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Application of near-infrared spectroscopy in detection of steroids adulteration in traditional Thai medicines

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Abstract

This study aimed to focus on applying near-infrared (NIR) spectroscopy to identify the adulteration of traditional Thai medicine products (TTM) with steroids. One hundred and ten samples were prepared with pure TTM and ten different steroid concentrations (0.25-5 mg steroid/g TTM). Fourier transform near-infrared (FT-NIR) spectrometer was used to scan TTM samples. The partial least squares (PLS) regression was used for the NIR spectroscopic model development to predict the level of steroid adulteration in TTM. For classification analysis, the principal component analysis (PCA) was used to discriminate 11 groups of raw TTM spectra (220 spectra). The developed PLS model accompanied by 3 latent variables (LVs) could predict the steroid content in TTM accurately with the coefficient of determination of prediction (r^2) of 98.20%, root mean square error of prediction (RMSEP) of 0.22 mg steroid/g TTM, and residual prediction deviation (RPD) of 7.46. Furthermore, the PCA approach was possible to discriminate among the groups of TTM. The study showed NIR spectroscopy's capability to be used as a powerful technique to evaluate the steroid adulterated in TTM. This report is useful for food and drug association, patients, pharmaceuticals, and medical sectors.

Keywords: Traditional Thai medicine, Herbal medicine, Adulteration, Steroids, Near-infrared spectroscopy

1. Introduction

Herbal medicines have been utilized to maintain good health and to treat diseases. Several conditions of some symptoms, such as stiffness, aches, obesity, or even high blood pressure, can be relieved using herbal medicine. Among these symptoms, high blood pressure or hypertension, which is the most common preventable risk factor for heart disease [1], has been paid attention to and accepted by some Asian people to the use of herbal medicines [2, 3]. Based on the fact that some herbs and spices may reduce blood pressure levels, this leads to lower risk of heart disease [2]. Nevertheless, the adulteration of herbal medicines has also been dispersing for many years around the world. For instance, Khazan et al. [4] studied Chinese herbal medicines adulteration sold as a medicine for weight loss. The presence of illegal substances and thyroid hormones were also investigated in this study. The results showed that the thyroid hormones and phencyclidine contained in the Chinese herbal slimming products without labeling their contents.

In Thailand, traditional Thai medicine products (TTM) are used widely in elderly patients. Since it was used in the therapy, the consumer accepted that it could safely heal the unhealthy. The TTM, which is bitter, is usually produced in bolus form using honey or syrup as a binder.

Adulteration of TTM with modern medicines is commonly found in herbal medicinal product (HMP) system of Thailand. Among the modern medicines detected (Steroids, NSAIDs, antihistamines, and psychotropic substance), steroids were found to be the most adulterated in tablet and capsule dosage forms [5]. By doing so, TTM manufacturers expected in order that within a few days, the patients feel much better, and their stiffness can be alleviated. Besides, the price of TTM (Adulterated with steroids), which has been advertised as a cure-all, is expensive. The purpose of adulterating steroids in TTM is to induce steroids' pharmacological activation, e.g., inhibiting the inflammatory mediator. Moreover, the most popular types of steroids adulterated in TTM were prednisolone and dexamethasone [6]. For safety monitoring of TTM and herbal medicines products in Thailand, the Health Product Vigilance Center (HPVC) under the Thai Food Drug and Administration (TFDA) is responsible to randomly collect the products and then send to laboratory to detect the fraudulence or adulteration using standard procedures like the thin-layer chromatography (TLC) technique.

There are some reports about the steroid in TTM. Arandorn et al. [7] studied the adulteration of steroids, prednisolone, and dexamethasone in TTM in Hat-Yai, Songkhla, Thailand by using the thin-layer chromatography (TLC) technique. The TTM samples had been compared with prednisolone and dexamethasone standards, in which the results showed that 2% of the samples were adulterated with steroids. Puripattanavong [6] reported the analysis of prednisolone and dexamethasone adulterations in TTM by the TLC technique. The author showed no adulteration in registered medicines; however, more than 50% of non-registered ones were found. The steroid adulteration in liquid herbal medicines was determined using quick, easy cheap, rugged, and safe (QuEChERS)

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technique and high-performance liquid chromatography [8]. These methods were allowed for the reliable detection of adulterated steroids, but it needs to be involved with chemical, sample preparation, and time-consuming.

Near-infrared (NIR) spectroscopy, widely used for the evaluation of chemical components in food and agricultural products as well as in medical sectors [9-12], involves the utilization of the electromagnetic spectrum across the wavelength range of 800-2500 nm, mainly related to vibrations of C-H, O-H, S-H and N-H bonds [13-15]. In the medical sectors, this technique, called functional near-infrared spectroscopy (fNIRS), showed the ability to aid in the diagnosis and prediction of the treatment response of major depressive disorder (MDD) [16, 17] and borderline personality disorder (BPD) [18]. The fNIRS technology could be used to study neurophysiology as it could continually monitor haemodynamic changes in the cerebral cortex using the NIR light [18]. Based on these, the NIR spectroscopy technique, if it could be a rapid method for adulterated steroid determination, is not only offer several significant advantages over traditional chemical methods, but also can apply to detect the effect of TTM usage on human responses. Its advantages include high precision and accuracy. In addition to this, it is non-destructive, non-chemical, and environmentally friendly. Also, it requires minimal or no sample preparation. In case study of using this technique with steroid, it was used to determine the content of a hormone steroid in single intact tablets. Partial least squares (PLS) regression model was constructed by correlating NIR spectra of the tablets with their corresponded steroid values determined by the high performance liquid chromatography (HPLC) method. The model could predict the steroid content in tablet with standard error of prediction (SEP) of 0.31 mg per tablet [19].

For application of the NIR technique to the determination of adulteration in some products, Haughey et al. [20] reported the application of NIR reflectance spectroscopy to detect the adulteration of melamine in soya bean meal, which provided the root mean square error of calibration (RMSEC) and prediction (RMSEP) of 0.081-0.276% and 0.134-0.368%, respectively. Dong et al. [21] applied the NIR technique to evaluate two adulterants in Cynanchum stauntonii. Three sample sets consisted of adulteration of either Cynanchum atrati (CA) or Cynanchum paniculati (CP) in Cynanchum stauntonii (CS), and that of both CA and CP in CS. The results for the PLS1 model showed that quantitative analysis of CS samples mixed with CA/CP adulterants provided RMSEP of 0.0097. Quantitative analysis of CS samples containing two adulterants identified that the radial basis function artificial neural network (RBF-ANN) model better than the PLS2 model with RMSEP of 0.0108. Ozdemir and Ozturk [22] reported NIR spectroscopy's use to determine sunflower and corn oil's adulteration in olive oil. The NIR spectrometer scanned pure olive oil and the adulterated one with varying sunflower and corn oil concentrations with a transmittance mode. Multivariate modeling with genetic inverse least squares (GILS) method was used to predict adulterants' concentration in the olive oil samples. Identifying a binary mixture of olive and sunflower oils exhibited the range of standard error of predictions between 2.49 and 2.88% (v/v). For the determination of the ternary mixtures of olive, sunflower, and corn oil, the error range was between 1.42 and 6.38% (v/v).

With no report on the steroid's determination adulterated in herbal medicine by using the NIR spectroscopic technique, therefore, this work aimed to study the application of this technique to detect steroids adulteration in traditional Thai medicines (TTM).

2. Materials and methods

2.1 Samples and sample preparations

Ingredients of traditional Thai medicines (TTM) and honey were purchased from traditional pharmacies (Ran-Khaai-Ya-Chao-Krom-Poe), Bangkok, Thailand, and Adrenocortical steroid (Prednisolone, Medic Pharma, Thailand) from Pattana Medical Center Clinic, Bangkok, Thailand. This TTM recipe was for curing body fatigue. Honey was used as the binding agent for pure TTM ingredients and those adulterated with steroids. The samples were TTM with 11 different concentrations of steroid (0, 0.25, 0.63, 1.00, 1.25, 1.88, 2.50, 3.13, 3.75, 4.38 and 5 mg steroid/g TTM). There were ten replicates. Therefore, there were one hundred and ten samples in total.

Ten grams of pure TTM or adulterated TTM was mixed thoroughly with 15.5 g of honey by hand in a mortar. The mixture was rolled into a rod and subjected to a wood granule extruder (Kritsada Samunprai Pharmacies, Bangkok, Thailand). This process provided 25 boluses of the TTM. Each bolus was approximately 7 mm in diameter. The process for making the TTM samples followed Homhual's method [23]. The samples were dried at an ambient temperature (about 30 ºC by average) for 48 hours to diminish the samples' moisture effect.

2.2 Near-infrared spectroscopy experiment

Ten boluses of each sample were put in a glass vial of 22 mm, as a measurement cell. They were scanned by a Multi-purpose analyzer (MPA) FT-NIR spectrometer (Bruker, Bremen, Germany) with a nominal resolution of 8 cm^{-1}, accumulating 32 scans per spectrum using a gold background. The spectral range of the scans was between 12500-3600 cm^{-1}. Each sample was scanned twice. Therefore, there were 220 spectra in total. The NIR measurement was done at a room temperature of 25±1 °C.

2.3 Spectral pre-processing and NIR model development

The partial least squares (PLS) regression was used for the NIR spectroscopic model development for steroids adulteration in TTM using the OPUS, v.7.0.129 multivariate analysis software package. Spectral data with their corresponding steroid concentrations was independently divided into a calibration set and a prediction set. Note that these two datasets were separated by the samples not spectrum. The NIR spectra of calibration set used for model development were pre-treated in the following ways; no pre-treatment, constant offset elimination, straight-line subtraction, vector normalization (SNV), min-max normalization, multiplicative scatter correction (MSC), first derivatives, second derivatives, first derivatives + straight line subtraction, first derivatives + SNV and first derivatives + MSC. The PLS model that exhibited the lowest root mean squares error of cross validation (RMSECV) was chose for the optimal one, accompanied by latent variables (LVs), spectral pre-preprocessing methods, and wavenumber range. The prediction models were developed by correlating the NIR spectral data with the corresponded steroid values. The models were validated using the prediction data set. An optimal model, along with a spectral range, was chosen based on the coefficient of determination (R^2), root mean square error of prediction (RMSEP), and the residual prediction deviation (RPD).

The statistical method of principal component analysis (PCA) without spectral pretreatments was used to discriminate among the groups of TTM. This method is based on the principal component (PC) score of different PCs developed by spectral data.

3. Results and discussion

Presentation of the averaged raw spectra of TTM with different levels of steroids is shown in Figure 1. The peaks at 8327, 6860, 6310, 5680, 5155, and 4755 cm^{-1} (1200, 1457, 1585, 1760, 1940, and 2103 nm), the vibration bonds of glucose, sucrose, water, and cellulose, were prominent. Appearances of glucose, sucrose, water, and cellulose were due to the main constituents of TTM as presented in Table 1. Glucose and sucrose were the components in honey, which was the binding material, whereas cellulose was the constituent of herbs having in TTM. However, the spectral absorbance was not changed in order of the concentration of steroids. This was because of the baseline shift due to the scattering effect of the different samples.

Statistical summary of the steroid values adulterated in TTM (mg steroid/g TTM) for the calibration and prediction approaches is shown in Table 2. As the PLS model developed, the optimum one for predicting the steroids' adulteration in TTM was from using the NIR spectral data, ranging from of 8883 to7498.3 cm^{-1} and pre-treated by the SNV means. The appropriate number of latent variables (LVs) of the model was only three. As the results shown in Table 3 for the calibration and prediction of the steroids adulterated in TTM, it could be seen that the models had a good performance, i.e., high coefficient of determination (R^2) while the root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) were low. This means that employing only 3 LVs, obtained from maximizing covariance between the NIR spectral and the steroid levels adulterated in TTM, for the modeling approach could account the variation of the steroid levels in the calibration and prediction datasets for 99.09 and 98.20%, respectively. Udompetaikul et al. [14] have explained in more details regarding the latent variables (LVs) via development of the PLS model to determine the soluble solids content of sugarcane billets on an elevator conveyor. Figure 2 shows a plot of validating the PLS model through the prediction dataset for the quantitative analysis of the steroids adulterated in TTM, which is useful for illustrating the calibration precision. The optimum model exhibited the predictive performance based on the error term (RMSEP) with the value of 0.22 mg steroid/g TTM, and residual prediction deviation (RPD) of 7.46. Based on this result, NIR models having the RPD value of 6.5-8.0 were usable in any application and process control [24]. However, this interesting result obtained in this study was just the preliminary research stage as the TTM samples adulterated with steroids were produced under laboratory circumstance. So, conducting the experimental research based on the TTM or other herbal medicine samples, which are gain from collecting the commercially medicinal products, is necessitated to obtain the reliable result and apply the NIR technique in real use for the detection of steroid content adulterated in the medicinal products.

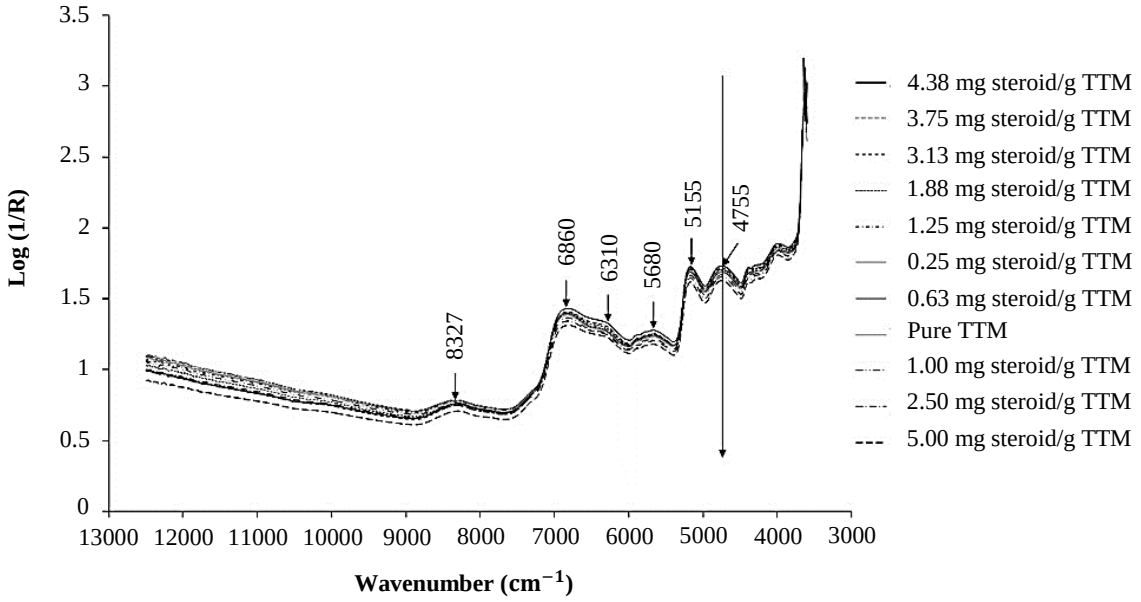
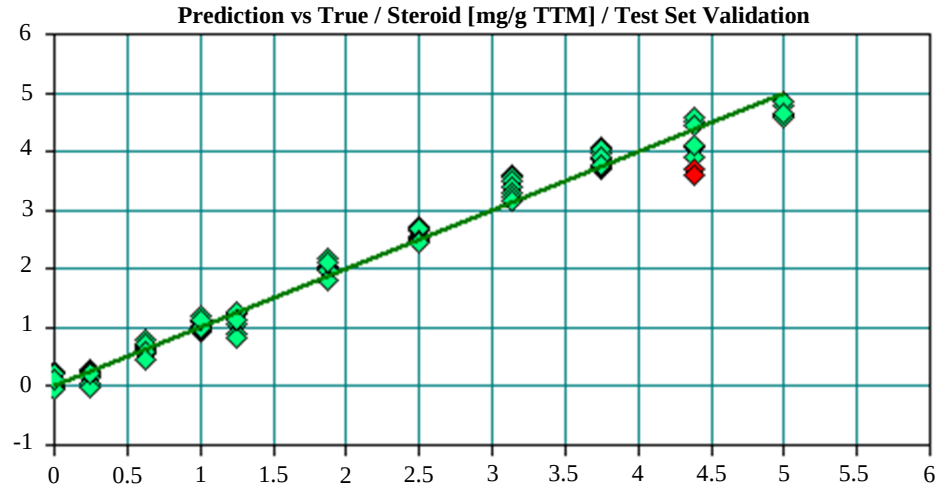


Figure 1 Raw spectra of traditional Thai medicine (TTM) with different levels of steroids

Figures 3 and 4 show the regression coefficient and X-loading weight plots of the optimal model. The high coefficients indicated that the bond vibration at the corresponding wavenumber had a large effect on predicting the dependent variable's value. There were many peaks illustrated in the figures that had the effect of bond vibrations on the prediction of steroid content in TTM. However, there were only four vibration bonds (Table 1) that were known [25] the peaks at 8694 cm^{-1} (1150 nm) and 8335 cm^{-1} (1199 nm), which might be the second overtone associated with C-H stretching of CH_3 (1152 nm and 1195 nm) and at 8254 cm^{-1} (1211 nm) and 7845 cm^{-1} (1274 nm) that might be the bond vibration of cellulose. According to a study of Broad et al. [19], whose work related to the application of NIR technique in determination of the hormone steroid content in single intact tablet, they reported that spectral characteristics of the steroid are corresponded to both the third overtone C-H stretching and second overtone O-H stretching vibrations. Based on these, the four vibration bonds mentioned above might be related to the vibration of steroid molecule adulterated in the TTM samples. Appearances of these peaks in both the regression coefficient and X-loading weight plots, especially in the regression, implied that the bond vibration of steroid had influence on the prediction of the developed model.



Rank: 3 R^2 = 98.2 RMSEP = 0.22 Bias: -0.00487 RPD: 7.46 Validation No 1+ method. Q2

Figure 2 The validation plot of adulterated steroid concentration with the predicted one in traditional Thai medicine samples (X axis presents the true TTM values and Y axis presents the predicted TTM values). Table 1 Bond vibration of some peaks at wavenumber and wavelength appeared on raw spectra of traditional Thai medicine, regression coefficient, and X-loading weight plot

Table 1 Bond vibration of some peaks at wavenumber and wavelength appeared on raw spectra of traditional Thai medicine, regression coefficient, and X-loading weight plot

Wavenumber (cm^{-1})	Wavelength (nm)	Wavelength (nm) [25]	Bond vibration	Structure	Source (Figure 1, 3 and 4)
8694,	1150	1152		CH_3	F3
8335, 8327	1199, 1200	1196	C-H str. second overtone	sucrose	RC, RS
		1198		glucose	RS
8254, 7845	1211, 1274	1207, 1278		cellulose	RC, F2
6860	1457	1450		H_2O	RS
6310	1585	1580	O-H str.first overtone	glucose	RS
		1584	O-H str.first overtone (intramol. H-bond)	cellulose	RS
		1586		sucrose	RS
		1589		glucose	RS
5680	1760	1762	O-H str.first+O-H def.	sucrose	RS
5155	1940	1940		H_2O	RS
4755	2103	2103	2xO-H def.+2xC-O str.	glucose	RS

F2 and F3 are PLS factor 2 and 3 in X-loading plot, respectively. RC is regression coefficient plot and RS is raw spectra of traditional Thai medicine.

Table 2 Steroid adulterated in traditional Thai medicine measured by the standard method of the calibration set and prediction set

No. samples	No. spectra	Max	Min	Mean	SD
Calibration set					
55	110	5	0.25	2.38	1.64
Prediction set					
55	110	5	0.25	2.38	1.64

Table 3 The results of the PLS regression model of adulterated steroid prediction

No. factors	Calibration		Prediction		
	R^2_{cal}	RMSEC	r^2_{pred}	RMSEP	RPD
3	99.09%	0.16%	98.20%	0.22%	7.46

Coefficient of determination for calibration model (R^2_{cal}), Coefficient of determination for prediction (r^2_{pred}), Root means square error of calibration (RMSEC), Root means square error of prediction (RMSEP) and Residual prediction deviation (RPD).

Besides the quantitative analysis, qualitative analysis by the PCA approach was performed in this study. Using the PCA, one of the projection methods [26], to group or classify the analytical data according to their similar elemental pattern is possible for our purpose to classify the groups of the TTM samples adulterated with different steroid levels. Phetpan et al. [27] applied the PCA concept for classifying the groups of sugarcane and non-sugarcane samples. As the mentioned concept, a score plot obtained after the PCA calculation was used for identifying the different samples' clusters and explaining their differences or similarities. Figure 5 shows the score plot for the first two principal components (PC) using steroid content as a variable. PC1 (horizontal axis) correlated to the steroid adulterated in TTM from the smallest to the largest (right to the left). Each group of the samples was separated.

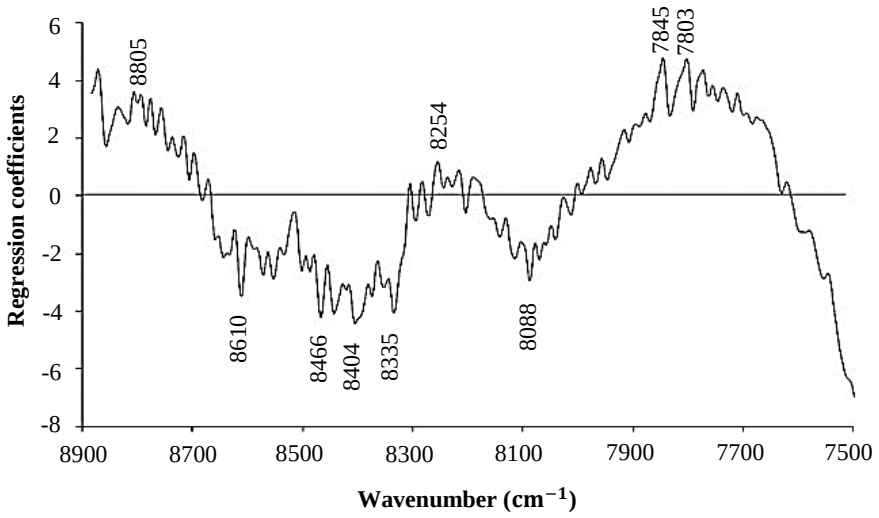


Figure 3 Regression coefficient plot of an optimum model for adulteration of steroids in traditional Thai medicine

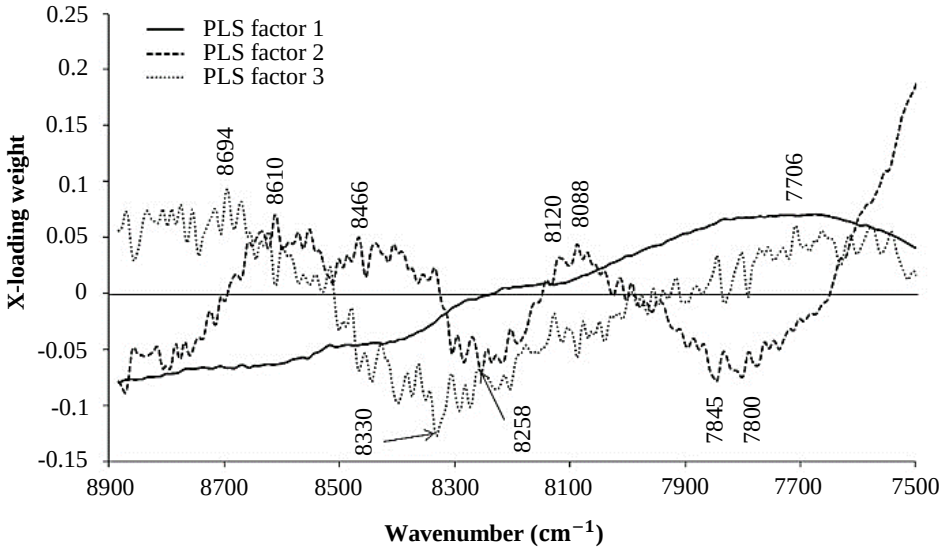


Figure 4 X-Loading weight plot of an optimum model for adulteration of steroids in traditional Thai medicine

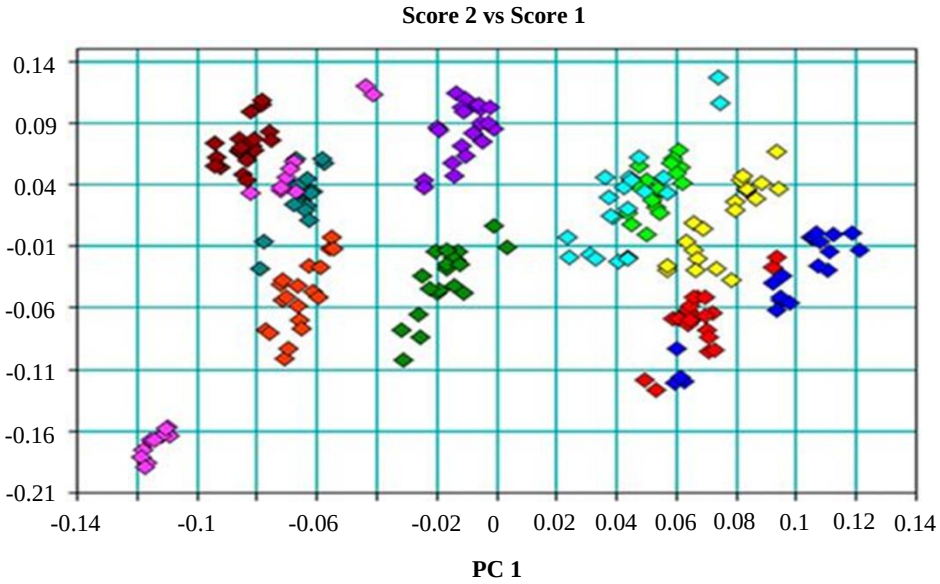


Figure 5 Score plot for the first two principal components. Blue, red, yellow, green, light blue, dark green, purple, orange, teal, pink and maroon indicates 0, 0.25, 0.63, 1, 1.25, 1.88, 2.5, 3.13, 3.75, 4.38, and 5 mg steroid/g tradition Thai medicine

4. Conclusions

This preliminary research provided the optimal NIR-based PLS model in detecting steroids adulteration in the TTM samples. The model employed the spectral range of 8883 to 7498.3 cm^{-1}, which was pre-treated by the SNV means, and employed 3 LVs for explaining the variance of steroid concentration in the sample. Prediction performance of the model, considered in terms of r^2, RMSEP, and RPD values were 98.20%, 0.22 mg steroid/g TTM, and 7.46, respectively.

As a result, the NIR spectroscopy can be used as a rapid and powerful technique to evaluate the steroid adulterated in TTM and to identify clusters of different groups of samples. The NIR-based protocol developed in the study was the first report on purpose, and it is useful for food and drug association, patients, pharmaceuticals, and medical sectors.

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