EURASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES
Innovative Academy Research Support Center
IF = 9.2 www.in-academy.uz/index.php/ejmns

EXTRACTION AND STANDARDIZATION OF LUTEOLIN FROM SALVIA OFFICINALIS L. RAW MATERIAL: A REVIEW OF SCIENTIFIC LITERATURE

Yakubov Shohzodbek Uktamboy ugli
Tashkent pharmaceutical institute, Tashkent, Uzbekistan
yaqubovshohzod@gmail.com
https://doi.org/10.5281/zenodo.18043374

ARTICLE INFO

Received: 16th December 2025
Accepted: 23rd December 2025
Online: 24th December 2025

KEYWORDS

Salvia officinalis, luteolin, extraction, standardization, HPLC-DAD, ultrasound, microwave, ICH Q2 (R1).

ABSTRACT

In this literature review, a systematic analysis of scientific sources on the isolation (extraction) of luteolin belonging to the flavone class from the raw material Salvia officinalis L. (medicinal wormwood, "sage") and standardization at the preparative/analytical level was conducted. The review discussed the pharmacological significance of luteolin as a bioactive substance, its presence in Salvia officinalis (aglycone and glycosidic forms), the main technologies of extraction (massage, Soxhlet, reversible condensation under boiling, ultrasonic extraction, microwave extraction, liquid pressure extraction, eye solvent and green solvents), as well as the chain of enrichment and purification of luteolin (liquid-liquid distribution, SPE/C18, polyamide). Quantitative analysis based on HPLC-DAD, assessment of the total flavonoid content by the AlCl3 colorimetry method, as well as solutions according to the validation criteria (linearity, accuracy, reproducibility, resistance) according to ICH Q2 (R1) were systematized. The results showed the importance of distinguishing between 60-80% ethanol-water mixtures, hydrolyzed/non-hydrolyzed fractions, pH, and particle size regulation as optimal solvents for luteolin. Mechanisms for reducing industrial safety (GMP, residual solvents), series-to-series compatibility, and raw material variability were presented. As a result, a clear and practical protocol for luteolin was proposed: authentication - optimized extraction - purification - HPLC-DAD quantification - validation - stability - series control.

Input

Luteolin (3',4',5,7-tetrahydroxyflavone), belonging to the flavonoid group, is considered a relevant bioactive marker in pharmacology due to its antioxidant, anti-

inflammatory, and neuroneuroprotective properties. In the leaves and young shoots of Salvia officinalis L., luteolin and its glycosides (for example, luteolin-7-O-glucoside) accumulate to a certain extent; the extraction and quantitative control of these substances are crucial for the qualitative management of plant preparations and pharmacological research (specialized code 15.00.02) [2]. Standardization of luteolin includes not only analytical quantitative control, but also reduction of the variability of the raw material, adaptation of production technologies, as well as ensuring the stability of the series in dosage forms. In this review, we systematize the scientific foundations, technological parameters, and validated analytical methods for the isolation of luteolin from Salvia officinalis L. raw materials in the IMRAD format, propose a practical protocol, and critically evaluate the available sources.

Materials and methods

For this analytical review, sources published in 2000-2024 were searched in the Web of Science, Scopus, PubMed, and Google Scholar databases using the keywords "Salvia officinalis luteolin extraction," "sage flavonoids HPLC," "luteolin standardization." Inclusion criteria: (i) presence of luteolin/glucosides in S. officinalis, (ii) methods of extraction and purification, (iii) quantitative analysis based on HPLC/UPLC or UV-Vis, (iv) validation parameters. Output criteria: religious, political, or non-relevant materials. Data were separated: type and concentration of solvent, strict ratios (solvent: raw material), time, temperature, particle size, method (massage, Soxhlet, ultrasound, microwave, under pressure), hydrolysis conditions (acid/alkali), enrichment (liquid-liquid distribution, SPE), analytical conditions (column type, mobile phase, DAD wavelength), validation (linearity, LOQ/LLOD, reduction).

Results

Occurrence and chemical forms of luteolin in S. officinalis. The genus Salvia is rich in polyphenols; derivatives of caffeic acid, along with rosmarinic acid, flavones (apigenin, luteolin) are also among the leading components [2]. "In representatives of Lamiaceae, luteolin is often found in the form of 7-O-glucoside and under hydrolysis converts to aglycone" [2, pp. 126-128]. In our opinion, this opinion necessitates the separation of two audit traces in pharmaceutical standardization - the indicators of "general luteolin (after hydrolysis) " and "free luteolin (without hydrolysis)," since the ratio of the bioactive form in the finished drug can affect the clinical effect.

Extraction solvents and technological parameters. Luteolin has a polyphenolic structure and dissolves well in medium-polar solvents. In the literature, it is noted that 60-80% ethanol-water mixtures or 50-70% methanol-water mixtures are optimal [5]. "Solvent-water mixtures often outperform pure organic solvents in the separation of phenols" [4, pp. 3275-3278]. We fully agree with this observation, since the presence of water increases matrix swelling and mass transfer rate, which makes luteolin extraction effective. Also, conditions of pH around 4-6 reduce the oxidative degradation of flavones; ascorbic acid can be added in trace amounts as an antioxidant stabilizer.

Masseration, Soxhlet, reversible condensation. Classic mastering (24-48 hours, room temperature, solvent: raw material 10:1-20:1) simple, but slow; In Soxhlet (6-8 hours) and reversible condensation (70-80°C, 1-2 hours), the time is reduced, but the risk

of adverse effects on heat-sensitive polyphenols increases[4]. Therefore, when working with heat, inert atmospheric (nitrogen) and short-term modes are recommended.

Ultrasonic extraction (UAE) and microwave extraction (MAE). Ultrasonic cavitation quickly ruptures the cell walls, deeply absorbing the solvent into the matrix; High reductions were noted at 30-45°C, 20-40 minutes, with 60-80% ethanol [5]. MAE provides short and efficient separation with pulses of 60-120 seconds through dielectric heating. "UAE and MAE are characterized by high efficiency for phenol extraction and require less solvent" [4, pp. 3286-3290]. We prefer these approaches as "green" and reversible methods for luteolin.

Pressure liquid extraction (PLE) and green solvents. PLE (50-100 bar, 40-80°C) accelerates extraction in water or ethanol-water mixtures. Eye CO2 is poorly suited for polyphenols, but provides a certain reduction when ethanol is added as a cofluid. Ionic liquids/DES (in particular, choline-chloride-based DES) are able to solvate phenols well; however, the residual solvent requires control and requires caution within the framework of pharmaceutical regulatory requirements.

Hydrolysis of glycosides. To obtain an aglycone from luteolin glycosides, mild acid hydrolysis (0.5-1.0 M HCl, 70-80°C, 30-60 minutes) is used; strong conditions can lead to polymerization and decomposition. Liquid-liquid separation after hydrolysis (bringing to pH 5-6 after ethanol decomposition, then extraction with ethyl acetate) enriches luteolin [2, 7]. After liquid-liquid treatment, purification through SPE (C18), elution with methanol-water gradient, followed by partial drying (under 40°C, in vacuum) are recommended.

Cleaning and enrichment. Polyamide columns strongly interact with flavonoids through hydrogen bonds; in this method, a slightly modified water-ethanol gradient with acid is effective [7]. "Polyamide adsorbents are useful in TLC and column chromatography due to their ability to selectively trap flavonoids" [7, pp. 198-200]. This idea is methodologically sound and helps to quickly achieve HPLC-level purity in practice.

Analytical standardization: HPLC-DAD quantification. The widely accepted analytical approach for luteolin is the inverse-phase HPLC-DAD. Typical conditions: column C18 (150 × 4.6 mm, 5 μm), mobile phase A: water (0.1% acetic or formic acid), B: acetonitrile; gradient: 15-35% B, 25-30 minutes; flow rate 1.0 mL/min; DAD: 254 nm (total), 340-350 nm (luteolin maximum) [2, 6]. Standard calibration showed luteolin with pure substance (≥98%) in the range of 1-50 μg/mL; linearity R2 ≥ 0.999, LOQ around 0.1-0.3 μg/mL [2, 5]. However, "HPLC conditions are sensitive to the matrix and polyphenol profile" [4, pp. 3281-3284]; therefore, system suitability (t^R repeatability, N, tailing) must be constantly monitored.

Supporting test: AlCl3 colorimetry. The total amount of flavonoids, forming a complex with AlCl3, is measured around 415 nm; expressed in luteolin or quercetin equivalents [6]. "The AlCl3 method is fast, but not selective" [6, pp. 179-181]. We fully support this criticism: the AlCl3 method should be used only as an auxiliary screening, without replacing HPLC quantification.

Validation. The main indicators for ICH Q2 (R1) are: selectivity (pix separation, DAD spectral correspondence), linearity (at least 5 points), accuracy (recovery 95-105%),

reproducibility (RSD ≤ 2%), LOQ/LLOD (signal/noise 10:1 and 3:1), durability (pH, gradient slope, temperature change) [8]. The system compatibility criterion for retention and absorption width must be recorded in the observation sheets.

Quality guarantee and stability. Luteolin solutions are light-sensitive; therefore, pitch-black containers, −20°C storage, antioxidant additives (0.1 mg/mL of ascorbic acid) are recommended. Test of raw materials: botanical authentication (macro/micro characteristics), moisture content (≤12%), ash (general/insoluble), foreign impurities, norms of heavy metals and pesticides, difference in the essential oil profile (chemotypes) [3]. In the EMA HMPC report, "There is sufficient evidence of historical use and safety for Salvia officinalis" [3, pp. 5-9]; however, considering that the report relies more on essential oil markers, we consider it relevant to introduce luteolin as an additional quality indicator as a polyphenolic marker.

Industrial application. Food-grade ethanol (Ph. Eur./USP); control of residual solvent norms (ICH Q3C) is mandatory. UAE/MAE scaling involves energy efficiency, safety, and CIP (clean-in-place) design. For series-to-series consistency, reference extracts, the addition of an internal standard (e.g., apigenin), and the "fingerprint" methodology of ICP and HPLC chromatograms can be applied[2].

Discussion

Analysis of the literature shows that the choice of 60-80% ethanol-water as a solvent for the isolation of luteolin from Salvia officinalis is the most balanced solution: it is toxicologically safe, fire hazard is controlled, and the solubility of polyphenols is high. UAE/MAE technologies are superior in terms of time-energy, piroq provides chemical stability; however, equipment capital requirements and scale extrapolation of the process require a thorough engineering approach. Soxhlet and reversible condensation are inexpensive, but long-term heating increases the risk of oxidation and degradation. We extend Lu and Fu's conclusion that "the genus Salvia has a high structural and functional diversity of polyphenols" [2, pp. 118-120] in the context of our review in two aspects: (i) evaluating luteolin not as a single marker, but as part of a multi-marker panel (luteolin, apigenin, rosmarinic acid) - better predicts the pharmacological effect; (ii) due to the sensitivity of the phenolic profile to terrain, harvesting, and drying conditions, it is necessary to install rigid SOP and IPC (in-process control) in the production-quality system.

The thesis emphasized by Stalikas that "solvent-water mixtures are often superior to pure organic solvents" [4, pp. 3275-3278] is supported by experimental advantages in the context of Salvia: matrix swelling increases, the kinetics of extraction accelerates, and the thermal load decreases. We also agree with the "fast, but not selective" [6, pp. 179-181] judgment of dust and co-authors regarding AlCl3 colorimetry; it is useful in preliminary screening and process control, but final quantitative control must rely on HPLC-DAD or LC-MS/MS.

In the EMA HMPC report, evidence of safety and conventional use for Salvia officinalis is presented in a balanced manner[3]. However, the clinical relevance of the luteolin polyphenol fraction is less covered in these reports. Therefore, the introduction of luteolin as an additional quality marker for pharmaceutical manufacturers - especially

for antioxidant/anti-inflammatory-oriented phytopreparations - increases competitiveness and clinical consistency.

The proposed standardization protocol is as follows: (1) authentication of raw materials and preliminary quality control (moisture, ash, contamination); (2) fine grinding (≤0.5 mm), storage in an inert atmosphere; (3) 70% ethanol-water, UAE 35°C, 30 minutes, 15:1 solvent: matrix; (4) filtration, partial evaporation of the solvent, bringing the pH to 5.0; (5) triple distribution with ethyl acetate (1:1), combination of the organic phase, drying; (6) Cleaning with SPE (C18); (7) Quantitative analysis in HPLC-DAD (340-350 nm) with luteolin reference standard; (8) validation according to ICH Q2 (R1); (9) control of stability and residual solvents; (10) Chromatographic fingerprint registration for interserial correspondence.

Restrictions. In the literature, measurements of luteolin content differ significantly between methods and samples; also, many works do not distinguish different chemotypes of S. officinalis. Hydrolysis conditions are not uniform, which makes it difficult to compare "free" and "general" luteolins. In the future, it is necessary to conduct comparative studies of both hydrolyzed and non-hydrolyzed multi-center protocols, combined with SOP.

Conclusion

Existing data on the isolation and standardization of luteolin from Salvia officinalis L. raw materials indicate the advantages of 60-80% ethanol-water mixtures, energy-saving technologies such as UAE/MAE, and quantitative control based on HPLC-DAD. Luteolin is most often found in the form of a glycoside, therefore, combining the indicators of "free" and "general" luteolin increases the pharmacological consistency. The quality system must comply with the requirements of ICH Q2 (R1) validation, stability testing, and GMP. Although the EMA HMPC justifies Salvia safety, the proportion of polyphenolic markers should be expanded in practical standardization. The proposed protocol provides a practical roadmap for production and scientific research and serves to raise phytopharmaceuticals to class quality standards within the framework of specialty 15.00.02.

References:

1. Kintzios, S. E. (ed.). Sage: The Genus Salvia. Boca Raton: CRC Press. - 296 p.
2. Lu, Y.; Foo, L. Y. Polyphenolics of Salvia - a review. Phytochemistry. Oxford: Elsevier Science Ltd., 2002, 59 (2): 117-140.
3. European Medicines Agency, Committee on Herbal Medicinal Products (HMPC). Assessment report on Salvia officinalis L., folium and aetheroleum. London: EMA, 2016. Pages 1-54.
4. Stalikas, C. D. Methods of extraction, separation, and detection of phenolic acids and flavonoids. Journal of Separation Science. Weinheim: Wiley-VCH. pp. 30 (18): 3268-3295.
5. Dai, J.; Mumper, R. J. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules. Basel: MDPI, 2010, 15 (10): 7313-7352.
6. Chang, C.-C.; Yang, M.-H.; Wen, H. M.; Chern, J.-C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis. Taipei: Taiwan FDA.

7. Wagner, H.; Bladt, S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. Berlin-Heidelberg: Springer-Verlag. Pp. 195-200.
8. ICH Q2 (R1). Validation of Analytical Procedures: Text and Methodology. Geneva: International Council for Harmonization. Pp. 1-17.