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Raman spectroscopy as a complementary tool to assess the content uniformity of dosage units in break-scored warfarin tablets

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ABSTRACT

Due to the side effects of overdosing, the therapeutic dose of warfarin preparations must be very strictly controlled. In order to make it easier for the patient to take the required dose, two different strategies can be followed: The medicine can be commercialized in different dosages and/or tablets can be scored in order to make them easy to split. The splitting of the tablets introduces the question of how to control that the fractions contain the desirable amount of warfarin. The regulations regarding the content uniformity of dosage unit for scored tablets have changed considerably in the last 10 years, and they are still evolving. Warfarin is commercialized under the trademark of Aldocumar in four different preparations, containing 1, 3, 5 and 10 mg sodium warfarin per tablet. All these tablets are also scored, thus suggesting the possibility of splitting. A quantitative Raman method has been developed for the determination of warfarin in tablets and in the potential fragments, taking into account the score lines on the tablet surface. This method is suggested as an auxiliary procedure to verify the uniformity of API distribution in dividable tablets. A combination of a second derivative and standard normal variate (SNV) was used as spectral pre-treatments, and partial least squares (PLS) as the regression algorithm. The relative standard deviation in API content among portions was found to be less than 5%. An HPLC procedure has been used as a reference analytical method.

1. Introduction

Tablets intended for oral administration are the most common pharmaceutical form, and most of them bear score mark. The presence of a score mark suggests that the tablet can be subdivided; even the role of the break-mark is not the splitting of the tablet for smaller doses. Its functions can be: (a) adjust the dose, which is the most important reason, especially for pediatric and geriatric patients, and for low international normalized ratio (INR) drugs, (b) ease swallowing and/or (c) save money (Van Santen et al., 2002).

In 2005 the European Medicines Agency (EMA) adopted a revision of the *guideline on summary of product characteristics* in which was written so that, in the case of tablets designed with score line, information should be given on whether or not reproducible dividing of the tablets has been shown (European Comission, 2005). The main question is how to demonstrate that each potential portion of the tablet (usually halves or quarters) actually contains the expected amount of API. In this regard, two points have to be considered. Probably the most important is to ensure a correct physical division i.e., the tablet should be easily split and the mass of the fragments should correspond to the expected mass (half or quarter of the total tablet mass). A second step would be to demonstrate that the amount of API in each portion is as expected (half or quarter of the total mass of API in the tablet). The balance between these two criteria has led to changes in the pharmacopoeia regulations. It is also clear that the total amount of API has to be taken into account, and a tablet with 500 mg of API, corresponding to a mass proportion of 50%, is not the same as a medicine containing 1 mg of API with a 1% of mass proportion. Clearly the regulations for medicines with a low API content need to be much stricter.

The criteria regarding dividable tablets and their equivalence with regular tablets have changed in the last decade, and are still evolving. In October 2001, an accuracy standard for subdivision of tablets was implemented for the first time by the European Pharmacopoeia (European Pharmacopoeia, 2002). It was stated that "*for tablets for which subdivision is authorized, it is demonstrated to the satisfaction of the competent authority that the subdivided parts comply with either Test A for uniformity of content of single-dose preparations (2.9.6) or with the test for Uniformity of mass (2.9.5), as appropriate*". Thus, there appears to be ambiguity on the choice of the test. In June 2004 the situation was clarified "*Uniformity of content test prevails if tablets with a content of active substance of tablet less than 2 mg or less than 2% of the total mass (. . .) uniformity of mass test prevails in all other instances.*" (European Pharmacopoeia, 2005). In December 2005 a profound change occurred

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(European Pharmacopoeia, 2006) the Uniformity of Content test was eliminated, and it was stated that in the case that the score mark is intended to comply with the posology (e.g. approved half-doses), the efficacy of the break-mark must be assessed during the development of the product in respect of uniformity of weight. Since then, European Pharmacopoeia has remained unchanged in regard of subdivision of tablets.

Despite that subdivision of tablets has still not been mentioned in current US Pharmacopoeia (US Pharmacopoeia, 2013), efforts has been made towards it. Thus, 2009 *Stimuli* (Green et al., 2009a) declared that the accuracy of division was the most important quality attribute, and that it could be tested either by uniformity of mass or uniformity of content. Also, it proposes the requirement of a uniformity test for every score-lined preparation, whether required by the posology or not.

Very recently, Guidance for Industry by FDA (CDER, 2013) proposes a philosophy change in the quality assurance. It states that "*The split tablet portions should meet the same finished-product testing requirement as for a whole-tablet product with equivalent strength*", requiring new tests such as friability, loss of mass in splitting, dissolution or stability tests. Also, uniformity of content of single dose is required for preparations below 25% w/w, or 25 mg of API and uniformity of mass for the remainder of the preparations.

Thus, although from the regulatory point of view only the uniformity of weight is still mandatory, it is quite clear that new analytical procedures to determine the API in different parts of a tablet in an easy and rapid way are needed. This determination can be critical for low content preparations.

The incorporation of Raman spectroscopy into quality control of pharmaceutical industries has followed the path of near infrared spectroscopy (NIR), but with a certain delay. The main problem has been the low reproducibility derived from the small laser spot area. In the classical set up of backscattering Raman spectroscopy, the illuminated area by the laser is too small to acquire representative spectra from particulate pharmaceutical sample, and leading to a classical sub-sampling issue. Hence, different strategies have been developed in order to acquire representative spectra from bulk pharmaceutical preparations. The most straightforward one is the acquisition of spectra at different places of the sample and their averaging (Kontoyannis, 1995; Szostak and Mazurek, 2002), although the sampling of large areas (such as a whole tablet) is very time-consuming. Recently, the development of large diameter Raman probes (Johansson et al., 2005) (up to several millimeter) has enabled a representative spectra of solid pharmaceutical preparation to be acquired in few seconds and, consequently, Raman applications in pharmaceutical analysis are growing rapidly, in some cases as a technique complementary to NIR (De Beer et al., 2009). Well-known advantages of Raman radiation is that it allows reliable information in aqueous media (Doub et al., 2007) or from encapsulated samples to be obtained (Niemczyk et al., 1998).

Furthermore, the signal arising from the Raman phenomenon is very well-suited to pharmaceutical analysis, because most of the active pharmaceutical ingredients (API) contain aromatic or conjugated chemical groups, which are strong Raman scatterers. Conversely, most excipients are aliphatic and exhibit weaker Raman intensity (Strachan et al., 2007). Hence, Raman permits the spectra of many samples with little matrix interference to be acquired, allowing straightforward data analysis (this fact is also linked to the narrow peaks of Raman spectroscopy that allow visual interpretation of spectra, unlike NIR spectroscopy).

Preparation of calibration samples for Raman quantitative purposes has been scarcely discussed in literature, mainly because the development of a calibration model is much more straightforward than in NIR spectroscopy; the peaks are much narrower, so the overlapping of bands is much less significant. Hence, a univariate calibration is usually sufficient to achieve reliable results when a peak of the analyte is found not to be overlapped by the contribution of other components of the mixture. Thus, the usual strategy to prepare a stable regression model involves the preparation of laboratory made samples by mixing API and excipients (Taylor and Zografi, 1998) and measuring the intensity of an API peak. The use of a multivariate regression procedure such as partial least squares (PLS) has shown to improve prediction (Everall et al., 1994).

Warfarin is an antagonist of vitamin K that acts decreasing the blood concentration of certain key proteins that ease blood clotting and is prescribed to patients with thrombosis or embolism risk. In order to reduce the overdose risk, patients take daily exact doses (Baglin et al., 2006). Hence, warfarin tablets bear score-marks very often and some warfarin formulations have been used to perform mass uniformity tests of halves or quarters of tablets (Hill et al., 2009; Polli et al., 2003).

The aim of this work is the development of a feasible Raman method (in terms of time required and precision of the method) to quantify sodium warfarin in the individual fractions of the four doses (one, three, five and ten milligram per tablet) of a commercial preparation (Aldocumar), as an easy way to ensure the homogeneity of content, not only between tablets, but also between the potential tablet fractions.

2. Material and methods

2.1. Materials

Sodium warfarin (WarfNa), the active pharmaceutical ingredient of Aldocumar, in the same crystalline form as is present in the commercial samples, chlatrate (Aldrich), alpha-D-lactose (Acros), and PH-101 microcrystalline cellulose (MCC, Fluka). The latter are the major excipients of the commercial preparation and were used to prepare the calibration samples. The HPLC method was developed using warfarin (Chem Service, 99.5% purity) as analytical standard, ACN (Fluka, HPLC grade) and H_3PO_4 50% (Panreac, Barcelona, Spain).

2.2. Instruments and software

Raman spectra were recorded on a Brüker MultiRam spectrometer (Brüker, Billerica, MA, USA) equipped with a nitrogen cooled Ge detector and a 1064 nm Nd:YAG laser source (500 μm spot size). Opus 6.5 (Brüker, Billerica, MA, USA) was used to control the instrument and export data.

HPLC reference method was performed by using an Agilent 1100 (Santa Clara, CA, USA), coupled to a G1315B DAD detector (λ = 280 nm) and a 25 cm long, 4.6 mm internal diameter *Symmetry-Shield* RP18 column. A Turbula T2C shaker mixer from WAB (Basel, Switzerland) was used for blending doped samples. Laboratory compressed tablets were prepared on a PerkinElmer 15.001 (Waltham, MA, USA) press, with a cross-section of 132.7 mm^2. Multivariate models were constructed with the aid of Unscrambler X (Trondheim, Norway).

2.3. Commercial samples

Aldocumar commercial preparations are available in four doses of, one (AC-1), three (AC-3), five (AC-5) and ten (AC-10) milligram of sodium warfarin per tablet. The first three have the same round shape and size (diameter of 8.5 mm) and are score-lined to obtain half fractions. AC-10 is larger than previous ones (12 mm), but also round, and is score-lined to be divided into quarters. The label claims that lactose is the major excipient, accounting for 60% w/w of the tablet mass in the four doses. The other main excipient is MCC. Samples from four batches were available for AC-3, AC-5 and AC-10,