Classification of aqueous-based ayurvedic preparations using synchronous fluorescence spectroscopy and chemometric techniques

Keshav Kumar[1], Partha Bairi[1], Krishnakanta Ghosh[1], Kishor Kumar Mishra[2] and Ashok Kumar Mishra[1,*]

[1]Department of Chemistry, Indian Institute of Technology-Madras, Chennai 600 036, India
[2]Ayurvedic Medical Officer, Government Ayurvedic Dispensary, Gaisima 768 040, India

The complexity of molecular compositions of ayurvedic preparations makes their classification or quality monitoring a difficult task. Classification of aqueous-based ayurvedic preparations with commonly used techniques such as Fourier transform infrared and near infrared spectroscopy is more difficult due to strong interferences from water signals. In the present work, by taking an aqueous-based ayurvedic preparation (jirakadyarista) as a test case, it has been shown that a simple, fast and efficient procedure for the classification of such preparations can be achieved by combining synchronous fluorescence spectroscopy with chemometric methods such as principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA). The PCA and PLS-DA models obtained were found to be sensitive and specific in classifying the jirakadyarista samples.

Keywords: Aqueous-based, ayurvedic preparation, chemometric techniques, synchronous fluorescence spectroscopy.

AN ayurvedic preparation (i.e. medicine) is essentially a complex mixture of a number of natural products. Maximum medicinal benefit from the ayurvedic preparations is achieved when the ingredients (i.e. natural products) of the preparations are present in a particular ratio. However, batch-to-batch compositional variation of ayurvedic preparations affecting their medicinal efficacy is often encountered. Generally, it happens if the method of preparation, conditions under which the preparations are made, and the storage process are not followed 'strictly' according to authentic ayurveda literature. The complexity in the molecular composition of such preparations limits the application of existing analytical techniques for the analysis of their individual constituents. Thus, unlike pharmaceutical drugs, quality control and classification of ayurvedic medicines is a difficult task. Classification attempts for some non-aqueous Chinese herbal preparations have been recently made, where Fourier transform infrared (FT–IR) and near infrared (NIR) spectroscopic techniques were used in combination with chemometric techniques[1–5]. Several review articles have been published in recent years, which summarize the application of spectroscopy and chromatographic techniques with chemometrics towards classifying herbal preparations[6–8].

Aristas and asavas are two different types of aqueous-based ayurvedic preparations which are used for the treatment of various ailments[9]. However, the classification of these aqueous preparations with commonly used FT–IR and NIR techniques is unreliable due to the significant interference from water signals. In this context the use of fluorescence spectroscopy could be an attractive proposition for the following reasons: (i) aqueous plant extracts are expected to contain a variety of fluorescent compounds like flavonoids, aromatic amino acids, vitamins, porphyrins, coumarins, alkaloids, etc.[10]; fluorescence of these compounds can act as a spectral fingerprint for a particular plant extract; (ii) as water itself is not a fluorophore in the UV–visible region of the electromagnetic spectrum, aqueous extracts can be conveniently analysed using fluorescence, and (iii) the significant difficulty encountered with electronic absorption spectroscopy for non-transparent samples (like most of the aristas) can be circumvented using fluorescence spectroscopy. The presence of a number of fluorophores at unknown concentrations with significant absorption and emission spectral profile makes the ayurvedic preparation a complex multifluorophoric system[11]. It is well established that such systems are difficult to analyse using conventional fluorescence techniques, i.e. study of single emission and/or excitation spectrum[11].

Synchronous fluorescence spectroscopy (SFS) is a technique which involves simultaneous scanning of excitation and emission monochromator with a constant wavelength offset ($\Delta\lambda = \lambda_{emission} - \lambda_{excitation}$)[11–13]. SFS has been successfully used for the analysis of various multifluorophoric mixtures such as petroleum products, essential oils, humic acids, etc.[14–17]. Selection of optimum wavelength offset is an essential requirement for the successful application of the SFS technique. An optimum offset is generally obtained by measuring SFS at various offsets.

*For correspondence. (e-mail: mishra@iitm.ac.in)

Excitation–emission matrix fluorescence (EEMF) spectroscopy is another technique which is commonly used for the analysis of multifluorophoric mixtures[18]. However, there are two issues with EEMF: (i) data acquisition is a time-consuming process, and (ii) problem of handling the Rayleigh scattering signals[19]. Therefore, in the present work SFS was preferred over EEMF spectroscopy.

The present work is aimed at achieving a simple and fast procedure for the classification of aqueous-based ayurvedic preparations by combining SFS with chemometric techniques. To the best of our knowledge there are no reports in the literature where any attempts have been made for the classifications of aqueous-based ayurvedic preparations using fluorescence spectroscopy and chemometric techniques. In the present work, jirakadyarista, an aqueous-based ayurvedic preparation used for treating puerperal disease, burning sensation in the extremities, diarrhoea, dyspepsia, etc. was taken as the test case. Further, jirakadyarista was chosen because it is available locally under several brand names. Specific objective of the present work is to achieve classification of jirakadyarista from non-jirakadyarista samples by combining SFS with chemometric methods such as principal component analysis (PCA; an unsupervized classification technique)[20–22] and partial least square discriminant analysis (PLS-DA; a supervized classification technique)[21,23–25]. Another minor objective of the present work is to achieve classification of various samples of the jirakadyarista group with respect to a standard reference sample by subjecting SFS data to PCA.

Materials and methods

Chemicals, sample preparation and need of reference (i.e. authentic) sample

For our study, ten jirakadyarista and seven non-jirakadyarista samples were collected. In this article, the names of the brands have been omitted; the jirakadyarista samples have been labelled as J1 to J10 and non-jirakadyarista samples as NJ1 to NJ7. The selected jirakadyarista and non-jirakadyarista samples are dark and highly concentrated liquids. In order to use fluorescence as a tool, it is necessary that light passes through the fluorescence cuvette (1 cm path length) with reasonable transparency of resolution in the UV–vis wavelength range. This was achieved by adjusting the optical density to 0.3 at 300 nm. Optical density normalization has certain advantages over uniform dilution, e.g. (i) it will reduce the chances of misclassification in the cases where two or more samples belong to the same group (i.e. relative ratio of the constituents are same), but differ in their level of dilution and (ii) adjusting optical density to 0.3 will reduce the inner filter effects.

Jirakadyarista sample J1 is an authentic reference sample, which was prepared by following the procedure given in the ayurvedic literature[9]. The composition of the reference sample and method of preparation are given in Appendix 1. In the present work, reference sample (J1) was used as a marker to find the group to which the jirakadyarista samples belong and it was also used to classify jirakadyarista samples obtained from different brands. The use of reference sample is necessary because in an unsupervized technique such as PCA, no a priori information is available about the group to which a sample belongs. Therefore, reference sample can be used as a marker to find the cluster of jirakadyarista samples. Moreover, distance (e.g. Euclidean distance) of various jirakadyarista samples from the reference sample could be used to find their relative quality.

Instrument and data acquisition

SFS of all the 17 samples (10 jirakadyarista + 7 non-jirakadyarista) was recorded using Hitachi F-4500 spectrofluorimeter with a 100 W xenon lamp as excitation source. Scan speed and PMT voltage were fixed at 240 nm s^{-1} and 700 V respectively. Band passes for excitation and emission monochromators were kept at 10 nm. Conventional right-angle geometry was used for all measurements. SFS was recorded in the excitation wavelength range from 200 to 550 nm, with a step size of 0.2 nm at $\Delta\lambda$ of 30–150 nm with a step of 10 nm. In order to find the optimum offset, PCA and PLS-DA were performed on SFS data collected at all the 13 different $\Delta\lambda$ values. It was found that PCA and PLS-DA models achieved highest sensitivity and specificity with SFS data collected at $\Delta\lambda$ of 110 nm. Therefore, in present work only the outcomes of PCA and PLS-DA of SFS data collected at $\Delta\lambda$ of 110 nm are reported.

Principal component analysis

PCA is an unsupervized pattern technique which reduces the dimension of a dataset without losing any important information[20–22]. Mathematically, PCA decomposes a data matrix X of dimension, $I \times J$, as the product of score matrix T of dimension, $I \times L$, and loading matrix P of dimension, $J \times L$.

$$X = TP^T + E, \tag{1}$$

where I, J, L and E are the number of samples, variables, number of factors (principal components; PC) and residual matrix respectively. The number of factors (L) should be less or equal to the minimum of sample (I) and variable (J), i.e. $L \leq \min\{I, J\}$. Columns of the score matrix are orthogonal to each other, i.e. $T^{\mathrm{T}}T = D$, where D is the diagonal matrix. Columns of the loading matrix are orthonomal to each other, i.e. $P^TP = I$, where I is the identity matrix. Superscript T indicates transpose of the

matrix. Score matrix contains information on how samples are related to each other and loading matrix contains information on how the variables are related to each other. First PC in the PCA contains maximum variation of the dataset followed by the second PC and so on. Score values of a PC can be plotted against the sample number or the score values of two or more PCs can be plotted against each other to see whether any pattern exists in the dataset.

Partial least square discriminant analysis

PLS-DA is a supervized pattern recognition technique[21,23–25] which is based on partial least square analysis (PLS) algorithm[20,21,26]. Unlike PCA which finds the factors that explain maximum variation of X block dataset (independent variables) without taking into account Y block dataset (dependent variables), PLS analysis finds the factors in such a way that it maximizes covariance between X and Y block datasets. When PLS is used for discriminant analysis, i.e. PLS-DA, Y matrix contains only logical values 1 and 0. The presence or absence of a sample in a group is indicated by 1 and 0 respectively. Mathematically, PLS analysis involves simultaneous decomposition of both X and Y matrices as given in eqs (2) and (3)

$$X = TP^T + E, \tag{2}$$

$$Y = UQ^T + F, \tag{3}$$

followed by a regression model relating T and U matrices, i.e.

$$U = TB + G, \tag{4}$$

where T and U are the score matrices, and P and Q are the loading matrices of X and Y matrices respectively. B is the regression matrix and E, F and G are the residual matrices. Dimensions of X, Y, T, P, U and Q are $I \times J$, $I \times K$, $I \times L$, $J \times L$, $I \times L$ and $K \times L$ respectively, where I, J, K and L are the number of samples, variables, groups and factors respectively. Matrix U does not contain logical values 0 and 1, which are used in Y matrix to indicate absence or presence of a sample in a group. Thus, a threshold value[21,24] is calculated using Bayes theorem[24,27] to discriminate one group from the other. Sensitivity and specificity are the measures of true positives (calculated using eq. (5)) and true negatives (calculated using eq. (6)) respectively[21,28]. An ideal PLS-DA model should have high sensitivity and specificity.

$$\text{Sensitivity} = \text{True positives}/(\text{true positives} + \text{false negatives}), \tag{5}$$

$$\text{Specificity} = \text{True negatives}/(\text{true negatives} + \text{false positives}). \tag{6}$$

Software used

PCA and PLS-DA were carried out using PLS_Toolbox 5.0.3 in MATLAB. Data were plotted using MATLAB R2008b.

Selection of samples and data arrangement for PCA and PLS-DA

For the classification of jirakadyarista from non-jirakadyarista samples using PCA in an unsupervized manner, a calibration set C1 consisting of all the 17 samples was used. For evaluating the relative quality of jirakadyarista samples with respect to the reference sample, PCA was performed on a calibration set C2 consisting of only jirakadyarista samples (i.e. 10 numbers). For the PLS-DA (supervized) technique, a calibration set C3 of 11 samples consisting of J1, J2, J3, J4, J9, J10, NJ1, NJ2, NJ3, NJ4 and NJ4 was prepared. In order to validate the PLS-DA model, a validation set V1 of six samples consisting of J5, J6, J7, J8, NJ6 and NJ7 was also prepared. Data of C1, C2, C3 and V1 were arranged in matrices of dimensions 17×1751, 10×1751, 11×1751 and 6×1751 respectively, where 17, 10, 11 and 6 are the number of samples and 1751 is the number of spectral variables.

Results and discussion

SFS of the jirakadyarista and non-jirakadyarista samples collected at $\Delta\lambda$ of 110 nm is shown in Figure 1. It can be seen that other than the variations in fluorescence intensities, there are no significant differences in SFS profiles which could be used to distinguish the jirakadyarista from the non-jirakadyarista samples or to achieve relative assessment of jirakadyarista samples. However, a reliable discrimination between the samples of different groups

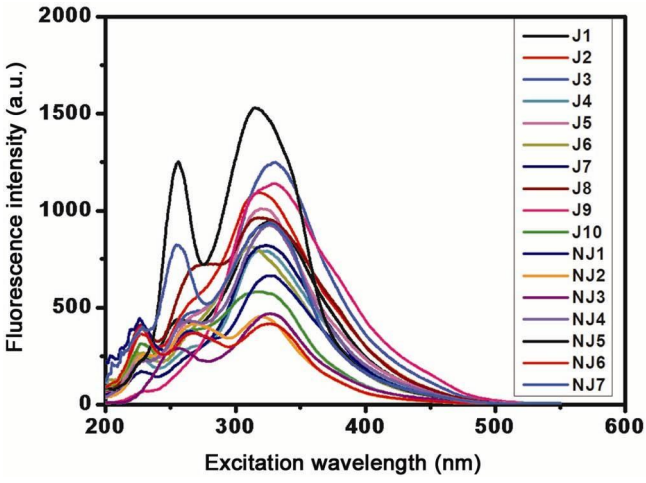

Figure 1. SFS of jirakadyarista (J1–J10) and non-jirakadyarista (NJ1–NJ7) samples collected at $\Delta\lambda$ = 110 nm.

can still be achieved successfully by analysing the spectral datasets with chemometric techniques such as PCA and PLS-DA. These techniques try to find the set of variables which can be used to discriminate the samples of different groups.

PCA to achieve unsupervized classification of jirakadyarista from non-jirakadyarista samples

In order to achieve classification between the jirakadyarista and non-jirakadyarista samples group the normalized SFS data matrix (17 × 1751; sample × wavelength) of set C1 was subjected to PCA. A three-PC model was made. PCA model explained 99.49% variance of the dataset. The first (PC1), second (PC2), and third (PC3) PCs individually explained 96.37%, 2.51% and 0.61% variance of the datasets. The score values of PC1 were plotted against the sample index. It was found that although PC1 captured maximum variance of the dataset of C1, it had no discriminating information which could be used to differentiate jirakadyarista from non-jirakadyarista samples. Similarly, PC2 and PC3 individually did not provide any discrimination between the samples of different groups. The score plots obtained from the different combinations of PCs were also analysed. Among the three combinations, i.e. (i) PC1 and PC2, (ii) PC1 and PC3 and (iii) PC2 and PC3, the score plot of PC1 versus PC3 (Figure 2), provides correct classification between the jirakadyarista and non-jirakadyarista samples. The samples J1–J10 are found to cluster in the score range 17.856 to 21.470 along PC1 and score range – 0.219 to 0.653 along PC3. The presence of reference sample (J1) verifies that clustered samples belong to the group of jirakadyarista. All the non-jirakadyarista samples NJ1–NJ7 are found to be scattered randomly around the cluster of jirakadyarista samples. There are no particular patterns in the score values of the non-jirakadyarista samples and this mainly due to the fact that they are as different from each other as they are from the jirakadyarista samples. Moreover, the extent of separations (i.e. distances) of all the non-jirakadyarista samples except for the NJ4 is found to be significantly large.

Relatively small separation of NJ4 from the group of jirakadyarista samples could be attributed to the similarity in their spectral profile in the wavelength range 250–550 nm (Figure 1). Classification results clearly show that the obtained PCA model is highly robust (i.e. sensitive and specific) in nature. Thus, it can be inferred that by combining SFS with PCA and using appropriate combinations of the PCs, it is possible to achieve classification of the aqueous-based ayurvedic preparations.

Classification of jirakadyarista samples with respect to the standard reference sample using PCA

Normalized SFS data of calibration set C2 were subjected to PCA. A three-component PCA model was used to classify various jirakadyarista samples with respect to a reference sample. The three components PC1, PC2 and PC3 of the PCA model captured the 98.05%, 1.56% and 0.21% variance of the dataset respectively. Among various possible combinations of PCs, combination of PC1 and PC3 was used to classify various jirakadyarista samples with respect to a reference sample. This particular combination of PCs is preferred because it provides successful classification of jirakadyarista from non-jirakadyarista samples. PC1 vs PC3 score plot of the PCA model of set C2 is shown in Figure 3. The Euclidean distance[21,28] between the reference sample and the other jirakadyarista samples in the score plot was calculated using

$$d_{\text{ref},\,j} = \sqrt{(t_{1\text{ref}} - t_{1j})^2 + (t_{3\text{ref}} - t_{3j})^2}, \qquad 7)$$

where $d_{\text{ref},j}$ is the Euclidean distance, $t_{1\text{ref}}$ and $t_{3\text{ref}}$ are the PC1 and PC3 score values of reference sample, and t_{1j} and

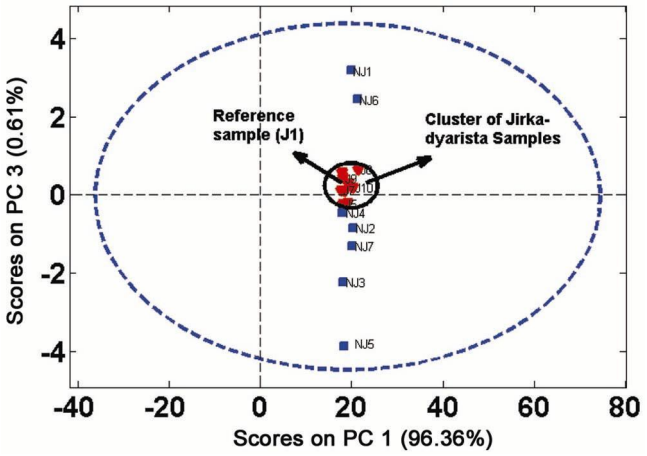

Figure 2. Classification of jirakadyarista samples (▼) from non-jirakadyarista samples (■) by PCA model.

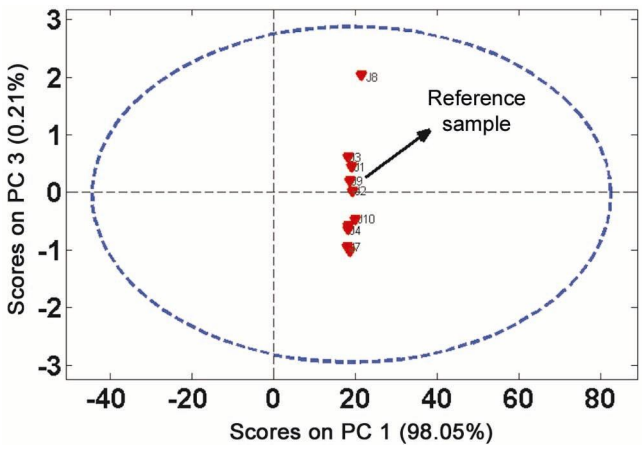

Figure 3. Classification of various jirakadyarista samples (J2–J10) with respect to reference jirakadyarista sample (J1) by PCA model.

t_{3j} are the PC1 and PC3 score values of the jth jirakadyarista sample. The calculated distances are summarized in Table 1.

It can be seen that samples J2, J3 and J9 are relatively close to reference sample J1. Therefore, it could be expected that the composition of these three jirakadyarista samples is similar to that of the reference sample. By contrast, Euclidean distance between J8 and J1 is highest; this probably indicates that the composition of J8 is different from the reference jirakadyarista sample. Distances of jirakadyarsita samples J2–J9 from the reference sample (J1) can be arranged in the following order J8 > J7 > J6 > J4 > J5 > J10 > J3 > J9 > J2. Euclidean distance of a test sample from the reference sample is expected to be correlated with the difference in the composition of the test sample from the reference. The implication of this observation towards the efficacy of the medicine can be a topic of further investigation. Nevertheless, here we propose a methodology for the classification of a particular ayurvedic preparation obtained from different brands.

PLS-DA to achieve supervized classification of jirakadyarista from non-jirakadyarista samples

PLS-DA (supervized classification technique) was performed to achieve classification of jirakadyarista samples from non-jirakadyarista samples. In this approach a calibration model has been made with a priori information of the group to which a particular sample belongs; therefore, the results obtained in the supervized classification analysis are expected to be much better than those obtained from unsupervized methods (i.e. PCA). To carry out this exercise, SFS spectral data matrix of set C3 and the Y matrix of dimensions 11×1751 and 11×1 respectively, were subjected to PLS-DA. The Y matrix contains logical values 1 and 0, the value of 1 indicates that the sample belongs to the jirakadyarista group and the value of 0 indicates that the sample does not belong to the jirakadyarista group. The spectral dataset of C3 was preprocessed using the combination of Savgol derivative[21,29] (polynomial order: 1, window: 15, derivative order: 1) and autoscale[21].

Table 1. Euclidean distance of various jirakadyarista samples (J2–J10) from the reference jirakadyarista sample (J1)

Sample	Euclidean distance
J2	0.480
J3	0.886
J4	1.487
J5	1.370
J6	1.574
J7	1.797
J8	2.698
J9	0.657
J10	1.225

In the present work, leave one out cross validation (LOOCV) approach[20,21] was used to find the number of latent variables required to make a robust PLS-DA model without over-fitting the data. In the LOOCV approach, calibration models with different number of latent variables are made using all the samples of the calibration set except one and the obtained model is then used to predict the group to which the left out sample belongs. This procedure is repeated, so that every sample of the calibration set is left out and predicted once. The number of latent variables giving the maximum sensitivity and specificity is typically used to fit the PLS-DA model. In the present case, sensitivity and specificity for a four latent variable PLS-DA model was found to be one. Thus, a four latent variable PLS-DA model was preferred to achieve supervized classification of jirakadyarista from non-jirakadyarista samples. The obtained PLS-DA model explained 86% variance of the spectral dataset. Using Bayesian approach a threshold value was calculated which separates the jirakadyarista from the non-jirakadyarista samples. The threshold value is calculated in such a way that the true positives (sensitivity) and true negatives (specificity) are maximized. The variation of sensitivity and specificity with threshold values was studied using the threshold plot (Figure 4 *a*). It can be seen that the PLS-DA model achieves maximum sensitivity and specificity at the threshold value of ~ 0.44. The receiver operating characteristic (ROC)[21,28] curve of the PLS-DA model, which represents the variation of sensitivity and specificity with

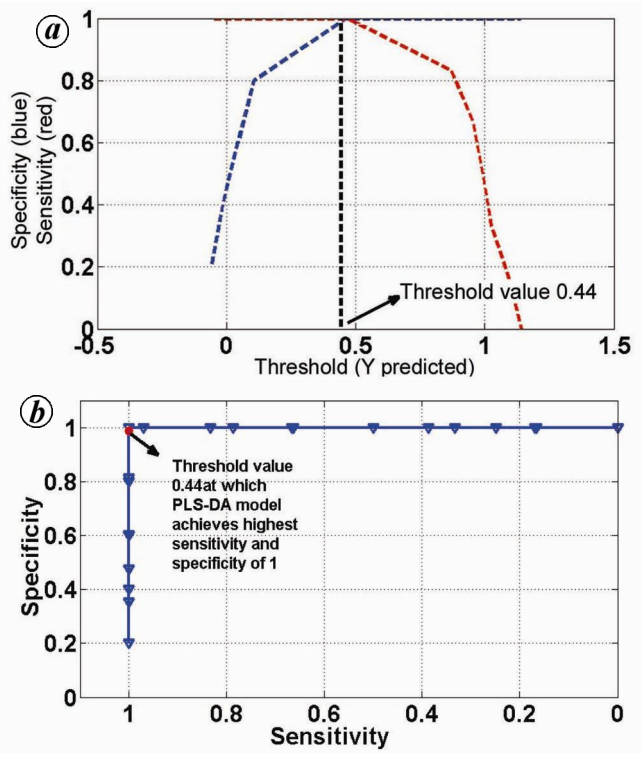

Figure 4. (*a*) Threshold plot and (*b*) ROC curve of PLS-DA model.

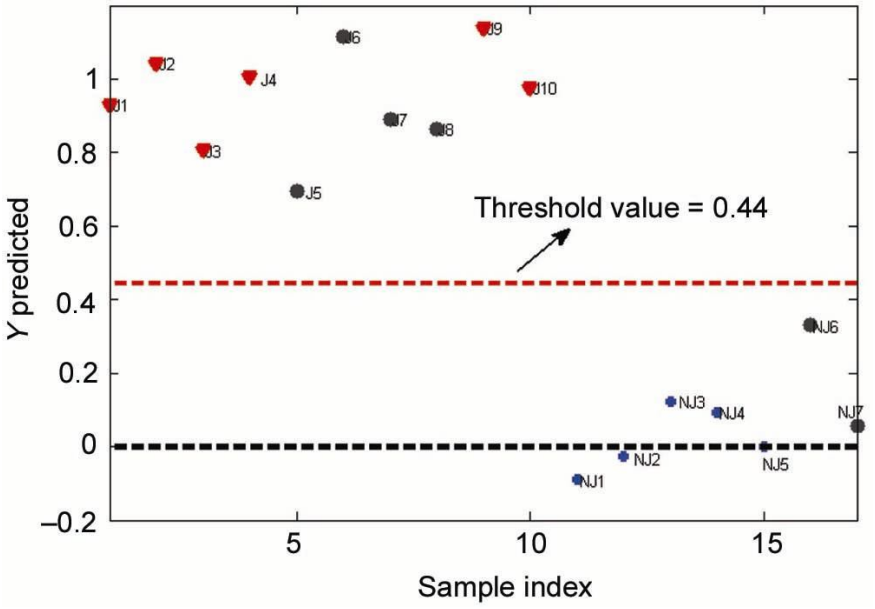

Figure 5. PLS-DA model for the supervised classification of jirakadyarista samples (▼) from non-jirakadyarista samples (■). Results of validation step are also shown; PLS-DA model made correct classification for all the samples (●) of validation set V1.

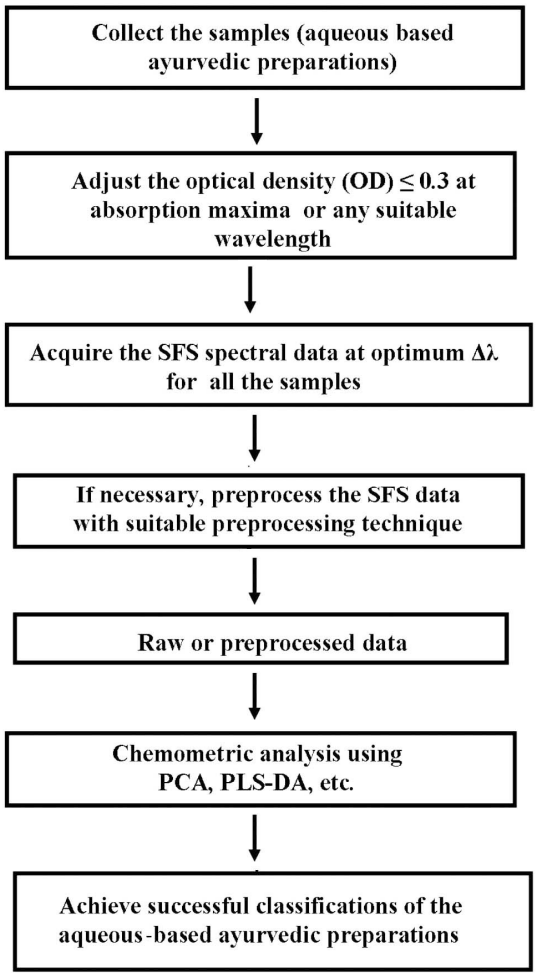

Figure 6. Scheme of achieving the classification of aqueous-based ayurvedic preparations using the combination of SFS and chemometric methods.

each other as the threshold value changes is shown in Figure 4 *b*. It can be seen that ROC curves of the present PLS-DA model mainly consist of vertical and horizontal lines with an area of close to one. It shows that *Y* values are not predicted randomly and the obtained PLS-DA model is a genuine classifier. The predicted *Y* values are plotted against the sample indices in Figure 5. Samples with predicted *Y* values greater than 0.44 are classified as jirakadyarista. The PLS-DA model made 100% correct classification for the jirakadyarista and non-jirakadyarista samples.

In order to test the classification ability of the PLS-DA model, a validation set V1 of six samples (J5, J6, J7, J8, NJ6 and NJ7) was used; results of the validation set are also given in Figure 5. For all the four jirakadyarista samples (J5, J6, J7 and J8) of the validation set, the predicted *Y* values are found to be greater than 0.65 and for both the non-jirakadyarista samples (NJ6 and NJ7) the predicted *Y* values are found to be less than 0.35. It shows that the obtained PLS-DA gives the correct classification for the validation set V1. Sensitivity and specificity of the PLS-DA model for both validation and calibration sets are found to be one. Thus, it can be inferred that the PLS-DA model is highly robust in nature and capable of classifying even those samples which are not present in the calibration set.

The proposed scheme to achieve classification of the aqueous-based preparations using the SFS and chemometric methods, i.e. PCA and PLS-DA has been summarized in Figure 6.

The results obtained clearly show that by combining SFS with chemometric methods, it is possible to achieve successful classification of jirakadyarista samples in supervized as well as in unsupervized manner.

Conclusions

In the present work, by combining SFS with chemometric methods such as PCA and PLS-DA aqueous-based ayurvedic preparations (jirakadyarista samples) have been successfully classified. Analysis of such aqueous-based preparations is otherwise difficult with commonly used techniques such as FT–IR and NIR due to significant interferences from water signal. In addition, Euclidean distance of a test sample from the reference sample in a score plot of PCA has been used to achieve the classification of various samples with respect to a reference sample.

Appendix 1

The ingredients mentioned below were coarsely powdered and kasaya was prepared. The kasaya was strained and kept in a fermentation pot. Honey (sugar or jaggery can also be used) was dissolved, boiled, filtered and added. The mouth of the pot, vessel or barrel was covered with an earthen lid and the edges sealed with clay-smeared cloth wound in seven consecutive layers. The container was kept either in a heap of paddy or in a special room to ensure that constant temperature was maintained during fermentation; since varying temperature may impede or accelerate fermentation. After the specified period, the lid was removed, and the contents examined to ascertain whether the process of fermentation has been completed. The fluid was first decanted and then strained after two or three days. When the fine suspended particles settled down, it was strained again and bottled.

Ingredients of jirakadyarista

Jiraka (*Cuminum cyninum*) 9.60 kg; water for decoction 49.152 l; guda (jaggery) 14.40 kg; dhataki (*Woodfordia fruticosa*) 768 kg; sunthi (*Zingiber officinale*) 96 kg; jatiphala (*Myristica fragrans*) 48 g; mustaka (*Cyperus rotundus*) 48 g; tvak (*Cinnamomum zeylanicum*) 48 g; ela (*Elettaria cardamomium*) 48 g; tejpatra (*Cinnamomum tamala*) 48 g; nagakesara (*Mesua ferrea* L.) 48 g; yamanika (*Trachyspermum ammi*) 48 g; kakkola (*Piper cubeba*) 48 g and devapuspa (*Syngium aromaticum*) 48 g.

1. Fu, H., Huang, D., Yang, T., She, Y. and Zhang, H., Rapid recognition of Chinese herbal pieces of *Areca catechu* by different concocted processes using Fourier transform mid-infrared and near-infrared spectroscopy combined with partial least-squares discriminant analysis. *Chin. Chem. Lett.*, 2013, **24**, 639–642.
2. Xu, L., Shi, P., Ye, Z., Yan, S. and Yu, X., Rapid analysis of adulterations in Chinese lotus root powder (LRP) by near-infrared (NIR) spectroscopy coupled with chemometric class modeling techniques. *Food Chem.*, 2013, **141**, 2434–2439.
3. Dharmraj, S., Jamaludin, A. S., Razak, H. M., Valliappan, R., Ahmed, N. A., Harn, G. L. and Ismali, Z., The classification of *Phyllanthus niruri* Linn. according to location by infrared spectroscopy. *Vib. Spectrosc*., 2006, **41**, 68–72.
4. Mao, J. and Xu, J., Discrimination of herbal medicines by molecular spectroscopy and chemical pattern recognition. *Spectrochim. Acta, Part A*, 2006, **65**, 497–500.
5. Wang, L., Lee, F. S. C. and Wang, X., Near-infrared spectroscopy for classification of licorice (*Glycyrrhizia uralensis* Fisch) and prediction of the glycyrrhizic acid (GA) content. *LWT–Food Sci. Technol.*, 2007, **40**, 83–88.
6. Bansal, A., Chhabra, V., Rawal, R. K. and Sharman, S., Chemometrics: a new scenario in herbal drug standardization. *J. Pharm. Anal.*, 2014; http://dx.doi.org/10.1016/j.jpha.2013.12.001
7. Gad, H. A., El-Ahmady, S. H., Abou-Shoer, M. I. and Al-Azizi, M. M., Application of chemometrics in authentication of herbal medicines: a review. *Phytochem. Anal.*, 2013, **24**, 1–24.
8. Rohman, A., Nugroho, A., Lukitaningsih, E. and Sudajadi, Application of vibrational spectroscopy in combination with chemometric techniques for authentication of herbal medicine. *Appl. Spectrosc. Rev.*, 2014, **49**, 603–613.
9. Shastri, A. and Shastri, R., *Bhaishajya Ratnavali*, Chowkkamba Sanskrit Academy, Varanasi, 1987.
10. Wolfbeis, O. S., *Molecular Luminescence Spectroscopy*, John Wiley, New York, 1985, pp. 167–370.
11. Patra, D. and Mishra, A. K., Recent developments in multicomponent synchronous fluorescence scan analysis. *Trends Anal. Chem*., 2002, **21**, 787–798.

12. Lloyd, J. B. F., Synchronized excitation of fluorescence emission spectra. *Nature Phys. Sci*., 1971, **231**, 64–65.
13. Dinh, T. V., Multicomponent analysis by synchronous luminescence spectrometry. *Anal. Chem*., 1978, **50**, 396–401.
14. Divya, O. and Mishra, A. K., Development of an analytical method combining chemometrics and synchronous fluorescence: analysis of diesel–kerosene mixtures. *Proc. Natl. Acad. Sci. India*, *Sect. A*, 2008, **78**, 115–122.
15. Divya, O. and Mishra, A. K., Combining synchronous fluorescence spectroscopy with multivariate methods for the analysis of petrol–kerosene mixtures. *Talanta*, 2007, **72**, 43–48.
16. Milano, T. M. and Senesi, N., Synchronous excitation fluorescence spectroscopy applied to soil humic substances chemistry. *Sci. Total Environ*., 1992, **117–118**, 41–51.
17. Dupuy, N., Dréau, Y. L., Ollivier, D., Artaud, J., Pinatel, C. and Kister, J., Origin of French virgin olive oil registered designation of origins predicted by chemometric analysis of synchronous excitation–emission fluorescence spectra. *J. Agric. Food. Chem*., 2005, **53**, 9361–9368.
18. Warner, I. M., Callis, J. B., Davidson, E. R., Gouterman, M. and Christian, G. D., Fluorescence analysis: a new approach. *Anal. Lett*., 1975, **8**, 665–681.
19. Kumar, K. and Mishra, A. K., Analysis of dilute aqueous multifluorophoric mixtures using excitation–emission matrix fluorescence (EEMF) and total synchronous fluorescence (TSF) spectroscopy: a comparative evaluation. *Talanta*, 2013, **117**, 209–220.
20. Kramer, R., *Chemometric Techniques for Quantitative Analysis*, Marcel Decker, New York, 1998.
21. Wise, B. M., Gallagher, N. B., Bro, R., Shaver, J. M., PLS Toolbox 4.0, Eigenvector Research Inc., WA USA, 2006.
22. Wold, S., Esbensen, K. and Geladi, P., Principal component analysis. *Chemometr. Intell. Lab. Syst*., 1987, **2**, 37–52.
23. Barker, M. and Rayens, W., Partial least squares for discrimination. *J. Chemometr.*, 2003, **17**, 166–173.
24. Pérez, N. F., Ferŕe, J. and Boqué, R., Calculation of the reliability of classification in discriminate partial least-squares binary classification. *Chemometr. Intell. Lab. Syst*., 2009, **95**, 122–128.
25. Louwere, D. J., Tates, A. A., Smide, A. K., Koot, G. L. M. and Berndt, H., PLS discriminant analysis with contribution plots to determine differences between parallel batch reactors in the process industry. *Chemometr. Intell. Lab. Syst*., 1999, **46**, 197–206.
26. Geladi, P. and Kowalski, B., Partial least square regression: a tutorial. *Anal. Chim. Acta*, 1986, **185**, 1–17.
27. Bishop, C. M., *Pattern Recognition and Machine Learning*, Springer, New York, 2006.
28. Brereton, R., *Chemometrics for Pattern Recognition*, John Wiley, UK, 2009.
29. Savitzky, A. and Golay, M. J. E., Smoothing and differentiation of data by simplified least squares procedures. *Anal. Chem*., 1964, **36**, 1627–1639.

ACKNOWLEDGEMENTS. K.K. thanks the Council of Scientific and Industrial Research (CSIR), New Delhi for providing Senior Research fellowship. We thank CSIR and RUTAG-IIT-Madras for financial support.

Received 5 July 2013; revised accepted 16 May 2014