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Chemometrics Assisted UV-Visible Spectrophotometric Method for Simultaneous Estimation of Ascorbic Acid and Gallic Acid from Herbal Formulations

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Abstract
In this work, numerical methods, based on the use of spectrophotometric data coupled to Partial Least Squares (PLS) and Principal Component Regression (PCR) is evaluated for the simultaneous determination of Ascorbic Acid (ACA) and Gallic acid (GAA) in bulk and formulations. Spectra of ACA and GAA were recorded at concentrations within the linear range of 0-3 µg/mL and 0-6 µg/mL respectively and were used to compute a total of 35 synthetic mixtures comprising of 25 calibration and 10 validation sets between the wavelength range of 200 – 350 nm with the wavelength interval of 1nm in hydroalcoholic solution (Water: methanol in the ratio 1:1). The suitability of the methods was assessed by means of root mean squares error (RMSE) for calibration and prediction sets. The analytical performance of these methods were evaluated by means of percentage recovery data. These methods were successfully applied to ayurvedic formulations and the drugs were evaluated without any interference from other compounds. The proposed methods are simple, rapid and can be easily used as an alternative tool for routine quality control analysis.

Keywords
Ascorbic Acid, Gallic Acid, Chemometrics, PLS, PCR, Herbal formulation.

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INTRODUCTION

Gallic acid (3, 4, 5 trihydroxy benzoic acid), a naturally occurring low molecular weight triphenolic compound, has emerged as a strong antioxidant and an efficient apoptosis inducing agent. Gallic acid is widely present in the plant kingdom and largely found in free form or as a derivative in different food sources such as nuts, tea and grapes. This secondary metabolite is known to exhibit a range of bioactivities including anti-oxidant, antimicrobial, anti-inflammatory and anticancer. Literature reveals several methods such as Spectrometry[1], chromatography[2-8] and Capillary electrophoresis[9] for quantitative estimation of gallic acid either alone or in combination with other substance.

Ascorbic acid or Vitamin C bearing the chemical name as (R)-5-((S)-1,2-dihydroxyethyl)-3,4-dihydroxy furan -2(5H) –one is a naturally occurring organic

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compound with antioxidant property. Ascorbic acid plays an important role in collagen biosynthesis, iron absorption and immune response activation. It also acts as a powerful antioxidant which fights against free radical induced diseases. Many analytical methods such as volumetric methods, spectrophotometric analysis[10-13] and chromatographic techniques[14] were reported for the estimation of ascorbic acid.

Literature survey revealed many analytical methods for evaluation of the selected constituents from polyherbal formulations. No method could justify the simultaneous estimation of Ascorbic acid and Gallic acid from herbal formulations. Hence we propose a rapid and specific method for simultaneous estimation of the afore-mentioned compounds either alone or in combination from different polyherbal formulations.

In recent years, multivariate calibrations such as Classical Least Squares (CLS), Inverse Least Squares (ILS), Partial Least Squares (PLS) and Principal Component Regression (PCR) have been started to apply to the analysis of data obtained in all instrumentation. It is perhaps the area within Chemometrics which has attracted the most interested so far. The approach is useful in the simultaneous spectrophotometric determination of two or more components in a pharmaceutical formulation with overlapping spectra. These are extensively applied in quantitative spectral analysis to get selective information from unselective data.

Intrument Employed:

A Shimadau UV-Visible spectrophotometer controlled by a computer and equipped with a 1-cm path length quartz cell was used for UV-VIS spectra acquisition. The computations were made with Microsoft Excel and Matlab 7.2 Software (Mathworks Limited). PLS calculations were carried out using the PLS Toolbox 5.0 demo version (Eigen Vector Technologies).

Commercial Products:

Tripala Churna and Chawanprash of three different brands were procured from the local market for the quantitative estimation of ascorbic acid and gallic acid.

Standard and Sample Solutions

Standard Stock Solutions (1000 µg/mL) of Ascorbic acid and Galiic acid were prepared separately in distilled water. Various aliquots of standard solutions were then diluted to 10 mL with water to get the desired analyte concentration.

For the preparation of sample solution, 4g of tripala churna (each from three different manufacturers) was macerated separately with hydro alcoholic solution (Water and Methnaol in the ratio of 1:1) for 24 hrs. The decant was then filtered and diluted suitably to get different analyte concentrations.

Individual Calibrations

To verify Beer's law, calibration graphs were prepared for the determination of Ascorbic acid and Gallic acid (Table 1). The correlation coefficients obtained indicate that the interaction between the two binary systems does not affect the linear correlation prevailing between the absorbance and concentration of each drug. The limit of detection (*LOD*) was determined using the formula: $LOD = kSDa/b$ where $k = 3.3$, SDa is the standard deviation of the intercept, and b is the slope.

Multivariate Calibration Methods (PLS and PCR)

Calibration and prediction sets were designed with 25 and 10 binary mixtures of thecited drugs, respectively. Concentrations of ACA and GAA in calibration and prediction solutions were in the range of 0-3 µg/mL of ACA and 0-6 µg/mL of GAA respectively. Each calibration or prediction mixture was prepared by diluting the appropriate aliquot of stock solution with distilled water. The spectrum of each solution was recorded against the blank in the range of 200–350 nm with a wavelength interval of 1 nm.

RESULTS AND DISCUSSION

Data Processing and Model Building

The UV spectra of both the drugs were overlapped to study the spectral characteristics. A complete overlap throughout the UV region was observed. To carry out multivariate analysis of these selected drugs, 25 different synthetic mixtures were prepared in the concentration ranges of 0-3 µg/mL of ACA and 0-6 µg/mL of GAA respectively (Table 1). The concentration range was set from zero in order to meet the requirement of utilizing the analysis of any one drug mentioned in the combination. The absorption values of spectra of the concentration set were measured at the 151 wavelength points with $\Delta\lambda = 1$ nm in the spectral region of 200 –350 nm. The concentration set and absorption data were considered as Y-block (25×3) and X-block (25×151) for the construction of PLS calibration using cross-validation procedure to reach the best calibration model. The calculations were done with PLS Toolbox 5.0 and the optimal factors were selected.

Statistical Parameters for PLS model

The mean % recovery ranges for both the drugs in the calibration set and in the prediction set were found to lie between 97 to 103%. RMSEC value ranges from 0.0116 – 0.0429 for all four drugs in the calibration set. RMSEP value ranges from 0.0443 – 0.1471 for all four drugs in the prediction set. The SD values were found to increase with increasing spectral mode in the calibration set but seem to decrease with increasing spectral mode in the prediction set. R^2 value ranges from 0.9986 – 0.9997 in the calibration set and from 0.9936 – 0.9996 in the prediction set.

Statistical Parameters for PCR model

The mean % recovery ranges for both the drugs in the calibration set and in the prediction set were found to lie between 97 to 103%. RMSEC value ranges from 0.0246 – 0.1537 for all three drugs in the calibration set. RMSEP value ranges from 0.0426 – 0.1691 for all three drugs in the prediction set. The SD values were found to increase with increasing spectral mode in the calibration set but seem to decrease with increasing spectral mode in the prediction set. R^2 value ranges from 0.9908 – 0.9988 in the calibration set and from 0.9921 – 0.9993 in the prediction set.

Table 1: Concentrations of Calibration and Prediction sets

Concentration Set (µg/mL)		**Prediction Set (µg/mL)**	
ACA	**GAA**	**ACA**	**GAA**
0.5	5	2	1
0.5	4	2.5	1.5
0.5	3	1	2.5
0.5	2	2	2
1	5	0	0
1	4	1	2
1	3	2	1
1	2	0	3
1.5	5	1	6
1.5	4	0	0
1.5	3	-	-
1.5	2	-	-
2	0	-	-
2	5	-	-
2	4	-	-
2	3	-	-
2.5	5	-	-
2.5	4	-	-
2.5	3	-	-
2.5	2	-	-
3	1	-	-
3	1.5	-	-
0	6	-	-
0	0	-	-
3	2	-	-

ACA – Ascorbic acid; GAA – Gallic acid

Table 2 - Statistical Parameters calculated from application of PLS and PCR methods to calibration samples

Drug	**Parameter**	**PLS**			**PCR**		
		D^0	**D^1**	**D^2**	**D^0**	**D^1**	**D^2**
ACA	Mean	97.091	98.032	98.216	100.11	99.741	99.292
	RMSEC	0.0260	0.0169	0.0126	0.0265	0.0246	0.0485
	R^2	0.9986	0.9994	0.9997	0.9986	0.9988	0.9954
GAA	Mean	99.645	100.24	100.23	99.991	98.980	100.729
	RMSEC	0.0428	0.0426	0.0272	0.1537	0.1274	0.1103
	R^2	0.9983	0.9988	0.9994	0.9908	0.9943	0.9953

D^0 – Fundamental mode; D^1 – First Derivative mode; D^2 – Second Derivative mode

Table 3 - Accuracy (% recovery) and Precision (SD) results from application of optimized PLS and PCR models on the prediction set

Drug	Parameter	PLS			PCR		
		D^0	D^1	D^2	D^0	D^1	D^2
ACA	Mean	99.456	98.847	101.119	96.692	104.390	103.728
	RMSEP	0.1142	0.0436	0.0489	0.0962	0.0803	0.0426
	SD	1.647	1.801	2.053	2.323	2.342	3.112
GAA	Mean	103.390	102.389	101.436	102.510	103.432	100.362
	RMSEP	0.1471	0.0980	0.0471	0.1651	0.0982	0.0750
	SD	2.103	1.560	1.543	1.254	1.667	3.256

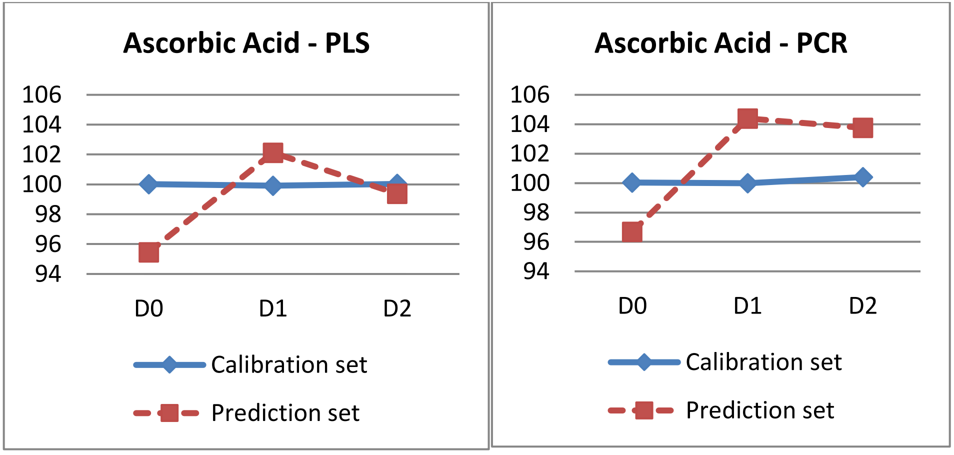
D^0 – Fundamental mode; D^1 – First Derivative mode; D^2 – Second Derivative mode

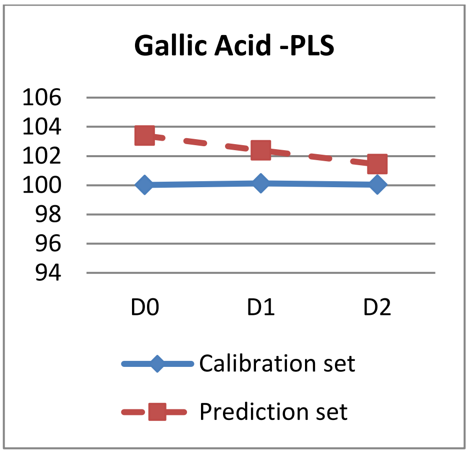
Table 4 – Assay results of PLS and PCR models in Herbal formulations

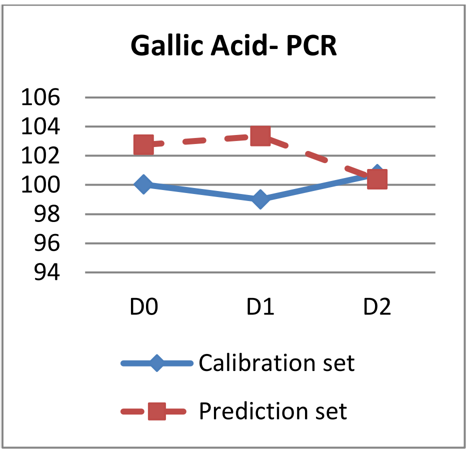
Drug (Ascorbic Acid)	Brands	PLS (Mean % Recovery)			PCR (Mean % Recovery)		
		D^0	D^1	D^2	D^0	D^1	D^2
Tripala Churna	T1	98.23	97.45	99.32	97.83	95.65	98.43
	T2	98.33	97.32	98.44	98.43	96.55	98.71
	T3	97.44	98.21	96.54	98.21	97.31	99.56
Chawanprash	C1	101.23	100.87	99.87	99.63	99.21	100.81
	C2	97.33	98.22	100.21	100.31	100.43	97.56
	C3	98.51	99.33	100.34	101.12	100.75	99.65
Drug (Gallic Acid)	**Brands**	**PLS (Mean % Recovery)**			**PCR (Mean % Recovery)**		
		D^0	D^1	D^2	D^0	D^1	D^2
Tripala Churna	T1	98.83	97.72	99.08	100.23	100.34	101.36
	T2	99.87	100.34	101.63	99.54	98.78	100.21
	T3	100.78	101.12	101.21	100.89	100.53	99.82
Chawanprash	C1	98.78	100.76	100.82	101.78	101.34	100.76
	C2	99.65	98.31	100.82	99.71	98.04	99.08
	C3	98.67	99.84	100.54	101.34	101.48	99.62

D^0 – Fundamental mode; D^1 – First Derivative mode; D^2 – Second Derivative mode

Figure 1. Comparison of Mean Recovery of PLS and PCR models for Fundamental (D0), First Derivative (D1) and Second Derivative (D2) Spectra







CONCLUSION

The combined use of derivative spectrophotometry and chemometric techniques has demonstrated to be a highly convenient choice in the determination of multi-component matrices presenting serious spectral overlapping, thanks to their common potential ability to exploit minor spectral features. This approach is simple in application, inexpensive and requires an easy treatment of the samples and provides reliable analytical results. Hence these methods can be effectively used in the routine quality control of ascorbic acid and galiic acid samples in pharmaceutical industries.

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