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Detection and quantification of active pharmaceutical ingredients as adulterants in *Garcinia cambogia* slimming preparations using NIR spectroscopy combined with chemometrics

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Abstract

A rapid, simple and efficient method with minimal sample treatment was developed for authentication of *Garcinia cambogia* fruit peel powder, along with determining undeclared active pharmaceutical ingredients (APIs) in its herbal slimming dietary supplements using near infrared spectroscopy combined with chemometrics. Five featured adulterants, including sibutramine, metformin, orlistat, ephedrine, and theophylline are selected as target compounds. The Near infrared spectral data matrix of authentic *Garcinia cambogia* fruit peel and specimens degraded by intentional contamination with the five selected APIs was subjected to hierarchical clustering analysis to investigate their bundling figure. SIMCA models were established to ensure the genuiness of *Garcinia cambogia* fruit peel which resulted in perfect classification of all tested specimens. Adulterated samples were utilized for construction of PLSR models based on different APIs contents at minute levels of fraud practices (LOQ < 0.2% w/w).The suggested approach can be applied to enhance and guarantee the safety and quality of *Garcinia* fruit peel powder as raw material and in dietary supplements.

Keywords: *Garcinia cambogia*; quality control; near infrared spectroscopy; chemometrics; Active pharmaceutical ingredients (APIs); herbal slimming.

1. Introduction

Obesity is among the major problems that might influence health and quality of life of many people. According to The World Health Organization investigations, obesity is boosting at a surprisingly elevated rate causing high incidence of health disorders including, type 2 diabetes, cardiovascular diseases, hypertension and cancer ([WHO], 2013; Coordinating Group of Nine Cities Study on the Physical Growth and Development of Children et al., 2008). The frustrations resulting from lack of success to lose weight, alongside the serious complications accompanying the use of pharmacologic weight loss drugs, opened windows for a statically growing market of alternative medicines, with herbal products topping the list. Herbal drugs are considered a core element of complementary and alternative medicine in clinical practice (Diamond, 2017). The popularity of incorporating natural products in slimming preparations, as a safe solution to obesity, is astonishingly rising for the reason of the belief that what is natural is always safe, even if not satisfyingly effective (Dastjerdi et al., 2018).

Garcinia cambogia L., family, Rutaceae, grown primarily in Southeast Asia, is one common example. *Garcinia cambogia* L. fruit peel extract is rapidly becoming a common component in many commercialized slimming preparations. The fruit is known to be rich in hydroxycitric acid, with a content up to 30% by weight in the dried and cured pericarp (T. et al., 2017). These rinds are applied in local culinary purposes and are assigned to make more filling meals (Raina et al., 2016). Having no brain accessibility, using hydroxycitric acid appears to be highly acceptable as it causes no CNS side effects. The mechanisms of action suggested by which hydroxycitric acid could reduce weight include suppression of de novo fatty acid synthesis, increase of lipid oxidation and reduction of food intake (Sripradha and Magadi, 2015). It is well agreed upon that rapid slimming results are not achievable from pure herbal slimming supplements. That is why active pharmaceutical ingredients are added to them (Deconinck et al., 2014). This ended up in illegal adulterations of synthetic anorexics by some manufacturers aiming at the magnification of the effectiveness of their slimming products which resulted into many serious health illnesses and eventually even death, as a consequence of the lack of awareness (Foroughi et al., 2017).

Some examples of synthetic drug classes which might be illegally incorporated in slimming herbal supplements include: e.g. stimulants and other anorexics, benzodiazepines, and antidepressants (Calahan et al., 2016). In fact, sibutramine and its analogues (stimulants), and orlistat (a lipase inhibitor) turned out to be the most frequently undeclared drugs to be detected in herbal slimming preparations, especially the ones exported from China (Deconinck et al., 2014). Furthermore, the hypoglycemic drug metformin was revealed to be another synthetic adulterant usually detected (Viana et al., 2013). Meanwhile, ephedrine is a natural antihistaminic drug, possesses various slimming enhancement properties. However, due to its structural similarity with adrenaline and its strongly reported sympathomimetic activity, its usage has been considered by the FDA since mid1990s, and finally its sale has been banned in the USA since 2004 (Lesiak et al., 2016). Theophylline is another representative of synthetic adulterants commonly incorporated in herbal slimming preparations with serious side effects (Al Lawati et al., 2017).

Not only do the adulterated products expose consumers' long term health to dangers, but they also encourage illegal acts. Thus, the development of a speedy screening, confirmation and quantitation method, with the purpose of identifying the synthetic weight-loss drugs in adulterated herbal slimming supplements, is inevitable to ensure human health and safety. Diverse studies applying various analytical techniques have been reported in the field of detection of synthetic anti-obesity drugs including TLC (Parodi et al., 1993), TLC-Micro IR (Csupor et al., 2013), HPLC (UPLC) (Bogusz et al., 2006), GC–MS (Haneef et al., 2013), NMR (Vaysse et al., 2010), immunochromatographic (Kanan et al., 2009) capillary zone electrophoresis (de Carvalho et al., 2010; Kanan et al., 2009) and infrared spectroscopic methods (da Silva et al., 2015; Feng et al., 2014).

From either a clinical or toxicological point of view, it seems to be extremely beneficial to develop a selective and specific analytical platform, which could detect synthetic adulterants in commonly used phytomedicines for weight loss such as *Garcinia cambogia* L. To reach that claimed goal, that developed platform should effectively distinguish and select the compound classes that might commonly show as synthetic adulterant in herbal slimming formulations.

NIR spectroscopy with chemometrics, proved to be an analytical tool of astonishing power in the world of pharmaceutical industry, and is in a non-stop growth as an striking complementary method for analysis of herbal drugs (Luo et al., 2015). When compared to other platforms of analysis, NIR spectroscopy reveals to have noticeable features especially its nondestructive nature of the analyzed sample and its capability of obtaining spectra for samples of different physical natures without or with only diminished pretreatment of such analyzed samples (Wang and Yu, 2015). There have been few previous reports for the application of NIR for the detection of synthetic adulterants in herbal slimming preparations (da Silva et al., 2015), yet these studies are limited to specific drugs such as sibutramine, or not displaying the ability of models in detection of low levels of adulteration by other possible APIs.

The present study aims at developing a rapid and simple method with minimal sample treatment for the authentication and quality control of *Garcinia cambogia* L. fruit peel powder, together with determining undeclared APIs in its herbal supplements using NIR spectroscopy with

multivariate data analyses. In this work, five featured adulterants, including sibutramine, metformin, orlistat, ephedrine, and theophylline are selected as target compounds.

Preprocessed NIR spectra data matrix of *Garcinia cambogia* L. fruit peel and samples adulterated by the five selected APIs was exposed to exploratory hierarchical cluster analysis (HCA) to investigate their clustering pattern. Soft independent modeling of class analogy (SIMCA) models were established afterwards for authentication of *Garcinia cambogia* L fruit peel. Lastly, adulterated samples were used for construction of partial least squares regression models (PLSR) in terms of their individual APIs contents.

There are no previous reports combining NIR fingerprints and chemometrics for authentication and quality control of *Garcinia cambogia* L. fruit peel in the literature as well as the inspection of possible APIs adulterants at low levels of adulteration not previously met.

2. Experimental

2.1.Materials and methods

2.1.1. Plant material

Fifteen samples (50 g each) of Garcinia cambogia fruit peel powder from different providers were supplied (Table S1). A 1 mm mesh sieve was used for sieving each sample.

2.2.Active pharmaceutical ingredients (APIs)

The reference materials sibutramine hydrochloride monohydrate, metformin hydrochloride, orlistat, ephedrine hydrochloride and theophylline anhydrous were purchased from Sigma-Aldrich (Germany) with a declared purity of 99.0%.

2.3. *Garcinia cambogia* fruit peel adulteration

Mixtures model were established by adding the individual active pharmaceutical ingredients (APIs); sibutramine, metformin, orlistat, ephedrine and theophylline to each of the individual fifteen *Garcinia cambogia* fruit peel powder samples at ratios of 0.5%, 1%, 2%, 5%, and 10% by weight for a sum of 375 samples added to the 15 unadulterated samples for a sum of 390 samples. The samples were randomly divided into calibration (300 samples) and test samples (90 samples). Pure APIs samples were also prepared (100%). The selection of the adulteration range was based on literature review of previous work on adulteration levels of slimming preparations (Vaysse et al., 2010; Xueyi et al., 2009). 1.5 g of each sample mixture was used for NIR analyses.

2.4. NIR Measurement

Amulti-purpose analyzer (MPA) FT-NIR spectrometer (Bruker Optics GmbH), with an InGaAs detector and an integrating sphere module by scanning over the wavenumber range of 8,000–4,000 cm^{-1} was used to obtain the NIR spectra. Spectra were collected using OPUS spectral acquisition software (ver. 6.5, Bruker Optics Inc.) at a resolution of 16 cm^{-1} per spectrum by averaging 64 scans. Samples were loaded in glass vessels of diameter 20 mm and height of 50 mm. 1 g of powder were used (covering the bottom of the vessel) for NIRS measurement.

Savitzky-Golay smoothing and first derivative to eliminate baseline drifts and enhancement of small spectral differences were applied for data preprocessing.

SIMCA-P+ 14.0 Software (Umetrics AB) was used for data pre-processing and multivariate statistical analyses.

2.5. Multivariate Statistical Analysis

2.5.1. Exploratory data Analyses

Dataset were subjected to unsupervised data analysis to explore the presence of potential patterns. The scores obtained through principle component analysis (PCA) were utilized to perform HCA (hierarchical clustering analysis) to generate a dendrogram using a similarity index that measured Euclidean distance.

2.5.2. Class-modelling of *Garcinia cambogia* samples using Soft Independent Modelling of Class Analogy (SIMCA)

SIMCA classification models were constructed for the different samples (*Garcinia cambogia* L. fruit peel, sibutramine, metformin, orlistat, theophylline and ephedrine). The models evaluation was achieved by investigating the R^2 as a measure of goodness of fit.

2.5.3. PLSR predictive modelling of adulterated *Garcinia cambogia* fruit peel in term of their APIs Content.

Prediction of the percentage of adulteration of *Garcinia cambogia* fruit peel by each of

sibutramine, metformin, orlistat, theophylline and ephedrine was achieved by construction of Partial Least Squares Regression (PLSR) models where the absorbance values at different wavelengths (data from NIR spectra) constituted the X-matrix while the content of each adulterant in the *Garcinia cambogia* fruit peel samples (0.5 % to 10 %) constituted the Y-matrix. Regression analysis was then performed. Before calibration curves construction, samples were divided into validation and calibration sets.

Root Mean Squared Error of calibration (RMSEC) and Root Mean-Squared Error of prediction (RMSEP) were calculated to test for the performances of the models (Rubingh et al., 2006). Fitting quality was measured using R^2 values to explain variance in the model, while future prediction was assessed using Q^2 values to explain predicted variance, values of 0.9 or higher are considered satisfactory (Lavine and Workman, 2013).

The Permutations Plots were used to assess the risk that the current PLSR model is spurious. The plot shows, for a selected Y-variable, on the vertical axis the values of R2 and Q2 for the original model (far to the right) and of the Y-permuted models further to the left. The horizontal axis shows the correlation between the permuted Y-vectors and the original Y-vector for the selected Y.

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using SEP (standard error of prediction) were LOD=3*SEP and LOQ=10* SEP.

3. Results and Discussion

3.1.NIR spectral analysis of Garcinia cambogia, hydroxycitric acid and APIs

NIR diffuse reflectance spectroscopy with chemometrics was exploited in this work for the authentication of *Garcinia cambogia* fruit peel powder, as well as detection and quantification of possible synthetic active pharmaceutical ingredients (APIs) as adulterants at low levels, in a short time of analysis. The NIR spectra of *Garcinia cambogia* fruit peel, sibutramine, metformin, orlistat, ephedrine and theophylline in the 8,000–4,000 cm^{-1} region are shown in Fig.1. In the sibutramine spectrum, the combination bands of C-H bonds were observed in the range of 4000–4700 per cm-1, while the first overtone bands were observed between 5670 and 5090 cm-1, Bands observed

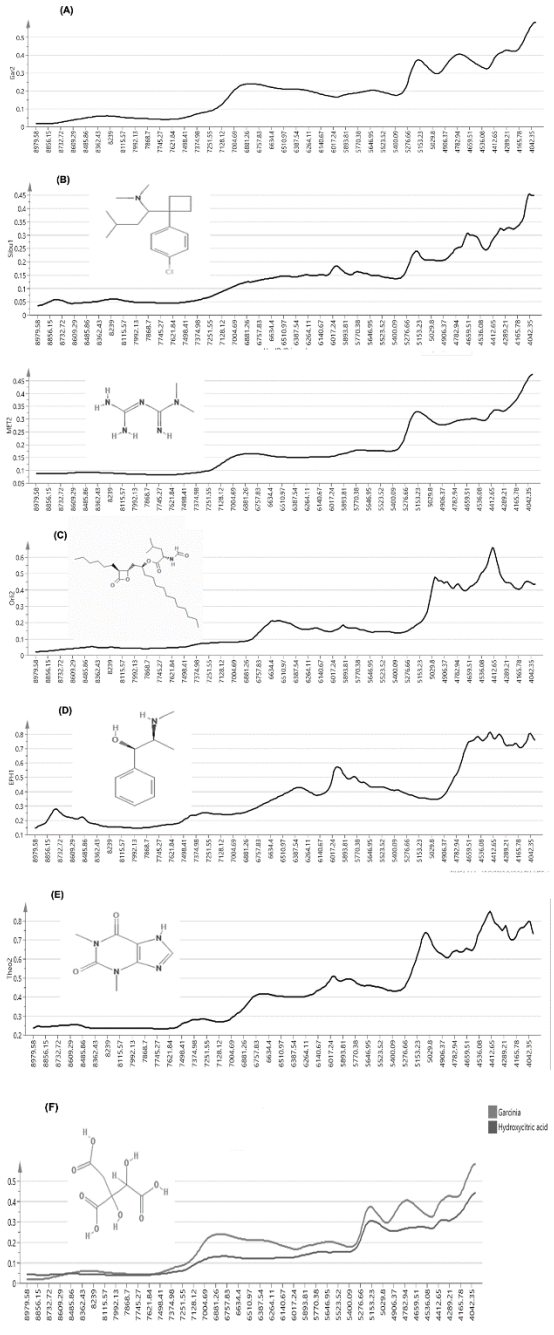

Fig. 1. Raw near infrared spectra (16 cm^{-1} resolution) acquired from 8000-4000 cm^{-1}, of representative samples of (A) *Garcinia cambogia* fruit peel powder, (B) sibutramine, (C) metformin, (D) orlistat, ephedrine, (E) theophylline, (F)Overlay of raw near infrared spectra (16 cm^{-1} resolution) acquired from 8000-4000 cm^{-1}, of *Garcinia cambogia* fruit peel powder and hydroxyl citric acid (all spectra offset on absorbance axis).

Table 1: Summary of five different SIMCA classification models using preprocessed NIR spectra in the range 8000 –4000 cm^{-1}.

Class #	Class name	Number of samples	R^2	Q^2	Number of components	PC1	PC2
1	Unadulterated *Garcinia cambogia*	15	0.999	0.999	2	0.67	0.18
2	Sibutramine-adulterated GCL	87	0.994	0.990	2	0.53	0.29
3	Metformin-adulterated GCL	87	0.991	0.991	2	0.74	0.13
4	Orlistat-adulterated GCL	87	0.997	0.991	2	0.51	0.24
5	Ephedrine-adulterated GCL	87	0.996	0.992	2	0.69	0.23
6	Theophylline-adulterated GCL	87				0.71	0.23
	Total number of samples	450					
	Correct classification	100 %					
	Fisher's probability	1.6e-021					
	Sensitivity	100 %					
	Specificity	100 %					

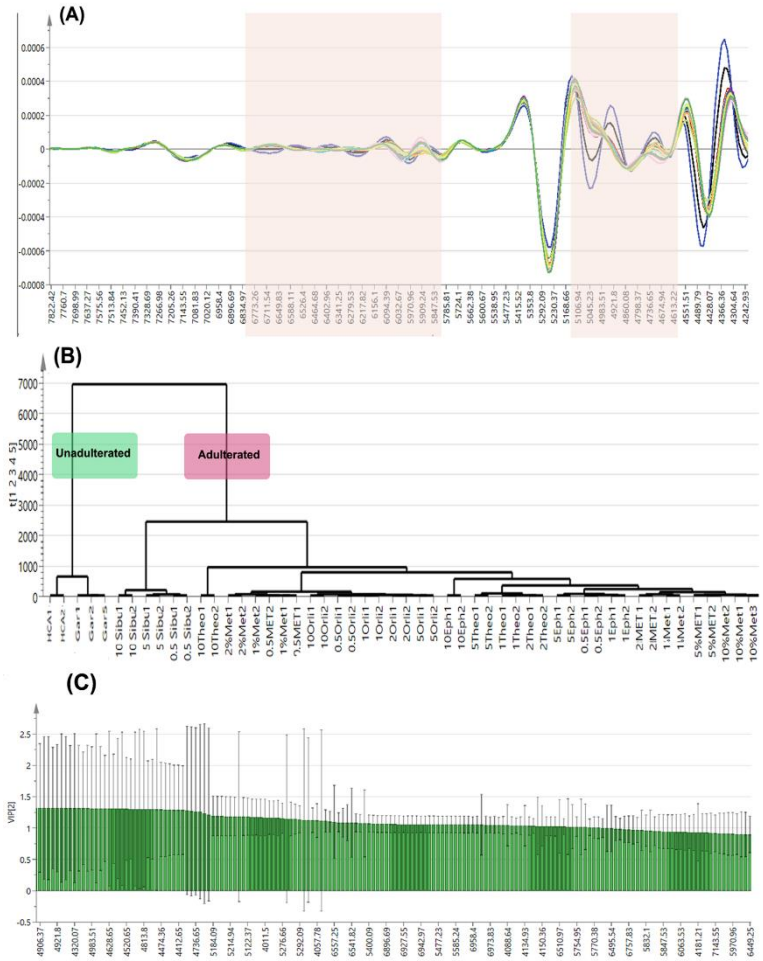

Fig. 2. (A) Overlay of first derivative spectra of pure *Garcinia cambogia* fruit peel powder and all adulterated samples. (B) Dendrogram of HCA (Ward's Algorithm) of representative unadulterated and adulterated test samples with different content of individual APIs.(C) Variables of importance to projection (VIP) plot of the HCA model.

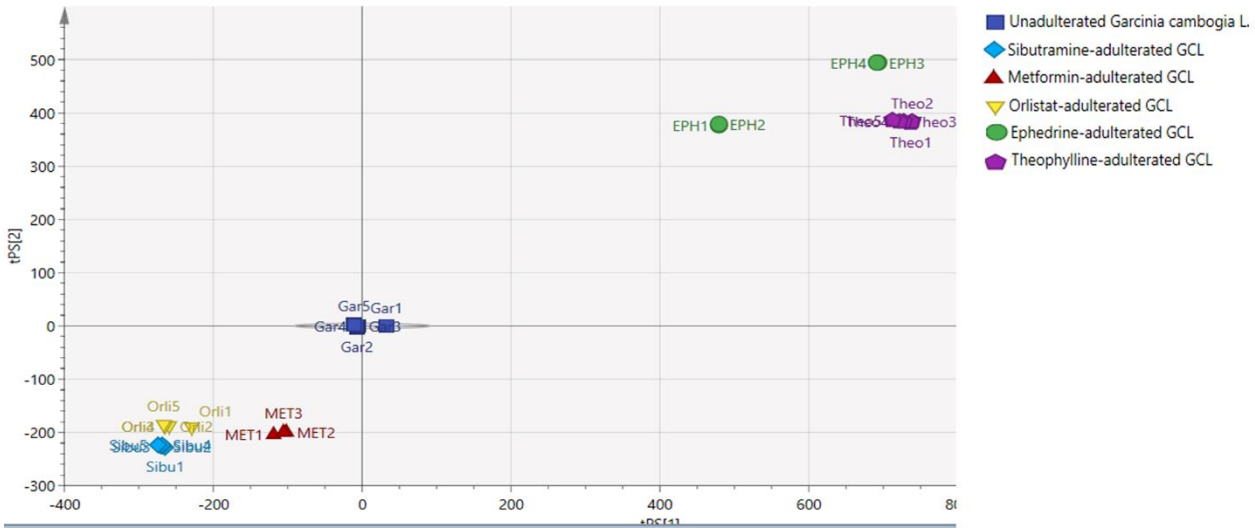

Fig. 3. PCA- SIMCA class model scatter plot of the different samples.

between 8200–8900 cm^{-1} were attributed to the second overtone bands. Meanwhile, in the spectra of metformin, the NH primary amine first overtone can be observed in the region 7,000-6,500 cm^{-1} (Westad et al., 2008) while the spectra of orlistat can be distinguished by the appearance of secondary amide stretching, first and second overtones observed in the regions 4800-4600 cm^{-1}, 6200-5700 cm^{-1} and 5100-5000 cm^{-1}, respectively. On the other hand, secondary amine first observed can clearly be observed in the NIR spectrum of ephedrine and theophylline in the range 6200-5700 cm^{-1} (Workman, 1996).

The overlaid NIR spectra of *Garcinia cambogia* fruit peel powder and its major constituent, hydroxycitric acid are presented in Fig. 1 which reveals a great resemblance between the two spectra. The main characteristic regions are observed at 5000-4500 cm^{-1} (C=O stretching second overtone of carboxylic group), 5300-5030 cm^{-1} (-CH aliphatic first overtone) and 7200-6600 cm^{-1} (-OH first overtone) (Westad et al., 2008).

3.2. Investigating the clustering pattern of unadulterated and adulterated Garcinia cambogia samples

Visual inspection and comparison of the spectra revealed some differences between the individual spectra of the samples considered in the study, especially in the regions 5,000-4,500 cm^{-1} and 6,500- 5,500 cm^{-1}. Consequently, implementing a suitable chemometric platform for the extraction of useful data in this spectral range, which in turn reflects the level of the adulteration is, with no doubt, of great value.

hierarchical cluster analysis (HCA) was first applied as an exploratory multivariate data analyses approach in order to study the grouping trends of the *Garcinia cambogia* fruit peel powder samples according to their level of adulteration by the different tested APIs,

Fig. 2A shows the derivative spectra of all adulterated samples at different levels of adulteration ranging from 0.5% to 10%, superimposed on that of *Garcinia cambogia* fruit peel powder derivative spectrum. The adulteration range was selected after consulting the literature for common adulteration ranges encountered in herbal medicines (Cheng et al., 2012; da Silva et al., 2015; Sarker, 2014). The best separation in HCA (Fig. 2B) was obtained using the regions highlighted in Fig. 2A (6800–5800 cm^{-1} and 5100–4600 cm^{-1}), for first derivative spectra obtained using the Savitzky-Golay smoothing. Meanwhile, variable importance for the projection (VIP) values were used to figure out the significant wavenumbers for the model, where the results were in quite agreement with above mentioned suggestions (Fig. 2C).

It can be deduced from the dendrogram that all *Garcinia cambogia* fruit peel unadulterated samples were clearly distinguished from those adulterated with different APIs. In the HCA dendrogram, two well-separated clusters were observed for each of adulterated and non-adulterated samples, where each of *Garcinia*

Table 2: Multivariate calibrations parameters for quantification models of adulterants; sibutramine, metformin, orlistat, ephedrine and theophylline in *Garcinia cambogia* powder expressed as % in 1 g.

(% in 1 g)	RMSEC	RMSECV	R^2	Q^2	Limit of detection	Limit of quantitation
Sibutramine model	0.030	0.037	0.995	0.992	0.11%	0.37%
Metformin model	0.031	0.050	0.992	0.96	0.15%	0.5%
Orlistat model	0.024	0.043	0.998	0.995	0.13%	0.43%
Ephedrine model	0.028	0.058	0.997	0.99	0.17%	0.58%
Theophylline model	0.034	0.071	0.995	0.98	0.21%	0.71%

cambogia fruit peel and hydroxyl citric acid formed a separate sub-cluster indicating that they hold chemical resemblance in comparison to other samples, yet they are readily distinguishable. This finding indicates that the spectra contain useful information in relative with the minute levels of adulteration of *Garcinia cambogia* fruit peel.

3.3. Class-Modeling for Authentication of Garcinia cambogia fruit peel

The class-modeling technique SIMCA (Frank and Lanteri, 1989; Tominaga, 1999) was then applied to model each class of the data set. For the selection of the appropriate number of principal components to which the data dimensionality can be reduced, a cross-validation procedure was implemented. The best SIMCA model (Fig. 3) was obtained using five principal components for class 1 (unadulterated *Garcinia cambogia* fruit peel or GCL powder), class 2 (sibutramine adulterated-GCL), and class 3 (metformin adulterated-GCL), four principal components for classes 4 (orlistat-adulterated GCL) and 6 (theophylline-adulterated GCL) and three principal components for class 5 (ephedrine).

The best number of components per model selection was aided by evaluation of the R^2 (for goodness of fit) and Q^2 (for the goodness of prediction) for each model to avoid the model overfitting.

The predictive ability of the equations was assessed by applying these equations to the test set (90 samples). Inspecting the results in Table 1 revealed that all classes were perfectly modelled since no misclassifications were detected either in the validation or prediction sets. A selectivity of 100 %, and a total specificity of 100 % were obtained at confidence level = 0.05.

3.4. PLSR Models for Prediction of the different APIs Content in adulterated Garcinia cambogia fruit peel samples

Predictive models for the ratio of APIs adulteration in *Garcinia cambogia* fruit peel samples as low as 0.5% were developed by applying PLSR for calibration. The best predictive models showed high coefficients of determination ranging between 0.992-0.998 for the calibration set and 0.969-0.995 for the prediction model. The RMSEC and RMSEP values were 0.024-0.034 and 0.037-0.1, respectively (Table 2). With such low error values, robustness of the regression model was deduced, indicating the reliability of the models when applied to samples outside the model. The permutation plots given in Fig. 4 strongly indicate that the original model is valid. The criteria for validity is that all blue Q2-values to the left are lower than the original points to the right.

External validation was attempted to check the performance of the PLSR calibration model where samples were randomly separated into calibration (300 samples) and test ones (90 samples). The designed models could successfully predict the % of each API in adulterated samples, with limit of detection and limit of quantification of 0.08% w/w and 0.26% w/w for sibutramine, 0.12% w/w and 0.36% w/w for metformin, 0.09% w/w and 0.28% w/w for orlistat, 0.07% w/w and 0.21% w/w for ephedrine and 0.09% w/w and 0.29% w/w for theophylline, respectively.

The determined LOQ are even lower than the typical concentrations of APIs in synthetic pharmaceutical products demonstrating the capability of constructed models to detect low levels of adulteration of *Garcinia cambogia* fruit peel powder samples by APIs that have not been described in literature before (Al Lawati et al., 2017; Calahan et al., 2016; Lesiak et al., 2016) The predicted values for the individual APIs concentration and the corresponding reference value are shown in Table 2.

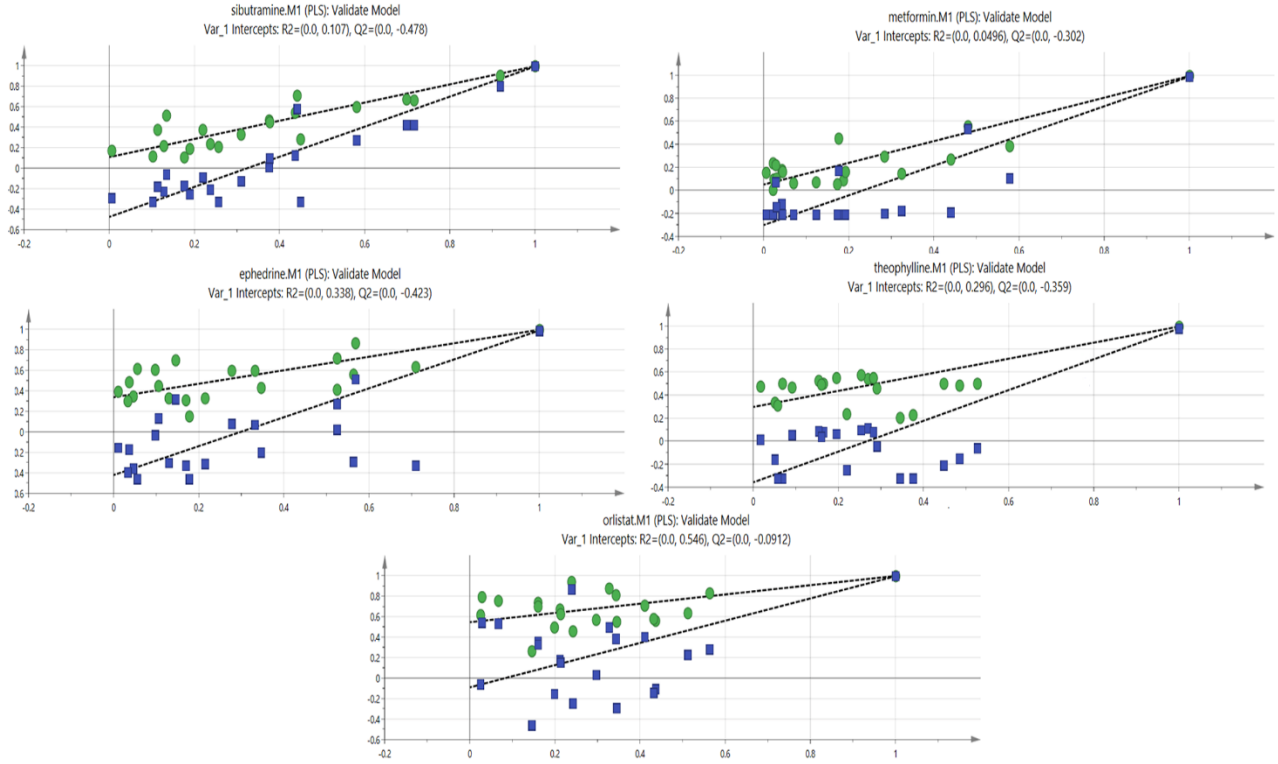

Fig. 4. Permutation plots of the models of adulterated samples by different APIs.

4. Conclusion:

A summary of the previously described results shows the practicability of the implementation of NIR diffuse reflectance spectroscopy aided with chemometrics for authentication of *Garcinia cambogia* fruit peel powder and efficient, rapid judgment of quality. Perfect discrimination of unadulterated and adulterated samples was achieved with respect to their APIs % content. The determined LOQ are even lower than the typical concentrations of APIs in synthetic pharmaceutical products. These results suggest that the developed technique is non-destructive and green since no solvents and reagents have been used during the study and no sample preparation steps are needed.

Declarations of interest: none

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