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UV Spectrophotometric Method Development And Validation For The Combined Plant Extract Traditionally Used In Arunachal Pradesh To Treat Malaria

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ABSTRACT

This study successfully developed and validated a simple, precise, and highly sensitive UV spectrophotometric method for the comprehensive analysis and quality control of combined plant extracts. Traditional medicine practitioners in Arunachal Pradesh, India, use a combination of *Coptis teeta wall*., *Andrographis paniculata*, and *Nyctanthes arbor-tris* to treat malaria. Hydroalcoholic extract of these three plants were prepared and combined in a 5:3:2 ratio. UV-visible double beam spectrophotometer method was used for determining maximum absorption wavelengths (λ_{max}) at 230nm for ethanol, 203nm for Phosphate buffer solution (PBs) (p^H 7.4), and 224nm for acidic buffer solution (ABs) (p^H 1.2). The developed method was rigorously validated according to ICH guidelines for linearity, precision, accuracy, LOD, and LOQ. The results have shown excellent linearity with high correlation coefficients (R^2> 0.9980 across various concentration ranges (5-60 µg/ml for ethanol and PBs, 5-90 µg/ml for ABs). Precision was confirmed through low relative standard deviation values for both intra-day and inter-day measurements. Accuracy was demonstrated by high percentage recovery values, affirming minimal error. The method also exhibited high sensitivity, with low LOD and LOQ values, enabling the detection and quantification of trace amounts of the analytes. The validated UV spectrophotometric method provides a rapid, cost-effective, and dependable tool for ensuring the identity, batch-to-batchg quality control evaluations and pharmacokinetic studies.

Keywords: *Medicinal plants, Formulation, Malaria, Phytochemistry.*

1. INTRODUCTION

Medicinal plants, also known as herbs, contain phytoconstituents with different therapeutic properties. Plant extracts have been utilized in traditional medicine systems, such as Ayurveda and Chinese medicine, to treat various diseases and illnesses [1]. With growing interest in exploring natural remedies, researchers are discovering new compounds from medicinal plants and investigating their potency for treating different diseases [2]. Medicinal plants like *Coptis teeta* wall. (CT), *Andrographis paniculata* (Burm.f.) wall. ex. Nees (AP) and *Nycthanthes arbor-tristis* (NA) are given in combination form by traditional medicine practitioners of Arunachal Pradesh to treat malaria, and many pharmacological activities of these plants have been reported. But using plant extract directly to treat various diseases has some drawbacks, such as low bioavailability, rapid metabolism, toxicity, and variability in composition [3]. To overcome the researcher are focusing on the development of plant extracts loaded nanoparticles (NPs) which will deliver them safely but will also protect sensitive phytochemicals from biological (such as enzymes and pH) and environmental degradation (such as light, temperature, humidity), and to enhance their therapeutic efficacy with better bioavailability and provide targeted delivery of phytochemicals to specific site with low toxicity [4, 5]. Evaluating plant extracts is crucial to maintaining the quality, effectiveness, and safety of herbal formulations used in pharmaceuticals, nutraceuticals, and cosmetics. These extracts contain various bioactive compounds responsible for their therapeutic properties, making their quantification essential for standardization and quality control [6]. Various analytical techniques, including chromatography and spectroscopic methods, have been employed to analyze plant-based products. Among these, UV spectrophotometry is most commonly and widely used due to its simplicity, cost-effectiveness, and rapid analytical performance [7,8].

In analytical chemistry, method development and validation play a vital role in ensuring the accuracy, reliability, and reproducibility of results [9]. Validation of analytical results involves evaluating crucial parameters such as specificity, linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ), following regulatory guidelines established by the International Council for Harmonisation (ICH, 2005) [10, 11]. A properly validated UV Spectrophotometric method is an effective and dependable tool for routine quality assessment of plant extract and herbal formulations.

Researchers simultaneously develop and validate the parameters for UV-visible Spectrophotometry to estimate and determine the phytoconstituents present in the plant extract by comparing with a pure chemical compound as a standard [12]. Since these plants are given in combined form by traditional medicinal practitioners, this study was not intended for the quantitative comparison or absolute quantification of a specific compound within the plant extract, but also to determine batch-to-batch quality consistency. To ensure and establish the identity, batch-to-batch consistency, and purity of the plant extracts, this study aimed to develop and validate a simple, precise, and extremely sensitive UV spectrophotometric method for the combined plant extract in the field of research and industrial applications, thereby supporting the standardization and quality control of herbal products.

The present study aims to develop and validate a simple, precise, and highly sensitive UV spectroscopy method for the combined plant extracts.

2. MATERIALS & METHOD

Instrumentation

A UV-visible double beam spectrophotometer (2206-TS, Systronics, India) was used for the analytical method development of plants' combined hydroalcoholic extracts (CE). Rotary Evaporator (IKA RV10, Germany) was used for recovering the solvent, and the Lyophilizer (SSI LYO, Southern Scientific, India) was used to lyophilize the plant extracts.

Materials

Plants were collected from three districts of Arunachal Pradesh, India. All the solvents and reagents used in the experiment were procured from Sigma-Aldrich Corporation (Germany), Hi-media (India), and Finar (India), and were of analytical grade.

Method

Preparation of combined plant extract

The hydroalcoholic extracts of CT, AP, and NA were prepared by cold maceration using a solvent-to-dried plant part ratio of 1:4. The solvent ratio was maintained at 1:1 (Water: ethanol) for 72 hours in a closed bottle container with frequent shaking [13]. Then, the extracts were filtered, and the solvent was recovered using a rotary evaporator. The extracts were dried in a Lyophilizer, and the lyophilized extracts were combined in a ratio of 5:3:2.

Preparation of Standard Stock Solution

Weigh 15 mg of combined extract (CE) accurately and then transfer it to a 50 mL centrifuge tube. Add 15 mL of ethanol to it, making the concentration 1 mg/mL (Stock-I). The prepared solutions were sonicated for 10 minutes with gentle heating and subjected to cold centrifugation at 5000 rpm for 5 minutes. Pipette out 10 mL of the above solution to a 100 mL volumetric flask, and then make up the volume to the mark with ethanol, making the concentration 100 µg/mL (Stock-II). The above process was repeated for phosphate buffer (pH 7.4) and acidic buffer solutions (pH 1.2) [14].

Determination of Wavelength Maximum (λ_{max})

CE mixtures were scanned using a UV-spectrophotometer between 200 nm and 400 nm wavelengths in three different concentrations, 10, 50, and 100 µg/mL. Ethanol, phosphate buffer pH 7.4, and acidic buffer solution pH 1.2 were used as a blank [15].

Preparation of Standard Calibration Curve

From the Stock-II solution, aliquots of 0.5, 1, 2, 3, 4, 5, and 6 ml were pipetted out and transferred to a 10 ml volumetric flask for each aliquot. The final volume was made up of Ethanol to obtain concentrations of 5, 10, 20, 30, 40, 50, and 60 µg/mL, respectively. The same procedure was performed for the required concentrations, both for the pH 7.4 phosphate buffer and pH 1.2 acidic buffer solutions, at concentrations ranging from 5 to 60 µg/mL and 5 to 90 µg/mL, respectively. Then, the standard calibration curve of CE was obtained by measuring the absorbance of CE solution in ethanol, PBS pH 7.4, and ABS pH 1.2 until the ideal range of absorbance values (0.1 to 1.0) was obtained [16].

Analytical method validation

According to the ICH guidelines, establishing documented proof that provides a high assurance that a particular activity will consistently generate a desired outcome or product that meets its set specifications and quality features is known as validation.

According to the ICH guidelines (Q2)R1, various parameters, including linearity, Accuracy, precision, Ruggedness, Robustness, Limit of Detection (LOD), and Limit of Quantification (LOQ), were validated [17].

Linearity & Range

To determine the linearity, the Stock-II solution was prepared. Seven aliquots (5, 10, 20, 30, 40, 50, and 60 µg/mL) were accurately pipetted into seven 10 mL volumetric flasks. Ethanol waswere added to increase the volume to 10 mL for both Ethanol and PBs, and the absorbance of each solution was measured at 230 and 203 nm, respectively. Ten aliquots (5, 10, 20, 30, 40, 50, 60, 70, 80, and 90 µg/mL) of ABs were pipetted as above, and absorbance was measured at 224 nm using the same solvent system as the blank by UV-visible spectrophotometer. Then, a calibration curve was established by plotting concentrations on the x-axis and absorbances on the y-axis. A calibration curve was established. The linearity was determined using a regression equation, indicated by the square of the correlation coefficient. The process was performed in triplicate [18].

Precision

Precision studies were conducted to determine the reliability and reproducibility of the proposed analytical method through intraday and interday variations for PBs and ABs. For intraday, three different aliquots, 5, 30, and 60 µg/ml from PBs and 5, 50, and 90µg/ml from Abs, were pipetted out from the stock-II solution, and absorbance was measured in the morning and evening within the same day to ensure intraday precision. Intraday and interday precision was evaluated by measuring the absorbance of the above aliquots daily for three consecutive days [19].

Accuracy

Accuracy is vital in confirming the planned method to evaluate how closely the measured value matches the actual or observed value. Percentage Recovery studies were carried out to determine the accuracy, and the technique was repeated three times for each concentration using the equation % Recovery = $(C_t/C_a) \times 100$, where C_a is the total CE concentration after addition and C_t is the CE Concentration in the test sample. [17].

LOD and LOQ

Limit of Detection (LOD) is the lowest concentration of the analyte that the instrument can reliably detect, but not quantify. LOD was calculated using the equation LOD = $3.3 \times \sigma/S$, where σ is the relative standard deviation of the response and s is the slope of the calibration curve. The limit of Quantification (LOQ) is the lowest concentration of the analyte that can be reliably measured or quantified with acceptable accuracy and precision. LOQ was calculated using the equation LOQ = $10 \times \sigma/S$, where σ is the relative standard deviation of the response and S is the slope of the calibration curve for the analyte [20].

3. RESULTS AND DISCUSSION

Determination of Maximum Wavelength

The wavelength of the CE for respective solvents was found at 230, 203, and 224 nm for Ethanol, PBs, and ABs, respectively (as shown in **Figures 1**, **2**, and **3**).

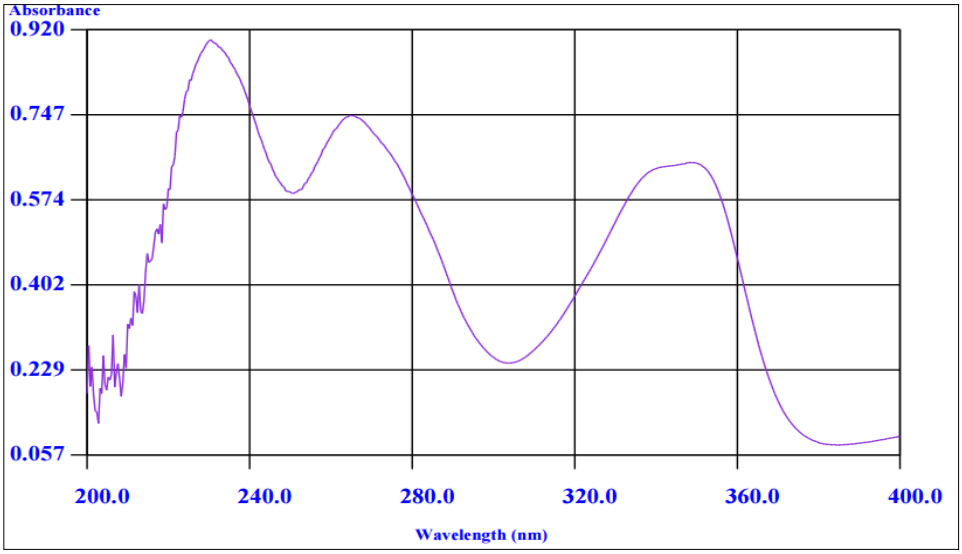

Figure 1: UV Spectrum of CE in Ethanol.

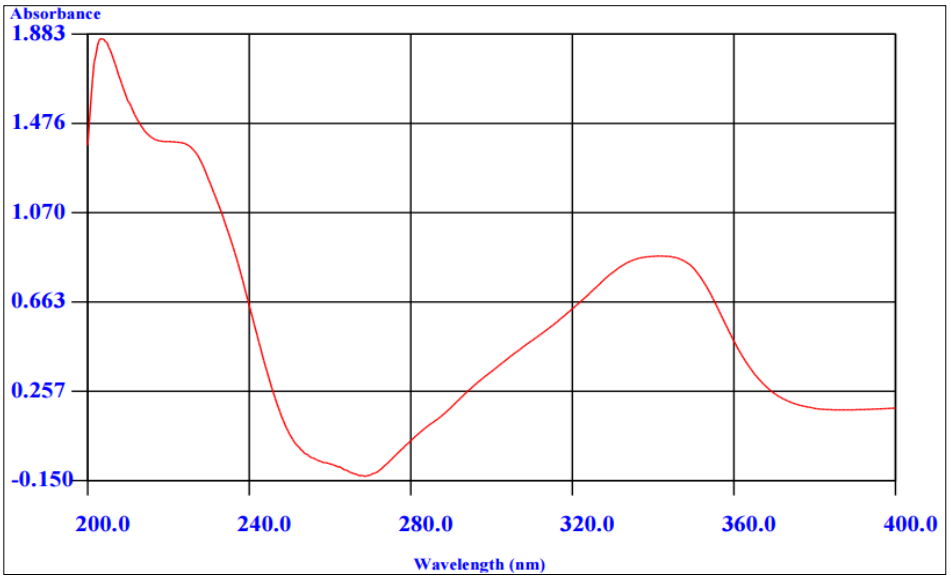

Figure 2: UV Spectrum of CE in Phosphate Buffer solution (PBs)

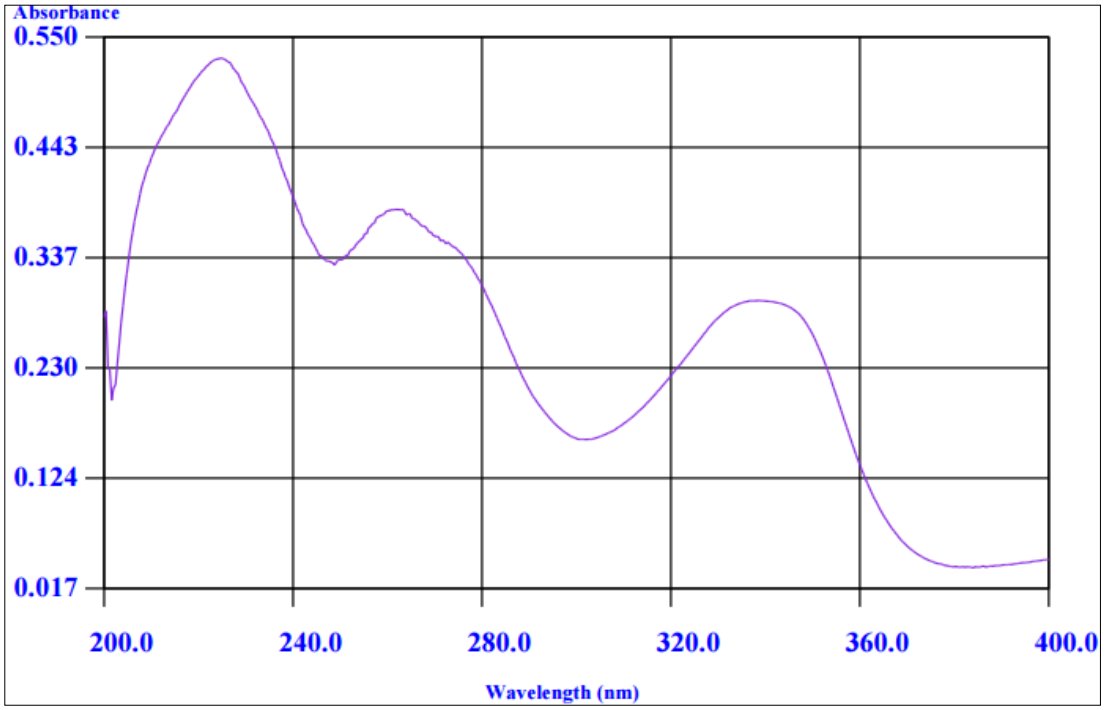

Figure 3: UV Spectrum of CE in Acidic Buffer solution (ABs)

Preparation of Standard Calibration Curve

The calibration plot of CE was found to be linear with correlation coefficients of 0.998, 0.9988, and 0.9985 for Ethanol, PBs, and ABs, respectively (as shown in **Figures 4, 5,** and **6**).

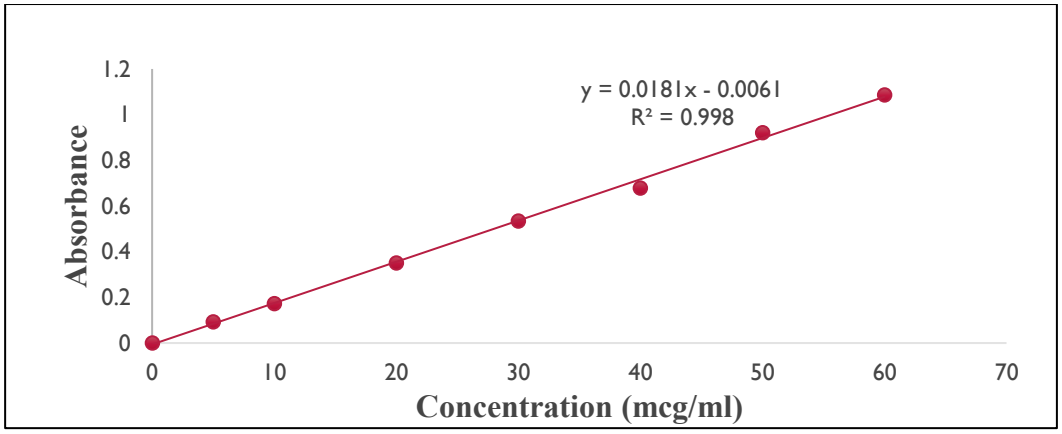

Figure 4: Standard Calibration Curve of CE in Ethanol

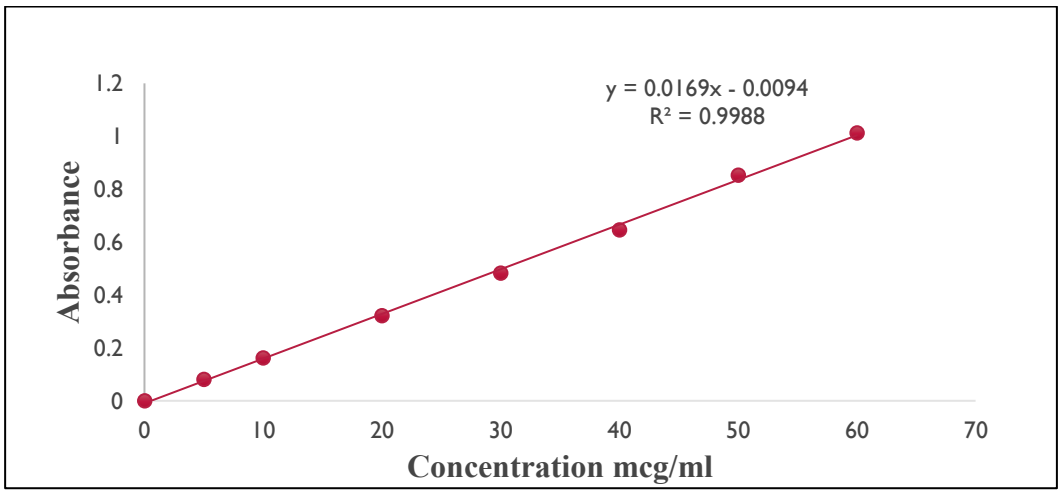

Figure 5: Standard Calibration Curve of CE in PBs

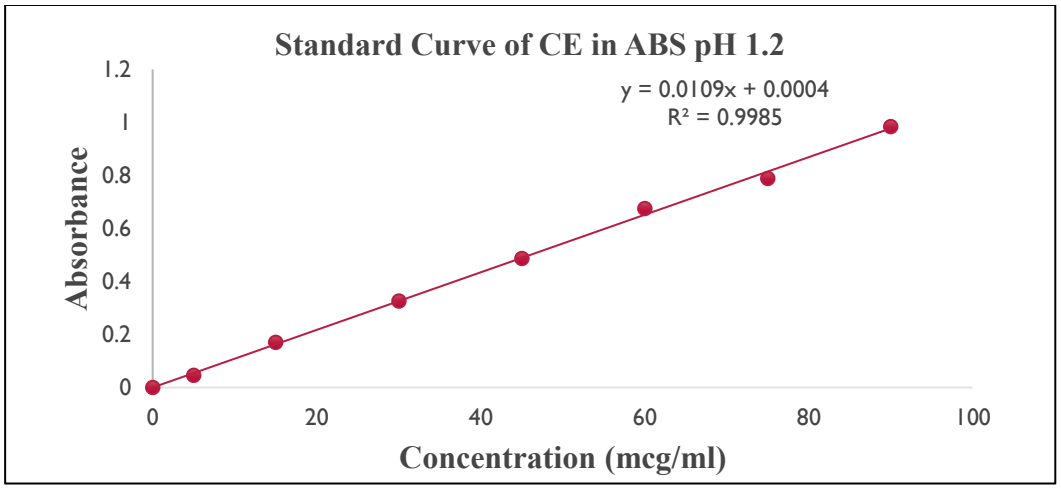

Figure 6: Standard Calibration Curve of CE in ABs

Method Validation

CE was dissolved in all the solvents to carry out simultaneous estimation. For this approach, analytical grade Ethanol, prepared PBs, and ABs were selected as the solvents for this method. As per the ICH guidelines, the proposed method was

validated. For the proposed study, the solutions were prepared according to the adopted procedure mentioned above, and then the method was validated in terms of linearity, precision, and accuracy. To ensure accurate quantification, the chosen wavelengths, 230 nm for Ethanol, 203 nm for phosphate Buffer Solution, and 224 nm for Acidic buffer Solution, were selected because they represent the point of maximum light absorption as shown in **Table 1**.

Table 1. Different parameters and their specifications.

Serial No.	Parameters	Specifications
1.	Analytes	Combined Extract (CE)
2.	Solvent	Ethanol, PBs pH 7.4, and ABs pH 1.2
3.	Maximum Absorbance of CE	206nm, 203nm, and 224nm

Linearity

The UV-Vis method's linearity for CE determination was evaluated over a 5 to 60 µg/ml concentration range for Solvents Ethanol and PBs, and 5 to 90 µg/ml for ABs. The calibration curve exhibited excellent linearity with R^2 values of 0.998, 0.9988, and 0.9985. The linear regression equation obtained was y = 0.0181x-0.0061, y = 0.0169x-0.0094, and y = 0.0109x + 0.0004 for Ethanol, PBs, and Abs, respectively. The reliably measured concentrations across a broad range prove that a strong linear relationship exists between concentration and absorbance, as shown in **Table 2**.

Table 2. Linearity of the CE in Ethanol, PBs, and ABs.

Sl. No.	Conc. EtOH (µg/ml)	Abs	Conc. PBs (µg/ml)	Abs.	Conc. Abs (µg/ml)	Abs
1.	0	0	0	0	0	0
2.	5	0.093	5	0.081	5	0.046
3.	10	0.173	10	0.161	15	0.171
4.	20	0.351	20	0.321	30	0.326
5.	30	0.534	30	0.482	45	0.487
6.	40	0.679	40	0.646	60	0.675
7.	50	0.921	50	0.852	75	0.789
8.	60	1.086	60	1.012	90	0.984
R^2	0.998		0.9988		0.9985	
Slope	0.0181		0.0169		0.0109	

Precision

The precision of the developed UV method for quantifying CE was evaluated for both repeatability and intra-day and inter-day precision. For repeatability, three replicates of three different concentrations of 5, 30, and 60 µg/ml for solvents ethanol and PBS, and 5, 50, and 90 µg/ml for solvent ABs, were analysed on three different days. The same concentrations in the same solvents were evaluated for intra-day and inter-day precision by analysing them on three different days. The mean, standard deviation, and % RSD of the repeatability and precision study are shown in **Tables 3** and **4**.

Table 3. Precision results showing repeatability of the CE in Ethanol, PBs, and ABs.

Serial No.	Conc. 30µg/ml (Ethanol)	Conc. 30µg/ml pH 7.4	Conc. 45µg/ml pH 1.2
1	0.537	0.481	0.487
2	0.539	0.479	0.485

3	0.533	0.486	0.489
4	0.538	0.475	0.483
5	0.538	0.476	0.487
6	0.536	0.479	0.481
Mean±SD	0.537±0.002	0.479±0.004	0.485±0.003
RSD	0.39%	0.82%	0.61%

Table 4. Precision results showing repeatability of the CE in Ethanol, PBs, and ABs.

Precision	Conc. µg/ml	Ethanol		PBS		Conc. µg/ml	AB	
		Morning	Evening	Morning	Evening		Morning	Evening
Intra-day Precision	5	4.572744	4.9042357	4.698225	4.974359	5	4.366972	4.88685
	30	29.434622	29.78453	28.643	29.13609	45	44.18349	43.72477
	60	58.127072	59.01105	58.24852	58.72189	90	89.74924	90.02446
Avg. %RSD		1.245831	0.91573	0.77229	0.808249	Avg. %RSD	1.257147	0.859901
Inter-day Precision	5	4.664825	4.9042357	4.974359	4.77712	5	4.764526	5.009174
	30	29.78453	30.042357	28.91913	29.37278	45	44.42813	44.18349
	60	58.974217	59.121547	58.70217	59.15582	90	90.20795	89.93272
Avg. %RSD		2.390612	1.064815	0.384433	0.60884	Avg. %RSD	0.948969	0.76529

Accuracy

The accuracy of the developed UV method was assessed through recovery studies at three different concentrations: low, medium, and high. The results showed excellent accuracy within the technique's operating range. In this development and validation process, potential sources of error, including volumetric fluctuations and standard purity, were carefully controlled.

Table 5. Data showing accuracy of the developed method.

Conc. µg/ml	Ethanol Avg % Recovery	PBS Avg % Recovery	Conc. µg/ml	ABS Avg % Recovery
5	91.45488029	93.96449704	5	87.33944954
30	98.11540823	95.47666009	45	98.18552497
60	96.87845304	97.08086785	90	99.72137275

Sensitivity

The developed UV method for the intended application showed adequate sensitivity. The LOD and LOQ for CE in different solvents are shown in the table. These values indicate that the developed method can detect trace amounts of analyte, which is crucial for monitoring potential impurities and ensuring reliable analyte quantification at concentrations relevant to the study. This shows that the approach is appropriate for the application where low concentrations of plant extracts have to be detected, including pharmacokinetic research or quality control evaluations of herbal products.

Table 6. Data showing LOD and LOQ of the Combined Extracts (CE).

Sl. No.	Solvents	LOD µg/ml	LOQ µg/ml
1.	Ethanol	0.28	0.85
2.	PBS	0.19	0.59
3.	ABS	0.30	0.91

4. CONCLUSION

This study successfully developed and validated a simple, precise, and highly sensitive UV spectrophotometric method for the comprehensive analysis of combined plant extracts. Leveraging the inherent advantages of UV spectrophotometry, namely its speed, cost-effectiveness, and widespread accessibility compared to more complex techniques like HPLC and electrochemical methods, this research provides a robust tool for the essential quality control of herbal products. The method demonstrated excellent linearity across various relevant concentration ranges in ethanol, phosphate buffer (pH 7.4), and acidic buffer (pH 1.2), with high correlation coefficients (R^2 >0.998). Precision studies confirmed the method's reliability and intra-day/ inter-day measurements. Furthermore, high percentage recovery values affirmed the method's accuracy, effectively minimizing potential sources of error in the quantification process. Crucially, the determined Limits of Detection (LOD) and Limits of Quantification (LOQ) for the combined extract in different solvents signify the method's ability to detect and quantify even trace amounts of the analyte. This enhanced sensitivity is particularly valuable for monitoring impurities, supporting pharmacokinetic investigations, and ensuring the consistent quality of herbal products in research and industrial settings. By precisely identifying the optimal wavelengths for maximum absorption, 230 nm for ethanol, 203 nm for phosphate buffer, and 224 nm for acidic buffer, this validated UV spectrophotometric method stands as an effective and dependable tool. It will play a vital role in ensuring the identity, batch-to-batch consistency, and overall quality of combined plant extracts, thereby supporting the advancement and standardization of traditional medicine and natural product development.

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Conflict of interest

The Authors declare that they don't have any potential conflict of interest.

Data availability

The data will be made available upon reasonable request.

REFERENCES

[1] Grover JK, Vats V. Shifting paradigm: from conventional to alternative medicines-an introduction on traditional Indian medicines. Asia-Pacific Biotech News. 2001 Jan 8;5(01):28-32.

[2] Nasim N, Sandeep IS, Mohanty S. Plant-derived natural products for drug discovery: current approaches and prospects. The Nucleus. 2022 Dec;65(3):399-411.

[3] Heinrich M, Jalil B, Abdel-Tawab M, Echeverria J, Kulić Ž, McGaw LJ, Pezzuto JM, Potterat O, Wang JB. Best practice in the chemical characterization of extracts used in pharmacological and toxicological research-the ConPhyMP-guidelines. Frontiers in Pharmacology. 2022 Sep 13; 13:953205.

[4] Husni P, Ramadhania ZM. Plant extract loaded nanoparticles. Indonas. J. Pharm. 2021; 3:38-49.

[5] Santhosh SB, Yuvarajan R, Natarajan D. Annona muricata leaf extract-mediated silver nanoparticles synthesis and its larvicidal potential against dengue, malaria and filariasis vector. Parasitology research. 2015 Aug;114:3087-96.

[6] Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants. 2017 Sep 22;6(4):42.

[7] Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY. Extraction, isolation and characterization of bioactive compounds from plants' extracts. African journal of traditional, complementary and alternative medicines. 2011;8(1).

[8] Gowthamarajan K, Jawahar N, Wake P, Jain K, Sood S. Development of buccal tablets for curcumin using Anacardium occidentale gum. Carbohydrate Polymers. 2012 May 16;88(4):1177-83.

[9] Mayuri D, Ravindranath S. Analytical Method Development and Validation: A Review. Journal of Drug Delivery & Therapeutics. 2019 May 1;9(3).

[10] Guideline IH. Validation of analytical procedures: text and methodology. Q2 (R1). 2005 Nov;1(20):05.

[11] Che Zain MS, Osman MF, Lee SY, Shaari K. UHPLC-UV/PDA method validation for simultaneous quantification of Luteolin and Apigenin derivatives from Elaeis guineensis leaf extracts: an application for antioxidant herbal preparation. Molecules. 2021 Feb 19;26(4):1084.

[12] Patil AK, Jalalpure SS, Chimagave SS, Kurangi BK. UV-Spectrophotometric Method Development and Validation for Piperine Estimation in Black Pepper, Ayurvedic Formulation and Novel Nano Formulation: A Perfect Quality Assessment Tool. Indian J. Pharm. Educ. Res. 2024;58(1):305-15.

[13] Abubakar AR, Haque M. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy and Bioallied Sciences. 2020 Jan 1;12(1):1-0.

[14] Bhusannavar D, Sharannavar B, Darbha NS, Suryawanshi S, Patil S, Bhandurge P. Development and Validation of UV-visible Spectrophotometry Method for Simultaneous Estimation of Rutin and Curcumin in Bulk Pharmaceuticals. International Journal of Pharmaceutical Investigation. 2024 Jul 1;14(3).

[15] Majumder KK, Sharma JB, Kumar M, Bhatt S, Saini V. Development and Validation of UV-Visible Spectrophotometric Method for The Estimation of Curcumin in Bulk and Pharmaceutical Formulation. Pharmacophore. 2020;11(1-2020):115-21.

[16] Chauhan I, Singh V, Majhi S, Verma M, Yasir M. Development and validation of UV-spectrophotometric method for berberine quantification. Journal of Applied Pharmaceutical Sciences and Research. 2024 Aug 1;7(2):28-33.

[17] Warule PS, Patel VP, Gosavi SA. Development and Validation of UV Spectrophotometric Method for the Estimation of Curcumin in an Ayurvedic Formulation Haridrakhand. Int J Pharm Drug Anal. 2017;5(5):193-7.

[18] Sharma JB, Bhatt S, Saini V, Gautam RK, Kumar M. Uv-visible spectrophotometric method development and validation for the estimation of curcumin and tetrahydrocurcumin. Research Journal of Pharmacy and Technology. 2022;15(2):650-4.

[19] Jain PS, Chaudhari AJ, Patel SA, Patel ZN, Patel DT. Development and validation of the UV-spectrophotometric method for determination of terbinafine hydrochloride in bulk and in formulation. Pharmaceutical methods. 2011 Jul 1;2(3):198-202.

[20] Kumari B, Khansili A. Analytical method development and validation of UV-visible spectrophotometric method for the estimation of vildagliptin in gastric medium. Drug Research. 2020 Sep;70(09):417-23.