STM JOURNALS

Research & Reviews: A Journal of
Pharmacognosy

ISSN: 2394-7276
Volume 12, Issue 3, 2025
September–December
DOI (Journal): 10.37591/RRJoPC

https://journals.stmjournals.com/rrjopc

Research RRJoPC

Analytical Method Development and Validation of Simultaneous Estimation of Natural Active Constituents Andrographolide and Piperine by UV Method

Vinay Kumar[1,*], Ankita Shukla[2], Bala Subramanyam[3]

Abstract

A simple, accurate, precise, and robust UV-visible spectrophotometric method was developed and validated for the simultaneous estimation of Andrographolide and Piperine in bulk and combined pharmaceutical dosage forms. Preformulation studies confirmed favorable physicochemical properties for both drugs, including acceptable solubility profiles, melting points, pH values, and partition coefficients. The method development involved the determination of λ_{max} values at 224 nm for Andrographolide and 288 nm for Piperine using ethanol as solvent. The proposed method showed excellent linearity over the concentration ranges of 10–50 µg/mL for Andrographolide and 2–10 µg/mL for Piperine, with correlation coefficients (R^2) of 0.999 for both drugs. Validation parameters demonstrated high precision, with %RSD values below 0.3% for repeatability, intraday, and interday precision studies. Recovery studies indicated accuracy within the range of 98%–102%. The LOD and LOQ values were 1.95 µg/mL and 5.92 µg/mL for Andrographolide, and 0.40 µg/mL and 1.24 µg/mL for Piperine, confirming method sensitivity. Ruggedness and robustness studies indicated the reliability of the method under varied analytical conditions. The method was successfully validated as per ICH Q2(R1) guidelines and is suitable for routine quality control and simultaneous quantification of Andrographolide and Piperine in pharmaceutical formulations.

Keywords: Andrographolide, piperine, UV-visible spectrophotometry, method validation, linearity, accuracy, precision, LOD, LOQ, simultaneous estimation

INTRODUCTION

Herbal medicines have garnered significant global interest due to their diverse therapeutic applications, cultural acceptance, and favorable safety profiles. Among the myriad of bioactive phytoconstituents, Andrographolide, a diterpenoid lactone derived from *Andrographis paniculata*, and Piperine, an alkaloid obtained from *Piper nigrum* and *Piper longum*, possess noteworthy pharmacological properties, including anti-inflammatory, hepatoprotective, antimicrobial, and bioenhancing activities [1]. These compounds are often coformulated in polyherbal products to achieve synergistic therapeutic outcomes. However, maintaining the quality, consistency, and efficacy of herbal formulations remains a critical challenge, primarily due to the complex nature of plant matrices and variability in active constituent concentrations [2]. While chromatographic techniques, such as HPLC and HPTLC, have been reported for their estimation, these methods often involve intricate sample preparation, higher operational costs, and longer analysis times, limiting their routine application in quality control laboratories. In contrast, UV-visible spectrophotometry offers a cost-effective, rapid, and reliable analytical alternative with minimal sample preparation requirements [3]. Despite its advantages, limited validated UV spectrophotometric methods are

***Author for Correspondence**
Sarbjot Singh
E-mail: vinay3989@gmail.com

[1–3]Research Scholar, Department of Pharmacy, Millenium College of Pharmacy, Bhopal, Madhya Pradesh, India

Received Date: July 07, 2025
Accepted Date: July 15, 2025
Published Date: August 13, 2025

Citation: Vinay Kumar, Ankita Shukla, Bala Subramanyam. Analytical Method Development and Validation of Simultaneous Estimation of Natural Active Constituents Andrographolide and Piperine by UV Method. Research & Reviews: A Journal of Pharmacognosy. 2025; 12(3): 33–41p.

available for the simultaneous estimation of Andrographolide and Piperine in combined herbal formulations. In view of this, the present study was undertaken to develop and validate a simple, precise, accurate, robust, and economical UV spectrophotometric method for the simultaneous estimation of Andrographolide and Piperine in marketed herbal formulations, adhering to ICH Q2 (R1) guidelines [4]. The proposed method aims to serve as a practical tool for routine quality control analysis, contributing to the standardization and regulatory compliance of herbal pharmaceuticals [5].

MATERIALS AND METHODS

Chemicals and Reagents

Andrographolide and Piperine reference standards were procured from a certified pharmaceutical supplier. Analytical-grade methanol, ethanol, distilled water, and other reagents used throughout the study were of UV-spectroscopy grade and obtained from SD Fine Chemicals, Mumbai, India. All solvents and reagents were employed without further purification.

Instrumentation

A UV-Visible Spectrophotometer (Shimadzu 1700) was employed for absorbance measurements. Other instruments included a boiling water bath (Universal), weighing balance (Sirtech), vortex shaker (Scientech SE-146), melting point apparatus (Amtech), FTIR spectrophotometer (Perkin Elmer Spectrum BX), hot air oven (Scientech), digital pH meter (Lab Junction), and micropipettes (Dragon Lab).

Pre-Formulation Studies

- *Organoleptic Evaluation:* The physical characteristics, such as color, odor, appearance, and state of both drugs, were assessed.
- *Melting Point Determination:* The melting points were determined using a digital melting point apparatus.
- *Solubility Studies:* The solubility of Andrographolide and Piperine was evaluated in various solvents, including water, methanol, ethanol, chloroform, acetone, DMSO, and DMF.
- *pH Determination:* A 1% w/v aqueous solution of each drug was prepared, and pH was measured using a calibrated digital pH meter.
- *Partition Coefficient Determination:* The n-octanol/water partition coefficient was determined using the standard shake-flask method.

Fourier Transform Infrared (FTIR) Analysis

The identity and purity of Andrographolide and Piperine were confirmed by FTIR spectroscopy (Shimadzu IR Affinity-1S, Japan) using the KBr pellet technique. Approximately 2 mg of each pure drug was triturated with 200 mg of dry KBr, compressed into a thin pellet, and scanned over the wavenumber range of 4000–400 cm^{-1}. The characteristic peaks of each compound were compared with standard reference spectra [6, 7].

Preparation of Standard Solutions

Accurately weighed 10 mg each of Andrographolide and Piperine were separately dissolved in methanol and sonicated to obtain stock solutions of 100 µg/mL. Subsequent serial dilutions were prepared using methanol to achieve final working concentrations ranging from 2 to 12 µg/mL for Andrographolide and 2–20 µg/mL for Piperine [8].

Selection of Wavelength

UV absorption spectra of Andrographolide and Piperine were recorded between 200 and 400 nm using methanol as a blank, which was employed for simultaneous quantification using the absorbance ratio method [9].

Preparation of Marketed Herbal Formulation Samples

Marketed herbal formulations containing both Andrographolide and Piperine were procured from local pharmacies. An accurately weighed quantity of the powdered sample equivalent to 10 mg of each

active constituent was extracted in methanol by sonication for 30 minutes, filtered through Whatman filter paper No. 41, and appropriately diluted for analysis [10].

Method Validation

- *Linearity:* Standard calibration curves for both Andrographolide and Piperine were constructed within their respective concentration ranges. The regression equation, correlation coefficient (r^2), and molar absorptivity were calculated.
- *Precision:* Intraday and interday precision were determined by analyzing three replicates of standard solutions at three concentration levels on the same and consecutive days, expressed as %RSD.
- *Accuracy (Recovery Study):* Recovery studies were carried out by spiking pre-analyzed samples with known amounts of standard drugs at 80%, 100%, and 120% of the target concentration. Percent recovery and %RSD values were computed.
- *Repeatability:* The absorbance of a single concentration was measured six times, and %RSD was calculated.
- *Ruggedness:* Method ruggedness was assessed by performing the analysis with different analysts and instruments under the same laboratory conditions.
- *Robustness:* The effect of deliberate variations in wavelength (±1 nm) and methanol concentration (±5%) on absorbance values was studied.
- *LOD and LOQ:* Limits of detection (LOD) and limits of quantification (LOQ) were determined based on the standard deviation of the response and slope of the calibration curve as per ICH guidelines [11–13].

RESULTS AND DISCUSSION

Organoleptic Evaluation

Both Andrographolide and Piperine were found to be crystalline powders with characteristic appearances as summarized in Table 1.

Table 1. Organoleptic properties of andrographolide and piperine.

Property	Andrographolide	Piperine
Appearance	White crystalline powder	Pale yellow powder.
Odour	Characteristic	Characteristic.
Taste	Bitter	Pungent.
Nature	Crystalline	Crystalline.

Melting Point Determination

The observed melting points were within officially reported ranges, confirming drug identity and purity (Table 2).

Table 2. Melting point determination.

Drug	Observed Melting Point (°C)	Reported Melting Point (°C)
Andrographolide	228–232	230
Piperine	128–132	130

Solubility Studies

Solubility studies showed Andrographolide was soluble in ethanol and methanol, while Piperine was freely soluble in methanol, ethanol, DMSO, and DMF (Table 3).

pH Determination

The pH of 1% w/v aqueous solutions was 6.1 for Andrographolide and 5.9 for Piperine (Table 4).

Table 3. Solubility profile.

Solvent	Andrographolide	Piperine
Water	Slightly soluble	Sparingly soluble.
Ethanol	Soluble	Soluble.
Methanol	Soluble	Soluble.
Acetone	Slightly soluble	Soluble.
Chloroform	Slightly soluble	Soluble.
DMSO	Freely soluble	Freely soluble.
DMF	Freely soluble	Freely soluble.

Table 4. pH of 1% drug solutions.

Drug	pH
Andrographolide	6.1
Piperine	5.9

Partition Coefficient

The n-octanol/water partition coefficient was found to be 1.68 for Andrographolide and 2.36 for Piperine (Table 5 and Figure 1).

Table 5. Partition coefficient.

Drug	n-Octanol/Water Partition Coefficient
Andrographolide	1.68
Piperine	2.36

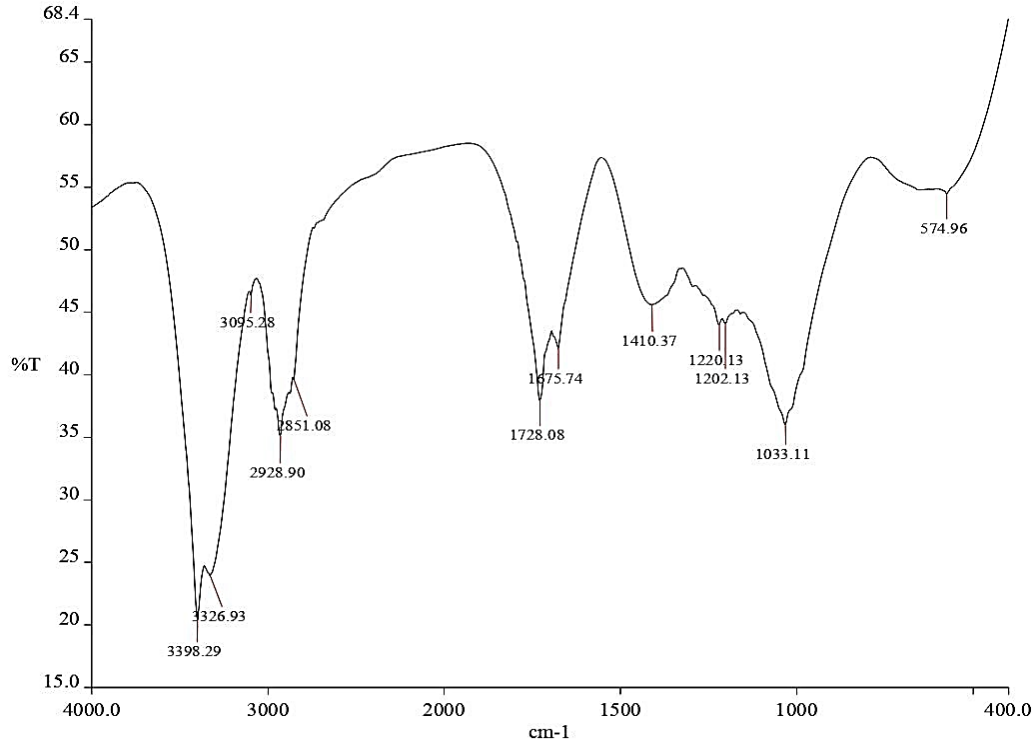

Figure 1. FTIR spectra of andrographolide.

FTIR Spectral Analysis

FTIR spectroscopy was carried out to identify the characteristic functional groups of Andrographolide and Piperine and to confirm their compatibility for simultaneous estimation. The FTIR

spectrum of Andrographolide exhibited prominent absorption bands at 3364 cm^{-1} corresponding to –OH stretching, 1665 cm^{-1} for C=O stretching of the lactone group, and 1603 cm^{-1} for C=C aromatic stretching. Piperine showed characteristic peaks at 2930 cm^{-1} for aliphatic C–H stretching, 1645 cm^{-1} for C=O stretching, and 1595 cm^{-1} for C=C aromatic stretching. No additional or missing peaks were observed in either spectrum, indicating the absence of incompatibility or chemical interaction between the drugs. The FTIR spectra are presented in Figure 1 for Andrographolide and Figures 2 and 3 for Piperine.

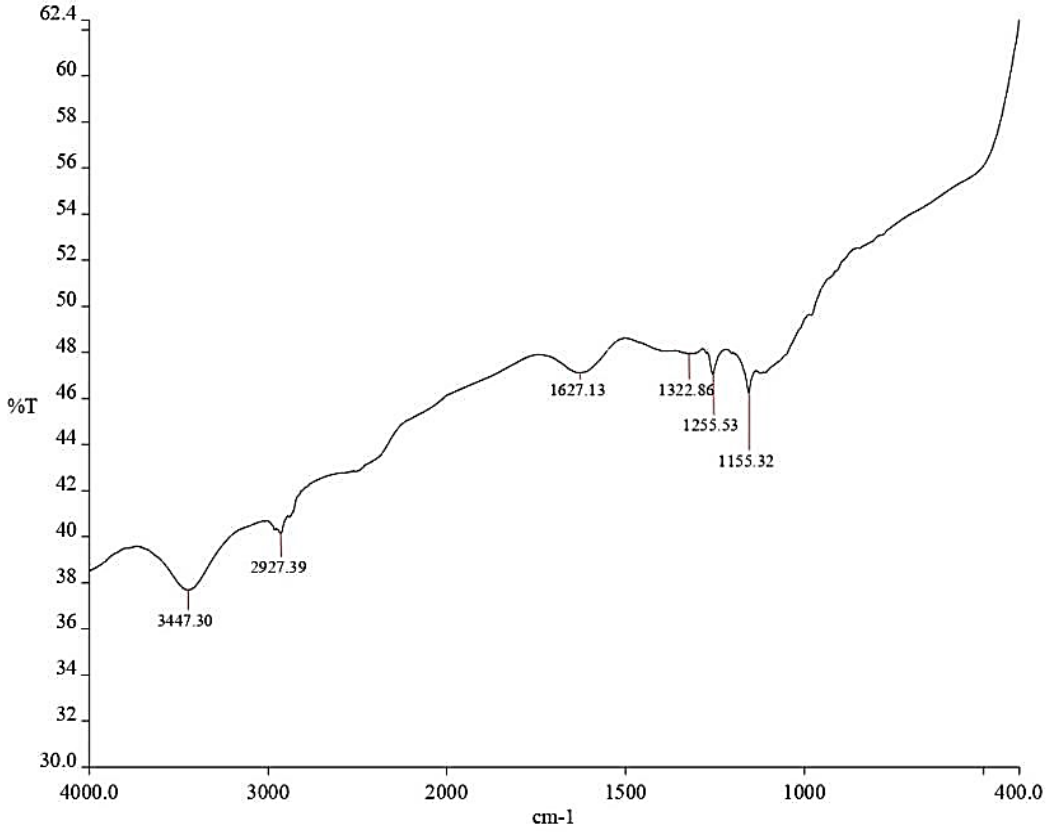

Figure 2. FTIR spectra of piperine.

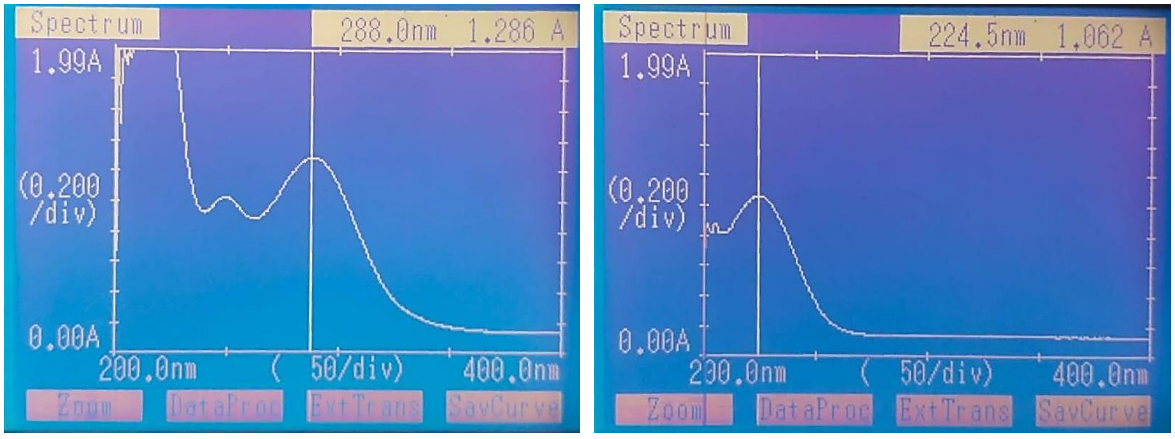

Figure 3. UV spectra of andrographolide and piperine.

METHOD DEVELOPMENT

The UV-Visible spectrophotometric method for the simultaneous estimation of Andrographolide and Piperine was developed by scanning standard solutions of both drugs in the wavelength range of 200–

800 nm using ethanol as the solvent. Andrographolide exhibited a maximum absorbance (λ_{max}) at 224 nm, while Piperine showed its λ_{max} at 288 nm. The UV spectra of Andrographolide and Piperine are presented in Figure 3.

Linearity

The developed method demonstrated excellent linearity for Andrographolide in the concentration range of 10–50 µg/mL and for Piperine in the range of 2–10 µg/mL. The calibration curves of both drugs showed a correlation coefficient (R^2) of 0.999, indicating a strong linear relationship between absorbance and concentration within the selected ranges. The calibration curves for Andrographolide and Piperine are presented in Figure 4.

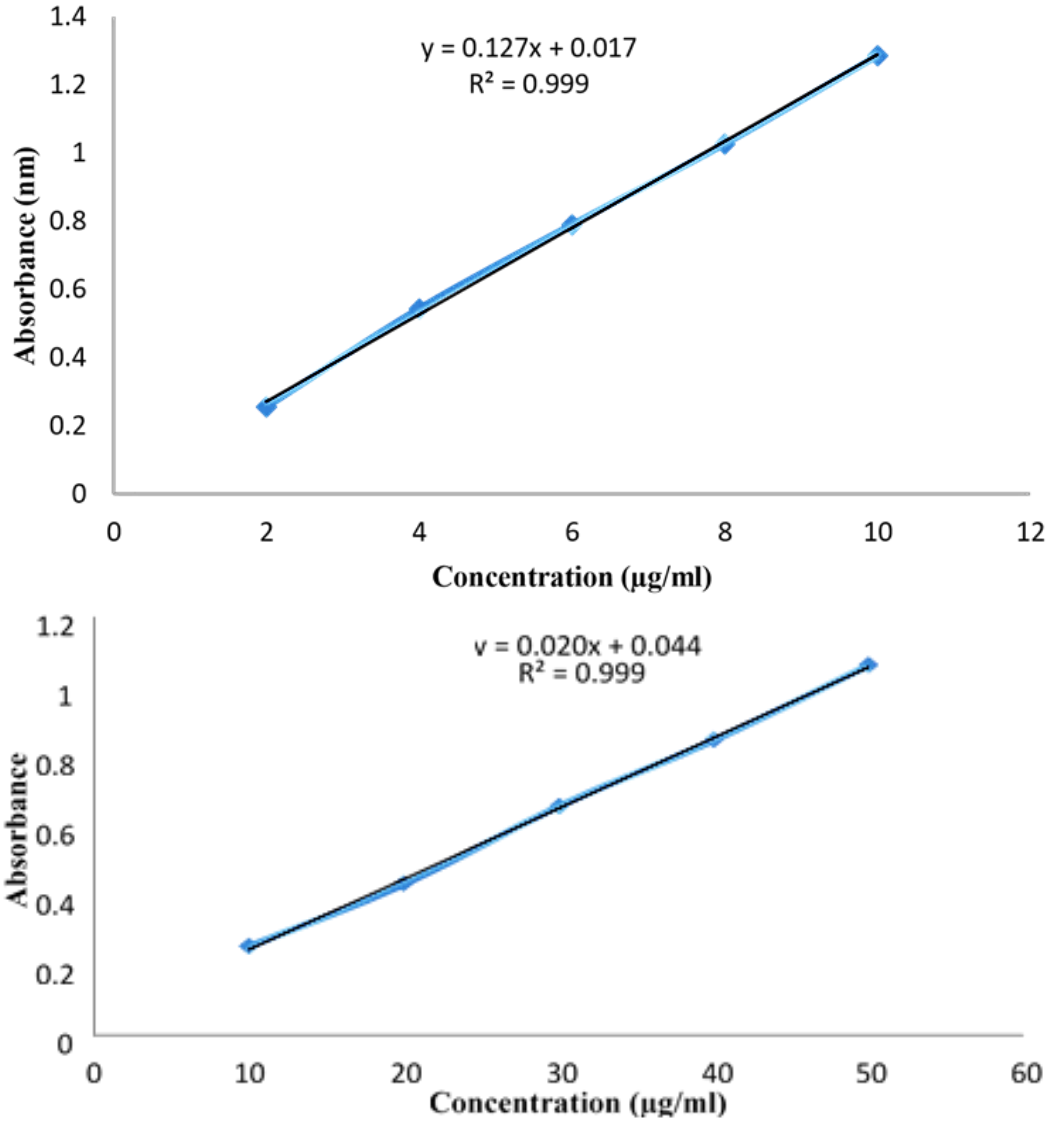

Figure 4. Calibration curves for andrographolide and piperine.

Precision

The precision of the developed method was evaluated through repeatability (intra-assay), intraday, and interday precision studies for both Andrographolide and Piperine. All %RSD values remained well below 2%, confirming the precision and reproducibility of the method. The results are summarized in Table 6.

Table 6. Precision data (%RSD).

Validation Type	Andrographolide (%RSD)	Piperine (%RSD)
Repeatability	0.262	0.237
Intraday	0.152	0.126
Interday	0.151	0.126

Accuracy

The accuracy of the developed UV spectrophotometric method was assessed by the standard addition method at three concentration levels for both Andrographolide and Piperine. The percentage recovery values ranged between 98%–102% for both drugs at all tested levels, confirming the accuracy and reliability of the proposed method for quantitative estimation in pharmaceutical formulations. The results are presented in Table 7.

Table 7. Accuracy (recovery study).

Drug	Recovery Range (%)
Andrographolide	98–102
Piperine	98–102

LOD and LOQ

The LOD and LOQ for Andrographolide were found to be 1.95 µg/mL and 5.92 µg/mL at a wavelength of 380 nm, while for Piperine, they were 0.40 µg/mL and 1.24 µg/mL at 230 nm, respectively. These values confirm the sensitivity of the developed analytical method for detecting and quantifying low concentrations of both compounds (Table 8).

Table 8. Results showing LOD and LOQ of andrographolide and piperine.

Drug	LOD (µg/mL)	LOQ (µg/mL)
Andrographolide	1.95	5.92
Piperine	0.40	1.24

Ruggedness

The ruggedness of the proposed method was evaluated by performing the analysis using two different analysts under the same laboratory conditions at concentrations of 30 µg/mL for Andrographolide and 6 µg/mL for Piperine. The %RSD values were well within acceptable limits, confirming the method's reproducibility across different analysts. Results are presented in Table 9.

Table 9. Ruggedness extract.

Analyst	Andrographolide (%RSD)	Piperine (%RSD)
Analyst 1	0.152	0.128
Analyst 2	0.156	0.126

Robustness

Robustness was assessed by introducing minor variations in analytical conditions, specifically altering the analysis temperature between 25°C and 30°C. The %RSD values remained below 2%, indicating the robustness of the method under these variations (Table 10).

Table 10. Robustness extract.

Temperature (°C)	Andrographolide (%RSD)	Piperine (%RSD)
25°C	0.151	0.127
30°C	0.151	0.192

CONCLUSIONS

The developed UV-visible spectrophotometric method for the simultaneous estimation of Andrographolide and Piperine was found to be simple, accurate, precise, and robust. Preformulation studies confirmed favorable physicochemical properties for both drugs, including good solubility in organic solvents, acceptable melting points, suitable pH values, and satisfactory partition coefficients. FTIR analysis confirmed the presence of characteristic functional groups with no incompatibility. The method demonstrated excellent linearity over concentration ranges of 10–50 µg/mL for Andrographolide and 2–10 µg/mL for Piperine, with correlation coefficients (R^2) of 0.999. Precision studies showed %RSD values below 0.3% for repeatability, intraday, and interday precision, confirming excellent reproducibility. Recovery values ranged from 98% to 102% for both drugs, indicating high accuracy. LOD and LOQ values of 1.95 µg/mL and 5.92 µg/mL for Andrographolide, and 0.40 µg/mL and 1.24 µg/mL for Piperine, reflected the good sensitivity of the method. Ruggedness and robustness studies demonstrated minimal variations (%RSD <2%) with changes in analysts and temperature, ensuring method reliability under varied conditions. Overall, the validated method is suitable for routine quantitative analysis and quality control of Andrographolide and Piperine in pharmaceutical formulations, complying with ICH Q2(R1) guidelines.

Acknowledgment

Correspondence authors sincerely acknowledge all authors for equal contribution.

Conflict of Interest

None.

Funding

Nil.

REFERENCES

1. Ravi K, Kumar VR, Rathi S, Singh S, Patel B, Singh S, et al. A comprehensive review on ulcer and their treatment. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2023;39:e20230006. doi:10.62958/j.cjap.2023.006. PMID: 38755116.
2. Rajput DS, Gupta N, Singh S, Sharma B. A comprehensive review: Personalized medicine for rare disease cancer Treatment. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2023;39:e20230008. doi:10.62958/j.cjap.2023.008. PMID: 38830754.
3. Singh S, Chaurasia A, Rajput DS, Gupta N. Mucoadhesive drug delivery system and there future prospective: Are a promising approach for effective treatment? Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2023;39:e20230005. doi:10.62958/j.cjap.2023.005. PMID: 38751344.
4. Patel S, Ismail Y, Singh S, Rathi S, Shakya S, Patil SS, et al. Recent innovations and future perspectives in transferosomes for transdermal drug delivery in therapeutic and pharmacological applications. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2024;40:e20240031. doi:10.62958/j.cjap.2024.031. PMID: 39442957.
5. Vaghela MC, Rathi S, Shirole RL, Verma J, Shaheen, Panigrahi S, et al. Leveraging AI and machine learning in Six Sigma documentation for pharmaceutical quality assurance. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2024;40:e20240005. doi:10.62958/j.cjap.2024.005. PMID: 39019923.
6. Patel S, Ismail Y, Singh S, Rathi S, Shakya S, Patil SS, et al. Recent innovations and future perspectives in transferosomes for transdermal drug delivery in therapeutic and pharmacological applications. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2024;40:e20240031. doi:10.62958/j.cjap.2024.031. PMID: 39442957.
7. Singh S, Chaurasia A, Rajput DS, Gupta N. An overview on mucoadhesive buccal drug delivery systems & approaches: A comprehensive review. Afr J Biol Sci (South Africa). 2024;6(5):522–41. doi:10.33472/AFJBS.6.5.2024.522-541.
8. Kumar S, Singh S, Rajput D, Sharma B, Chaturvedi K, Singh N, et al. Pharmacological approaches and herbal interventions for Alzheimer's disease. Nat Prod J. 2024;14(8):e220124225945. https://doi.org/10.2174/0122103155275266231123090138.

9. Ravikkumar VR, Patel BD, Rathi S, Parthiban S, Upadhye MC, Shah AM, et al. Formulation and evaluation of drumstick leaves tablet as an immunomodulator. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2024;40:e20240004. doi:10.62958/j.cjap.2024.004. PMID: 38902996.
10. Sharma A, Bara G, Keshamma E, Sharma B, Singh S, Singh SP, et al. Cancer biology and therapeutics: A contemporary review. J Cardiovasc Dis Res. 2023;14(10):1229–47.
11. Singh S, Chaurasia A, Gupta N, Rajput DS. Effect of formulation parameters on enalapril maleate mucoadhesive buccal tablet using quality by design (QbD) approach. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2024;40:e20240003. doi:10.62958/j.cjap.2024.003. PMID: 38925868.
12. Patel SK, Prathyusha S, Kasturi M, Godse KC, Singh R, Rathi S, et al. Optimizing irbesartan fast dissolving tablets using natural polysaccharides for enhanced drug delivery and patient compliance. Int Res J Multidiscip Scope. 2025;6(1):1181–90. https://doi.org/10.47857/irjms. 2025.v06i01.02542.
13. Patel P, Jain P, Patel H, Tiwari A, Rathi S, Singh S. Formulation, optimization and evaluation of mucoadhesive buccal tablets of ondansetron for enhanced bioavailability and sustained drug release. Biochem Cell Arch. 2025;25:1063–9. doi: https://doi.org/10.51470/bca.2025.25.1.1063.

