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# Screening suspected counterfeit Viagra® and imitations of Viagra® with near-infrared spectroscopy

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**Abstract**

We describe a near-infrared spectroscopy (NIRS) method for fast-screening Viagra® tablets, counterfeit Viagra tablets, and imitations of Viagra. The method can (1) check the homogeneity of a batch; (2) distinguish counterfeits and imitations from authentic Viagra®; (3) screen for the presence of sildenafil citrate, the pharmacologically active substance in Viagra®, irrespectively of the excipients present; (4) and detect whether similar samples have been previously analysed. We applied the method to 103 samples with a diversity of appearance, chemical composition, and origin. Other analytical methods confirmed the positive screening results for sildenafil citrate and the presence of other pharmacological active substances. The NIRS screening indicated the absence of sildenafil citrate in the presence of another pharmacological substance for only 2 samples, where the reference methods showed the presence of sildenafil citrate in addition to that of clomifene citrate. Otherwise, the method gave no false positive or negative results. The NIRS screening method is very fast and reliable for detecting counterfeits and imitations, and it correctly predicts the presence or absence of sildenafil citrate in 98% of the samples.


*Keywords:* Viagra®; Counterfeit; NIRS; Screening; Sildenafil

## 1. Introduction

Since the introduction of Viagra®[1] for erectile dysfunction, this medicament has been counterfeited and imitated. This is a risk to human health [1], due to the fact that there is no quality control for the production and distribution of counterfeits and imitations [2].

Viagra® counterfeits are tablets intentionally made to look like Viagra®. These tablets may or may not contain the active pharmacological ingredient of Viagra®, sildenafil citrate, in quantities and qualities similar to or different from those in Viagra®. This is also true of the excipients [3]. Sometimes the tablets contain a different pharmacologically active substance.

Imitations of Viagra® are not made to look like Viagra®, but these tablets, capsules, or other pharmaceutical dosage forms claim or imply that they contain sildenafil citrate. This is not always the case. Most imitations are produced in Asia. India and China do not recognise the European and American patent laws so that products manufactured legally in such countries are illegal in Europe, the USA, and other countries.

When a suspect sample is analysed, the following questions have to be answered. Is it an original Viagra® tablet? If not, does it contain sildenafil citrate and/or other pharmacologically active substances? If the sample consists of more than one tablet: is the sample homogeneous? Has a similar sample previously been analysed?

Due to its discriminating power, near-infrared spectroscopy (NIRS) is an attractive analytical technique [4] for answering these questions. It is very suitable for many individual dosage forms, because sample pretreatment is unnecessary and the analysis time is short. In addition, the method is non-destructive. The NIR spectrum reflects both chemical and physical parameters and therefore serves as a 'fingerprint', making this technique the method of choice for detecting counterfeit drugs.

Scafi and Pasquini [5] used a wide variety of counterfeit drugs to test the ability of NIRS to detect counterfeit drugs. They reported an application able to recognise all the counterfeits that

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[1] In this article, Viagra® refers only to genuine Viagra® tablets known to be manufactured by Pfizer.

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differed in composition from the original drug. Olsen et al. [6] used Prozac® as a model drug to demonstrate the flexibility of NIRS in screening for counterfeits in general.

We describe a fast screening method using NIRS for Viagra® tablets, counterfeits, and imitations of Viagra®. Two chemometric algorithms are performed to process the spectra. Wavelength Correlation (WC) is chosen because it is fast, simple and libraries can be updated easily. Principal components analysis (PCA) is chosen because it is able to compare the spectral information in a large dataset and to detect any clusters of unknown tablets.

The method has been tested for its ability to: (1) check the homogeneity of a sample; (2) distinguish counterfeits and imitations from Viagra®; (3) screen for the presence of sildenafil citrate, irrespective of the excipients; (4) determine whether a similar sample has been analysed previously, in order to get insight into possibly related sources [7].

## 2. Materials and methods

### 2.1. Instrumental

All NIR spectra were recorded on a Spectrum Identicheck FT-NIR system (Perkin-Elmer, Beacconsfield, Bucks, England) with an IdentiCheck Reflectance Accessory (ICRA) with the standard Spectrum Identicheck software including Wavelength Correlation, version 2.00 and QUANT+ software, version 4.10 to acquire and process the data. The WC was always applied with the default filter setting. These settings include a resolution weighting, an intensity weighting and a noise weighting. The resolution weighting is a weighted derivative function that emphasizes the influence with widths of approximately 8–16 $cm^{-1}$, corresponding to absorption bands. It discriminates against both broad baseline features and high frequency noise. The intensity weighting is a *black body* filter that reduces the influence of data in those regions at the ends of the spectrum where signal-to-noise ratio is lower. The noise weighting is a *black body* filter that reduces the influence of regions where the transmission is low because they have high noise in absorbance. Measurements were carried out with an optical resolution of 16 $cm^{-1}$ over the spectral range of 12,000–3000 $cm^{-1}$ and 64 scans were co-added. A PbS detector was used. Spectralon was used as a background reference for solid samples. The spectra were recorded in the diffuse reflection mode.

### 2.2. Measurements

Samples were measured as received. The NIR spectra were recorded from both sides for at least five tablets (if available) of every sample. A tablet holder with a 5-mm hole was used for the analysis of the 25-mg tablets or other small tablets. The 50- and 100-mg tablets were measured without a tablet holder. Solids (1 g) were measured as delivered in a 4-ml glass vial with screw cap (Alltech/Applied Science Group, Hoogeveen, The Netherlands).

### 2.3. Reference materials

Pfizer (Netherlands) kindly donated four different batches of reference tablets of 50-mg Viagra® in closed blisters made in Europe, three different batches of reference tablets of 50-mg Viagra® in closed jars made in the USA and sildenafil citrate.

The Dutch Health Care Inspectorate provided eight batches of Viagra® tablets of 25-, 50- and 100-mg strength without original packaging. These tablets were used as references but the spectra were not included in the reference library.

### 2.4. Libraries

Two libraries were constructed to screen the unknown tablets. The reference library consisted of ten NIR spectra of five 50-mg Viagra® tablets (top and bottom) from each of seven samples of European and American manufacture. Principal components analysis was applied to the Viagra® tablet spectra to investigate the similarity between the European and American samples [8,9]. The 95% probability ellipses were calculated around the scores of the European and American samples.

Similar calculations on spectra of Viagra® tablets of 25-, 50- and 100-mg were used to determine whether the strengths gave rise to differences in their NIR signals. NIR spectra of independent 50-mg tablets were used to validate the library.

The second library containing spectra of all measured samples was used to check if a new sample had been analysed previously.

### 2.5. Chemometrics

WC was used to compare the NIR spectra (10,000–4000 $cm^{-1}$) of unknown tablets to the reference library. WC was applied without any spectrum pretreatment. Mean correlation coefficients were calculated from the spectra of all tablets of one sample.

For PCA, the NIR spectra were exported to Matlab (Matlab Release 13, Servicepack 1, August 2003; The MathWorks, Natick, USA). The PCA was applied to the NIR spectra in the frequency range 10,000–4000 $cm^{-1}$ without any spectrum pretreatment. A homemade toolbox was used (Probell Toolbox Version 2.5 June, 2003) to calculate the 95% probability ellipses [10] around the scores of the Viagra® spectra.

### 2.6. Samples

Among the 103 different samples of unknown tablets (originals, imitations, and counterfeits) from the Dutch Health Care Inspectorate, the sample size varied from 1 to 30 tablets. Data regarding appearance, labelled strength, and product name of a representative set from the 103 samples are shown in Table 1. The tablets were delivered in closed or open jars, in blisters, in plastic bags or without any packaging. All samples, as received, were stored in the dark at ambient temperature and humidity.

Table 1
Results of NIRS screening and reference methods for a representative set of Viagra counterfeits and imitations

| Class | (Subclassnumber) Subclass | Amount of samples in subclass | Sample code | Appearance (shape, colour, inscription) | Product name | NIRS screening results | | | Amount of active substances determined with reference method (in mg/dosage unit) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Homogeneous *n*=(number of spectra) | Similarity to Viagra® (50 mg) | Sildenafil citrate[a] | Sildenafil[b] | Others | Reference method[c] |
| Genuine Viagra®? | (1) Indistinguishable from Viagra® | 4 | 5109 | Diamond, blue; Pfizer/VGR 100 | Viagra™ | Yes($n = 16$) | Identical | + | 100 | | 1; 2; 3 |
| | | | 5159 | Diamond, blue; Pfizer/VGR 50 | Viagra™ | Yes($n = 16$) | Identical | + | 47 | | 1; 2; 3 |
| | | | 5162 | Diamond, blue; Pfizer/VGR 50 | Viagra™ | Yes($n = 16$) | Identical | + | 48 | | 1; 2; 3 |
| | | | 6726 | Diamond, blue; Pfizer/VGR 50 | Viagra® 50 | Yes($n = 16$) | Identical | + | 49 | | 1; 2 |
| Counterfeit | (2) Amount of sildenafil conform Viagra® | 23 | 6916 | Diamond, blue; Pfizer/VGR 50 | Not specified | Yes ($n = 16$) | Dissimilar | + | 49 | | 4 |
| | | | 6918 | Diamond, blue; Pfizer/VGR 50 | Not specified | Yes ($n = 16$) | Dissimilar | + | 49 | | 4 |
| | (3) Amount of sildenafil not conform Viagra®; no other substances found | 17 | 6817 | Diamond, blue; Pfizer/VGR 100 | Viagra™ | Yes ($n = 16$) | Dissimilar | ? | 65 | | 1; 2 |
| | | | 6922 | Diamond, blue; Pfizer/VGR 100 | Not specified | Yes ($n = 16$) | Dissimilar | + | 88 | | 4 |
| | | | 6923 | Diamond, blue; Pfizer/VGR 100 | Not specified | Yes ($n = 16$) | Dissimilar | + | 87 | | 4 |
| | (4) No sildenafil; but other substance | 3 | 5471 | Diamond, blue; Pfizer/VGR 50 | Not specified | No ($n = 10$) | Dissimilar | – | n.d. | Yohimbine, 10 | 3 |
| | | | 6531 | Diamond, blue; Pfizer/VGR 50 | Not specified | No ($n = 10$) | Dissimilar | – | n.d. | Yohimbine, 12 | 1; 3 |
| | | | 6816 | Diamond, blue; Pfizer/VGR 100 | Viagra™ | Yes ($n = 8$) | Dissimilar | ? | n.d. | Kinine, 7 | 1; 4 |
| | (5) Contains sildenafil | 43 | 6175 | Round, blue; none | Edegra 50 | Yes ($n = 4$) | Dissimilar | + | 47 | | 1; 2 |
| Imitation | | | 6232 | Triangular, red; 100 | Caverta 100 | Yes ($n = 2$) | Slightly similar | + | 102 | | 1; 2; 3 |
| | | | 6459 | Triangular, red; 50 | Caverta 50 | Yes ($n = 8$) | Slightly similar | + | 50 | | 1; 2; 3 |
| | | | 6398A[d] | Diamond. blue; 100 | Sildenafil citrate | No ($n = 6$) | Slightly similar | + | 73 | | 1; 2; 3 |
| | (6) Contains sildenafil and other substance(s) | 2 | 6398B[d] | Diamond. blue; 100 | Sildenafil citrate | No ($n = 6$) | Dissimilar | – | 21 | Clomifene citrate, 64 | 1;3 |
| | | | 6649 | Diamond, blue; 100 | Not specified | Yes ($n = 2$) | Slightly similar | – | 20 | Clomifene citrate, 64 | 3 |
| | (7) No active substance found | 1 | 7228 | Ovale, grayish; none | Penis XL | Yes ($n = 10$) | Dissimilar | – | n.d. | | 4 |
| | | | 5472 | Diamond, pink; Pfizer/VGR50 | Not specified | Yes ($n = 10$) | Dissimilar | – | n.d. | Amphetamine, 12; yohimbine, 0.4 | 3 |
| | (8) No sildenafil; other substance(s) | 10 | 6755 | Diamond, blue; Pfizer/VGR50 | Not specified | Yes ($n = 6$) | Dissimilar | – | n.d. | Dipyrone, 317 | 3 |

Definitions:
*Counterfeit:* appearance as Viagra®, diamond shaped, blue, inscription Pfizer on one side, VGR 25 (or 50 or 100) on other side.
*Imitation:* appearance different from Viagra®, but claiming or suggesting a similar effect.
*Similarity:* >0.998 identical, >0.99 strongly similar, >0.98 similar, >0.95 slightly similar, <0.95 dissimilar.
[a] (+) Present; (−) absent; (?) not obvious.
[b] n.d., not detectable.
[c] 1, TLC; 2, UV–vis; 3, HPLC–DAD; 4, LC–DAD–$MS^2$.
[d] Samples 6398A en B were sent in as one sample, consisting of 6 tablets.

### 2.7. Method development

#### 2.7.1. Spectral signal of tablets

We investigated whether the NIR signals in the spectra were not only caused by the coating but also by the core of the tablets by powdering some 100-mg Viagra® tablets in an agate mortar and doing NIR analysis.

#### 2.7.2. Addition of sildenafil citrate

A quantity of 10-mg of sildenafil citrate was added to a powdered 50-mg Viagra® tablet and the ingredients were mixed in a Vortex. This was repeated five times. After each addition, a NIR spectrum was recorded. The second-derivative spectra were calculated to explore the accumulation of sildenafil citrate in the mixture of excipients and coating materials.

#### 2.7.3. Influence of the storage conditions on the analytical signal

As unknown samples are not usually delivered in closed blisters or closed jars, the effects of humidity and temperature on Viagra® tablets were studied. The NIR spectra of 10 American Viagra® tablets (50-mg) in closed jars and 10 European Viagra® EU tablets (50-mg) in blisters were recorded immediately after they were unpacked ($t = 0$). Five tablets of each sample were subjected to three sets of storage conditions:

Condition A: room temperature (RT) and 4% relative humidity (RH) for 14 day.
Condition B: RT and 80% RH for 7 days, followed by RT and 4% RH for another 7 days.
Condition C: 100 °C and no control of RH for 7 days followed by RT and 4% RH for another 7 days.

NIR spectra were recorded after 7 days ($t = 1$) and after 14 days ($t = 2$). PCA was performed on the second-derivative spectra (10,000–4000 $cm^{-1}$) measured at the start and after the 7 days. A second PCA was performed on all data collected after 14 days.

### 2.8. Screening samples

NIR-spectra were recorded immediately after unpacking the tablets. The homogeneity of the sample was examined by visual comparison of the spectra of all tablets to each other. The spectra of a sample were compared to the reference library by WC. The mean correlation coefficient indicated the similarity with the Viagra® tablets. The limit for the predicate 'identical' was set at 0.998: higher values indicated that a tablet could not be distinguished from the Viagra® tablets. This criterion was used for a single tablet as well as for a whole sample. Other indications of similarity used were: >0.99 strongly similar, >0.98 similar, >0.95 slightly similar, and <0.95 dissimilar. The similarity with any other NIR spectrum previously measured was explored by comparing each spectrum to the second library. A positive indication of the presence of sildenafil citrate was determined by visual inspection of the second-derivative spectra of the sample tablet and reference Viagra® tablet (50-mg) in the spectral range 6200–5700 $cm^{-1}$.

### 2.9. Reference methods

After NIR analysis, the presence of sildenafil citrate and/or other active ingredients was confirmed by chemical analysis in most cases. The following methods were used:

a. Thin-layer chromatography (TLC) for identification.
b. Ultraviolet–visible (UV–vis) spectrophotometry for identification and quantitation.
c. High-performance liquid chromatography, diode-array detection (HPLC–DAD) for identification and quantitation.
d. Liquid chromatography, diode-array detection, mass spectrometry (LC–DAD–$MS^2$) for identification and quantitation [11].

## 3. Results and discussion

### 3.1. Measurements

No differences were found in the NIR spectra of the top (with Pfizer stamp) and the bottom of the 25-, 50- and 100-mg Viagra® tablets. However, sometimes different spectra were obtained from the top and bottom of the counterfeit tablets. Therefore, all tablets were measured on both sides. The use of a tablet holder with a narrow opening for 25-mg tablets lead to a reduction of about 50% of the spectral signal and caused a difference in the height of the baseline. Thus, when small tablets have to be screened, reference spectra should be acquired in a similar way.

### 3.2. Libraries

The PCA was performed on the spectra of 50-mg Viagra® tablets of European and American manufacture. Both European and American tablets were used to cover possible differences in place of manufacture. The scoreplot (PC1/PC2) showed two clusters with the 95% probability ellipses, which partially overlap (Fig. 1). There seemed to be small differences between the European and American Viagra® tablets. A correction for water by removing the wavenumber ranges (5600–5100 $cm^{-1}$,7450–6950 $cm^{-1}$ and 8600–9100 $cm^{-1}$) had no effect on the position of the points in the plot. We did expect that the water content would not differ very much because all tablets were genuines from Pfizer made under controlled circumstances and delivered in closed blisters or jars.

The average correlation coefficient, the range and the standard deviation of five Viagra® tablets (50-mg) each of the European and American samples were calculated by WC. The in-between correlation coefficients were all higher than 0.998 and the spectra were all 'identical' by the standard for such assessment. Thus, European Viagra® tablets could not be distinguished from American Viagra® tablets which justifies the construction of one reference library of the spectra of 50-mg Viagra® tablets.

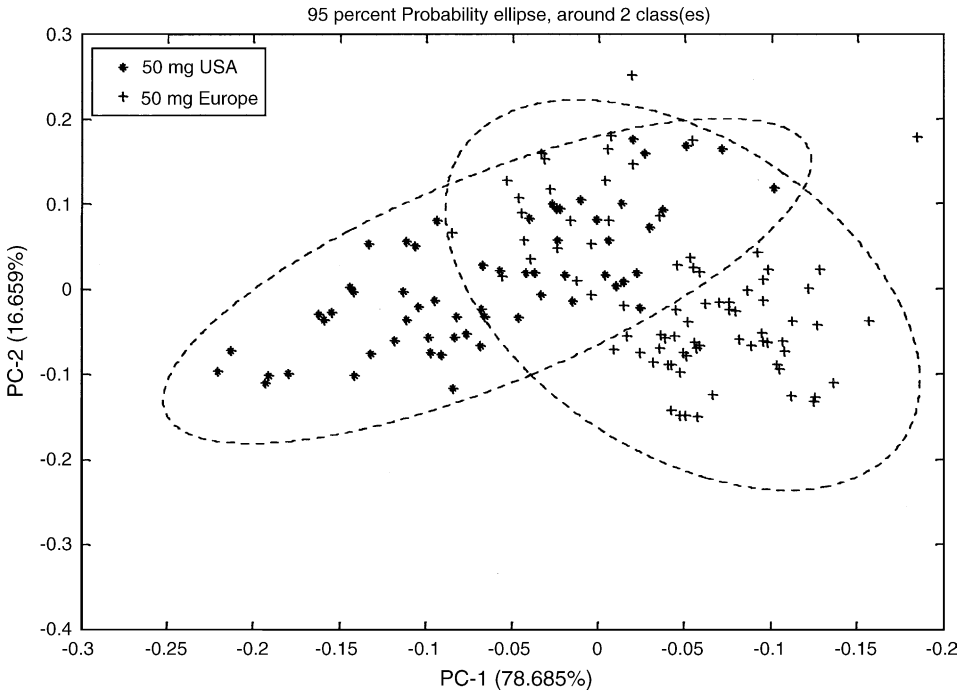


Fig. 1. Similarity of American and European Viagra®, PCAplot (PC1/PC2) of NIR spectra.

The influence of tablet size was examined with PCA of spectra of Viagra® tables with 25-, 50- and 100-mg strengths. These tablets all have the same chemical composition, all are blue coated and diamond-shaped. The 50-mg tablets are twice the weight of the 25-mg tablets and 100-mg tablets are twice the weight of the 50-mg tablets. The tablets are proportional analogues. The score plot (PC1/PC2) showed no difference between 50- and 100-mg tablets (Fig. 2). The scores of the spectra of the 25-mg tablets measured with a tablet holder with a 5 mm hole form a separate cluster. Therefore, the reference library was used only for screening tablets large enough to be measured without a tablet holder.

A small library of spectra of 25-mg Viagra® tablets measured with a tablet holder was set up. However, it was not

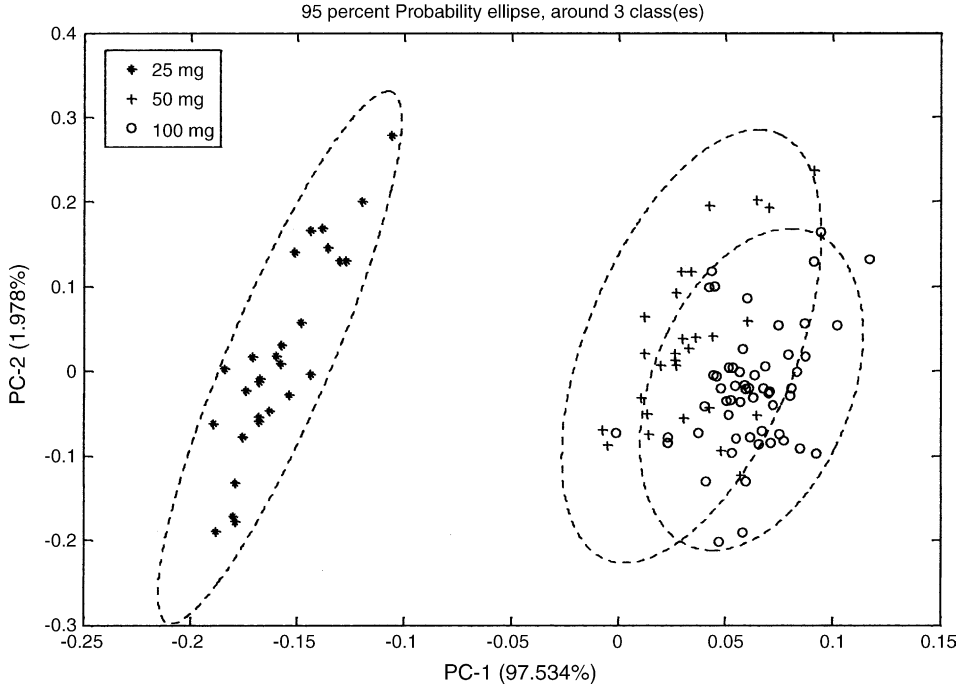


Fig. 2. PCA plot (PC1/PC2) of raw NIR spectra of Viagra® tablets, 25-mg tablets recorded with a tablet holder and 50- and 100-mg tablets recorded without a tablet holder.

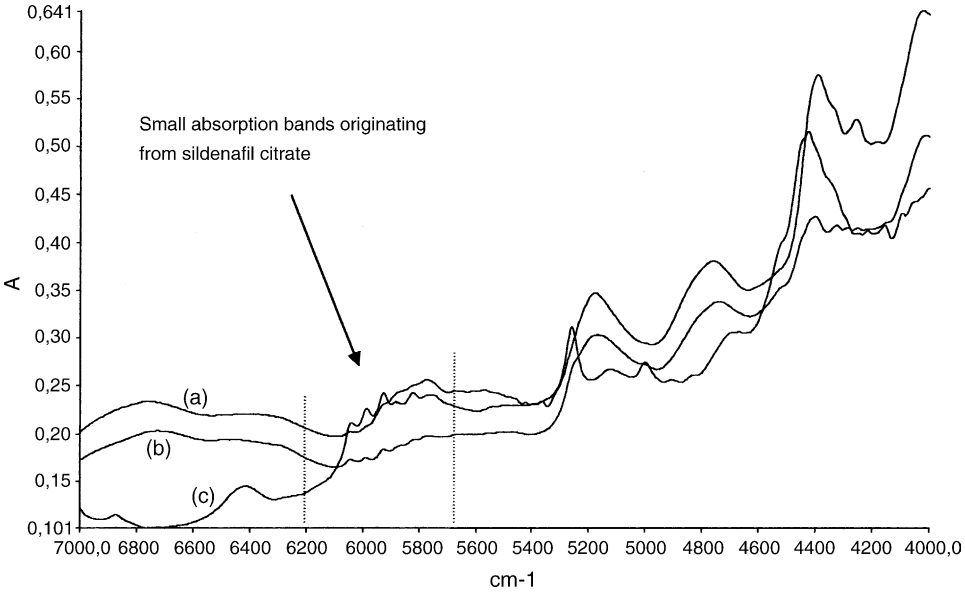


Fig. 3. NIR spectra of an intact 50-mg (a) and a powdered 50-mg Viagra® tablet (b) and of sildenafil citrate (c).

used because there were no requests for analyses of such small tablets.

### 3.3. Method development

#### 3.3.1. Spectral signal of tablets

The spectra of the intact tablets and of powdered tablets show small differences in absorption over the whole spectral range (Fig. 3). These differences are mainly caused by a shift in the proportion of absorption by the coating and by the core of the tablets. The absorption bands from the core increases in the spectra of powdered tablets, while the absorption bands caused by the dominant presence of the coating of the intact tablets decreases. We conclude that the penetration depth of the NIR signal is great enough to reflect the whole composition of a tablet, i.e. the coating and the core. Small peaks in the spectral region 6200–5700 $cm^{-1}$ originate from sildenafil citrate so that the active ingredients can be measured in the analysis of intact tablets. Absorption bands due to glass were ignored.

#### 3.3.2. Addition of sildenafil citrate

The additions of sildenafil citrate to a powdered tablet showed an increase of some absorption bands in the spectra. Clear absorption bands at 6046, 5990, and 5929 $cm^{-1}$ could be assigned to sildenafil citrate in the second-derivative spectra (Fig. 4). A second-derivative spectrum of an intact and a powdered 50-mg Viagra® tablet showed the same absorption bands.

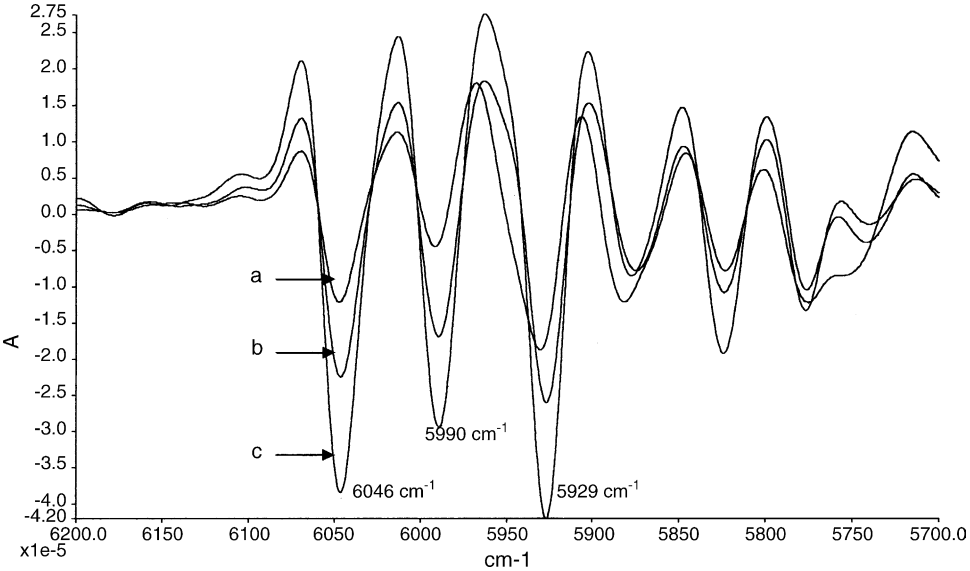


Fig. 4. Absorption bands of sildenafil citrate in second-derivative spectra of an intact 50-mg Viagra® tablet (a); a powdered tablet (b) and a powdered tablet with addition of 60-mg of sildenafil citrate (c).

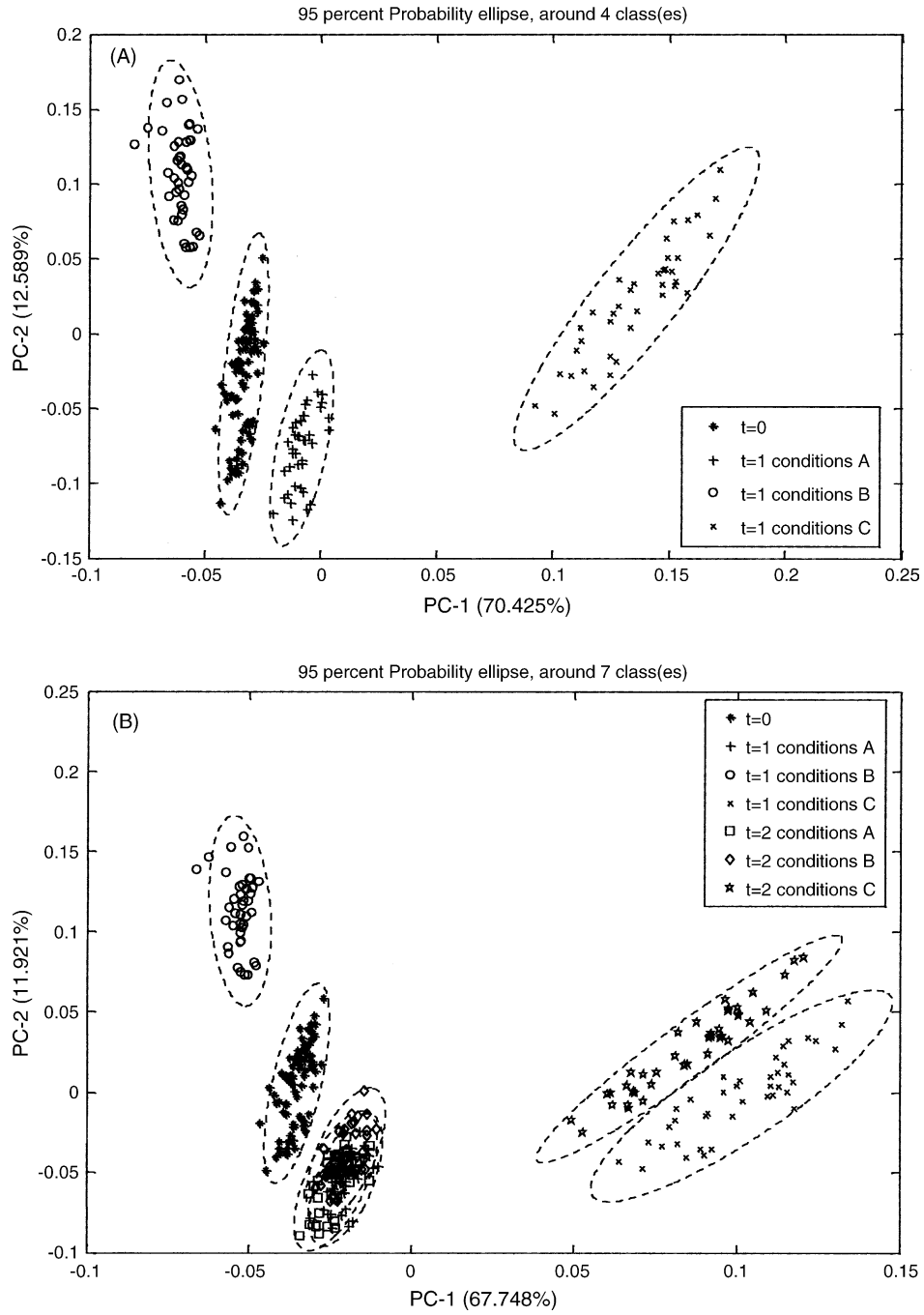


Fig. 5. PCA score plot (PC1/PC2) of second-derivative spectra. (A) Recorded at $t = 0$ (untreated tablets) and at $t = 1$ week after storage under conditions A, B, and C. (B) recorded at $t = 0$ (untreated tablets), $t = 1$ week and $t = 2$ weeks after storage under conditions A, B, and C.

The presence of these absorption bands during screening indicates the presence of sildenafil citrate in the tablet.

### 3.3.3. Storage conditions

The effects of storing tablets for 7 days at RT and 80% RH or for 7 days at 100 °C are clearly visible in the NIR spectra. The intensity of the absorption band of water at 5165 $cm^{-1}$ increased with high humidity, decreased with low humidity, and sharply decreased after storage at a high temperature. This is also evident in the PCA of the second-derivative spectra. The effects of 1 week of storage is shown by the position of the four clusters in the score plot (PC1/PC2) in Fig. 5(A).

After storage of all the tablets at RT and 4% RH for another 7 days, PCA was again performed on the second-derivative spectra. The score plot (PC1/PC2) of all recorded spectra is shown in Fig. 5(B). The scores of the tablets stored previously at 80% RH now cluster with the scores of the tablets stored at 4% RH. This shows that storage at a high RH is a reversible process. The scores of the tablets stored previously at 100 °C do not cluster with the scores of the tablets stored at 4% RH, showing that

drying at 100 °C is an irreversible process. We do not know why this process is irreversible, but a significant increase of the intensity of the absorption bands at 4330 and 4258 $cm^{-1}$ was seen in the spectra of tablets that were dried at 100 °C. It might be that changes occurred in the structure of one of the organic compounds (degradation caused by heat). American and European Viagra® tablets gave analogous results.

These extreme storing conditions were chosen just to explore the influences of water on the tablets. The amount of water in tablets of unknown samples can differ because of storage or because the tablets are delivered unpacked. Although this can affect the results of WC, tablets were not conditioned at a uniform RH. In daily practice, all samples were kept in a storage room from receipt until the moment of measurement, usually just a few days. Storage room conditions were ambient temperature and humidity (approximately 18 °C and 40% RH).

### 3.4. Screening samples

On the basis of visual inspection of the tablets, the unknown Viagra samples were divided into two classes. Half of the samples (47) were suspected of being counterfeits, as they had the appearance of Viagra® (blue, diamond shaped, inscription 'Pfizer' on one side and the inscription 'VGR 25' or 'VGR 50' or 'VGR 100' on the other side) and 56 samples were indicated as imitations.

After NIRS screening a large number of different spectra, caused by the use of different excipients and/or other active ingredients, was obtained. Variations in quantity of the tablet ingredients in one sample and inhomogeneous samples, showing different spectra from bottom and top, were detected. On the basis of these results all Viagra samples were divided into 8 subclasses (Table 1).

The NIRS analysis of the 4 samples in subclass 1 shows that the mean correlations of these spectra met the criterion of 0.998 for 'identical', that the samples were very homogeneous, and that they contained sildenafil citrate. Chemical analysis proved that the tablets indeed contained the amount of sildenafil citrate declared. The tablets in subclass 1 could not be distinguished from Viagra®. Until now, it was impossible to determine whether such samples are extremely good counterfeits (which would mean the appearance of false positives) or whether they are original Viagra® tablets that somehow got lost in the chain from manufacture to retail sale and entered the illegal circuits.

A large party of counterfeit tablets consisting of 23 different samples was assigned to subclass 2. Again NIRS indicated that these tablets did contain sildenafil citrate, and the presence of the declared amount was proved by chemical analysis. However, the correlation coefficients were below 0.99, meaning that other excipients or different materials for the coating [12] were used in the production of these tablets. NIRS revealed that the tablets of 17 subclass 3 samples contained the active ingredient

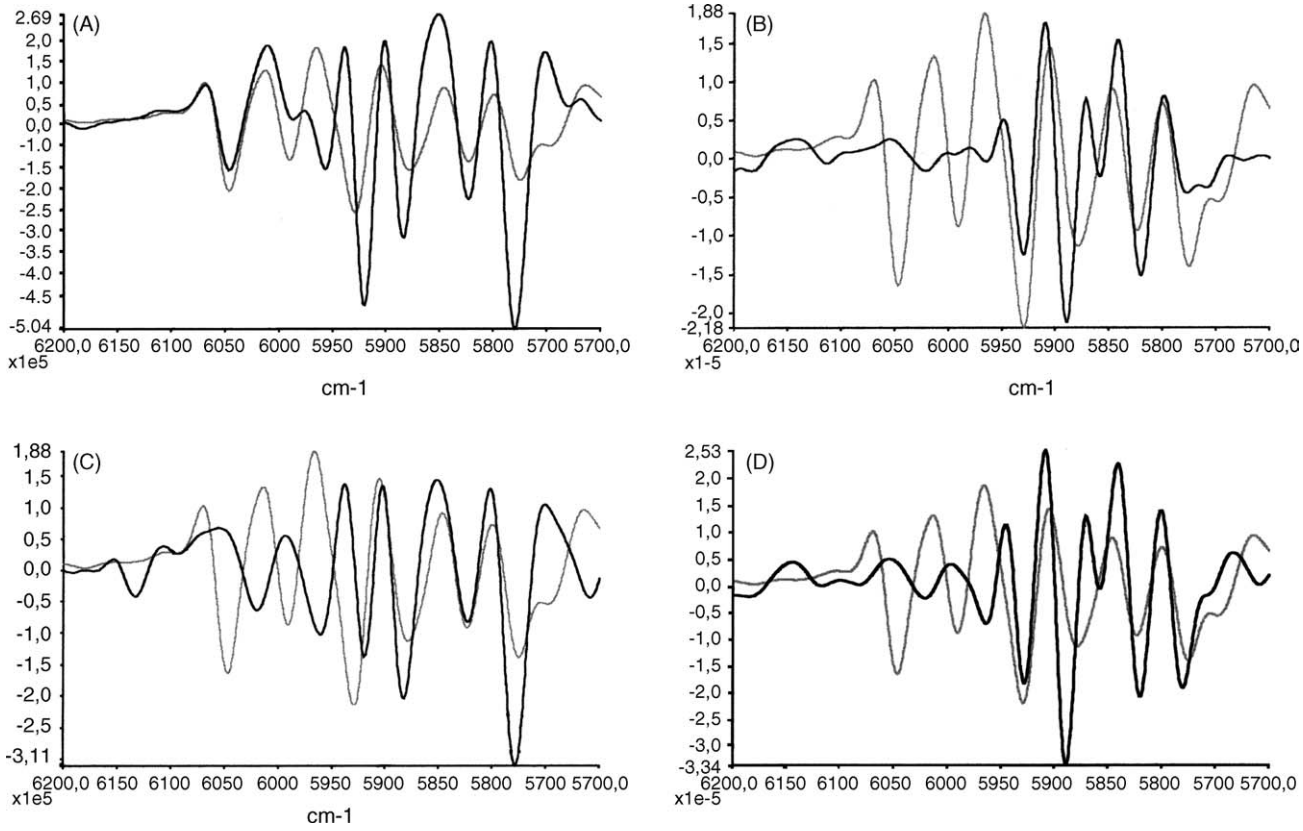


Fig. 6. Second-derivative spectra of a reference Viagra® tablet (dashed) and of four different sample tablets, sample code 6817 (A); sample code 5471 (B); sample code 6816 (C); and sample code 5472 (D).

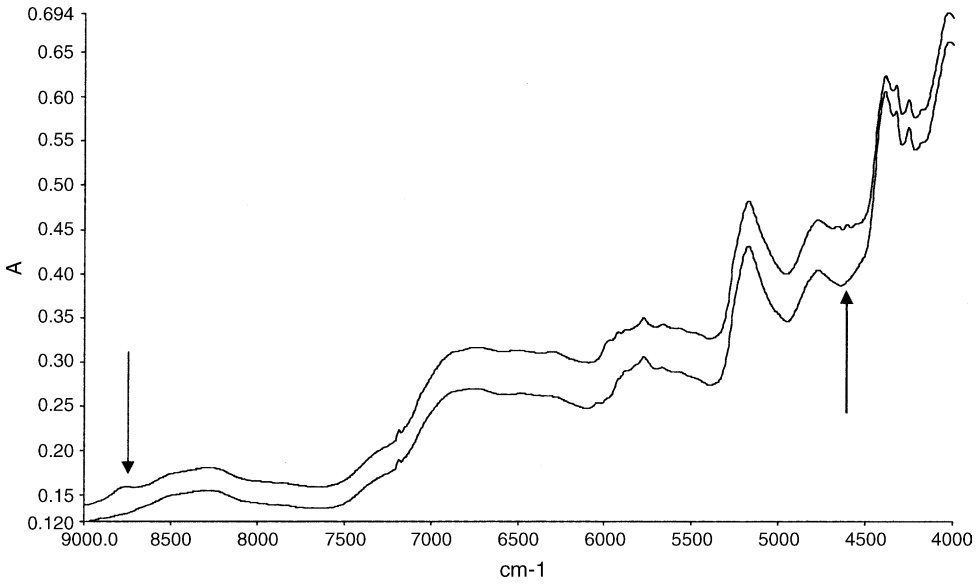


Fig. 7. NIR spectra of tablets (sample codes 6398A and 6398B) with identical appearances. Arrows indicate inhomogeneities in the spectra due to different active ingredients.

sildenafil citrate, but chemical analysis showed that the amount was not as declared The presence of sildenafil citrate in one sample (code 6817) could not indisputably be determined with NIRS due to an interference in the spectra in the frequency range 6200–5700 $cm^{-1}$ (see Fig. 6(A)).

NIRS detected inhomogeneity of the samples with code 5471 and 6531 in subclass 4 meaning that the chemical composition of the tablets in one sample was not consistent. No sildenafil citrate was detected, but chemical analyses revealed that these tablets contained yohimbine. We could not determine whether sildenafil citrate was present in the tablets of one sample (code 6816) with NIRS, but chemical analysis identified quinine in these tablets. The spectra of tablets with sample code 5471 and 6816 are shown in Fig. 6(B and C). The NIR spectra show that the quality of the tablets in this subclass is rather poor.

It is surprising that the correlation coefficients of all 43 counterfeited samples in subclasses 2, 3, and 4 were smaller than 0.95 when compared to the reference library even though they looked like Viagra®.

From the imitation tablets a large group of 43 different samples was assigned to subclass 5, and a part of these samples were regularly produced medicines from India. Although six tablets from one sample (code 6398) did not differ in appearance, NIRS revealed differences in the spectra (Fig. 7) due to different active ingredients. Chemical analyses proved that three tablets contained only sildenafil citrate (coded 6398A), while the other three tablets also contained clomifene citrate (coded 6398B). NIRS indicated that the tablets of all other samples contained sildenafil citrate, and the amount was quantified by chemical analyses.

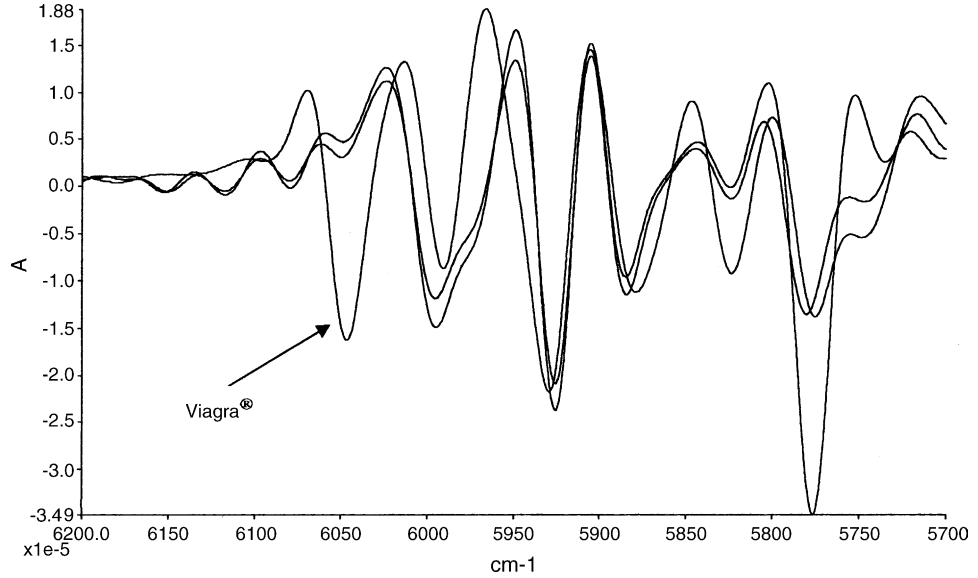


Fig. 8. Second-derivative spectra of Viagra® tablets and of two subclass 6 samples.

We concluded from the second-derivative spectra of two samples in subclass 6 that the tablets did not meet the criteria for positive identification of sildenafil citrate (Fig. 8). However, chemical analyses proved that the two samples contained both sildenafil citrate and clomifene citrate. Thus, they were two false negative NIRS results in the presence of clomifene citrate.

NIRS did not detect sildenafil citrate in the only sample in subclass 7. Chemical analysis confirmed this result, and no other active ingredient was found.

NIRS correctly indicated no sildenafil citrate in 10 samples in subclass 8, chemical analyses revealed the presence of other active substances such as amphetamine and dipyrone. A typical example of a second-derivative spectrum of a tablet containing amphetamine (code 5472) is shown in Fig. 6(D).

## 4. Conclusions

NIRS combined with WC has proven to be a good technique for screening Viagra samples for authenticity. For the use of WC, the threshold of 0.998 for 'identical' is adequate as a criterion to distinguish counterfeit tablets from genuine Viagra®. Among 48 unknown samples that looked like Viagra® in shape, colour, and inscription, NIRS easily identified 44 samples as counterfeit tablets. Four samples were indistinguishable from genuine Viagra®.

Screening for the presence of sildenafil citrate gives good results when second-derivative spectra and selection of specific absorption bands are used. 103 samples were screened and the NIRS results were correct in 99 cases. NIRS did not indicate the presence of sildenafil citrate in only two samples. This was due to the presence of another pharmacological substance. Screening also revealed the presence of other substances, identified by chemical analyses as yohimbine, quinine, clomifene citrate, amphetamine, and dipyrone.

In general, the variation in quality of the samples was great; inhomogeneous samples and different compositions of tablets within one sample were found. The quality of products manufactured in India (Caverta, Edegra) was much better than the quality of most counterfeit tablets. The assignment of related sources can be difficult, due to the large intra-batch variability.

Finally, the NIR spectra provide a lot of information that is most valuable to guide further chemical and/or forensic analyses.

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