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Development and Validation of UV-Spectrophotometric method for the Estimation of *Saraca asoca*, *Bauhinia variegata Linn*, and *Commiphora mukul* in standardized Polyherbal Formulation

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Abstract

Researchers aimed to develop a simple UV-spectrophotometric method relates to the estimation of *Saraca asoca (SA)*, *Bauhinia variegata Linn (BV)*, and *Commiphora mukul (CM)* in standardized polyherbal formulations. The method validation parameters were evaluated as per International Conference on Harmonization (ICH) guidelines. Further, this method was applied for the assay of *SA*, *BV*, and *CM* using UV-spectrophotometric method. Specificity, linearity, range, precision, accuracy, and robustness were proven to be within the boundaries of acceptance requirements set by ICH standards based on the findings of method validation. In conclusion, the developed approach was shown to be specific, linear, exact, accurate, and robust enough to be used for routine analysis of polyherbal formulations containing *SA*, *BV*, and *CM* as their main constituents in the extracts.

Key words: UV-spectrophotometric method, *Saraca asoca*, *Bauhinia variegata Linn*, and *Commiphora mukul*, ICH Guidelines.

INTRODUCTION

In the post-genomic era, World Health Organization (WHO) estimated about 80% of the world population uses herbs and other traditional medicines for their primary health care needs [1]. Marvelous rise in the use of herbal medicine is leading to a fast-growing market of Polyherbal formulation worldwide [2]. Whereas, according to WHO guidelines, standardization of herbal products is essential to assess the quality, clinical safety, and efficacy before releasing into the market [3]. *SA*, *BV*, and *CM* individually belonging to the family *Leguminosae* [4], *Fabaceae* [5] and *Burseraceae* [6]. Numerous clinical trials over the past two decades have addressed the safety, and efficacy of this nutraceutical against multiple diseases including cancer, tumor, PCOS, hormonal imbalance, inflammations, etc [7, 8, 9]. Acute toxicity studies have indicated the safety of *SA*, *BV*, and *CM* at doses as (1000 mg/kg) over 14 days. In comparison to other marketed formulations, the combination of (*SA*, *BV*, and *CM*) the Polyherbal formulation (phytosomes) have a significant role in the treatment of ovarian cyst [10, 11, 12]. Several methods were reported for the estimation of *SA*, *BV*, and *CM* in different pharmaceutical and herbal formulations by using ultra-violet (UV) [13], High performance liquid chromatography (HPLC) [14], Fourier Transform Infrared Spectroscopy (FTIR) [15], High-performance thin-layer chromatography (HPTLC) [16], and other hyphenated methods [17, 18]. However, these techniques are not suitable for analyzing compounds in a combination of Polyherbal formulations like Ayurvedic Chinese medicinal products, since they contain more than one herb. While UV-spectrophotometric methods are more suitable for this objective, studies on dedicated UV-spectrophotometric methods to quantify the *SA*, *BV*, and *CM* in Polyherbal formulations are very limited [19]. Therefore, in the present study, a simple UV method was developed and validated according to international conference harmonization (ICH) guidelines for the quantitative estimation of *SA*, *BV*, and *CM* in Polyherbal formulation. Literature survey also revealed that, to date, No UV method has been proposed using buffer 7.4 pH as a solvent for the assay of *SA*, *BV*, and *CM* in Polyherbal formulations.

MATERIALS AND METHODS

Instruments

Shimadzu double beam UV-Vis spectrophotometer (Model UV-1700) with 1 nm spectral bandwidth using 10 mm matched quartz cuvettes. Data acquisition was performed by using spectra manager software version UV Probe software SHIMADZU double beam UV-Vis spectrophotometer with 1 nm spectral bandwidth using 10 mm matched quartz cuvettes. All weight was taken on an electronic analytical balance.

Chemicals

Dry powdered extracts of *SA*, *BV*, and *CM* individually purchased from Vital Herbs Z-26/27 Commercial Enclave Mohan Garden Uttam Nagar Delhi -110059. Laboratory grade Potassium di Hydrogen Phosphate, Sodium chloride, di Sodium Hydrogen phosphate, Soy lecithin, acetone, N-Hexane.

UV SPECTROPHOTOMETRIC STUDIES

Preparation of standard solution

A standard stock solution of *SA*, *BV*, and *CM* were prepared individually with the concentration of containing 1mg/ml (100 mg in 100 ml). 100 mg of *SA*, *BV*, and *CM* were taken individually accurately weighed, and transferred into 100 ml. volumetric flask and made up to the mark with phosphate buffer (PBS) with (pH 7.4), standard solution was prepared in the concentration range of 1 mg/ml.

Determination of wavelength of maximum absorbance (λ_{max}) of *SA*, *BV*, and *CM*

The wavelength of maximum absorbance (λ_{max}) was determined by scanning individually 1mg/ml solution of *SA*, *BV*, and *CM* using UV-visible double beam spectrophotometer from 200-400 nm using PBS (7.4 pH) as blank.

Preparation of standard calibration curve

The absorbance of the standard solution in PBS (7.4 pH) at 100-500 µg/ml ranges were measured at 245, 279, and 336 nm for *SA*, *BV*, and *CM* respectively. The standard calibration curve was prepared by plotting average (n=3) maximum absorbance (λ_{max}) versus concentration. Linearity was studied using the regression equation.

Method validation

The method was validated according to the ICH Q2 (R1) guideline for the validation of analytical procedures. Typical validation characteristics such as specificity, linearity, precision, accuracy was considered for evaluation. These measurements regarded as the most important for the validation of assay type analytical procedure.

Specificity

Specificity was confirmed by UV-spectrophotometric scanning of each *SA*, *BV*, and *CM* standard solution (1 mg/ml) in the range of 200-400 nm against (PBS) pH 7.4 as blank.

Linearity

The linearity was determined by analyzing the absorbance of each of the *SA*, *BV*, and *CM* standard concentrations (10-50 µg/ml) at 245, 279, and 336 nm against (PBS) Ph 7.4 as blank. The calibration curve was plotted using concentration against absorbance. A regression equation and correlation coefficient were determined individually for *SA*, *BV*, and *CM* at standard concentration ranges (100-500 µg/ml).

Precision

Precision was evaluated by using repeatability and intermediate precision. Repeatability was analyzed using *SA*, *BV*, and *CM* individually six times in the day (Intra-day). The intermediate precision was analyzed using three standard concentrations (100, 300, and 500 µg/ml) of *SA*, *BV*, *and CM* individually three times on three consecutive days (inter-day).

Accuracy

Accuracy was established by percentage recovery of known added concentrations individually of *SA*, *BV*, and *CM* to the pre-analyzed sample solutions (2 µg /ml). The method was repeated three times for each concentration.

% Recovery = [Ct / Ca] ×100

Ca is the total *SA*, *BV*, and *CM* concentration after standard addition.

Ct is *SA*, *BV*, and *CM* concentration in the test sample.

Robustness

Robustness was a measure for *SA*, *BV*, and *CM* individually standard solutions (100 µg/ml) by different analysts and different instruments. The percentage relative standard deviation (%RSD) values for different analysts (analyst 1 and 2) and different instruments (UV-Vis spectrophotometer (Model UV-1700) and Shimadzu (Mode UV-2600i) were calculated.

RESULTS

Method development

Analytical method was developed and validate according to ICH Q2 (R1) guideline Table 1.

Method validation

Specificity

According to ICH Q2 (R1) guidelines, specificity is the ability to assess unequivocally the analyte in the presence of a component that may be expected to be present. Results of specificity are shown in figure 1.

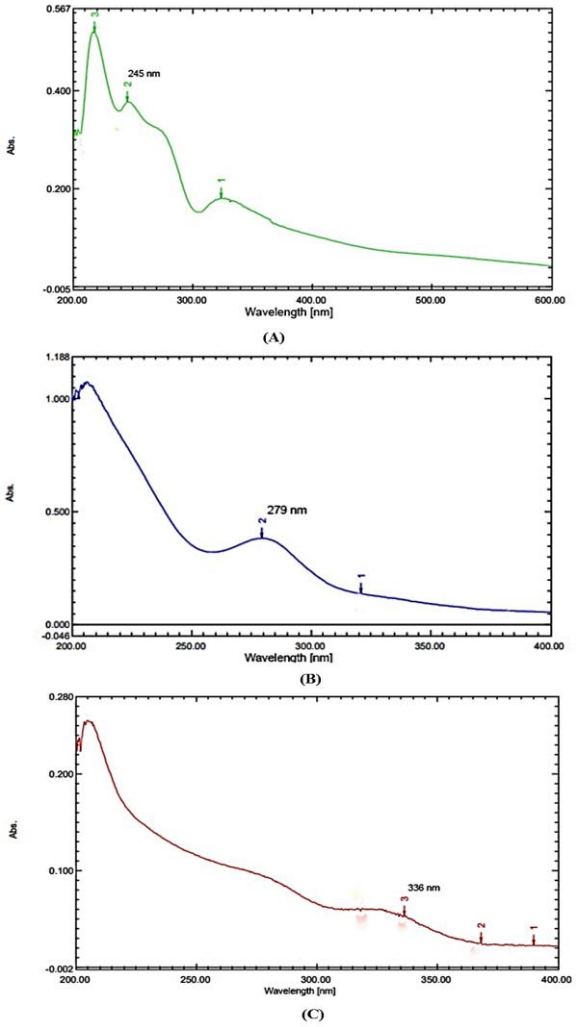

Figure 1: UV spectroscopic study of (A) *SA*, (B) *BV*, (C) *CM* in 7.4 pH Buffer.

Linearity

As per ICH Q2 (R1) guidelines, the linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample. Results of linearity are shown in table 2.

Precision

Based on the ICH Q2 (R1) guidelines, the precision of an analytical procedure expresses the closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate

precision, and reproducibility. Results of precision (repeatability and intermediate precision) are shown in Table 3.

Accuracy

Established ICH Q2 (R1) guidelines specified that the accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Results of accuracy are shown in table 4.

Robustness

In agreement with ICH Q2 (R1) guidelines, the robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variation in method parameters and indicates its reliability during normal usage. Results of robustness are shown in table 5.

Table 1: Analytical method development

Parameters	*SA*	*BV*	*CM*
Absorption maximum (λ_{max})	245 nm	279 nm	336 nm
Regression equation (y=mx+c)	Y= 0.0011x+0.2662	Y=0.0005x+0.0054	Y= 0.0002x+0.0048
Slope	0.0011	0.0005	0.0002
Intercept	0.2662	0.0054	0.0048
Coefficient of correlation	0.999	0.999	0.998
Repeatability (%RSD), (n=6)	0.9613	0.9995	1.0060
Precision (%RSD)	Intra-day = 0.961 Inter-day= 0.958,0.990,0.875	Intra-day = 0.999 Inter-day= 1.923,1.036,0.413	Intra-day = 1.006 Inter-day= 1.589,1.124,1.363
Robustness (% RSD)	82.72-92.72	93.2-97.2	81-96

Table 2: Linearity and range of the proposed UV method for herbal extracts at 245, 279 and 336 nm respectively.

Concentrations (µg/ml)	Absorbance (λ_{max}) (mean±SD) (n=3)			% RSD		
	***SA* (245 nm)**	***BV* (279 nm)**	***CM* (336 nm)**	***SA***	***BV***	***CM***
100	0.3527±0.0015	0.0520±0.0010	0.0220±0.0010	0.4331	1.9231	4.5455
200	0.4973±0.0012	0.1020±0.0010	0.0360±0.0010	0.2322	0.9804	2.7778
300	0.6020±0.0010	0.1450±0.0010	0.0523±0.0015	0.1661	0.6897	2.9188
400	0.7120±0.0010	0.1950±0.0010	0.0670±0.0010	0.1404	0.5128	1.4925
500	0.8340±0.0010	0.2420±0.0010	0.0840±0.0010	0.1199	0.4132	1.1905

Table 3: Table showing intra-day and inter-day precision study of herbal extracts using UV method

Concentrations (µg/ml)	Repeatability (Intra-day precision) (n=6)					
100	Absorbance (λ_{max}) (mean±SD) (n=3)			% RSD		
	***SA* (245 nm)**	***BV* (279 nm)**	***CM* (336 nm)**	***SA***	***BV***	***CM***
	0.3765±0.0036	0.0517±0.0005	0.0200±0.0010	0.9613	0.9995	4.5455
	Intermediate precision (Inter-day precision) (n=3)					
100	0.3760±0.0036	0.0520±0.0010	0.0363±0.0006	0.9589	1.9231	1.5890
300	0.6083±0.0060	0.1473±0.0015	0.0513±0.0006	0.9909	1.0368	1.1247
500	0.8423±0.0074	0.2420±0.0010	0.0847±0.0012	0.8751	0.4132	1.3638

Table 4: Accuracy studies of the proposed UV method.

Initial amount (µg/ml)	Added amount (µg/ml)	Predicted concentration (µg/ml)	Observed concentration (µg/ml)			Residual concentration (µg/ml)			% Mean Recovery			%RSD		
			SA	*BV*	*CM*	*SA*	*BV*	*CM*	*SA*	*BV*	*CM*	*SA*	*BV*	*CM*
2	1(50%)	3	2.8835	2.6789	1.9998	0.1165	0.3211	1.0002	96.1167	89.2967	66.66	1.4295	1.6312	1.8600
2	2(100%)	4	3.8999	3.9876	3.8878	0.1001	0.0124	0.1122	97.4975	99.6900	97.195	0.9186	1.2883	1.5587
2	3(150%)	5	4.9235	4.8967	4.6078	0.0765	0.1033	0.3922	98.4700	97.9340	92.156	1.6153	0.7235	1.7100

Table 5: Robustness of absorbances at absorption maxima of 245, 279 and 336 nm using UV method.

Variable parameters	Absorbance (Mean±SD)			%RSD			Mean % recovery		
	***SA* (245 nm)**	***BV* (279 nm)**	***CM* (336 nm)**	***SA***	***BV***	***CM***	***SA***	***BV***	***CM***
Analyst 1	0.3717±0.0035	0.0527±0.0006	0.0213±0.0006	0.9449	1.0962	2.7063	92.72	93.2	81
Analyst 2	0.3577±0.0035	0.0543±0.0006	0.0237±0.0006	0.9819	1.0626	2.4395	82.72	97.2	96

DISCUSSION

The method was validated according to ICH guidelines. Specificity was confirmed by comparing the UV-scan obtained from the *SA, BV,* and *CM* standard concentration individually (100-500 µg/ml), all runs exhibited a prominent peak at 245nm, 279nm, and 336nm individually and these were confirmed as an average wavelength of maximum absorbance (λ_{max}). Therefore, these were selected for linearity studies Table 2. The regression plots showed compliance with Beer Lambert's Law in the concentration range of 100-500 µg/ml with a correlation coefficient (r) of 0.9993, 0.9998, and 0.9985 individually indicate good linearity between absorbance and concentration. Range (working range) were confirmed by the results where UV-absorbance's were directly proportional to the true concentration of the analyte were predefined by the method of goodness-of –fit test at 245nm, 279nm, and 336nm individually. The range of individually analyte concentrations that the proposed method can directly measure was (100-500 µg/ml) individually and these were confirmed by accuracy studies as 50-150 % of the test concentration. The individual analyte concentration was in the measurable range of the instrument. The repeatability (%RSD) and intermediate precision (%RSD) were observed for analysis of three independent triplicate samples of each three herbs. The repeatability %RSD values were found to be 0.9613, 0.9995, and 1.0060 individually of *SA, BV, and CM* in buffer 7.4 pH table 3, and intermediate precision %RSD values for select sample in three consecutive different days were found to be 0.9589,0.9909, and 0.8751% for *SA,* 1.9231, 1.0368, and 0.4132% for *BV* and 1.5890, 1.1247, and 1.3638% for *CM* in buffer 7.4 pH table 4, respectively. The low values of %RSD in both intra-day and an inter-day analysis were found to be less than <2% hence, the precision was confirmed. The accuracy of the proposed method was established by standard addition method Table 5, estimated by analyzing individually samples of *SA, BV,* and *CM* spiked at three different concentrations (low, medium, high) covering the working range (100-500 µg/ml). The data revealed the closeness of the observed values to the true values for the sample. Accuracy was assessed as the % recovery of the added *SA, BV,* and *CM* concentration. Recovery values for the standard addition method followed for the *SA, BV,* and *CM* analysis range from 96.1167 to 98.4700%, 89.2967 to 97.4975%, and 66.66 to 97.156 % Ensure an accurate method as well as non-interference with the excipients of formulation table 5. The method was found to be robust as indicated by reliable and consistent absorbance with change method concerning the analyst (analyst 1 and 2) and instrument (UV-Vis spectrophotometer (Model UV-1700 and Shimadzu (Mode UV-2600i)), triplicate determination at selected concentration (100 µg/ml) of the *SA, BV, and CM* were carried out and the % RSD values in both the parameters were appeared to be <2% and the percentage recovery was appeared for *SA, BV,* and *CM* individually in the ranges 82.72 to 92.72%, 93.2 to 97.2 % and 96 to 81% table 6. Hence robustness was established.

CONCLUSION

Method developed and validated was found to be eco-friendly, simple, cost-effective, and was successfully applied for the *SA, BV,* and *CM* without the involvement of any interference. Based on the results and statistical parameters demonstrate that this method could be specific and remarkable for the analysis of *SA, BV,* and *CM* in Polyherbal formulation.

Conflicts Of Interest- None

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