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DEVELOPMENT OF THE METHOD FOR CENTAURY HERB IDENTIFICATION BY THIN LAYER CHROMATOGRAPHY FOR THE STATE PHARMACOPOEIA OF UKRAINE MONOGRAPH

Abstract. This article describes experimental results of comparing the pharmacopoeia requirements concerning "Identification. TLC" test of Centaury herb on 7 series of raw material. Swertiamarin and rutin were selected as standard samples (marker substances). It is shown that PhEur 8.4 requirements to chromatographic conditions are the most appropriate for the separation of these markers. A new method for Centaury herb BAS identification was developed by the method of TLC after replacement of expensive marker substance swertiamarin by more affordable SPhU CRS Centaury extract, and expensive reagents – ethyl formate by more affordable solvent – ethyl acetate. Harmonization of the SPhU requirements with PhEur 8.4 was carried out to develop national requirements concerning the quality of domestic raw material for the SPhU monograph «Centaury herb»

Keywords: Centaury herb, identification, thin layer chromatography, State Pharmacopoeia of Ukraine.

Common centaury *(Centaurium erythraea Rafn.)* and Centaurium pulchellum *(Centaurium pulchellum (Sw.) Druce)* are typical representatives of domestic medicinal herbal material (MHM) Centaury grass (Centaurii herba); its other species are rare and do not have any commercial value. Therefore, there is a need for standardization of MHM [5-10], which implies, above all, a reasonable choice of criteria for the quality of MHM.

In Ukraine, Centaury is not cultivated, but the need for this kind of material is satisfied by wild plants gathering. The raw material is harvested at the beginning or during the flowering period (July-August), because in this period it is the maximum accumulation of biologically active substances (BAS) which belong to the class of iridoids – sekoiridoide glycosides. Iridoids is a group of monoterpene compounds of herbal origin, which are most often found in the form of glycosides. The main substance of the sekoiridoide glycosides group, presenting in the Centaury herb and causes its pharmacological effect, is swertiamarin [1-4].

The requirements to the quality of Centaury herb are displayed in the monographs of the leading pharmacopoeias: European Pharmacopoeia (PhEur 8.4) monograph «Centaury», German Pharmacopoeia (DAB 10) monograph «Tausendgüldenkraut», British herbal Pharmacopoeia (BHP) monograph «Centaury», and the USSR State Pharmacopoeia (SP XI) article «Herb centaury» [7, 11-13]. Currently, in the State Pharmacopoeia of Ukraine (SPhU) the monograph on the Centaury herb does not exist.

Thin layer chromatography (TLC) method is one of rather sensitive and acceptable methods for identification of Centaury herb in the mentioned monographs [8-10, 14, 15, 18-21]. In SP XI identification of Centaury herb by TLC is not represented [7].

The aim of the work was to compare chromatography conditions of methods for identification of Centaury herb by TLC method, described in the monographs of the leading pharmacopoeias, to select the optimal conditions of analysis (identification), and to develop new methods for identification of Centaury herb BAS using TLC for implementation of national quality requirements to the domestic herbal drugs in the SPhU monograph «Centaury herb».

Materials and methods. The raw material for research was 7 types of Centaury herb, collected in 2014 in different regions of Ukraine.

A comparative analysis results of the pharmacopoeial requirements to Centaury herb in terms of «Identification. TLC method» in accordance with the PhEur 8.4, DAB 10 and BHP, are presented in Table 1.

Table 1 – Requirements of the PhEur 8.4, DAB 10 and BHP to identification of Centaury herb by TLC method

	PhEur 8.4	DAB 10	BHP
Sample preparation	25 mL of methanol is added to 1.0 g of powdered herbal material, shaken for 15 minutes and filtered. The filtrate was evaporated to dryness under vacuum at a temperature not higher than 50 C. The resulting residue was taken up in small amounts of methanol to obtain 5 ml of solution, which can contain precipitate.	20 ml of methanol is added to 1.0 g of of powdered herbal material, heated for 10 minutes under reflux to boiling, and is filtered after cooling.	10 ml of methanol is added to 1.0 g of of powdered herbal material, heated for 10-15 minutes in a warm water bath and is filtered after cooling.
Markers	Rutin, swertiamarin	Rutin	Rutin
Mobile phase	Water – formic acid anhydrous – ethyl formate (4:8:88)	Water – glacial acetic acid – ethyl acetate (16:16:69)	Ethyl acetate – formic acid anhydrous – glacial acetic acid – water (100:11:11:27)
Detection (visual assessment)	**A.** The plate is browsing in UV light at a wavelength of 254 nm. **B.** Spray with solution of anisaldehyde, heated at a temperature of 100-105 °C and browsing in daylight.	**A.** The plate is browsing in UV light at a wavelength of 254 nm. **B.** Spray with solution of anisaldehyde, heated at a temperature of 100-105 °C and browsin in UV light at a wavelength of 365 nm.	**A.** The plate is is heated at a temperature 100-105 °C. Spray with 1% solution of boric acid monoethyl diphenyl ether in methanol, and then with 5% solution of polyethylene glycol 4000 in ethanol. The plate is browsing in UV light at a wavelength of 366 nm.

TLC plates with layer thickness 5 ÷ 40 μm (Supelco Silicagel 60 F_{254} firm «Sigma-Aldrich») and high performance thin layer chromatography (HPTLC) with a particle size from 2 μm to 10 μm (Silicagel 60 F_{254} firm «Merck») were used during the Centaury herb identification by TLC method, as PhEur 8.4 required. Since different types of TLC plates were used in the analysis, both the volumes of the solutions applied to the plate and the level of the solvent front from the starting line were different, e.g: 10 μl and 12 cm – for conventional TLC plate and 5 μl and 6 cm – for HPTLC.

During the identification of Centaury herb by the method of TLC conventional TLC plates were used, as DAB 10 required. Volumes of solutions applied on the plate were 30 μl for test solution and of 10 μl for the reference solution.

Under the BHP conditions, both test solutions and reference solution were applied to conventional TLC plate in volume of 20 μl. The level of the solvent front from the starting line was 15 cm.

In the monograph DAB 10 «Tausendgüldenkraut», TLC identification of herbal material is carried out using only one marker – rutin, against which zone the description of areas including swertiamarin area in test solution chromatogram is carried out. In the monograph BHP identification of herbal material is carried out using only one marker – rutin, against which the main chromatographic zones are identified. Since one of the main BAS in the studied herb are sekoiridoide glycosides [11-13], PhEur 8.4 reference solution was offered for identification. Thus, swertiamarin and rutin were chosen as the marker substances.

Results and discussion. Under PhEur 8.4 conditions of analysis, on the chromatograms of the reference solutions swertiamarin and rutin zones completely separated (Figure 1–4). All test samples of herbal material have distinct specific zones at the level of swertiamarin, which confirms both specificity of the method and quality of the analyzed Centaury herb.

During chromatography analysis under DAB 10 conditions only one zone was found in the reference solution (rutin, swertiamarin) area. This fact indicates that under mentioned conditions zones of rutin and swertiamarin are not separated. Besides, it takes three times longer to carry out chromatography analysis under mentioned conditions comparing to PhEur 8.4 (Figure 5, 6).

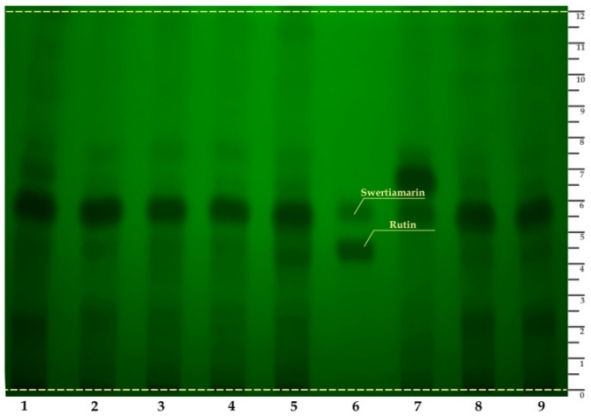

Figure 1 – Chromatogram in UV light at 254 nm, obtained under the conditions of PhEur 8.4 analysis on a conventional plate: 1 – Common Centaury (CC) series 15; 2 – CC series 16; 3 – CC series 17; 4 – CC series 18; 5 – CC series 19; 6 – standard samples (SS) of swertiamarin and rutin; 7 – Centaurium pulchellum (CP); 8 – CP series 20; 9 – CP series 21

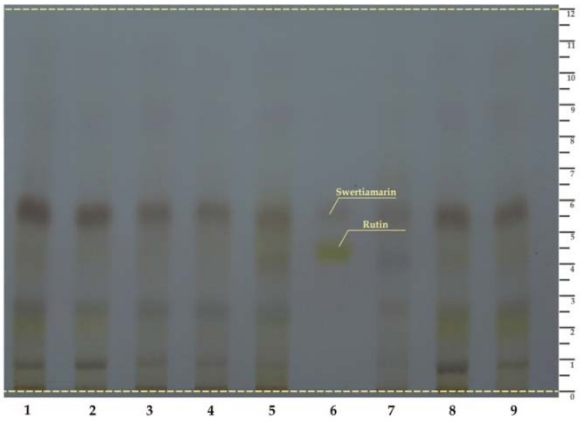

Figure 2 – Chromatogram in daylight, obtained under the conditions of PhEur 8.4 analysis on a conventional plate

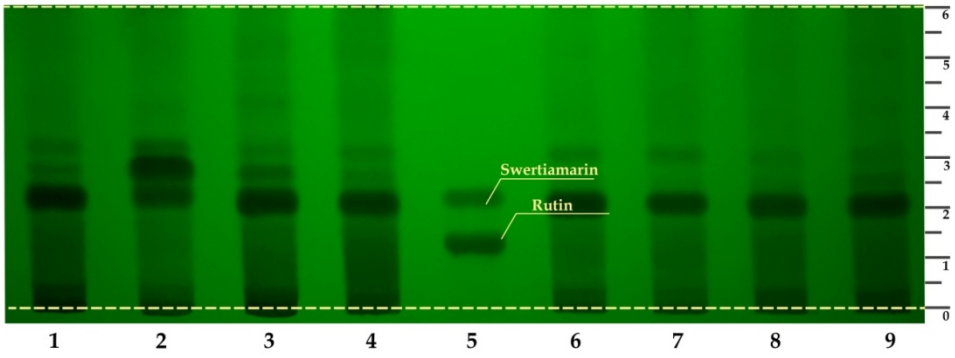

Figure 3 – Chromatogram in UV light at 254 nm, obtained under the conditions of PhEur 8.4 analysis on HPTLC-plate: 1 – CC series 15; 2 – CP; 3 – CC series 16; 4 – CC series 17; 5 – SS of swertiamarin and rutin; 6 – CC series 18; 7 – CC series 19; 8 – CC series 20; 9 – CC series 21

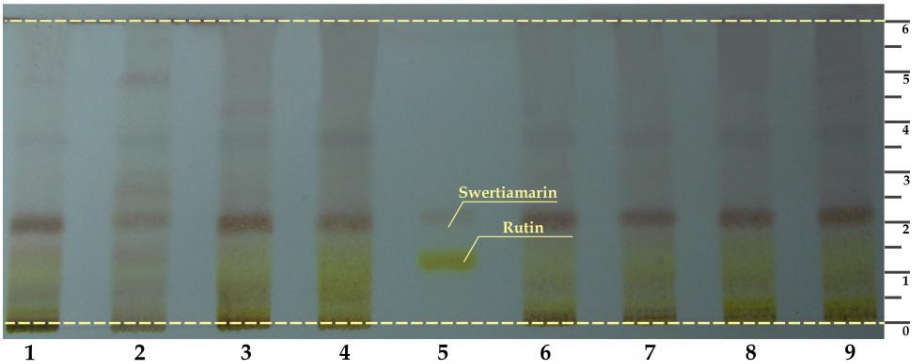

Figure 4 – Chromatogram in daylight after processing with anisaldehyde, obtained under the conditions of PhEur 8.4 analysis on HPTLC-plate

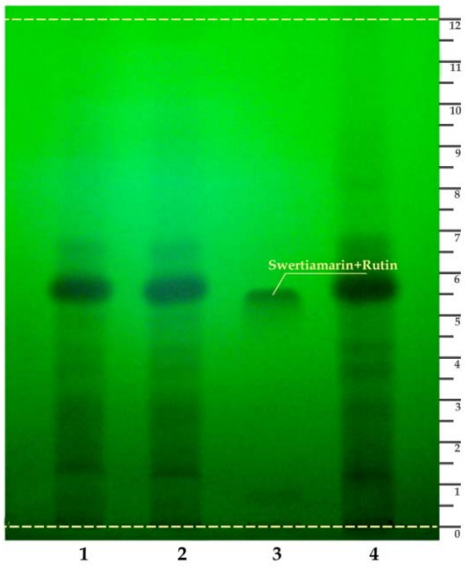

Figure 5 – Chromatogram in UV light at 254 nm, obtained under the conditions of DAB 10 analysis on conventional analytical plate: 1 – CC series 15; 2 – CC series 18; 3 – SS of swertiamarin and rutin; 4 – CC series 21

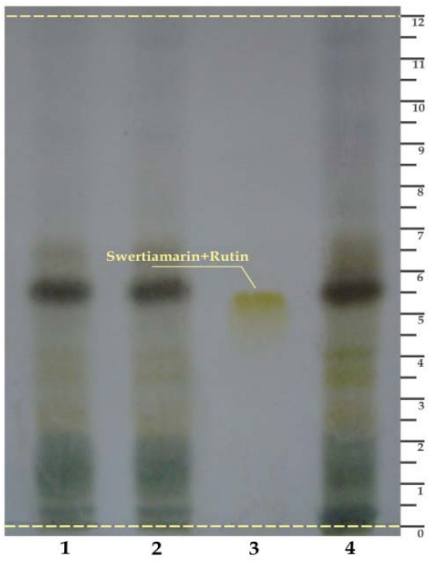

Figure 6 – Chromatogram in daylight after processing with anisaldehyde, obtained under the conditions of DAB 10 analysis on conventional plate

In conditions of identification method by BHP, a yellow-orange fluorescent zone was found in the reference solution area. It shows that mentioned chromatographic conditions are not suitable for the separation of specific absorption bands of rutin and swertiamarin (Figure 7). Turquoise fluorescent band in the test solution area with R_f about 1,5, and seven different closely located yellow-orange fluorescent zones, with R_f from 0,3 to 1,1 were observed, that meets the requirements of BHP. Under BHP method conditions, a long chromatography time compared with PhEur 8.4 considered as disadvantage (almost 3 times longer).

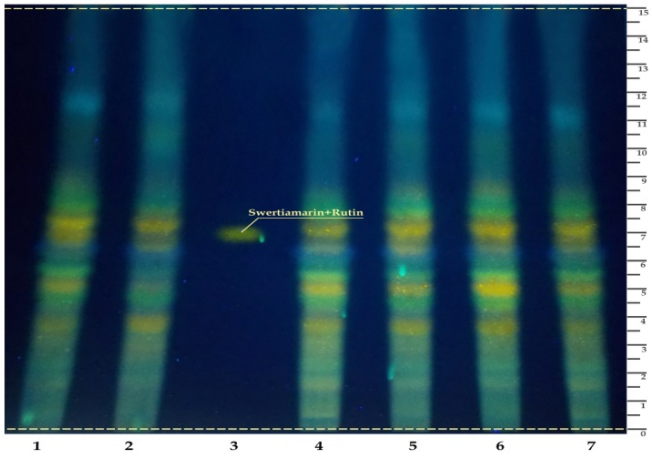

Figure 7 – Chromatogram in UV light at 365 nm, obtained under the conditions of BHP analysis on a conventional plate: 1 – CC series 15; 2 – CC series 16; 3 – SS of swertiamarin and rutin; 4 – CC series 17; 5 – CC series 18; 6 – CC series 19; 7 – CC series 20

Based on the aforementioned studies, the most appropriate chromatographic conditions for swertiamarin and rutin separation are PhEur 8.4 analysis conditions. It should be noted that under these conditions a reliable identification of specific biologically active substances, which are part of the MHM of Centaury herb, is carried out.

To implement in the SPhU national quality requirements to domestic MHM, a new method for identification of biologically active substances in Centaury herb using TLC method was developed.

In Ukraine, quality control of herbal material and remedies on its basis is provided by using respective pharmacopoeial standard samples as marker substances. As is known, respective standard samples are quite expensive, that threatens pharmaceutical quality control of MHM actual carrying out. One of important areas of standardization in the SPhU is a certification of standardized herbal extracts as pharmacopoeial standard samples (PSS), containing all the necessary substances and markers, that can reduce reference materials acquisition costs to a reasonable level [16].

Using described algorithm of PSS SPhU development for herbal extracts [16], in State Enterprise «Pharmacopoeial Center» the PSS SPU of Centaury extract was developed, for which it has been studied such issues as component composition of the PSS under the conditions of method used; selection of extract concentration before using in the method; development of sample preparation to ensure reproducible results. Using the given PSS SPhU allowed to replace the expensive reference substance (swertiamarin), providing accurate identification of the same classes of compounds in the raw material, as in conditions of PhEur 8.4 (Figure 8–11).

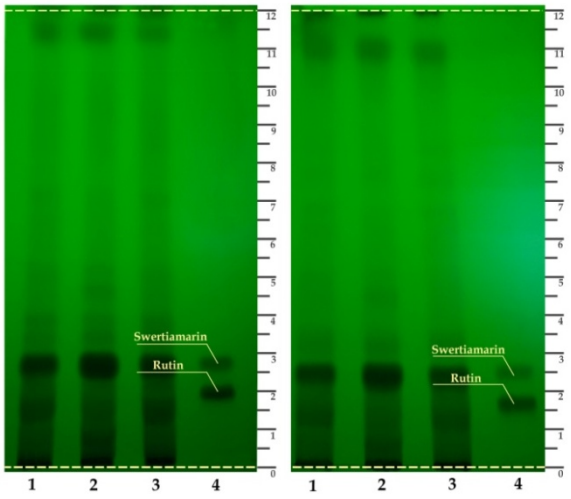

Figure 8 –Chromatogram in UV light at 254 nm on a conventional plate with aluminum and glass substrates (from left to right): 1 – CP; 2 – CC; 3 – PSS SPhU Centaury extract; 4 – SS of swertiamarin and rutin

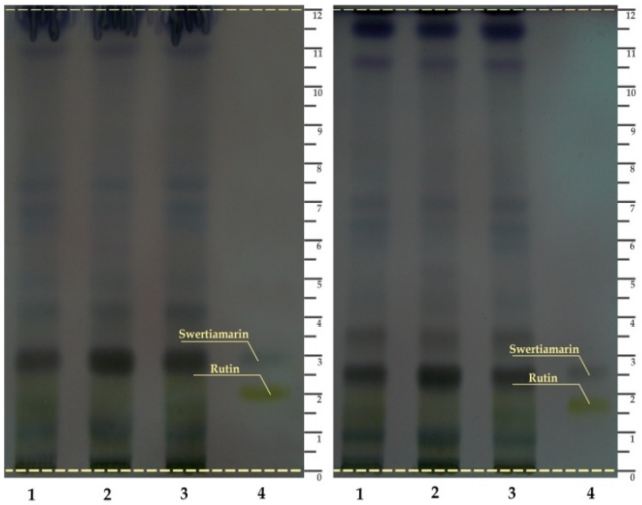

Figure 9 – Chromatogram in daylight after treatment with anisaldehyde on a conventional plate with aluminum and glass substrates (from left to right)

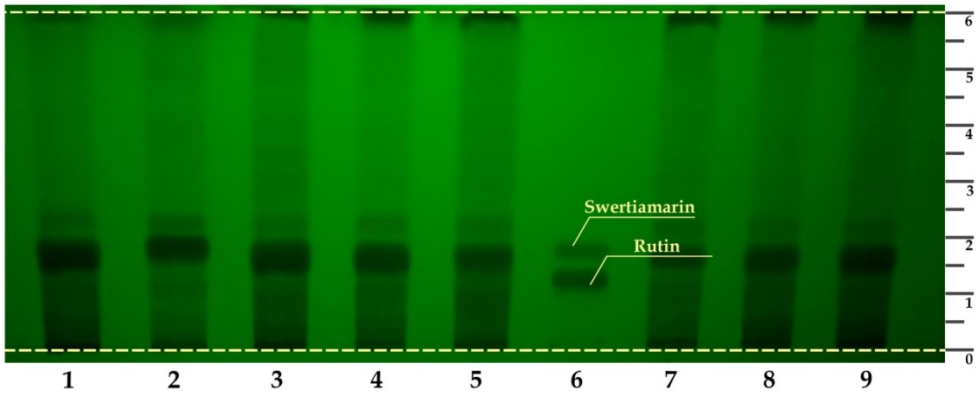

Figure 10 – Chromatogram in UV light of 254 nm, obtained under the conditions of analysis on HPTLC-plate: 1 – CC series 15; 2 – CP, 3 – CC series 2; 4 – CC series 17; 5 – PSS SPU Centaury extract; 6 – SS of swertiamarin and rutin; 7 – CC series 18; 8 – CC series 19; 9 – CC series 21

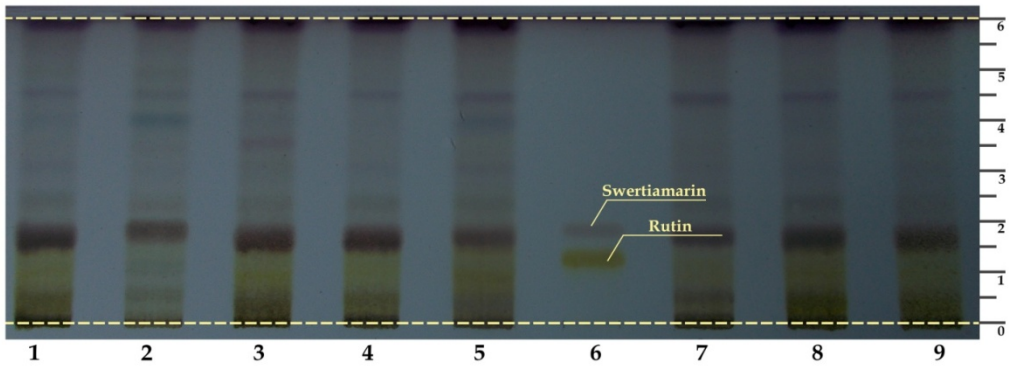

Figure 11 – Chromatogram in daylight after treatment with anisaldehyde, obtained in the conditions of the developed method on HPTLC-plate

According to preliminary investigations, the optimal conditions for Centaury herb chromatographic research have been chosen in accordance with the requirements of PhEur 8.4. In the context of PhEur 8.4 analysis mobile phase of water – formic acid anhydrous – ethyl formate (4:8:88) was used. During the new method development, the expensive reagent – ethyl formate was proposed to be replaced by more affordable solvent – ethyl acetate, which had a significant advantage in terms of chromatography – chromatography time reduction.

Conventional plates for TLC and HPTLC were used for research. The volume of solution applied to a conventional TLC plates with aluminum (AS) and glass substrate (GS) was 10 µl (on HPTLC-plate – 5 µl), the level of solvent front from the starting line was 12 cm (for HPTLC – 6 cm). Identification of the specific zones was performed under UV light (at 254 nm) and in the daylight after processing with anisaldehyde solution and heating (t = 105 ˚C).

It was found that all studied samples of MHM had the same chromatographic profile which matches the profile of PSS SPhU Centaury extract. Swertiamarin Rf zone value in the chromatogram of solution, prepared from the standard sample of swertiamarin, coincided the R_f value of the same zone of herbs Centaury solutions and PSS SPhU Centaury extract. Determination results are shown in Table 2.

Table 2 – Comparison of Rf zone of swertiamarin

TLC plates	Rf value of swertiamarin zone		
	AS	PSS SPhU extract	MHM
With AS	0,23	0,23	0,23
With GS	0,21	0,21	0,21
HPTLC	0,12	0,12	0,12

The given PSS SPhU Centaury extract contains essential substance-marker – swertiamarin and allows for reliable identification of herbal drugs in accordance with the approaches of standardization, acceptable for PhEur 8.4.

Thus, harmonization of the SPhU requirements with PhEur 8.4 requirements was carried out, which allowed to develop national quality requirements of MHM for the monograph of SPhU «Centaury herb».

Conclusion.

1. A comparative analysis of chromatographic conditions of methods for Centaury herbs identification using TLC in accordance with the requirements of the monographs of the leading pharmacopoeias showed that the most appropriate chromatographic conditions for swertiamarin and rutin separation are PhEur 8.4 requirements, wherein a reliable identification of specific biologically active substances, which are part of MHM Centaury herb, takes place.

2. When developing the new method of identification BAS of Centaury herb by TLC method, the approach of SPhU to standardization of MHM was used: it was suggested to replace expensive standard sample of swertiamarin by PSS SPhU Centaury extract, which ensures optimal and acceptable conditions

for the quality control of domestic MHM Centaury herb. Expensive reagent – ethyl formate was replaced by more affordable solvent – ethyl acetate.

3. A new method for identification of Centaury herb BAS by TLC method was offered for the implementation of national quality requirements of MHM in the monograph of SPhU «Centaury herb».

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УКРАИНА МЕМЛЕКЕТТІК ФАРМАКОПЕЯСЫ МОНОГРАФИЯСЫНА АРНАЛҒАН ЖҰҚА ҚАБАТТЫ ХРОМАТОГРАФИЯ ӘДІСІМЕН ШАТЫРША ТОЛҒАҚШӨБІНІҢ НЕГІЗДЕНДІРУ ӘДІСТЕМЕСІН ЖАСАУ

Аннотация. Мақалада Шатырша толғақшөбінің шикізаттың 7 топтамасында «Негіздендіру. ЖҚХ Әдісі» көрсеткіші бойынша фармакопеялық талаптарын салыстыру тәжірибе нәтижелері келтірілген. Стандартты үлгі ретінде свертиамарин және рутин таңдалды. Алынған маркерлердің бөліп алу хроматографиялық жағдайлары европалық фармакопея PhEur 8.4. талаптары бойынша ең қолайлы болып табылды.

Шатырша толғақшөбінің ББЗ негіздеу ЖҚХ әдісімен қымбат бағалы свертиамарин маркер-затты тиімді УМФ ҰФС Шатырша толғақшөбінің экстрактіне және қымбат бағалы этилформиат реактивін тиімді этилацетат еріткішіне ауыстырып жаңа әдістемесі жасалған. Шатырша толғақшөбінің УМФ монографиясына отандық шикізаттың сапасына мемлекеттік талаптар өңдеу мақсатында УМФ мен PhEur 8.4 талаптарының үйлестіру жүргізілді.

Түйін сөздер: шатырша толғақшөбі, негіздендіру, жұқа қабатты хроматография, Украина мемлекеттік фармакопеясы.

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РАЗРАБОТКА МЕТОДИКИ ИДЕНТИФИКАЦИИ ТРАВЫ ЗОЛОТОТЫСЯЧНИКА МЕТОДОМ ТОНКОСЛОЙНОЙ ХРОМАТОГРАФИИ ДЛЯ МОНОГРАФИИ ГОСУДАРСТВЕННОЙ ФАРМАКОПЕИ УКРАИНЫ

Аннотация. В статье представлены экспериментальные результаты сравнения фармакопейных требований по показателю «Идентификация. Метод ТСХ» травы золототысячника на 7 сериях сырья. В качестве стандартных образцов (веществ-маркеров) были выбраны свертиамарин и рутин. Показано, что наиболее приемлемыми хроматографическими условиями разделения этих маркеров являются требования PhEur 8.4. Разработана новая методика идентификации БАВ золототысячника методом ТСХ с заменой дорогостоящего вещества-маркера свертиамарина на более доступный ФСО ГФУ экстракт золототысячника и дорогого реактива – этилформиата на более доступный растворитель – этилацетат. Проведена гармонизация требований ГФУ с PhEur 8.4 с целью разработки национальных требований к качеству отечественного сырья для монографии ГФУ «Золототисячнику трава».

Ключевые слова: золототысячник, трава, идентификация, тонкослойная хроматография, Государственная фармакопея Украины.

CONTENT

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