

Research Article

Simultaneous Spectrophotometric Estimation of Curcuminoids and Gallic Acid in Bulk Drug and Ayurvedic Polyherbal Tablet Dosage Form

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ABSTRACT:

In the present study, an attempt has been made to develop an analytical method for the simultaneous estimation of curcuminoids and gallic acid in commercially marketed ayurvedic polyherbal formulation Nisha Amalaki vatti by spectrophotometric method. Simultaneous equations (Vierodt's method) were performed by UV/Visible spectrophotometric. Curcuminoids has absorbance maxima at about 427nm and Gallic acid maxima at about 227nm in methanol. The linearity was obtained in the concentration range of 10-50 mcg/mL for both curcuminoids and gallic acid. The results were of the analysis were validated statistically and the recovery studies were carried out as per ICH guidelines.

Key words:

Curcuminoids, gallic acid, Nisha amalaki vatti.

INTRODUCTION

Traditional medicine 'Ayurveda' since antiquity it has been in serving the mankind in defending against chronic conditions. Nisha Amalaki Vatti is a polyherbal formulation containing Curcuma longa and Phyllanthus emblica used as anti-diabetic agents marketed by Ayush, India. Amla (*Emblica officinalis*) and turmeric (*Curcuma longa*) have long been known in India and many other countries as important dietary sources in addition to their use in traditional medicine for wound healing, inflammation and stomach acidity. The active phytoconstitution of *Curcuma longa*¹⁷ contains (Curcumin, demethoxycurcumin, bisdemethoxycurcumin) and *Emblica Officinalis*¹⁸ (gallic acid and tannins). Several investigators have determined the efficacy of both amla and turmeric for their medicinal activities¹². It has now been well established that oxidative stress plays an important role in these disorders¹². In India, 19 species of *Curcuma longa* and 29 species of *Emblica officinalis* were found. Standardization of these compounds were reported for individual phytoconstituents by UV spectroscopy^{5,8,11}, HPLC^{3,15}, HPTLC^{1,2,4,9,19}, and gravimetric analysis methods.

Nisha amalaki vatti is a polyherbal tablet marketed by Ayush India contains *Curcuma longa* and *Emblica officinalis* used for the treatment of diabetes.

Standardisation by UV, HPLC and HPTLC method has not been reported for the simultaneous analysis of curcuminoids and gallic acid in combined polyherbal formulation. Thus the present study was designed to develop new analytical method to estimate the polyherbal drugs containing curcuminoids and gallic acid by simultaneous equation method¹⁰ using UV spectrophotometer.

MATERIALS AND METHOD

Chemical and reagents:

Curcumin and gallic acid were purchased for S.D fine chemicals and Merck chemicals (Mumbai, India). *Curcuma longa* and Amla (*Emblica officinalis*) raw materials were obtained from local market of Nalgonda, A.P. India, standard extracts were obtained from Chemiloids Vijayawada. Methanol analytical grade solvent was obtained from Merck (Mumbai, India), Polyherbal formulation Nisha Amalaki Vatti (Ayurveda tablet) obtained from Ayush (Hyderabad A.P, India).

Instrumentation

Spectroscopic analysis was carried out using Elico SL-197 UV/Vis-Double beam spectrophotometer with Spectra treaties software. Spectrophotometer with spectral width 2nm, wavelength accuracy of 0.5nm and a pair of 10mm matching quartz cells was used to measure absorbance of the resulting solutions.

Preparation of Standard stock solution:

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Standard stock solution (A) of curcuminoids and gallic acid was prepared by dissolving 100mg of each drug in 100mL volumetric flask separately by using methanol.

From the stock solution final concentration (100 µg/mL) of the individual working standards were prepared with methanol.

Table: 1 Optical characteristics and Validation date of Curcuminoids and Gallic acid

Parameters	Gallic acid	Curcuminoids
Working λ_{max}	227 nm	427 nm
Beer-Lamberts Law	10-50	10-50
Range(µg/mL)		
Molar absorptivity		
Regression Values:	42.08×10^4	62.37×10^4
Slope	0.51750	0.5292
Regression	0.9999	0.9998
Mean	0.9816	0.9816
Intercept	-0.0012	-0.0098
Precision:	0.056	0.1237
Interday (%CV)	0.234	0.1067

Procedure

Simultaneous Equation Methods:

Working standards solution was scanned in the range of 200 to 600 nm to determine the λ_{max} of both drugs using methanol as a blank. The λ_{max} of curcuminoids and gallic acid were found to be 427nm and 227nm respectively. From the stock solution (A) 10ml was taken and diluted to 100ml with methanol (B), form this solution (B) 1ml, 2ml, 3ml, 4ml, and 5ml were taken and volume is made up to 10ml in volumetric flask to get a concentration of 10, 20, 30, 40 and 50µg/ml. The absorbance of the resulting solution was measured at 427 nm and 227 nm respectively and a calibration curve were plotted at these wavelength. A set of two simultaneous equations were established using the mean absorptivity values of curcuminoids and gallic acid¹⁰. $A_1=42.08 C_{gallic\ acid} + 22.13 C_{curcuminoids}$ (i), $A_2=12.39 C_{gallic\ acid} + 62.37 C_{curcuminoids}$ (ii). Where- 42.08 and 12.39 are the mean absorptivity of gallic acid at λ_1 and λ_2 respectively and 22.13 and 62.37 are the mean absorptivity of curcuminoids at λ_1 and λ_2 respectively. A_1 and A_2 are the

absorbance of the mixed standard and the solution at λ_1 and λ_2 respectively. $C_{gallic\ acid}$ and $C_{curcuminoids}$ are the concentration in gm/L. The concentration of $C_{gallic\ acid}$ and $C_{curcuminoid}$ in mixed standard and the sample solution can be obtained by solving equation (i) and (ii).

Procedure for Analysis of Ayurvedic Polyherbal tablets (Nisha Amalaki Vatti):

Twenty tablets (Nisha Amalaki vatti) were weighed accurately and the average weight was determined and then grounded to fine powder. Quantity equivalents to 0.1 g were transferred to 100 mL of volumetric flask and volume is adjusted with 100ml with methanol. The solution was centrifuged for five minutes at 3000 rpm. Centrifugation was found to be faster and more effective than filtration. Centrifugation forms a cake of excipients at the bottom of the test tube, which is not disturbed while drawing out the supernatant solution. This supernatant solution was pipette out and diluted appropriately with methanol to obtain the concentration of 10 µg/mL concentration of curcuminoids and gallic acid. For forming simultaneous equation, the solution was scanned from 200-600 nm. The absorptivity value at 227 nm and 427nm for both the drug were determined by checking the absorbance values of over a concentration range 10-50 µg/mL for working standards

Table: 2 Regression Analysis of Calibration curve

Gallic acid		Curcuminoids	
Conc. (µg/mL)	Absorbance (at 227 nm)	Conc. (µg/mL)	Absorbance (at 427 nm)
10	0.327	10	0.329
20	0.654	20	0.659
30	0.982	30	0.989
40	1.309	40	1.318
50	1.636	50	1.673

shown in table.2.

Validation of proposed method

The proposed method was validated by studying several parameters such as accuracy, precision, and linearity.

Precision:

The repeatability of the sample application was calculated by repeating the assay six times for each

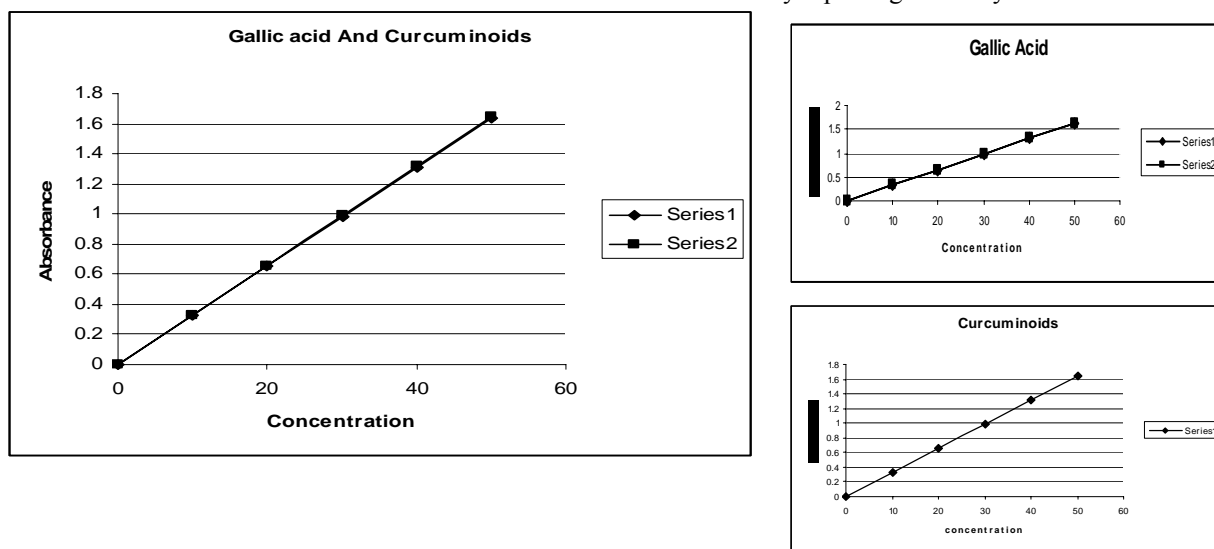


Fig:1 Showing the calibration curves and the linearity for curcuminoids and gallic acid

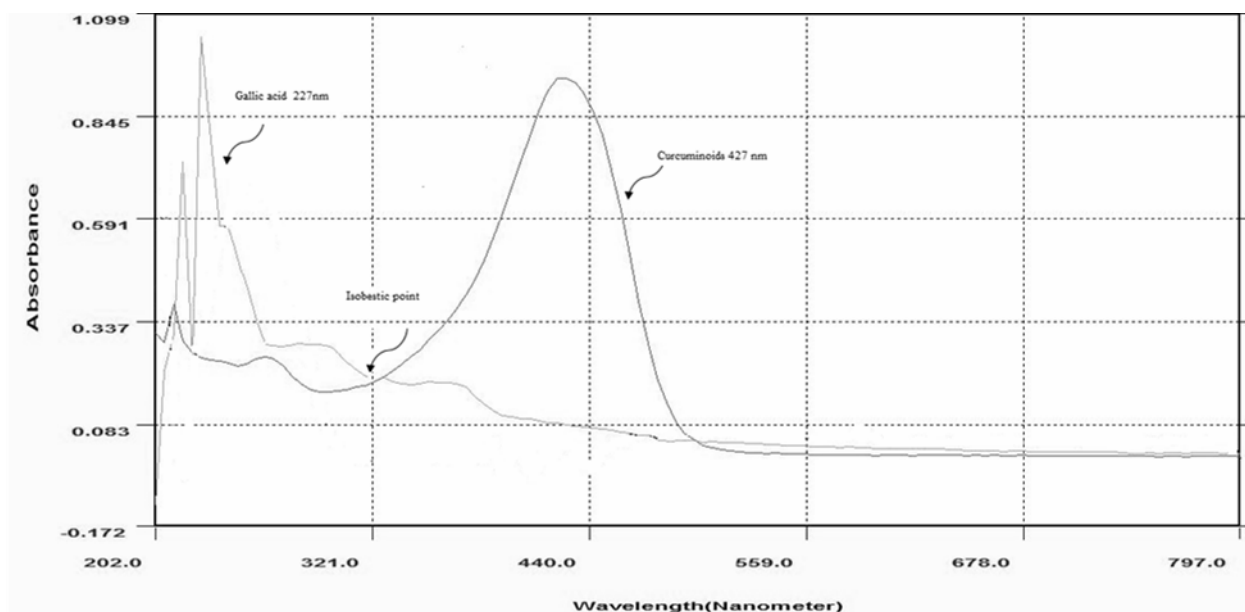


Fig: 2 Over lapping spectra showing Curcuminoids and Gallic acid

concentration. Intraday precision were performed by analyzing sample solution on the same days on the different days at specific time intervals. The results of the same are show in table 3.

Accuracy:

To check the accuracy of the proposed method, recovery studies were carried out at 80,100 and 120% of the test concentration as per ICH guidelines. The recovery studies were performed three times at each level. Results of the formulation analysis recovery studies along with its statistical validation data are given in table 3.

Linearity:

The linearity of the measurement was evaluated by analyzing different concentration of the solution of curcuminoids and gallic acid. For the simultaneous equation method the Beer-Lambert's concentration ranges was found to be from 10-50 µg/mL for curcuminoids and gallic acid respectively. The standard calibration curve and standard table for curcuminoids and gallic acid is given in fig.1 and table.1 respectively. Hence the proposed method can be used for the routinely employed for the estimation of curcuminoids and gallic

acid in bulk and pharmaceutical ayurvadic dosage forms.

RESULTS

The proposed method was found to be simple, sensitive, accurate, precise, economical and rapid for the routine simultaneous estimation of these two phytoconstituents in a combined dosage form. The value of the standard deviation and coefficient of variation were satisfactory shown in table 1, 2 and 3. In the simultaneous equation method tow wavelength of respective absorbance maxima i.e. 227 nm for gallic acid and 427 nm for curcuminoids were used for the analysis of the phytoconstitution in the standard and tablet.

CONCLUSION

The method described in this paper for the simultaneous estimation of curcuminoids and gallic acid are found to be simple, sensitive, accurate ,precise, rapid and economical. Hence the method could be used for the routine analysis of this phytoconstitution in their combined bulk and ayurvadic pharmaceutical dosage.

Table 3: Analysis Date of formulation, Statistical validation and recovery studies

Drugs	Label claim (mg)	Amount Found (mg)	Label claim (%)	Standard Deviation	Coefficient of variation	Standard error	Amount Added At (%)	mg	% Recovery
Curcuminoids	250	248.9	99.70	31.74	0.9998	0.2453	80	400	98.97
							100	500	100.65
							120	600	99.79
Gallic acid	250	237.56	97.62	28.618	0.9999	0.1234	80	400	98.68
							100	500	99.72
							120	600	100.95

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