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Comparison of genuine, generic and counterfeit Cialis tablets using vibrational spectroscopy and statistical methods

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ABSTRACT

The dubious online market in phosphodiesterase type 5 inhibitors is growing on a global scale. Counterfeit medical products can represent health issues for the user and cause medical mistrust. Within this work, genuine Cialis containing the active pharmaceutical ingredient (API) tadalafil, its generics available in the Czech Republic and the Cialis tablets from questionable online pharmacies were analysed. The methods of infra-red and Raman spectroscopy were used for the identification of the counterfeit tablets and for the verification of their API and excipients. All 9 tablets from online pharmacies were counterfeit with 2 of them even containing a different API (sildenafil, vardenafil). In addition, Raman mapping was used to determine the API and excipients' distribution and, in combination with multivariate data analysis, to separate similar tablets in clusters and to identify the outliers. Scanning electron microscopy of the samples revealed that the process of a wet granulation of micronized API was used during the formulation of the tablets. This comprehensive approach of analysis can be used for advanced exploration of the dubious samples of various medical products.

1. Introduction

According to the OECD [1] and World Health Organization (WHO) [2], medicines for erectile dysfunction treatment are among the most frequently counterfeited. The main representatives of these lifestyle drugs, which act as inhibitors of phosphodiesterase type 5 (PDE5), are primarily Viagra with sildenafil citrate as its active pharmaceutical ingredient (API), Cialis with tadalafil as its API, Levitra with vardenafil and Spedra with avanafil. The advantage of Cialis over other PDE5 inhibitors is in the duration of action in the human organism – it has a terminal half-time of approximately 17.5 h in comparison to approximately 4–6 h for Viagra, Levitra and Spedra. The increasing phenomenon of counterfeit medicines is caused mostly by illegal online pharmacies where everyone can buy those prescription-only medicines without the embarrassment of consulting a doctor. The second advantage of ordering those counterfeits via online pharmacies for patients is a lower price than the price of the genuine medicine in a regular pharmacy but in comparison with the generic equivalent tablets, the price is similar. However, their unknown origin and composition may cause a potential health risk [3].

Substandard and counterfeit medical products, as defined by the WHO in 2017 [4], have an impact not only on the individual's health, but also on the economy, families, national health systems as well as on pharmaceutical companies. Moreover, the poor-quality of such medical products often caused by not following good manufacturing practice (GMP) could arouse mistrust for medicines in general. A comprehensive analysis of these dubious medical products is highly desirable as well as education of general public about the dangers purchasing medicines online.

In general, experts in the pharmaceutical field in cooperation with the WHO and national institutes of health are suggesting simple and also sophisticated forensic chemistry techniques to

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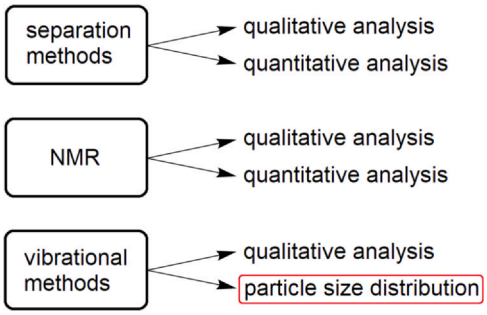

Fig. 1. The decision tree for appropriate methods of required pharmaceutical analysis.

detect, screen and analyse counterfeit medical products [5,6]. Beside visual inspection [7], the methods of thin layer chromatography (TLC) [8], colorimetry [9], chromatographic techniques [10,11], nuclear magnetic resonance (NMR) [12,13], X-ray fluorescence spectrometry [14], X-ray powder diffraction (XRD) analysis [15] and infra-red (IR) and Raman spectroscopy [16–22] are highly recommended. Several analytical methods have also been used to distinguish between counterfeit medical products of PDE5 inhibitors and the genuine products. Vibrational spectroscopy is one of those with the highest application potential in pharmacy for its reliability, speed, minimal (or no) need for sample preparation and non-destructiveness [23,24]. Another promising tool for the analysis of the counterfeit tablets is Raman mapping, which enables detailed analysis of the tablet composition and spatial distribution [25]. Moreover, in combination with a multivariate data analysis, it could help to reveal more significant modifications during the counterfeiting of tablets [26,27]. Furthermore, when deciding which analytical method to choose (Fig. 1), vibrational spectroscopy combined with statistical methods is unique among separation methods and NMR in its capability of components and particle size distribution, which is highly desirable for the pharmaceutical industry. This method has already been used for the analysis of the counterfeit Viagra tablet core [28,29] and provided information on the particle distribution, size as well as the excipients used and even for a simple analysis of two counterfeit Cialis tables [30]. However, there is still a lack of detailed studies on other PDE5 inhibitors using Raman mapping.

Our intent was to combine the common methods of IR and Raman spectroscopy and explore the possibilities of an innovative approach for reliable Raman imaging of the Cialis tablets in connection with the statistical method based on using the principal component analysis (PCA) and soft independent modelling of class analogy (SIMCA). The aim was to distinguish between the counterfeit medical products and the genuine or generic medicines, to examine their quality, to identify the APIs and the excipients used, to determine the particle size distribution and to inspect the dubious online market in Cialis which is a prescription-only medical product in the Czech Republic. Furthermore, we analysed the samples using scanning electron microscopy (SEM) to confirm the mechanisms of their formulation and to verify the API distribution from Raman mapping.

2. Material and methods

2.1. Samples and visual inspection

One original batch (with imprint of batch number on the secondary package) of genuine Cialis and generics available in the Czech Republic – Gerocilan, Rakifre, Tadalafil Accord, Tadalafil Mylan, Tadalafil Teva, Tadilecto and Zenavil – containing 20 mg of tadalafil were purchased from a local pharmacy in Prague.

Cialis is a prescription-only drug in the Czech Republic, but some online pharmacies offer it without the need for a prescription. We ordered 9 batches of Cialis (20 mg of tadalafil) from different dubious online pharmacies assuming that they were counterfeits, which was subsequently confirmed by the analyses below. These samples were labelled with the letters A to I in this work.

First, the tablets were photographed on both sides using an iPhone 6S (Apple Inc., USA) with a forensic ruler and then weighed on analytical balances (Denver Instrument, USA).

All the samples were stored at ambient temperature and protected from light.

2.2. Infra-red spectroscopy

To measure the IR spectra, a Nicolet 6700 Fourier-transform infra-red (FT-IR) spectrometer (Thermo Fisher Scientific, USA) equipped with a DTGS (deuterated triglycine sulphate) detector and an ZnSe attenuated total reflection (ATR) accessory was used. The coating of the tablets was removed using a razor blade and the uncoated tablet was then ground with an agate mortar with a pestle into a powder. The spectra were recorded in a spectral region of 4000–600 cm^{-1} with an accumulation of 32 scans and a resolution of 4 cm^{-1} at ambient temperature. The final spectrum was averaged from three individual measurements of the ground uncoated tablet powder. Before each sample measurement, the IRE was cleaned with ethanol and the background was measured.

2.3. Raman spectroscopy

The Raman spectra were recorded using the MultiRAM FT-Raman spectrometer (Bruker, Germany) equipped with an Nd:YAG laser (1064 nm) and a Ge detector at liquid nitrogen temperature in the spectral region of 4000–100 cm^{-1} with an accumulation of 64 scans and a resolution of 4 cm^{-1}. The laser power on the sample was approximately 180 mW. The powder of the ground uncoated tablet was analysed in glass cell three times and those spectra were averaged to gain the final spectrum.

2.4. Raman mapping

The Raman maps were recorded using the dispersive inVia Reflex Raman microscope (Renishaw, UK) coupled with an integrated Leica microscope and equipped with a high sensitivity ultra-low noise CCD detector Renishaw RenCam (Renishaw, UK), an XYZ motorised stage and an excitation diode laser at a wavelength of 785 nm. Each tablet was sealed in the paraffin block and cut on a Leica RM2255 rotary microtome (Leica Biosystems Nussloch GmbH, Germany) several times with a step of 10 µm to reach approximately the middle of the tablet core and to obtain a section as smooth as possible to avoid spectral artefacts. The measurements were performed using a lens with a magnification of 50 (0.75 N.A.) to display even small particles in the formulations, a monochromator utilised dual gratings of 1200 grooves/mm, a resolution of 0.5–1 cm^{-1} and an exposure time of 0.1 s. The incident laser power on the sample was approximately 30 or 15 mW (because of high fluorescence of tablets B, D, G, E). Approximately an 860 × 550 µm sample area was scanned with a step size of 4 µm, and the spectral range was set to 727–1812 cm^{-1}. More than 30 000 spectra were collected during approximately a one-hour measurement at one area of the tablet core, while measuring every tablet core at three different areas to avoid distorted information.

The data pre-processing including the normalisation of the raw spectra to the intensity of the highest band to equal 1 and the cosmic ray removal of spectral artefacts were performed in a WiRE 4.1 program (Renishaw, UK). No additional smoothing or baseline correction was performed.

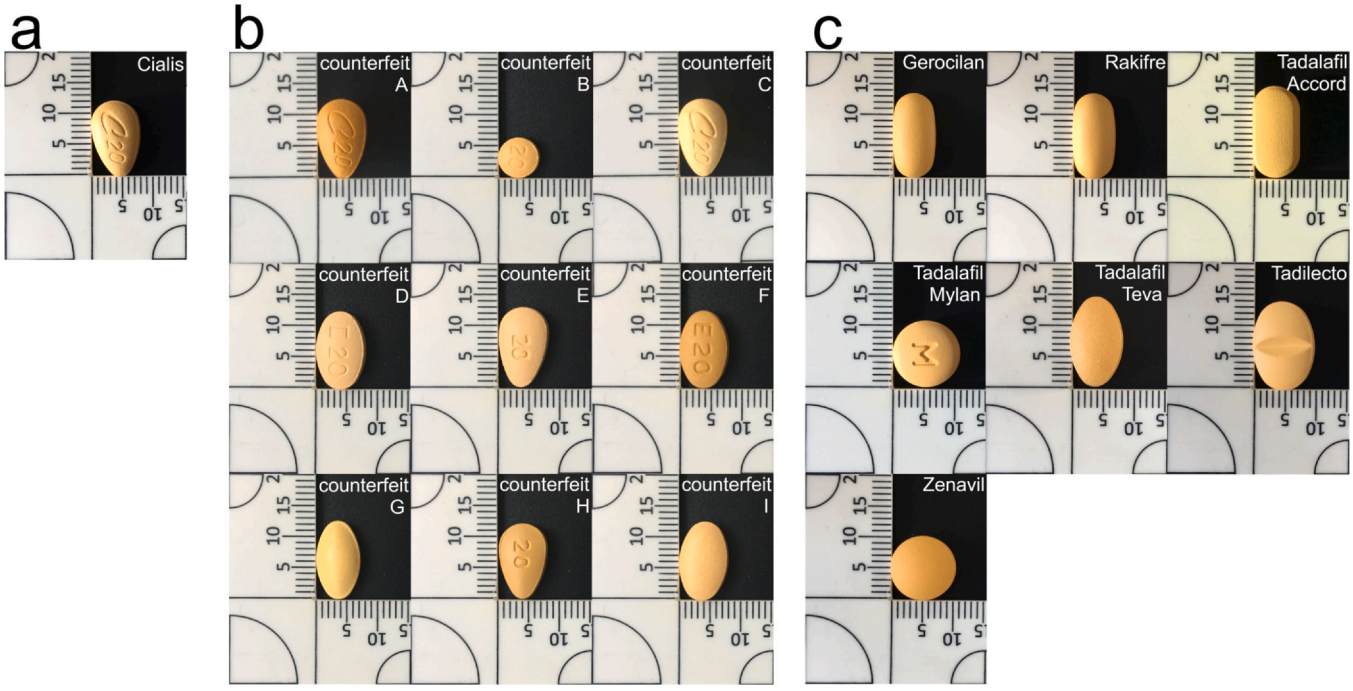

Fig. 2. Photo documentation of the front side of original Cialis (a), and the counterfeit Cialis tablets A to I (b) and generics of Cialis available in the Czech Republic – Gerocilan, Rakifre, Tadalafil Accord, Tadalafil Mylan, Tadalafil Teva, Tadilecto and Zenavil (c).

Table 1
The composition of the genuine and counterfeit Cialis tablets A to I. MCC = microcrystalline cellulose, HPC = hypromellose, SLS = sodium lauryl sulphate.

	Cialis	A	B	C	D	E	F	G	H	I
Tadalafil	x			x	x	x	x	x	x	x
Sildenafil		x								
Vardenafil			x							
MCC	x	x	x	x	x	x	x	x	x	x
Lactose monohydrate	x		x		x	x	x	x	x	x
HPC	x		x				x	x	x	
Croscarmellose Na	x		x		x	x	x	x	x	x
Stearate Mg	x	x	x	x	x	x	x	x	x	x
SLS	x		x		x	x	x	x	x	x
Starch		x			x	x				
Povidone										x
Amberlite					x	x				
$CaCO_3$		x			x	x				
Unknown		x			x					

2.5. Data processing and statistical analysis

To evaluate the Raman maps, a direct classical least squares (DCLS) algorithm, one of the most used chemometric method in the imaging processing [27,29], was used. For this method, only the reference spectra of the pure APIs and excipients were necessary, without a complete calibration process [27]. The APIs and excipients were identified in all tablets during the previous IR or Raman spectroscopy analysis. Then, the DCLS method sought the linear combination of those spectra to find the best match with each point (each spectrum) in the map. In this way, we were able to construct the Raman maps of the tablets while taking into consideration also the spatial distribution of the components and particle size.

To carry out the statistical analyses, individual Raman spectra were extracted from the Raman maps. Due to the large amount of data, we were forced to reduce the number of input data from the Raman mapping of one tablet, which was approximately around 90,000 (3 × 30,000) Raman spectra. To do so, approximately 30,240 Raman spectra from one Raman map were divided into 100 individual segments, each with the same number of spectra. Then, the spectra from each segment were averaged using the in-house built program SpectraHelper [31] resulting in 100 new spectra in total. That means that 300 (3 × 100) averaged spectra were obtained for the constitution of 3 maps of each tablet.

As is usual for multivariate image analysis, it has to deal with a large number of variables with a high correlation between them. To overcome this obstacle, PCA is usually the first choice method reducing the number of variables of a data set while preserving as much information as possible in the form of new latent uncorrelated variables – principal components (PCs) [32,33]. Those PCs describe most of the variability in the multivariate image while the remaining

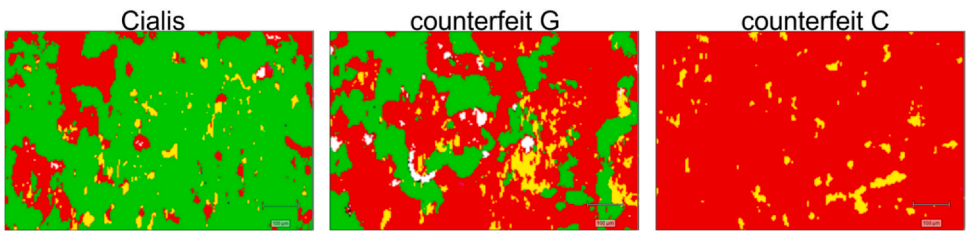

Fig. 3. A comparison of the Raman maps of genuine Cialis (left) and the counterfeit tablets G (middle) and C (right) with 50× magnification and 4 µm step used. The Raman maps consist mainly of yellow parts for tadalafil, green parts for lactose monohydrate, red parts for MCC and white parts for croscarmellose sodium. Very small parts of maps are labelled dark blue for HPC, magenta for stearate magnesium and cyan for SLS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

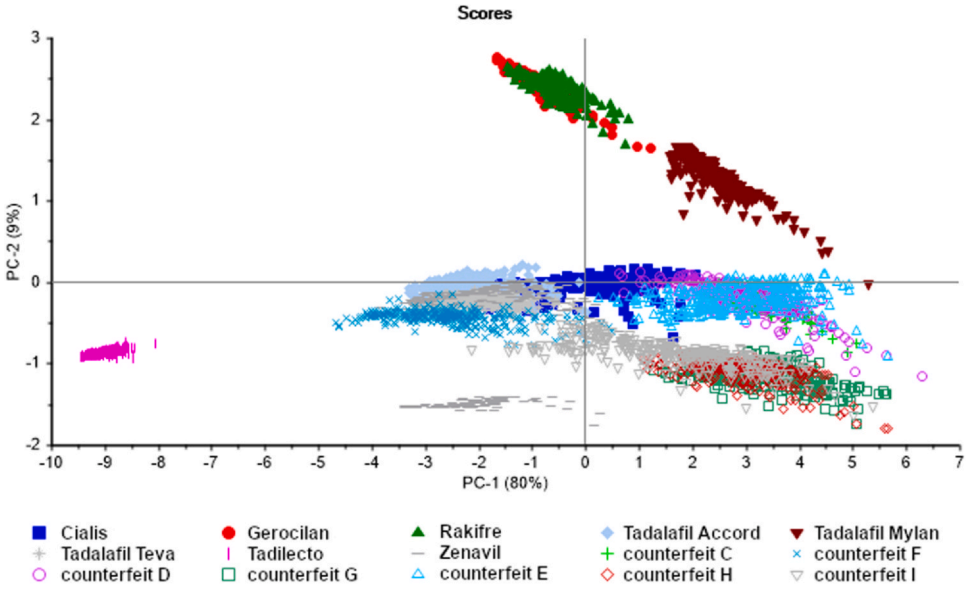

Fig. 4. The PCA scores plot for genuine Cialis, the generic tablets (Gerocilan, Rakifre, Tadalafil Accord, Tadalafil Mylan, Tadalafil Teva, Tadilecto, Zenavil) and the counterfeit tablets (C, D, E, F, G, H, I) with the labelled clusters.

variables can be eliminated as noise. To quantify the differences between the input data, a supervised classification algorithm SIMCA (constructing multidimensional space around the model of the target class derived by PCA) was employed [34]. The individual PCA models for each sample of the counterfeit, generic and genuine tablets were made and their mutual distances from each other were evaluated by SIMCA. This approach allowed us to check the similarity of the studied tablets. All statistical methods were processed in The Unscrambler X 10.5.1 (CAMO Software, Norway).

2.6. Scanning electron microscopy

For the sample analysis, the tablets in the paraffine blocks were cut on a microtome or a small amount of powder from the ground tablets was fixed on an adhesive carbon tape on an aluminium disc. Subsequently, the samples were coated by a layer of platinum using a Q150R ES Plus sputter coater (Quorum, UK) and the final thickness of the Pt layer (5 nm) was measured by an integrated film thickness monitor (FTM) crystal. The morphological evaluation of the samples by

Table 2
The SIMCA result for the individual PCA models as a distance matrix with a unit diagonal for genuine Cialis, the generic tablets (Gerocilan, Rakifre, Tadalafil Accord, Tadalafil Mylan, Tadalafil Teva, Tadilecto, Zenavil) and the counterfeit tablets (C, D, E, F, G, H, I).

	Cialis	Gerocilan	Rakifre	Tadalafil Accord	Tadalafil Mylan	Tadalafil_ Teva	Tadilecto	Zenavil	C	F	D	G	E	H	I
Cialis	**1**	356	375	31	268	83	726	59	149	16	47	16	14	32	47
Gerocilan	356	**1**	9	597	83	689	1870	244	521	464	176	351	179	358	237
Rakifre	375	9	**1**	633	70	708	2277	295	443	531	177	342	169	351	263
Tadalafil_Accord	31	597	633	**1**	449	85	1397	67	314	10	71	19	15	15	13
Tadalafil_Mylan	268	83	70	449	**1**	485	2461	213	227	399	93	219	88	219	150
Tadalafil_Teva	83	689	708	85	485	**1**	908	126	722	61	263	281	136	347	162
Tadilecto	726	1870	2277	1397	2461	908	**1**	401	2992	628	530	980	447	1120	439
Zenavil	59	244	295	67	213	126	401	**1**	158	57	77	92	53	85	58
C	149	521	443	314	227	722	2992	158	**1**	259	44	133	29	172	113
F	16	464	531	10	399	61	628	57	259	**1**	73	15	18	12	16
D	47	176	177	71	93	263	530	77	44	73	**1**	38	13	40	43
G	16	351	342	19	219	281	980	92	133	15	38	**1**	16	16	14
E	14	179	169	15	88	136	447	53	29	18	13	16	**1**	22	26
H	32	358	351	15	219	347	1120	85	172	12	40	16	22	**1**	7
I	47	237	263	13	150	162	439	58	113	16	43	14	26	7	**1**

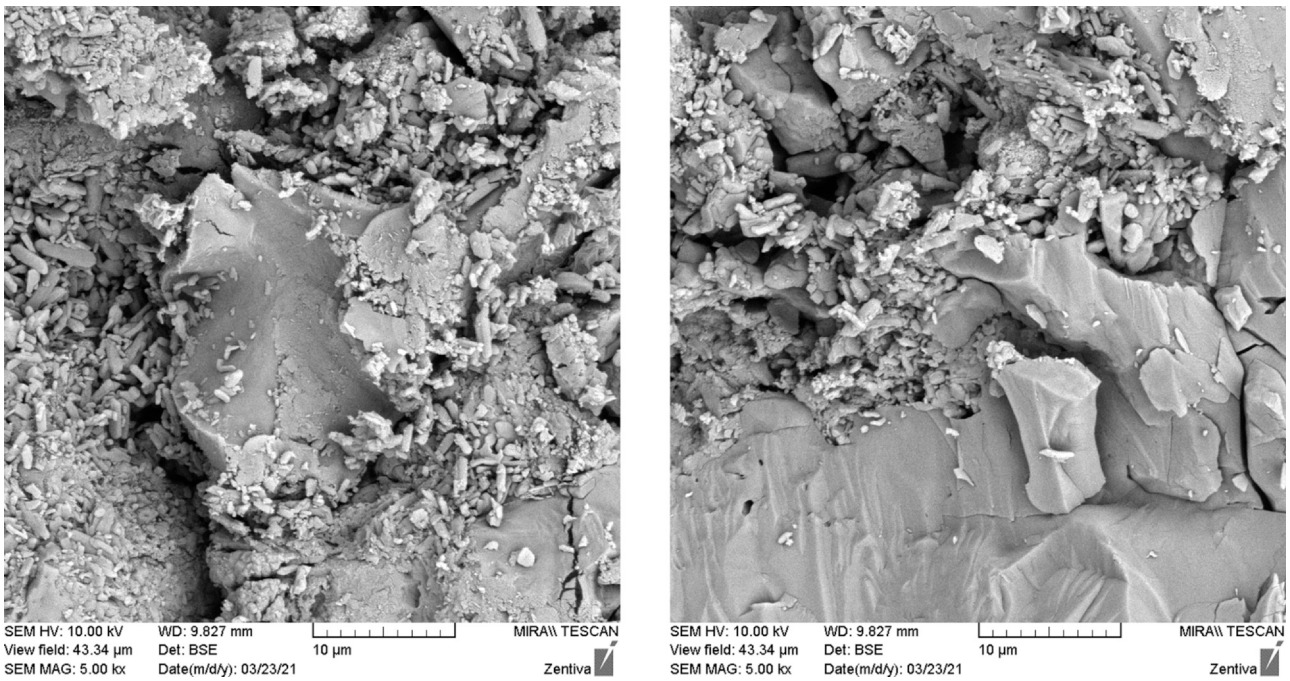

Fig. 5. The SEM images of the counterfeit Cialis tablet F at 5000× magnification. Example of the detailed image of the API separated clusters with lactose measured at two different positions of the same tablet.

SEM was performed using a Mira/Tescan II LM (Tescan a.s., Czech Republic) with a backscattered-electron (BSE) detector. Magnification of 1000× and 5000× were used for the final imaging of the SEM photos.

3. Results and discussion

3.1. Visual inception of the tablets

From all the studied counterfeit tablets (Fig. 2, b and Supplementary material – Fig. S1, b), only tablets A and C corresponded to genuine Cialis (Fig. 2, Fig. S1, a) with the almond shape, size and imprint. However, their colour shade was slightly different. The counterfeit tablets E and H were of a similar shape to genuine; the size and imprint were different. The counterfeit tablets B, D, F, G and I were of an elliptical shape. The generics of Cialis (Figs. 2, S1, c) were of an elliptical or circular shape.

3.2. Infra-red and Raman spectroscopy

Based on the Raman and IR spectroscopy analysis (Fig. S2), the API and the main excipients were identified in all the counterfeit tablets (Table 1) by comparing the spectra of the tablets with the spectra of the individual substances measured previously in-house by employing the in-house built spectral library. Comparisons were carried out by assigning the individual vibrational bands in the Raman spectra of the tablets to the vibrational bands of API and each excipient (demonstrated in Fig. S3). Those excipients were subsequently verified by Raman mapping. Instead of the API tadalafil, sildenafil (both citrate and base) and vardenafil were found in the counterfeit tablets A and B, respectively. For this reason, those tablets were excluded from further statistical analyses. The most similar counterfeit tablets to genuine Cialis according to the Raman and IR spectra include tablets F, G, H and I with the same composition differing in a maximum of one component. Using Raman mapping, also a small amount of additional excipients could be identified: amberlite in the counterfeit tablets D and E, and $CaCO_3$ in the counterfeit tablets A, D and E. In the core of the tablets A and D, an identical component has not been identified yet.

3.3. Raman mapping

The Raman mapping provided a powerful method for analysing the counterfeit Cialis tablets and their discrimination from genuine Cialis. Even if the composition of the counterfeit tablet was very similar to genuine Cialis, the Raman maps of those tablets were different. In Fig. 3, there is an example of the Raman spectra of genuine Cialis and the counterfeit tablets G (very similar composition to the genuine) and C (only similar composition to the genuine). The difference between genuine Cialis and the counterfeit tablet G was noticeable at a glance due to the different spatial distribution of the API and the main excipients (MCC and lactose monohydrate). Raman maps of the rest of the counterfeit Cialis tablets can be found in Supplementary material (Fig. S4). In comparison with the consistent distribution of the particles and good homogeneity of the genuine Cialis tablet, the API in the counterfeit tablets was in separated clusters with lactose. Using Raman mapping, a small amount of $CaCO_3$ was found in the counterfeit tablets A, D and E and a negligible amount of amberlite in tablets D and E. One unknown substance in a very small amount was found in tablets A and D and has not yet been identified.

3.4. Principal component analysis

The data from the Raman maps processed with the DCLS method were used for PCA to provide a reliable comparison of the genuine, generic and counterfeit tablets. First, the PCA explained variance plot (Supplementary material, Fig. S5) gave us information on how many PCs should be used for further evaluation. The first and second PC explain 80% and 9% of variance, respectively. In general, 89% of total variability are enough for our purpose. The loading plots of PC-1 and PC-2 (Supplementary material, Fig. S6) showed the most relevant variables for the variance explanation, in this case it means mostly the bands reflecting the API and main excipients: lactose monohydrate and MCC. Positive and negative correlation between bands of PC-1 showed that the content of MCC depended on the content of lactose. If the content of MCC grew, the content of lactose decreased. In the PCA score plot (Fig. 4), each score represented one averaged

spectrum and the scores located near each other meant that the spectra have a similar pattern while the distant scores suggest differences [35]. This helps to reveal the similar patterns, clusters and also outliers. The scores of genuine Cialis could be found in the middle of the four quadrants. The cluster of the generics Rakifre and Gerocilan was separated above, along PC-2. The scores of Tadalafil Mylan could be found close to it. The cluster of two generics (Tadalafil Accord and Zenavil) together with the counterfeit tablet F was separated along the PC-1 towards the negative values. The last cluster consisted of the counterfeit tablets D, E, G, H and I and was shifted to the more positive values of PC-1 and negative values of PC-2 in relation to genuine Cialis. The most outlying were the scores of generic Tadilecto separated mostly along PC-1 towards the negative values. This separation was caused by the presence of the different excipients hypromellose (HPMC) and mannitol instead of MCC and lactose.

According to the PCA, the most similar to genuine Cialis were the counterfeit tablets D and E. In general, the counterfeit tablets were more like Cialis than generics as was expected since they try to mimic the genuine tablet. Clusters differences of generic Cialis tablets from original Cialis show the effort of generic pharma companies to produce bioequivalent products by patent non-infringing formulations. On the other hand, the overlap of counterfeit clusters with genuine Cialis is significant. Therefore, the SIMCA being a supervised method was applied on the data to analyse possible differences of counterfeits and Cialis. The SIMCA compares the distances of the individual cluster centres of tablet's spectra. These determined distances of the cluster centres from each other therefore show the similarity of individual tablets. If the distances are small, the models are close together or even overlap. Large distances between models indicate that they are different.

3.5. Soft independent modelling of class analogies

SIMCA was used as a dependent method on the previous PCA models of the individual tablets to provide a quantitative classification of those models. The result is a matrix with a unit diagonal (Table 2) based on the measurement of the distances between the individual PCA models to find the similarities between them. Lower distances (values in units, dozens) mean greater mutual similarity, higher distances (hundreds, thousands) then lesser similarity of the PCA models. In our case, it was obvious that the distances of the counterfeit tablets F, D, G, E, H, I were low, specifically in dozens, reflecting their mutual similarity. To compare, the distances of the generics were in thousands as generic pharma companies try to set themselves apart from the original. For Cialis, the distances from the other models were very different. The lowest distances were observed for the counterfeit medicines F, D, G, E, H, I. However, even such small distances (shown in Table 2) enabled us to differentiate counterfeits from genuine Cialis. The highest distance of the Cialis PCA model was observed in comparison to the generic Tadilecto PCA model.

3.6. Scanning electron microscope

To gain more information about the tablets, SEM was used allowing us to verify that crystalline and micronized tadalafil was incorporated into genuine Cialis by the method of a wet granulation [36] resulting in tablets with good homogeneity. Inhomogeneous blends can evince discrepancy in the content of the API and possibly affect the quality of the final product. The wet granulation is based on the production of the granules by wet massing of the excipients and API with granulation liquid with or without binder and subsequent drying of the mass [37]. Based on the results from Raman mapping and SEM, formulations of the counterfeit Cialis tablets were probably also made by a wet granulation from micronized tadalafil with particle size of ~2 μm as well and the API was mostly in the bounded clusters with lactose (Fig. 5). This formulation is evident due to the API particles being stuck to larger lactose particles and the crystals not having regular and sharp shapes.

4. Conclusion

In this study, we have focused on an analysis of genuine Cialis, its generics available in the Czech Republic and the Cialis tablets purchased from dubious online pharmacies. The aim was to investigate the online market with Cialis, detect the counterfeit tablets, identify the APIs and excipients used in those tablets and compare them with the genuine medicines also containing 20 mg of tadalafil as an API.

Our comprehensive approach embraced the methods of IR and Raman spectroscopy, SEM and Raman mapping along with the multicomponent statistical method PCA to explore each tablet. All the Cialis tablets from dubious online pharmacies were counterfeit while two (tablets A and B) of the nine samples even contained a different API (sildenafil and vardenafil). Those tablets were excluded from further PCA. Even if the IR and Raman spectra of the ground tablets were very similar to genuine Cialis in four cases, Raman mapping provided reliable discrimination of all counterfeit tablets. Besides, PCA supported by SIMCA applied to the Raman mapping data allowed separation of the individual clusters of the generics and counterfeit Cialis tablets according to their different chemical profiles and distinguished successfully between the genuine, generic and counterfeit Cialis samples. Using SEM, the wet granulation of micronized tadalafil was identified as the formulation method for the counterfeit tablets.

A routine analysis is usually not able to detect well-made counterfeit medical products. The proposed combination of several analytical methods together with statistical evaluation is much more reliable. Moreover, the information obtained by Raman mapping is important not only for the identification of the counterfeit, but also for determining the formulation process used, the distribution and the particle size or clusters of API and excipients. This information can be especially valuable for generic pharmaceutical companies looking to draw ideas for their manufacturing process. In addition, the knowledge of particle distribution can help to identify unauthorised products.

The proposed combination of different methods for the analysis of the dubious medical products resulted in a comprehensive approach for gaining maximum information about the samples.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

We prefer colour images for the online version and black-and-white images for the printed version.

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CRediT authorship contribution statement

Dita Spálovská: Conceptualization, Formal analysis, Writing – original draft, Methodology. **Tomáš Pekárek**: Conceptualization, Methodology, Writing – review & editing. **Martin Kuchař**: Conceptualization, Writing – review & editing. **Vladimír Setnička**: Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpba.2021.114383.

References

[1] OECD/EUIPO, Trade in Counterfeit Pharmaceutical Products, Illicit Trade, OECD Publishing, Paris, 2020.
[2] WHO, Global Surveillance and Monitoring System for substandard and falsified medical products, World Health Organization, Geneva, 2017.
[3] K. Dégardin, Y. Roggo, P. Margot, Understanding and fighting the medicine counterfeit market, J. Pharm. Biomed. Anal. 87 (2014) 167–175, https://doi.org/10.1016/j.jpba.2013.01.009
[4] WHO, Member State mechanism on substandard/spurious/falsely-labelled/falsified/ counterfeit medical products 2017. In *A70/23*, Seventieth world health assembly. ⟨https://apps.who.int/gb/ebwha/pdf_files/WHA70/A70_23-en.pdf⟩ (accessed 31 May 2021).
[5] IOM (Institute of Medicine), Countering the Problem of Falsified and Substandard Drugs, National AcademiesPress, Washington D.C., 2013.
[6] L. Roth, K.B. Biggs, D.K. Bempong, Substandard and falsified medicine screening technologies, AAPS Open 5 (2019) 2, https://doi.org/10.1186/s41120-019-0031-y
[7] International Pharmaceutical Inspection, Tool for visual inspection of medicines. ⟨https://www.whpa.org/sites/default/files/2018–12/Toolkit_BeAware_Inspection.pdf⟩ (accessed 8 April 2021).
[8] E.A. Abourashed, M.S. Abdel-Kader, A.-A.M. Habib, HPTLC determination of sildenafil in pharmaceutical products and aphrodisiac herbal preparations, JPC-J. Planar Chromat. 18 (2005) 372–376, https://doi.org/10.1556/JPC.18.2005.5.7
[9] A.L. Rodomonte, M.C. Gaudiano, E. Antoniella, D. Lucente, V. Crusco, M. Bartolomei, P. Bertocchi, L. Manna, L. Valvo, F. Alhaique, N. Muleri, Counterfeit drugs detection by measurement of tablets and secondary packaging colour, J. Pharm. Biomed. Anal. 53 (2010) 215–220, https://doi.org/10.1016/j.jpba.2010.03.044
[10] M.A. Abdelshakour, R.A. Abdel Salam, G.M. Hadad, D.M. Abo-ElMatty, E.A. Abdel Hameed, HPLC-UV and UPLC-MS/MS methods for the simultaneous analysis of sildenafil, vardenafil, and tadalafil and their counterfeits dapoxetine, paroxetine, citalopram, tramadol, and yohimbine in aphrodisiac products, RSC Adv. 11 (2021) 8055–8064, https://doi.org/10.1039/D0RA10324A
[11] B.J. Venhuis, G. Zomer, M.J. Vredenbregt, D. de Kaste, The identification of (−)-trans-tadalafil, tadalafil, and sildenafil in counterfeit Cialis® and the optical purity of tadalafil stereoisomers, J. Pharm. Biomed. Anal. 51 (2010) 723–727, https://doi.org/10.1016/j.jpba.2009.08.010
[12] P.H.J. Keizers, F. Bakker, J. Ferreira, P.F.K. Wackers, D. van Kollenburg, E. van der Aa, A. van Beers, Benchtop NMR spectroscopy in the analysis of substandard and falsified medicines as well as illegal drugs, J. Pharm. Biomed. Anal. 178 (2020) 112939, https://doi.org/10.1016/j.jpba.2019.112939
[13] S. Wilczyński, M. Petelenz, M. Florek-Wojciechowska, S. Kulesza, S. Brym, B. Błońska-Fajfrowska, D. Kruk, Verification of the authenticity of drugs by means of NMR relaxometry—Viagra® as an example, J. Pharm. Biomed. Anal. 135 (2017) 199–205, https://doi.org/10.1016/j.jpba.2016.12.018
[14] R.S. Ortiz, K.C. Mariotti, N.V. Schwab, G.P. Sabin, W.F.C. Rocha, E.V.R. de Castro, R.P. Limberger, P. Mayorga, M.I.M.S. Bueno, W. Romão, Fingerprinting of sildenafil citrate and tadalafil tablets in pharmaceutical formulations via X-ray fluorescence (XRF) spectrometry, J. Pharm. Biomed. Anal. 58 (2012) 7–11, https://doi.org/10.1016/j.jpba.2011.09.005
[15] J.K. Maurin, F. Pluciński, A.P. Mazurek, Z. Fijałek, The usefulness of simple X-ray powder diffraction analysis for counterfeit control—The Viagra® example, J. Pharm. Biomed. Anal. 43 (2007) 1514–1518, https://doi.org/10.1016/j.jpba.2006.10.033
[16] T. Sanada, N. Yoshida, R. Matsushita, K. Kimura, H. Tsuboi, Falsified tadalafil tablets distributed in Japan via the internet, Forensic Sci. Int. 307 (2020) 110143, https://doi.org/10.1016/j.forsciint.2020.110143
[17] R.S. Ortiz, Kd.C. Mariotti, B.; Fank, R.P. Limberger, M.J. Anzanello, P. Mayorga, Counterfeit Cialis and Viagra fingerprinting by ATR-FTIR spectroscopy with chemometry: Can the same pharmaceutical powder mixture be used to falsify two medicines? Forensic Sci. Int. 226 (2013) 282–289, https://doi.org/10.1016/j.forsciint.2013.01.043
[18] S. Singh, B. Prasad, A.A. Savaliya, R.P. Shah, V.M. Gohil, A. Kaur, Strategies for characterizing sildenafil, vardenafil, tadalafil and their analogues in herbal dietary supplements, and detecting counterfeit products containing these drugs, TrAC Trends Anal. Chem. 28 (2009) 13–28, https://doi.org/10.1016/j.trac.2008.09.004
[19] P.-Y. Sacré, E. Deconinck, T. De Beer, P. Courselle, R. Vancauwenberghe, P. Chiap, J. Crommen, J.O. De Beer, Comparison and combination of spectroscopic techniques for the detection of counterfeit medicines, J. Pharm. Biomed. Anal. 53 (2010) 445–453, https://doi.org/10.1016/j.jpba.2010.05.012
[20] D. Custers, S. Vandemoortele, J.-L. Bothy, J.O. De Beer, P. Courselle, S. Apers, E. Deconinck, Physical profiling and IR spectroscopy: simple and effective methods to discriminate between genuine and counterfeit samples of Viagra® and Cialis®, Drug Test. Anal. 8 (2016) 378–387, https://doi.org/10.1002/dta.1813
[21] Z. Tománková, V. Setnička, M. Urbanová, P. Matějka, V. Král, K. Volka, P. Bouř, Conformational flexibility of corey lactone derivatives indicated by absorption and vibrational circular dichroism spectra, J. Org. Chem. 69 (2004) 26–32, https://doi.org/10.1021/jo035099h
[22] O. Julínek, V. Setnička, A. Řezáčová, J. Dohnal, V. Vosátka, M. Urbanová, Product of alaptide synthesis: determination of the absolute configuration, J. Pharm. Biomed. Anal. 53 (2010) 958–961, https://doi.org/10.1016/j.jpba.2010.07.007
[23] A.S. Gilbert, R.W. Lancaster, IR and Raman spectroscopy, industrial applications, in: J.C. Lindon, G.E. Tranter, D.W. Koppenaal (Eds.), Encyclopedia of spectroscopy and spectrometry, third ed., Academic Press, Oxford, 2017, pp. 394–407.
[24] S. Šašić, Pharmaceutical Applications of Raman Spectroscopy, John Wiley & Sons, Inc, Hoboken, 2007.
[25] A. Paudel, D. Raijada, J. Rantanen, Raman spectroscopy in pharmaceutical product design, Adv. Drug Deliv. Rev. 89 (2015) 3–20, https://doi.org/10.1016/j.addr.2015.04.003
[26] R.A. Storey, I. Ymén, Solid State Characterization of Pharmaceuticals, Wiley-Blackwell, Hoboken, 2011.
[27] L. Zhang, M.J. Henson, S.S. Sekulic, Multivariate data analysis for Raman imaging of a model pharmaceutical tablet, Anal. Chim. Acta 545 (2005) 262–278, https://doi.org/10.1016/j.aca.2005.04.080
[28] P.-Y. Sacré, E. Deconinck, L. Saerens, T. De Beer, P. Courselle, R. Vancauwenberghe, P. Chiap, J. Crommen, J.O. De Beer, Detection of counterfeit Viagra® by Raman microspectroscopy imaging and multivariate analysis, J. Pharm. Biomed. Anal. 56 (2011) 454–461, https://doi.org/10.1016/j.jpba.2011.05.042
[29] H. Rebiere, M. Martin, C. Ghyselinck, P.-A. Bonnet, C. Brenier, Raman chemical imaging for spectroscopic screening and direct quantification of falsified drugs, J. Pharm. Biomed. Anal. 148 (2018) 316–323, https://doi.org/10.1016/j.jpba.2017.10.005
[30] K. Kwok, L.S. Taylor, Analysis of counterfeit Cialis® tablets using Raman microscopy and multivariate curve resolution, J. Pharm. Biomed. Anal. 66 (2012) 126–135, https://doi.org/10.1016/j.jpba.2012.03.026
[31] T. Čapková-Helešicová, T. Pekárek, M. Schöngut, P. Matějka, New designed special cells for Raman mapping of the disintegration process of pharmaceutical tablets, J. Pharm. Biomed. Anal. 168 (2019) 113–123, https://doi.org/10.1016/j.jpba.2019.02.019
[32] P. Geladi, H.F. Grahn, Multivariate and hyperspectral image analysis, in: R.A. Meyers (Ed.), Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation, John Wiley & Sons, Hoboken, 2008, , https://doi.org/10.1002/9780470027318.a8106.pub2
[33] J.B. Cooper, Chemometric analysis of Raman spectroscopic data for process control applications, Chemom. Intell. Lab. Syst. 46 (1999) 231–247, https://doi.org/10.1016/S0169-7439(98)00174-9
[34] S. Wold, M. Sjöström, SIMCA: a method for analyzing chemical data in terms of similarity and analogy, in: B.R. Kowalski (Ed.), Chemometrics: Theory and Application, American Chemical Society, Washington, 1977, pp. 243–282.
[35] J. Aaltonen, K.C. Gordon, C.J. Strachan, T. Rades, Perspectives in the use of spectroscopy to characterise pharmaceutical solids, Int. J. Pharm. 364 (2008) 159–169, https://doi.org/10.1016/j.ijpharm.2008.04.043
[36] European Medicines Agency, Scientific discussion, ⟨https://www.ema.europa.eu/en/documents/scientific-discussion-variation/cialis-h-c-436-ii-46-epar-scientific-discussion-variation_en.pdf⟩ (accessed 12 May 2021).
[37] S.K. Niazi, Handbook of Pharmaceutical Manufacturing Formulations: Compressed Solid Products, second ed...., CRC Press, Boca Raton, 2009.