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Short communication

The usefulness of simple X-ray powder diffraction analysis for counterfeit control—The Viagra® example

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Abstract

Counterfeit and illegally manufactured drugs become a very important problem all over the world, hence, a search for new fast, easy, reliable and not expensive methods of drugs screening is essential. We describe the use of X-ray powder diffraction method for the fast screening of tablets for counterfeit medicines identification. The original Viagra® tablets and some counterfeit and/or imitations of Viagra were used as example. We demonstrate the application of diffraction method for discrimination of tablets coatings and for identification of differences in drug composition. The X-ray diffraction method turns out to be very fast and reliable for detecting counterfeits and imitation, and it correctly predicts the presence or absence of active substance and/or particular excipients.

Keywords: X-ray powder diffraction; Counterfeit medicines; Fast screening; Viagra®; Sildenafil

1. Introduction

Counterfeit drugs, usually illegally marketed and most probably illegally manufactured have become a noticeable problem in most countries recently [1]. As it might be expected, the spectrum of fake pharmaceuticals ranged from antimicrobial, antihistaminic, hormone through anti-sexual dysfunction remedies [2]. The scope of illegally marketed drugs is different in poor, developing countries and these in North America and Europe. Still in many countries the health care authorities are not fully aware of the problem and health risk for the population [3], however, several initiatives to change this situation have been taken lately [4–6]. The lack of specific regulations concerning Internet marketing and the lack of systematic control of the pharmaceutical market, both legal and illegal, increases the risk. In such case, the need of fast, easy, reliable and not expensive methods of drugs screening is essential. No wonder that the NIR (near infrared) spectroscopy became popular in this respect [7–9].

Here, we would like to present an alternative, and in our opinion much more informative and even simpler in use, method for counterfeit drugs identification. From our long experience in the Drug Institute in Warsaw Poland, we found that the proposed by us X-ray phase analysis is popular in pharmaceutical industry only for polymorph identification of the pharmaceutically active ingredients, but not for analysis of final drugs. This is probably because the classical Bragg–Brentano method used for such purposes is not very fast method and needs flat, powdered samples. The development of new X-ray optics, new, more intensive X-ray sources and new ways of radiation detection during last 10–15 years makes crystallographic methods much faster and easier to use. We demonstrate results of analyses of original and counterfeit Viagra® showing differences in tablet coatings and their interior. No prior preparation, apart from eventual coating removal (when necessary), was applied.

2. Experimental

2.1. Materials and methods

Samples from two production batches of original Viagra® 100 mg were obtained free of charge from Pfizer Ltd. Great Britain. Also samples of Viagra® 50 mg and Viagra® 25 mg orig-

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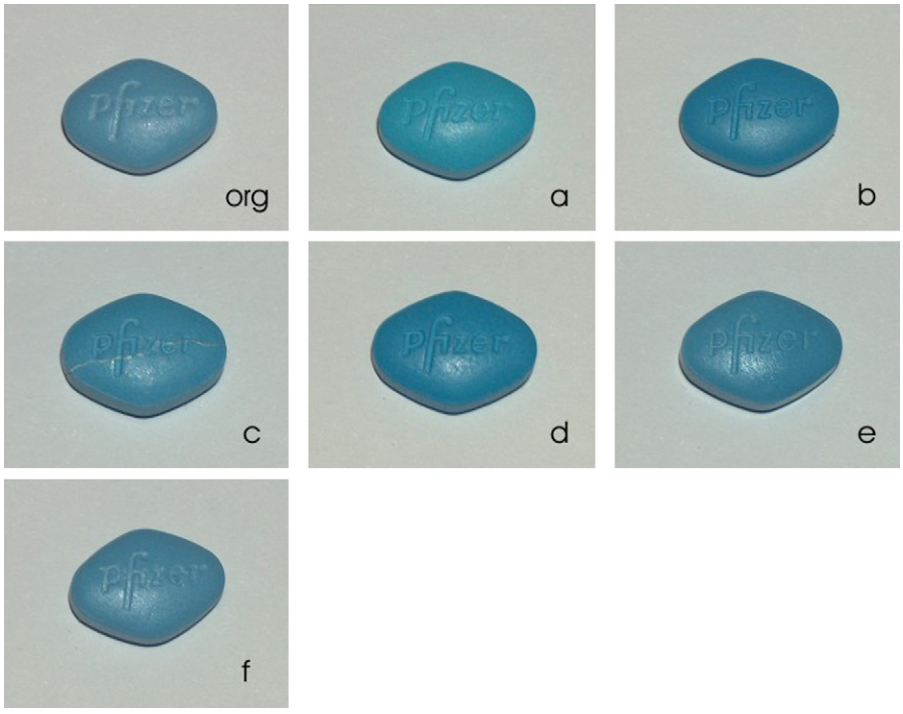

Fig. 1. The view of samples taken to the study. "Org" states for original Viagra® 100 mg, whereas, (a–f) marked counterfeit samples.

inated from the same source. Six samples of Viagra® 100 mg of questionable provenience were secured by either police or custom authorities in different places in Poland. All of them were coated, diamond shape, blue tablets marked as original Pfizer samples (see Fig. 1).

For all X-ray experiments Bruker AXS D8 Advance powder diffractometer was used. A special configuration consisting of X-ray tube, multilayer X-ray mirror (Göbel mirror), sample holder and gaseous, position sensitive Våntec detector was applied. Cu Kα radiation and a θ–θ scan mode were used to collect diffraction data. Samples were mounted in sample holder using modeling clay (plasticine).

2.2. Data

At the beginning, coated tablets were studied, starting from original samples of different sildenafil doses. Then the same procedure was applied to six counterfeit tablets coming from different sources. The consecutive step was to collect diffraction data after removing of coatings from tablets—usually, we found sufficient to take off coating from an area ca. 2 mm × 14 mm of a tablet which would be exposed to the X-ray radiation. In this part of the study, only tablets containing a nominal dose 100 mg of sildenafil were examined.

To verify the scan-speed dependence of diffraction pattern quality, we performed data collection for original Viagra® 100 mg sample using three different scan speeds.

3. Results and discussion

The comparison of diffraction patterns of three original Viagra® samples of 100, 50 and 25 mg doses of sildenafil showed expected result that no differences in coated tablet patterns are visible apart from proportionally lower intensity of all picks for the lowest dosage resulting from smaller dimensions of the tablet.

The studies, however, of counterfeit drugs samples showed crucial differences in diffraction patterns of coated tablets despite of seemingly no differences in appearance (see Fig. 1). Fig. 2a–f shows comparisons of diffraction patterns registered for six counterfeit samples with that of original Viagra® 100 mg, respectively. Here, for all but not the last sample several differences could be seen leading to the conclusion of at least not identical origin of samples. Fig. 2f may suggest the same coating and possibly similar interior composition.

Fig. 3a–f shows similar comparisons of diffractograms obtained for samples with coatings removed. Here, once more, one can easily discriminate counterfeit and original tablets. Similarly as before the last sample (Fig. 3f) looks identical to the original Viagra® 100 mg.

Talking about feasibility of a method for testing pharmaceuticals for counterfeits, illegal and sometimes dangerous products it is essential to show not only an analytical value of a method to discriminate original and illegal products – there are several methods which could be used for this purpose – but also to show how reliably, fast and easy this task could be performed. In this respect, several authors postulate the NIR spectroscopy to be the method of choice, since it does not require any prior preparation of samples, is fast and easy to perform. Unfortunately, NIR spectra at first glance are very often very similar and need application of advanced mathematical and statistical methods to be of any use. Furthermore, they are very equipment dependent, and hence, data are not transferable between laboratories. Here, the above examples show, even for not professionals, that

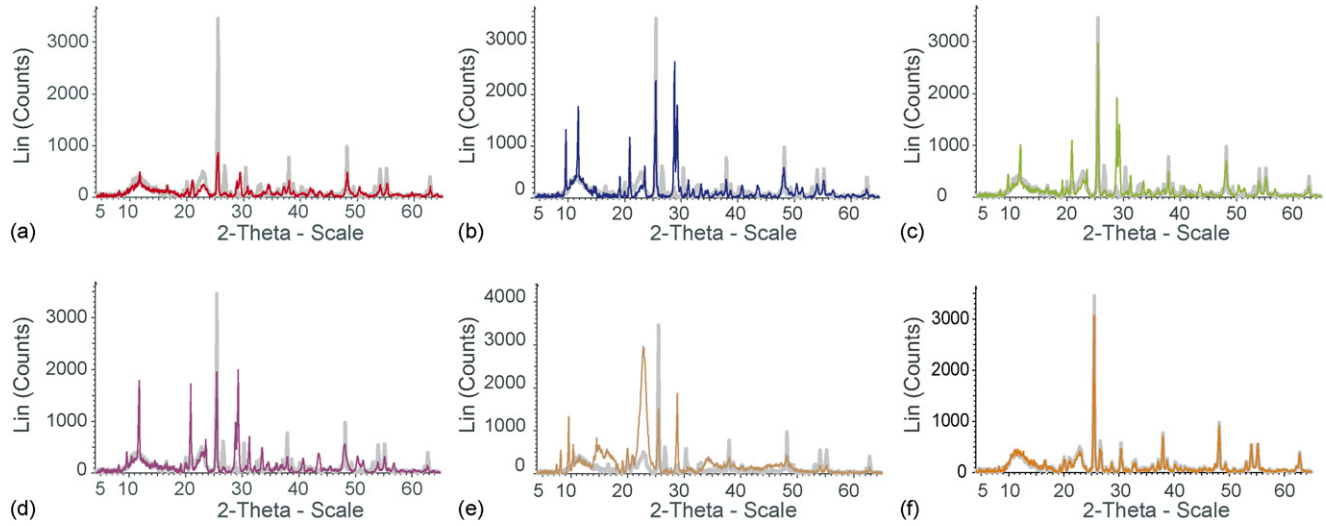

Fig. 2. The comparison of diffraction patterns of six counterfeit "Viagra" tablets and the original drug (shown as the thick lines in grey) taken without any prior preparation.

diffraction patterns have many good resolved peaks, which may be used as fingerprints of a sample and selection of counterfeits could be done even visually, comparing diffraction patterns.

Other question is, how fast and easy the analysis can be done. We showed already that using abovementioned configuration of a diffractometer diffraction patterns can be registered for tablets without any prior preparations. The above-cited diffraction data were collected in 2θ range 2–60° in 45 min, however, for screening purposes this time can be much shortened. Below in Fig. 4, the scan speed dependence of diffraction patterns quality is demonstrated. On the left-side view, uncorrected patterns obtained using three different scan speeds are superimposed. On the right-side view, the same superposition of three diffraction patterns after basic numerical smoothing is shown. As it could be expected, the fastest scan-speed gives the most noisy diffraction pattern; however, its appearance can be improved by simple smoothing. It is also obvious, however, that very weak reflections could be lost in noises during fast scanning and therefore for very accurate measurements the slow scan will be needed.

It is worth mentioning that a diffraction pattern characteristic for a given pharmaceutical is a superposition of diffraction patterns from all crystalline components of this product and that intensities of characteristic reflections for a given ingredient are related to its concentration. Hence, diffraction pattern is a fingerprint of a product and even small changes of composition – even changes in excipients – can be identified, what can be utilized for manufacturer identification.

Taking into account our subject Viagra® from Pfizer, we could expect that in the diffraction pattern we should see reflections from sildenafil citrate – the active ingredient – and for both microcrystalline cellulose and anhydrous calcium hydrogen phosphate—the excipients being in higher concentrations. Since, different crystalline substances have different diffraction power, what is a result of a crystal structure and atomic compo-

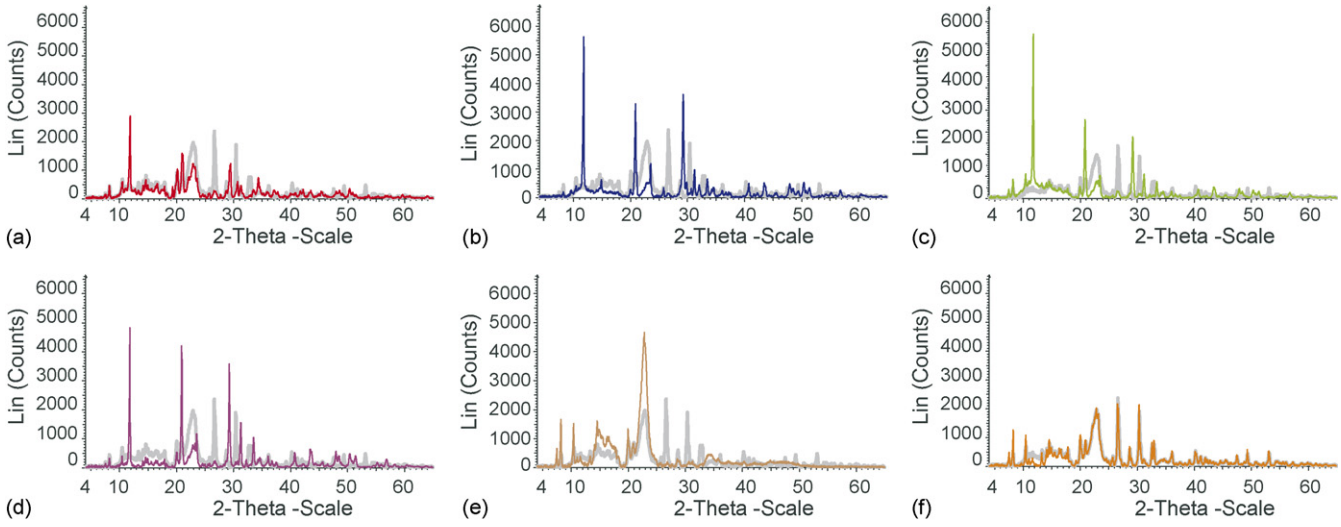

Fig. 3. The comparison of diffraction patterns of six counterfeit "Viagra" tablets and the original drug (shown as the thick lines in grey) taken with the coatings removed.

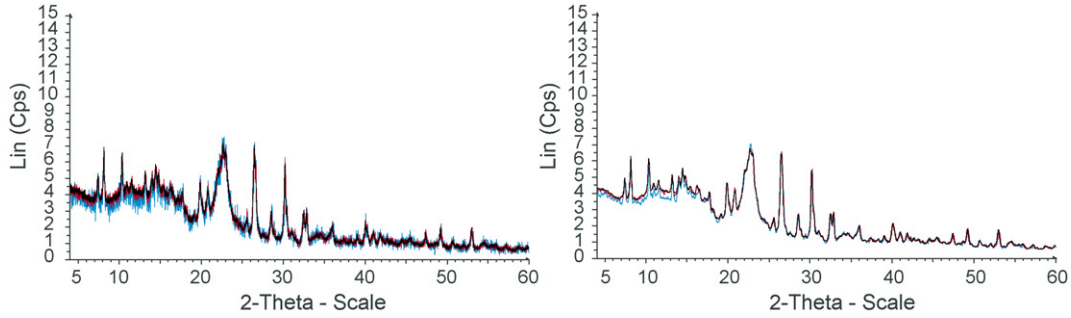

Fig. 4. The scan-speed dependence of diffraction patterns quality. On the left-side view, the not corrected patterns are visualised (the intensities are shown in counts/s). On the right-side view, the simple smoothing was applied. The blue diagram was obtained in 5 min, whereas, red and black were registered in 15 and 45 min, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

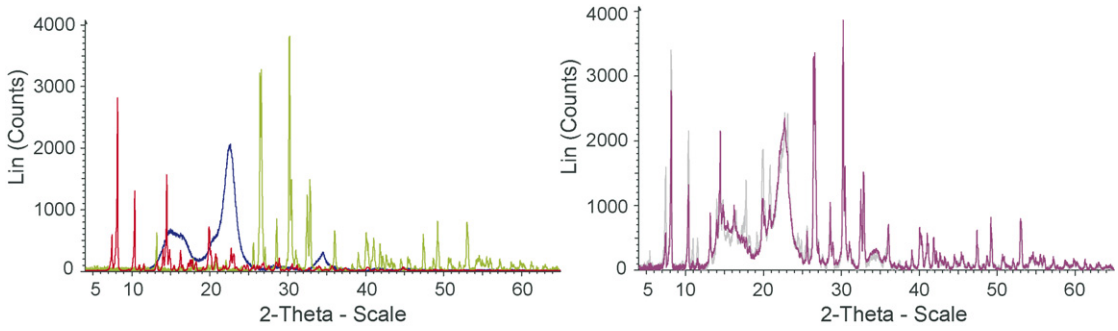

Fig. 5. The superposition of diffraction patterns for three major ingredients of Viagra (left) and the comparison of a "model" diffraction pattern and a diffractogram of Viagra®. An arbitrary scaling was used. Color coding: red – sildenafil citrate, dark blue – microcrystalline cellulose, pale green – anhydrous calcium hydrogen phosphate, violet – "model" pattern, grey – Viagra® powder. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

sition, the resultant pattern is not an effect of simple summation. Below in Fig. 5, we show a result of superposition of diffraction patterns of three ingredients (left-side view) and a comparison of such "model" pattern, obtained by their numerical addition with the pattern obtained for Viagra® (right-side view). The comparison is unexpectedly good.

4. Conclusions

The presented results obtained for counterfeit samples of Viagra® show that the X-ray powder diffraction, especially when using new, fast diffraction techniques, including multilayer mirrors and position-sensitive counters, is a method suitable for pharmaceutical market screening control for counterfeit drugs. This method can easily discriminate fake and original samples, even by visual examination of diffraction patterns, what can be done by not highly experienced employees. All statistical methods for principal components analysis, etc., usually utilized for spectroscopic data evaluation, e.g., NIRS, can be also employed, however, are not necessary. Well-resolved picks and their characteristic 2θ values can be used for examining powder diffraction databases (pdf-2 and/or pdf-4) [10] in qualitative composition analysis. Diffraction patterns can serve as fingerprints of manufacturers – both legal and illegal – since even small changes in composition are visible. It is worth noting, however, that X-ray powder diffraction, same as NIR spectroscopy, is not a method of trace analysis, and that for those purposes other methods are more sensitive, and hence, more reliable. These commonly applied and simultaneously time-consuming methods are GS–MS, HPLC and HPLC-MS. The numerous examples of application of these methods to investigation of counterfeit drugs are described in literature [11,12].

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