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SIMULTANEOUS ESTIMATION OF CURCUMIN AND ASCORBIC ACID IN POLYHERBAL FORMULATION USING UV SPECTROSCOPY

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Keywords:

Polyherbal formulation, Curcumin, Ascorbic acid, Simultaneous estimation

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ABSTRACT: Nowadays, polyherbal formulations are in demand for managing and treating many diseases owing to their less side effects. Thus, qualitative and quantitative standardization of these herbal formulations as per WHO and ICH guidelines is essential to ensure the quality and efficacy of the formulations. The present study involves the development of a simple and precise UV/Visible spectrophotometric method for simultaneous estimation of curcumin and ascorbic acid in the polyherbal antidiabetic formulation at their respective λ_{max} 422 nm and 266 nm. The method obeys Beer's law in the concentration range of 2 to 10 µg/mL for both curcumin and ascorbic acid with a correlation coefficient of 0.9992 and 0.999, respectively. The limits of detection (LOD) and quantitation (LOQ) were found to be 0.225 µg/mL and 0.675 µg/mL for curcumin and 0.12 µg/mL and 0.36 µg/mL for ascorbic acid, respectively. The obtained results demonstrated that the proposed method is simple, sensitive, and cost-effective and can conveniently be employed for the routine analysis of any polyherbal formulations containing curcumin (Curcuma) and ascorbic acid (Amla). The method was validated as per ICH Q2R1 guidelines.

INTRODUCTION: Currently, herbal formulations are most popular for treating and managing many diseases, including diabetes, cancer, inflammation, and asthma. Thus, it is important to increase the popularity and global acceptance of these herbal formulations, providing them with better quality and efficacy. Hence the standardization of herbal formulations as per WHO and ICH guidelines is essential to ensure their quality and efficacy. It is required to develop methods for their standardization essentially based on the quantity of the active principles [1].

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Curcuma and amla are the key herbs of many polyherbal formulations. These play a vital role in ensuring physiological health benefits. In combination, they have anti-inflammatory and immune-boosting properties. These herbs also improve digestion, control blood sugar and blood pressure and reduce congestion [2]. Rajanyamalakadi Life Herbs Bipha diabetic care is one of the Ayurvedic polyherbal tablet dosage forms manufactured by Bipha Ayurveda - a dietary supplement used in managing diabetes. It helps to prevent complications due to diabetes.

It includes three herbs curcuma, amla, and salacia, among which curcuma and amla are well-known herbs. Curcuma consists of dried rhizomes of *Curcuma longa* (family: Zingiberaceae) and contains 2–6% curcuminoid compounds and 1.5-3.14% curcumin [3-5]. Amla consists of dried fruits of *Emblica officinalis* (Family: Euphorbiaceae), is a

natural source of ascorbic acid, and contains 600-750 mg per 100 g of fruit [3, 6]. As curcumin **Fig. 1** and ascorbic acid, **Fig. 2** are the main active constituents of curcuma and amla, respectively, they can be used as marker compounds for their estimation in polyherbal formulations [2].

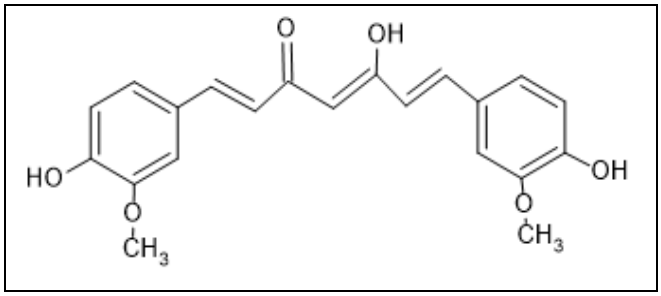

FIG. 1: CHEMICAL STRUCTURE OF CURCUMIN

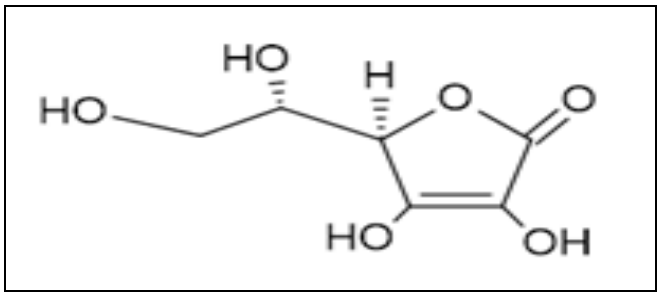

FIG. 2: CHEMICAL STRUCTURE OF ASCORBIC ACID

A wide range of analytical methods *viz.*, UV-spectrophotometry, HPTLC and HPLC have been reported for the estimation of many marker compounds in various herbal extracts and herbal formulations individually or in combination. HPLC-PDA method was used to simultaneously quantify eight marker compounds in the traditional herbal drug Haepyoyijin-Tang [7]. A simple HPTLC method was developed to simultaneously estimate three marker compounds such as kaempferol, rutin and quercetin in the marketed formulations and extracts of Bhuiamla and amla [8]. Valerenic acid was determined using the RP-HPTLC method in methanolic extract of *Valeriana officinalis* and commercial herbal products [9]. Simultaneously, 6-shogaol and 6-gingerol was determined using the RP-HPTLC-densitometry method in various ginger extracts and commercial formulations [10].

In similar way the UV- spectrophotometric method was developed and validated for the estimation of curcumin in a bulk drug, pharmaceutical and polyherbal formulation [11-13] and also in combination with piperine and quercetin [14].

Simultaneous estimation of curcumin with berberine [15], gallic acid [16-17] and quercetin [18-19] were developed using UV-spectrophotometric HPTLC and HPLC 20 methods in polyherbal formulations. Methods are available for the estimation of ascorbic acid using UV-spectrophotometry, UPLC and HPLC methods [21-23].

However, there are no reports on the simultaneous estimation of curcumin and ascorbic acid in bulk drug, pharmaceutical and herbal formulations. Hence, the present study adopted a simultaneous equation method to quantify the marker compounds, curcumin and ascorbic acid in the polyherbal formulation-Rajanyamalakadi tablets. The method was validated according to ICH guidelines [24-25].

MATERIAL AND METHOD: Curcumin, a marker compound, is obtained as a gift sample from Himalaya Drug Company, Bengaluru, India. Methanol and ascorbic acid, the analytical grade, was purchased from Himedia, Mumbai, India. Spectrophotometric analysis was carried out using Shimadzu UV/Visible -1800, UV/Visible probe, Kyoto, Japan, and a pair of 10 mm matching quartz cells was employed to measure the absorbance of the solutions. The commercial Rajanyamalakadi Life Herbs Bipha diabetic care- apolyherbal tablets is manufactured by Bipha Ayurveda, Bipha Drug laboratories, Kerala. It consists of 125 mg of *Curcuma longa*, 125 mg of *Emblica officinalis*, and *Salacia perinoids* – 250 mg per tablet.

Simultaneous Equation Method for Curcumin and Ascorbic Acid:

Preparation of Standard Stock Solution and Determination of λ_{max}: 10 mg of each curcumin (CU) and ascorbic (AA) acid were accurately weighed and transferred to separate 100 mL of volumetric flask and dissolved in 30 mL of methanol. The flasks were sonicated for 15 min and diluted up to the mark with methanol (100µg/mL).

From the CU and AA stock solution, 1 mL each was transferred into separate 10 mL volumetric flasks, and the volume was made up to the mark with methanol (10µg/mL). Each solution was scanned in UV and visible range to measure the λ_{max}. The overlain zero-order spectra of curcumin

and ascorbic acid displayed an absorption maximum at 422 nm and 266 nm, respectively.

Preparation of Sample Solution of Polyherbal Tablet Formulation: Twenty tablets were weighed accurately, and the average weight was taken and powdered. 10 mg powder was transferred into a 100 mL volumetric flask, and the volume was made up to the mark with methanol. The solution was sonicated for 10 minutes, filtered, and 1 mL of the filtrate was diluted to 10 mL (10µg/mL) with methanol. The solution was scanned in UV, and the visible range and absorbance were measured at 422 nm and 266 nm, considered A1 and A2, respectively and substituted in the below equation to calculate the CU and AA [21-22] concentrations.

$$Cx = (A_2\ ay_1 - A_1\ ay_2) / (ax_2\ ay_1 - ax_1\ ay_2)$$

$$Cy = (A_1\ ax_2 - A_2\ ax_1) / (ax_2\ ay_1 - ax_1\ ay_2)$$

Where, A_1 and A_2 are the absorbance of tablet solution at 422 and 266nm. ax_2 and ax_1 are the absorptivity of CU at 422 and 266nm ax_2 and ax_1 are the absorptivity of CU at 422 and 266nm. ay_1 and ay_2 are the absorptivity of AA 422 and 266nm. ay_1 and ay_2 are the absorptivity of AA acid 422 and 266nm.

Validation of the Proposed UV/Visible Spectrophotometric Method: The proposed method was validated for linearity, accuracy, precision, Limit of Detection (LOD), and Limit of Quantification (LOQ) as per ICH guidelines [20-21].

Linearity and Range: From the stock solution of curcumin and ascorbic acid, aliquots of 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL and 1.0 mL were withdrawn and transferred into 10 mL volumetric flasks and made up the volume with methanol to get the concentration of 2, 4, 6, 8 and 10 µg/mL, respectively. We scanned the solutions at 422 nm, and 266 nm for CU and AA, respectively and absorbance values were recorded and plotted the calibration graphs with concentrations *vs* absorbance. All the measurements were performed in triplicate.

Precision: We determined the intra-day precision of the proposed method for CU and AA, was determined by estimating the corresponding response three times on the same day for three different concentrations, 4, 6 and 8 µg/mL, respectively, in triplicate (n=3). The same procedure was followed for three days over one week to determine inter-day precision. The results were reported as a percentage RSD.

Accuracy: Accuracy of the method for CU and AA was performed by the standard addition method at three different levels, 80%, 100% and 120%. The amount of tablet solution was kept constant, *i.e.*, 4 µg/mL and the standard solution of CU and AA were varied at three different levels, *i.e.*, 3, 4, and 5 µg/mL. The percentage recovery was calculated. Each determination was done in triplicate.

Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ were performed on standard solutions of CU and AA containing very low concentrations, 5 µg/mL in 5 replicates.

LOQ and LOD were determined using the following equation:

$$LOD = 3.3 \times \sigma / S$$

$$LOQ = 10 \times \sigma / S$$

Where, σ = Standard deviation of response, S = Slope of the calibration curve.

RESULTS AND DISCUSSION: UV/Visible spectrophotometric methods are well known for regular investigation of pharmaceutical preparation owing to their easy, rapid, cheap and reproducible results. The proposed method was found to be simple, accurate and rapid for the routine simultaneous analysis of polyherbal formulations containing marker compounds, CU and AA.

Simultaneous Equation Method (SEM): SEM was developed to determine CU and AA acid in the polyherbal formulation and was found to be sensitive and satisfactory. The zero-order overlain UV/Visible spectra **Fig. 3** showed the highest absorbance at 422 and 266 nm for CU and AA, respectively. The overlapping zero-order UV/Visible spectra of CU and AA facilitate simultaneous estimation of both in the polyherbal formulations. The amount of marker compounds in the mixture was calculated using a simultaneous equation. Method validation parameters are shown in **Table 1**.

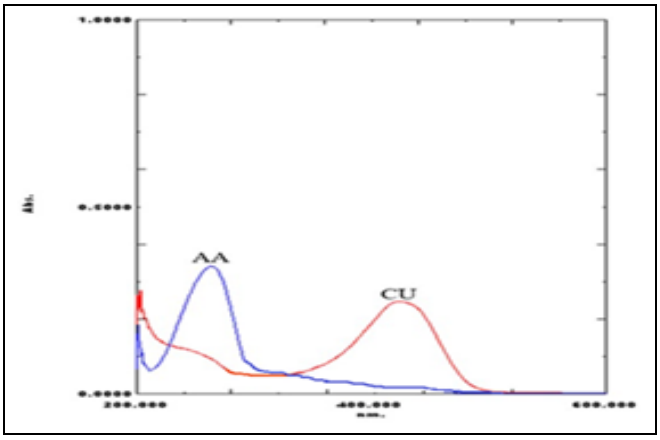

FIG. 3: OVERLAIN SPECTRA OF CURCUMIN AND ASCORBIC ACID AA = ASCORBIC ACID AND CU = CURCUMIN

TABLE 1: METHOD VALIDATION PARAMETERS OF PROPOSED METHOD

Parameters	Results obtained	
	Curcumin	Ascorbic acid
Absorption maxima ($\lambda_{max.}$)	422nm	266nm
Beer's law limit (µg/mL)	2-10	2-10
Regression equation (y=mx+c)	y = 0.0205x +0.0285	y= 0.11x - 0.062
Slope	0.0205	0.11
Intercept	0.0285	-0.062
Correlation coefficient (r^2)	0.9992	0.999
Accuracy (%RSD)	95.83-98.86	98.61-101.13
Precision (%RSD)	< 2 (%RSD)	< 2 (%RSD)
LOD (µg/mL)	0.225	0.12
LOQ (µg/mL)	0.675	0.36

Linearity: The linearity range for CU and AA was found to be 2-10 µg/mL at 422 nm and 266 nm, respectively, and obeys Beer's law. Calibration graphs were plotted for CU at 422 nm and AA at 266 nm and are shown in **Fig. 4** and **Fig. 5.**

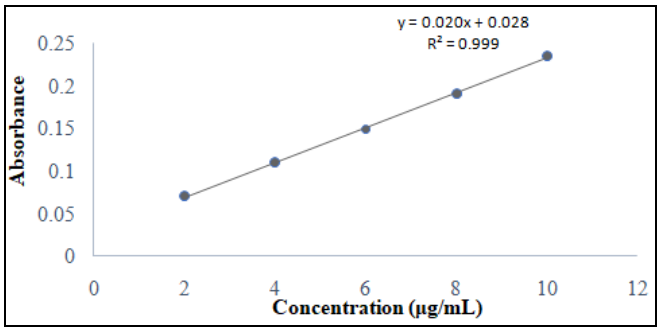

FIG. 4: CALIBRATION CURVE OF CURCUMIN

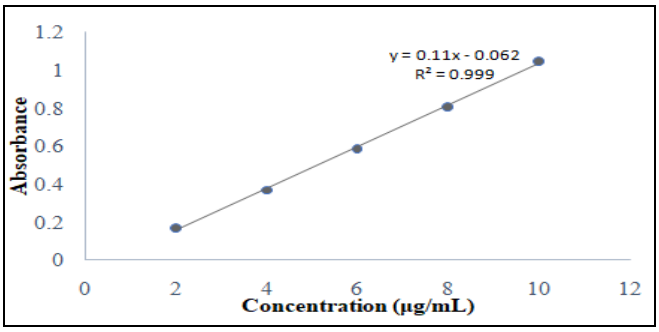

FIG. 5: CALIBRATION CURVE OF ASCORBIC ACID

Accuracy: The accuracy of the proposed method was based on the % recovery by the standard addition method. The results were found to be in the range of 95.83 - 98.86 and 98.61 - 101.13% for CU **Table 2** and AA **Table 3**, marker compounds, respectively, indicating the accuracy of the proposed method.

TABLE 2: ACCURACY DATA OF CURCUMIN

Spiking level (%)	Conc. of sample (µg/mL)	Conc. of std. spiked (µg/mL)	Actual amount (µg)	Amount recovered (µg) (AM±SD) (n=3)	% Recovery	%RSD
80	4	3.2	7.2	6.9±0.02	95.83	0.28
100	4	4	8	7.8±0.026	97.50	0.33
120	4	4.8	4.8	8.7±0.012	98.86	0.13

% Recovery for curcumin was found to be 95.83- 98.86%

TABLE 3: ACCURACY STUDIES OF ASCORBIC ACID

Spiking level (%)	Conc. of sample (µg/mL)	Conc. of std. spiked (µg/mL)	Actual amount (µg)	Amount recovered (µg) (AM±SD) (n=3)	% Recovery	%RSD
80	4	3.2	7.2	7.1± 0.04	98.61	0.56
100	4	4	8	7.9± 0.038	98.75	0.48
120	4	4.8	8.8	8.9± 0.044	101.13	0.49

% Recovery for ascorbic acid found to be 98.61- 101.13%

Precision: The precision study is indicated in % RSD and reported in **Table 4** and **Table 5** and was found to be less than 2%. It confirms the fewer intra and inter-day changes of the proposed method.

TABLE 4: PRECISION DATA OF CURCUMIN

Actual conc. (µg/mL)	Intra-day precision*		Inter-day precision	
	Conc. found (µg/mL) (AM±SD) (n=3)	%RSD	Conc. found (µg/mL) (AM± SD) (n=3)	%RSD
4	3.8± 0.001	0.02	3.8± 0.002	0.05
6	5.9± 0.004	0.06	5.8± 0.0026	0.04
8	8.12± 0.003	0.03	8.2± 0.001	0.01

The %RSD of intra-day and inter-day precision for curcumin was < 2.

TABLE 5: PRECISION DATA OF ASCORBIC ACID

Actual conc. (µg/mL)	Intra-day precision		Inter-day precision	
	Conc. found (µg/mL) (AM± SD) (n=3)	%RSD	Conc. found (µg/mL) (AM±SD) (n=3)	%RSD
4	3.8± 0.020	0.53	3.9± .027	0.69
6	5.8± 0.036	0.62	5.9± .033	0.56
8	8.2± 0.026	0.32	8.1± .040	0.49

The %RSD of intra-day and inter-day precision for ascorbic acid was < 2.

LOD and LOQ: The quantity of LOD and LOQ for the proposed method was found to be much less, demonstrating the extent of sensitivity of the proposed method **Table 1**.

Assay of Polyherbal Formulation: The % assay of CU and AA was found to be 4.51 % and 0.8%, respectively, as shown in **Table 6**.

TABLE 6: ESTIMATION OF CURCUMIN AND ASCORBIC ACID IN TABLET FORMULATION

Formulation	% w/w of marker compound Mean ± SD (n=3)	
	Curcumin	Ascorbic acid
Rajanyamalakadi-Bipha diabetic care	4.51±0.21%	0.8±0.52%

CONCLUSION: The developed UV/Visible spectrophotometric method is simple, precise, and accurate for simultaneous estimation of curcumin and ascorbic acid in polyherbal antidiabetic tablet dosage form and thus can be used as a routine method for the analysis of these marker compounds in the polyherbal formulations including bulk drug and marketed pharmaceutical.

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CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

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