INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

Review Article

Recent Advances in Analytical Method Development and Validation of Phenolic Compounds Using UV–Visible Spectroscopy and HPTLC

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ARTICLE INFO

Published: 04 July 2026
Keywords:
Phenolic Compounds, UV–Visible Spectroscopy, HPTLC, Method Validation, Phytochemical Standardization.
DOI:
10.5281/zenodo.21194594

ABSTRACT

Phenolic compounds are important plant-based bioactive molecules that possess antioxidant, anti-inflammatory, antimicrobial, and therapeutic properties. Accurate identification and quantification of these compounds are essential for ensuring the quality, safety, and effectiveness of herbal medicines and pharmaceutical formulations. Among the available analytical techniques, UV–Visible spectroscopy and High-Performance Thin-Layer Chromatography (HPTLC) are widely used because they are simple, reliable, economical, and suitable for routine analysis. This review highlights recent advances in analytical method development and validation of phenolic compounds using UV–Visible spectroscopy and HPTLC. It discusses the principles of both techniques, method optimization, and validation parameters such as specificity, linearity, accuracy, precision, robustness, limit of detection, and limit of quantification according to international guidelines. The review also summarizes the applications of these methods in phytochemical standardization, quality control, and quantitative estimation of phenolic compounds in herbal extracts and formulations. Overall, these analytical techniques remain valuable tools for pharmaceutical research and herbal drug standardization.

INTRODUCTION

Medicinal plants are widely used throughout the world because they contain many bioactive compounds that help prevent and treat various diseases. Among these compounds, phenolic compounds are one of the most important groups due to their antioxidant, anti-inflammatory, antimicrobial, anticancer, and cardioprotective properties. They are naturally present in fruits, vegetables, cereals, herbs, and medicinal plants and contribute significantly to the therapeutic value of herbal medicines. Because the concentration of phenolic compounds can vary depending on plant source, cultivation conditions, extraction method, and storage, accurate analytical

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

methods are essential for their identification and quantification.[1–3]

Analytical method development plays an important role in pharmaceutical research and quality control. It involves selecting appropriate analytical conditions to accurately identify and quantify chemical constituents in a sample. A well-developed analytical method ensures reliable, reproducible, and accurate results, which are essential for maintaining the quality, safety, and efficacy of herbal medicines and pharmaceutical formulations. Standardized analytical methods also help detect impurities, degradation products, and adulterants present in herbal preparations.[4,5]

Method validation is an equally important step after method development. Validation confirms that the developed analytical method consistently produces accurate and reliable results for its intended purpose. According to the International Council for Harmonisation (ICH) Q2(R2) guideline, validation parameters include specificity, linearity, accuracy, precision, robustness, detection limit, quantitation limit, and system suitability. Proper validation improves confidence in analytical results and ensures compliance with regulatory requirements.[6,7]

Among various analytical techniques, UV–Visible spectroscopy is one of the simplest and most widely used methods for the quantitative estimation of phenolic compounds. It is based on the absorption of ultraviolet or visible light by chemical compounds. Many phenolic compounds contain aromatic rings and conjugated double bonds that absorb light at specific wavelengths, allowing their identification and quantification. UV–Visible spectroscopy is rapid, economical, easy to perform, and suitable for routine quality control analysis. It requires minimal sample preparation and provides good sensitivity for the estimation of phenolic markers in herbal extracts.[8–10]

Another important analytical technique is High-Performance Thin-Layer Chromatography (HPTLC)**,** which is widely used for the separation, identification, and quantification of phytoconstituents. HPTLC is an advanced form of conventional thin-layer chromatography that offers improved resolution, accuracy, reproducibility, and sensitivity. It allows simultaneous analysis of multiple samples with low solvent consumption and short analysis time. HPTLC is particularly useful for herbal drug standardization because it provides characteristic fingerprint profiles that help authenticate medicinal plants and detect adulteration.[11–13] The combination of UV–Visible spectroscopy and HPTLC provides a reliable approach for the qualitative and quantitative analysis of phenolic compounds. UV spectroscopy offers rapid estimation, whereas HPTLC provides precise separation and fingerprint analysis of individual phytoconstituents. These complementary techniques are widely employed in pharmaceutical industries, research laboratories, and quality control laboratories for the standardization of herbal drugs and herbal formulations.[14]

Recent advances in analytical instrumentation, image analysis, densitometry, software integration, and automated sample application have significantly improved the sensitivity, precision, and reproducibility of UV–Visible spectroscopy and HPTLC methods. These developments have enhanced their applications in phytochemical analysis, stability studies, herbal quality control, and pharmaceutical research. Furthermore, validated analytical methods contribute to regulatory acceptance, product consistency, and consumer safety by ensuring that

herbal products contain the desired amount of bioactive compounds.[15]

UV–Visible spectroscopy and HPTLC remain valuable analytical techniques for the development and validation of methods used in the analysis of phenolic compounds. Their simplicity, cost-effectiveness, accuracy, and wide applicability make them essential tools for phytochemical standardization, quality assurance, and pharmaceutical research. Continued improvements in analytical technologies are expected to further strengthen their role in herbal drug analysis and natural product research.

ANALYTICAL METHOD DEVELOPMENT

Analytical method development is a systematic process used to establish a reliable method for the identification and quantification of chemical compounds in pharmaceutical and herbal samples. The primary objective is to obtain accurate, precise, sensitive, and reproducible results. In herbal drug analysis, method development is particularly important because plant extracts contain a complex mixture of phytochemicals that may interfere with the estimation of the target compound. Therefore, careful optimization of analytical conditions is necessary to achieve proper separation and accurate quantification of phenolic compounds.[16,17]

During method development, several factors are optimized depending on the analytical technique. For UV–Visible spectroscopy, important parameters include solvent selection, wavelength (λmax), sample concentration, pH, and calibration range. The selected solvent should completely dissolve the analyte without causing interference, while the wavelength should provide maximum absorbance and minimum background noise. Calibration curves are prepared using standard solutions to establish the relationship between concentration and absorbance.[18]

For HPTLC analysis, method development involves selecting a suitable stationary phase, mobile phase composition, saturation time, application volume, migration distance, detection wavelength, and densitometric scanning conditions. Optimization of the mobile phase is one of the most critical steps because it determines the separation efficiency and resolution of the analyte from other phytoconstituents. Proper optimization results in well-defined, sharp, and reproducible peaks with suitable Rf values.[19–21]

METHOD VALIDATION

After developing the analytical method, validation is performed to confirm that the method is suitable for its intended purpose. Analytical method validation ensures that the method consistently produces reliable and reproducible results. The ICH Q2(R2) guideline recommends validating analytical methods using parameters such as specificity, linearity, accuracy, precision, detection limit, quantitation limit, robustness, range, and system suitability.[22,23]

1. **Specificity** evaluates the ability of the method to measure the analyte accurately in the presence of impurities, degradation products, or other phytochemicals.
2. **Linearity** determines the relationship between analyte concentration and analytical response over a specified concentration range.
3. **Accuracy** measures how close the experimental value is to the true value and is usually determined through recovery studies.

4. **Precision** evaluates the repeatability and reproducibility of the analytical method under similar operating conditions.

5. **Limit of Detection (LOD)** is the lowest concentration of analyte that can be detected but not necessarily quantified.

6. **Limit of Quantification (LOQ)** is the lowest concentration that can be accurately and precisely quantified.

7. **Robustness** determines the effect of small deliberate changes in analytical conditions such as mobile phase composition, wavelength, temperature, or flow rate on the analytical performance.

System suitability tests verify that the analytical system is functioning properly before routine sample analysis by evaluating parameters such as peak symmetry, resolution, repeatability, and theoretical plates.[24–26]

APPLICATIONS IN HERBAL DRUG STANDARDIZATION

Validated UV–Visible spectroscopy and HPTLC methods are widely used for the standardization of herbal drugs and herbal formulations. These techniques help identify and quantify phenolic compounds, flavonoids, alkaloids, glycosides, tannins, and other phytochemicals present in medicinal plants. Standardization ensures batch-to-batch consistency, product quality, safety, and therapeutic effectiveness.[27]

HPTLC fingerprint profiling has become an important tool for authentication of medicinal plants. Each plant produces a unique chromatographic fingerprint that helps distinguish genuine plant materials from adulterated or substituted samples. This technique is widely used by pharmaceutical industries for raw material evaluation and quality assurance.[28]

UV–Visible spectroscopy is frequently employed for routine estimation of phenolic compounds because it is rapid, economical, and requires minimal sample preparation. HPTLC complements UV analysis by providing simultaneous separation and quantitative estimation of multiple phytoconstituents. Together, these techniques improve the reliability of phytochemical analysis and support regulatory compliance for herbal medicines.[29]

Recent technological advancements, including automated sample applicators, digital image processing, densitometric scanning, and computer-assisted data analysis, have significantly improved the sensitivity, accuracy, and reproducibility of HPTLC methods. These developments have expanded their applications in pharmaceutical research, stability studies, herbal formulation development, and natural product analysis.[30]

CURRENT CHALLENGES AND FUTURE PERSPECTIVES

Although UV–Visible spectroscopy and HPTLC are widely used for the analysis of phenolic compounds, several challenges still exist. One of the major challenges is the complexity of herbal extracts, which contain a large number of phytochemicals that may interfere with the identification and quantification of the target compound. Variations in plant species, geographical location, harvesting season, extraction method, and storage conditions can also affect the concentration of phenolic compounds, making standardization difficult.[31,32]

Another challenge is the lack of standardized analytical procedures for many medicinal plants.

Differences in solvent systems, extraction techniques, mobile phase composition, and detection wavelengths often result in variations between laboratories. Therefore, validated and harmonized analytical methods are necessary to ensure consistent and reproducible results. The availability of high-purity reference standards for all phenolic compounds is another limitation in routine herbal analysis.[33] Despite these challenges, continuous advancements in analytical instrumentation have significantly improved the performance of UV–Visible spectroscopy and HPTLC. Automated sample application, digital densitometry, image analysis, software-assisted data processing, and high-resolution plates have increased the accuracy, sensitivity, and reproducibility of HPTLC methods. These developments have expanded their applications in pharmaceutical quality control and herbal drug standardization.[34]

Future research should focus on developing rapid, sensitive, environmentally friendly, and cost-effective analytical methods for the estimation of phenolic compounds. Integration of UV–Visible spectroscopy and HPTLC with advanced analytical techniques and digital technologies is expected to improve analytical performance and support regulatory compliance. Such advancements will strengthen the quality assurance of herbal medicines and promote the global acceptance of plant-based pharmaceutical products.[35]

CONCLUSION

Validated analytical methods are essential for the accurate identification and quantification of phenolic compounds in medicinal plants and herbal formulations. Among the available analytical techniques, UV–Visible spectroscopy and High-Performance Thin-Layer Chromatography (HPTLC) remain reliable, simple, economical, and effective tools for phytochemical analysis. UV–Visible spectroscopy provides rapid quantitative estimation, whereas HPTLC offers excellent separation, fingerprint profiling, and simultaneous analysis of multiple samples. Together, these techniques play a vital role in herbal drug standardization, quality control, and pharmaceutical research. Analytical method development and validation according to ICH Q2(R2) guidelines ensure the accuracy, precision, specificity, robustness, and reproducibility of analytical methods. Proper validation increases confidence in analytical results and supports regulatory acceptance of herbal products. Recent technological advancements have further improved the sensitivity, efficiency, and reliability of UV–Visible spectroscopy and HPTLC, making them suitable for routine laboratory analysis as well as industrial quality assurance.

Although challenges such as variability of herbal materials, complex phytochemical composition, and lack of standardized protocols remain, continuous improvements in analytical technologies are expected to overcome these limitations. Future research should focus on developing more sensitive, rapid, and environmentally sustainable analytical methods for herbal drug evaluation. Overall, UV–Visible spectroscopy and HPTLC will continue to serve as indispensable analytical tools for the quality assessment, standardization, and pharmaceutical development of phenolic compounds and other bioactive constituents.

REFERENCES

1. Dai J, Mumper RJ. Plant phenolics: Extraction, analysis and their antioxidant properties. Molecules. 2010;15(10):7313–7352.

2. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants. Oxidative Medicine and Cellular Longevity. 2009;2(5):270–278.
3. Shahidi F, Ambigaipalan P. Phenolics and polyphenolics in foods. Journal of Functional Foods. 2015;18:820–897.
4. Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. 3rd ed. Wiley; 2010.
5. Swartz ME, Krull IS. Analytical Method Development and Validation. Marcel Dekker; 2012.
6. International Council for Harmonisation. ICH Q2(R2): Validation of Analytical Procedures. 2023.
7. Ermer J, Miller JHM. Method Validation in Pharmaceutical Analysis. Wiley-VCH; 2005.
8. Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. 7th ed. Cengage Learning; 2018.
9. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed. CBS Publishers; 2004.
10. Harris DC. Quantitative Chemical Analysis. 10th ed. W.H. Freeman; 2020.
11. Sherma J. Modern HPTLC in pharmaceutical analysis. Journal of AOAC International. 2010;93(3):754–764.
12. Reich E, Schibli A. High-Performance Thin-Layer Chromatography for the Analysis of Medicinal Plants. Thieme Medical Publishers; 2007.
13. Srivastava MM. High-performance thin-layer chromatography (HPTLC). Springer; 2011.
14. Patel PM, Patel NM, Goyal RK. HPTLC method development and validation in herbal analysis. Journal of Pharmaceutical Analysis. 2012;2(2):134–141.
15. Poole CF. Thin-layer chromatography: Challenges and opportunities. Journal of Chromatography A. 2003;1000:963–984.
16. Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. 3rd ed. Wiley; 2010.
17. Dong MW. Modern HPLC for Practicing Scientists. Wiley-Interscience; 2006.
18. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed. CBS Publishers; 2004.
19. Reich E, Schibli A. High-Performance Thin-Layer Chromatography for the Analysis of Medicinal Plants. Thieme; 2007.
20. Sherma J. Planar chromatography in pharmaceutical analysis. Journal of AOAC International. 2010;93(3):754–764.
21. Poole CF. Instrumental thin-layer chromatography. Journal of Chromatography A. 2003;1000:963–984.
22. International Council for Harmonisation. ICH Q2(R2): Validation of Analytical Procedures. 2023.
23. Ermer J, Miller JHM. Method Validation in Pharmaceutical Analysis. Wiley-VCH; 2005.
24. Swartz ME, Krull IS. Analytical Method Development and Validation. Marcel Dekker; 2012.
25. Kazakevich Y, Lobrutto R. HPLC for Pharmaceutical Scientists. Wiley; 2007.
26. Harris DC. Quantitative Chemical Analysis. 10th ed. W.H. Freeman; 2020.
27. WHO. Quality Control Methods for Herbal Materials. World Health Organization; 2011.
28. Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd ed. Springer; 2009.
29. Mukherjee PK. Quality Control and Evaluation of Herbal Drugs. Elsevier; 2019.
30. Srivastava MM. High-Performance Thin-Layer Chromatography (HPTLC). Springer; 2011.
31. Mukherjee PK. Quality Control and Evaluation of Herbal Drugs. Elsevier; 2019.
32. Kunle OF, Egharevba HO, Ahmadu PO. Standardization of herbal medicines – A review. International Journal of Biodiversity and Conservation. 2012;4(3):101–112.
33. Reich E. Modern HPTLC for quality control of herbal drugs. Journal of Planar Chromatography. 2014;27(5):313–320.
34. Sherma J. Advances in HPTLC instrumentation and pharmaceutical applications. Journal of AOAC International. 2015;98(5):1323–1328.

35. Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. 3rd ed. Wiley; 2010.

HOW TO CITE: Dr. Samrat Khedkar, Dr. Nitin Mali, Amit Chaugule, Recent Advances in Analytical Method Development and Validation of Phenolic Compounds Using UV–Visible Spectroscopy and HPTLC, Int. J. of Pharm. Sci., 2026, Vol 4, Issue 7, 1024-1030. https://doi.org/10.5281/zenodo.21194594