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The effect of amorphous and crystal sodium warfarin and its content uniformity on bioequivalence of tablets

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ABSTRACT

Warfarin is intensively discussed in terms of generic substitution due to particular cases of bleeding, which are attributable to fluctuations in API content or the substitution of crystalline (WSC) for amorphous (WSA) warfarin. The aim of this study was to assess to what extent the *in vitro* release was affected by the form of API depending on the composition and technology. Bioequivalent tablets containing 5 mg of WSA or WSC prepared by wet granulation or direct compression were used. Furthermore, tablets of the same composition with WSC or WSA prepared by direct compression were evaluated. Raman spectroscopy was used to confirm the presence of WSA or WSC. The dissolution was more influenced by the technology than by the form of API but even tablets with dissimilar profiles were bioequivalent. This is probably due to the precipitation of WSA and WSC in the stomach on a poorly soluble acidic form, which subsequently dissolves in the neutral environment of the small intestine. Recrystallization was demonstrated in the *in vitro* assay at a pH of 1.2 and 4.5 using Raman spectroscopy and X-ray diffraction. In summary, the content uniformity appears to be the main factor affecting the safety of the treatment.

1. Introduction

Warfarin has been used since the 1950s to prevent thrombosis and thromboembolism (Tadros and Shakib, 2010). According to the Ph. Eur. 9, it is available as an amorphous (WSA) or crystalline sodium salt (WSC) where isopropyl alcohol is trapped in the WSC structure in a ratio of 2:1. WSC irreversibly converts to a more soluble WSA due to elevated temperature during a longer period of time, which may affect its release (Gao and Maurin, 2001). The substance has a narrow therapeutic index (NTI) (Kellner et al., 1981), hence the patient's titration is typically increased or decreased by only 5–15% of the daily dose. Therefore the content uniformity (CU) is an important parameter of the tablets (Wittkowsky, 1997).

In the 1980s in a Boston hospital, substitution of the original formulation (Coumadine®) containing WSC substance for generic (Panwarfarine®), containing WSA, was the cause of bleeding (Vercaigne and Zhanel, 1998). The reason was probably inappropriate CU of the generic (Wittkowsky, 1997). Nevertheless, a different form of active substance (API) was also discussed (Jaffer and Bragg, 2003). Although the cause of the problems in Boston was unclear (Richton Hewett et al., 1988), Coumadine® and Panwarfarine® could not be considered as therapeutically equivalent (Haines, 2011). Unfortunately the problem was not isolated and therefore for example in Denmark is stricter requirements for bioequivalence and one producer was banned from the local market (Hellfritzsch et al., 2016). In Kentucky and North Carolina generic switching of warfarin tablets is still prohibited (Vivian, 2008).

Hence the quality assurance of producers should take into account both – the CU and quality of API requirements. A number of producers solved problems with NTI of warfarin with the stricter CU criterion (*e.g.* DuPont, Taro Pharmaceuticals and Apotex) introducing a narrower limit for individual values within the scope of 92.5–107.5% of the average and RSD lower than 3% (Wittkowsky, 1997). The eventual influence of the substance, or the dosage form, and quality could be indicated by a suitable dissolution method (Franc et al., 2016). While a number of additional statistical tools can be used to assess CU, a qualitative comparison of dissolution profiles can be difficult. The USP

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method using an aqueous medium is not discriminatory (Ali and Krämer, 1999; Nguyenpho et al., 2015; USP, 2016) and is not applicable to estimate *in vivo* behavior (O'Reilly et al., 1966). The medium with a pH of 1.2 causes the transition of the sodium salt of warfarin to an acidic form, which has a poor solubility, leading to a failure to respect the "sink conditions" (Stella et al., 1984). Similarly, at a pH of 4.5, the amount of dissolved API is too low and the standard limit for immediate release dosage forms (Qureshi, 2004) is reached only in some cases (Nguyenpho et al., 2015; Zhang et al., 2017). The use of two step mediums with a pH of 1.2 increased to a pH of 7.5 (Wagner et al., 1971) or two-phase dissolution (bottom 0.1 M HCl phase and upper *n*-octanol phase) is demanding and it is not clear whether their discriminatory capability will be manifested *in vivo* (Franc et al., 2016). The medium with a pH of 6.8 was found as the most relevant condition to bioavailability (Zhang, 2016). These existing paddle methods do not use a lesser speed than 50 rpm, while some new studies suggest that for well soluble APIs (BCS I) a reduced paddle speed of 25 rpm could be used (Lukášová et al., 2017; Qureshi, 2004).

The aim of this work was to investigate the extent to which the API quality or physical parameters of the tablets could affect the bioequivalence of the product and retrospectively assess whether the form of API (WSA and WSC) or CU of the tablets was responsible for the problems with generic substitution. To evaluate the effect of API form (WSC/WSA), CU and physical properties of tablets on dissolution results, four types of tablets of strength 5 mg (A, B, C and D) were compared. Two bioequivalent commercial products containing WSA (A) or WSC form (B) were produced by wet granulation or direct compression. In order to distinguish the influence of preparation technology from the type of used substance, two other formulations containing the WSC (C) or WSA (D) were produced by the same direct compression technology according to the patented procedure (Franc and Muselík, 2013). The process capability index (Cpk) and Bergum method, were used to evaluate the CU of the tablets (Muselík et al., 2014). The tablets were also evaluated for the usual physical parameters. Warfarin release kinetic was evaluated in water and phosphate buffer of a pH of 6.8 at paddle speed of 50 and 25 rpm. The mediums used ensured that the "sink conditions" were maintained. The dissolution profiles were compared by factors of similarity (f_2) and difference (f_1), the MDT and area under the dissolution curve (AUC) (Anderson et al., 1998; Costa and Lobo, 2001). In addition, qualitative changes of API in *in vitro* conditions simulating fasting of the stomach (pH 1.2) and after eating or using antacids (a pH of 4.5) were studied. Qualitative changes of API were studied to clarify WSC and WSA behavior in the *in vivo* conditions and its potential effect on the bioequivalence. The quality of the API (amorphous/crystal) and the particle size were assessed using Raman spectroscopy and X-ray or laser diffraction.

2. Materials and methods

2.1. Preparation of tablets

For the evaluation, the two commercial and bioequivalent tablets "A" and "B" from the Czech market were used, with "C" and "D" tablets being prepared. Tablets "A" contained a WSA with the content of 5 mg in one tablet. Tablets B contained a WSC of 5.40 mg in one tablet that was an equivalent to 5.00 mg of warfarin sodium. For the preparation of "C" tablets the warfarin sodium isopropanol clathrate (Pliva, Croatia) was used. The WSA substance for tablet "D" was obtained by dissolving approximately 20.0 g of WSC in 300 ml of purified water. After complete dissolution when no visible traces of powder particles were present, the prepared solution was dried in a hot air oven at 60 ± 1 °C. The residue after evaporation was pulverized in a rough porcelain mortar. The prepared WSA was then sieved with a 250-μm sieve. The sieved powder was used to prepare the mixture designated for direct compression. Common excipients for "C" and "D" are Di-Cafos® 92–14 (Budenheim KG, Germany), Avicel® PH 101 (FMC BioPolymer, USA), Ac-Di-Sol® (FMC BioPolymer, USA) and Magnesium stearate (Peter Greven, Germany). Tablets "C" and "D" were prepared according to the already described methodology (Franc and Muselík, 2013). Turbula (T2C, Switzerland) homogenizer was used to homogenize the powders at the speed of 40 rpm. The weight of one batch was 500.0 g. Flat tablets with a diameter of 10 mm weighed about 270 mg and had hardness about 50 N. These were produced using an eccentric press (Korsch EK0, Germany). The composition of the tablets is stated in Table 1.

Table 1
Composition of tablets.

A	B	C/D
WSA	WSC	WSC/WSA
Lactose monohydrate	Lactose monohydrate	Calcium phosphate anhydrous
Corn starch	Cellulose microcrystalline	Cellulose microcrystalline
Gelatin	Corn starch	Croscarmellose sodium
Magnesium stearate	Colloidal silicon dioxide	Magnesium stearate
	Magnesium stearate	

2.2. Physical testing of tablets

Tablet thickness, diameter, hardness and weight (n = 20 tablets) were measured automatically on Pharmatest WHT-1 (Pharmatest, Germany). Mass uniformity was evaluated according to EP 2.9.5 and the resistance to crushing of tablets was performed according to EP 2.9.8. Friability was tested according to EP 2.9.7, using TAR 10 (Erweka, Germany). Disintegration was tested according to EP 2.9.1, using ZT 4 (Erweka, Germany).

2.3. The measurement of warfarin content in tablets

For the CU, 10 commercial tablets A and B were randomly selected. Each of the tested tablets was weighed on an analytical balance Kern 870-13 (Gettl. Kern & Sohn, Germany) and placed in a 100 ml flask. The tablets were then allowed to dissolve for 12 h in a 9:1 (V/V) mixture of water and methanol. By the same procedure, samples of tablets C and D (10 tablets from each batch) were prepared and were taken at regular intervals during the compression process. An analysis of content uniformity by HPLC, including sample treatment, was described in detail in a previous paper (Muselík et al., 2014).

2.4. The dissolution test

For the dissolution testing, SOTAX (AT7 Donau Lab, Switzerland) was used. With respect to the USP monograph Warfarin Sodium Tablets, water was the dissolution medium. Furthermore, EP phosphate buffer (pH 6.8) was used. The volume of dissolution medium was 900 ml and the temperature was maintained at 37.0 ± 0.5 °C. The test was performed using an EP 2.9.3 Apparatus II (paddles, 50 rpm) as well as a modified method at 25 rpm. Twelve tablets containing 5 mg of warfarin sodium (A, B, C, D) were used for the test. The dissolved amount of the active substance was measured by HPLC according to the methodology of Muselík et al. (2014) at the following time intervals: 5, 10, 20, 30, 60, and 120 min.

2.5. Statistical evaluation of the results

For each batch from the content found in samples of tablets, the average content, RSD and Cpk with respect to EP 2.9.6 limits (85–115%) were calculated. The tablets were evaluated according to an EP of 2.9.40 and Bergum method. Dissolution profiles were compared using the difference factor (f_1) and similarity factor (f_2) and values of DE and MDT according to published methodology (Costa and Lobo,