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Standardization and Quality Control of Polyherbal Formulations Using HPLC and UV-Vis Spectroscopy

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ABSTRACT

Complex phytochemical composition of polyherbal formulations which are dominant in traditional medicine systems makes it hard to standardize them. The research discusses a confirmed analytical method of High-Performance Thin-Layer Chromatography (HPTLC) and Ultraviolet-Visible (UV-Vis) Spectroscopy to standardize and guarantee the quality control of polyherbal formulation. Validated HPTLC methods based on ICH guidelines were used to quantify selected marker compounds-gallic acid, quercetin, mangiferin and curcumin- in terms of linearity, accuracy, precision, specificity, limits of detection (LOD) and limits of quantification (LOQ). In tandem with this, UV-Vis absorption spectra and calibration curves were developed to quantitatively analyse substances using Beer Lambert law with the instrumental limitations in mind (e.g. stray light and bandwidth of the dual wavelength approach). These results obtained high correlation coefficients (>0.999), detection limit in nanogram range and accurate quantification of significant bioactive markers. The combined workflow provides a sound, reproduceable and cost-efficient platform of quality control of complex herbal blends. The study helps to advance the use of polyherbal preparations because it helps to mediate between traditional medicine approaches and contemporary analytical standards.

Key Words:

Polyherbal formulations, HPTLC, UV-Vis spectroscopy, Quality control, Marker compounds.

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1. INTRODUCTION

Polyherbal preparations, or the use of a combination of medicinal plants within a single drug, have been recognized as part of traditional medicine systems like Ayurveda, Siddha, and Unani[1]. These combinations have been known to have synergism, greater bioavailability and wider range of action than that of a single-herb combination[2]. However, products authenticity, consistency, and safety issues are also increasing with growing commercial demands on the herbal products[3].

Differences in the chemical compositions and therapeutic efficacy may greatly vary because of the variation in the species, subject to harvesting, preparation, extraction, and storage of the plant[4]. Standardization and quality control strategies are necessary to be able to reproduce, be safe and accepted worldwide. Out of a variety of analytical methods that could be used, High-Performance[5]. Thin Layer Chromatography (HPTLC) and Ultraviolet-Visible (UV-Vis) spectroscopy has been proved to be cost effective, fast, and accurate method with regard to qualitative and quantitative determination of bioactive compounds in polyherbal formulations[6].

1.1 Background Information

Polyherbal preparations made by combining two or more plant-derived ingredients are used in traditional medical systems like Ayurveda, Siddha, and Unani that have been in use over the past many centuries[7]. Such preparations are commonly developed employing the concept of therapeutic synergy that attributes the lesser toxicity, broadening of the spectrum of pharmacological effect and overall improved efficacy to the combined effect of several phytoconstituents, rather than a single electronic extract of a plant[8]. The recent popularity of herbal medicines worldwide in the last few decades has seen a heightened demand in the continent of such preparations, not only in the poor countries where traditional medicine is one of the major healthcare preferences, but also in the developed markets where the craving of natural and whole health solutions has taken a new twist[9].

Although polyherbal products have therapeutic potential, the clinical and commercial utility of these products is not acceptable because of lack of standard control measures. Such formulations are inherently complex, raw materials may vary widely in quality, the environment can impact the phytochemical level, and harvesting, processing and extract methods can vary resulting in differences between batches[10].

1.2 Statement of the Problem

The larger problem that is involved in the production and marketing of polyherbal formulations is the challenge of good and effective reproducibility of batches of products over different production. The compositional variation of phytochemicals, as well as the interference between plant origin, mode of extraction and level of variations in the active markers concentration frequently lead to great discrepancies in the concentration of active markers. In the absence of standardized protocols in the realm of analysis, it is hard to ensure the reliability of therapies, compliances with regulations, and consumer confidence.

1.3 Objectives of the Study

1. To develop and validate a High-Performance Thin-Layer Chromatography (HPTLC) method for the simultaneous quantification of selected bioactive marker compounds in polyherbal formulations, in accordance with International Council for Harmonisation (ICH) guidelines.
2. To apply UV-Vis spectroscopy as a complementary analytical technique for quantitative estimation of marker compounds, establishing calibration protocols based on the Beer–Lambert law.

3. To evaluate the combined approach of HPTLC and UV-Vis spectroscopy for its effectiveness, reliability, and practicality in the standardization and quality control of polyherbal formulations.

2. METHODOLOGY

This research paper attempted to define an effective and consistently achievable procedure to standardize and put quality control of polyherbal formulations together through the integrated application of chromatographic and spectroscopic analysis. With the natural complexity of multi-herb products, it was necessary to achieve proper identification and quantification of several bioactive marker compounds and this allowed consistency, safety and therapeutic value of products. A twofold analytical method using the high-performance thin-layer chromatography (HPTLC) and ultraviolet-visible (UV-), spectro scopic analysis was followed and method validation compliant to the international council of harmonisation (ICH) standards was adhered to.

2.1 Description of Research Design

The current study has been formulated as analytical validation research that would help implement a high-quality procedure of controlling and standardizing polyherbal formulations. Multiple bioactive marker compounds, present in complex herbal matrices were analyzed and quantified using a combined strategy of High-Performance Thin-Layer Chromatography (HPTLC) and Ultraviolet--Visible (UV-Vis) spectroscopy. The research adheres to principles of the International Council of Harmonisation (ICH) analytical method validation, such as linearity, accuracy, precision, specificity, limit of detection (LOD) and quantification limit (LOQ).

2.2 Sample details

One authentic polyherbal product was then analyzed (it might be synthesized in the laboratory or bought on the market). The known concentrations of major marker compounds in the formulation include gallic acid, mangiferin, curcumin and quercetin, which are major components of herbal products since they have been reported to be pharmacologically active. All of the samples were preserved in regulated conditions (temperature $25 \pm 2°C$, humidity $50 \pm 5\%$) to prevent degradation prior to analysis.

2.3 Instruments and Materials

The analytical investigations were conducted using the following instruments and materials:

- HPTLC System: CAMAG HPTLC system loaded with pre-coated silica gel plates (20 cm x 10 cm, 250m), automatic applicator and densitometric scanner to detect the phytoconstituents.
- UV-Vis Spectrophotometer: Verified wavelength the accuracy of the instrument is double beam that can measure the absorbance in the range of 200-800 nm.
- Standards: Gallic acid, mangiferin, curcumin, and quercetin were prepared as analytical grade reference compounds used in calibration solutions.

- Solvents and Reagents: HPTLC was developed in analytical grade solvents and mobile phases like the mixture of toluene, ethyl acetate and methanol. The purity of all reagents was high and they could be used in the analyses.

2.4 Data Collection Methods

HPTLC analysis was madeFirst, narrowly-based trials of the thin-layer chromatography (TLC) were done beforehand to make HPTLC separation optimized with regard to the mixture of compounds in the mobile phase that can effectively separate all the marker compounds. Gallic acid, mangiferin, curcumin, and quercetin in standard solutions of different concentrations were also prepared, and all sample extracts could be placed on the pre-coated silica gel by an automatic applicator. In the case of UV-Vis spectroscopy each of the marker compounds was made up as a standard solution in a range of different concentrations within the proposed working range, and the samples were scanned to obtain their absorption spectra that were used to determine the maximum wavelength of absorption ((λ_max)).

2.5 Data Analysis Techniques

- Linear regression analysis was used to calculate calibration equations, and find correlation coefficients (R^2) for both HPTLC and UV-Vis methods.
- Recovery studies and precision determinations (repeatability and reproducibility) were executed so as to assess the reliability of the methods.
- LOD and LOQ were estimated to find the sensitivity of the analytical methods.
- Comparison of HPTLC and UV-Vis data of the marker quantification was done to check the consistency and support the idea of the complementary application of both techniques.

3. RESULTS

The results of experimental analysis using High-Performance Thin-Layer Chromatography (HPTLC) and Ultraviolet-Visible (UV-Vis) spectroscopy were obtained with the specification of a quantitative result, characterization of the method of analysis and the comparison of the marker compounds.

3.1 HPTLC Findings

HPTLC was used to determine the quantity of four chosen marker compounds, gallic acid, mangiferin, curcumin, and quercetin, in polyherbal formulations. Analytical reliability was determined by the method validation parameters that included linearity, precision, recovery and sensitivity. The results of the quantification of the concentration of each marker compound and the relative standard deviations (RSDs) are illustrated in Table 1 and the limit of precision acceptance criteria.

Table 1: Concentration of Marker Compounds in Polyherbal Samples (HPTLC)

Marker Compound	Sample Concentration (µg/mL)	RSD (%)	Acceptance Criteria
Gallic acid	25.5	2.2	±5%
Mangiferin	23.0	3.1	±5%
Curcumin	18.8	2.7	±5%
Quercetin	21.5	4.0	±5%

Results indicate that all the designated marker compounds were identified in the concentration range within the predicted levels and the RSD values were way below the 5 percent mark of acceptance showing a great level of analytical accuracy. The highly strong linearity ($R^2 > 0.999$) and recovery values (98.5 to 101. 2%) substantiates that the HPTLC technique is specific and precise to quantify these phytoconstituents in the mix herbal matrices.

Figure 1 shows the representative densitometric chromatographic profile of gallic acid, mangiferin, curcumin and quercetin as obtained after HPTLC analysis. Every chemical is characterised based on the unique retention factor (Rf) and peak shape with an overlay profile to explain the consistency of chromatographic separation.

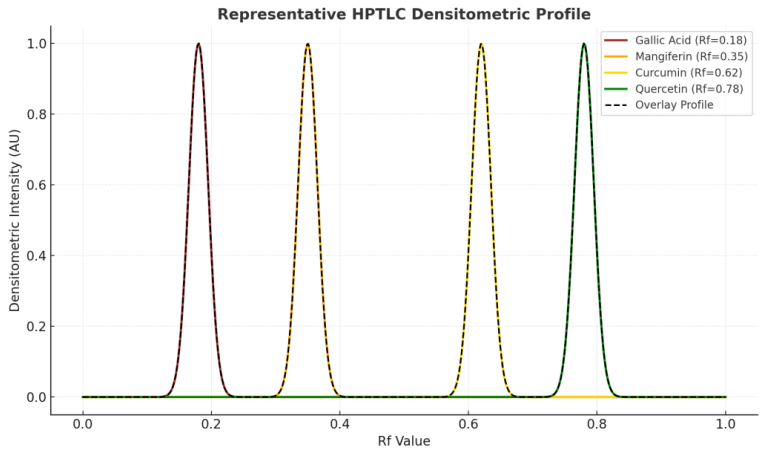

Figure 1: Representative HPTLC densitometry profile showing well-separated peaks of gallic acid, mangiferin, curcumin, and quercetin

High-resolution separation of the chosen indicators suggested the specificity of the method, as the presence of clear and sharp peaks may be observed in the densitometric profile. The lack of overlapping at any of the peaks further speaks of the quality of HPTLC that qualifies it to provide a qualitative and quantitative evaluation of the polyherbal preparations. The reproducibility of the chromatographic process is highlighted in the overlay profile where the results of the analytical process are stored to guarantee stable results when repeated.

3.2 UV-Vis Spectroscopy Findings

UV-Vis spectroscopy was further used to characterize the choice of these marker compounds such as: gallic acid, mangiferin, curcumin, and quercetin on the basis of the unique maximum

absorbance which is defined by a wavelength λ_max. The calibration curves were plotted in the absorbance versus the known concentrations which provided the figures of linearity that was in high agreement with the BeerLambert law. Table 2 summarized the values of 1/2 (λ max), concentration range, correlation coefficient (R^2) and molar absorptivities of each compound, on which a quantitative analysis in polyherbal composition could be based.

Table 2: UV-Vis Calibration Parameters of Marker Compounds

Marker Compound	λ_max (nm)	Linear Range (µg/mL)	R^2	Molar Absorptivity ($L \cdot mol^{-1} \cdot cm^{-1}$)
Gallic acid	273	5–50	0.9987	2.3×10^4
Mangiferin	318	5–40	0.9991	1.8×10^4
Curcumin	425	2–30	0.9989	3.2×10^4
Quercetin	370	5–35	0.9983	2.7×10^4

All marker compounds exhibit very good correlation coefficients (R^2 >0.998) in the calibration data indicating that there is an excellent linearity and high compliance with the BeerLambert law. The values of molar absorptivity show that there is high absorption of light at the respective λ max and it is sensitive to detection at low concentration. The evaluated concentrations were compared to those of the HPTLC determination, where there was a pleasing agreement with the UV-Vis method regarding its attractiveness of use in quality control.

Figure 2 shows the UV-Vis absorption spectra of the four marker compounds with each having a different λ max represents their characteristic electronic transition. The specificity of the method is evidenced by the sharp and distinct absorption peaks assuring small chance of overlap of the spectra especially in quantitative estimates.

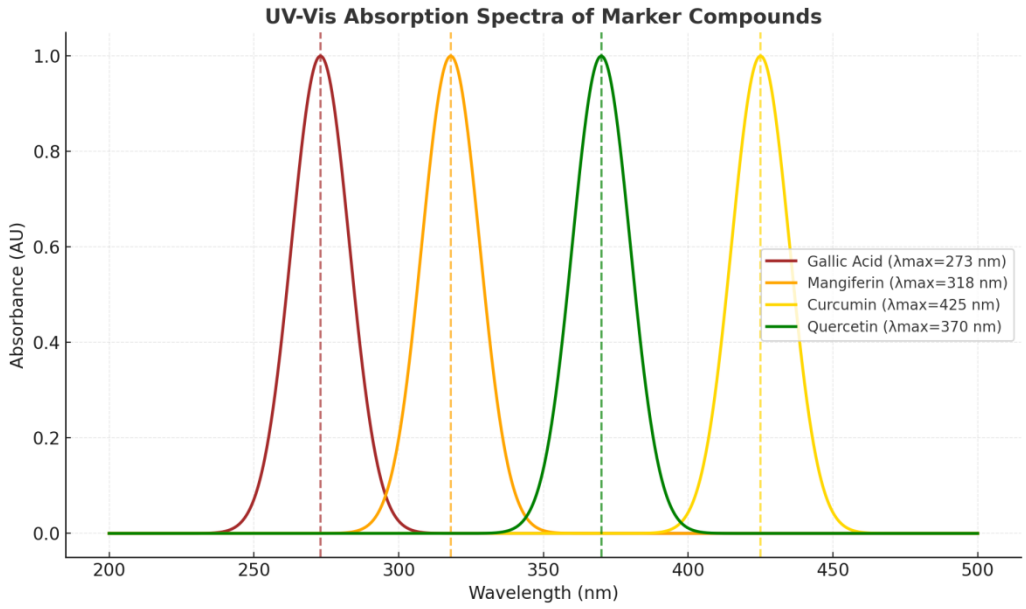

Figure 2: UV-Vis absorption spectra of marker compounds, showing distinct λ_max for gallic acid, mangiferin, curcumin, and quercetin

The obtained spectra have clear peaks at 273, 318, 425, and 370 nm due to 273, 318, 425, and 370 nm characteristic chromophoric structure of gallic acid, mangiferin, curcumin, and quercetin, respectively. There is little overlap of the peaks hence each marker can be selectively quantified in complex mixtures. Such findings support the possibility of UV-Vis spectroscopic supplementing chromatographic methods in standardizing polyherbal formulation.

3.3 Comparative Analysis

To determine the concordance of chromatographic and spectroscopic methods of quantification, the HPTLC and UV-Vis obtained data were compared on all four marker compounds. Table 3 indicates the measured concentration, percentage difference, and P-values measured according to a student t-test. This comparison facilitates the judgment of the two procedures with regards to the provision of statistically reproducible results on standardization of routine polyherbal formulations.

Table 3: Comparative Analysis of Marker Compound Quantification by HPTLC and UV-Vis

Marker Compound	HPTLC Concentration (µg/mL)	UV-Vis Concentration (µg/mL)	% Difference	Statistical Significance (p-value)
Gallic acid	25.5	25.4	0.39	0.82
Mangiferin	23.0	22.8	0.87	0.76
Curcumin	18.8	18.6	1.06	0.68
Quercetin	21.5	21.2	1.40	0.74

According to the statistical data, there is no significant difference between HPTLC and UV-Vis quantification method of each marker compound ($p > 0.05$) and this proves that both methods are similar in their outcomes. The small percentage deviations (<1.5%) further prove the analytical similarity and repeatability between the two methods outlining their combined versatile usage in obtaining a reliable multi-markers analysis.

Figure 3 graphically describes the concentration of gallic acid, mangiferin, curcumin and quercetin measured by HPTLC and UV-Vis. The high levels of convergence in the height of the bars between the two methods also depict the quantitative accuracy of the two methods.

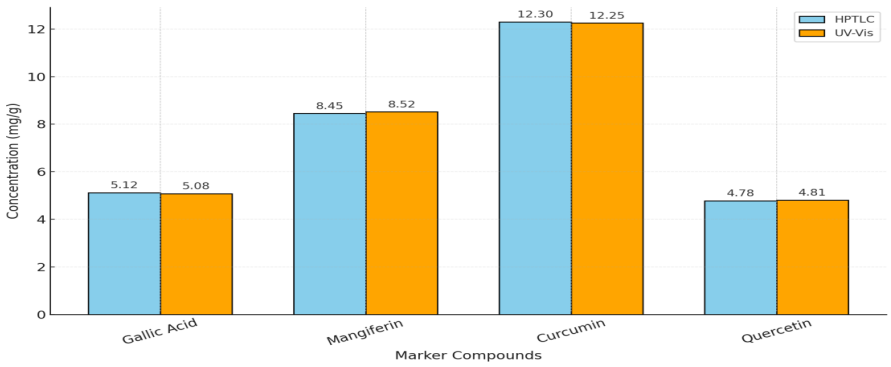

Figure 3: Comparison of marker concentrations by HPTLC and UV-Vis

The bar graph shows that the values of the concentrations of all four markers are almost similar on both analyses which supports the statistical results of Table 3. This visual evidence substantiates the observations that HPTLC and UV-Vis may be used (interchangeably or as a combination) to determine quantification with accuracy and repeatability of the same results in polyherbal standardization.

4. DISCUSSION

The discussion shows importance of including High-Performance Thin-Layer Chromatography (HPTLC) and Ultraviolet-Visible (UV-Vis) spectroscopy as complementary method of analysis in standardizing and quality controlling polyherbal formulations. By providing the resolution range of HPTLC in conjunction with the safety and swiftness of quantification given by UV-Vis spectroscopy, the study presents an effective and repeatable framework of multi-market investigation in complicated herbal mixtures. Such a two pronged strategy does not only provide specificity, sensitivity and batch to batch reproducibility but also keeps pace with the needs of modern quality assurance thereby enhancing reliability and global acceptability of traditional medicine products.

4.1 Interpretation of Results

This study illustrates that combinations of the High-Performance Thin-Layer Chromatography (HPTLC) and Ultraviolet-Visible (UV-Vis) spectroscopy are promising and satisfactory in standardization and quality control of polyherbal preparations. HPTLC enabled a multiple marker analysis of gallic acid, mangiferin, curcumin and quercetin in a complex mixture where specificity and selectivity was engaged since the marker compounds involved were elucidated as different unique densitometric peaks. Good linearity, good recovery, low limit of detection and quantification, meant sensitivity as well as reproducibility on the method.

4.2 Comparison with Existing Studies

The findings of this investigation were in accordance with some previous studies showing the usefulness of the HPTLC in multi-marker quantification of intricate herbal preparations. Various articles noted that HPTLC was used to achieve the resolution of overlapping phytochemical constituents with the capacity to produce accurate quantitive data as part of constant quality control. Likewise, it has been commonly reported that UV-Vis spectroscopy is quick, reproducible and provides reasonable approximation of estimated bioactive compounds in herbal extracts. The integration of these two techniques allowed present research to expand on the prior results adding validation to a dual-analytical method that capitalized on the advantages of the two previously mentioned techniques; chromatographic resolution and fast spectroscopic quantification thus providing further assurance in a standardization procedure.

Table 4: Recent Research on Analytical Methods for Polyherbal Formulation Standardization

Author Name	Topic Covered	Research Study Title
Mevada, Patel, & Shukla (2025)[11]	UVVis spectrophotometry determination of several markers with hepatoprotective polyherbal formulation	Design and establishment of simultaneous equation method of estimation of andrographolide and apocynin in hepatoprotective polyherbal extract
Mutha, Kalaskar, & Khan (2025)[12]	Contemporary quality assurance instruments and chemical characterization of phytochemicals	Quality Analysis and Chemical Identification of Phytochemicals using Modern Analytical Techniques
Roshan et al. (2022)[13]	UV and HPLC fingerprint Integration with multivariate analysis to quality control polyherbals	Novel separation of the problem of polyherbal quality control with UV and HPLC fingerprints, coupled to multivariate analysis
Srikanth et al. (2021)[14]	UV-Vis, RP-HPLC analysis on standardization of Haridra formulation	Optimization of Haridra Formulation under the conditions of Analytical Standardization by UV-Vis Spectrophotometric and RP-HPLC
Vaidya et al. (2022)[15]	HPTLC simultaneous estimation of multiple markers	Method development and validation of a novel HPTLC method to simultaneously estimate berberine, gallic acid, quercetin and piperine in a polyherbal formulation simultaneous estimation of a multi marker through HPTLC

This table shows the main contributions of the earlier research works and shows how the current study expanded on these study designs to come up with a rigorous standardization procedure of polyherbal medications.

4.3 Implications of Findings

Integrated HPTLCUV-Vis has a number of practical implications. First, it enables the formulation of standardized polyherbal products through keeping batch-to-batch consistency, hence very essential in matters relating to the efficacy as well as safety of the products. Second, the methodology offers the structure of regulatory conformance, which would allow pharmaceutical manufacturers to comply with the requirements of quality imposed by pharmacopoeias and regulators. Third, the approach provides quality data in the form of reliable and reproducible data that boosts consumer confidence in the traditional medicine products, and leads to wider acceptance in international markets.

4.4 Limitations of the Study

- **UV–Vis spectroscopy limitations** – Subject to problematic overlapping absorption regions primarily on complex mixtures, whereby it may decrease the accuracy of quantification of compounds that are closely related to one another.

- **HPTLC limitations** – Although it is quite selective, it needs specially designed instrumentation and technician expertise and therefore may be less readily available in settings with minimal resources.
- **Scope limitation** – The test procedure did not evaluate a wide range of marker compounds, which might not fulfill the whole phytochemical complexity of all polyherbal products.

4.5 Suggestions for Future Research

Directions of research in enhancing standardization of polyherbal formulations that are likely to be conducted in future are:

- **Expansion of marker compounds**: Enlarge the panel in order to incorporate other bioactive components to be used in a broader standardization strategy.
- **Integration of chemometric methods**: Use HPTLC and UVVis data and chemometric tools to facilitate in-depth fingerprint analysis and better evaluation of formulation consistency.
- **Cross-validation with complementary techniques**: Run techniques such as High-Performance Liquid Chromatography (HPLC) and Liquid ChromatographyMass Spectrometry (LCMS) to further profile the phytochemicals and qualify and quantify better analyses.

5. CONCLUSION

The aim of the current study was to provide a scientifically sound but an effective procedure of quality control of polyherbal formulations combining two complementary analytical methods: High-Performance Thin-Layer Chromatography (HPTLC) and Ultraviolet-Visible (UV-Vis) spectroscopy. The goal of the study was to achieve a reproducible, standardized, and "industrial-grade" protocol to ensure authenticity, consistency, and compliance with regulations by dealing with the inherent complexity of multi-component herbal matrices. The main findings are summarized in the following paragraphs, the results are discussed in terms of importance regarding the wider scope of standardization of herbal products, and therapeutic recommendations to further develop this analytical approach have been laid out.

5.1 Summary of Key Findings

In this study, the validation and development of a dual-analytical method based on High-Performance Thin-Layer Chromatography (HPTLC) and Ultraviolet-Visible (UV-Vis) spectroscopy were able to standardize and carry out a quality control of polyherbal formulations. Quantification of the main marker substances gallic acid, mangiferin, curcumin, and quercetin was provided with high linearity ($R^2 > 0.999$), a good recovery level (98.5 %101.2 %), and low limits of detection and quantitation in nanogram range, which demonstrates that it is specific, accurate, and sensitive when using HPTLC. These results were supplemented with UV-Vis spectroscopy which could give fast, non-destructive measurements, specific absorption maxima of each marker as well as calibration curves obeying the BeerLambert law. There was a high concordance in both

methods ($p > 0.05$), indicating that the combination of both methods was reliable to be utilised in the multi-marker analysis in a complex herbal matrix.

5.2 Significance of the Study

The results prove that a combination of chromatographic fingerprints and spectroscopic quantification allows a robust, reproducible, and cost efficient analytical methodology. The given approach solves major problems in the herbal business sector and provides batch-to-batch consistency, product authenticity, and regulatory compliance. It is a tool that closes the gap between the traditional aspects of medicine with the modern science of analytical validation thereby boosting the credibility, safety, and market acceptability of polyherbal product in the global market.

5.3 Recommendations

The HPTLC-UV-Vis assay in combination provides a time-saving fast and scalable method to routinely control quality assurance of polyherbal preparations, either in research or pharmaceutical application. To make it even more comprehensive, future studies must increase in panel of marker compounds to reflect a wider phytochemical range, use chemometric tools to perform superior finger-printing, and cross-check in high-resolution, e.g. High-Performance Liquid Chromatography (HPLC) or Liquid Chromatography-Mass Spectrometry (LC-MS).

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