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Ensuring environmental safety during express determination of individual components of plant raw materials in aqueous solutions

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Abstract. In the paper, using the method of thin-layer chromatography the peculiarities of determining individual components of plant raw materials- flavonoids were investigated. The test samples were solutions of quartzetin, rutin, kaempferol, hyperoside, caffeic acid, luteolin and chlorogenic acid. Cetylpyridinium chloride, Tween 80 and their modifications with propionic acid were used as the mobile phase. The quantitative determination parameters were retention coefficients and retention factors. It was shown that the presence of a modifier in the mobile phase, cetylpyridinium chloride, increases the retention factor for caffeic and chlorogenic acids, and in the mobile phase, Tween 80 decreases it for these substances. It was found that the use of a mobile phase with Tween 80 (C = 1.57 g/l) ensures the identification of the largest number of investigated substances for which the retention factor increases in a number of hyperosides, chlorogenic acid, caffeic acid, rutin (from 3 to 11). It is proposed to use this variant for the development of a method of rapid identification of these flavonoids in natural waters. It was noted that the use of the proposed eco-friendly methodology will contribute to increasing the level of environmental safety of both individual chemical-ecological studies and territories as a whole.

1. Introduction

Today, modern anthropogenic activity has a significant impact on natural and marine ecosystems, causing environmental pollution and significant climate changes [1, 2]. Active development of industry, irrational management of agriculture, emergence of new chemical compounds of synthetic origin lead to disturbance of soil [3, 4], deterioration of water resources [5, 6, 7], air [8], reduction of biodiversity. Emergency situations are also additional factors affecting the environment. In particular, fires [9, 10, 11], pandemics [12], military conflicts [13, 14] make a dangerous contribution to the deterioration of

the environment. At the same time, both manifestations of direct negative impact and long-term indirect consequences should be noted.

In turn, awareness of the dangers that threaten humanity and existence of life on the planet as a whole gave the world community an impetus to change the paradigm of interaction between a human and nature. This led to a series of international and national political decisions. In particular, the UN proposed the so-called Sustainable Development Goals [15] whose achievement is an important element of the politics and economy of modern states. As a result, there is a transformation of the energy industry and a transition to diversified renewable energy sources [16], the use of "green" [17] and "smart" [18, 19, 20] technologies and ecological approaches in industry, agriculture, and the communal sphere, which are aimed at minimizing the environmental pollution and rationalizing the use of natural resources.

Control of the content of chemical compounds, both in the process of their production and when they enter the environment, is a necessary element of ensuring the ecological safety of the environment. Modern methods of analysis involve the use of various electrochemical, spectrophotometric, chromatographic and other methods based on the physical and chemical characteristics of substances or their combination. The priority of research goals determines the methodology of applying these methods. Modern focus on "green" technologies makes it urgent to search for new methods of analyzing substances that are also ecologically friendly to the environment [21].

Chromatographic analysis and its variations are currently one of the most widely used methods due to high selectivity, the ability to analyze a wide range of both organic and inorganic compounds. The rather high cost of the equipment and its specificity, the need for personnel training, and the mandatory use of chemical reagents of various hazard classes can be noted as disadvantages. Thin-layer chromatography is the simplest and most accessible variation of this method, which allows rapid identification and determination of the quantitative content of chemical compounds [22]

The tendency to use more ecologically safe substances is supported by the use of compounds of plant origin in medicine, cosmetology, food, textile and other industries. This, in turn, indicates the need to implement modern, simple and environmentally friendly methods of their determination. In particular, flavonoids [23], substances of plant origin that are widely used in the production of food, medical and cosmetic products [24, 25] have become widespread. They are natural polyphenolic compounds [24] contained in vegetables, fruits, medicinal plants. Their direct use also provides a protective effect against some toxic substances (pesticides) due to antioxidant, anti-inflammatory, antimutagenic, anti-stress properties and stimulation of immunity [26].

It is common to use chromatography methods for their identification and determination. Thus, thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) are proposed for the analysis of *Cirsium vulgare (Savi) Ten*. and identification and determination of such compounds as linarin, luteolin-7-O-β-D-glucopyranoside, apigenin-7-O-β-D-glucopyranoside, apigenin, kaempferol-3-O-methyl ester, hyperoside, luteolin, rutin, quercetin, luteolin-5-O-β-D-glucopyranoside, gispidulin-7-O-β-D-glucopyranoside, chlorogenic acid, but the TLC method uses such dangerous compounds as benzene, ethyl acetate, acetic acid, formamide, chloroform [27]. Combining TLC with other methods, for example, vibrational spectroscopy or 2D TLC-Raman allows detection of individual groups of flavonoids [23], but the question of the cost of such studies arises. High-speed countercurrent chromatography [28] is also a promising method for separating and determining individual groups of flavonoids in various plant products, but, as in previous cases, the question of the cost of research and the use of hazardous solvent systems (such as methanol, acetic acid, etc.) arises. HPLC with mass spectrometry (MS) has also been used to determine the groups of polyphenolic plant compounds in *Butia catarinensis (Butiá da Praia), Butia eriospatha (Butiá da Serra)* and *Opuntia elata (Arumbeva)*, but the experimental procedure is quite complex, with applications as liquid phases of acetonitrile, formic acid [29]. The same components of the liquid phase were used in the determination of flavonoids by HPLC with mass spectrometry in *Lupinus Angustifolius L* [30]. Electrospray ionization in combination with HPLC-MS was used for the analysis of flavonoids in green leafy vegetables [31], formic acid, acetonitrile, methanol, and acetic acid were noted as the liquid phase. Also, HPLC in various variations is proposed for the determination of a number of flavonoids in honey [32] The use of

HPLC, TLC for the determination of flavonoids in the medicinal plant *Glehnia littoralis* is shown, but it is proposed to use methanol, acetonitrile (HPLC) or petroleum ether with ethyl acetate (TLC) as part of the carrier phase [33]. Thus, the question of finding inexpensive, express and environmentally friendly approaches to the determination of components of plant origin remains open.

The purpose of this work is to investigate the possibility of environmentally friendly determination of the components of plant raw materials using the method of thin-layer chromatography.

2. Methods

2.1. Reagents

Aqueous solution of Tween 80 with a concentration of 0.16 g/l, aqueous solution of cetylpyridinium chloride (CPCh) with a concentration of 3.06 g/l, aqueous solution of Tween 80 with a concentration of 0.16 g/l with the addition of propionic acid (0.1%), aqueous solution of cetylpyridinium chloride (CPCh) with a concentration of 3.06 g/l with the addition of propionic acid (0.1 %), aqueous solution of Tween 80 with a concentration of 1.57 g/l, ethanol 96 %. Propionic acid was used as a modifier.
Standard samples of components of plant raw materials: quartzetin, rutin, kaempferol, hyperoside, caffeic acid, luteolin, chlorogenic acid.

2.2. Equipment

Sorbfil-PTSHAF-A chromatographic plates with an aluminum substrate and an applied working layer of porous silica gel with a thickness of 90-120 microns were used in the work. Chromatographic chamber - capacity 19x19.5x6.5 cm made of chemically resistant glass with a separate recess at the bottom for fixing plates and saving eluent. Thermostat MIZMA TC-20 with a temperature adjustment range of +5 ...+60 °C, measuring ruler (0-250 mm, Δ=±0.1 mm). Samples were weighed on electronic analytical balances with an accuracy of 0.0001 g AS60/220.R2 manufactured by Radwag.

2.3. Experiment methods

The mobile phase (MF) is an aqueous solution of nonionic surfactant (surfactant) Tween 80 and a solution of cationic surfactant - cetylpyridinium chloride.

All solutions were prepared in distilled water. Solutions were prepared from exact measurements of standard samples or reagent measurements (not lower than "cfa"). Test solutions of plant components were prepared by dissolving aliquots of solutions of quartzetin, rutin, kaempferol, hyperoside, caffeic acid, luteolin, chlorogenic acid with a concentration of 1 mg/ml in 96 % (*v/v*) ethanol.

Before work, the chromatographic plates were washed with pure ethanol 96 % (*v/v*). and activated in an oven at 110 °C for 1 hour.

Spots of the analyzed solutions were applied at a height of 1-2 cm from the edge of the plate. The diameter of the sample spots was 2-4 mm. Samples of the analyzed solutions were applied in the following sequence: 1. Quercetin; 2. Routine; 3. Kaempferol; 4. Hyperoside; 5. Caffeic acid; 6. Luteolin; 7. Chlorogenic acid. The study was conducted in a thermostatic environment (T = 25°C).

The run of the front ended 5 mm from the edge of the plate. After fixed time, visual detection of the analytical signal was carried out. The distance of the course and the size of the spot were fixed with a measuring ruler. Each study was carried out 3 times (n=3), the obtained results were processed using standard statistical approaches.

3. Results and discussion

The first stage of the study was qualitative and included the influence of the mobile phase on the determination of test samples. Alcoholic solutions of individual components of plant raw materials - flavonoids quartzetin, rutin, kaempferol, hyperoside, caffeic acid, luteolin, chlorogenic acid - were used as test samples. The analysis of test samples was carried out by applying the method of thin-layer chromatography in the medium of alcoholic solutions of Tween 80 (polysorbate 80, nonionic surfactant) [34], CPCh (cationic surfactant) [35, 36], and the same solutions modified with propionic acid. These compounds are able to form micelles, which allows us to speak about the use of the micellar thin-layer

chromatography method in the determination of the indicated flavonoids. In particular, the critical micelle formation concentration (CMC) of CPCh and Tween 80 is 0.0009 M and 0.001%, respectively. These substances are used in the medical [35], food and cosmetic [34] industries, they can be classified as substances with a low toxic effect (CPCh) or non-toxic (polysorbate 80), respectively. And the used method itself can be classified as environmentally friendly. The general view of the chromatographic plate with applied samples is shown in figure 1.

The obtained results are shown in table 1.

Figure 1. General view of a chromatographic plate with applied alcohol solutions of test samples. The mobile phase is an aqueous solution of CPCh (C_{CPCh} = 3.06 g/l).

Table 1. Results of investigations of solutions of test samples in the environment of various types of mobile phases.

Mobile phase	Research results
$C_{(CPCh)}$ = 3.06 g/l	Stains of rutin and caffeic acid were detected
$C_{(Tween\ 80)}$ = 0.16 g/l	Caffeic and chlorogenic acid stains were detected
$C_{(CPCh)}$ = 3.06 g/l + 0.1% C_2H_5-COOH	Stains of rutin, caffeic and chlorogenic acids were detected
$C_{(Tween\ 80)}$= 0.16 g/l + 0.1% C_2H_5-COOH	Stains of rutin, caffeic and chlorogenic acids were detected
$C_{(Tween\ 80)}$= 1.57 g/l	Spots of rutin, hyperoside, caffeic and chlorogenic acids were detected

According to the obtained results (table 1), the introduction of a modifier to the mobile phase increases the selectivity and number of determinations of the components of plant raw materials and unifies this determination compared to individual solutions of CPCh and Tween 80. These solutions separately allow the determination of only two components (at concentrations of these solutions of 3.06 g/l and 0.16 g/l, respectively). But an order-of-magnitude increase of the concentration of Tween 80 in the mobile phase (up to 1.57 g/l) allows the simultaneous identification of 4 components (rutin, hyperoside, caffeic acid, chlorogenic acid) and is the most promising direction for further research.

The next stage of the study included the determination of the quantitative characteristics of the experiment - the retention coefficient of the substance R_f (the ratio of the distance the spot of substance l moved to the distance traveled by the solvent front L) and the retention factor k (indicates how well the component is retained in the stationary phase) [22, 37]:

$$R_f = l / L \quad (1)$$

$$k = R_f / (1 - R_f) \quad (2)$$

The results of the study are presented in table 2.

Table 2. Results of determination of retention coefficients R_f and retention factors k of the investigated components of plant raw materials with different mobile phases.

Mobile phase	Retention coefficient (R_f)	Retention factor (k)
$C_{(CPCh)}$ = 3.06 g/l;	$R_{f(caffeic\ acid)}$ = 0.876 $R_{f(chlorogenic\ acid)}$ = 0.853	$k_{(caffeic\ acid)}$ = 7 $k_{(chlorogenic\ acid)}$ = 6
$C_{(Tween\ 80)}$ = 0.16 g/l;	$R_{f(rutin)}$ = 0.861 $R_{f(chlorogenic\ acid)}$ = 0.888	$k_{(rutin)}$ = 6 $k_{(chlorogenic\ acid)}$ = 8
$C_{(CPCh)}$ = 3.06 g/l + 0.1% C_2H_5-COOH	$R_{f(rutin)}$ = 0.827 $R_{f(caffeic\ acid)}$ = 0.945 $R_{f(chlorogenic\ acid)}$ = 0.870	$k_{(rutin)}$ = 5 $k_{(caffeic\ acid)}$ = 17 $k_{(chlorogenic\ acid)}$ = 7
$C_{(Tween\ 80)}$ = 0.16 g/l + 0.1% C_2H_5-COOH	$R_{f(rutin)}$ = 0.823 $R_{f(caffeic\ acid)}$ = 0.900 $R_{f(chlorogenic\ acid)}$ = 0.855	$k_{(rutin)}$ = 5 $k_{(caffeic\ acid)}$ = 9 $k_{(chlorogenic\ acid)}$ = 6
$C_{(Tween\ 80)}$ = 1.57 g/l	$R_{f(rutin)}$ = 0.914 $R_{f(hyperoside)}$ = 0.767 $R_{f(caffeic\ acid)}$ = 0.902 $R_{f(chlorogenic\ acid)}$ = 0.854	$k_{(rutin)}$ = 11 $k_{(hyperoside)}$ = 3 $k_{(caffeic\ acid)}$ = 9 $k_{(chlorogenic\ acid)}$ = 6

According to the obtained data (table 2), the highest retention coefficient is observed for rutin in the medium of the mobile phase Tween 80 ($C_{(Tween\ 80)}$ = 1.57 g/l). The presence of the modifier has different effects on the retention of components of plant raw materials. In particular, it increases the retention factor for caffeic acid and chlorogenic acid with the mobile phase of CPCh (by 10 and 1, respectively). At the same time, for the Tween 80 mobile phase, the introduction of the modifier, on the contrary, reduces the retention factor for these substances, and increasing the concentration of Tween 80 in the mobile phase increases the selectivity of the determination (in terms of increasing the components that can be identified) and increases their retention factor (except for chlorogenic acid). It can also be assumed that the additional use of the modifier also allows the improvement the shape of the chromatographic zones.

The proposed approach refers to the methods of green chemistry, is inexpensive and easy to use, it can be considered in the future as an alternative for the express identification of residues of components of plant raw materials used in anthropogenic activities in natural waters, which, in turn, will contribute to increasing the level of environmental safety of chemical-ecological research and the territories for which research is conducted, in general. As a result, this will provide an additional contribution to achieving the goals of sustainable development in terms of preserving natural ecosystems (Sustainable Development Goals SDGs 14 and 15). Further avenues of research include the development of methods for their quantitative determination by thin-layer chromatography with Tween 80 ($C_{(Tween\ 80)}$ = 1.57 g/l as the mobile phase).

4. Conclusions

Thus, the work investigated the peculiarities of identification of individual components of plant raw materials using the thin-layer chromatography method and determined the influence of various types of mobile phases on their determination.

The possibility of express identification of such plant raw material samples as quartzetin, rutin, kaempferol, hyperoside, caffeic acid, luteolin, chlorogenic acid) with alcoholic solutions of Tween 80 and cytylpyridinium chloride, including those ones modified with propionic acid, as mobile phases is shown. It was found that in the medium of Tween 80 ($C_{(Tween\ 80)}$ = 1.57 g/l) the identification of the largest number of studied substances (4) is observed, for which the retention factor increases in the series of hyperoside, chlorogenic acid, caffeic acid, rutin (3<6<9<11). The presence of the modifier - propionic acid - with the mobile phase of CPCh increases the retention factor for caffeic acid and chloragenic acid, and with the mobile phase Tween 80, on the contrary, reduces the retention factor for these substances. Increasing the concentration of Tween 80 in the mobile phase increases the selectivity of the determination (in terms of increasing the components that can be identified) and increases their retention factor (with the exception of chlorogenic acid). It was noted that the proposed approach is simple, inexpensive, can be considered as one of the methods of green chemistry. Its further implementation for the express identification of flavonoids as residues of components of plant raw materials in natural waters will contribute to increasing the level of environmental safety of chemical-ecological research and territories in general, which corresponds to the goals of sustainable development 14 and 15 (preservation of natural ecosystems).

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