4; 125.7; 129.7; 130.1; 130.5; 137.8; 138.8; 158.6; 159.2; 168.7 (d, $^1J_{CF}$ = 252 Hz, C^4); 169.5. FT-IR (KBr), ν, cm^{-1}: 3061 (O-H); 1702 (C=O); 1667 (C=O); 1630 (C=O); 1573; 1590; 1506; 1335; 1299; 1226; 1096 (C-F); 852; 775. MS (FAB), m/z: 274 [M+H]$^+$.

The procedure for the synthesis of N-(2-carboxyethyl)-4-fluoro-1,8-naphthalimide (9b)

Compound **9b** was prepared similarly to compound **9a** using 4-fluoro-1,8-naphthalic anhydride (0.1 g, 0.46 mmol) and β-alanine (0.2 g, 2.3 mmol) in acetic acid (20 mL), the mixture was refluxed for 32 h. The title compound was obtained as a white solid.

Yield – 0.11 g (83%). M. p. 236–238 °C. Anal. Calcd for $C_{15}H_{10}FNO_4$, %: C 62.72; H 3.51; F 6.61; N 4.88. Found, %: C 62.70; H 3.52; F 6.59; N 4.89. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.61 (2H, t, J = 6.8, CH_2); 4.26 (2H, t, J = 6.8, CH_2); 8.00 (1H, dd, J = 7.8, 9.8 Hz, H^3); 8.04 (1H, dd, J = 7.8, 8.2 Hz, H^6); 8.43 (1H, d, J = 7.8 Hz, H^5); 8.59 (1H, d, J = 7.8 Hz, H^2); 8.61 (1H, d, J = 8.2, H^7); 12.34 (1H, s). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 31.2; 36.5; 112.1; 120.8; 125.4; 125.7; 129.8; 130.1; 130.4; 137.9; 138.9; 158.7; 159.2; 168.8 (d,

$^1J_{CF}$ = 254 Hz, C^4); 171.5. FT-IR (KBr), ν, cm^{-1}: 3058 (O-H); 2922; 1703 (C=O); 1668 (C=O); 1630 (C=O); 1590; 1440; 1348; 1233; 1221; 1102 (C-F); 853; 781. MS (FAB), m/z: 288 $[M+H]^+$.

The procedure for the synthesis of N-(5-carboxypentyl)-4-fluoro-1,8-naphthalimide (9c)

Compound **9c** was prepared similarly to compound **9a** using 4-fluoro-1,8-naphthalic anhydride (0.1 g, 0.46 mmol) and 6-aminocaproic acid (0.3 g, 2.3 mmol) in acetic acid (20 mL), the mixture was refluxed for 30 h. The title compound was obtained as a white solid.

Yield – 0.08 g, 57%. M. p. 176–178 °C. Anal. Calcd for $C_{18}H_{16}FNO_4$, %: C 65.65; H 4.90; F 5.77; N 4.25. Found, %: C 65.63; H 4.91; F 5.79; N 4.24. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.52–2.54 (6H, m, $(CH_2)_3$); 2.60 (2H, t, CH_2, J = 6.4 Hz); 4.26 (2H, t, CH_2 J = 6.8 Hz); 8.04 (1H, dd, J = 7.6, 8.4 Hz, H^6); 8.14 (1H, dd, J = 7.7, 9.8 Hz, H^3); 8.42 (1H, d, J = 7.6 Hz, H^5); 8.58 (1H, d, J = 7.7 Hz, H^2); 8.61 (1H, d, J = 8.4 Hz, H^7); 12.4 (1H, s). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 24.5; 26.4; 30.5; 34.4; 40.4; 112.5; 120.9; 125.5; 125.8; 129.9; 130.2; 130.5; 137.8; 139.1; 158.9; 159.1; 168.7 (d, $^1J_{CF}$ = 254 Hz, C^4); 177.5. FT-IR (KBr), ν, cm^{-1}: 3060 (O-H); 2940; 1702 (C=O); 1667 (C=O); 1628 (C=O); 1573; 1506; 1437; 1335; 1226; 1103 (C-F); 861; 777. MS (FAB), m/z: 330 $[M+H]^+$.

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The Study of the Elemental and Amino Acid Composition of *Cetraria Islandica* (L.) Ach. Thalli Batches Harvested in Ukraine

Abstract

Aim. To study the elemental and amino acid composition of *Cetraria islandica* (L.) Ach. thalli batches harvested in Ukraine.
Materials and methods. Seven batches of *C. islandica* thalli harvested in different regions of Ukraine in late summer/early fall 2019 were used for the study. The elemental composition of the raw material was studied by atomic absorption spectrometry with photographic registration. The component composition of free and bound amino acids in the raw material was determined by the HPLC method.
Results and discussion. The presence of at least 19 macro-, trace and ultra-trace elements was determined in 7 batches of the raw material. The predominance of the following elements was found: potassium 190 mg/100 g (batch 6) – 325 mg/100 g (batch 7); silicon 30 mg/100 g (batch 1) – 115 mg/100 g (batch 4); calcium 37 mg/100 g (batch 6) – 86 mg/100 g (batch 4). It was determined that the quantitative content of molybdenum and cobalt did not exceed 0.03 mg/100 g, the content of cadmium, astatine and mercury did not exceed 0.01 mg/100 g. The quantitative content of lead met the requirements of the monograph of the State Pharmacopoeia of Ukraine (SPhU) 2.0. The total ash content in the batches of the raw material ranged from 0.61 ± 0.02 % to 1.43 ± 0.05 %, meeting the requirements of the monograph of the SPhU 2.0. The presence of 7 amino acids was determined; 5 of them were nonessential (aspartic and glutamic acids, serine, arginine, and alanine) and 2 were essential (threonine and valine). The quantitative content of the total amount of free amino acids was 0.794 μg mg^{-1}, the total amount of bound ones was 3.276 μg mg^{-1}.
Conclusions. For the first time, the elemental composition of 7 batches of *C. islandica* thalli harvested in Ukraine was determined. In each batch, potassium was the predominant element (from 190 mg/100 g to 325 mg/100 g depending on the batch). The component composition of free and bound nonessential and essential amino acids was determined (the quantitative content of the total amount of free amino acids was 0.794 μg mg^{-1}, the total amount of bound ones was 3.276 μg mg^{-1}); alanine (0.289 μg mg^{-1}) was the dominant component among free amino acids, while arginine (0.993 μg mg^{-1}) prevailed among bound ones. The results obtained will be used for further studies of the raw material of *C. islandica* harvested in Ukraine.
Keywords: *Cetraria islandica*; thalli; mineral composition; free and bound amino acids

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Вивчення елементного та амінокислотного складу серій слані *Cetraria islandica* (L.) Ach., заготовлених в Україні

Анотація

Мета. Дослідити елементний та амінокислотний склад серій слані *Cetraria islandica* (L.) Ach., заготовлених в Україні.
Матеріали та методи. Для дослідження було використано 7 серій слані *C. islandica*, заготовлених у різних регіонах України наприкінці літа – восени 2019 року. Елементний склад сировини досліджували методом атомно-абсорбційної спектрометрії з фотографічною реєстрацією. Компонентний склад вільних і зв'язаних амінокислот у сировині визначали методом ВЕРХ.
Результати та їх обговорення. Визначено наявність у 7 серіях сировини щонайменше 19 макро-, мікро- та ультрамікроелементів. Серед елементів виявлено переважання калію: 190 мг/100 г (серія 6) – 325 мг/100 г (серія 7); силіцію: 30 мг/100 г (серія 1) – 115 мг/100 г (серія 4); кальцію: 37 мг/100 г (серія 6) – 86 мг/100 г (серія 4). Визначено, що кількісний вміст молібдену та кобальту не перевищував 0,03 мг/100 г, вміст кадмію, астату та ртуті не перевищував

0,01 мг/100 г. Кількісний вміст плюмбуму відповідав вимогам монографії Державної фармакопеї України (ДФУ) 2.0. Вміст загальної золи в серіях сировини становив від 0,61 ± 0,02 % до 1,43 ± 0,05 %, що відповідає вимогам монографії ДФУ 2.0. Визначено наявність 7 амінокислот, з яких 5 замінні (аспарагінова і глутамінова кислоти, серин, аргінін та аланін) та 2 незамінні (треонін і валін). Кількісний вміст суми вільних амінокислот становив 0,794 мкг мг$^{-1}$, суми зв'язаних – 3,276 мкг мг$^{-1}$.

Висновки. Уперше визначено елементний склад 7 серій слані *C. islandica*, заготовлених в Україні. У кожній серії домінантним елементом був калій (від 190 мг/100 г до 325 мг/100 г залежно від серії). Визначено компонентний склад вільних і зв'язаних замінних і незамінних амінокислот (кількісний вміст суми вільних амінокислот – 0,794 мкг мг$^{-1}$, суми зв'язаних – 3,276 мкг мг$^{-1}$), домінантним компонентом серед вільних амінокислот був аланін (0,289 мкг мг$^{-1}$), серед зв'язаних – аргінін (0,993 мкг мг$^{-1}$). Отримані результати будуть використані для подальших досліджень сировини *C. islandica,* заготовленої в Україні.

***Ключові слова*:** *Cetraria islandica*; слань; мінеральний склад; вільні та зв'язані амінокислоти

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■ Introduction

Cetraria islandica (L.) Ach. is a foliose lichen of the *Parmeliaceae* family, which has been used in the European and Asian folk medicine for centuries, in particular for the respiratory and digestive diseases [1]. Due to the content of many groups of biologically active substances (BAS) in the raw material and a wide spectrum of their pharmacological activity, including antibacterial, expectorant, antioxidant and anti-inflammatory, the study of the raw material of *C. islandica* does not lose its relevance nowadays [2, 3].

As of May 2022, the raw material of *C. islandica* is a component of 5 medicinal products presented at the pharmaceutical market of Ukraine, such as Pectolvan phyto Iceland moss (JSC Farmak, Ukraine); pastilles Isla-Moos and Isla-Mint (Engelhard Arzneimittel GmbH& Co. KG, Germany), Herbion Iceland moss syrup (KRKA, Slovenia) and Complex expectorant extract (Phytopharm Klenka SA, Poland) [4]. It is also contained in dietary supplements in the form of tablets, pastilles, syrups and herbal teas, which are used for the inflammatory processes of the respiratory system treatment and help to regulate the respiratory function [5].

The pharmacological action of lichens is due to the ability of myco- and photobiont to form primary and secondary metabolites belonging to different groups of BAS [6]. A comprehensive study of their component composition is important for the systematic study of the raw material and for the search for optimal ways of their complex processing as well [3]. Lichens have a high ability to absorb mineral substances from the lichen substrate, air, and precipitation [7]. Mineral substances accumulated by the mycobiont can form complex compounds with such primary lichen metabolites as amino acids, proteins, polysaccharides, vitamins [8].

The study of the elemental composition of the raw material not only allows to prevent the use of the raw material which can be exposed to environmental pollution, but it is also important for determining the influence of elements on the pharmacological effect of the drugs based on this raw material [8, 9].

The monographs on *C. islandica* are included in the pharmacopoeias of many countries, including the State Pharmacopoeia of Ukraine (SPhU) 2.0 [10]. The monograph "Cetraria islandica" of the SPhU 2.0 does not contain a national part and does not regulate the quantitative content of BAS in the raw material. In the "Tests" section of the monograph of the SPhU 2.0, it is specified that the content of lead in the raw material of *C. islandica* must not exceed 0.0010% (10.0 ppm) [10].

In one of the first studies of the elemental composition of *C. islandica* thalli, Finnish scientists (*Airaksinen* et al., 1986) studied the toxicity of the raw material of lichens *C. islandica* and *Cladonia rangiferina.* It has been traditionally used in this region in the food industry, as emergency food and as livestock fodder. In the study, a high content of heavy metals, in particular lead, was determined, and the necessity of additional processing of the raw material for further safe application was shown [11].

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The results of the elemental analysis of the raw material of *C. islandica* of Italian origin (*Meli* et al., 2017) demonstrate a high content of calcium, silicon, and potassium in the raw material [12]. Similar data on the elemental composition of *C. islandica* thalli were obtained for the raw material harvested in the South-Eastern Siberia, the Pre-Urals, Karelia and other regions of the boreal forest and tundra (*Vershinina* et al., 2010), as well as on the Taymyr Peninsula (*Kaiser* et al., 2020) where the dominance of potassium and calcium was noted [13–14]. Ukrainian scientists (*Vladymyrova* et al., 2019) also found the predominance of potassium, silicon, and calcium in the raw material of *C. islandica* when studying the elemental composition of the raw material of various medicinal plants in thyroid gland diseases [15]. The analysis of the raw material of *C. islandica* and medicines and food supplements on its basis represented at the European market showed the absence of a strong correlations between the origin of the raw material and its elemental composition (*Giordani* et al., 2017) [16].

Recent data on the study of the amino acid composition of *C. islandica* thalli are mainly devoted to the research of the raw material harvested in different regions of the boreal forest and tundra. Their results demonstrate the presence of 14 to 16 amino acids in the raw material, including a high content of glutamine, phenylalanine, and valine (*Kaiser* et al., 2020); alanine, ornithine (*Tabalenkova* et al., 2017); aspartic and glutamic acids, alanine, lysine, and leucine (*Vershinina* et al., 2010) [13, 14, 17].

An earlier study conducted by Serbian scientists (*Grujic-Injac*, 1976) demonstrated the presence of 17 amino acids in the raw material of *C. islandica*, among which aspartic and glutamic acids, alanine and leucine predominated [18].

Therefore, in the literature sources available to us no data on the elemental and amino acid composition of *C. islandica* thalli harvested in Ukraine were found. For this reason, the aim of the current work was to determine the elemental and amino acid composition of *C. islandica* (L.) Ach thalli batches harvested in Ukraine.

■ Materials and methods

For the study, 7 batches of the raw material of *C. islandica* harvested in late summer/early fall 2019 were used (batch 1 – Volyn region; batch 2 – Zakarpattia region; batch 3 – Zakarpattia region; batch 4 – Zakarpattia region; batch 5 – Ivano-Frankivsk region; batch 6 – Chernivtsi region; batch 7 – Rivne region).

The total ash content in the batches of the raw material was determined according to the requirements of the monograph 2.4.16 "Total ash" of the SPhU 2.0 [19].

The elemental composition of the raw material was studied by atomic absorption spectrometry with photographic registration according to the generally accepted method [20]. The research was conducted in the SSI "Institute of Single Crystals" of the National Academy of Sciences of Ukraine (Kharkiv).

The component composition of free and bound amino acids in the raw material was determined by the HPLC method in batch 2 of the raw material. The chromatographic separation was performed on an Agilent 1200 liquid chromatograph (Agilent technologies, USA). A Zorbax AAA column, 150 mm long, with a 4.6 mm internal diameter and 3 μm sorbent grain diameter was used. The mobile phase A was 40 mM Na_2HPO_4 with pH 7.8; B – MeCN/MeOH/H_2O (45:45:10, *v*/*v*/*v*). The separation mode was gradient with a constant flow rate of 1.5 mL min^{-1}:

Chromatography time, min	Mobile phase A	Mobile phase B
0:00	100	0
2:00	100	0
18:00	43	57
19:00	0	100
23:00	0	100
26:00	100	0

The temperature of the thermostat column was 40 °C. The pre-column derivatization was performed in the automatic programmed mode with the FMOC reagent (Agilent 5061-3337) and the OPA reagent (Agilent 5061-3335). The derivatized amino acids was detected with a fluorescent detector [21].

Sample preparation

For the extraction of free amino acids, a portion of the sample of the raw material ground to a powdery state was placed in a vial, 2 mL of 1N hydrochloric acid was added, and the vial was kept in an ultrasonic bath at 50°C for 3 hours. For the extraction of general amino acids, a portion of the sample of the raw material ground to a powdery state was placed in a vial, 2 mL of 6N hydrochloric acid was added, and the vial was placed in a thermostat at 110 °C. Hydrolysis was carried out for 24 hours.

Then 0.5 mL of the centrifuged extract was evaporated on a rotary evaporator, and the residue was washed with three portions of distilled water to eliminate hydrochloric acid. The extract was re-suspended in 0.5 mL of distilled water and filtered through regenerated cellulose membrane filters with 0.2 µm pores. The fluorescent derivatives were obtained in an automatic programmed mode before the sample injection into the chromatographic column.

The identification of amino acids was performed by comparing retention times with a mixture of amino acid standards (Agilent 5061-3334). The quantitative content of amino acids was calculated from the value of the peak area of the amino acids comparing to that one of the amino acid standards. The quantitative content of bound amino acids was determined by subtracting the value of the quantitative content of free amino acids from the value of their total quantitative content [22].

■ Results and discussion

The results of the determination of the total ash content (%) in the batches of the raw material are shown in Figure 1. The total ash content was ranged from 0.61 ± 0.02% (batch 5) to 1.43 ± 0.05% (batch 4) and did not exceed 3% specified in the monograph of the SPhU 2.0 [10].

The presence of at least 19 macro-, trace, and ultra-trace elements was determined in the batches of the raw material. The results of the determination of the macroelemental composition of the batches of the raw material of *C. islandica* harvested in Ukraine are shown in Figure 2. The quantitative content of macroelements, such as potassium, calcium, silicon, magnesium, sodium, and phosphorus was determined. The potassium content was the highest among the macronutrients in all batches and ranged from 190 mg/100 g (batch 6) to 325 mg/100 g (batch 7). There was a sufficiently high content of silicon – from 30 mg/100 g (batch 1) to 115 mg/100 g (batch 4) and calcium – from 37 mg/100 g (batch 6) to 86 mg/100 g (batch 4) (Figure 2).

The results of determining the trace elemental composition of the batches of the raw material are shown in Figure 3. Among the trace elements the quantitative content of zinc, aluminum, manganese, iron, strontium, copper, and molybdenum was determined.

Compared to other trace elements, a significant part was occupied by zinc – from 5.4 mg/100 g (batch 7) to 28.6 mg/100 g (batch 4) and aluminum – from 4.6 mg/100 g (batch 6) to 12.2 mg/100 g (batch 5).

The pattern of accumulation of the elements for the each batch of the raw material studied was as follows:

- batch 1: K > Ca > Si > Mg > Na > P > Zn > Al > Mn > Fe > Sr > Cu > Mo;
- batch 2: K > Ca > Si > Mg > P > Na > Zn > Al > Fe > Mn > Sr > Cu > Mo;
- batch 3: K > Si > Ca > Mg > Na > P > Zn > Fe > Al > Mn > Sr > Cu > Mo;
- batch 4: K > Si > Ca > Mg > Na > P > Zn > Al > Mn > Fe > Sr > Cu > Mo;
- batch 5: K > Si > Ca > Na > Mg > P > Al > Zn > Fe > Mn > Cu > Sr > Mo;
- batch 6: K > Si > Ca > Mg > Na > P > Zn > Al > Fe > Mn > Sr > Cu > Mo;
- batch 7: K > Ca > Si > Mg > P > Na > Al > Zn > Fe > Mn > Sr > Cu > Mo.

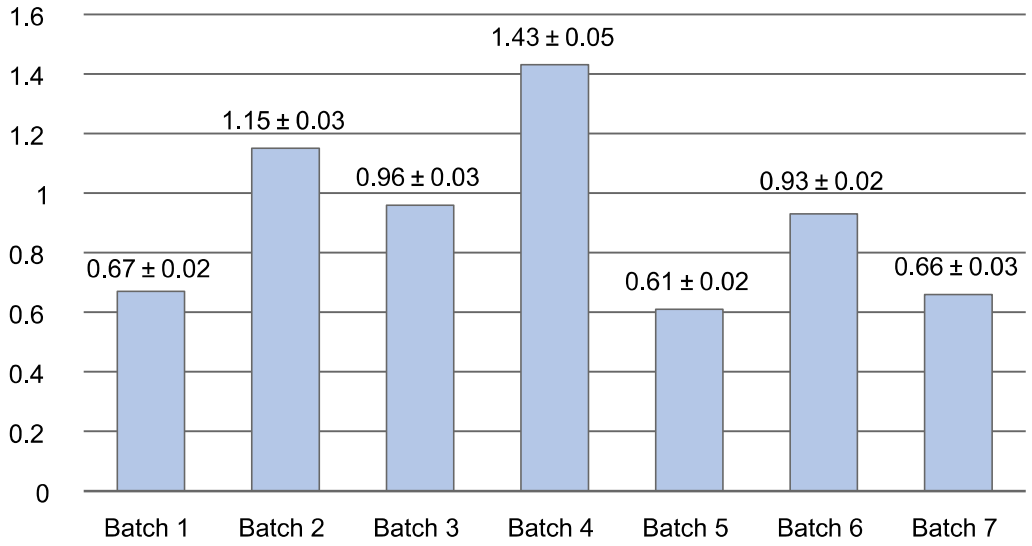

Figure 1. The total ash content (%) in the batches of the raw material of *C. islandica* harvested in Ukraine

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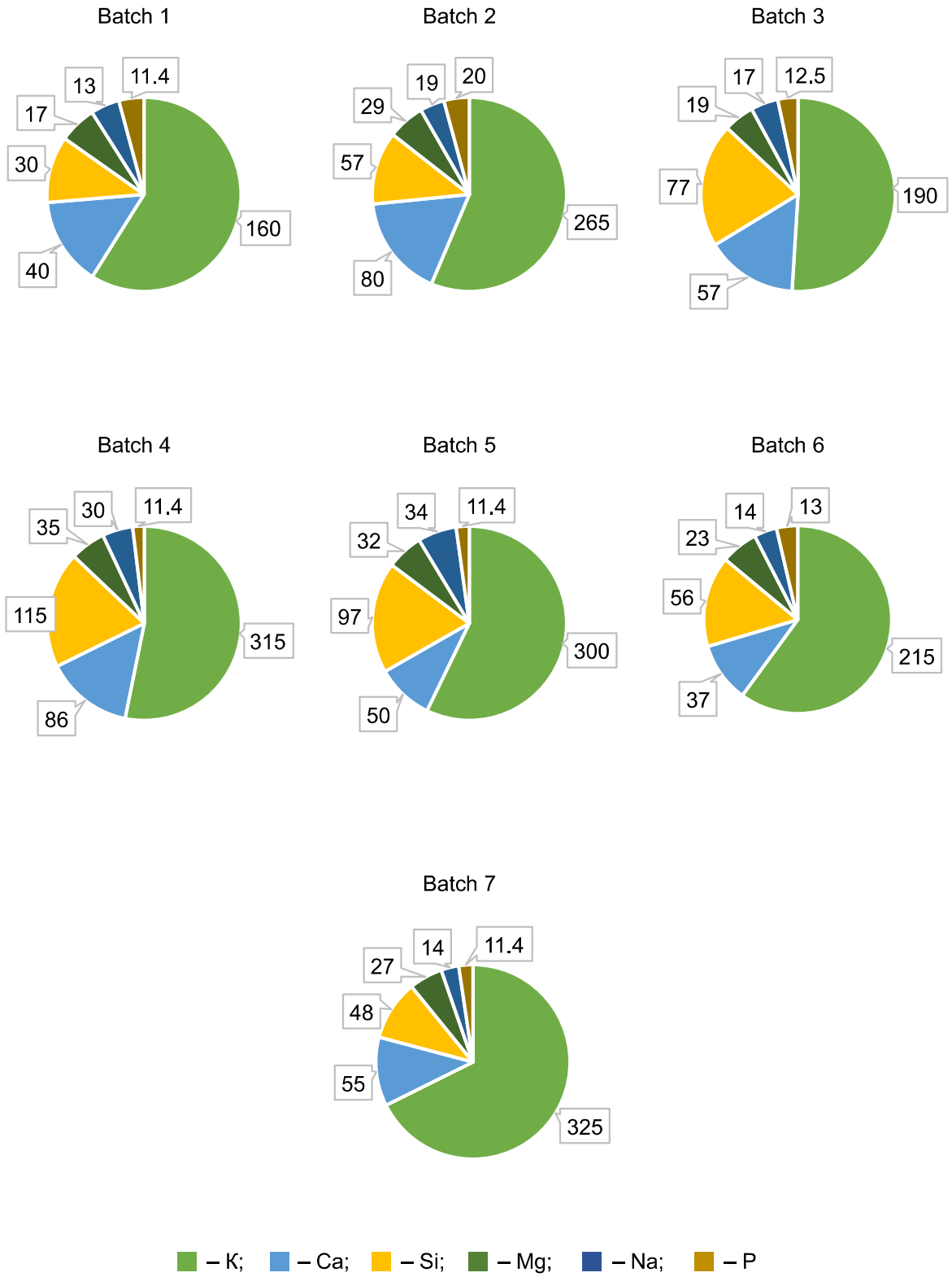

Figure 2. The macroelemental composition of the batches of the raw material of *C. islandica* (mg/100 g)

In all batches of the raw material the quantitative content of molybdenum and cobalt was less than 0.03 mg/100 g, the content of cadmium, astatine and mercury was less than 0.01 mg/100 g. The quantitative content of nickel did not exceed 0.128 mg/100 g.

It is important to note that the quantitative content of lead in the raw material ranged from 0.067 mg/100 g or 0.67 ppm (batch 1) to 0.18 mg/100 g or 1.8 ppm (batch 4), which did not exceed 10.0 ppm specified in the monograph of the SPhU.2.0 [10].

The results of determination of the component composition of free and bound nonessential and essential amino acids by the HPLC method are shown in Table 1 and Figure 4.

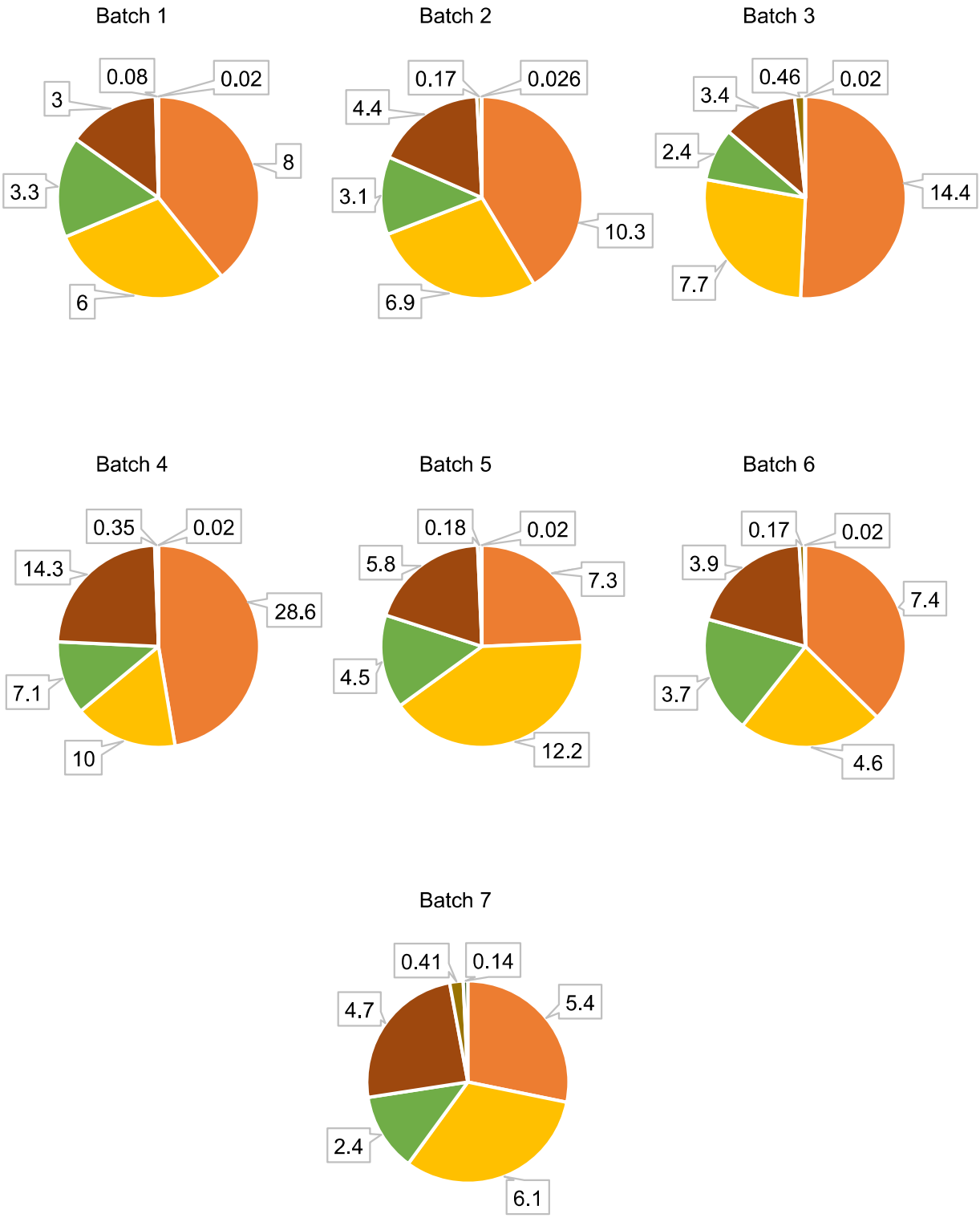

Figure 3. The trace element composition of the batches of the raw material of *C. islandica* (mg/100 g)

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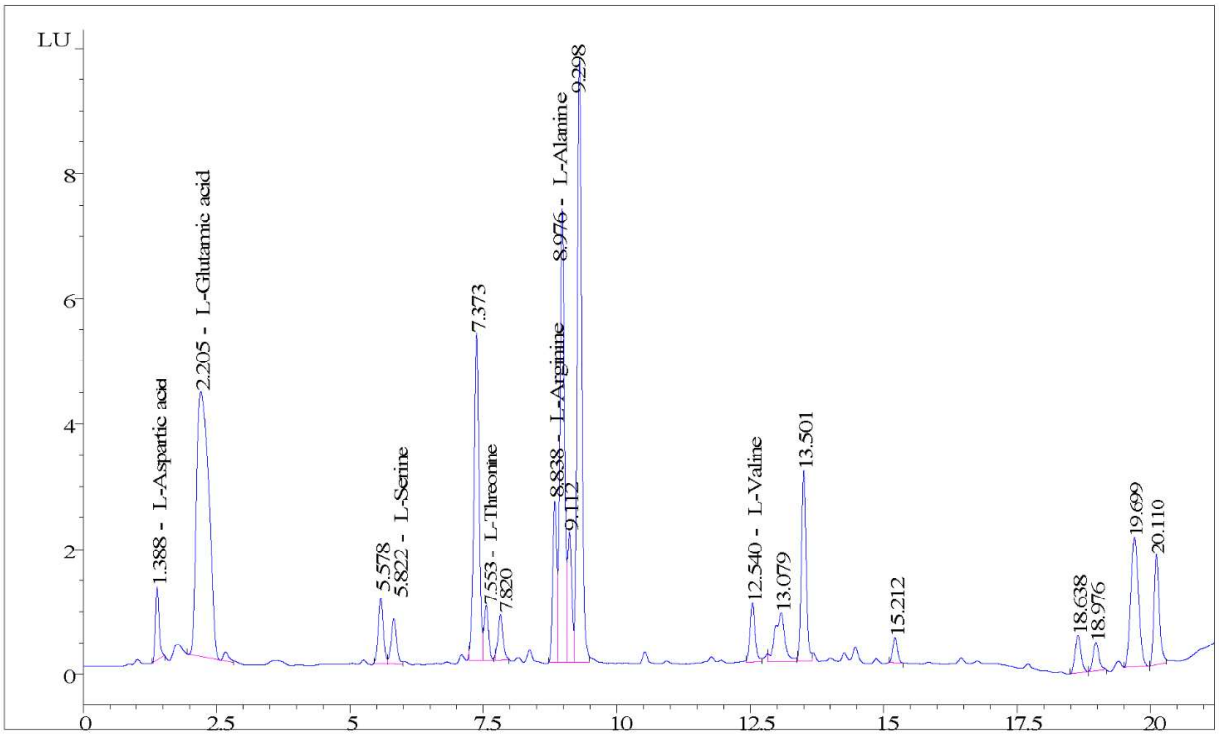
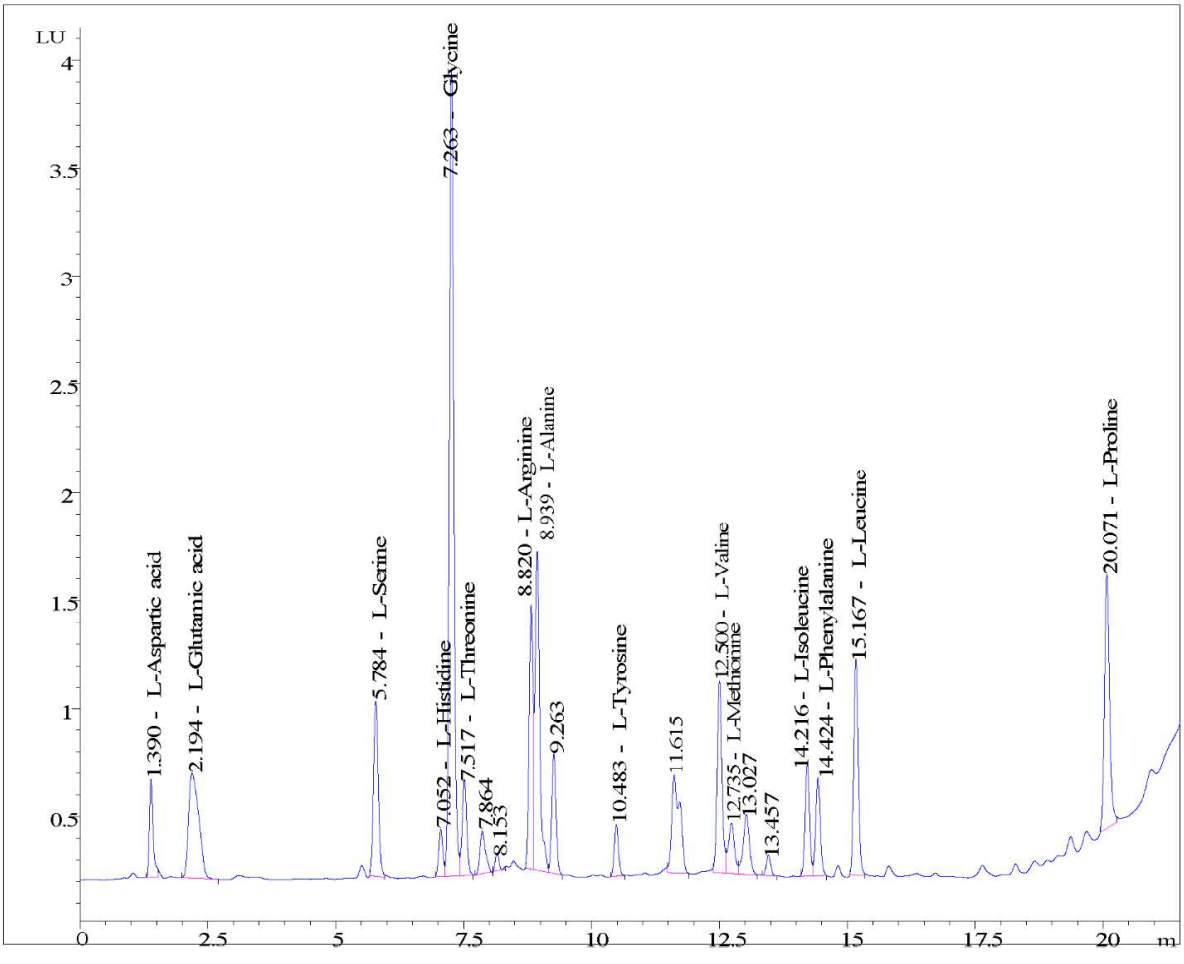


A



B

Figure 4. The HPLC chromatogram of free (A) and bound (B) amino acids in the raw material of *C. islandica*

Table 1. The quantitative content of amino acids in the raw material of *C. islandica*

	Free, μg mg^{-1}	Bound, μg mg^{-1}
Nonessential amino acids		
Aspartic acid	0.075	0.326
Glutamic acid	0.115	0.029
Serine	0.043	0.550
Glycine	–[a]	–
Arginine	0.175	0.993
Alanine	0.289	0.556
Tyrosine	–	–
Proline	–	–
Essential amino acids		
Threonine	0.050	0.304
Valine	0.047	0.518
Methionine	–	–
Histidine	–	–
Phenylalanine	–	–
Isoleucine	–	–
Leucine	–	–
Lysine	–	–
In total	0.794	3.276

Note: [a] "–" means that a component was not found

The presence of at least 7 amino acids was determined in the raw material studied, 5 of them were nonessential (aspartic acid, glutamic acid, serine, arginine, and alanine) and 2 acids were essential (threonine and valine). The quantitative content of the total amount of free nonessential amino acids in the raw material was 0.697 μg mg^{-1}, the bound ones – 2.454 μg mg^{-1}. The quantitative content of the total amount of free essential amino acids was 0.097 μg mg^{-1}, the bound ones – 0.822 μg mg^{-1}. The total quantitative content of the total amount of free amino acids in the raw material of *C. islandica* was 0.794 μg mg^{-1}, the total amount of bound amino acids was 3.276 μg mg^{-1}.

■ Conclusions

For the first time, the elemental composition of 7 batches of *C. islandica* thalli harvested in Ukraine was determined. In each batch, potassium was the predominant element (from 190 mg/100 g to 325 mg/100 g depending on the batch). The component composition of free and bound nonessential and essential amino acids was determined (the quantitative content of the total amount of free amino acids was 0.794 μg mg^{-1}, the total amount of bound ones was 3.276 μg mg^{-1}). Alanine (0.289 μg mg^{-1}) was the dominant component among free amino acids, while arginine (0.993 μg mg^{-1}) prevailed among bound ones. The results obtained will be used for further studies of the raw material of *C. islandica* harvested in Ukraine.

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Standardization of Dry Extracts from Large Cranberry Leaves

Abstract

Aim. To determine the parameters of standardization of a dry extract from large cranberry (*Oxycoccus macrocarpus* (Ait.)) leaves and a dry extract modified with arginine and develop projects of Drug Quality Control Methods (DQCM) for these substances.

Materials and methods. The study object was dry extracts from large cranberry leaves. Leaves were harvested in October 2021 in the Zhytomyr region (Kostivtsi village, 50.326862437345945, 29.54310845594284). The extracts were obtained with a 50 % solution of ethyl alcohol in the ratio of 1:30 by double maceration. Half of the combined extract was dried to a dry extract (Extract 1), and the other half was modified with arginine in the threefold equimolar amount relative to the total amount of phenolic compounds and evaporated to a dry extract (Extract 2). Standard pharmacopoeial methods were used to determine standardization parameters. The quantitative determination was carried out using the spectrophotometric method by the content of flavonoids calculated with reference to hyperoside and hydroxycinnamic acids calculated with reference to chlorogenic acid on an Evolution 60S spectrophotometer (Thermo Scientific Spectronic, USA).

Results and discussion. The parameters of standardization of dry extracts from large cranberry leaves were determined. The project of DQCM was proposed according to the following indicators: description, solubility, identification using the thin-layer chromatography method (by the content of flavonoids, hydroxycinnic acids and arginine), loss on drying, the residual amount of organic solvents (ethanol), microbiological purity, and the content of heavy metals. The assay was carried out using spectrophotometry by the content of flavonoids and derivatives of hydroxycinnic acids. Three batches of the extracts obtained, which fully corresponded to the projects of DQCM developed, were analyzed.

Conclusions. The parameters of standardization of dry extracts from large cranberry leaves have been determined, and projects of DQCM for the substances obtained have been developed. It is the basis for creating new medicines for the correction of insulin-resistant conditions in Type 2 diabetes mellitus.

Keywords: cranberry; leaves; dry extract; standardization

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Стандартизація журавлини великоплодої листя сухих екстрактів

Анотація

Мета. Визначити параметри стандартизації екстракту сухого з листя журавлини великоплодої (*Oxycoccus macrocarpus* (Ait.)) і сухого екстракту, модифікованого аргініном, та розробити проєкти методів контролю якості (МКЯ) на ці субстанції.

Матеріали та методи. Об'єктом дослідження були сухі екстракти з листя журавлини великоплодої. Листя було заготовлено в жовтні 2021 р. в Житомирській області (с. Костівці, 50.326862437345945, 29.54310845594284). Екстракти одержано 50 % розчином спирту етилового у співвідношенні 1:30, методом двократної мацерації. Половину об'єднаного витягу було висушено до сухого екстракту (екстракт 1), а іншу половину модифіковано аргініном у трикратній еквімолярній кількості щодо суми фенольних сполук та упарено до сухого екстракту (екстракт 2). Визначаючи параметри стандартизації, використовували стандартні фармакопейні методики. Кількісне визначення здійснювали спектрофотометричним методом за вмістом флавоноїдів у перерахунку на гіперозид та гідроксикоричних кислот у перерахунку на хлорогенову кислоту на спектрофотометрі Evolution 60S (Thermo Scientific Spectronic, США).

Результати та їх обговорення. Визначено параметри стандартизації сухих екстрактів з журавлини великоплодої листя. Запропоновано проєкт методів контролю якості за такими показниками: опис, розчинність, ідентифікація за допомогою методу тонкошарової хроматографії (за вмістом флавоноїдів, гідроксикоричних кислот і аргініну), втрата в масі під час висушування, залишкова кількість органічних розчинників (етанолу), мікробіологічна чистота, вміст важких

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металів, а кількісне визначення рекомендовано виконувати за допомогою спектрофотометрії за вмістом флавоноїдів та похідних гідроксикоричних кислот. Проаналізовано три серії одержаних екстрактів, які цілком відповідали розробленим проєктам МКЯ.

Висновки. Визначено параметри стандартизації сухих екстрактів журавлини великоплодої листя та розроблено проєкти МКЯ на отримані субстанції, що є основою для створення нових лікарських засобів для корекції інсулінорезистентних станів у разі цукрового діабету 2 типу.

Ключові слова: журавлина; листя; сухий екстракт; стандартизація

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■ Introduction

Compared to 2011, the number of people suffering from diabetes increased by 31% in 2021 [1]. Complications of diabetes can include kidney failure, heart attacks, strokes, amputation of limbs, and blindness. Type 2 diabetes develops as a result of inefficient use of insulin by the body, namely the development of the insulin resistance state. This type of disease affects more than 90% of diabetic patients. Symptoms may be similar to those of Type 1 diabetes, but less pronounced. Because of this, the disease is often diagnosed only a few years after its occurrence and manifestations of complications. Until recently, this type of diabetes was observed only among adults, but now it is increasingly diagnosed in children [2].

Combating the spread and complications of Type 2 diabetes is one of the most urgent tasks in the world [3]. Despite the sufficient number of synthetic drugs for the treatment of this disease, it is necessary to note a limited range of herbal products although they have a number of advantages, including effectiveness, fewer side effects and the possibility of combined therapy with other groups of drugs [4, 5]. Plants of the *Vaccinium* genus are a promising source for creating hypoglycemic and hypolipidemic agents [6–9]. Earlier studies have already shown that an extract from large cranberry leaves obtained with a 50% solution of ethyl alcohol and its modified extract with arginine have a positive effect on the correction of insulin-resistant conditions [10].

Amino acids are able to form conjugants, complexes, amides and imides with other substances, including phenolic compounds. These interactions lead to changes in the physical and chemical properties, in particular solubility, bioavailability of these substances, potentiation and emergence of new aspects of the pharmacological action. Taking this into account, a dry extract of large cranberry leaves was modified with arginine and showed higher hypoglycemic and hypolipidemic activity [10].

A dry extract of large cranberry can be both a substance for the development of new dosage forms, and for the production of a modified extract; therefore, it needs standardization.

With this in mind, to create new medicines based on large cranberry leaves, it is necessary to standardize and develop projects of Drug Quality Control Methods (DQCM) for both substances obtained since they have proven to be promising agents for correcting insulin resistance.

Therefore, the aim of the work was to determine the parameters of standardization of a dry extract from large cranberry (*Oxycoccus macrocarpus* (Ait.)) leaves and a dry extract modified with arginine and develop projects of DQCM for these substances.

■ Materials and methods

The study objects were a dry extract from large cranberry (*Oxycoccus macrocarpus* (Ait.)) leaves and a dry extract modified with arginine. Leaves were harvested in October 2021 in the Zhytomyr region (Kostivtsi village, 50.326862437345945, 29.54310845594284).

The extracts were obtained with a 50% solution of ethyl alcohol in the ratio of 1:30 by double maceration. Combined extracts were filtered through a paper filter. Half of the combined extract

was dried to a dry extract (Extract 1), and the other half was modified with arginine in the threefold equimolar amount relative to the total amount of phenolic compounds and evaporated to a dry extract (Extract 2).

Weighing was performed using the AN100 digital analytical balances (AXIS, Poland) with d = 0.0001 g. "Merck Silica gel F254" plates were used for chromatography. Solvents for the preparation of chromatographic systems were of "pure for analysis" or "chemically pure" grade; the solvent ratios indicated by numbers were taken in volume units. The optical density was measured on an Evolution 60S spectrophotometer (Thermo Scientific Spectronic, USA).

Various standard pharmacopoeial methods were used as the basis for the development of quality control methods for the extracts obtained [11]. Since large cranberry leaves are not represented in monographs of the State Pharmacopeia of Ukraine (SPhU), the monograph "European blueberry leaves" was taken as the basis.

■ Results and discussion

Taking into account the recommendations of the SPhU and modern approaches to standardization the following indicators were proposed.

Description. Dry extracts of large cranberry leaves are hygroscopic amorphous powders of brown color with a reddish tint with a faint specific odor.

Solubility. Before performing various tests it was necessary to determine the solubility of the extracts. The tests were performed in accordance with the requirements of the SPhU [11]. Extract 1 is easily soluble in 50% ethyl alcohol, moderately soluble in 96% ethyl alcohol, methanol and water, very slightly soluble in chloroform and ether. A modified Extract 2 is moderately soluble in 50, 96% ethanol and methanol and water, readily soluble in ethyl alcohol/water (70:30), slightly soluble in chloroform and ether.

Identification. Large cranberry leaves are not included in the SPhU. Therefore, a modified pharmacopoeial method of thin-layer chromatography for the flavonoid content given in the monograph "European blueberry leaves N" of the SPhU (2.2.27) was used to identify dry extracts from cranberry leaves.

Method A. Identification of major flavonoids and hydroxycinnamic acid derivatives [12].

Test solution. Dissolve 50 mg of Extract 1 in 10 mL of 96% ethanol or methanol, filter through a paper filter, distill the solvent and dissolve in 1 mL of methanol; dissolve 50 mg of a modified Extract 2 in 10 mL of 96% ethanol or methanol and water (70:30), then filter through a paper filter, distill the solvent and dissolve in 1 mL of methanol.

Reference solution. Dissolve 1.0 mg of hyperoside *R*, 1.0 mg of chlorogenic acid *R* in 10 mL of methanol *R*.

Plate: TLC plate with silica gel *R* layer.

Mobile phase: ethyl acetate *R* – water *R* – anhydrous formic acid *R* – anhydrous acetic acid *R* (72:14:7:7).

Injection volume: 10 μL, in bands.

Distance that the mobile phase must move: 10 cm from the start.

Drying: at a temperature of 100 °C to 105 °C.

Detection: spray with the solution of 10 g L^{-1} aminoethyl ether of diphenylboric acid *R* in methanol *R*. Then spray the plate with the solution of 50 g L^{-1} macrogol 400 *R* in methanol *R*, dry in the air for 30 min and view in the ultraviolet light at a wavelength of 366 nm.

Results: below is the sequence of zones on the chromatograms of *Test solutions* and *Reference solution* (Figure 1). Other fluorescent zones may also be detected on the chromatograms of *Test solutions*.

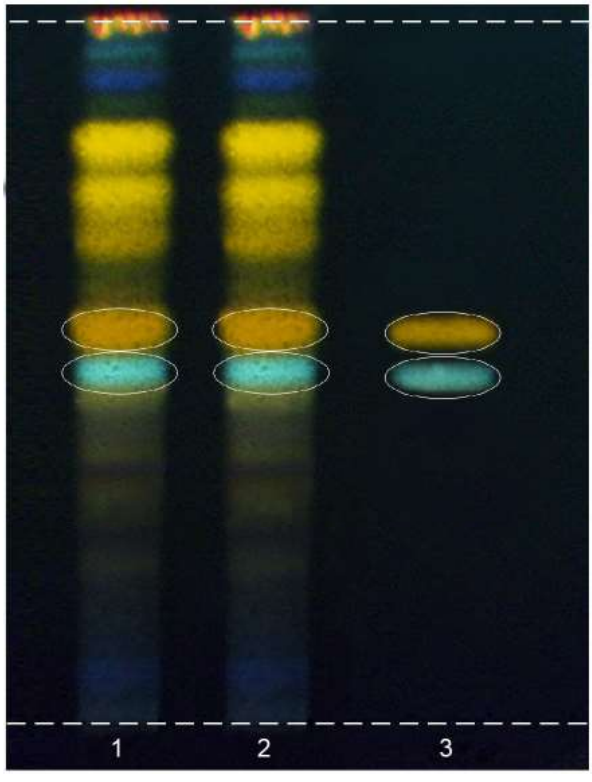

Figure 1. The chromatogram of dry extracts from large cranberry leaves: 1 – Extract 1; 2 – a modified Extract 2; 3 – *Reference solution*: hyperoside and chlorogenic acid

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In the middle part of the chromatogram of *Reference solution*, an orange fluorescent zone corresponding to hyperoside is detected. A blue fluorescent zone corresponds to chlorogenic acid, which is lower than hyperoside. On the chromatogram of *Test solution*, an orange fluorescent zone is detected at the level of *Reference solution* of hyperoside, and a blue fluorescent zone is detected at the level of *Reference solution* of chlorogenic acid. Other fluorescent zones may also be detected on the chromatogram of *Test solution*.

Method B. Identification of arginine.

Test solution. Dissolve 50 mg of the extract in 10 mL of 96% ethanol or methanol, filter through a paper filter, distill the solvent and dissolve in 1 mL of methanol.

Reference solution. Dissolve 10 mg of the reference standard (*RS*) of arginine hydrochloride in water *R* and dilute the volume to 50 mL with the same solvent.

Mobile phase: concentrated concentrated ammonia solution *R* – 2-propanol *R* (30:70).

Plate: TLC plate with silica gel *R* layer.

Injection volume: 10 μL of each solution, in bands.

Drying: dry at a temperature of 100 °C to 105 °C until the smell of ammonia disappears and spray with ninhydrin solution *R*. Heat the plate at a temperature of 100 °C to 105 °C for 15 min.

Apply 10 μL of each solution to the start line of the chromatographic plate. Dry the plate in the air and place in a chamber with a mixture of solvents, including the concentrated solution of ammonia *R* – 2-propanol *R* (30:70). When the solvent front passes 15 cm from the start line, remove the plate from the chamber, dry at a temperature of 100 °C to 105 °C until the smell of ammonia disappears and spray with ninhydrin solution *R*. Heat the plate at a temperature of 100 °C to 105 °C for 15 min. On the chromatogram of *Test solution* a spot at the level with arginine *RS* of *Reference solution* is identified.

Tests

Heavy metals. For the purpose of toxicological safety, it is necessary to control the content of heavy metals in extracts according to the requirements of the SPhU *(2.4.8)*. Their content should not exceed 100 ppm [11].

Microbiological purity. It is necessary to control the total aerobic bacteria and fungi count in the extract since phytochemicals are characterized by microbial contamination. Tests were carried out in accordance with the requirements of the SPhU, *2.6.12, 2.6.13*.

The *loss on drying* should not exceed 20.0 %. It is determined according to the SPhU 2.0 *(2.8.17)*. Place 0.50 g of the extract in a weighing bottle and dry in a drying cabinet at a temperature of 100 °C to 105 °C for 3 h. Cool in a desiccator and weigh [11].

Residual amount of organic solvents (ethanol). The content of ethyl alcohol in extracts of large cranberry leaves should not exceed 1.0 %. Place approximately 1.0 g (accurate weight) of the extract to a 10 mL measuring flask, dissolve in 7 mL of water, add 1 mL of acetone and 1,2-dichloroethane (internal standards), dilute the solution to the volume with water and mix.

Chromatograph 1 μL of the resulting solution and *Reference solution* of ethyl alcohol *RS* on a gas chromatograph with a flame-ionization detector, obtaining at least 5 chromatograms.

The results of the analysis are considered reliable if the requirements of the Chromatographic System Suitability Test are met.

Quantification. The flavonoid content in extracts of large cranberry calculated with reference to hyperoside was determined in accordance with the monograph "European blueberry leaves N" of the SPhU by the spectrophotometric method [11–13].

Place 200 mg (accurate weight) of large cranberry leaf extracts in a 100 mL round-bottomed flask, add 1 mL of a 5 g L^{-1} hexamethylenetetramine solution *R*, 20 mL of acetone *R*, 2 mL of hydrochloric acid *R*, boil at reflux for 30 min, and filter through a cotton swab into a 100 mL flask. Add a cotton swab to the residue in a round-bottomed flask, and extract in 2 portions of acetone *R*, 20 mL each; each time boil at reflux for 10 min, cool to room temperature, filter each extract through a cotton swab into the flask. Filter the resulting cooled combined acetone extracts through a paper filter into a measuring flask, dilute the volume of the solution to 100 mL with acetone *R*, rinsing the flask and the paper filter. Place 20.0 mL of the solution obtained into a separation funnel, add 20 mL of water *R*, extract the mixture with 15 mL, and then with 3 portions of ethyl acetate *R*, 10 mL each. Combine the resulting ethyl acetate extracts in a separation funnel, wash with 2 portions of water *R*, 50 mL each, filter over 10 g of anhydrous sodium sulfate *R* into a 50 mL measuring flask, and dilute the volume of the solution to 50.0 mL with ethyl acetate *R* (Solution A and B, respectively).

Test solution. Add 1 mL of aluminum chloride reagent *R* to 10.0 mL of Solution A (B) and

dilute the volume to 25.0 mL with the solution of 5 % (*v*/*v*) glacial acetic acid *R* in methanol *R*.

Compensation solution. Dilute 10.0 mL of the initial solution to 25.0 mL with the solution of 5 % (*v*/*v*) glacial acetic acid *R* in methanol *R*. Measure the optical density (2.2.25) of *Test solution* in 30 min compared to *Compensation solution* at a wavelength of 425 nm.

The content of flavonoids calculated with reference to hyperoside was calculated as a percentage by the formula:

$$X = \frac{A \times 1.25}{m}$$

where: *A* – is the optical density of *Test solution* at a wavelength of 425 nm;
m – is the weighed sample of the extract, g.

The specific index of hyperoside absorption equal to 500 was used.

The total amount of hydroxycinnamic acid derivatives was determined using the spectrophotometric method calculated with reference to chlorogenic acid [14, 15].

Dissolve approximately 100 mg (accurate weight) of dry extracts of large cranberry leaves, constantly stirring, in 5 mL of a 50% solution of ethyl alcohol. Repeat the procedure three times with a new portion of the solvent. After that, combine the solutions, filter through a paper filter, and place quantitatively into a 25.0 mL measuring flask, dilute the solution in the flask to the volume with the same solvent, and mix (Solution C and D).

Add 1.0 mL of Solution C (D) to a 25 mL measuring flask, then dilute to the volume with 50% alcohol, and mix. As *Reference solution,* a 50% ethanol solution is used. Measure the optical density of the resulting solution on an Evolution 60S spectrophotometer (USA) at a wavelength of 327 nm.

As *Reference solution,* the solution of chlorogenic acid is used. To prepare it, weigh 0.05 g (accurate weight) of chlorogenic acid *RS*, place it into a 100 mL measuring flask, dissolve in a 50% ethyl alcohol solution, dilute the solution to the volume with the same solvent, and mix.

Table 1. The results of the analysis of large cranberry leaf extracts according to the projects of DQCM

Quality indicator	Requirements	Extract 1 (batch)			Extract 2 (batch)		
		1101	1701	1902	1309	2001	2102
Description	according to the requirements of the project of DQCM	+[a]	+	+	+	+	+
Identification (TLC)	according to the project of DQCM (Method A)	+	+	+	+	+	+
	according to the project of DQCM (Method B)	–	–	–	+	+	+
Tests							
Loss on drying	Not more than 5 %	4.8	4.3	4.1	4.2	4.6	4.3
Residual amount of organic solvents (ethanol)	Not more than 1.0 %	0.6	0.4	0.7	0.5	0.6	0.8
Heavy metals	Not more than 100 ppm	+	+	+	+	+	+
Microbiological purity	In 1 g of the drug there are no more than 100 CFU (bacteria and fungi in total). The presence of enterobacteria and some other gram-negative bacteria; *Pseudomonas aeruginosa, Staphylococcus aureus* in 1 g is not allowed	+	+	+	+	+	+
Assay							
The content of the total amount of flavonoids calculated with reference to hyperoside	not less than 4 % (for Extract 1); not less than 2 % (for Extract 2)	5.17 ± 0.06	4.62 ± 0.04	5.04 ± 0.05	2.60 ± 0.05	2.22 ± 0.03	2.05 ± 0.04
The content of the total amount of hydroxycinnamic acid derivatives calculated with reference to chlorogenic acid	not less than 10 % (for Extract 1); not less than 3 % (for Extract 2)	11.47 ± 0.07	11.60 ± 0.05	11.56 ± 0.03	3.07 ± 0.04	3.09 ± 0.05	3.15 ± 0.03

Note: [a] «+» – the extract meets the requirements of the projects of DQCM

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After that add 1.0 mL of chlorogenic acid *RS* to a 50.0 mL measuring flask, dilute to the volume with 50% ethyl alcohol, mix, and measure the optical density under the same conditions as *Test solution*. As *Reference solution,* a 50% ethanol solution is used.

The content of the total amount of hydroxycinnamic acid derivatives calculated with reference to chlorogenic acid in the samples studied was calculated as a percentage by the formula:

$$X = \frac{A_1 \times a_0 \times 25 \times 1 \times 25 \times 100 \times 100}{A_0 \times a_1 \times 100 \times 1 \times 50 \times (100 - w)}$$

where: A_1 – is the optical density of *Test solution* of the extract;
A_0 – is the optical density of *Reference solution* of chlorogenic acid *RS*;
a_1 – is the weighed sample of the extract, g;
a_0 – is the weighed sample of chlorogenic acid *RS* (SPhU), g;
w – is the loss on drying, %.

Three batches of each dry extract from large cranberry leaves, which fully corresponded to the projects of DQCM proposed, were analyzed (Table 1).

Based on the results of the analysis, it can be concluded that all dry extracts of large cranberry leaves meet the requirements of the projects of DQCM developed.

■ Conclusions

The parameters of standardization of dry extracts of large cranberry leaves have been determined, and projects of DQCM for the substances obtained have been developed. It is the basis for creating new medicines for the correction of insulin-resistant conditions in Type 2 diabetes mellitus.

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