RESEARCH ARTICLE

UV Spectrophotometric Studies of Ashwagandha, Chamomile and Fever Few Flowers Oil: Method Development and Validation

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ABSTRACT

Using the UV-Vis spectrophotometry technique is a simple way to estimate or quantify any pharmacological compounds. Present research focused on development and validation of UV-visible method for feverfew (FFW), chamomile (CMM), and ashwagandha (AGA) flower oils. This method was also applied in conjunction with the UV spectrophotometric analysis of AGA, CMM, and FFW. Method validation parameters revealed that specificity, accuracy, linearity, precision and robustness met the acceptance criteria of ICH standards. It was concluded that developed. UV spectroscopic method proved to be sufficiently precise, robust, linear, and accurate for routine analysis of AGA, CMM, and FFW in polyherbal formulations.

Keywords: Feverfew, chamomile, and ashwagandha, UV spectrophotometer, Method validation etc.

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INTRODUCTION

Global demand for polyherbal formulations is growing quickly as a result of the striking rise in the use of herbal therapy.[1,2] On the other hand, standardising herbal products is necessary in order to evaluate their clinical safety, efficacy, and quality prior to their release onto the market, in compliance with WHO guidelines.[3] AG belongs to family Solanaceae[4], while CM)[5], FFW belongs to Asteraceae.[6] Pharmacological investigations have shown that ashwagandha plant preparations has anti-inflammatory, antioxidant, anticancer, anxiolytic, and immunomodulatory qualities. Ashwagandha has also been demonstrated to have effects on the neurological, endocrine, and cardiovascular systems. This herb has been utilised for millennia and has shown neuroprotective, relaxing, and antidepressant properties in stressed animals. The active ingredients in chamomile include flavonoids, coumarins, sesquiterpenes, and polyacetylenes. The blue essential oil that is produced from the blossoms includes coumarin, flavonoids including luteolin, patuletin, apigenin, and quercetin, and terpenes like bisabolol, farnesene, and chamazulene. A popular herb used to both prevent and treat migraine headaches is feverfew.[7,8] Various methods have been reported for estimating AGA, CMM, and FFW in different pharmaceutical and herbal formulations.[10] Nevertheless, these methods are not appropriate for examining the constituents of polyherbal formulations, such those seen in Chinese and Ayurvedic medicines, which include many different herbs.[11] More suitable for this purpose is UV spectrophotometry, there are very few studies on dedicated UV-spectrophotometric methods for quantifying AGA, CMM, and FFW in polyherbal formulations.[12-15] Therefore, for the quantitative assessment of AGA, CMM, and FFW in polyherbal formulations, a straightforward UV technique was created and validated in the current study in accordance with International Conference on Harmonisation (ICH) recommendations.[16] A review of the literature revealed that no UV method using buffer with a pH of 6.8 as a solvent has yet been proposed for the evaluation of AGA, CMM, and FFW in polyherbal formulations.

Instruments

Shimadzu double beam UV-vis spectrophotometer (Model UV-1700) was used for data acquisition using the UV Probe software from the spectra Manager program. An electronic analytical balance was employed for weight measurements.

Chemicals

The herbal oils AGA, CMM, and FFW were acquired from Vital Herbs, Delhi -110059. sodium hydroxide, potassium dihydrogen phosphate, sodium chloride, and so forth of laboratory grade.

UV Spectrophotometric Studies

5 ml of AGA, CMM, and FFW were dissolved in 50 ml of phosphate buffer 6.8 individually and scanned in the range of 200 – 400nm in basic spectrum mode. λ_{max} were recorded and compared with literature value.

Calibration curve preparation

Ten milliliters each of AGA, CMM, and FFW were dissolved in 100 ml of pH 6.8 phosphate buffer with

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Table 1: The suggested UV technique for herbal extracts at 329, 312, and 250 nm, respectively, and its linearity and range.

Concentrations (µl/ml)	Absorbance (λ_{max}) (mean±SD) (n=3)			% RSD		
	AGA (329 nm)	CMM (312 nm)	FFW (250 nm)	AGA	CMM	FFW
2	0.185± 0.0115	0.025± 0.0115	0.045± 0.0021	1.1112	0.36464	1.0123
4	0.325± 0.0057	0.104± 0.0053	0.104± 0.0010	0.03077	0.4900	0.5379
6	0.506± 0.0058	0.194± 0.0025	0.194± 0.0015	0.3009	1.280	2.926
8	0.653± 0.0015	0.299± 0.0020	0.299± 0.0021	0.3176	0.664	0.511
10	0.804± 0.0053	0.396± 0.0015	0.396± 0.0010	0.1242	0.384	1.323
12	0.941± 0.0015	0.513± 0.0021	0.513± 0.0025	0.2667	0.408	0.298

Table 2: Table displaying precision study of AGA, CMM, and FFW using UV technique both during and between days.

Concentrations (µl/ml)	Repeatability (Intra-day precision) (n=3)					
	Absorbance (λmax) (mean±SD) (n=3)			% RSD		
	AGA (329 nm)	CMM (312 nm)	FFW (250 nm)	AGA	CMM	FFW
12	0.947±0.0046	0.0507±0.0005	0.510±0.0010	0.26	0.51	0.35
Intermediate Precision (Inter- day precision) (n=3)						
2	0.185±0.0115	0.025±0.0115	0.045±0.0021	1.12	0.364	0.30123
8	0.653±0.0015	0.299±0.0020	0.299±0.0021	0.3176	0.664	0.511
12	0.941±0.0015	0.513±0.0021	0.513±0.0025	0.2667	0.408	0.298

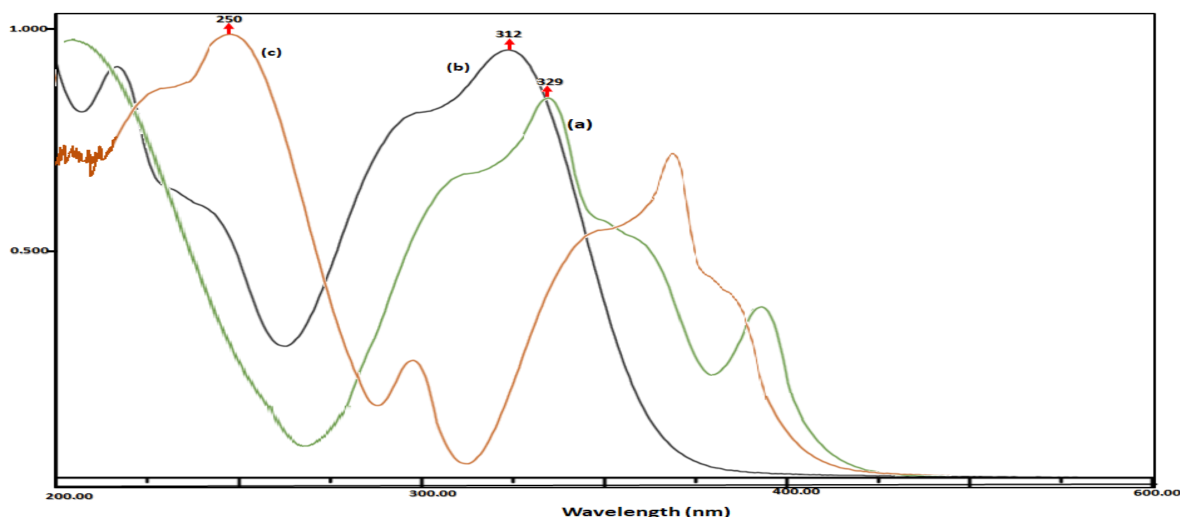

Figure 1: λ_{max} scanning through UV-vis spectrophotometer of (A) AGA, (B) CMM, (C) FFW in 6.8 pH Buffer.

concentration of 100 µl/ml. For every sample oil, aliquots of the stock solutions i.e. 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml were transferred to 10 ml volumetric flasks individually and diluted by pH 6.8 phosphate buffer upto the mark for preparation of concentrations ranging from 2-12 µl/ml.[17]

The concentrations of AGA, CMM, and FFW were measured at wavelengths of 329 nm, 312 nm, and 250 nm, respectively.

Validation of Calibration Curve

As per the requirements for the validation of analytical techniques provided by ICH Q2 (R1), the method was validated. Accuracy, precision, linearity, specificity, and other crucial validation characteristics were evaluated. For the validation of analytical techniques such as assays, these requirements are deemed necessary.[18]

Specificity

Specificity in method validation pertains to the ability of an analytical technique to accurately identify the analyte, even when potentially interfering substances are present, including degradants, contaminants, or elements from the matrix. When a technique correctly detects and measures the target analyte without the interference of other chemicals in the sample, it is said to be specific. To guarantee that the procedure accurately detects the target analyte even in complicated combinations, specificity is very important.[11-15] The method used to confirm the

Table 3: Studies on the accuracy of the suggested UV technique.

Initial amount (µl/ml)	Added amount (µl/ml)	Predicted concentration (µl/ml)	Observed concentration (µl/ml)			Residual concentration (µl/ml)			% Mean Recovery			%RSD		
			AGA	CMM	FFW	AGA	CMM	FFW	AGA	CMM	FFW	AGA	CMM	FFW
2	1(50%)	3	2.35	2.89	1.98	0.65	0.11	1.02	96.67	89.67	66.4	1.05	1.12	1.00
2	2(100%)	4	3.99	3.76	3.78	0.01	0.24	0.22	97.75	99	97.1	0.91	1.03	1.07
2	3(150%)	5	4.35	4.67	4.78	0.65	0.33	0.22	98	97.4	92.1	1.03	0.35	1.00

Table 4: Robustness of absorbances using UV technique at absorption maxima of 329, 312, and 250 nm.

Variable parameters	Absorbance of 12 µl/ml (Mean±SD)			%RSD			Mean % recovery		
	AGA (329 nm)	CMM (312 nm)	FFW (250 nm)	AGA	CMM	FFW	AGA	CMM	FFW
Analyst 1	0.997±0.108	0.0612±0.911	0.596±0.891	0.8449	1.0862	1.7063	91.72	90.2	81.3
Analyst 2	1.001±0.901	0.0732±0.954	0.551±0.921	0.9719	1.0326	1.4395	92.72	98.2	97.1

specificity involved three UV spectrophotometric scans of individual AGA, CMM, and FFW standard solutions (1 mg/mL) spanning a wavelength range of 200–400 nm, with phosphate buffer pH 6.8 serving as the blank.

Linearity

By measuring each standard concentration of AGA, CMM, and FFW (2–12 µl/ml) at 329, 312, and 250 nm and comparing the results to blank (pH 6.8), the linearity was ascertained. For each of the three substances at conventional concentration ranges (2–12 µl/ml), regression equation and correlation coefficient were derived from the standard curve.

Precision

To assess the accuracy of the current procedure, a repeatability study (both intra- and inter-day) was carried out .[10,14] Intra-day precision was evaluated by measuring the absorbance three times in a single day at a concentration of 12 µl/ml, and the average Relative Standard Deviation (RSD) percentage was calculated from the data. To assess inter-day accuracy, absorbance measurements were taken over three consecutive days at three different sample concentrations (2, 8, and 12 µl/ml), allowing for the calculation of the average RSD percentage.

Accuracy

The degree to which the theoretical and experimental values closely agree with the data is how the method's accuracy is measured. Preciseness is frequently expressed as the percentage of the known analyte quantity recovered.[9-13] The percentage difference between the experimental and theoretical concentrations was used to compute the recovery. The pre-analyzed sample solutions (1, 2, and 3 µl/ml) were spiked with known addition quantities of AGA, CMM, and FFW, and the % recovery was used to assess accuracy.

$$\% \text{ Recovery} = \frac{\text{Ct}}{\text{Ca}} \text{ x } 100$$

Ct= AGA, CMM, and FFW concentration in the test sample

Ca= the total AGA, CMM, and FFW concentration after standard addition.

Robustness

Robustness in method validation refers to the method's capability to maintain reliability under normal conditions, even when minor, intentional variations in parameters are introduced. Evaluating robustness is a crucial part of method validation, as it helps ensure that the method can perform consistently under different conditions, which may occur in routine analysis.[13,16] By utilising distinct analysts and tools, robustness was assessed for the AGA, CMM, and FFW standard solutions (100 µl/ml).

Sensitivity

The sensitivity evaluation involved determining the limit of detection (LoD) and limit of quantitation (LoQ) by calculating the standard deviation (σ) of the responses and the slope (S) of the linearity plot[2,5], derived from AGA, CMM, and FFW. Sample absorbance was measured three times, and the σ of the answers was computed to calculate the standard deviation. Next, the following formula was used to determine the LoD and LoQ:

$$\text{LoQ} = 10 * \frac{\sigma}{\text{S}}$$

$$\text{LoD} = 3.3 * \frac{\sigma}{\text{S}}$$

RESULTS AND DISCUSSIONS

Specificity

Specificity was verified from the individual standard concentrations of AGA, CMM, and FFW (100-500 µl/ml). Each scan showed a distinct peak at 329 nm, 312 nm, and 250 nm, respectively, confirming these as the average wavelengths of maximum absorbance (λ_{max}). As a result, as indicated in Table 1, these wavelengths were chosen for linearity investigations. Figure 1 displays the specificity analysis's findings.

Limits of quantification and detection

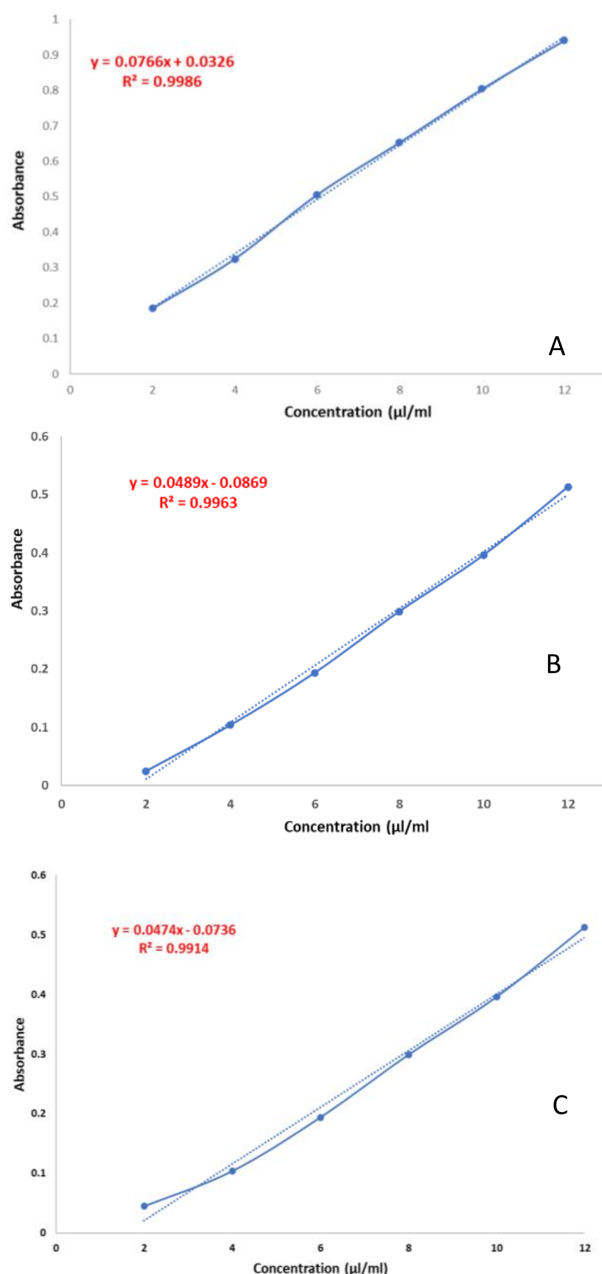

Figure 2: Calibration curve of AGA, CMM and FFW using average range of absorbance (n=3).

Detection and quantitation limits were measured to be 0.469 µl/ml, 0.512 µl/ml, 0.603 µl/ml and 1.58 µl/ml, 1.023 µl/ml, 1.004 µl/ml for AGA, CMM and FFW respectively.

Linearity

The regression graphs showed good linearity between absorbance and concentration, with correlation coefficients (r^2) of 0.9986, 0.9963, and 0.9914, respectively for AGA, CMM and FFW (Figure 2), suggesting conformity with Beer-Lambert's Law across the concentration range of 2–12 deviations from linearity were observed across the concentration ranges, suggesting that the method is reliable across this range without any expected issues at specific concentrations.

Precision

Three levels can be used to evaluate accuracy: reproducibility, intermediate precision, and repeatability. Repeatability and intermediate precision accuracy findings showed a %RSD of less than 2%, as Table 2 illustrates.

Accuracy

The method demonstrated the ability to directly measure analyte concentrations within the range of 2-12 µl/ml, which was further validated by accuracy studies covering 50-150% of the test concentration. Recovery percentages were as follows: for AGA, 96.67% to 98.00%; for CMM, 89.67% to 99.00%; and for FFW, 66.4% to 97.10%. The recovery percentages in these data were within ±2% of the actual concentration, which satisfied the acceptability requirements as per the ICH recommendations. The accuracy findings are detailed in Table 3.

Robustness

An analytical technique is considered robust if it can sustain its dependability even in the face of slight, deliberate alterations in method parameters.[14] This characteristic is crucial to ensure that the method can tolerate normal variations in conditions, thereby providing consistent and reliable results in routine analysis.[18] The method demonstrated robustness, as evidenced by consistent and reliable absorbance measurements despite variations in the analyst and the instrument. Triplicate determinations at a selected concentration of 12 µl/ml for AGA, CMM, and FFW were conducted, with % RSD values under 2% for both parameters. Additionally, the percentage recovery for AGA, CMM, and FFW was within the appropriate range, as shown in Table 4. Therefore, the robustness of the method was confirmed. A straightforward, quick, precise, and affordable UV-spectrophotometric technique has been created for the method development of AGA, CMM, and FFW flower oils (Table 5).

CONCLUSION

The particular acceptance requirements for the UV spectroscopic technique are satisfied in terms of specificity, linearity, accuracy, precision, and LoD and LoQ on both intraday and interday basis. It was discovered that this approach of quantifying the herbal oils of AGA, CMM, and FFW was straightforward, repeatable, accurate, and exact.

Table 5: Analytical method development.

Parameters	AGA	CMM	FFW
λmax	329 nm	312 nm	250 nm
Slope	0.0766	0.0489	0.0474
Intercept	0.0326	0.0869	0.0736
Correlation coefficient	0.9986	0.9963	0.9914
Repeatability (% RSD)	0.3585	0.5984	1.1013
Precision (%RSD)	Intra-day = 0.26	Intra-day = 0.51	Intra-day = 0.35
Precision (%RSD)	Inter-day= 0.5681	Inter-day= 0.47866	Inter-day= 0.3700
Robustness (% RSD)	0.9084	1.0594	1.5729

The method's cost-effectiveness, which results from using fewer chemicals and apparatus, is its greatest significance. This approach might serve as a substitute for estimating the amount of oils in medicinal formulations. The proposed and validated method was found to be simple, economical, and environmentally friendly. It was successfully implemented for the AGA, CMM, and FFW without requiring any outside intervention. This approach may be exceptional and special for the examination of AGA, CMM, and FFW in polyherbal formulation, as indicated by the findings and statistical parameters.

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