Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 46 (2008) 303–309
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Detection of counterfeit Viagra® with Raman spectroscopy

Marleen de Veij [a], Annelien Deneckere [a], Peter Vandenabeele [a,*], Dries de Kaste [b], Luc Moens [a]

[a] *Ghent University, Laboratory of Analytical Chemistry, Proeftuinstraat 86, B-9000 Ghent, Belgium*
[b] *National Institute for Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands*

Received 10 August 2007; received in revised form 20 September 2007; accepted 9 October 2007
Available online 23 October 2007

Abstract

During the last few years, counterfeiters have become increasingly sophisticated by falsifying drugs and making them look identical to genuine tablets. In this paper, Raman spectroscopy is proposed as a fast and reliable method for the detection of counterfeit Viagra® tablets. This technique can easily differentiate genuine from counterfeit tablets without the need of sample preparation. In total 18 tablets were analysed which all contained the active ingredient sildenafil, but different excipients were used, as could be observed in the Raman spectra between 1150 and 700 cm^{-1}. So, the spectra could be divided into genuine or counterfeit. Additionally, principal component analysis (PCA), combined with hierarchical cluster analysis (HCA), was used to establish an automated approach for the discrimination of counterfeit from genuine Viagra® tablets. Raman spectroscopy, combined with principal components analysis, could be used in the future by customs or in the field to identify counterfeit tablets on the spot without involvement of trained chemists.

Keywords: Viagra®; Counterfeit drug detection; Raman spectroscopy; Principal components analysis (PCA)

1. Introduction

Since the introduction of Viagra® for erectile dysfunction by Pfizer in 1998 [1], numerous websites have been appearing, were people easily and anonymously can purchase Viagra® tablets. Viagra® has been falsified numerous times [2] and has been put on the National Specified List of Susceptible Products by the National Association of Boards of Pharmacy [3] in the USA. The products on this list are popular among counterfeiters and could therefore pose a potential risk for public health. According to the World Health Organization (WHO) a counterfeit medicine is one which is deliberately and fraudulently mislabelled, with respect to its identity and/or source [4]. This includes products with correct ingredients or with the wrong ingredients, without active ingredients, with insufficient active ingredient or with fake packaging.

So far, different methods have been used for the analysis of sildenafil in Viagra® tablets. These methods include capillary gas chromatography [5], polymer membrane sensors [6], NMR (^{1}H, ^{13}C, ^{15}N) [7], UV-VIS [8,9], infrared spectroscopy [9,10], Micellar electrokinetic capillary chromatography [11], high-performance liquid chromatography with diode-array detection [9,12,13], liquid chromatography coupled with mass spectrometry (LC-DAD-MS [9], LC-ECI-MS/MS [12] and LC-MS/MS [14]) and thin-layer chromatography [9,13]. The disadvantage of many non-spectroscopic methods is their need for sample preparation, e.g. by pulverizing the tablet and dissolving it. Until now, in literature only two methods have been used, specifically to detect counterfeit Viagra®, namely X-ray powder diffraction analysis [15] and near-infrared spectroscopy (NIRS) [9]. With X-ray diffraction only six Viagra® tablets were investigated and compared with a genuine Viagra® tablet. Based on the information it could predict if the active ingredient and/or particular excipients were present, so the analysis with X-ray diffraction is only qualitative [15]. A downside of this method is that the coating of the Viagra® tablet had to be removed before analysis. For the analysis of the Viagra® with NIRS, a total of 103 samples were analysed. The method can be used to check homogeneity of a batch, distinguish counterfeits and imitations from authentic Viagra® and screen

* Corresponding author. Tel.: +32 9 264 66 15; fax: +32 9 264 66 99.
E-mail address: Raman@UGent.be (P. Vandenabeele).

0731-7085/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2007.10.021

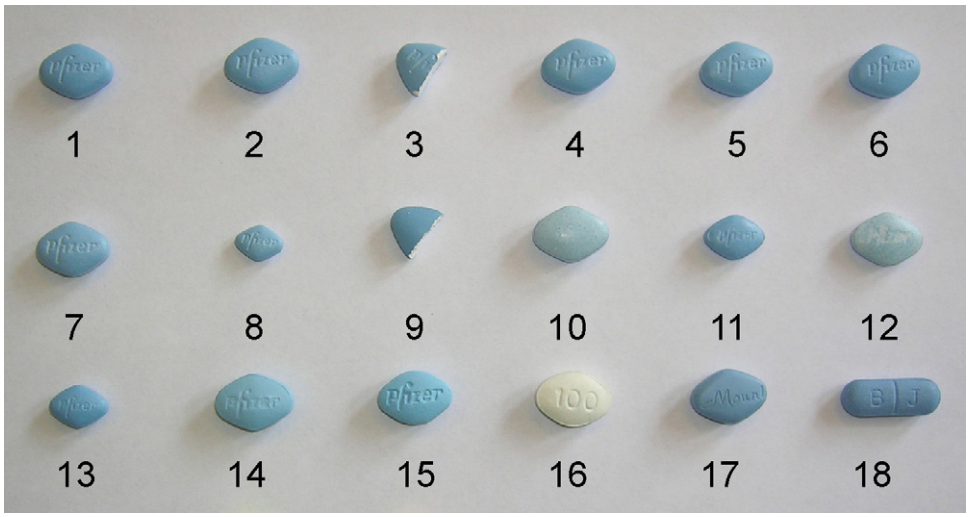

Fig. 1. Picture of the 18 tablets used in this research. In some cases, tablets are broken, for reason of differentiation of the coating and the bulk of these tablets.

for the presence of sildenafil citrate [9]. NIRS can therefore give an indication if sildenafil citrate is present in the tablet but for definitive confirmation LC-MS analysis are necessary [9].

Lately, it has become very difficult to distinguish counterfeit from genuine drugs, based on their appearance [16]. So it is necessary to develop a fast and easy method, in order to identify these counterfeit drugs in the field. In this paper, we propose a Raman spectroscopy method for the detection of counterfeit Viagra® tablets. Pharmaceutical analysis by Raman spectroscopy is becoming a commonly accepted approach as illustrated by the rising number of research papers that are published in this field [17]. Raman spectroscopy has been used for drug identification of active ingredients as well as excipients [18], polymorphism [19], imaging of tablets [20], quality control [21] and industrial applications [22]. This technique requires no sample preparation; tablets can even be analysed through their coating, while non-destructive character and high speed of analysis make Raman spectroscopy well-suited for investigating possible counterfeit drugs [23,24]. Moreover, by using chemometric techniques to compare the recorded spectra with those in a reference library, instrument operation and spectral interpretation can be automated, in order to give a pragmatic answer.

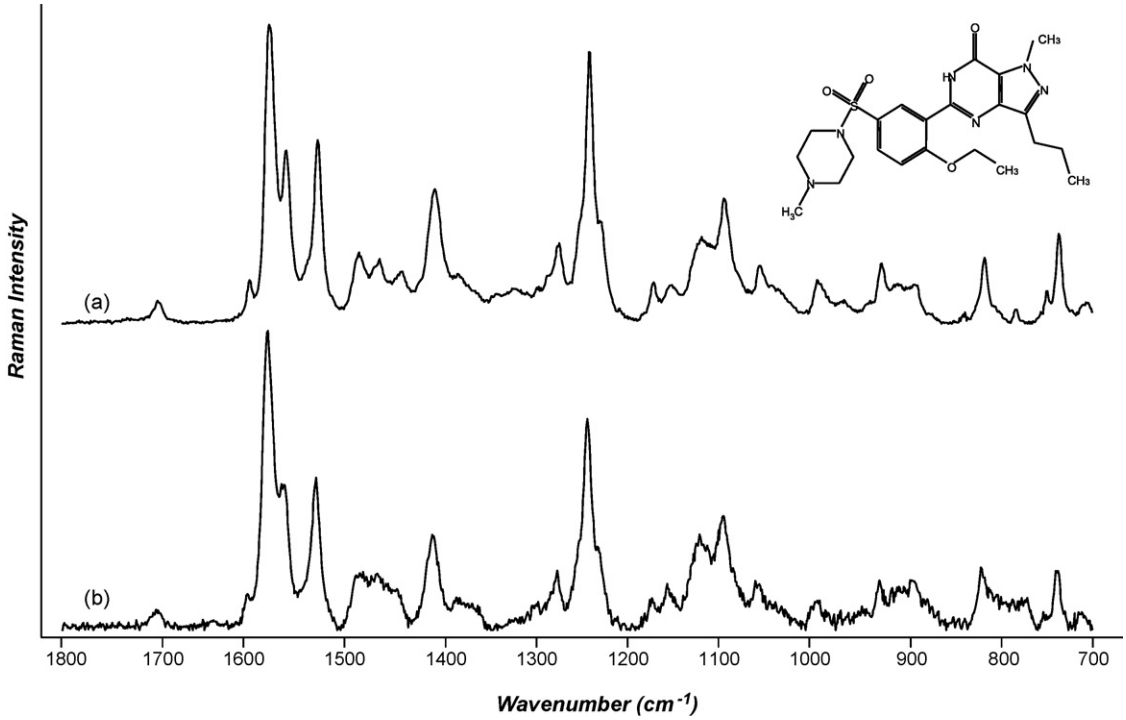

Fig. 2. Raman spectrum of (a) sildenafil and its molecular structure and (b) genuine Viagra® tablet (5X objective, 10 accumulations of 30 s, 700–1800 cm^{-1}, 785 nm laser wavelength, after baseline correction).

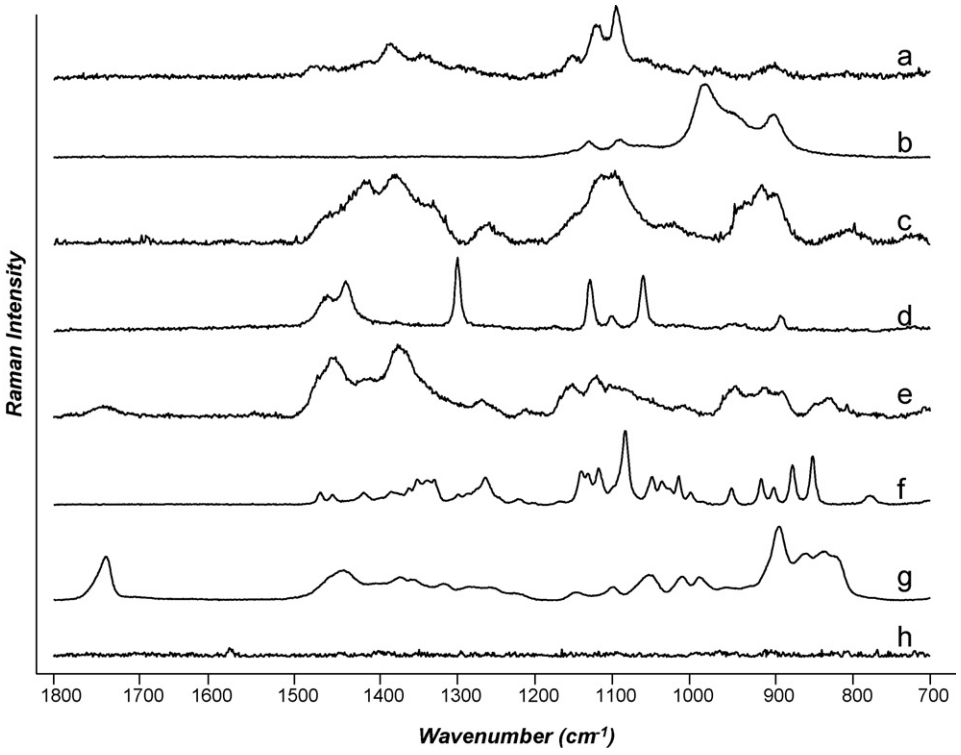

Fig. 3. Raman spectrum of (5X objective, 700–1800 cm^{-1}, 785 nm laser wavelength, after baseline correction) of the excipients used in a Viagra® tablet, (a) microcrystalline cellulose (10 accumulations of 30 s), (b) calcium hydrogen phosphate (5 accumulations of 30 s), (c) croscarmellose (50 accumulations of 60 s), (d) magnesium stearaat (5 accumulations of 30 s), (e) hypromellose (15 accumulations of 60 s), (f) lactose (5 accumulations of 30 s), (g) triacetin (5 accumulations of 30 s) and (h) indigo carmine (40 accumulations of 30 s).

2. Experimental

The analyses of the Viagra® tablets were carried out with a Renishaw System-1000 spectrometer (Wotton-under-Edge, UK) which is connected to an Olympus BH-2 microscope. The laser used is a diode laser with a wavelength of 785 nm and a power of 50 mW (DL 100, TuiOptics GmbH, Martinsried/Munich, Germany). A 5X objective lens (Olympus MDPlan50, 0.75 NA, Omnilabo, Aartselaar, Belgium) was used to focus the laser light on the sample and to collect the scattered light. The collected Raman radiation was dispersed with a 1200 lines mm^{-1} grating (focal length 250 mm) and focussed on a Peltier-cooled CCD detector, allowing to obtain a spectral resolution of ca. 1 cm^{-1}.

Eighteen tablets were recorded in the spectral window of 700–1800 cm^{-1}, with 10 accumulations of 30 s each. Besides the 18 tablets, a genuine Viagra® tablet (provided by pharmacy, Ghent, Belgium), the active ingredient (provided by the National Institute for Public Health and the Environment, RIVM, Bilthoven, the Netherlands) and inactive components of a genuine Viagra® tablet were analysed as well. These are: microcrystalline cellulose (Sigma–Aldrich, Belgium), calcium hydrogen phosphate (Sigma–Aldrich, Belgium), croscarmellose (Certa, Belgium), magnesium stearaat (Sigma–Aldrich, Belgium), hypromellose (Shin Etsu, Japan), lactose (DMV international, the Netherlands), triacetin (Sigma–Aldrich, Belgium) and indigo carmine (Fluka, Belgium). Additionally, several reference products were analysed, namely: barium sulphate (UCB, Belgium), calcium sulphate (Fluka, Switzerland), calcium carbonate (Merck, Belgium), mannitol (Cerestar, Belgium) and sucrose (Merck, Belgium). Three spectra were recorded, averaged and baseline-corrected before presentation, to eliminate the influence of broadband fluorescence.

Eighteen "Viagra" tablets (Fig. 1) were purchased in local pharmacies in China (tablet 1 and 3 from Shanghai, tablet 2 from Beijing and tablet 4, 5, 6, 17 and 18 from Suzhou) and Mexico (tablet 7 from Cancun). Other "Viagra" tablets were donated by Public Health and Environment in the Netherlands (tablet 8, 9, 10, 11, 12 and 13), customs at Brussels Airport (tablet 14 and 16) and by Federal Agency for Medicines and Health Products in Belgium (tablet 15).

Data processing was performed with Matlab 6.5 (The MathWorks inc. Natick, MA) and the PLS toolbox, version 3.0 (Eigenvector Research inc., Manson, Washington). The average of the three or five spectra (depending on tablet or pure substance) and a manual baseline correction was performed by using ACD/Specmanager (version 9.13, Advanced Chemistry Development, inc. Toronto, Canada).

3. Results and discussion

3.1. Visual inspection of the tablets

The first step in detecting counterfeit drugs is based on the appearance of the tablet. Viagra® is known for its distinct blue diamond shape, with on one side the Pfizer-logo and on the other side the abbreviation "VGR" plus the amount of sildenafil in mg

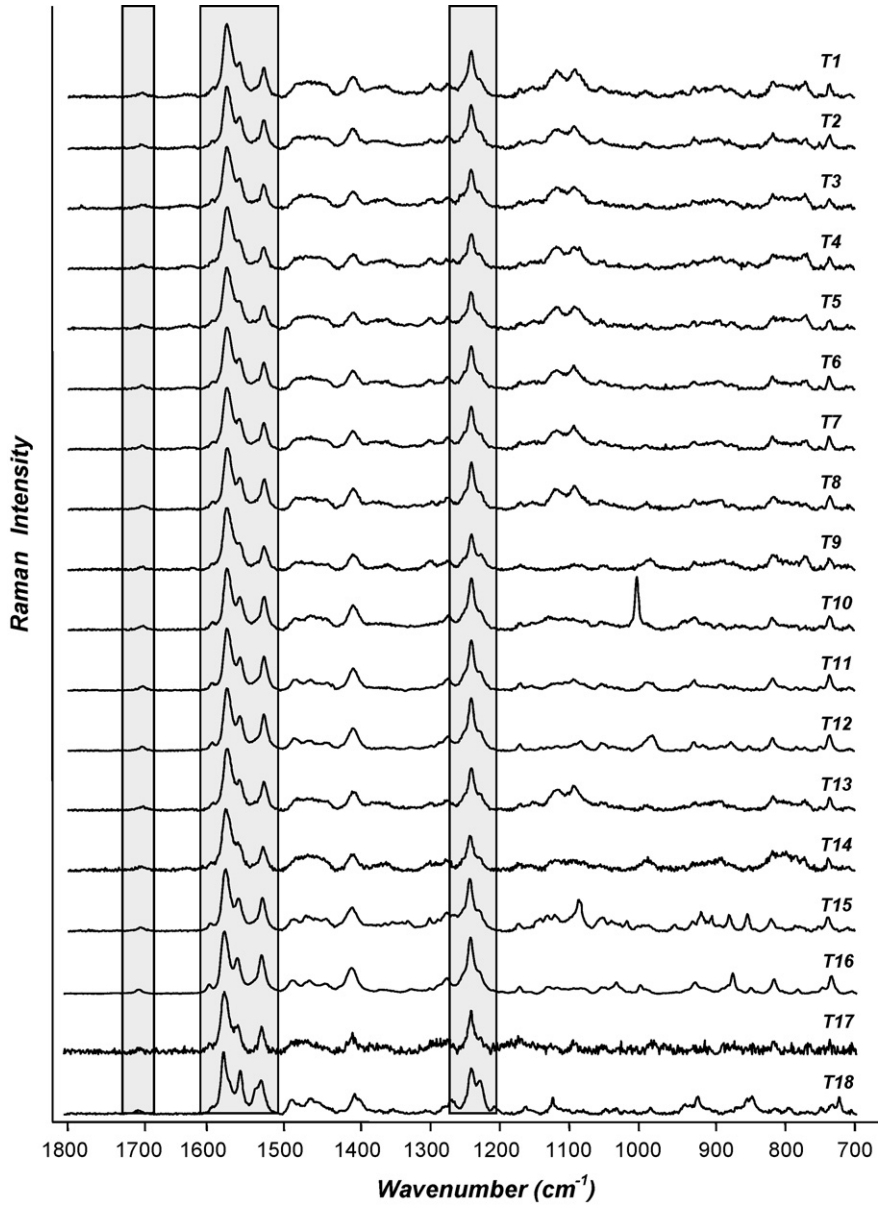

Fig. 4. Raman spectra of the 18 tablets (5X objective, 10 accumulations of 30 s, 700–1800 cm^{-1}, 785 nm laser wavelength, after baseline correction).

(25, 50 or 100). Based on this information the tablets 16, 17 and 18 can be eliminated as genuine Viagra® tablet, since tablet 16 lacks the blue colour, tablet 17 has different names pressed in the tablet and tablet 18 has a different shape. Tablet 1 to 15, based on their appearance, look similar to genuine Viagra® tablet and for these tablets Raman analysis is necessary.

3.2. Visual inspection of the Raman spectra

To be able to differentiate the 18 tablets based on their Raman spectrum, a Raman spectrum of a genuine Viagra® tablet is necessary. Fig. 2 shows the spectrum of pure sildenafil (a), its chemical structure and the spectrum of genuine Viagra®(b). The majority of the Raman bands in the genuine Viagra® spectrum (Fig. 2b) can be assigned to the corresponding functional groups [25] of the active ingredient (Fig. 2a). A Raman band at the far end of the spectrum at 1699 cm^{-1} can be assigned to the stretching vibration of the C=O group, additionally the doublet at 1580/1562 cm^{-1} is attributed by the C=C bond. Raman bands at 1528 cm^{-1} (ν(N–C=N), 1238 and 1272 cm^{-1} (ν(C=N)) and the Raman band at 927 cm^{-1} (ν(C–N)) are attributed to nitrogen containing bonds. The Raman bands at 1170, 1057 and 652 cm^{-1} can be assigned to the symmetric $\nu(SO_2)$, the asymmetric $\nu(SO_2)$ and the ν(C–S) stretching vibrations, respectively.

There are some additional Raman bands present in the spectrum of genuine Viagra® which cannot be attributed to the active ingredient, but can be assigned to one of the excipients (769, 847, 873, 1117, 1294 and 1352 cm^{-1}, weakly, not always visible in Fig. 2b). A Viagra® tablet consist of a white core and a blue coating. The core of the tablet consists of the active ingredient sildenafil, microcrystalline cellulose, calcium hydrogen phosphate, croscarmellose and magnesium stearate. The coating of

the tablet consists of hypromellose, lactose, triacetin, titanium dioxide and indigo carmine for its distinct blue colour [26]. The Raman spectra of these excipients are shown in Fig. 3, the spectrum of titanium dioxide is not presented since this spectrum only consist of three very strong Raman bands below 700 cm^{-1} (394, 514 and 635 cm^{-1}) [27]. Based on these spectra, the additional bands in the spectrum (Fig. 2b) of the genuine Viagra® tablet can be assigned to microcrystalline cellulose (1352 and 1117 cm^{-1}) [28], magnesium stearate (1294 and 769 cm^{-1}) and lactose (873 and 847 cm^{-1}) [28].

The spectrum of genuine Viagra® (Fig. 2b) is compared to the Raman spectra of the 18 tablets in Fig. 4. The strong Raman bands at 1699 cm^{-1} (ν(C=O) stretch vibration), 1580 and 1562 cm^{-1} (ν(C=C) doublet), 1528 cm^{-1} (ν(C=N) stretch) and at 1238 cm^{-1} (ν(C–N) stretch), which are grey highlighted, were used to check for the presence of the active ingredient. It can be concluded that all 18 tablets contain the active ingredient. However, when focussing on the Raman bands between 1150 and 700 cm^{-1}, differences between these tablets can be noticed.

Tablet 1–8 and tablet 13 show the same spectrum as the genuine Viagra®, including the Raman band of microcrystalline cellulose at 1117 cm^{-1} (Fig. 3a). This Raman band is clearly lacking in the tablets 9 till 12 and 14 till 18, which therefore can be considered as counterfeits. These 9 tablets (9–12 and 14–18) do not show certain Raman bands which are present in the spectrum of genuine Viagra® and/or have additional Raman bands of other excipients.

Five tablets have extra Raman bands which are attributed by different excipients not present in genuine Viagra®. Tablet 9 has an additional Raman band at 987 cm^{-1} which can be assigned to barium sulphate (Fig. 5a) [29] whereas tablet 10 has an extra Raman band at 1005 cm^{-1} attributed by calcium sulphate (Fig. 5b) [29]. Raman bands at 711 and 1084 cm^{-1} which are specific for calcium carbonate (Fig. 5c) [30] can be observed in the spectrum of tablet 15. Mannitol (Fig. 5d) [31] is present in tablet 16 according to the three Raman bands at 782, 872 and 1034 cm^{-1}. Tablet 18 shows three additional Raman bands at 846, 921 and 1123 cm^{-1} which can be assigned to sucrose (Fig. 5e) [31].

The three tablets 11, 12 and 14 lack the Raman band at 1117 cm^{-1}, as do all 9 counterfeits, but show no obvious Raman bands of other excipients besides the excipients used in genuine Viagra®. Finally, besides the Raman bands of sildenafil (Fig. 2a) no other Raman bands can be observed in the Raman spectrum of tablet 17, not even Raman bands from any of the excipients used in the genuine Viagra®. In stead of using Raman bands of the active ingredient as distinction between genuine and counterfeit Viagra®, the tablets could be divided into these two categories based on the excipients used.

3.3. Chemometric analyses

When considering an analytical technique for routine or screening investigations, this technique has to fulfil several requirements. These include, among others, a high speed of analysis, easiness to operate and the technique should be robust. It is clear that the accuracy of the applied method is also critical. Raman spectroscopy seems to meet many of these conditions. However, if this technique has to be operated by non-specialist users (e.g. at customs), there is need for an automated spectral interpretation. Here a chemometric approach combined with an elaborated database with Raman spectra of genuine pharmaceutical products has to be considered.

Here, in an exploratory approach, we use a combination of principal components analysis (PCA) and hierarchical cluster analysis (HCA). However, it is not the aim of this work to select the most appropriate chemometric techniques for this discrimination. The dataset, consisting of the Raman spectra of 19 Viagra® tablets is arranged in a 19 by 1205 data matrix (1205 data points, between 700 and 1800 cm^{-1}). In order to avoid contributions from the broad-featured fluorescence background, the second derivative of all spectra was taken by using the Savitsky–Golay algorithm [32]. A 13-point window-function and the use of a third order polynomial happened to yield the

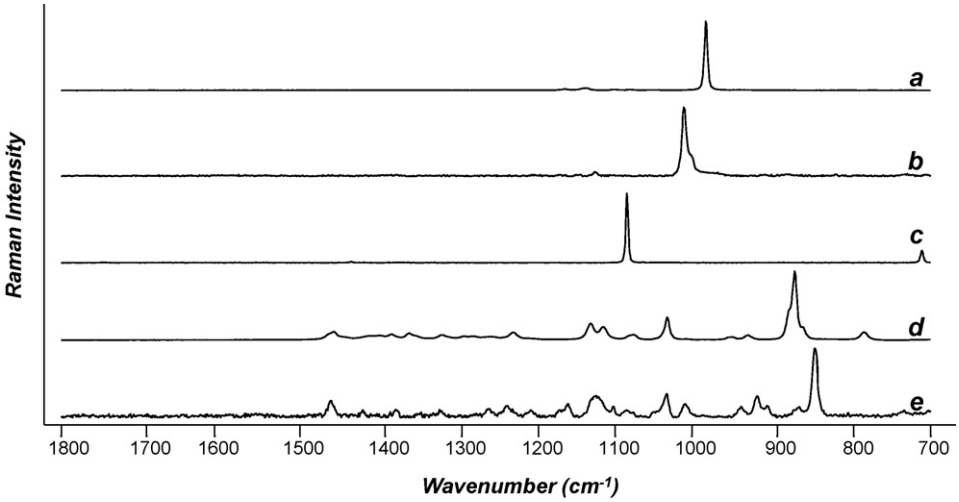

Fig. 5. Raman spectra excipients found in the analysed "Viagra" tablets, (a) barium sulphate (1 accumulations of 30 s), (b) calcium sulphate (20 accumulations of 30 s), (c) calcium carbonate (1 accumulation of 30 s), (d) mannitol (5 accumulations of 120 s) and (e) sucrose (20 accumulations of 30 s).

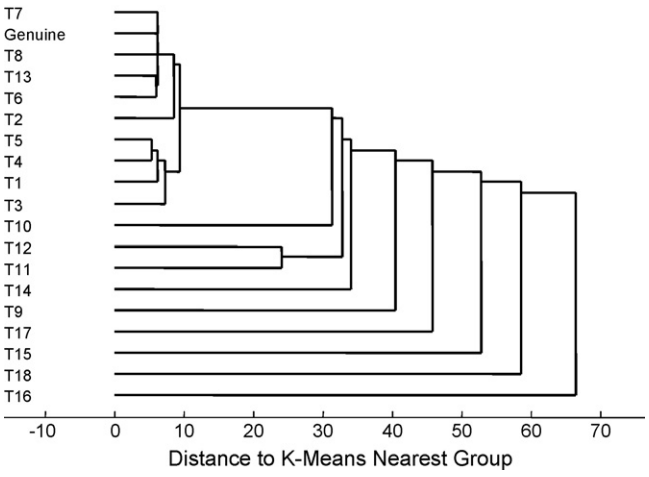

Fig. 6. Dendrogram of the 18 Viagra® tablets and genuine Viagra® tablet.

best results. The mean-centered dataset was reduced, retaining the first eight principal components. Subsequently, K-means clustering was applied, by using the nearest group clustering algorithm and Euclidean distance in the eight-dimensional principal component space. The resulting dendrogram is presented in Fig. 6.

In this dataset, a clear cluster is observed containing the spectra of tablets 1–8, tablet 13 and the genuine tablet. This group contains Raman spectra of the genuine Viagra® tablets. The other tablets are on a distinct distance from this group, showing that chemometric methods are able to discriminate genuine from counterfeit Viagra® tablets. Amongst the counterfeit spectra, the dendrogram seems to show some tailing effect which is a consequence from the minute differences that are observed between these falsifications. Most of these counterfeits are different from each other. Only tablets 11, 12 and 14 resemble in the sense that they all lack the cellulose additive, which is present in genuine Viagra®. Apparently, from the cluster diagram tablets 11 and 12 seem to be more closely related than 14. Although, large-scale research is needed before final conclusions can be drawn, indication of relationships between the counterfeit tablets might contain useful information in forensics in order to track down the origin of these products.

4. Conclusion

Raman spectroscopy has been proposed as a fast and reliable qualitative technique for the detection of counterfeit Viagra® tablets. In this work, 18 tablets were examined. From visual inspection, 3 tablets (16–18) could be assigned as counterfeit, whereas Raman spectroscopy was able to detect 9 counterfeit tablets (9–12 and 14–15) from our test set. Although, these tablets contain the active ingredient sildenafil citrate, they can be considered as counterfeit, since they contain less or other inactive compounds. In the spectra of counterfeit Viagra® tablets, Raman bands of barium sulphate, calcium sulphate, calcium carbonate, mannitol and sucrose could be detected. The other tablets (1–8 and 13) show an identical Raman spectrum compared to the Raman spectrum of the genuine Viagra®. By using a combined approach of principal components analysis (PCA) and hierarchical cluster analysis (HCA), with Raman spectroscopy it was possible to design an automated approach to distinguish between genuine and counterfeit tablets. The latter turns out to be useful when considering this technique for screening by non-specialist operators, for instance at customs.

Acknowledgements

The authors wish to thank Roy Vancauwenbergh from the FAGG (Federal Agency for Medicines and Health Products), Belgium and Pol Meuleniere from customs at Brussels Airport, Belgium for their cooperation with this project by supplying the seized Viagra® tablets. Peter Vandenabeele wishes to acknowledge the Research Foundation—Flanders (FWO-Vlaanderen) for his postdoctoral Grant.

References

[1] Patient Information Sheet Viagra®, US. Department of Health and Human Services, Food and Drug Administration, Rockville MD, USA, June 2005.
[2] L. Blok-Tip, H. Vogelpoel, M.J. Vredenbregt, D.M. Barends, D. de Kaste, RIVM Report 267041001/2005, Bilthoven, The Netherlands.
[3] National Specified List of Susceptible Products, National Association of Boards of Pharmacy, Mount Prospect II, USA, December 28th 2004.
[4] Counterfeit Drugs, guidelines for the development of measures to combat counterfeit drugs, Department of Essential Drugs and Other Medicines, World Health Organization, Geneva, Switzerland, 1999.
[5] J.J. Berzas, J. Rodríguez, M.J. Villaseñor, A.M. Contento, M.P. Cabello, Chromatographia 55 (2002) 601–606.
[6] A.M. Othman, N.M.H. Rizk, M.S. El-shahawi, Anal Chim Acta 515 (2004) 303–309.
[7] I. Wawer, M. Pisklak, Z. Chilmonczyk, J. Pharm. Biomed. Anal. 38 (2005) 865–870.
[8] N.D. Dinesh, P. Nagaraja, N.M. Made Gowda, K.S. Rangappa, Talanta 57 (2002) 757–764.
[9] M.J. Vredenbregt, L. Blok-Tip, R. Hoogerbrugge, D.M. Barends, D. de Kaste, J. Pharm. Biomed. Anal. 40 (2006) 840–849.
[10] Y. Wang, G. Chen, Z. Zhu, J. Zhu, W. Lu, Int. J. Infrared Milli. W. 24 (7) (2003) 1177–1185.
[11] J. Rodriguez Flores, J.J. Berzas Nevado, G.C. Castañeda Peñalvo, N. Mora Diez, J. Chromatogr. B 811 (2004) 231–236.
[12] P. Zhou, S.S.Y. Oh, P.L. Hou, M.Y. Low, H.L. Koh, J. Chromatogr. A 1104 (2006) 113–122.
[13] E. Mikami, T. Ohno, H. Matsumoto, Forensic Sci. Int. 130 (2002) 140–146.
[14] Q. Liang, J. Qu, G. Luo, Y. Wang, J. Pharm. Biomed. Anal. 40 (2006) 305–311.
[15] J.K. Maurin, F. Pluciński, A.P. Mazurek, Z. Fijalek, J. Pharm. Biomed. Anal. 43 (2007) 1514–1518.
[16] P.N. Newton, R. McGready, F. Fernandez, M.D. Green, M. Sunjio, C. Bruneton, S. Phanouvong, P. Millet, C.J.M. Whitty, A.O. Talisuna, S. Proux, E.M. Christophel, G. Malenga, P. Singhhasivanon, K. Bojang, H. Kaur, K. Palmer, N.P.J. Day, B.M. Greenwood, F. Nosten, N.J. White, Plos. Med. 3 (6) (2006) 752–755.
[17] M. de Veij, P. Vandenabeele, L. Moens, Eur. Pharm. Rev. 3 (2005) 86–89.
[18] A.N. Ghebremeskel, C. Vemavarapu, M. Lodaya, Int. J. Pharm. 328 (2007) 119–129.
[19] I. Karabas, M.G. Orkoula, C.G. Kontoyannis, Talanta 71 (2007) 1382–1386.
[20] S. Sasic, Appl. Spectrosc. 61 (3) (2007) 239–250.
[21] J.F. Kauffman, M. Dellibovi, C.R. Cunningham, J. Pharm. Biomed. Anal. 43 (2007) 39–48.

[22] G.J. Vergote, T.R.M. De Beer, C. Vervaet, J.P. Remon, W.R.G. Baeyens, N. Diericx, F. Verpoort, Eur. J. Pharm. Sci. 21 (2004) 479–485.
[23] M. de Veij, P. Vandenabeele, K. Alter Hall, F.M. Fernandez, M.D. Green, N.J. White, A.M. Dondorp, P.N. Newton, L. Moens, J. Raman Spectrosc. 38 (2) (2007) 181–187.
[24] M.R. Witkowski, Am. Pharm. Rev. 8 (1) (2005) 56–60.
[25] G. Socrates, Infrared and Raman Characteristic Group Frequencies, 3rd ed., John Wiley and Sons, Chichester, 2001.
[26] LAB-0221-2.4, NDA 20-895/S-021, US. Department of Health and Human services, Food and Drug Administration, Rockville MD, USA, June 2005.
[27] A. Gajović, K. Furić, N. Tomaši, S. Popović, Ž. Skoko, S. Musić, J. Alloy. Compd. 398 (2005) 188–199.
[28] J. De Gelder, K. De Gussem, P. Vandenabeele, L. Moens, J. Raman Spectrosc. 38 (2007) 1133–1147.
[29] B. Wehling, P. Vandenabeele, L. Moens, R. Klockenkämper, A. von Bohlen, G. van Hooydonk, M. de Reu, Microchim. Acta 130 (1999) 253–260.
[30] I. Lee, S.W. Han, H.J. Choi, K. Kim, Adv. Mater. 13 (21) (2001) 1617–1620.
[31] B. O'Sullivan, P. Barrett, G. Hsiao, A. Carr, B. Glennon, Org. Process Res. Dev. 7 (2003) 977–982.
[32] A. Savitzky, M.J.E. Golay, Anal. Chem. 36 (1964) 1627–1639.