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Challenging Near InfraRed Spectroscopy discriminating ability for counterfeit pharmaceuticals detection

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ABSTRACT

This study was initiated by the laboratories and control department of the French Health Products Safety Agency (AFSSAPS) as part of the fight against the public health problem of rising counterfeit and imitation medicines. To test the discriminating ability of Near InfraRed Spectroscopy (NIRS), worse cases scenarios were first considered for the discrimination of various pharmaceutical final products containing the same Active Pharmaceutical Ingredient (API) with different excipients, such as generics of proprietary medicinal products (PMP). Two generic databases were explored: low active strength hard capsules of Fluoxetine and high strength tablets of Ciprofloxacin. Then 4 other cases involving suspicious samples, counterfeits and imitations products were treated. In all these cases, spectral differences between samples were studied, giving access to API or excipient contents information, and eventually allowing manufacturing site identification.

A chemometric background is developed to explain the optimisation methodology, consisting in the choices of appropriate pretreatments, algorithms for data exploratory analyses (unsupervised Principal Component Analysis), and data classification (supervised cluster analysis, and Soft Independent Modelling of Class Analogy). Results demonstrate the high performance of NIRS, highlighting slight differences in formulations, such as 2.5% (w/w) in API strength, 1.0% (w/w) in excipient and even coating variations (<1%, w/w) with identical contents, approaching the theoretical limits of NIRS sensitivity. All the different generic formulations were correctly discriminated and foreign PMP, constituted of formulations slightly different from the calibration ones, were also all discriminated. This publication addresses the ability of NIRS to detect counterfeits and imitations and presents the NIRS as an ideal tool to master the global threat of counterfeit drugs.

1. Introduction

The counterfeit drugs issue constitutes a major public health challenge and has become a priority as the lives of increasing numbers of consumers are endangered. The prevalence of counterfeit drugs appears to be rising [1] and currently makes up 1% of the pharmaceutical market in industrialised countries [2]. This proportion is 20% in many of the former Soviet republics, and 30% in Africa, parts of Asia and Latin America [2]. The World Health Organisation (WHO) reports that the largest faked drugs market is that of the medicines purchased over the Internet [2,3]. Two years ago, more than 2.7 millions of counterfeited medicine units were seized by the European customs, which represented an annual increase of 384% [4]. Counterfeit medicines are, by definition, not manufactured under Good Manufacturing Practices mastered processes and/or sold fraudulently. Therefore, different qualities of counterfeits may be found on these illegal markets, from extremely toxic substances to inactive preparations. In general, counterfeit products are presented like authentic medicines but they may contain the correct ingredients in fake packaging, they may be formulated with wrong ingredients, without any active ingredients or with insufficient active ingredients. Imitation products, on the other hand, are not intended to ressemble their corresponding authentic PMP but they are presented as if they could generate the same pharmacodynamic effects. The WHO has a launched taskforce to fight counterfeit drugs by creating a global coalition of stakeholders called IMPACT (International Medical Products Anti-Counterfeiting Taskforce). This taskforce, created in 2006, has been active in forging international collaborations to seek solutions to this global challenge and to raise awareness of the dangers of counterfeit medical products [2,5]. The fight against this public health problem is considered seriously by the authorities but it has been difficult to find good tools for discriminating counterfeits, which may ressemble both branded and generic products. Many analytical methods have been described for counterfeit drug detection, such as High Performance Liquid Chromatography with UV detection (HPLC-

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UV) [6–8], and mass spectrometry detection (HPLC–MS) [7,8], ^{1}H Nuclear Magnetic Resonance (NMR) [9], Raman spectroscopy [9,10], X-ray powder diffraction [11], refractometry and colorimetry [12]. Recently, Near InfraRed Spectroscopy (NIRS) has also been introduced in this domain for counterfeits [13–15] and/or imitations [3,16–18] investigations. This technique offers real potential for screening suspected samples due to the rapidity of the analyses and their non-destructive character allowing subsequent investigations. Among these publications, some deal with a wide variety of different PMP and counterfeits or imitations but little samples per PMP [3,13,18], others focus on only one or two PMP with corresponding counterfeits or imitations including a large number of samples [14,16,17]. NIR spectra are influenced by the chemical and physical properties of the samples. Then, calculated from NIR spectra, appropriate chemometric models can highlight differences in chemical formulations, but physical quality differences in relation with the manufacturing process (e.g. manufacturing sites) can also be distinguished when comparing different batches of an authentic PMP. The present study was initiated to assess the limits of NIRS for the comparison of similar formulations in the case of the two most common solid forms for oral route: hard capsules and tablets.

Generics are characterised by a pharmaceutical quality comparable to the corresponding genuine medicines, but they do not necessary contain the same excipients. From a spectral point of view, discrimination of genuines and generics is not always successful [16,17] and certainly more difficult compared to discrimination of genuines and counterfeits. This is why we first chose to optimise NIRS discriminating models from close formulations of Good Manufacturing Practices (GMP) pharmaceutical qualities, such as generics, with a high number of samples, a worse case scenario test for specificity of the technique.

Two generic cases were first considered to explore the discriminating capacity of the NIRS, integrating different pharmaceutical forms, with different compositions of API and excipients. These two cases were composed of hard capsules containing 10% of Fluoxetine (case 1) and tablets containing 75% of Ciprofloxacin (case 2). They were thereby chosen to represent a large variability of samples, to potentially extrapolate the results to different counterfeits forms. The second part of the study (cases 3–6) challenged the screening ability of NIRS when faced with samples suspected to be counterfeited, actual counterfeits and imitations.

2. Materials

2.1. Types of drugs

Two classes of generics were studied. Among them, 28 different generics were evaluated together with the two corresponding reference PMP. Hence, the specificity of the technique was challenged with a large set of samples, including maximum industrial process variability (4–6 batches per PMP including different expiratory dates, and different manufacturing sites when possible). Considering the generics available on the French market, formulations very close to each PMP were available and also authentic copies of each. These latest are called the “copies/copies” as they are composed of exactly the same formulations, but made by a different supplier. Such samples were tested as “identities” in an external validation set, to challenge the developed method with independent batches (batches not included in the calibration set) of substances that are included in the calibration. Cypriot and Swedish generics were also included in our discrimination projects to test their capacity to detect different origins of formulations. These samples were tested as “nonidentities”, to challenge the developed method with substances that are not included in the calibration but whose formulations are very close to those included in the calibration.

Among the counterfeit medicines detected on the international market, 4 different cases were considered in this study. We had at our disposal 3 kinds of samples:

- counterfeit or imitation samples,
- suspect samples having the same batch number than the counterfeit samples and coming from a batch recall from the French market (withdrawn as a precaution),
- authentic samples obtained from the manufacturers.

The challenge of this real life study was on one hand to identify the counterfeit samples, and on the other hand to ensure that suspect samples coming from the French market are safe.

For each formulation, a large set of authentic batches (7–15) was collected in order to define the manufacturing process variability (several batches from different manufacturing sites, including several samples manufactured with different batches of drug substances and excipients, etc.). All the NIRS results were confirmed with reference methods.

In this study all the PMP formulations were obtained by the AFSSAPS, and in order to protect trade secrets, their composition was abstracted.

2.2. NIR equipment

Measurements were performed on a Büchi N500 FT® reflectance spectrometer equipped with an InGaAs detector. Measurements were carried out with an optical resolution of 8 cm^{-1} over the spectral range 10,000–4000 cm^{-1} and 64 scans were accumulated for each spectrum. Samples were placed centrally on the sample plate.

Data acquisition was performed with Nirware software suite (Büchi, Flawil, Switzerland), and treatments were optimised with NIRCal® 5.0 Chemometric Software (Büchi, Flawil, Switzerland) and SIMCA-P® 11 Software (Umetrics, Umeå, Sweden).

3. Chemometric background and methodology

A suitable combination of pretreatments is generally required to give access to the relevant information from the spectra. By applying appropriate pretreatments, it becomes possible to minimise the physical effects included in NIR spectra [19]. For example, Standard Normal Variate (SNV) is a classical pretreatment to remove the multiplicative interferences of scatter and particle size [20]. The SNV is applied spectrum per spectrum, and consist of a subtraction of the mean and division by the standard deviation over each object. Also widely used, first derivative pretreatments may also be applied when the objective is to enhance API bands from spectra originating from powder compressed samples.

After mathematical transformation of the data, an algorithm for discriminating spectra must be chosen. A brief comparison between unsupervised and supervised algorithms is proposed to argue the choice of methodology applied to the different sets of data.

Unsupervised analyses, such as Principal Component Analyses (PCA), are data exploratory analyses. PCA is a mathematical procedure that transforms the spectral variables into orthogonal components accounting for the largest variance in the dataset. These components form the axis of a multidimensional space. PCA calculation results in two matrix: scores and loadings matrix. The scores are the coordinates of spectra in this Principal Component (PC) space and inform about the distances between spectra. The loadings express the weight of the original (spectral) variables. The most important original variables have the higher loading values. When PCA model show discriminating tendencies but do not lead to effective discriminations between classes, then supervised analyses may enhance these discriminations. Among the super-