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Determination of counterfeit medicines by Raman spectroscopy: Systematic study based on a large set of model tablets

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ABSTRACT

In the last decade, counterfeit pharmaceutical products have become a widespread issue for public health. Raman spectroscopy which is easy, non-destructive and information-rich is particularly suitable as screening method for fast characterization of chemicals and pharmaceuticals. Combined with chemometric techniques, it provides a powerful tool for the analysis and determination of counterfeit medicines. Here, for the first time, a systematic study of the benefits and limitations of Raman spectroscopy for the analysis of pharmaceutical samples on a large set of model tablets, varying with respect to chemical and physical properties, was performed. To discriminate between the different mixtures, a combination of dispersive Raman spectroscopy performing in backscattering mode and principal component analysis was used. The discrimination between samples with different coatings, a varying amount of active pharmaceutical ingredients and a diversity of excipients were possible. However, it was not possible to distinguish between variations of the press power, mixing quality and granulation. As a showcase, the change in Raman signals of commercial acetylsalicylic acid effervescent tablets due to five different storage conditions was monitored. It was possible to detect early small chemical changes caused by inappropriate storage conditions. These results demonstrate that Raman spectroscopy combined with multivariate data analysis provides a powerful methodology for the fast and easy characterization of genuine and counterfeit medicines.

1. Introduction

Counterfeit medicines have become a widespread issue for public health in the last years [1–3]. The consequences associated with the intake of counterfeit pharmaceuticals are dramatic [4,5]. Since illegal medicines are produced under inappropriate conditions, no guarantee concerning the quality of such products can be given. Furthermore, correct storage and manufacturing conditions are important for the stability of pharmaceutical products [6–9]. Chemical changes caused by inadequate storage conditions can be considered as a clear hint for illegal repacking or manufacturing.

The detection of substandard medicines becomes increasingly more difficult, caused by the high quality of these products. A major problem in most cases is that there is no available information about the suspected product [5]. In order to detect counterfeit products, a qualitative and quantitative analysis of different compounds is beneficial. Spectroscopic techniques, which are easy and non-destructive, are particularly suitable as screening methods for the fast characterization of pharmaceuticals. Furthermore, these techniques require only little (or no) sample preparation. Especially, Raman spectroscopy provides several important benefits, such as the opportunity to measure through coatings, as well as through packaging materials [5,10–16]. The technique is applicable to solids, liquids and gaseous samples including transparent and non-transparent samples [13,17–20]. The obtained Raman spectrum contains unique and detailed features of the analyzed sample [12,21]. Combined with chemometric techniques, Raman spectroscopy provides a powerful tool for the identification of suspected medicines.

Raman spectroscopy has been successfully applied to detect counterfeit antimalarial tablets [10,21–23], Viagra® or Cialis® tablets [5,11,16,24–28], Lipitor® tablets [7] and a variety of other counterfeit pharmaceuticals [12,15,29,30]. The aim of these studies has been to establish Raman spectroscopic methods combined with data analysis, in order to distinguish counterfeits from genuine products.

Abbreviations: API, active pharmaceutical ingredient; CCD, charge-coupled device; HPLC, high-performance liquid chromatography; NIR, near-infrared; PCA, principal component analysis; PC, principal component; SORS, spatially offset Raman spectroscopy.

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Here, for the first time, the benefits and limitations of Raman spectroscopy for the analysis of pharmaceutical products were systematically studied. In order to cover a wide range of appearing types of counterfeits, a large set of model tablets (38,000) were produced. The model tablets differed in their chemical and physical parameters. The set consisted of samples with a varying amount of active pharmaceutical ingredients (APIs), diverse excipients, different coatings, varying press power and different compression procedures. In order to discriminate between the different mixtures, principal component analysis (PCA) was performed. Furthermore, in many cases, counterfeits were received unpacked (single tablets or blisters without packaging) and without any information about the storage history. In order to study the influence of different storage conditions on the Raman spectra, a set of acetylsalicylic acid effervescent tablets, which were stored under different conditions over 4 weeks, were analyzed.

2. Materials and methods

2.1. Materials

2.1.1. Model tablets

For the preparation of different mixtures, two APIs, eight excipients, two coating materials and one organic dye were used. The model APIs were quinine sulfate dihydrate (Ph. Eur.) and caffeine (Ph. Eur.) obtained from Fagron GmbH & Co. KG (Barsbüttel, Germany).

The eight excipients were Aerosil® 200 (Ph. Eur.) obtained from Overlack GmbH & Co. KG (Mönchengladbach, Germany), Avicel® (Ph. Eur.) received from Sigma-Aldrich (Steinheim, Germany), Kollidon® VA 64 and Ludipress® obtained from BASF (Ludwigshafen, Germany), magnesium stearate (Ph. Eur.) received from AppliChem GmbH (Darmstadt, Germany), talc (techn.) obtained from VWR International GmbH (Darmstadt, Germany), maize starch (Ph. Eur.) received from Colorcon (Kent, United Kingdom) and lactose monohydrate (Ph. Eur.) obtained from DMV-Fonterra Excipients GmbH & Co. KG (Goch, Germany).

As organic dye, erythrosine (AL 38–42%) obtained from Colorcon was used. Kollicoat® IR Yellow obtained from BASF and Opadry® (Ph. Eur.) received from Colorcon were used as coating materials.

The test set consisted of five different mixtures (Table 1). Mixtures A and B contained the same ingredients, but differed in the mixing quality. In comparison with A and B, mixture C contained different pharmaceutical excipients, but the same concentration of the APIs. In mixture D, the used excipients were identical to mixture A/B, however the amount of APIs varied. The amount of APIs differed fundamentally from the authorized range (90–110%). The aim of the study was the discrimination capabilities of the different spectroscopic techniques along with the detection of counterfeits, which contain a potential harmful amount of API. Mixture D showed a decrease in the amount of caffeine on a level of 30% and an increase of quinine sulfate dihydrate four times higher, compared with all other mixtures. The last mixture E contained the same ingredients as mixture A/B and, in addition, a small amount of an organic dye. Some samples from mixture A were covered with two different coating materials (Kollicoat® IR Yellow and Opadry®). Moreover, a variation of the physical parameters, press power and granulation, was applied. For the uncoated tablets from mixtures A, B, C and D, the press power was varied over four levels: very low, low, optimal and high. Furthermore, for the coated tablets from mixture A, optimal press conditions were chosen. For these samples, the pure compounds were compressed directly. In addition, two different types of granulates (KG1 and KG2) for mixtures A and E were used for the tablet manufacturing. Here, the press power was modified over four levels, too. To sum up, there were 14 different batches from mixture A, four different batches from mixtures B, C and D and eight different batches from mixture E (Table S1).

The model tablets were designed within the collaborative project MIME (Multimodales Mustererkennungssystem zum Schutz der Bevölkerung vor organisierter Arzneimittelkriminalität und zur Bekämpfung des Internationalen Drogenhandels) with the objective to combine spectroscopic (Raman, NIR, induced fluorescence, ultraviolet and visual spectroscopy) and physical methods for a fast screening platform for counterfeit detection. The composition of the model tablets were chosen so that (i) typical pharmaceutical compounds and (ii) different spectroscopic techniques could be applied. Thus, the model tablets also contain some Raman-inactive compounds such as talc or maize starch (Table S2). The model tablets were produced by the project partner Glatt GmbH Systemtechnik (Dresden, Germany) according to the requirements in the pharmaceutical industry. Each batch consisted of 1000 tablets. The tablets were packed into blisters and distributed between the different project partners.

2.1.2. Samples used in the storage experiments

As samples for the storage experiments, effervescent tablets (ASS + C-ratiopharm®) with an amount of 600 mg acetylsalicylic acid and 200 mg ascorbic acid (vitamin C) obtained from ratiopharm (Ulm, Germany) were used. Adipic acid, sodium bicarbonate, citric acid, sodium dihydrogen citrate, povidon (K25), lemon flavor and sodium saccharin were used as excipients in the tablets. The tablets were stored under five different conditions over 28 days: with and without daylight at room temperature, in the fridge, in the drying chamber (45 °C) and with exclusion of water in the desiccator (desiccant silica gel).

2.2. Raman spectroscopy

The different Raman measurements were performed on a TIDAS S Raman from J & M Analytik AG (Aalen, Germany) equipped with a 785-nm diode laser and a maximum laser power of 350 mW. The collected Raman radiation was dispersed with an 1200 lines mm^{-1} grating and focused on a thermoelectrically cooled charge-coupled

Table 1
Detailed composition of the individual mixtures including the declaration of weight (mg) for each ingredient with regard to one tablet.

	Raw material	Mixture A	Mixture B	Mixture C	Mixture D	Mixture E
API	Caffeine	30.67	30.67	30.67	21.47	30.67
	Quinine sulfate dihydrate	3.07	3.07	3.07	12.27	3.07
Excipient	Aerosil®	1.53	1.53		1.53	1.53
	Avicel® PH 101			30.67		
	Kollidon® VA 64	15.33	15.33	14.07	15.33	15.33
	Lactose monohydrate	210.08	210.08		210.08	210.08
	Ludipress®			226.67		
	Magnesium stearate	1.53	1.53	1.53	1.53	1.53
	Maize starch	30.67	30.67		30.67	30.67
	Talc	13.79	13.79		13.79	13.79
	Total	306.67	306.67	306.67	306.67	306.67