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The use of hyperspectral imaging in the VNIR (400–1000 nm) and SWIR range (1000–2500 nm) for detecting counterfeit drugs with identical API composition

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ABSTRACT

The risk of death from taking counterfeit drugs is now greater than the probability of dying from malaria and AIDS combined (at least half a million deaths each year). At the same time, counterfeit medicines are falsified more and more "skillfully". According to WHO about 10% of counterfeit drugs are copies of original products.

The methods of hyperspectral imaging and image analysis and processing were used to detect counterfeit drugs.

Original Viagra® (Pfizer) and counterfeit tablets were compared. Hyperspectral imaging was used to acquire hyperspectral data cubes from both original and counterfeit tablets in the spectral range of 400–2500 nm. Spectral parameters for both the original Viagra® and counterfeit drugs were compared. Grey-Level Co-Occurrence Matrix (GLCM) analysis and Principal Component Analysis (PCA) were performed.

Hyperspectral analysis of the surface of the original Viagra® and counterfeit tablets demonstrates significant differences in reflectance (maximum difference for 1619.75 nm). The GLCM contrast for the falsified drug is on average higher than for the original one $16 \pm 4\%$.

GLCM contrast analysis enables to quantify homogeneity of distribution of tablet ingredients and enables to distinguish tablets with identical chemical composition. SWIR (1000–2500 nm) hyperspectral imaging has a definite advantage over imaging in VNIR (400–1000 nm) – higher wavelength is less sensitive to non-uniform illumination.

1. Introduction

The WHO (World Health Organisation) and FDA (Food and Drug Administration) estimate that counterfeit products can constitute about 10% of the global drug market: from about 1% in developed countries to 10–30% in some African, Asian and South American countries [1].

What is important is a sharp increase in the counterfeit medicine trade from approximately USD 35 billion in 2005 to about USD 75 billion in 2010 [1]. This phenomenon is particularly pronounced in the EU where in the years 2005–2006 there was a 384% increase in the number of seized counterfeit drugs and illegal medicinal products [2].

It should also be noted that the risk of death from taking counterfeit drugs is now greater than the probability of dying from malaria and AIDS combined (at least half a million deaths each year) [1].

In order to protect themselves from counterfeiting their medicinal products, manufacturers use techniques of labelling the packaging or the drug itself, which is to reduce the scale of the phenomenon [3,4]. In addition, different laboratory techniques are used to identify counterfeit drugs such as ATR-FTIR [5], UPLC-MS [6], XRF spectrometry [7], HPLC [8], Raman spectroscopy [9] and near infra-red spectroscopy [10–16]. The techniques used on site are also becoming more and more popular [17].

The methods of near-infra-red spectroscopy are becoming more readily used in pharmacy, inter alia, to detect counterfeit drugs [10–16]. However, attention should be paid to the

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differences between hyperspectral imaging and near-infra-red spectroscopy. Hyperspectral imaging enables not only to acquire the spectrum of a small tablet area but also to image the entire tablet surface at the same time. In addition, it allows for the spectral analysis of each pixel of the image separately, enabling among others, analysis of the homogeneity of ingredient distribution, which may be an additional diagnostic criterion in distinguishing between genuine and counterfeit drugs. At the same time, the use of image analysis and processing methods, including the applied method of principal component analysis (PCA), enables effective visualisation of the results.

The applied hyperspectral imaging technique enables to determine both morphological and molecular properties of the analysed objects. A hyperspectral camera performs image acquisition by recording the radiation of defined energy intensity (I) and the wavelength (λ) in a specific range of spatial coordinates (m, n) where m = row of the image matrix, n=column of the image matrix. The values Δm and Δn determine the spatial resolution of the image and the value Δλ determines the spectral resolution. The result is a series of images of the same object, each registered at a different wavelength. This allows for complex analysis of the optical spectra of the analysed object as well as the examination of the whole object using the methods of image analysis and processing in a wide spectral range. This enables, among others, preliminary identification of drug ingredients, defect detection, determination of the spatial distribution of the active ingredients [18,19].

The colour of the object being imaged, e.g. a solid dosage form, has important diagnostic significance. Importantly, the colour of the analysed object (pixels) depends on the properties of incident light, but mostly its reflective properties. In hyperspectral imaging, the colour is represented by the vector of reflectivity over a wide wavelength range [18]. This provides much more information than the three-component RGB system and allows for the multivariable analysis of analysed objects and, thus, far higher effectiveness in identifying counterfeit drugs.

In turn, molecular hyperspectral imaging is a technique in which each image pixel that includes the complete spectral cross-section (spectral signature) in the whole spectral range is additionally subjected to analysis [19]. This provides not only the image of an object (e.g. a tablet) in the wide, discrete spectral range, but also spectral information that allows for the quantitative analysis of the active pharmaceutical ingredient (API) and excipients [20]. The use of methods of image analysis and processing, such as those used herein, allows for the precise quantitative determination of the homogeneity of ingredient distribution.

The disadvantage of hyperspectral imaging is small penetration depth due to the scattering of photons on the object surface. The average depth of penetration depends on the imaging wavelength - the longer the wave, the deeper the penetration [21]. However, in the differential analysis of genuine and counterfeit drugs this limitation is not, in most cases, a significant disadvantage because the outer layers of original and counterfeit drugs have different hyperspectral properties [11,14,16].

In the case of uncoated tablets, where lactose, carboxymethylcellulose (CMC) or starch is the most common main excipient, identification of hyperspectral parameters followed by discriminant analysis is even simpler than in the case of coated tablets. Spectroscopic signatures of uncoated tablets are less homogeneous than those of coated tablets. Therefore, homogeneity of spectroscopic parameters is lower, which is their more unique feature. Greater heterogeneity of hyperspectral parameters of the surface of uncoated tablets may result, inter alia, from: the use of components having different degrees of fineness, a larger variety of components than in the case of the coating ingredients, the use of excipients from different suppliers, different degrees of hydration, a smaller proportion of components reflecting infra-red radiation than in the coating (such as titanium dioxide, zinc oxide) [22,23].

2. Material and method

2.1. Material

Original and counterfeit tablets of one of the most counterfeited drugs – Viagra® manufactured by Pfizer – were compared. The original drug was purchased at a pharmacy in Poland. The study used Viagra® tablets containing 100 mg of sildenafil citrate. The counterfeit drug was purchased on the black market from a seller who advertised on the Internet. According to the information given on the counterfeit drug packaging it should also contain 100 mg of sildenafil citrate. The presence of sildenafil citrate were chemically verified using NMR relaxometry and FTIR spectroscopy. 8 counterfeit tablets and 4 original Viagra® tablets were examined using a hyperspectral camera. Both original and counterfeit tablets had almost identical mass: 620 ± 15 mg and 624 ± 20 mg respectively. Also the size and shape of the tablets were very similar.

Since the aim of analysis is to determine hyperspectral properties of the original and falsified drug, the analyses were limited to the areas covering the test object. Due to the existence of embossments on the test tablets ("100" on the front side of the counterfeit tablet and "VGR100" on the original drug), the spectral analysis area was determined in such a way so as to avoid the embossments and the tablet edges. Refraction of electromagnetic radiation at the embossment edges or the edges of the tablet could distort some parameters of the spectra. This primarily concerns analysis with the use of the GLCM (Gray-Level Co-Occurrence Matrix). Portions of original and counterfeit tablets, which were subjected to spectral analysis, were determined manually in such a way so that they would have the same area and cover the same portion of both the original and counterfeit tablet. This portion covered each time about 35% of the tablet surface free of the embossment.

2.2. Hyperspectral imaging

The block diagram of performed analyses is presented in Fig. 1 in supplementary material. SisuCHEMA hyperspectral imaging instrument (SPECIM, Spectral Imaging Ltd. Oulu, Finland) equipped with a SPECIM mercury cadmium tellurium (MCT) based Spectral Camera was used to acquire hyperspectral data cubes from both original and counterfeit tablets. In this work the spectral range between 921 and 2544 nm was recorded with a step of 6.30 nm. Then, the wavelength range was limited to 1000–2500 nm in accordance with the camera effective range. It enables to achieve image cubes of $M \times N = 384 \times 844$ pixels (where M=number of rows, N=number of columns) at 256 wavelength channels acquiring 324,096 spectra.

The imager was mounted on a motorised linear stage and attached to an optical rail structure through a lockable universal joint. As the linear stage translates at the designated step size and speed, the line imager acquires a hyperspectral data cube within a designated two-dimensional field of view. SPECIM's diffuse line illumination unit was used to provide stable illumination.

All images were achieved in raw signal format representing A/D sensor counts which then subsequently were converted to reflectance spectra/image. The unit was converted to reflectance. The hypercube data containing the reflectance spectra was calibrated and normalised. All data acquisition and pre-processing and multivariate analysis were performed using the Evince Image