

## ORIGINAL ARTICLE

# UV-Visible Chemometrics Approach for the Determination of Selected Adulterants in Claimed Premixed Coffee

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## ABSTRACT

**Introduction:** Adulterated premixed coffees have turned into an issue in Malaysia lately and have caught the eye of the authorities due to death reports linked to these products. The major cause of this issue is reported that these premixed coffees have passed food inspection test and eventually released to the market for public consumption. These coffees were claimed to be spiked with several sexual enhancers like sildenafil, tadalafil, and vardenafil, which are common drugs used to treat erectile dysfunction. **Methods:** Chemometrics approach using UV-Vis spectroscopy was developed to detect the selected sexual enhancer drugs found in commercial coffees by employing SIMCA-P software for the multivariate statistical analysis. Seven brands of coffee samples were purchased from local stores, and 30 sachets each were tested, hence totalling to 210 samples. Each sample was named H, J, G, W, N, T, and K, respectively. **Results:** Three multivariate models were generated, namely principal component analysis (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA), and partial least squares discriminant analysis (PLS-DA). OPLS-DA was selected as the best model for the overall results as it displayed minimal discriminate. Sildenafil, tadalafil, and vardenafil were detected in sample H, while vardenafil in brand J, and none in samples G, W, N, T, and K. **Conclusion:** OPLS-DA analysis showed discrimination for the sexual enhancer drugs in two brands of premixed coffee. The UV-Vis spectroscopy-based chemometrics method proved to be reliable and efficient in determining the selected drugs, as well as in saving time and cost.

**Keywords:** Chemometrics, UV-Vis, Premixed coffee, Adulterants, SIMCA-P

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## INTRODUCTION

Coffee is known to be mildly acidic with a pH value of 5-6. Unlike tea, it has caffeine with a very positive and stimulating effect to humans as booster. Coffee itself can be used for other industries, such as insect repellent, fertiliser, and medicine. However, coffees at these present days are mixed with adulteration as enhancers. "Enhancement Coffee" or "Herbal Coffee" is widely consumed by South East Asians, especially Malaysians. These coffees are in high demand due to their ability in enhancing their libido, vitality, and overall health. The claimed coffees are also consumed as beauty products amongst women.

To avoid contaminated coffee from being priced similar to that of genuine coffee, the coffee quality is checked thoroughly before being released into the market and contaminated coffee is always rejected (1). Most

coffee dealers in Malaysia seem to abuse the property of coffee by spiking adulterants (libido drug) into the original coffee. There were reports on health issues when consuming such coffee. In most cases, these illegal coffees were spiked with certain drugs, such as sildenafil, vardenafil, and tadalafil. These drugs are used as sexual enhancers for humans as they contain active pharmaceutical ingredients found in Viagra (2-3).

The critical issue regarding these adulterated coffees has caught the attention of authorities with reports on the drug content in the spiked coffees. Early 2018, the authorities apprehended several companies selling contaminated coffee due to reported death cases in Malaysia (4). The Ministry of Health Malaysia has been alerted on this issue due to the loose inspection of the permit approval system. Therefore, this study looked into the adulterants (sildenafil, tadalafil, and vardenafil) in selected premixed claimed coffees bought from local markets via UV-Vis spectroscopy-based chemometrics approach.

## MATERIALS AND METHODS

Seven selected products of premixed coffee were

purchased from various brands and shops located throughout Malaysia. Absolute ethanol (VMR Chemicals, France) was used as the solvent to dissolve the coffee premix.

**Sample preparation**

All the premixed coffees were in powdered form; therefore, extraction process was not required. The premixed coffees were readily dissolved in ethanol and the suspended particles were filtered using filter paper, grade 1 (Whatman, Kent, UK). They were mixed in a centrifuge tube (2 mL) and vortexed for a minute. The concentration of sample was 3 mg/mL. 30 replicates of each premixed coffee were taken from each brand, hence totalling to 210 samples. Pure forms of sildenafil, vardenafil, and tadalafil were purchased (Sigma-Aldrich, New York, US) and prepared in ethanol at the concentration of 1 mg/mL.

**UV-Vis**

The experimental method involved a UV-Visible Spectroscopy (Perkin Elmer, Massachusetts, USA) for the scanning range of 0 nm - 600 nm with 1 nm resolution. The cells used were rectangular cuvettes measuring 2 mm (UVette ® 220-1600 nm disposable single sealed cuvettes, Eppendorf, Germany). Absolute ethanol was used as blank. All the samples and drugs were dissolved in ethanol and analysed.

**Multivariate data analysis (MVDA)**

All the spectra were baseline corrected and smoothed before conversion to ASCII format and then converted into Excel format. The processed data were then imported into SIMCA-P version 13.0 (Umetrics, Umee, Sweden) for the multivariate statistical analysis in a scaling parameter of unit variance (UV). PCA was adopted for the initial exploratory data analysis, whereby the score plot reflected separation among the samples. Orthogonal partial least squares discriminant analysis (OPLS-DA) and PLS-DA were later applied to observe discrimination of the samples in accordance with their contents or adulterants. The employed model was described by the criteria of R<sup>2</sup>, which indicated the goodness of fit, and Q<sup>2</sup>, the goodness of prediction. Variables that influenced the projection (VIP) values exceeding 1.0 were selected (5). The significance of each model established was validated by P value (P <0.05) of CV-ANOVA as supporting information.

**RESULTS**

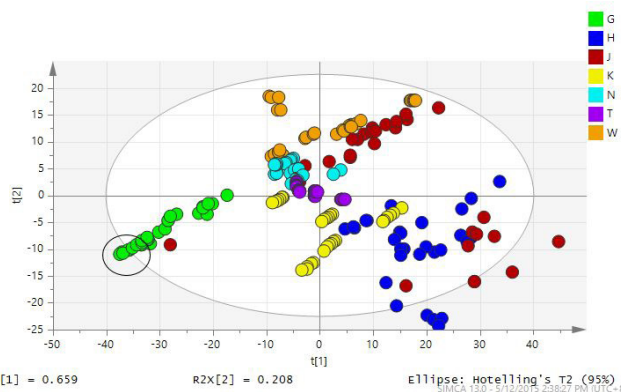
**PCA**

**Score scatter plot**

Score scatter plots are orthogonally independent to one another with t[1] and t[2] to indicate the concluded X variables. The scatter plot of t[1] vs t[2] shows a plane in the X variable space, showing t[1] score to be a significant variation in the X plane accompanied by t[2]. Outliers, similarities, and grouping indicate a map of the

observations, as shown in Fig 1. With a two-dimensional score plot, SIMCA (Umetrics, Umee, Sweden) draws the tolerance ellipse based on Hotelling's T<sup>2</sup>.

From the PCA model, it was observed that the J coffee component is scattered all over the plot and some appear to be outliers. In fact, the H component also has several outliers. Meanwhile, the G group exhibits a very tight and good clustering with minimal outliers.



**Figure 1:** Score scatter plot mapping of all 210 samples in the PCA model with outliers

**Biplot**

A two-variable scatter plot makes up a biplot in the simplest possible way, displaying the information needed of two different plots on a similar plane where it is presented as a vector, linear axes or a nonlinear trajectory (5). It interprets the data easily by not changing any vector, but instead, it forces the large magnitude element of each column to be positive that actually flips the vector.

The biplot displays the cluster of grouping with outliers. Inconvincible data interpretation is displayed for this model as the components cannot directly reflect each cluster of group efficiently as of those outliers from the group J. However, it clearly shows that the components in the PCA biplot linger near the cluster of group for H and a bit of J (Fig 2).

**OPLS-DA**

**Score scatter plot**

The OPLS-DA score scatter plot model illustrates a significant clustering group where the groups are close to each other distributed by their components and form a pattern of alignment within the group itself. The components do not stray further out from each clustering as in the PCA model. It is shown that the components are packed together at the middle within each other, which may be due to the presence of caffeine since all the components used are coffee samples, except for G that is still on the left side out of reach from the other clustering groups and this is explainable due to the fact that this coffee brand has the presence of collagen. This explains the shift of brand G to the left side away from the group, but still within the ellipse due to its caffeine base (Fig 3).

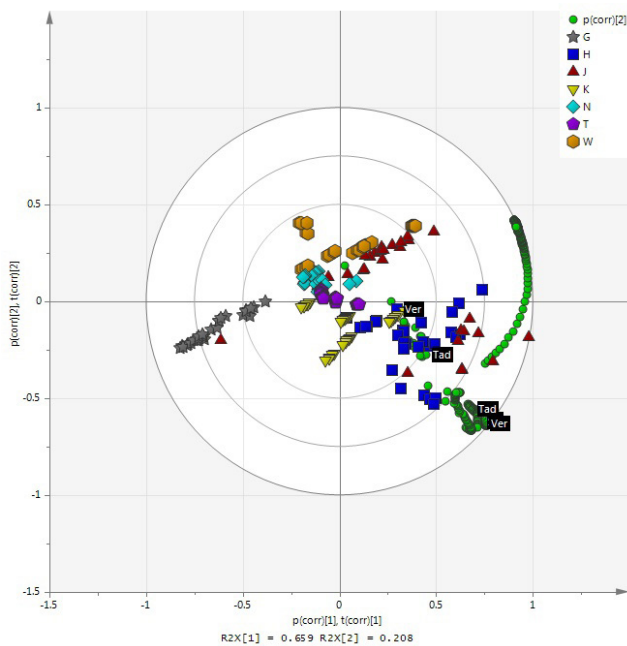


Figure 2: Biplot of PCA model, showing discriminant over the plot

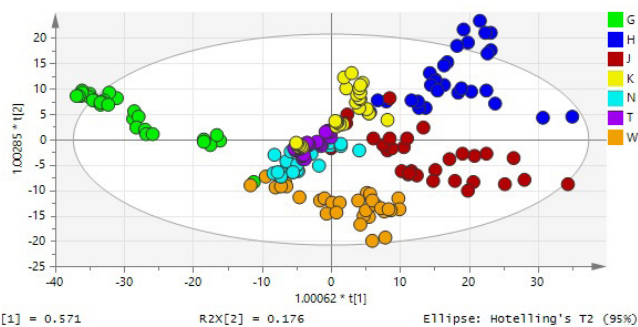


Figure 3: Score scatter plot for OPLS-DA with a better group clustering

### Loading column and scatter plot

The OPLS-DA loading plot displays the relationship between the X and Y variables for the predictive components. It shows that p corresponds to the X part of the model and the loading q to the Y part of the model. In order to facilitate interpretation, this plot is by default colour-coded according to the model terms. The p q plot is a superimposition of the p plot and the q plot, for the first and second predictive components of the OPLS-DA model (6). Loading p corresponds to the co-variances between the X variable and the predictive score vector t, whereas loading q corresponds to the co-variances between the Y variable and the predictive score vector u. X and Y variables with large p or q contribute strongly to the model. Hence, it is disclosed how the Y variable varies in relation to each other, as well as those that gave similar information and their relationship to the X variables in the model.

Fig 4 portrays the relation of the average plot of samples for each grouping onto the plane to the corresponding component used to detect the availability of the said component (sildenafil, tadalafil, and vardenafil). Each

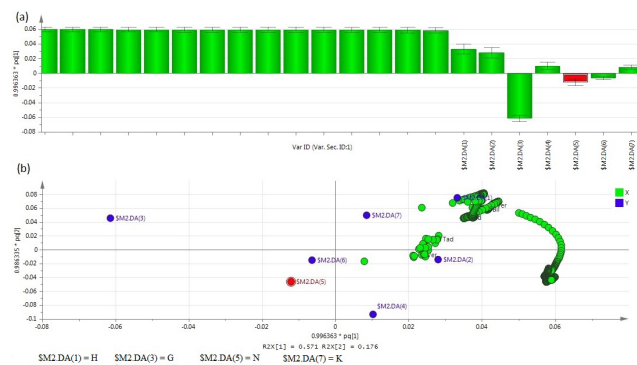


Figure 4: (a) Loading column plot (b) Loading scatter plot of OPLS-DA showing the red marked area from the column as a corresponding coordinate on the score loading plot as shown, which in this case is group N

\$M2.DA refers to each group: \$M2.DA(1) for group H, \$M2.DA(2) group J, \$M2.DA(3) group G, \$M2.DA(4) group W, \$M2.DA(5) group N, \$M2.DA(6) group T, and \$M2.DA(7) group K. Based on the OPLS-DA model, both H and J groups are situated near and around the components. This indicates the potential presence of the said components in the coffee samples and further interpretation is required for the complete mapping of both components and groups.

### Biplot

A very neat plotting was noted for the OPLS-DA model, where the significance of each component corresponding to the group is presented clearly in an orderly manner. The H group has all sildenafil, tadalafil, and vardenafil, whereas in J group, the dominating component is vardenafil. The G group was also expected to contain the components used, but surprisingly, it does not even have one and has its own clustering on the left side of the plot with nil outlier. Each \$M2.DA refers to each group: \$M2.DA(1) for group H, \$M2.DA(2) group J, \$M2.DA(3) group G, \$M2.DA(4) group W, \$M2.DA(5) group N, \$M2.DA(6) group T, and \$M2.DA(7) group K (Fig 5).

### PLS-DA

#### Score scatter plot

The PLS-DA model of scatter plot does not show good clustering, when compared to OPLS-DA. The grouping in the scatter plot is similar as those of PCA where the components of each group in the supposed clustering are scattered across the plane. It can be seen by the J group, which appears to be within the G and H clusters. H shows the most outliers in this model itself, whereas only one outlier in plane J. Unfortunately, this model does not show proper clustering according to group (Fig 6).

After further studies on the score scatter plot, the OPLS-DA was taken to be the best representative for this study as it clearly shows the clustering of each group in the plot and it can be distinguished easily with the corresponding

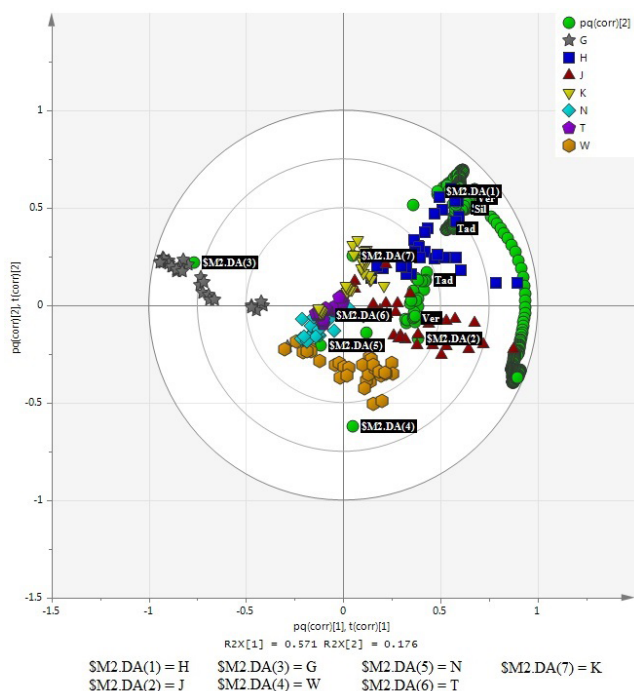


Figure 5: Biplot of OPLS-DA with low discriminant

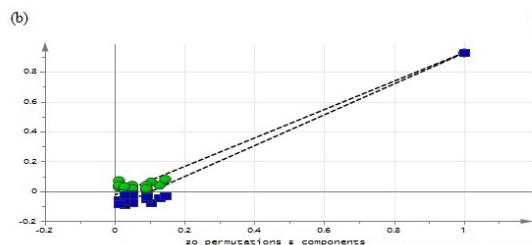
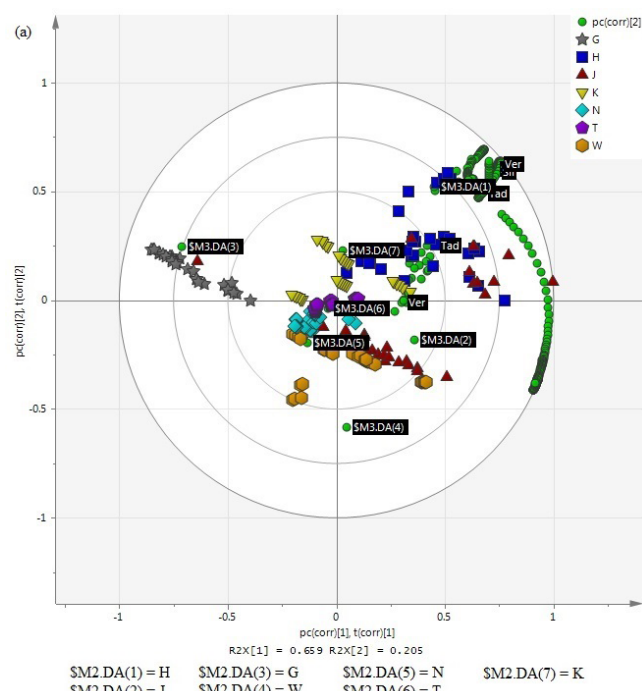


Figure 7: (a) PLS-DA biplot with high discriminant. Each \$M3.DA refers to each group: \$M3.DA(1) for group H, \$M3.DA(2) group J, \$M3.DA(3) group G, \$M3.DA(4) group W, \$M3.DA(5) group N, \$M3.DA(6) group T, and \$M3.DA(7) group K (b) Permutation plot of PLS-DA model, where the 2 points meet in a perfect manner and the rule of thumb is followed

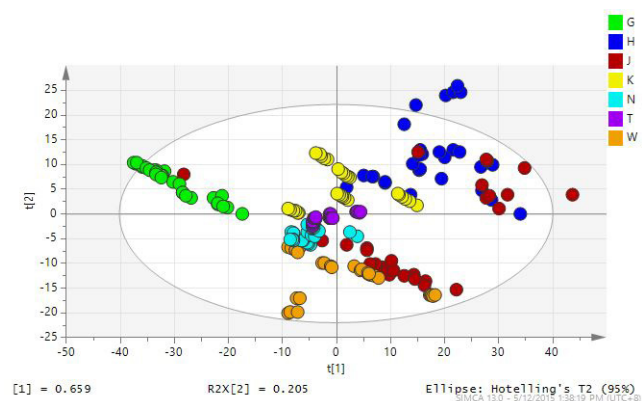


Figure 6: Scatter plot of PLS-DA with moderate to high outliers

component that exists between them. Even though they are outliers for the OPLS-DA model, they are still taken into account for this study as discussed earlier; the outliers maybe due to the presence of noise during the sample processing in the UV-Vis spectrometer. Nevertheless, repeating the sample process might alter the whole data and the grouping of each sample to a different style as it is calculated as whole (6-7).

**Biplot and permutation**

Since the outliers in the PLS-DA model are quite a number, it does not clearly state the components of the particular group precisely. Most of the outliers derived from the H and J groups, which were suspected to contain adulterants. When compared to the OPLS-DA biplot that displayed better plotting, the OPLS-DA biplot is preferred in this study and as a final result, the OPLS-DA model is considered as the precise distribution of group clustering with components (Fig 7a).

Permutation in MVDA determines overfit in PLS, OPLS,

and PLS-DA models. It means by the converging of the 2 lines, as shown in the figure below, to a point where they meet. This shows that the model is a perfect fit to one another and the data are a good model. The rule of thumb of permutation is that Y axis intercepts  $R^2 < 0.3$  and  $Q^2 < 0.05$ . Permutation test is usually useful for models with vast amounts of variables and a low amount of observation.  $Q^2$  value, as shown, ranged between 0 and -0.1, which shows that the  $Q^2$  is an overfit, similar to  $R^2$  with values less than 0.3. This test is closely related to the diagnostic of CV-ANOVA obtained for this model (CV-ANOVA is given in appendix).  $R^2Y$  and  $Q^2$  of the non-permuted Y model is actually compared by the test estimate of  $R^2Y$  and  $Q^2Y$  of the distribution when the permutation test randomly permuted the Y-vector (8) (Fig 7b).

**DISCUSSION**

As this study was progressing to the final stages during the usage of the MVDA, the OPLS-DA model was selected

as the best model to fit this study case as it shows a good clustering of groups within the plots as compared to the other two models (PCA and PLS-DA). This is because the components that correspond to the group are shown clearly in the OPLS-DA model. Brands H and J showed significant amounts of adulterants present in their coffee mixtures. H has all three types of adulterants; sildenafil, tadalafil, and vardenafil in its mixture, whereas J was only seen with the component vardenafil to be present in its mixture.

The G brand was suspected to contain adulterants because its ingredients are similar to those of brands H and J. Surprisingly, brand G showed no close relation to any of the said components tested to detect adulteration. The G brand had an extra ingredient of collagen present in the pre-mix of each sachet. This may be the determination factor of why the brand shifted to the left of the plot on all 3 models, rather than being in the heavy cluster in the middle.

The other brands, namely N, W, K, and T, were the normal safe brand of pre-mixed coffee used and consumed by many with no adulterant suspected. However, brands K and T were believed to have natural boosting herbs for the overall health booster, but the plots show K tends to shift near to the components of adulterants, whereas brand T is similar to brand N. Brand N is a normal pre-mixed coffee with no additional ingredient. The price of both coffee brands differs as brand T claims to contain special ingredients. Brand W, on the other hand, is specially brewed with a different blend, thus making it shift downwards, as seen in the plots. Brand W displayed the most outliers, but they did not stray far out of the cluster as compared to those of brands J and H. This shows that the adulteration of the suspected brands were not all true, but two out of three did contain adulteration. On the other hand, this method proved to be time-efficient and low in cost.

Many pre-mixed coffees in Malaysia are suspected to be spiked with adulterants and this has been a serious issue over the years. The locals prefer to consume these types of coffees, instead of costly supplements in boosting their overall health and libido. Detection of this mass outbreak could be challenging if the normal detection method is employed. A new unique approach, such as the chemometrics approach, seems to be useful and effective for detection within a short period of time and with less complication (9). Not only that, it is cost-effective and the accuracy is unquestionable. Many models can be used to present the findings so as to easily convince the public on the adulteration components spiked into pre-mixed coffees. This method can be used in opting out not only adulterated coffee, but also other sources of consumables suspected to be contaminated with such properties.

## CONCLUSION

Most of the pre-mix coffees are suspected to be spiked with adulterants in rural areas with increasing cases reported over the years. The locals prefer these coffees rather than costly supplements in boosting their health and libidos. Thus, UV-Vis based chemometrics approach has shown useful and efficient in detecting adulterants within short period. Many models can be used to present the findings to educate the public on the adulteration components spiked into the pre-mixed coffee. This method is potentially applied to other products for validation in future.

## ACKNOWLEDGMENT

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