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Chemometrics assisted uv-vis spectrophotometric method for simultaneous estimation of gallic acid, ascorbic acid, embelin and piperine from ayurvedic formulations

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Abstract: The use of herbal medicines has increased remarkably in line with the global trend of people returning to natural therapies. Herbal formulations have reached widespread acceptability as therapeutic agents for diabetics, arthritics, liver diseases, cough remedies, memory enhancers and adoptogens. Herbal medicine products are dietary supplements that people take to improve their health and are sold as tablets, capsules, powders, tea, extracts, fresh and dried plants. Though herbals are traditionally considered to be harmless, some may cause health problems, some are not effective or some may react with other drugs. Hence standardization of herbal formulations is essential in order to assess the quality of drugs based on the concentration of their active principles.

Various advanced methods such as chromatographic, spectrophotometric and combination of these methods, electrophoresis, polarography and the use of molecular biomarkers are currently employed in standardization of herbal drugs. Chemical fingerprints obtained by these methods have become the powerful tools for the authenticity and quality control of traditional herbal medicine. However, there is a need for a rapid and specific method allowing the manufacturers to set quality standards and specifications so as to seek marketing approval from regulatory authorities for therapeutic efficacy, safety and shelf life of herbal drugs. Chemical markers play a pivotal role at various stages of the development and manufacturing of a herbal medicine such as authentication and differentiation of species, stability assessments and quality control of herbal medicines. An attempt is proposed to develop a method for simultaneous estimation of four chemical markers that are commonly present in many of the poly herbal formulations. The proposed methods offer rapid and cost-effective analysis of Gallic acid, Ascorbic acid, Embelin, and Piperine either individual or in combination from different ayurvedic formulations.

Introduction

According to an estimate of the World Health Organization (WHO), about 80% of the world population still uses herbs and other traditional medicines for their primary health care needs. WHO stresses the importance of the qualitative and quantitative methods for characterizing the samples, quantification of the biomarkers and or chemical markers and the fingerprint

profiles. Chemical fingerprinting has been demonstrated to be a powerful technique for the quality control of herbal medicines. A chemical fingerprint is a unique pattern that indicates the presence of multiple chemical markers within a sample. The European Medicines Agency (EMEA) defines chemical markers as chemically defined constituents or groups of constituents of a herbal medicinal product which are of interest for quality control purposes regardless whether they possess any therapeutic activity [2].

Chemometrics is widely applied tool for Herbal Drug Standardization (HDS). Chemometrics can be applied in HDS for authenticity, efficacy and chemical analysis of herbal drugs. Some of the common methods applied for quality evaluation of medicinal plants include similar analysis (SA), principle component analysis (PCA), cluster analysis (CA), discriminate analysis (DA) and Pattern recognition [3-5].

National Status

In India, traditional medicine is governed by the Drugs and Cosmetics Act, 1940 and the provisions of the act are implemented by the state governments. The first Indian National Health Policy 1983 claims that India is the richest source of herbs and the drugs should be standardized [17]. The department of AYUSH, Government of India, launched a central scheme to develop standard operating procedures for the manufacturing process to develop pharmacopeial standards for ayurvedic preparations [18].

Literature Survey

Following are the literature related to application of Chemometrics on HDS.

- Lai YH et al [6] have developed method for authentication of cassia seeds on the basis of two wavelength HPLC fingerprinting with the use of Chemometrics.
- Abdul Rohman [7] has utilized Fourier Transform Infrared Spectroscopy (FTIR) using attenuated total reflectance as sample accessory and in combination with chemometrics, he developed a method for detection and quantification of some vegetable oils as adulterants in cod liver oil.
- Gong et al [8] and Xu et al [9] applied similarity analysis method of peak height, peak area and ratio between peaks of the fingerprint for quality evaluation of medicinal plants.
- Tian et al [10] evaluated Chaihu (Bupleuri Radix) by both High Performance Liquid Chromatography – Evaporative light scattering detector (HPLC-ELSD) and High Performance Thin Layer Chromatography (HPTLC) analyses of its principal bioactive components (saikosaponins).
- Lin et al [11] developed enhanced fingerprints of various Artemisia selengensis Turcz by means of HPLC-Diode Array Detector (HPLC-DAD). The results were analyzed by SA, HCA and PCA. It provided comprehensive information for matching and discrimination of the fingerprints, and appeared to be suited for quality assurance purposes of medicinal plants.
- Guo et al [12] established HPLC-DAD method to simultaneously determine 10 triterpenoid acid and used HCA and PCA to differentiate and classify the samples based on the contents of the 10 triterpenoid acids.

- Kong et al [13] developed Ultra performance liquid chromatography with photodiode array detector (UPLC-PAD) method for quantitative analysis of five active alkaloids and chemical fingerprint analysis.
- Wang et al [14] determined trace and toxic element concentrations in *Paris polyphylla* from china by flame and graphite furnace atomic absorption spectrophotometry to evaluate and classify their quality. The results were analyzed with PCA and HCA which support important information for safety evaluation.
- Kolasani et al [15] determined mineral elemental concentrations of 50 chinese medicinal herbs in acid digests with flame atomic absorption spectrophotometry. The data were analyzed by PCA and HCA to understand the association between the elements and to classify the herbal samples.

Scartezzini et al [16] proposed a reliable method for the identification and quantification of ascorbic acid and further indicated that high antioxidant activity is due to a large percentage of the presence of ascorbic acid.

Following are the literature search for the evaluation of selected phytoconstituents from different herbal preparations.

1. Ascorbic Acid

The fruits of *Emblica ofiicinalis* are widely used in the Ayurveda and are believed to defense against diseases. *Emblica officinalis* primarily contains tannis (gallic acid and Ellagic acid), 1-O-galloyl-beta-D-glucose, 3, 6-di-Ogalloyl-Dglucose, chebulinic acid, quercetin, alkaloids, phenolic compounds, amino acids and carbohydrates, chebulagic acid. It contains highest source of Vitamin C or ascorbic acid. Vitamin C present in amla is one of the main factors that help to retrieve or refill the energy lost by body [19, 20]. Ascorbic acid is official in Indian [21], British, European and Japanese Pharmacopoeias. For the estimation of ascorbic acid few analytical methods such as UV [22], HPLC [23], TLC [24], Gas Chromatography (GC), HPTLC [25, 26] Gas Chromatogaphy -Mass Spectropmetry (GC-MS), Capillary electrophoresis [27], FTIR, Nuclear Magnetic Resonance (NMR), Nuclear Inductive Resonance (NIR) [28], Differential Scanning Calorimetry (DSC), amperometric and voltametric methods were reported. A quantitative HPLC analysis was reported for estimation of ascorbic acid and gallic acid in *Phyllanthus Emblica* by Sawant L et al [29].

2. Embelin

Embelia ribes Burm F. also known as Vidanga is one of the oldest herbs in Indian traditional medicine. Embelia ribes have a long history of use in ayruvedic system of medicine in various forms like churna, asava, aristha, lauha amd taila. It is an Indo-Malaysian species mainly found in India, Singapore, Sri Lanka and Malaysia. The main active component is Embelin, chemically 2,5-dihydroxy-3-undecyl-1,4-benzoquinone. It is mainly used as an anthelmintic, carminative and stimulant. It is also used in the treatment of abdominal disorders, lung diseases, constipation, indigestion, fungus infections, mouth ulcer, sore throat, pneumonia, heart disease and obesity [30]. Few analytical methods such as Spectrophotometry [31] HPLC [32 - 39], HPTLC [40-42] HPLC-PDA and HPTLC [43] were reported. Kachhadiya KH et al [44] have reported a RPHPLC method for simultaneous estimation of embelin and curcumin in polyherbal formulations. Imran

Khan Pathan et al [45] have developed and validated a spectrophotometric method for simultaneous estimation of embelin and gallic acid in ayurvedic churna formulation. Rakesh K Patel et al [46] have reported a method for simultaneous estimation of embelin, Rottlerin and ellagic acid in vidangadi churna using RPHPLC. Rajiv Kukkar et al have estimated the Embelin and Strychnine in Krimimudgara Rasa by HPTLC method [47].

3. Piperine

Piperine 1-[5-(1, 3 Benaodioxol-5-yl)1-oxo-2,4 pentadienyl] piperidine is the alkaloid a biomarker constituent of Piper nigrum and Piper longum responsible for the pungency of Piper nigrum and Piper longum [48]. Piperine is official in Indian Pharmacopoeia [49] which describes Liquid chromatographic method for its estimation. Literature survey reveals HPLC [50-53], UV spectrophotometry [54-57] and HPTLC [58-60] methods for the determination of pipierine. Literature survey also reveals different methods for determination of piperine in combination with other drugs [61-65]. Sunita et al have reported a HPTLC method for estimation of piperine from complex matrix of Trikatu Churna [66]. Piperine is basically used in preparations intended for stomach and digestive disorders, cold, bronchitis, neuralgia, scabies, piles, various skin diseases etc [67].

4. Gallic Acid

Gallic acid is a poly phenolic compound having an antioxidant property. Chemically it is a 3,4,5-trihydroxy benzoic acid. Gallic acid and its derivatives are a group of naturally occurring polyphenol antioxidants which have recently been shown to have potential health benefits. Gallic acid and its derivatives have antioxidant activities and neuro protective effects with free radical scavenging effects [68]. Literature survey reveals UV Spectrophotometric [69, 70] HPLC [71-77], RP-UFLC [78], HPTLC [79-85] methods for quantitative estimation of gallic acid from poly herbal formulations.

Methodology

Importance of Evaluation of Herbal drug formulation

Evaluation of herbal drug is an important tool in the formulation of high quality herbal products. Quality of herb depends upon many factors like cultivation, collection, drying storage, processing for market etc. Owing to medicinal properties attributed to an herb, it is necessary to maintain its quality and purity in the commercial market. Herbal drugs or its extracts or pure active compound needs analytical techniques to confirm its identity, quality, purity, potency, safety and efficacy of the plant. Standardization if the system that ensures a predefined amount of quantity, quality and therapeutic effect of ingredients in each dose. Herbal product cannot be considered scientifically valid if the drug tested has not been authenticated and characterized in order to ensure reproducibility in the manufacturing of the product.

Quality control of Herbal Formulation

According to WHO standardization and quality control of herbals is the process involved in the physiochemical evaluation of crude drug covering aspects such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion.

Chemical and Instrumental analysis is routinely used for analyzing synthetic drugs to confirm its authenticity. In the case of

herbal drugs, however the scene is different, especially for polyherbal formulation, as there are no chemical or analytical methods available. The combination of qualitative fingerprinting and quantitative multicomponent analysis is the novel and rational method to address the key issues of quality control of herbal medicines. The advancement of analytical techniques will serve as a rapid and specific tool in herbal research thereby allowing the manufacturers to set quality standards and specifications so as to seek marketing approval from regulatory authorities for therapeutic efficacy, safety and shelf life of drugs.

Chemical Markers

The European Medicine Agency (EMEA) defines chemical markers as chemically defined constituents or group of constituents of an herbal medicinal product which are of interest for quality control purposes regardless whether they possess any therapeutic activity. The quantity of chemical marker can be an indicator of the quality of the herbal medicine. The study of chemical markers is applicable to many research areas, including authentication of genuine species, search for new resources or substitutes of new raw materials, optimization of extraction and purification methods, structure elucidation and purity determination. Systematic investigations using chemical markers lead to discoveries and development of new drugs. The EMEA categorizes chemical markers in to analytical marker and active markers.

Analytical markers are the constituents that are solely used for analytical purpose. Active markers are the constituents that contribute to therapeutic activity.

Selected Chemical constituents for the Study

S.No.	Name of the Chemical compound	Major Plant Source
1	Ascorbic Acid	*Emblica officinalis*
2	Gallic acid	*Phyllanthus emblica* *Terminalia bellirica* *Terminalia chebula* *Terminalia arjuna*
3	Embelin	*Embelia ribes*
4	Piperine	*Piper nigrum*

Formulations selected for the study

Code	Name of the formulation	Ascorbic Acid (ACA)	Embelin (EMB)	Gallic Acid (GAA)	Piperine (PIP)
P1	Triphala churna (for weight loss)	×		×	
P2	Trikatu Churna (for digestion)				×

P3	Vidangadi churna (anthelmentic)		×	×	
P4	Vidang Churna (germicidal, bactericidal)		×		
P5	Ashta Churna (for digestive disorders)				×
P6	Chyawanprash (Immuno modulator)	×		×	×
P7	Brahma rasayana (for stress and tiredness)	×	×	×	×
P8	Sanjeevani Vati (for cough and fever)	×	×	×	×

Above mentioned formulations were selected based on their predominant use in India. These formulations are commonly used in many households and regularly consumed by all age groups.

Instrument to be Employed

- UV-Vis Spectrophotometer
- Chemometric Software – PLS Tool box and Matlab

Theoretical Background

Principal components regression (PCR)

In the spectral work, the following steps can explain the fundamental concept of PCR. The original data obtained in absorbances (A) and concentrations (C) of analytes were reprocessed by mean-centring as A_0 and C_0, respectively. Using the ordinary linear regression

$$C = a + b \times A \quad (1)$$

the coefficients a and b:

$$b = P \times q \quad (2)$$

where P is the matrix of eigenvectors and q is the C-loadings given by

$$q = D \times T^T \times A_0 \quad (3)$$

Here T^T is the transpose of the score matrix T.

D is the diagonal matrix having on the components the inverse of the selected eigenvalues.

Knowing b one can easily find a by using the formula

$$a = C_{mean} - A^T_{mean} \times b \quad (4)$$

where A^T_{mean} represents the transpose of the matrix having the entries of the absorbance values and C_{mean} is the mean concentration of the calibration set.

Partial least-squares (PLS)

In the UV-Vis spectra, the absorbance data (A) and concentration data (C) are mean centered to give data matrix A_0 and vector C_0. The orthogonalized PLS algorithm has the following steps:

The loading weight vector W has the following expression:

$$W = \frac{A^T_0 C_0}{C_0^T C_0} \quad (5)$$

The scores and loadings are given by:

$$t_1 = A_0 W \quad (6)$$

$$P_1 = \frac{A^T_0 t_1}{t_1^T t_1} \quad (7)$$

$$q_1 = \frac{C^T_0 t_1}{t_1^T t_1} \quad (8)$$

The matrix and vector of the residuals in A_0 and C_0 are:

$$A_1 = A_0 - t_1 P_1^T \quad (9)$$

$$C_1 = C_0 - t_1 q_1^T \quad (10)$$

From the general linear equation, the regression co-efficients were calculated by:

$$b = W (P^T W)^{-1} q \quad (11)$$

$$a = C_{mean} - A^T_{mean}\, b$$

The built up calibration equations were used for the estimation of the compounds in the samples.

Calibration matrix and selection of spectral region for PLS and PCR

The quality of multicomponent analysis is dependent on the wavelength range and spectral mode used. PLS procedures are designated to be full spectrum computational procedures; however using highly noisy, scarcely informative wavelengths detracts from precision. This can be lessened, by discarding particularly noisy wavelengths.

Selection of optimum number of factors

An appropriate choice of the number of principal components or factors is necessary for PCR and PLS calibrations. The number of factors should account as much as possible for the experimental data without resulting in over fitting. Various criteria have been developed to select the optimum number. Cross validation methods leaving out one sample at a time is the most commonly employed technique for selection of optimum factors. The predicted concentrations are compared with the known concentrations of the compounds in each calibration sample. The Root Mean Squares Error of Cross Validation (RMSECV) is calculated for each method as follows:

$$RMSECV = \sqrt{\frac{PRESS}{n}} \quad (12)$$

where n is the number of training samples and

$$PRESS = \sum (Ypred - Ytrue)^2 \quad (13)$$

where Y_{pred} and Y_{true} are predicted and true concentrations in µg/ml respectively.

The RMSECV was used as a diagnostic test for examining the errors in the predicted concentrations. It indicates both of the precision and accuracy of predictions. The optimum model is selected with the fewest number of factors which yield the lowest RMSECV.

Steps to be followed for development of chemometric models:

- Design of calibration sets based on the MRLs -Number of samples in calibration set can be designed based on the factorial design.
- Preparation of samples as per calibration sets using reference

standards procured from manufacturers.

- Scanning of calibration samples throughout the spectral range using UV-Vis Spectrophotometer.
- Collection of data points in the full spectral range or in the selected spectral wavelength range at regular wavelength intervals.
- Transferring the data to PLS Toolbox via Matlab for further data analysis.
- Optimization of calibration model can be achieved by employing different pre-processing and cross validation procedures.
- To investigate the prediction ability of the developed calibration model, separate mixtures called prediction set can be employed.
- All the developed chemometric methods should be validated before proceeding with the analysis of real samples.
- Validation of all proposed methods can be performed using pharmaceutical dosage forms for different parameters such as
    - Accuracy
    - Precision – Inter-day and Intra-day
    - Mean Recovery
    - Correlation Coefficient and range
    - Limit of Detection
    - Limit of Quantification
    - Standard Error of Calibration
    - Standard Error of Prediction
    - Robustness
    - Ruggedness
    - Procurement of Herbal Formulation – Three different brands of stated herbal formulations will be procured.
    - Preparation of sample mixtures – Each formulation will be subjected to methanolic extraction and stored in deep freezer for further analysis.
    - Quantitative Estimation – Simultaneous estimation of phyto-constituents using the proposed analytical methods.
    - Comparison of results for proposed PLS and PCR models.

Results and Discussion

Spectral characteristics

The UV spectra of all four drugs were overlapped to study the spectral characteristics. A complete overlap thorughout the UV region was observed. To carry out multivariate analysis of the selected drugs, 25 different synthetic mixtures were prepared in the concentration ranges of 0-3 µg/ml of ACA and EMB, 0-6 µg/ml of GAA and 0.5-2.5 µg/ml of PIP respectively (Table 1). The concentration range was set from zero in order to meet the requirement of utilizing the analysis of any two drugs mentioned in the combination

Table 1: Concentrations of Calibration and Prediction sets in µg/ml

Calibration Set (µg/ml)				Prediction Set (µg/ml)			
ACA	**EMB**	**GAA**	**PIP**	**ACA**	**EMB**	**GAA**	**PIP**
0.5	0.5	5	0.5	2	0.5	1	0.5
0.5	1	4	0.5	2.5	1	1.5	1.5
0.5	1.5	3	0.5	1	1.5	2.5	2
0.5	2	2	0.5	2	2	2	1
1	0.5	5	0.5	0	2.5	0	1

1	1	4	1	1	3	2	2
1	1.5	3	1	2	3	1	1
1	2	2	1	0	0	3	2.5
1.5	0.5	5	1	1	1	6	1.5
1.5	1	4	1	0	2	0	2
1.5	1.5	3	1.5	-	-	-	-
1.5	2	2	1.5	-	-	-	-
2	0	0	1.5	-	-	-	-

Optimized conditions for PLS model

Wavelength range : 200-350 nm
Data Points : 151 nm (Δ1 nm)
Preprocessing : Mean Centre and orthogonal signal correction
Cross Validation : Leave one out
Latent Variables : 13

Table 2: Estimated Concentrations of Ascorbic Acid (ACA) in the calibration set under optimized PLS model

Amount Present (µg/ml)	D0		D1		D2	
	Amount found (µg/ml)	**% recovery**	**Amount found (µg/ml)**	**% recovery**	**Amount found (µg/ml)**	**% recovery**
0.5	0.489	97.96	0.4921	98.42	0.4865	97.3
0.5	0.492	98.52	0.4883	97.66	0.4821	96.42
0.5	0.504	100.9	0.5183	103.66	0.5084	101.68
0.5	0.491	98.36	0.515	103	0.4961	99.22
1	1.020	102.02	0.9838	98.38	0.9758	97.58
1	1.053	105.3	1.0137	101.37	0.9779	97.79
1	0.997	99.75	1.0035	100.35	1.0298	102.98
1	0.960	96.05	1.0084	100.84	1.0687	106.87
1.5	1.526	101.76	1.5117	100.78	1.5051	100.34
	Mean	**97.0914**	**Mean**	**98.0324**	**Mean**	**98.2167**

Statistical Parameters for PLS model

The mean % recovery ranges for all the four 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.

Table 3: Statistical Parameters calculated from application of PLS model to spectral data of calibration and prediction sets

	Calibration set				Prediction set			
Drug	**Mean**	**SD**	**R^2**	**RMSEC**	**Mean**	**SD**	**R^2**	**RMSEP**
ACA-D0	97.091	2.405	0.9986	0.0260	99.456	1.647	0.9936	0.1162
ACA-D1	98.032	2.231	0.9994	0.0169	98.847	1.801	0.9989	0.0476
ACA-D2	98.216	1.723	0.9997	0.0116	101.119	2.003	0.9988	0.0489
EMB-D0	97.017	1.324	0.9992	0.0265	95.543	2.345	0.9957	0.0672
EMB-D1	98.866	1.112	0.9993	0.0234	102.124	2.630	0.9981	0.0606
EMB-D2	98.974	2.321	0.9994	0.0223	99.368	2.341	0.9981	0.0443
GAA-D0	99.645	1.654	0.9993	0.0428	103.390	2.103	0.9958	0.1471
GAA-D1	100.28	1.208	0.9993	0.0429	102.389	1.560	0.9986	0.0980
GAA-D2	100.26	2.343	0.9997	0.0282	101.436	1.543	0.9996	0.0471
PIP-D0	99.946	2.311	0.9999	0.0061	101.323	1.234	0.9918	0.0546
PIP-D1	99.972	2.325	0.9997	0.0128	102.852	1.110	0.9954	0.0453
PIP-D2	99.988	2.118	0.9997	0.0125	99.749	1.231	0.9979	0.0310

Optimized conditions for PCR model

Wavelength range : 200-350 nm
Data Points : 151 nm (Δ1 nm)
Preprocessing : Mean Centre and orthogonal signal correction
Cross Validation : Leave one out
Number of PCs : 13

Statistical Parameters for PCR model

The mean % recovery ranges for all the three drugs in the calibration set and in the prediction set were found to lie betweeen 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 4: Statistical Parameters calculated from application of PCR model to spectral data of calibration and prediction sets

	Calibration set				Prediction set			
Drug	**Mean**	**SD**	**R^2**	**RMSEC**	**Mean**	**SD**	**R^2**	**RMSEP**
ACA-D0	100.1142	2.312	0.9986	0.0265	96.692	2.323	0.9921	0.0962
ACA-D1	99.7415	1.231	0.9988	0.0246	104.390	2.342	0.9975	0.0803
ACA-D2	99.2956	2.342	0.9954	0.0485	103.728	3.112	0.9985	0.0426
EMB-D0	99.9862	2.132	0.9973	0.0474	96.654	2.345	0.9957	0.0871
EMB-D1	99.9319	1.452	0.9976	0.0450	104.029	3.321	0.9951	0.0899
EMB-D2	100.258	1.267	0.9968	0.0523	100.018	3.245	0.9981	0.0438
GAA-D0	99.9981	2.321	0.9908	0.1537	102.750	1.234	0.9941	0.1691
GAA-D1	98.9809	2.432	0.9943	0.1274	103.342	1.567	0.9993	0.0992
GAA-D2	100.7295	2.541	0.9953	0.1103	100.3760	3.456	0.9983	0.0770
PIP -D0	100.127	2.113	0.9964	0.0425	101.975	1.333	0.9955	0.0615
PIP -D1	100.031	3.421	0.9968	0.0401	102.599	2.444	0.9959	0.0436
PIP-D2	99.670	3.652	0.9979	0.0327	100.697	3.656	0.9973	0.0332

Application of PLS and PCR models in Ayurvedic Preparations

Optimized PLS and PCR models were applied to commercial formulations and the results are tabulated in Table 20 and 21. The percentage recovery was found to be from 97 – 103% for all the four drugs. These methods can be successfully applied for formulations containing either two or three or four drugs.

Table 5: Assay results of PLS model (D0) in Marketed preparations

Code	**Name of the formulation**	**Ascorbic Acid (ACA) % Recovery**	**Embelin (EMB) % Recovery**	**Gallic Acid (GAA) % Recovery**	**Piperine (PIP) % Recovery**
P1	Triphala churna (for weight loss)	98.23	-	98.83	-

P2	Trikatu Churna (for digestion)	-	-	-	97.65
P3	Vidangadi churna (anthelmentic)	-	97.84	98.55	-
P4	Vidang Churna (germicidal, bactericidal)	-	99.56	-	-
P5	Ashta Churna (for digestive disorders)	-	-	-	99.67
P6	Chyawanprash (Immuno modulator)	101.23	-	98.78	99.67
P7	Brahma rasayana (for stress and tiredness)	99.54	102.34	98.67	99.12
P8	Sanjeevani Vati (for cough and fever)	103.21	101.34	100.67	99.89

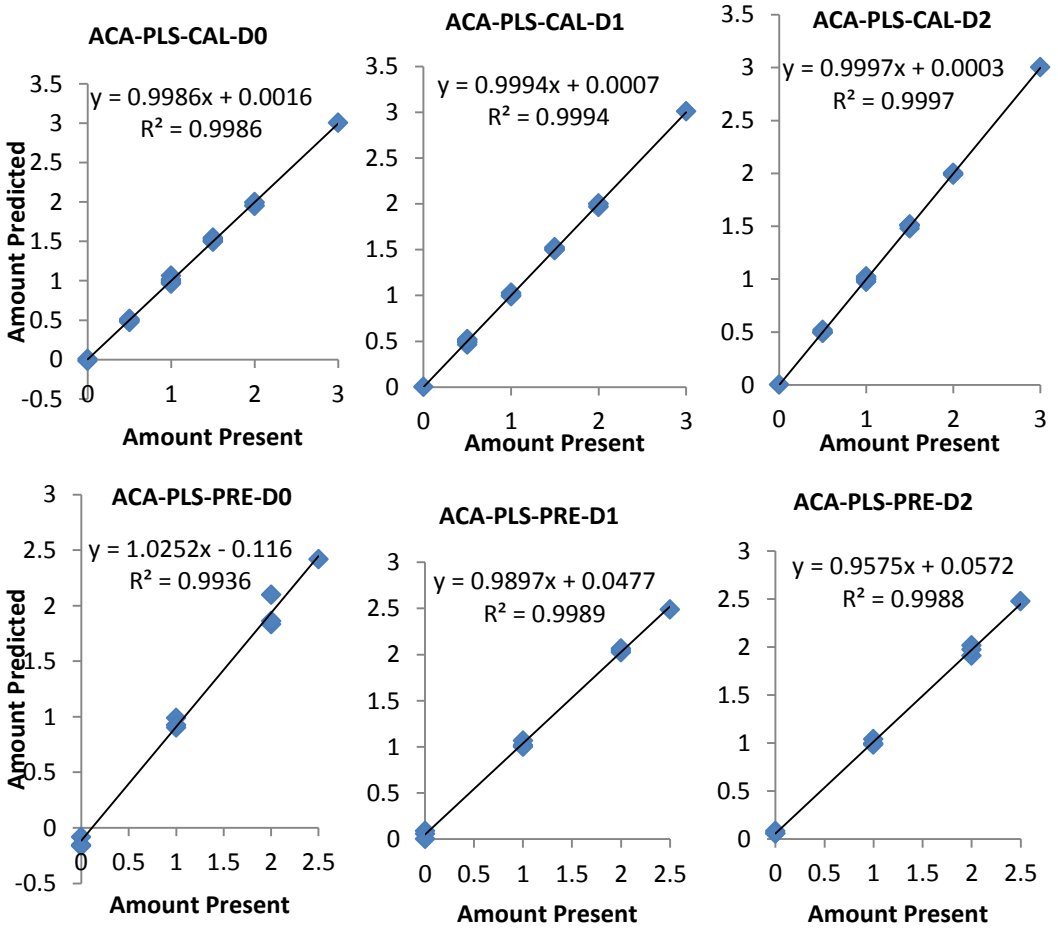
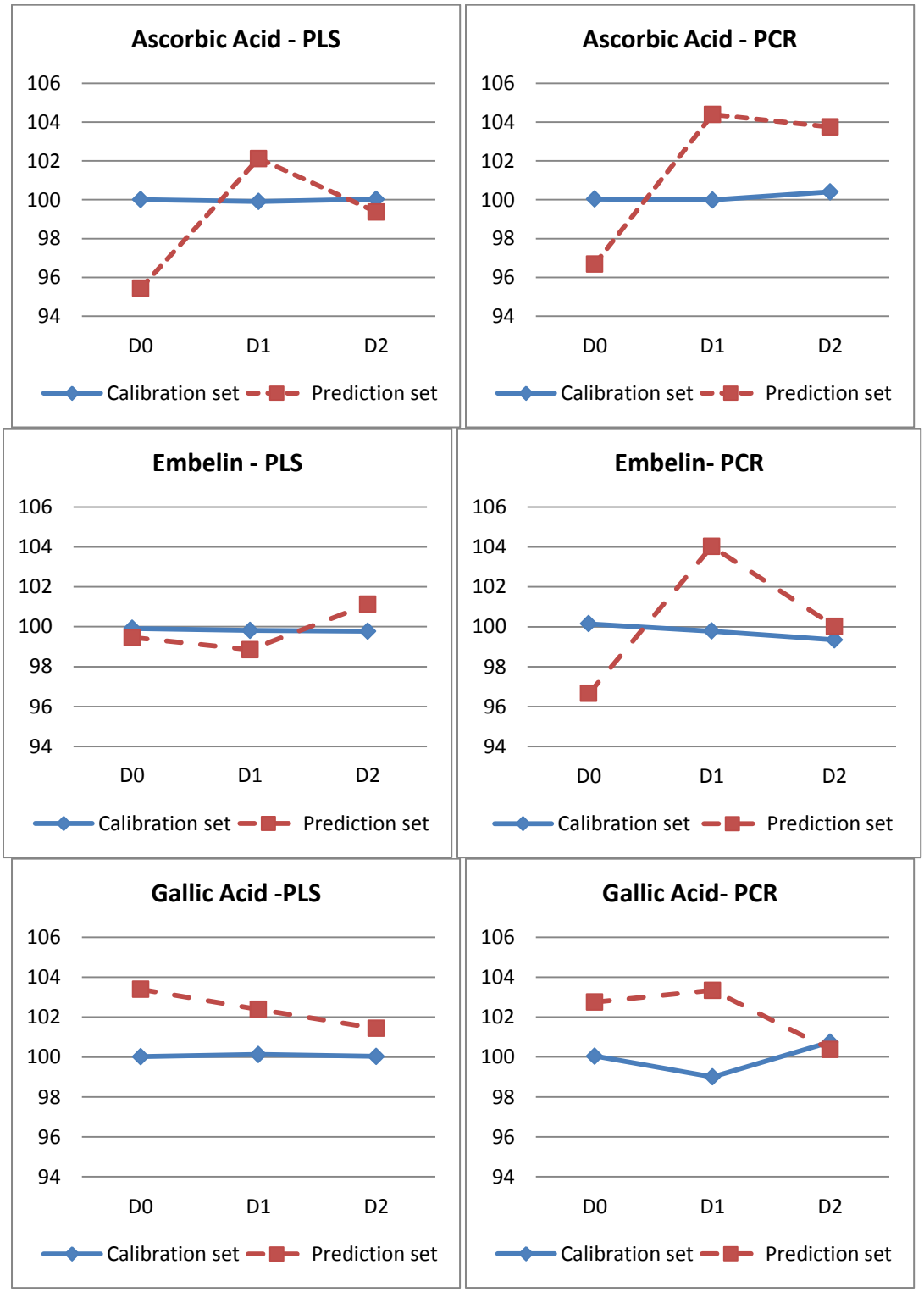


Figure 1: Plots of calibration and prediction sets of Ascorbic Acid (ACA) in PLS model

Comparison of results



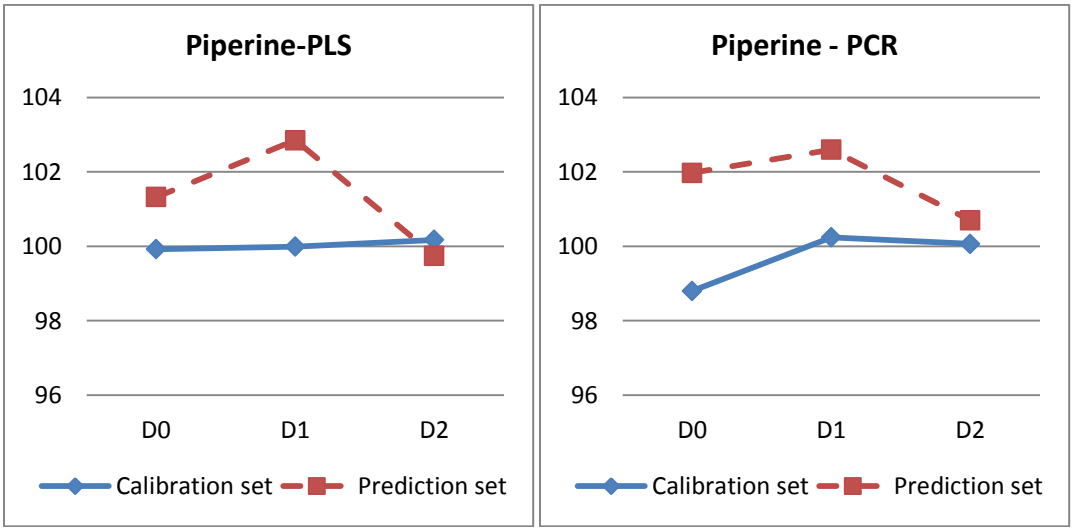


Figure 2: Comparison of Mean Recovery of PLS and PCR models for Fundamental (D0), First Derivative (D1) and Second Derivative (D2) Spectra

Conclusion

Herbal medication usage has increasing as individuals revert to natural remedies globally. Herbal formulations are widely used as diabetic, arthritic, hepatic, and cough, memory, and adoptogen treatments. Herbal supplements are offered as pills, capsules, powders, tea, extracts, fresh and dried plants to promote health. Some herbals may create health concerns, be ineffective, or interact with other medications. Standardizing herbal formulations is vital for assessing medicine quality based on active ingredient concentration.

Standardization of herbal medications uses chromatographic, spectrophotometric, electrophoresis, polarography, and molecular biomarkers. Chemical fingerprints created by these procedures are important instruments for herbal authenticity and quality control. There is a need for a speedy and specific approach for producers to define quality standards and criteria for therapeutic effectiveness, safety, and shelf life of herbal medications. Chemical markers are used in the authentication and separation of species, stability evaluations, and quality control of herbal medicines. A technique for simultaneously estimating four chemical indicators prevalent in poly herbal mixtures is provided. The suggested techniques give quick and cost-effective Gallic acid, Ascorbic acid, Embelin, and Piperine analysis from distinct ayurvedic formulations.

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