Comparison and combination of spectroscopic techniques for the detection of counterfeit medicines

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Abstract

During this study, Fourier transform infrared spectroscopy (FT-IR), near infrared spectroscopy (NIR) and Raman spectroscopy were applied to 55 samples of counterfeit and imitations of Viagra® and 39 samples of counterfeit and imitations of Cialis®. The aim of the study was to investigate which of these techniques and associations of them were the best for discriminating genuine from counterfeit and imitation samples. Only the regions between 1800-400 cm^{-1} and 7000-4000 cm^{-1} were used for FT-IR and NIR spectroscopy respectively. Partial Least Square analysis has been used to allow the detection of counterfeit and imitation tablets. It is shown that for the Viagra® samples, the best results were provided by a combination of FT-IR and NIR spectroscopy. On the other hand, the best results for the Cialis® samples were provided by the combination of NIR and Raman spectroscopy (1400-1190 cm^{-1}). These techniques permitted a clear discrimination between genuine and counterfeit or imitation samples but also the distinction of clusters among illegal samples. This might be interesting for forensic investigations by authorities.

Keywords:

counterfeiting; phosphodiesterase type 5 inhibitors; IR-spectroscopy; partial least squares.

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Introduction

Counterfeit medicines are more and more present since the last decade. This is mostly due to the extension of the internet and the apparition of numerous fraudulent websites where anyone can easily and anonymously buy prescription only medicines [1,2]. In developed countries, the most popular counterfeit drugs are lifestyle medicines like the phosphodiesterase type 5 (PDE-5) inhibitor drugs: sildenafil citrate (Viagra®), tadalafil (Cialis®) and more recently vardenafil hydrochloride (Levitra®) [3].

The internationally recognized definition of a counterfeit medicine is the one of the World Health Organization (WHO) [4]:

> "A counterfeit medicine is one which is deliberately and fraudulently mislabelled with respect to identity and/or source. Counterfeiting can apply to both branded and generic products and counterfeit products may include products with the correct ingredients or with the wrong ingredients, without active ingredients, with insufficient active ingredient or with fake packaging."

However, the most encountered illegal drugs in Belgium do not exactly correspond to this definition because most of them do not copy the packaging and brand names of the genuine products. This is why the classification proposed by the Dutch National Institute for Public Health and the Environment (RIVM) [3] was applied. They make the distinction between counterfeits, which appearance is in conformity with genuine medicines and imitations which do not look like genuine (table1). In fact these imitations come in most cases from Asia (mainly India and China) where they do not recognize European and American patent laws. So they are legally manufactured in those countries but illegally imported in Europe.

Several techniques have been used for the analysis of erectile dysfunction drugs [5]. Among these, colorimetry [6,7], TLC [8], NMR (^{1}H, ^{13}C, ^{15}N) [9], NMR (^{1}H, ^{2}D DOSY ^{1}H) [10], HPLC-UV [11], LC-ESI-MS-MS [12], extracted ion LC–MS/TOF [13], LC-DAD-CD [14]. For the specific detection of counterfeit drugs, spectroscopic techniques are preferred because they are fast and need only a little sample preparation or no preparation at all. Raman spectroscopy has been used to detect counterfeit Viagra® by de Veij et al. [15], counterfeit Cialis® by Trefi et al. [10] Roggo et al. [16] used Raman spectroscopy for the identification of pharmaceutical tablets and Vajna et al. [17] used Raman spectroscopy for the identification of different manufacturing technologies. Vredenbregt et al. [8] used the near infrared (NIR)

spectroscopy to check the homogeneity of a batch of genuine Viagra® and to screen for the presence of sildenafil citrate. Storme-Paris et al. [18] and Chong et al. [19] also used the NIR spectroscopy for the detection of counterfeit drugs and the identification of antibiotics respectively. A comparison of NIR and Raman spectroscopy for the detection of counterfeit Lipitor® has been realised by de Peinder et al. [20]. It has been demonstrated that NIR-Chemical imaging was able to detect counterfeit medicines [21, 22, 23]. Finally, Maurin et al. [24] permitted the prediction of the presence of sildenafil citrate and/or particular excipients in counterfeit Viagra® by the mean of X-ray powder diffraction (XRD).

In this study, 55 counterfeit and imitations of Viagra®, 9 genuine Viagra®, 39 counterfeit and imitations of Cialis® and 4 genuine Cialis® were analysed by Raman-, NIR- and FT-IR-spectroscopy. It has been investigated which technique or combination of these techniques was the best to (1) detect counterfeit Viagra® and counterfeit Cialis® and (2) to make clusters in illegal medicines which can be useful for forensic investigations by authorities.

1. Experimental

1.1. Samples

The counterfeit and imitation tablets of Viagra® and Cialis® were donated by the Federal Agency for Medicines and Health Products in Belgium (AFMPS/FAGG). They all come from postal packs ordered by individuals via internet sites. All samples were delivered in blisters or closed jars with or without packaging. All samples, once received, were stored at ambient temperature and protected from light. The samples have been divided in groups according to their visual aspect. Table 2 and 3 shows the groups of Viagra®-like and Cialis®-like samples respectively.

Pfizer SA/NV (Belgium) kindly provided one batch of each different dosage of Viagra® (25mg, 50mg, 100mg). Two other batches of each dosage were purchased in a local pharmacy in Belgium.

Eli Lilly SA/NV (Benelux) kindly provided one batch of commercial packaging of Cialis® (10mg and 20mg). Two other batches of Cialis® 20mg were purchased in a local pharmacy in Belgium.

All references were delivered in closed blisters with packaging and were stored protected from light at ambient temperature.

1.2. Instrumental

1.2.1. Raman spectroscopy

A RamanRxn1 spectrometer (Kaiser Optical Systems, Ann Arbor, MI, USA), equipped with an air-cooled charge coupled device (CCD) detector (back-illuminated deep depletion design) was used in combination with a fiber-optic non-contact probe to collect Raman spectra from the core of the tablets. The laser wavelength during the experiments was the 785 nm line from a 785 nm Invictus NIR diode laser. All spectra were recorded in the range of 0-3500cm^{-1} with a resolution of 4 cm^{-1} using a laser power of 400 mW. Data collection, data transfer, and data analysis were automated using the HoloGRAMS^{TM} (Kaiser Optical Systems, USA, version 2.3.5) data collection software, the HoloREACT^{TM} (Kaiser Optical Systems, USA, version 2.3.5) reaction analysis and profiling software, the Matlab software (The Matworks, Natick, MA, USA, version 7.7), and the Grams/AI-PLSplusIQ software (Thermo Fisher Scientific, Waltham, MA, USA, version 7.02). Ten second exposures were used for spectral acquisition. Spectra were collected at 3 locations per tablet. Spectra were preprocessed by baseline correction (Pearson's method, [25]), mean centered and averaged before data-analysis.

1.2.2. NIR spectroscopy

Diffuse reflectance NIR spectra were collected per tablet using a Fourier-Transform NIR spectrometer (Thermo Fisher Scientific, Nicolet Antaris II near-IR analyzer) equipped with an InGaAS detector, a quartz halogen lamp and an integrating sphere, which was used for NIR spectra collection from the tablets. Data analysis was done using Thermo Fisher Scientific's Result software, SIMCA-P (Umetrics AB, Kinnelon, NJ, USA, version 11) and Matlab (The Matworks, Natick, MA, USA, version 7.7). Each spectrum was collected in the 10000 – 4000 cm^{-1} region with a resolution of 16 cm^{-1} and averaged over 16 scans. All spectra were preprocessed using standard normal variate transformation (SNV) and mean centered before data-analysis. Each spectrum was performed on the core of the tablet.

1.2.3. FT-IR spectroscopy

A Spectrum 1000 (Perkin Elmer, Waltham, MA, USA) FT-IR spectrometer with a DTGS detector was used. All spectra were recorded from the accumulation of 16 scans in 4000-400 cm^{-1} range with a 4 cm^{-1} resolution. Samples were prepared by compressing a 0.3% mixture

of pulverised tablet with spectral grade KBr (Merck, Germany). Three spectra of each sample were obtained, normalized and averaged.

Once recorded, the spectra were normalized with the Spectrum software (Perkin Elmer, Waltham, MA, USA, version 5.0.1.).

1.3. Chemometrics

1.3.1. PCA

PCA is a variable reduction technique, which reduces the number of variables by making linear combinations of the original variables. These combinations are called the principal components and are defined in such way that they explain the highest (remaining) variability in the data and are by definition orthogonal.

The importance of the original variables in the definition of a principal component is represented by its loading and the projections of the objects on to the principal components are called the scores of the objects. [26] In this investigation, it was decided to conduct the research only on the three first PC's, since in all cases more than 95% of the variation in the data was explained by them.

1.3.2. PLS

PLS is based on exactly the same principles as PCA. The difference is situated in the definition of the latent variables, here called PLS-factors. The PLS-factors, also linear combinations of the original explanatory variables in the data set, are defined in such a way that they maximize the covariance with the response variable. In this way latent variables are obtained that are more directly related to the response variable than, for example, those obtained in PCA. In this study, a discrete response variable was chosen (0 for illegal samples and 1 for genuine samples). This is justified since the genuinity of the reference samples is certified.

The scores of the objects on the different PLS-factors were used in this study as tool to distinguish clusters of the different samples. [26]

1.3.3. Data processing

The data pre-processing was performed using HoloREACT^{TM} software. For NIR and FT-IR spectroscopy, the three spectra of a sample were normalized and averaged. For Raman spectroscopy, the three spectra of a sample were baseline corrected using the Pearson's method. All calculations were done with Matlab (The Matworks, Natick, MA, version 7.9.0).

The Principal component analysis (PCA) of the data has been performed with algorithms based on Kernel PCA [27]. The Partial Least Squares (PLS) analysis of the data has been performed with the algorithms described by de Jong [28]. The algorithms are part of the ChemoAC toolbox (Freeware, ChemoAC Consortium, Brussels, Belgium, version 4.0). For each method, the dataset consist of a matrix with a number of columns equal to the number of wavelengths measured and a number of rows equal to the number of samples studied. The combination of the techniques has been performed by addition of the matrixes obtained for each technique. The combination matrix was then autoscaled in order to eliminate the influence of the differences of scaling.

2. Results and Discussion

2.1. Measurements

All IR measurements were performed in triplicate on the pulverised tablet. All NIR measurements were performed once on the core of three different tablets of each sample and all Raman measurements were performed on three different locations of the core of one tablet of each sample. Only the fingerprint region of the IR spectra (1800-400 cm^{-1}) and the 7000-4000 cm^{-1} region of the NIR spectra were used because of their high variability and their richness of information. The Raman spectra were taken with an exposure time of ten seconds on the core of the tablets at three different locations per tablet.

2.2. Case one: Viagra®

2.2.1. PCA

First, a PCA analysis was performed. No separation or not enough separation was seen between genuine and counterfeit or imitation samples. FT-IR provided the best results for the PCA analysis. It was decided to perform a PLS analysis. This choice was based on the supervised character of this chemometric method.

2.2.2. PLS

2.2.2.1. FT-IR spectroscopy

Figure 1 shows the three dimensional PLS plot of the analysis of the FT-IR spectra of the Viagra®-like samples. As can be seen, a good distinction between genuine and counterfeit or imitations is obtained. Previous inspection of the loading plots confirms that almost the complete fingerprint region is needed for classification. Most of the separation is probably

due to the differences in chemical composition of the tablets: combinations of different API, differences in excipients used, presence of contaminants or both of them.

The counterfeit samples are quite close to each other (cluster 1) except two of them. Cluster one contains also 4 imitations; this indicates that their chemical composition is presumably similar to the one of the counterfeits. Among the different clusters identified, cluster 3 is very far from the other ones (along the axes PLS 2 and PLS 3 on figure 2). No reason has been identified for that huge separation. But it can be observed that cluster 3 is always separated from other samples by each technique or combination of techniques except for Raman spectroscopy and the combination of Raman and FT-IR spectroscopy. Clusters 4 and 5 can easily be confirmed by visual examination. Each cluster contains tablets originating from the same manufacturer. Cluster 2 contains two samples visually different but having the same packaging (same brand name and dosage: Nizagara 25mg). The fact that these two samples are close to each other indicates that they probably have the same chemical composition. They may be manufactured in two different laboratories but with the same raw material.

Other samples are widespread and no relationship between these samples is seen. After inspection of the loading plots, no specific wavenumbers corresponding to an excipient or the sildenafil were correlated to the separation.

2.2.2.2. NIR spectroscopy

Figure 2 shows the three dimensional PLS plot of the analysis of the NIR spectra of the Viagra®-like samples. As with FT-IR, a good separation between genuine and imitations or counterfeit is obtained. Some clusters are observed. No obvious reason has been found as an explanation of these clusters except for clusters 4 and 5. NIR cluster 4 contains the same samples as the FT-IR cluster 5 and these samples are from the same manufacturer. NIR cluster 5 contains chewing tablets of three different manufacturers. Their classification in the same cluster is probably the consequence of a same manufacturing process. This is in line with the principle of NIR spectroscopy which is dependant of the physical properties of the sample such as particle size, density and morphology [29]. After visual inspection of the NIR spectra, it appears that the major variability is present between 5500-5000 cm^{-1}.

After plotting the loadings in function of the wavenumbers (Fig 3a), it has been found that the separation in groups by PLS factor 1 was due to microcrystalline cellulose (4732 cm^{-1}), sodium laurylsulfate (5130 cm^{-1}) and sildenafil citrate (5250 cm^{-1}) (fig 3b). The separation by PLS factor 2 was due to cellulose derivatives (5180 cm^{-1}) (fig 4 and 3b). The whole spectrum

was taken into account for the separation by PLS factor 3. It can therefore be postulated that there are differences in the chemical composition of the tablets.

2.2.2.3. Raman spectroscopy

Raman spectroscopy was able to distinguish genuine from illegal medicines (fig 5). Illegal samples were widespread and no cluster has been identified except two samples that are apart from other ones. One of these two samples may be separated from other ones because it contains both sildenafil and tadalafil.

No satisfying explanation has been found for the separation of the other sample and the loading plots don't give more information.

The Raman spectra also have been evaluated in smaller spectral regions, but the conclusions were similar.

2.2.2.4. Combination of techniques.

The association of Raman and FT-IR spectroscopy and the association of Raman and NIR spectroscopy allow the distinction of 5 and 6 clusters respectively (results not shown). No reason has been found for this distinction. Neither the visual aspect nor the loading plots permits an explanation.

Fig 6 shows the three dimensional PLS plot of the analysis of the combination of the NIR and FT-IR spectra of the Viagra® samples. A good separation between genuine and counterfeit or imitation samples can be observed. The illegal samples are divided in 4 clusters. These clusters are the most relevant for a forensic investigation because the classification realised by the combination of the two techniques shows clusters that are the most different according to both physical and chemical properties. These differences should be relatively little among samples from the same manufacturer. Cluster 3 contains the same samples as cluster 3 in Fig 1 and 2. Cluster 4 contains some samples from group 2 and 4 (see table 2). Samples from group 2 are all manufactured by Axon Drugs Pvt Ltd. No reason has been found for the presence of samples from group 4 because no manufacturer name was present. They may be manufactured by Axon Drugs but this can not be confirmed. No satisfying reason has been found to explain the cluster 2. Once again, no specific wavenumbers corresponding to an excipient of the genuine tablets or the sildenafil were correlated to the separation according to the loading plots.

Once Raman spectroscopy data are introduced in the analysis, clusters are no more coherent with the visual aspect of the tablets. As it cannot be ruled about the relevance of the

information provided by the Raman spectroscopy, the combination of NIR and FT-IR spectroscopy will be preferred.

2.3. Case two: Cialis®

2.3.1. PCA

As for Viagra®, the PCA analysis of Raman spectroscopy didn't allow to distinguish genuine from imitations or counterfeit samples. So this technique was abandoned to the advantage of NIR and FT-IR.

PCA analysis of the Cialis® FT-IR dataset (fig 7) permitted to clearly separate the counterfeit samples (cluster 2 in figures 7 and 8) from the imitations and from the genuine samples. Cluster 3 contains the remaining samples. However, a group of imitations is not separated from genuine samples (cluster 1 in figures 7 and 8). The same observation is done with the NIR dataset (Fig 8). The imitations (group 5 in table 3) have different brand names but are visually similar and can be easily identified as being only one group. The qualitative and quantitative analysis by HPLC and dissolution of some of them indicates that they are of good quality. This might result of a chemical composition and a manufacturing process very close to the original Cialis®. So, the PLS analysis was needed to distinguish these imitations from the originals.

The samples of clusters 4 are coherent with visual inspection. Cluster 3 and 5 in figure 8 cannot formally be explained. Cluster 2 contains the counterfeit samples.

2.3.2. PLS

2.3.2.1. FT-IR spectroscopy

The PLS analysis permitted a very good separation of the imitations and the counterfeits from the genuine samples. FT-IR also permitted to see 5 clusters of samples (fig 9). Cluster 1 shows the imitations samples that weren't separated from the genuine samples by PCA. It contains imitations that are visually similar: oval shape with E20 embossed; without distinction between conventional tablets and chewable tablets (see table 3). It can therefore be postulated that only few chemical differences are present (flavouring agents such as menthol). After qualitative and quantitative HPLC analysis and according to the classification of the RIVM [3], they can be called "professional imitations".

Cluster 2 contains the counterfeit samples. It is very clearly separated from other samples. A HPLC analysis showed that they contain both sildenafil and tadalafil. So they can be called "mixed counterfeit" according to the RIVM classification [3]. This combination of API may

explain this clear separation. Cluster 3 contains samples from the same manufacturer (according to the packaging) that are visually similar: round, orange and without film-coat. In this case, the chewable tablets were separated from other samples (arrow 3).

Cluster 4 contains samples that are neither counterfeit nor imitation samples. Their packaging is totally different from genuine samples but their shape, film-coat colour and engraving C20 are similar. However, they really seem manufactured by amateurs so we classed them among imitations. Once again, the chewable version of these tablets is not comprised in the cluster (arrow 4).

Cluster 5 contains the other samples except three of them that are widespread in the plot. They are not similar between them but quite close to each other. They probably have the same chemical composition and the same manufacturer.

The examination of the loading plots did not permit to identify which component was correlated with this separation. After visual inspection of the FT-IR spectra of each cluster, it can be observed that almost the whole spectrum is different between each sample except between cluster one and genuine samples which is in agreement with the fact that they weren't separated by PCA.

2.3.2.2. NIR spectroscopy

NIR spectroscopy shows a clear separation between genuine samples and counterfeit or imitation ones (fig 10). Clusters 1 and 2 are very clearly separated from other samples but the clustering of the illegal samples is not clear (clusters 3, 4). These 2 clusters are very close to each other. Once again, the counterfeit samples (cluster 2) are clearly separated from other samples. The visual inspection of the NIR spectra of each cluster shows no clear difference between the different clusters.

2.3.2.3. Raman spectroscopy

Raman spectroscopy permits the distinction between genuine and illegal samples. This distinction was greater when the region between 1400-1190 cm^{-1} was studied. So this region has been selected for the rest of the analysis with Raman spectroscopy on Cialis®-like samples. Figure 11a shows the separation of the illegal samples in 3 clusters. Cluster 1 contains the imitations from the group 5 (see table 3) and one sample from the group 7. This sample wasn't classified in any cluster by both NIR and FT-IR spectroscopy. Cluster 3 contains two samples from the same group but no reason has been found for their separation from the other ones. The loading plots didn't permit to identify which component was

correlated with these clusters. As shown on figure 11b, the Raman spectra of the three clusters are different in particular in the studied region (grayed area).

2.3.2.4. Combination of techniques.

The NIR and the FT-IR data were associated to obtain figure 12. This plot shows a separated cluster of the genuine samples and four other clusters. Cluster 1 contains the professional imitations identified by both FT-IR and NIR (group 5 of table 3) and it contains also a sample that was not included in a cluster by each technique separately except by the Raman spectroscopy. Cluster 2 contains the counterfeit samples and cluster 3 contains the remainder samples. Only one sample stand alone (cluster 4), this sample was included in cluster 5 by both techniques separately (fig 9 and 10). It couldn't be explained why the association of the techniques isolated it.

Figure 13 shows the combination of the NIR and the Raman spectra. Three clusters are observed. These clusters are the same as for the association of NIR and FT-IR spectroscopy except that the sample from group 7 that was in cluster one is now separated in cluster 5. The sample of cluster 4 in fig 12 is still isolated.

Figure 14 shows the association of the FT-IR and the Raman spectra. This time, illegal samples are divided into 4 clusters. Clusters 2 and 3 are coherent with visual inspection of the tablet. Cluster 4 contains all the samples from group 3 except a tablet from group 4. This sample was already classified close to group 3 by FT-IR spectroscopy (see fig 9). Cluster 1 contains the remainder samples.

The association of the three techniques permitted exactly the same separation as the association of NIR and Raman spectroscopy.

3. Conclusion

The aim of this study was to establish which technique or combination of techniques was the most powerful to distinguish counterfeits from genuine samples of Viagra® and Cialis®. The spectroscopic techniques investigated comprised FT-IR, NIR and Raman spectroscopy.

FT-IR is a widely spread and relatively cheap technique, used for decades and present in each analytical laboratory. The main drawback of this technique is its destructive character.

NIR and Raman techniques are more and more used in the pharmaceutical industry because of their easiness of use, their rapidity and the fact that it is non-destructive. So, any further analysis can still be done on the tablets analysed by NIR or Raman spectroscopy which is very important for an official analytical laboratory.

The ability of NIR and Raman spectroscopy separately to make the distinction between genuine and counterfeit or imitation samples has already been demonstrated [8,10,15]. PCA analysis of the data was insufficient to achieve complete separation of the samples. Hence, PLS analysis was preferred because it is a powerful tool for a discrimination study with reference samples.

For the Viagra® samples, after investigation, the conclusion is that the association of NIR and FT-IR spectroscopy provides the best results (see table 4a). Indeed, the many clusters observed with NIR and FT-IR alone were reduced to 4 clusters showing the most variability between the samples. This variability is correlated to both physical and chemical information. This is very useful for a forensic investigation because it takes into account both chemical composition and the manufacturing process. This information is useful for characterizing a manufacturer.

For the Cialis®-like samples, each technique separately permitted a classification of the samples and the distinction between genuine and illegal samples (see table 4b). But this classification was insufficient for the Raman spectroscopy or incomplete for the FT-IR and the NIR spectroscopy. It is concluded that the association of NIR spectroscopy (region between 7000-4000 cm^{-1}) and Raman spectroscopy (region between 1400-1190 cm^{-1}) was the most useful association of techniques. This association permitted a very good separation between genuine and counterfeit or imitation samples. The classification performed allows the distinction between very bad counterfeits, very good imitations and other samples from genuine samples. This classification is also very easy even by visual inspection of a non-trained operator. This is useful for its application in control laboratory. The association of FT-IR and Raman spectroscopy has not been considered as optimal because some groups were not separated and there were samples misclassified. For these reasons, this association seems to be less useful than the association of NIR and Raman spectroscopy.

This study was performed on a limited number of samples. If those techniques are applied to a routine forensic laboratory working on counterfeits drugs, it is clear that the constitution of a library of genuine and illegal samples would allow a sharper classification of samples.

The use of spectroscopic tools allows an objective distinction between legal and illegal tablets based on chemical and physical information of the tablets. This distinction sometimes confirms the visual classification of the samples but most of the time it completes this classification. As it has been demonstrated, it is frequent that many visually similar samples are finally classified in the same cluster which indicates that they have similar physico-

chemical properties. That kind of objective classification is the most useful for any further investigation.

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497 Table 1: Classification of illegal medicines according to the RIVM [3]

498

Main category	Subcategory	Inclusion and exclusion criteria
Counterfeit	Professional	Appearance in conformity with genuine medicine; Content of correct API within 90 - 110 % of declared value; No other APIs; not genuine medicine.
	Non-professional	Appearance in conformity with genuine medicine; Content of correct API outside 90 - 110 % of declared value; No other APIs.
	Mixed	Appearance in conformity with genuine medicine; Contains correct API and another, known API
	Fraudulent	Appearance in conformity with genuine medicine; Contains a different, known API.
	Analog	Appearance in conformity with genuine medicine, Contains other, unapproved API
	Placebo	Appearance in conformity with genuine medicine; Does not contain APIs.
Imitation	Professional	Appearance not in conformity with genuine medicine; Content of correct API within 90 - 110 % of declared value; No other APIs.
	Non-professional	Appearance not in conformity with genuine medicine; Content of declared API outside 90 - 110 % of declared value; No other APIs.
	Mixed	Appearance not in conformity with genuine medicine; Contains declared API and another API.
	Fraudulent	Appearance not in conformity with genuine medicine; Contains an undeclared API.
	Analog	Appearance not in conformity with genuine medicine; Contains other, unapproved API
	Placebo	Appearance not in conformity with genuine medicine; Does not contain APIs.

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501 Table 2 : Viagra®-like samples. (For RIVM classes see table 1)
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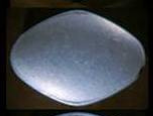
Group number	Sample photo	Symbol in plots	RIVM Class	Number of Samples
1		•	Non-professional counterfeits	6
2		X	Professional imitation	8
3		□	Professional imitations and one non-professional imitation	4
4		*	Professional imitation	23
5		Δ	Professional imitation	4
6		☆	Professional imitation and one mixed imitation	5
Other		◊	Non-professional imitation and one professional imitation	5
genuine		○		9

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505 Table 3: Cialis®-like samples. (For RIVM classes see table 1)
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Group number	Sample photo	Symbol in plots	RIVM Class	Number of samples
1		•	Mixed counterfeits	5
2		X	Professional imitation	4
3		□	Professional imitation	5
4		*	Non-professional imitation	3
5		Δ	Professional imitation	13
6		☆	Non-professional imitation and two mixed imitations	4
7		✡	Non-professional imitation	2
other		◊	Professional imitation and mixed imitations	3
genuine		○		4

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Table 4: (a) Summary table of the results obtained with the different techniques and associations analysed by PLS for the Viagra®-like samples. The best method is in bold font.

Viagra®-like samples	Genuine discrimination	Counterfeit-Imitations discriminations	Clusters number	Explained clusters	Unclassified samples
FT-IR (1800-400cm^{-1})	Yes	No	5	4	33
NIR (7000-4000 cm^{-1})	Yes	No	5	2	31
Raman (1800-200cm^{-1})	Yes	No	-	-	55
FT-IR+NIR	**Yes**	**No**	**4**	**2**	**0**
FT-IR+Raman	Yes	No	5	1	2
NIR+Raman	Yes	No	6	1	2
FT-IR+NIR+Raman	Yes	No	6	1	2

(b) Summary table of the results obtained with the different techniques and associations analysed by PLS for the Cialis®-like samples. The best method is in bold font.

Cialis®-like samples	Genuine discrimination	Counterfeit-Imitations discriminations	Clusters number	Explained clusters	Unclassified samples
FT-IR (1800-400cm^{-1})	Yes	Yes	5	4	3
NIR (7000-4000cm^{-1})	Yes	Yes	5	4	3
Raman (1400-1190cm^{-1})	Yes	Yes	3	2	1
FT-IR+NIR	Yes	Yes	3	2	1
FT-IR+Raman	Yes	Yes	4	3	0
NIR+Raman	**Yes**	**Yes**	**3**	**2**	**2**
FT-IR+NIR+Raman	Yes	Yes	3	2	2

Fig 1: 3D PLS score plot of the FT-IR spectra (region between 1800-400cm^{-1}) of the Viagra®-like samples. For symbol caption see table 2.

Fig 2: 3D PLS score plot of the NIR spectra (region between 7000-4000cm^{-1}) of the Viagra®-like samples. For symbol caption see table 2.

Fig 3: (a) Loadings scores of the PLS 1 of the NIR spectra of the Viagra®-like samples. Three major peaks are observed at 4732, 5130 and 5250 cm^{-1}.

(b) NIR spectra of the excipients linked to the peaks identified in (a).

Fig 4: Loadings scores of the PLS 2 of the NIR spectra of the Viagra®-like samples. The major peak observed is at 5180 cm^{-1}. This wavenumber can be linked to the cellulose derivates excipients.

Fig 5: 3D PLS score plot of the Raman spectra (region between 1800-200cm^{-1}) of the Viagra®-like samples. For symbol caption see table 2.

Fig 6: 3D PLS score plot of the association of the FT-IR spectra (region between 1800-400 cm^{-1}) and the NIR spectra (region between 7000-4000 cm^{-1}) of the Viagra®-like samples. For symbol caption see table 2.

Fig 7: 3D PCA score plot of the FT-IR spectra (region between 1800-400cm^{-1}) of the Cialis®-like samples. For symbol caption see table 3.

Fig 8: 3D PCA score plot of the NIR spectra (region between 7000-4000cm^{-1}) of the Cialis®-like samples. The results show no separation of genuine samples. For symbol caption see table 3.

Fig 9: 3D PLS score plot of the FT-IR spectra (region between 1800-400cm^{-1}) of the Cialis®-like samples. For symbol caption see table 3.

Fig 10: 3D PLS score plot of the NIR spectra (region between 7000-4000cm^{-1}) of the Cialis®-like samples. For symbol caption see table 3.

Fig 11: (a) 3D PLS score plot of the Raman spectra (region between 1400-1190cm^{-1}) of the Cialis®-like samples. For symbol caption see table 3.

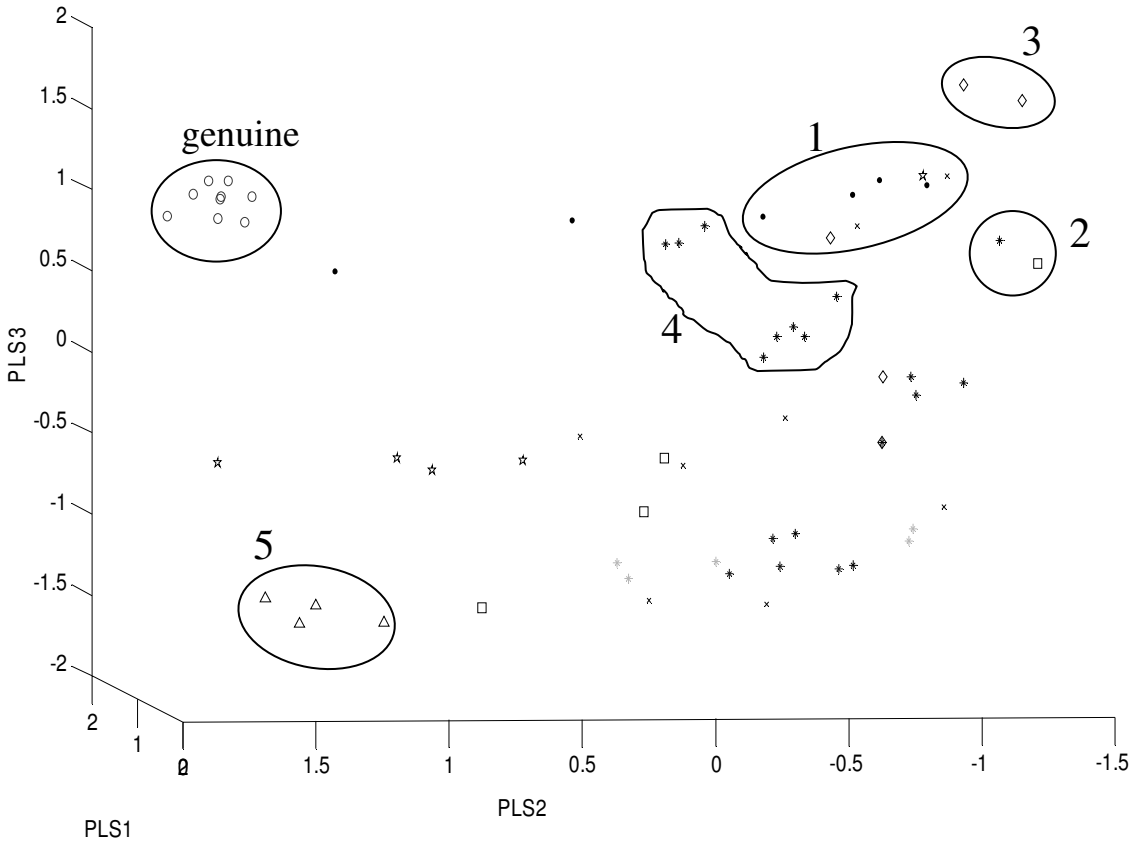
(b) Mean Raman spectrum of each cluster. The region between 1400-1190cm^{-1} is grayed.

Fig 12: 3D PLS score plot of the association of the FT-IR spectra (region between 1800-400 cm^{-1}) and the NIR spectra (region between 7000-4000 cm^{-1}) of the Cialis®-like samples. For symbol caption see table 3.

Fig 13: 3D PLS score plot of the association of the Raman spectra (region between 1400-1190 cm^{-1}) and the NIR spectra (region between 7000-4000 cm^{-1}) of the Cialis®-like samples. For symbol caption see table 3.

Fig 14: 3D PLS score plot of the association of the FT-IR spectra (region between 1800-400 cm^{-1}) and the Raman spectra (region between 1400-1190 cm^{-1}) of the Cialis®-like samples. For symbol caption see table 3.

Fig 1



621 Fig 2:

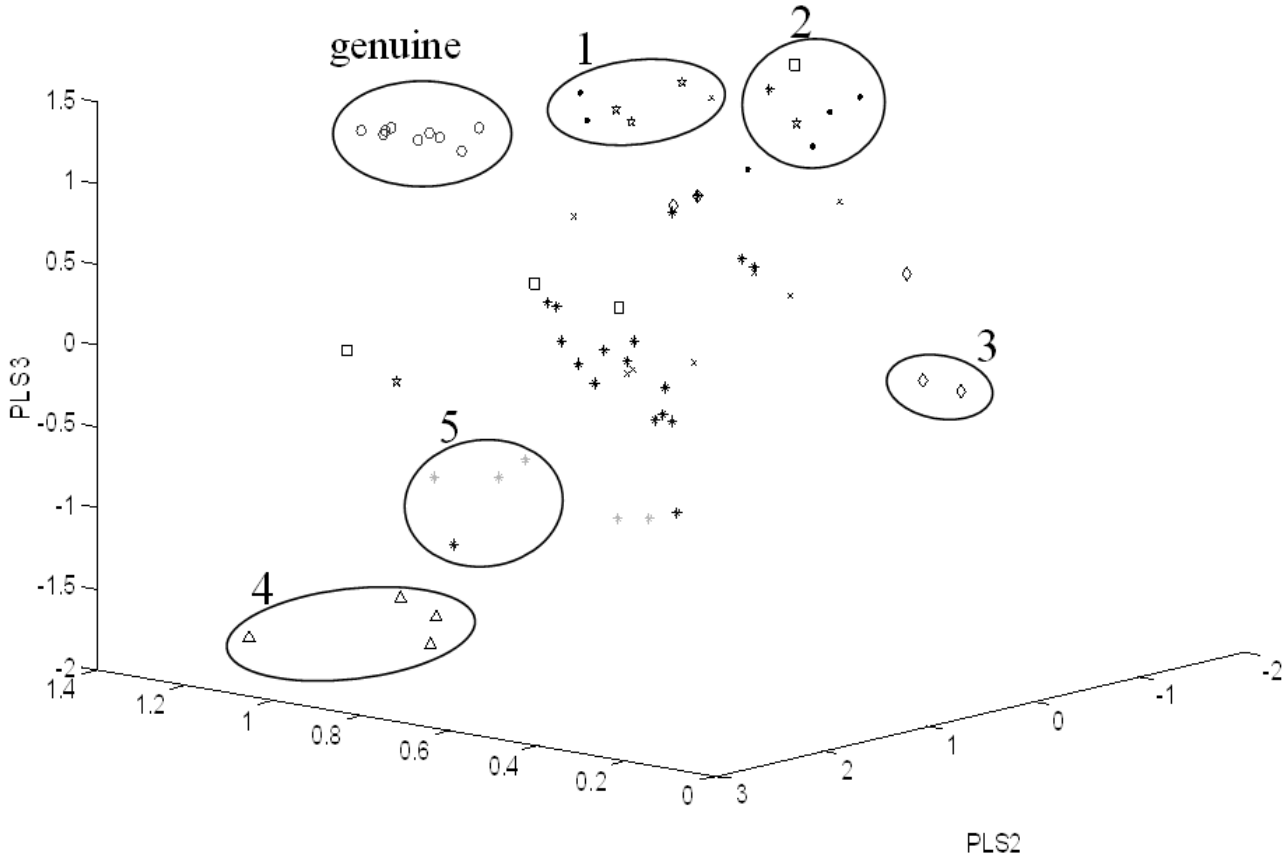


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Fig 3a:

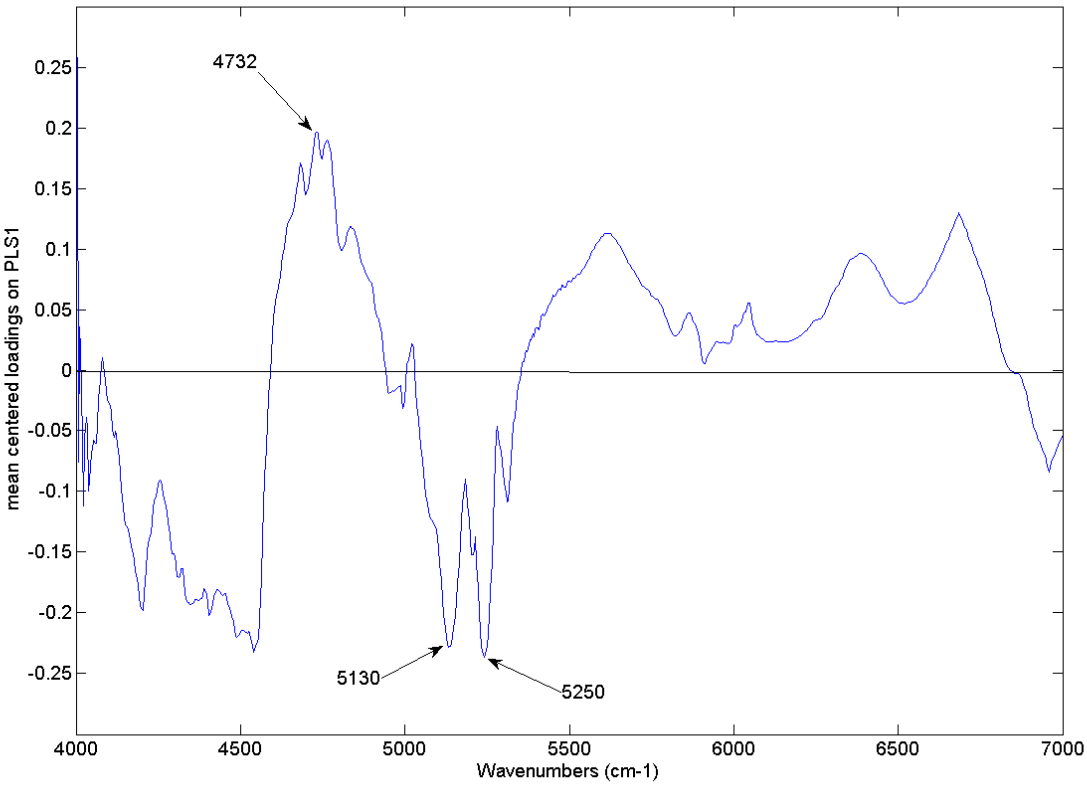
4732
5130
5250
mean centered loadings on PLS1
Wavenumbers (cm-1)
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
4000
4500
5000
5500
6000
6500
7000

Fig 3b:

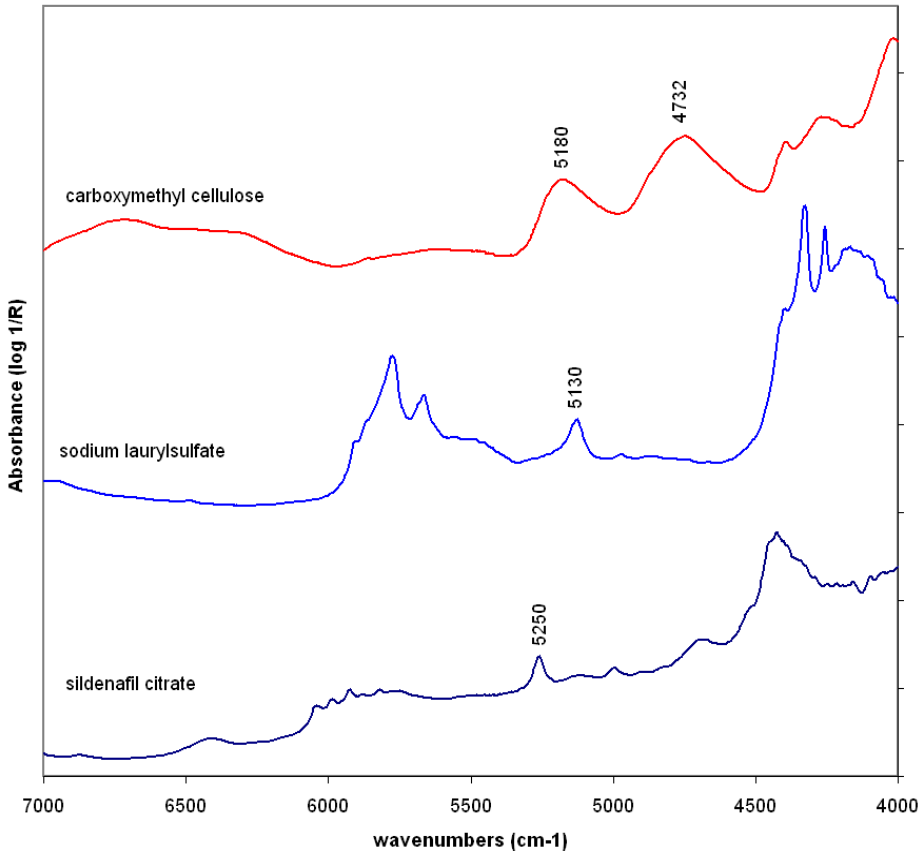
carboxymethyl cellulose
sodium laurylsulfate
sildenafil citrate
5180
4732
5130
5250
Absorbance (log 1/R)
wavenumbers (cm-1)
7000
6500
6000
5500
5000
4500
4000

Fig 4:

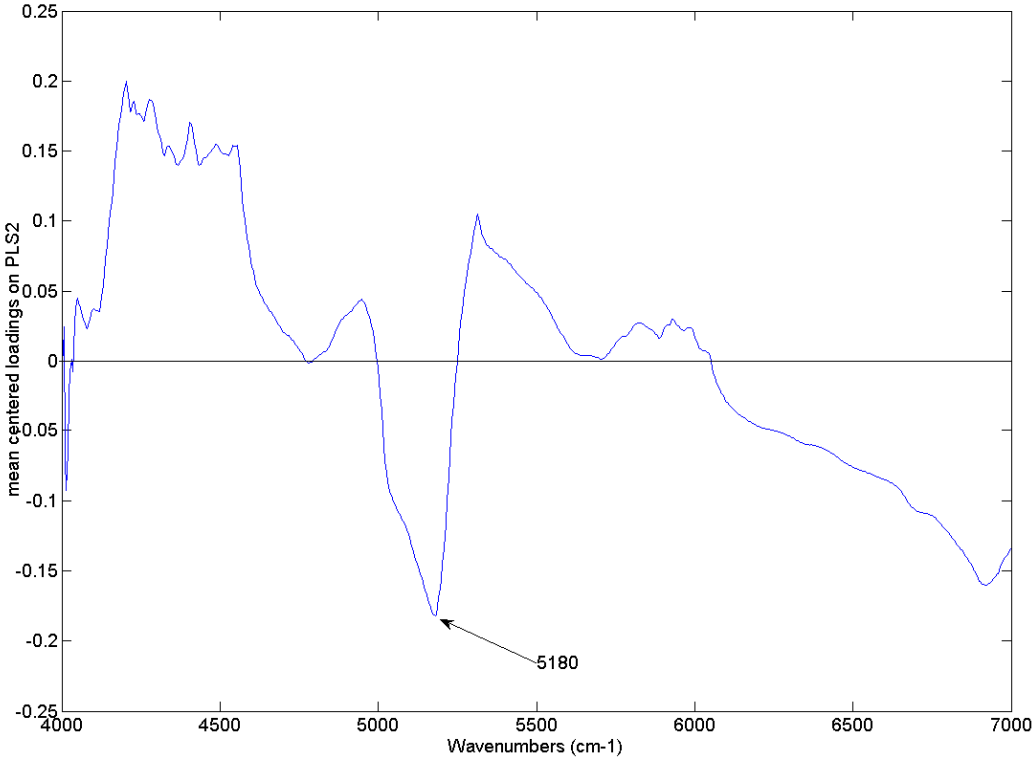
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
mean centered loadings on PLS2
5180
4000
4500
5000
5500
6000
6500
7000
Wavenumbers (cm-1)

Fig 5:

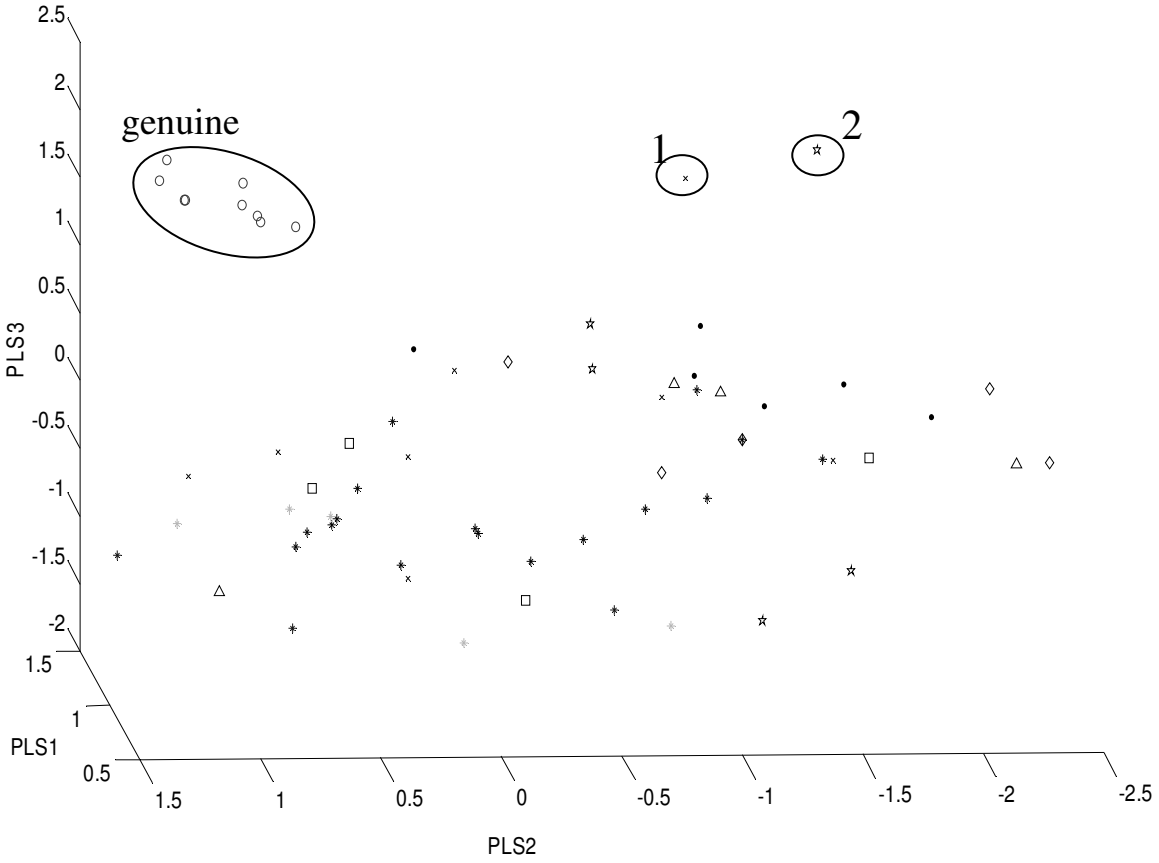

Fig 6:

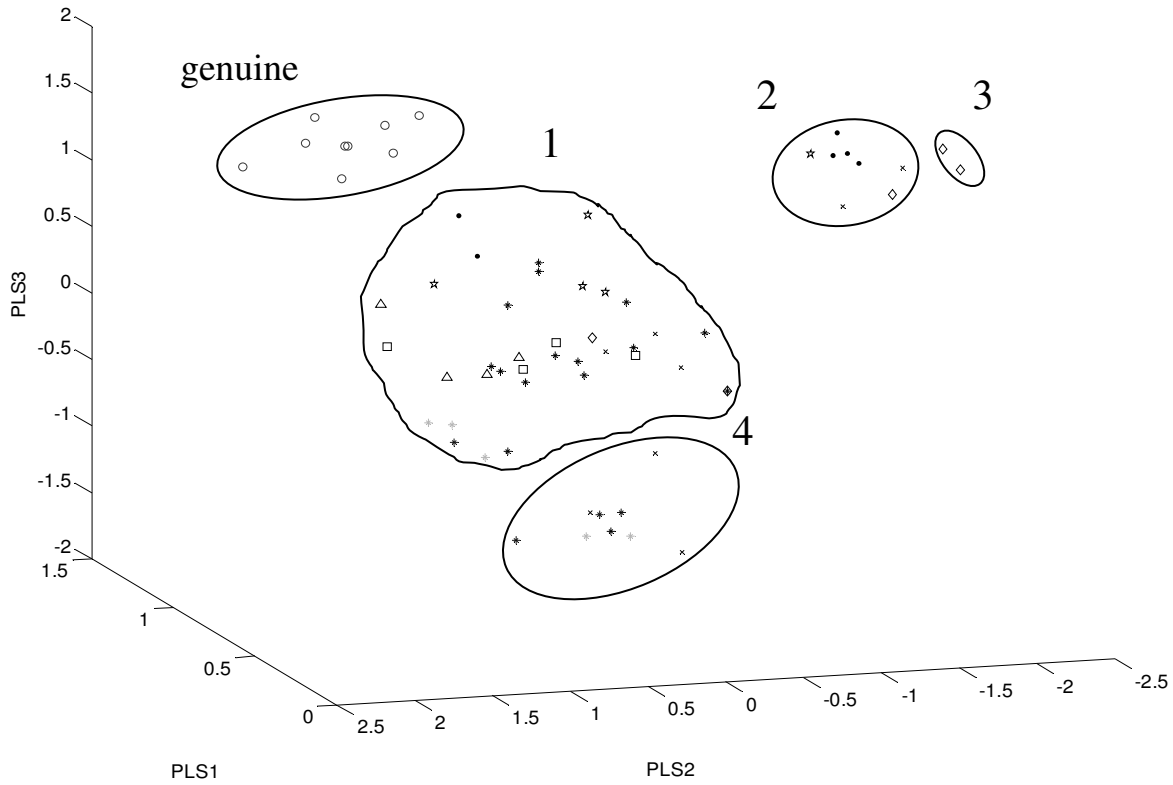

Fig 7:

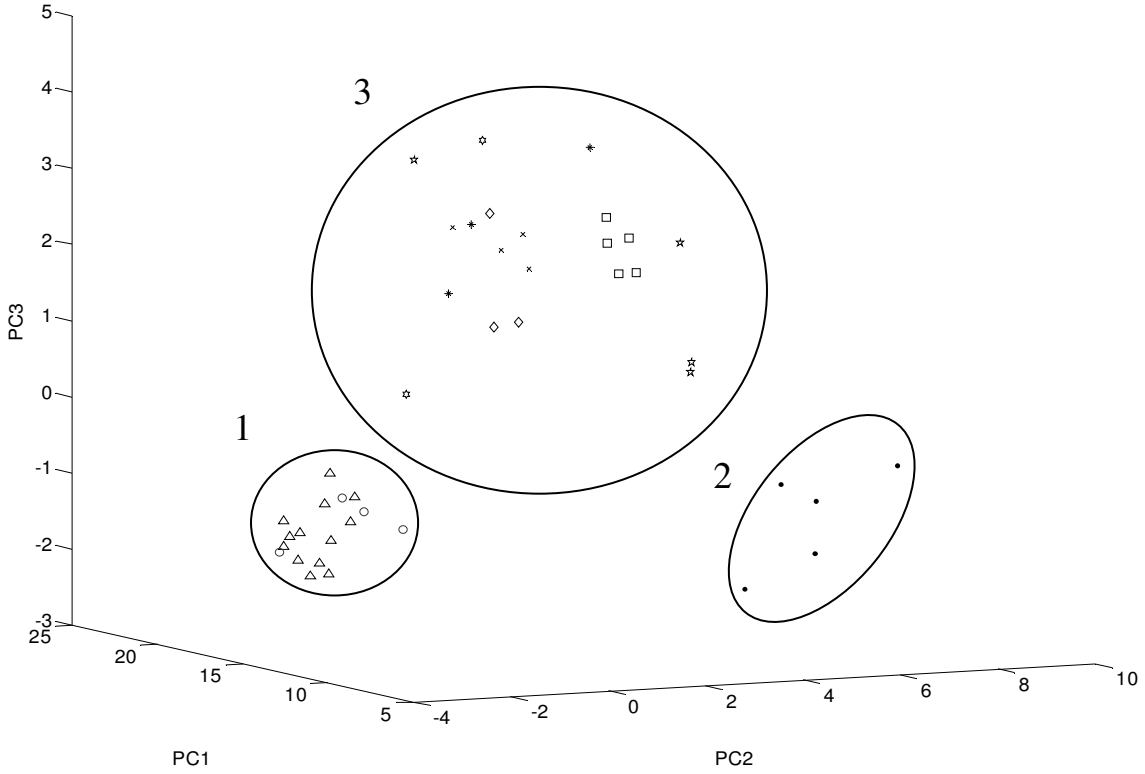

Fig 8:

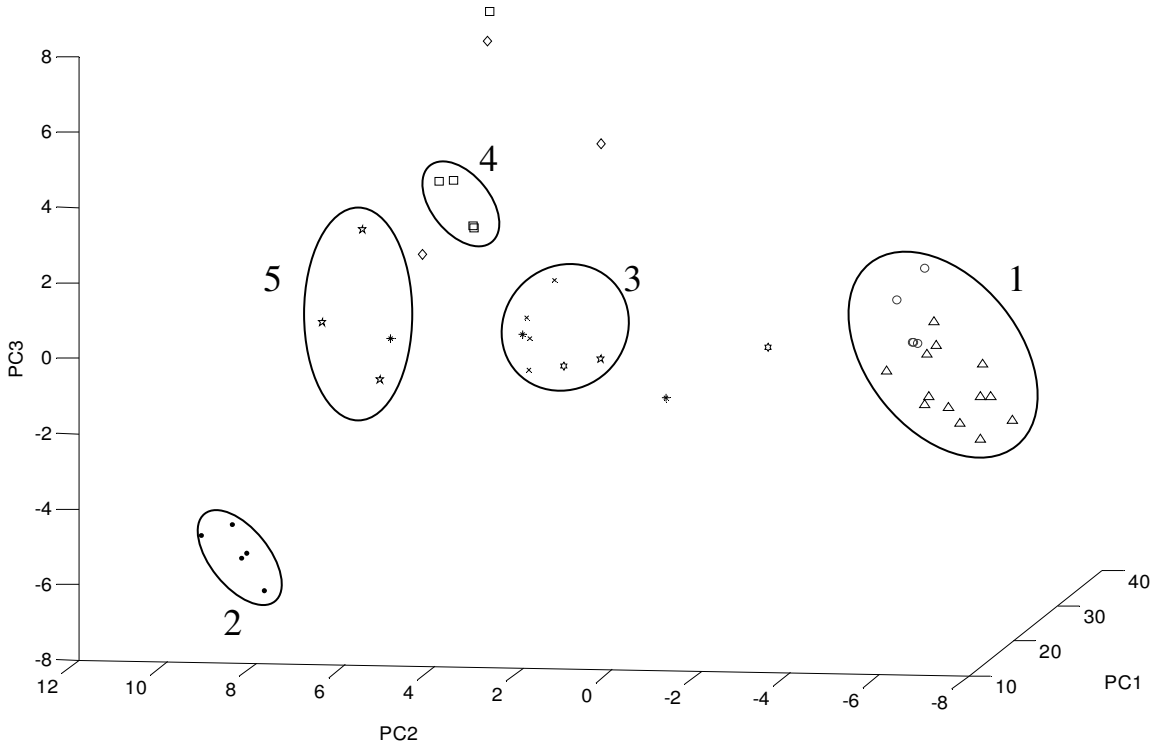

Fig 9:

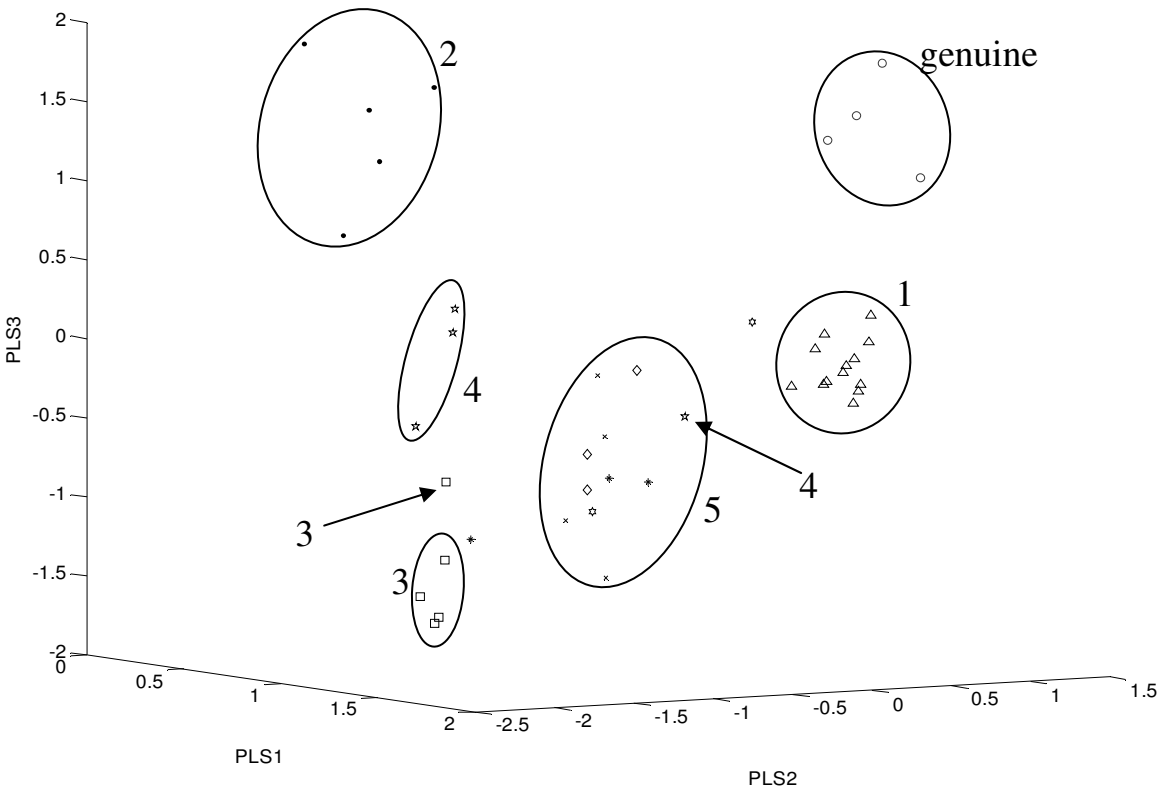
2
genuine
1
4
3
4
5
3
PLS3
PLS1
PLS2
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
0.5
1
1.5
2
-2.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5

Fig 10

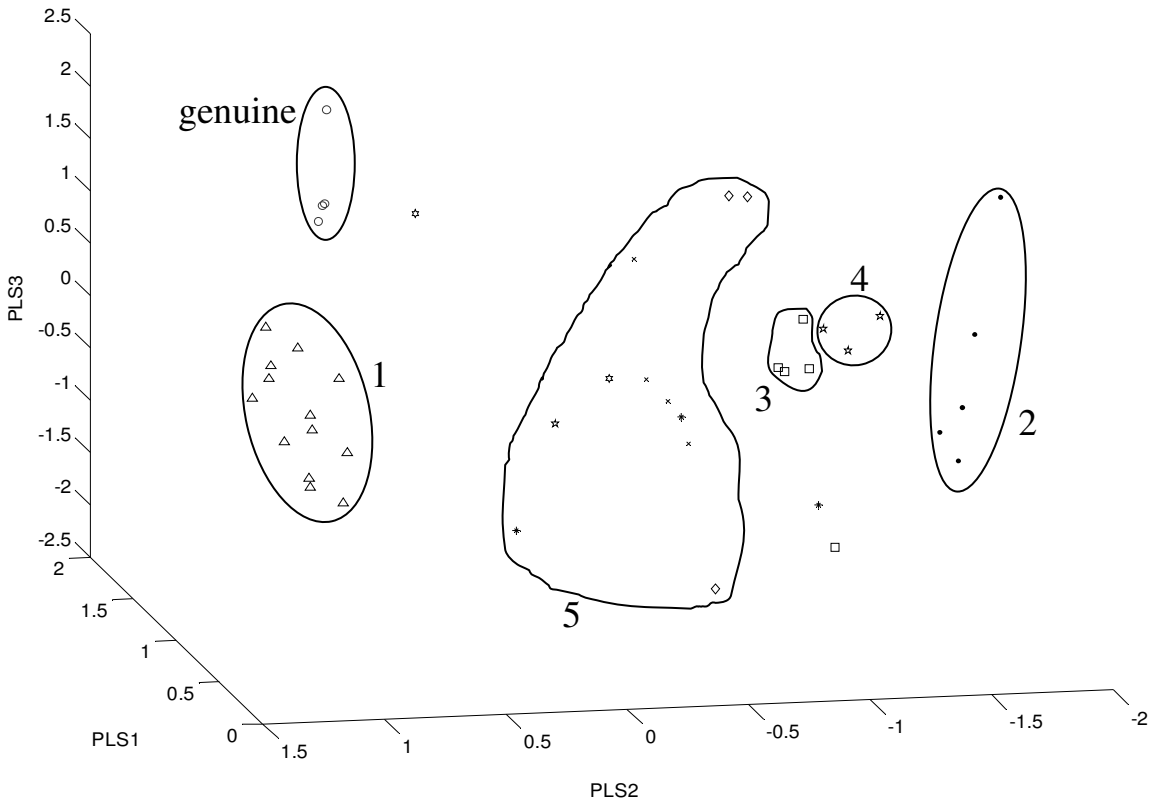

Fig 11a:

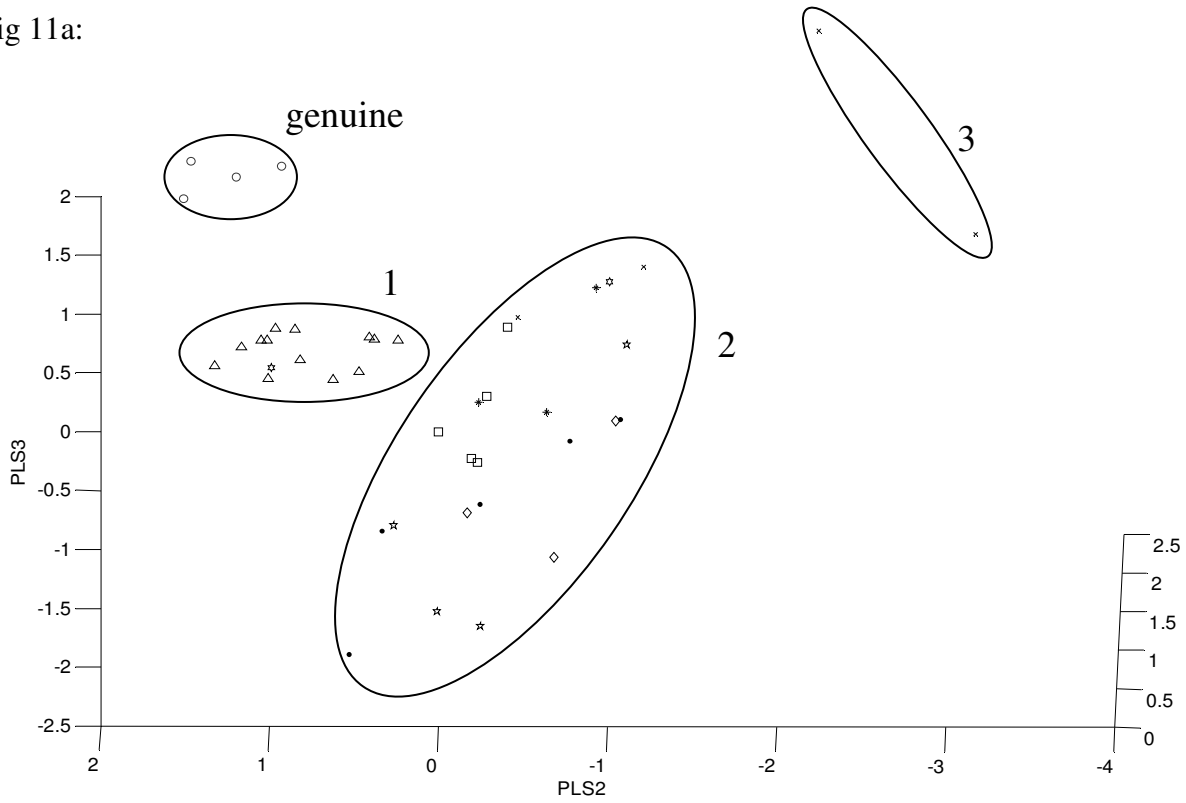

Fig 11b:

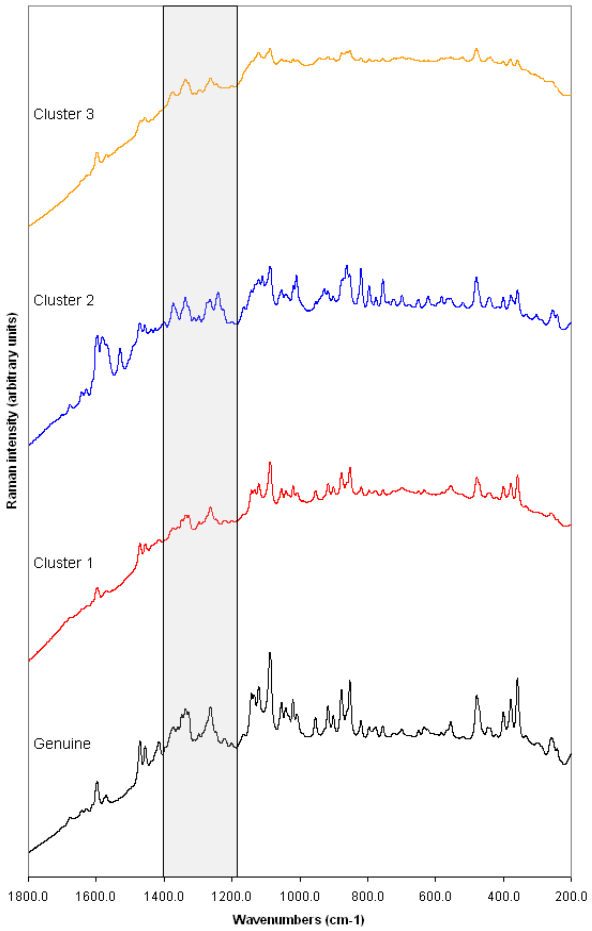

Fig 12:

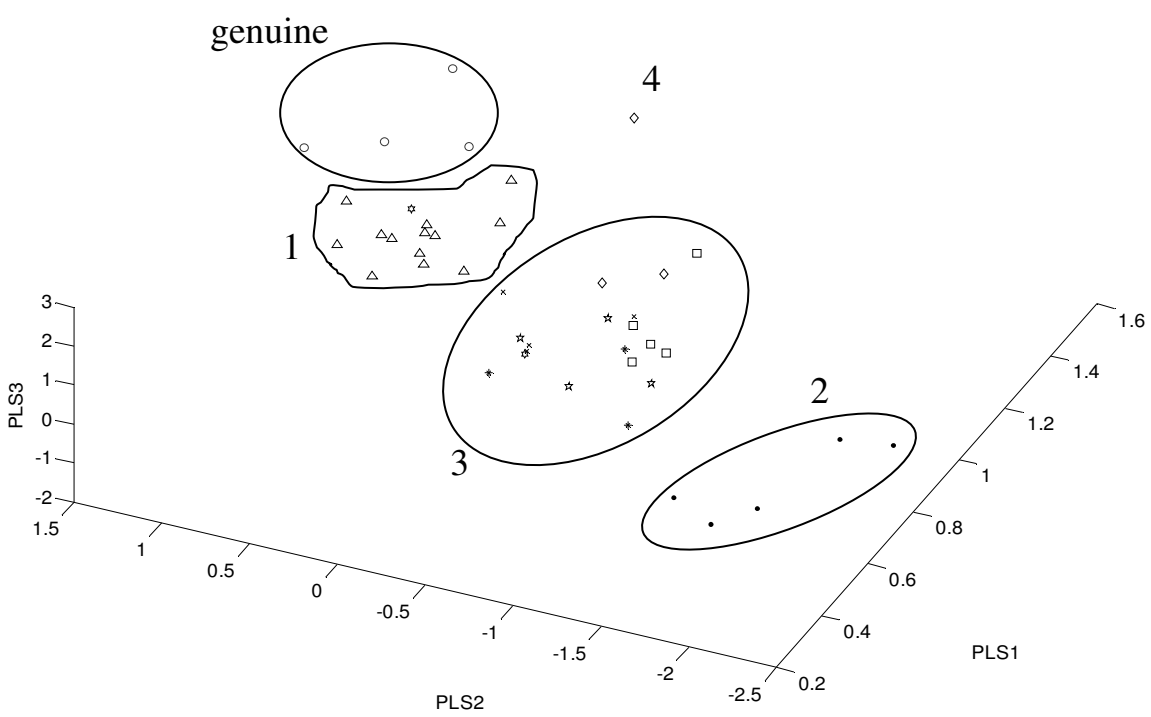
genuine
4
1
2
3
PLS3
PLS2
PLS1
3
2
1
0
-1
-2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6

Fig 13:

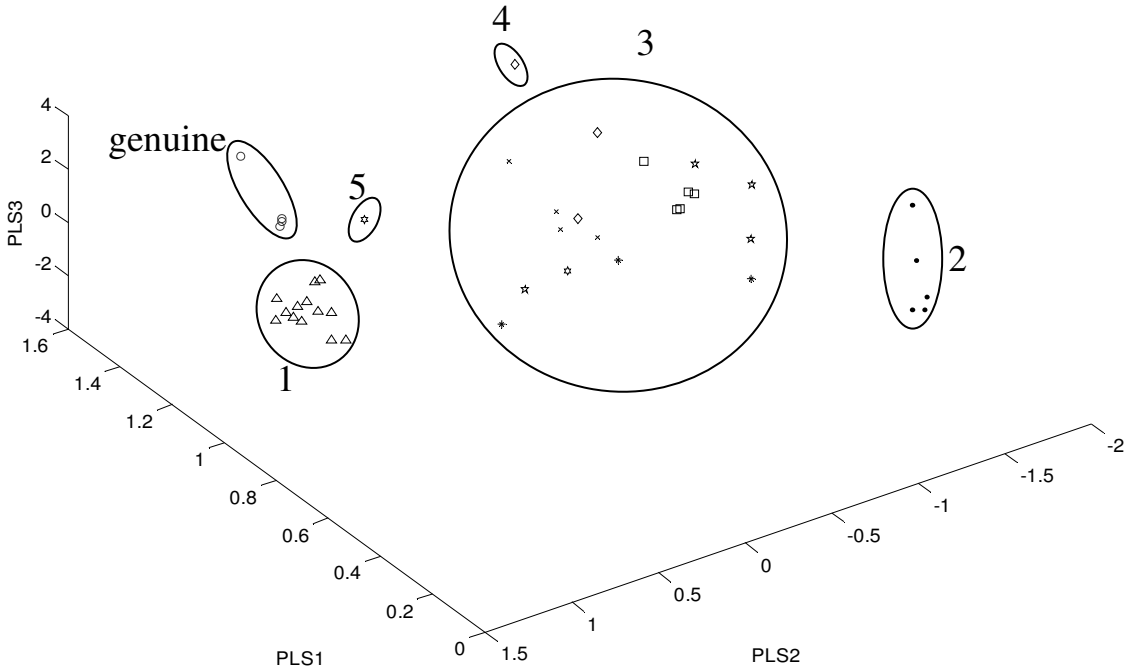
4
3
genuine
5
2
1
PLS3
4
2
0
-2
-4
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
PLS1
1.5
1
0.5
0
-0.5
-1
-1.5
-2
PLS2

Fig 14: