Modern Methods of Standardization of Biologically Active Compounds of Medicinal Plant Raw Materials Calendulae Flos: Chemical Technologies, Analytical Indicators

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Abstract. Herbal preparations are an important source of biologically active compounds, have a wide range of pharmacological activity. Calendula officinalis L. is the most popular plant and is widely used in medicine. The medicinal plant raw material of Calendula officinalis L. is Calendulae flos. The composition of biologically active compounds includes flavonoids, carotenoids, saponins, essential oils and triterpene compounds. Preparations based on Calendulae flos have anti-inflammatory, antiseptic, wound-healing and antispasmodic properties. An important component of the effectiveness and safety of phytotherapy is considered to be the proper quality of the derived medicinal plant raw material Calendulae flos, modern chemical technologies for obtaining biologically active compounds, issues of standardization and quality control. Modern methods of standardization for Calendulae flos raw material are presented in the national monograph State Pharmacopoeia of Ukraine on "Nahidok kvitky" and the monograph European Pharmacopoeia on "Calendula flower". In both monographs, the standardization of Calendulae flos is carried out according to the quantitative content of the sum of flavonoids in terms of hyperoside with regulation (not less than 0.4%). Recent advances in analytical chemistry, such as HPLC-UV/Vis, HPTLC densitometry and hyphenated LC-MS techniques, allow rapid fingerprinting of marker flavonoids and triterpenoids, supporting batch-to-batch reproducibility. Good Agricultural and Collection Practice guidelines now recommend controlled cultivation of certified cultivars under defined pedoclimatic conditions to maximize calenduloside and faradiol ester yields and to minimize pesticide residues and heavy metals. Clinical observations also suggest adjunctive use of standardized calendula extracts accelerates epithelialization in radiation-induced dermatitis and post-surgical wounds, highlighting the translational relevance of rigorous phytochemical standardization.

Keywords: Calendula officinalis L., Calendulae flos, Calendula flower, standardization of raw materials, State Pharmacopoeia of Ukraine, European Pharmacopoeia.

Introduction. Pharmacotherapy with herbal medicines (phytopreparations) remains relevant in the world against the background of Covid, post-Covid, long-Covid, chronic, comorbid disorders in accordance with the ICD-11 [1-7].

Herbal medicines are an important source of biologically active compounds, have a wide range of pharmacological activity [8-10].

In recent decades, interest in herbal medicine has increased significantly due to the increasing resistance of pathogenic microorganisms to synthetic drugs, as well as the trend towards the use of environmentally safe and bioavailable treatments. In many countries of the world, herbal medicines are used both in official medicine and in traditional treatment systems [11-13].

However, despite the popularity of herbal medicine, one of the key challenges remains the provision of modern chemical technologies for obtaining biologically active compounds, methods of quality control and standardization of medicinal plant raw materials. Standardization is a critically important aspect, since the lack of clear regulations for determining the quality indicators of raw materials can lead to variability in the chemical composition, and therefore to changes in the therapeutic efficacy of herbal medicines. The quality of medicinal plant raw materials is determined not only by their botanical authenticity, but also by the content of biologically active compounds that provide pharmacotherapeutic effects [14-16].

The main criteria for standardizing herbal medicines are organoleptic, physicochemical and microbiological indicators, as well as determining the content of the main biologically active compounds. The regulatory documents regulating the quality of medicinal plant raw materials include

such methods of analysis as thin-layer chromatography, liquid and gas chromatography, UV spectrophotometry, etc. These methods allow not only to confirm the identity of medicinal raw materials, but also to detect possible impurities, determine the concentration of active substances, which directly affects the effectiveness of the drug [17-21].

The source for obtaining herbal medicines is plant raw materials, which may include various vegetative organs of officinal and non-officinal medicinal plants [22-24].

Interdisciplinary research in cooperation between research institutions, organizations, enterprises, laboratories, and centers plays an important role in innovative developments in phytotherapy. They provide prevention of medical errors and risks in the field of health care and pharmacy [25-28].

One of the most popular and widely used plants in medicine is calendula (Calendula officinalis L.). The medicinal plant raw material of Calendula officinalis L. is Calendulae flos. It contains biologically active compounds flavonoids, carotenoids, saponins, essential oils, and triterpenoids. Calendula-based preparations have anti-inflammatory, antiseptic, wound-healing, and antispasmodic properties. That is why Calendula officinalis L. is used in dermatology, gastroenterology, dentistry, gynecology and many other areas of medicine [29].

However, the quality control of calendula preparations has certain peculiarities. One of the main aspects is the standardization of medicinal plant raw materials, which includes determining the quantitative and qualitative composition of active components, controlling impurities and ensuring compliance with the requirements of pharmacopoeial articles. Monitoring the storage and transportation conditions of raw materials is also important, since the instability of biologically active compounds can lead to the loss of pharmacological properties of the finished product. [29].

Thus, the issue of standardization of medicinal plant raw materials of calendula is extremely relevant in the context of ensuring the effectiveness and safety of herbal medicines. Improving quality control methods, implementing modern analytical techniques and harmonizing regulatory requirements will contribute to increasing the level of standardization of medicinal plant raw materials and guaranteeing the stability of the therapeutic properties of herbal medicines based on calendula.

The purpose of the study was to review the methods of standardization of Calendulae flos in accordance with the requirements of the State Pharmacopoeia of Ukraine and the European Pharmacopoeia.

To achieve this goal, the following tasks were solved (Fig. 1) [30, 31].

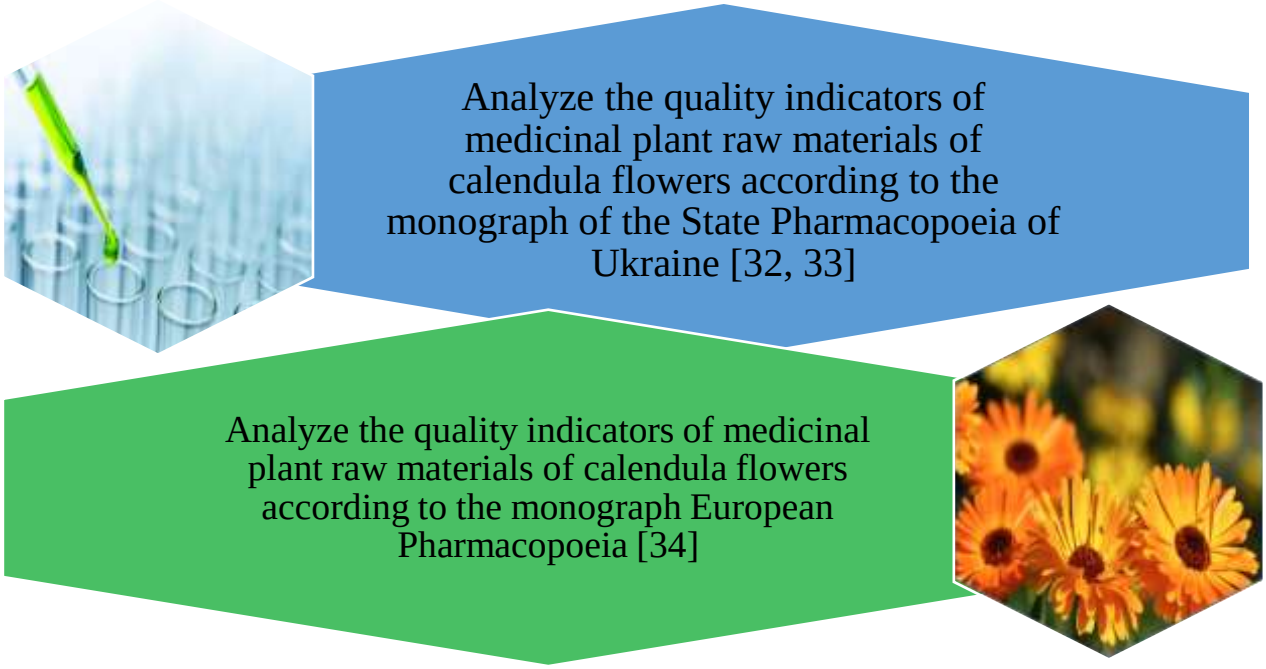

Fig. 1. Research objectives [30, 31].

Materials and methods. Research period – January-March 2025. The main research material is Calendula officinalis L., Calendulae officinalis flos, Calendula officinalis flowers, Calendulae officinalis flos, Marigold, Marygold, Garden marigold, Pot marigol. Asteraceae [35]. Pharmacopoeial standard samples (caffeic acid, chlorogenic acid, rutin, calendulosides) used in the standardization of Calendula officinalis flos raw materials [32, 33].

The following materials were used to study Calendula officinalis flos raw materials:

- State Pharmacopoeia of Ukraine 2nd Edition [32, 33];
- European Pharmacopoeia 11th ed. [34].

Research methods: chemical-technological, normative, documentary, retrospective, bibliographic, comparative, graphic, physical and chemical methods of analysis.

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Results and discussion. Preparations from Calendula officinalis L. are widely popular in phytotherapy. An important component of the effectiveness and safety of phytotherapy using calendula preparations is considered to be the proper quality of the derived medicinal plant raw materials, the effectiveness of chemical technologies for obtaining biologically active compounds, standardization and quality control of intermediate and final products. The main physicochemical methods of standardization (thin-layer chromatography, liquid and gas chromatography, UV spectrophotometry) are included in the regulatory documents. They allow you to control the release and quality of biologically active compounds. The source for obtaining calendula herbal preparations is medicinal plant raw materials obtained from the flowers of medicinal plants. Their quality control has certain features [29].

Modern methods of standardization for raw materials Calendula officinalis flos are presented in the national monograph State Pharmacopoeia of Ukraine on "Calendula flos" and the monograph European Pharmacopoeia on "Calendula flower. Calendula flos". In both monographs, standardization of Calendula flos is carried out by the quantitative content of the sum of flavonoids in terms of hyperoside with regulation (not less than 0.4%) [32, 33, 34].

The results of comparing the requirements for the quality of raw materials Calendula flos are given in Tables 1-8.

Table 1. Calendula flos: quality indicator macroscopic examination (Identification A) in comparison in the State Pharmacopoeia of Ukraine and European Pharmacopoeia.

Quality indicators	State Pharmacopoeia of Ukraine National monograph Calendulae flos	European Pharmacopoeia Calendula flower Calendulae flos
Macroscopic examination (Identification A)	Whole or those whose flowers have partially fallen off, baskets up to 5 cm in diameter, without peduncles or with remnants of peduncles no more than 3 cm long. The involucre is gray-green, single- or double-rowed, of linear, pointed, densely pubescent leaves. The base of the basket is somewhat convex, bare. The marginal flowers are false-ligulate, reddish-orange, orange, bright or pale yellow in color, (15-28) mm long, (3-5) mm wide, arranged in (2-3) rows in non-double forms and in (10-15) rows in double	The ligulate florets consist of a yellow or orange-yellow ligule, about 3-5 mm wide and about 7 mm in the middle part, with a 3-toothed apex and a hairy, partly sickle-shaped, yellowish-brown or orange-brown tube with a projecting style and a bifid stigma occasionally with a partly bent yellowish-brown or orange-brown ovary. The tubular florets, about 5 mm long, are present and consist

	forms. The corolla of false-ligulate flowers has a curved, short, pubescent tube and a tridentate, with (4-5) veins, which is twice as long as the involucre. The pistil of these flowers has a bent lower, single-celled ovary, a thin column, and a two-lobed stigma. The central flowers are tubular with a five-toothed corolla, orange, yellowish-brown, or yellow.	of the yellow, orange-red or reddish-violet 5-lobed corolla and the yellowish-brown or orange-brown tube, hairy in its lower part, mostly with a partly bent yellowish-brown or orange-brown ovary.

Table 2. Calendulae flos: quality indicator microscopic examination (Identification B) in comparison with the State Pharmacopoeia of Ukraine and the European Pharmacopoeia.

Quality indicators	**State Pharmacopoeia of Ukraine National monograph Calendulae flos**	**European Pharmacopoeia Calendula flower Calendulae flos**
Microscopic examination (Identification B)	The raw material is ground into powder. The powder is yellowish-brown. Examine under a microscope using chloral hydrate solution. The powder shows the following diagnostic characters: fragments of epidermises of false ligulate or tubular flowers from elongated, covered with a folded cuticle cells with orange chromoplasts; fragments of epidermises of involucre leaves from elongated cells with straight or sinuous membranes and stomata of anomocytic type; integumentary hairs of false ligulate or tubular flowers multicellular, single-double row; integumentary hairs of involucre leaves long, single-double row; glandular hairs single-double row with a head of 2 or 4 cells; essential oil glands with a multicellular two-row stem and a large ovoid two-row multicellular head; spherical pollen grains, with a spiny exine.	The powder is yellowish-brown. Examine under a microscope using chloral hydrate solution. The powder shows the following diagnostic characters: fragments of epidermises of the corolla [C, F, K] containing light yellow oil droplets, some with fairly large anomocytic stomata (2.8.3) [Fa, Ka]; covering trichomes biseriate, multicellular and conical [G], usually fragmented, and glandular trichomes with a multicellular stalk [E], very abundant on the base of the corolla [D]; fragments of parenchyma of the corolla [B] containing prisms and very small cluster crystals of calcium oxalate [Ba, Da] and small vessels [Bb]; spherical pollen grains up to about 40 μm in diameter with a sharply spiny exine and 3 germinal pores [A, J]; occasional fragments of the stigmas with short, bulbous papillae [H] (see Fig. 2).

Comparative analysis of data (Tables 1, 2) revealed the following:

- *Macroscopic examination* (identification A) and *Microscopic examination* (Identification B) described in the monograph State Pharmacopoeia of Ukraine [33] and European Pharmacopoeia [34] are somewhat different. This may be due to different environmental factors, climatic conditions and soil in which Calendulae flos raw material grows. It should also be noted that the monograph State Pharmacopoeia of Ukraine provides a more detailed description for macroscopic features of the raw material. The monograph European Pharmacopoeia [34] for microscopic examination provides detailed diagnostic structures (Fig. 2).

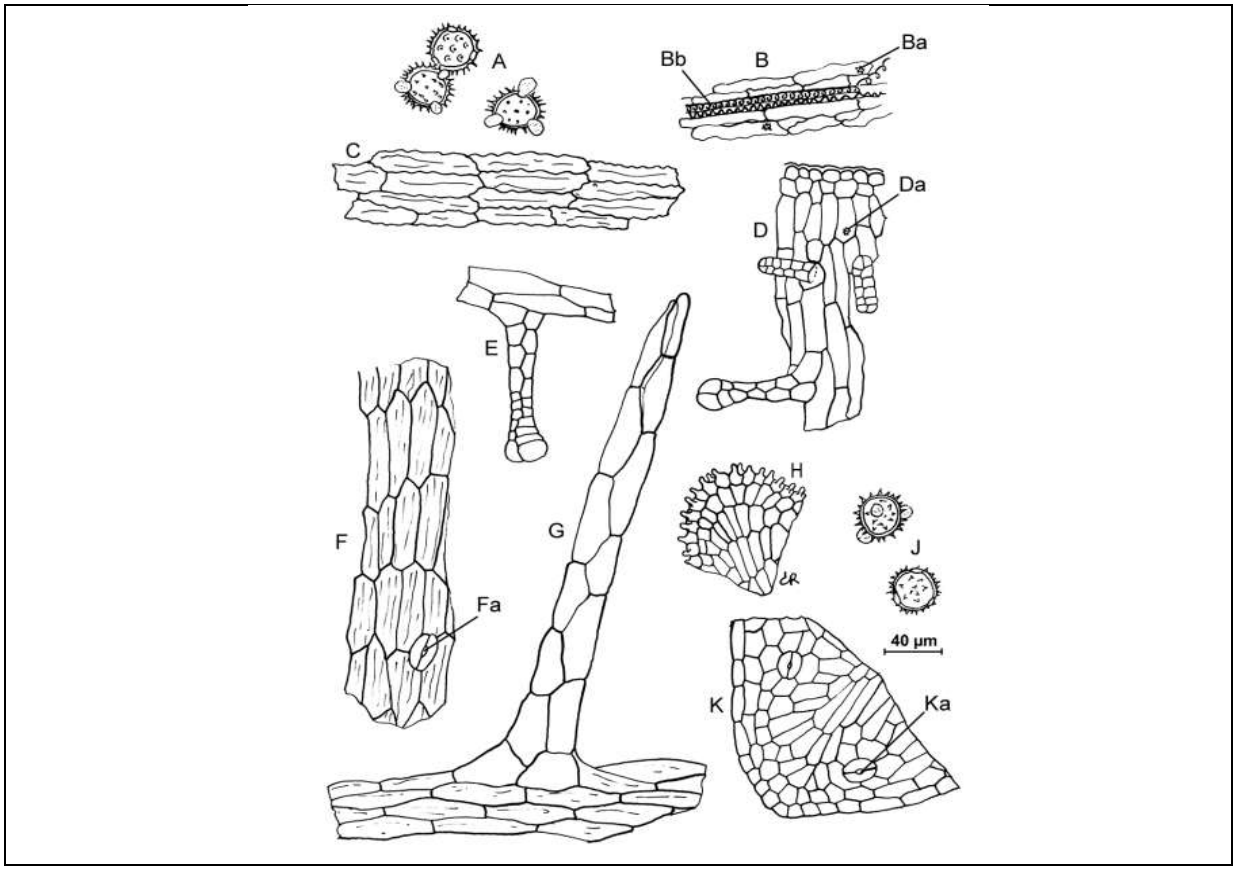

Fig. 2. Microscopic examination, diagnostic characters (illustration for identification B of powdered herbal drug of Calendula flos). [34]

Table 3. Calendulae flos: quality indicator Identification of flavonoids (Identification C) in comparison with the State Pharmacopoeia of Ukraine and the European Pharmacopoeia.

Quality indicators	**State Pharmacopoeia of Ukraine National monograph Calendulae flos**	**European Pharmacopoeia Calendula flower Calendulae flos**
Identification of flavonoids (Identification C)	Thin-layer chromatography: • Test solution. To 1.0 g of the powdered herbal drug (500) add 10 mL of methanol, heating on a water-bath under a reflux condenser for 10 min, cool and filter; • Reference solution. Dissolve 1.0 mg Chemical reference standard of State Pharmacopoeia of Ukraine caffeic acid, 1.0 mg chlorogenic acid and 2.5 mg rutoside trihydrate in 10 mL of methanol; • Plate: TLC silica gel plate; • Mobile phase: anhydrous formic acid, water, ethyl acetate (10:10:80); • Application: 20 µL of Test solution and 10 µL of Reference solution, as bands; • Development: 10 cm from the lower edge of the plate. • Drying: at temperature 100-105°C • Detection: spray the warm plate with a 10 g/L solution of diphenylboric acid	High-performance thin-layer chromatography. • Test solution. To 0.5 g of the powdered herbal drug (355) add 5.0 mL of methanol. Sonicate for 15 min, filter or centrifuge and use the filtrate or supernatant. • Reference solution (a). Dissolve 3.0 mg of chlorogenic acid and 4.0 mg of isorhamnetin-3-O-rutinoside in methanol and dilute to 10.0 mL with the same solvent. • Reference solution (b). Dilute 2.5 mL of reference solution (a) to 10.0 mL with methanol. • Reference solution (c). Dissolve 2.5 mg of hyperoside and 3 mg of chlorogenic acid in methanol

	aminoethyl ester in methanol, then with a 50 g/L solution of macrogol 400 in methanol; allow to dry in air for about 30 min and examine in ultraviolet light at 365 nm. • Results: the chromatogram of the reference solution shows: in the lower part — yellow-brown fluorescence zone (rutoside trihydrate), in the middle part — light blue fluorescence zone (chlorogenic acid), in the upper part – light blue fluorescence zone (caffeic acid). • The chromatogram of the test solution shows a yellow-brown fluorescence zone at the level of the rutoside trihydrate on the chromatogram obtained with reference solution; • lower and directly above it have to be yellowish-green fluorescence zone and light blue fluorescence zone (chlorogenic acid) present in the chromatogram obtained with reference solution; • above it – yellowish-green fluorescence zone and light blue fluorescence zone slightly below the zone corresponding to caffeic acid in the chromatogram obtained with reference solution. • Furthermore, in the chromatogram obtained with the test solution, other zones may be present.	and dilute to 10 mL with the same solvent. • Intensity marker: isorhamnetin-3-O-rutinoside. • Plate: TLC silica gel F254 plate (2-10 μm). • Mobile phase: anhydrous formic acid, water, ethyl acetate (10:10:80 V/V/V). • Application: 4 μL as bands of 8 mm. • Development: 70 mm from the lower edge of the plate. • Drying: in a current of air at room temperature for 5 min. • Detection: heat at 100-105ºC for 5 min; spray the warm plate with a 10 g/L solution of diphenylboric acid aminoethyl ester in methanol, then with a 50 g/L solution of macrogol 400 R in methanol or, alternatively, dip the warm plate in a 5 g/L solution of diphenylboric acid aminoethyl ester in ethyl acetate and then in a 50 g/L solution of macrogol 400 in methylene chloride; allow to dry in air for about 1 min and examine in ultraviolet light at 366 nm. • System suitability: reference solution (c): – the chromatogram shows in the middle third 2 distinct zones, which may be touching; the lower zone (chlorogenic acid) shows a light blue fluorescence and the upper zone (hyperoside) shows a yellow or orange fluorescence. • Results: see below the sequence of fluorescent zones present in the chromatograms obtained with reference solution (a) and the test solution. Furthermore, in the chromatogram obtained with the test solution, other faint to very faint blue, brown or orange fluorescent zones may be present.

Comparative analysis of the data presented in Table 3 revealed the following:

- Identification of flavonoids (Identification C): carried out by the Thin-layer chromatography (TLC) method [36] according to the monograph of the State Pharmacopoeia of Ukraine [33];
- the monograph of the European Pharmacopoeia [34] carries out the analysis by the more modern and multifunctional method of High-performance thin-layer chromatography [37];
- the difference in methods affects the conditions for carrying out the identification of flavonoids; the method of preparing the test solution in both methods is somewhat different. Although the concentration remains the same;
- for the preparation of the reference solution according to the State Pharmacopoeia of Ukraine [33], the Chemical reference standard of the State Pharmacopoeia of Ukraine caffeic acid, the Chemical reference standard of the State Pharmacopoeia of Ukraine chlorogenic acid and the Chemical reference standard of the State Pharmacopoeia of Ukraine rutoside trihydrate are used;
- several reference solutions are prepared according to the method of the European Pharmacopoeia [34]. The reagents used are chlorogenic acid, isorhamnetin-3-O-rutinoside, hyperoside, of which isorhamnetin-3-O-rutinoside is an intensity marker. Different plates are used for applying solutions according to the Thin-layer chromatography [36] and High-performance thin-layer chromatography [37] methods; the sample volume and the distance that the mobile phase must travel are also different (Table 1);
- the mobile phase, drying and detection are identical in both monographs;
- the results after the test are provided by the State Pharmacopoeia of Ukraine [33] in the form of a description and location of the test solution zones in relation to the reference solution. In the European Pharmacopoeia [34] monograph, the results are presented in the form of a table (Fig. 3) with the corresponding description and location of the zones.

Top of the plate	
	2 blue zones, faint to equivalent
_____	_____
	A greenish-yellow zone, faint
Chlorogenic acid: a light blue zone	A light blue zone, faint to equivalent
_____	_____
Isorhamnetin-3-*O*-rutinoside: a greenish-yellow zone	A greenish-yellow zone (isorhamnetin-3-*O*-rutinoside) A brown or orange zone, faint to equivalent
	A greenish-yellow zone
	A brownish-orange zone, very faint to faint
Reference solution (a)	**Test solution**

Fig. 3. Picture of the results of chromatography of Test solution and Reference solution (a), Identification of flavonoids (Identification C) according to the monograph of the European Pharmacopoeia "Calendulae flos" [34].

Comparative analysis of the data given in Table 4 revealed the following:

- section Identification of calendulosides (Identification D): carried out by the method Thin-layer chromatography (2.2.27) [36] according to the monograph [33]. The methodology describes the conditions for carrying out the identification of calendulosides.

  In the monograph European Pharmacopoeia [34] there is no such section.

According to the results of comparing the quality indicators of Calendulae flos given in the monograph State Pharmacopoeia of Ukraine [33] and the monograph European Pharmacopoeia [34] (Tables 3, 4), some differences were found, namely the identification of Biologically Active Compounds as flavonoids in Calendulae flos. The use of the method High-performance thin-layer chromatography [37] according to the monograph European Pharmacopoeia [34] is currently the most modern and effective method for determining Biologically Active Compounds, which allows not only to identify substances, but also to carry out their quantitative determination. The disadvantage of using this method is the high cost of equipment.

Table 4. Calendulae flos: quality indicator Identification of calendulosides (Identification D) in comparison with the State Pharmacopoeia of Ukraine and the European Pharmacopoeia.

Quality indicators	**State Pharmacopoeia of Ukraine National monograph Calendulae flos**	**European Pharmacopoeia Calendula flower Calendulae flos**
Identification of calendulosides (Identification D)	Thin-layer chromatography: • Test solution. To 1.0 g of the powdered herbal drug (500) add 20 mL of ethanol (50% V/V) heating on a water-bath under a reflux condenser for 30 min, cool and filter; The filtrate is evaporated under residual pressure to a volume of about 5 ml, cooled and filtered through a paper filter. The precipitate on the filter is washed with 5.0 ml of water and washed with 5.0 ml of methanol. The resulting methanol filtrate is used. • Reference solution. To 5.0 mg of the Chemical reference standard of State Pharmacopoeia of Ukraine calendulosides add 5 mL of, mix, settle and use the supernatant; • Plate: TLC silica gel plate; • Mobile phase: chloroform, glacial acetic acid, methanol, water (35:16:6:4); • Application: 20 µL as bands; • Development: 10 cm from the lower edge of the plate. • Drying: in a current of air for 10 min. • Detection: spray with anisaldehyde solution, and heat at 100-105°C for 10 min, examine in daylight. • Results: the chromatogram of the reference solution shows: in the middle part – two main clearly separated blue-violet zones. The chromatogram of the test solution shows: at least two clearly separated blue-violet zones at the level of the corresponding zones on the	-

	chromatogram of the reference solution (calendulosides).	

Thin-layer chromatography [36] method, which is described in the monograph State Pharmacopoeia of Ukraine [33] is still relevant and effective enough for the determination of Biologically Active Compounds, while not requiring expensive equipment. It should also be noted that in the monograph State Pharmacopoeia of Ukraine [33] an additional method for the identification of calendulosides, Biologically Active Compounds Calendulae flos, the standardization of which can reach 2-10% [29].

Table 5. Calendulae flos: quality indicator Tests (Foreign matter) in comparison in the State Pharmacopoeia of Ukraine and the European Pharmacopoeia.

Quality indicators	**State Pharmacopoeia of Ukraine National monograph Calendulae flos**	**European Pharmacopoeia Calendula flower Calendulae flos**
Foreign matter	Maximum 6% of flower stalk residues, including those separated during analysis; no more than 20% of baskets without flowers (bed of baskets with wrappers); no more than 3% of browned baskets; no more than 3% of other parts of the plant (pieces of stems and leaves); no more than 1% foreign parts, no more than 0.5% of impurities of mineral origin.	Maximum 5% of bracts and maximum 2% of other foreign matter

Comparative analysis of the data presented in Table 5 revealed the following:

- Tests (Foreign matter) quality indicator [38], according to the monograph State Pharmacopoeia of Ukraine [33] foreign matter regulation is more extensive (Table 2), gives different standardization depending on the nature of the impurity. The monograph European Pharmacopoeia [34] defines impurities according to two criteria and gives standardization of no more than 5% for bracts and no more than 2% for other foreign matter.

Table 6. Calendula flos: Tests (Loss on drying) quality indicator in comparison in the State Pharmacopoeia of Ukraine and European Pharmacopoeia.

Quality indicators	**State Pharmacopoeia of Ukraine National monograph Calendulae flos**	**European Pharmacopoeia Calendula flower Calendulae flos**
Loss on drying	Maximum 14.0%, determined on 1.000 g of the powdered herbal drug by drying in an oven at 105ºC	Maximum 12.0%, determined on 1.000 g of the powdered herbal drug by drying in an oven at 105ºC for 2 h.

Comparative analysis of the data presented in Table 6 revealed the following:

- the quality indicator Tests (Loss on drying) [39], which is described in the monograph State Pharmacopoeia of Ukraine [33], gives a regulation of maximum 14.0% by drying in an oven at 105°C. The monograph European Pharmacopoeia [34] gives a regulation of maximum 12.0% by drying in an oven at 105ºC for 2 h.

Table 7. Calendulae flos: quality indicator Tests (Total ash) in comparison in the State Pharmacopoeia of Ukraine and the European Pharmacopoeia.

Quality indicators	State Pharmacopoeia of Ukraine National monograph Calendulae flos	European Pharmacopoeia Calendula flower Calendulae flos
Total ash	Maximum 11.0%	Maximum 10.0%

Comparative analysis of the data presented in Table 7 revealed the following:

- the quality indicator Tests (Total ash) [40] according to the monograph State Pharmacopoeia of Ukraine [33] and the monograph European Pharmacopoeia [34] has almost the same regulation with a difference of 1% (Table 7);

Table 8. Calendulae flos: quality indicator ASSAY (Content of flavonoids) in comparison in the State Pharmacopoeia of Ukraine and the European Pharmacopoeia.

Quality indicators	State Pharmacopoeia of Ukraine National monograph Calendulae flos	European Pharmacopoeia Calendula flower Calendulae flos
ASSAY (Content of flavonoids)	Absorption spectrophotometry, ultraviolet and visible. • Stock solution. Into a 100 mL round-bottomed flask introduce 0.800 g of the powdered herbal drug (500) (2.9.12), 1 mL of a 5 g/L solution of hexamethylenetetramine R, 7 mL of hydrochloric acid R1 and 20 mL of acetone R. Boil the mixture under a reflux condenser for 30 min. Filter the liquid through a plug of absorbent cotton into a 100 mL volumetric flask. Add the absorbent cotton to the residue in the round-bottomed flask and extract with 2 quantities, each of 20 mL, of acetone R, each time boiling under a reflux condenser for 10 min. Allow to cool to room temperature, filter the liquid through a plug of absorbent cotton, then filter the combined acetone solution through a filter-paper into the volumetric flask, and dilute to 100.0 mL with acetone R by rinsing the flask and filter. Introduce 20.0 mL of this solution into a separating funnel, add 20 mL of water R and extract the mixture with 1 quantity of 15 mL and then with 3 quantities, each of 10 mL, of ethyl acetate R. Combine the ethyl acetate extracts in a separating funnel, rinse with 2 quantities, each of 50 mL, of water R, filter the extract over 10 g of anhydrous sodium sulfate R into a 50 mL	Absorption spectrophotometry, ultraviolet and visible. • Stock solution. Into a 100 mL round-bottomed flask introduce 0.800 g of the powdered herbal drug (500) (2.9.12), 1 mL of a 5 g/L solution of hexamethylenetetramine R, 7 mL of hydrochloric acid R1 and 20 mL of acetone R. Boil the mixture under a reflux condenser for 30 min. Filter the liquid through a plug of absorbent cotton into a 100 mL volumetric flask. Add the absorbent cotton to the residue in the round-bottomed flask and extract with 2 quantities, each of 20 mL, of acetone R, each time boiling under a reflux condenser for 10 min. Allow to cool to room temperature, filter the liquid through a plug of absorbent cotton, then filter the combined acetone solution through a filter-paper into the volumetric flask, and dilute to 100.0 mL with acetone R by rinsing the flask and filter. Introduce 20.0 mL of this solution into a separating funnel, add 20 mL of water R and extract the mixture with 1 quantity of 15 mL and then with 3 quantities, each of 10 mL, of ethyl acetate R. Combine the ethyl acetate extracts in a separating funnel, rinse with 2 quantities, each of 50 mL, of water R, filter the extract over 10 g

	volumetric flask and dilute to 50.0 mL with ethyl acetate R. • Test solution. To 10.0 mL of the stock solution add 1 mL of aluminium chloride reagent R and dilute to 25.0 mL with a 5% V/V solution of glacial acetic acid R in methanol R. • Compensation liquid. Dilute 10.0 mL of the stock solution to 25.0 mL with a 5% V/V solution of glacial acetic acid R in methanol R. • Measure the absorbance (2.2.25) of the test solution after 30 min, by comparison with the compensation liquid at 425 nm. • Calculate the percentage content of flavonoids, expressed as hyperoside, taking the specific absorbance of hyperoside to be 500. • Content: minimum 0.4% of flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; Mr 464.4) (dried drug).	of anhydrous sodium sulfate R into a 50 mL volumetric flask and dilute to 50.0 mL with ethyl acetate R. • Test solution. To 10.0 mL of the stock solution add 1 mL of aluminium chloride reagent R and dilute to 25.0 mL with a 5% V/V solution of glacial acetic acid R in methanol R. • Compensation liquid. Dilute 10.0 mL of the stock solution to 25.0 mL with a 5% V/V solution of glacial acetic acid R in methanol R. • Measure the absorbance (2.2.25) of the test solution after 30 min, by comparison with the compensation liquid at 425 nm. • Calculate the percentage content of flavonoids, expressed as hyperoside, taking the specific absorbance of hyperoside to be 500. • Content: minimum 0.4% of flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; Mr 464.4) (dried drug).

Comparative analysis of the data given in Table 8 revealed the following:

• section ASSAY (Content of flavonoids), standardization of raw materials is carried out by the method of Absorption spectrophotometry, ultraviolet and visible [41] by quantitative Content of flavonoids expressed as hyperoside with regulation (minimum 0.4%) in accordance with the monograph of the State Pharmacopoeia of Ukraine [33] and the monograph of the European Pharmacopoeia [34]. The methods are identical.

Modern chemical technologies play a role in the standardization of Biologically Active Compounds of medicinal plant raw materials. Development of sensor means for monitoring the air environment at pharmaceutical enterprises is impossible without modern physicochemical methods (spectrophotometric, electrochemical, quantum-chemical) [42-46]. Further research in this direction is ongoing.

Conclusions. The review of methods for standardization of Calendulae flos (Calendula officinalis L.) in accordance with the requirements of the State Pharmacopoeia of Ukraine and the European Pharmacopoeia was conducted.

The comparative analysis of the quality indicators of medicinal plant raw materials Calendulae flos according to the monographs of the State Pharmacopoeia of Ukraine and the European Pharmacopoeia revealed the following:

- macroscopic examination and microscopic examination have some differences, which may be due to different environmental factors, climatic conditions and the soil in which the calendula raw materials grow;
- needs to note that the monograph of the State Pharmacopoeia of Ukraine gives a more detailed description for the macroscopic examination of the raw materials, and the monograph of the European Pharmacopoeia for microscopic examination provides detailed diagnostic characters;
- identification of flavonoids (Identification C) is carried out by the method of Thin-layer chromatography according to the monograph of the State Pharmacopoeia of Ukraine, and the monograph of the European Pharmacopoeia is carried out by the method of High-performance

thin-layer chromatography. The difference in methods affects the conditions for the identification of flavonoids.

The use of the High-performance thin-layer chromatography method according to the European Pharmacopoeia monograph is currently the most modern and effective method for determining Biologically Active Compounds, which allows not only to identify substances, but also to carry out their quantitative determination. The disadvantage of using this method is the high cost of equipment.

- The thin-layer chromatography method, which is described in the monograph of the State Pharmacopoeia of Ukraine, still remains relevant and quite effective for determining Biologically Active Compounds. It does not require expensive equipment. It should also be noted that the monograph of the State Pharmacopoeia of Ukraine additionally developed the method of Identification of calendulosides (Identification D), Biologically Active Compounds Calendulae flos, the standardization of which can reach 2-10%.
- The quality indicator Tests (Foreign matter), according to the monograph of the State Pharmacopoeia of Ukraine, the regulation is more extensive, gives different standardization depending on the nature of the impurity; the European Pharmacopoeia monograph defines impurities according to two criteria and gives a standardization of no more than 5% for bracts and no more than 2% for other foreign impurities;
- The quality indicator Tests (Loss on drying) according to the monograph State Pharmacopoeia of Ukraine gives a regulation of no more than 14.0%, while the European Pharmacopoeia monograph gives a regulation of no more than 12.0%, the difference is 2%;
- The quality indicator Tests (Total ash) according to the monograph State Pharmacopoeia of Ukraine and the monograph European Pharmacopoeia has almost the same regulation with a difference of 1%;
- Assay: Calendulae flos is carried out by the method of Absorption spectrophotometry, ultraviolet and visible by quantitative content of flavonoids expressed as hyperoside with a regulation (minimum 0.4%) according to the monograph State Pharmacopoeia of Ukraine and the monograph European Pharmacopoeia. The methods are fully harmonized and describe the same conditions.

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