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X-ray diffraction characterization of different polymorphyc forms of clopidogrel bisulphate in substance and pharmaceutical dosage form

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Abstract

The present study deals with the application of X-ray powder diffraction (XRPD) analysis as a technique for identification of the forms of clopidogrel bisulphate (CLP) present in both the active pharmaceutical ingredients (API) and tablets, specifically addressing the question of whether API converts to another form after 12 months of storage. The investigation of the possibility of phase transitions occurring over a temperature range spanning from room temperature to the melting point, both in tablets and pure CLP, were also performed. Tablet samples were observed for the changes in their structure using polarizing optical microscopy, which was also used to determine the melting points of tablet samples. The results gained during this work confirm that XRPD is applicable for API and tablets testing. This is particularly important if we take into account that the method can be used during stability studies, i.e. in order to test the quality of tablets during the validity period.

Key words: Drug polymorphs, clopidogrel bisulphate; X-ray powder diffraction analysis, polarizing optical microscopy

1. Introduction

Clopidogrel bisulphate $C_{16}H_{17}Cl\ N\ O_2S{\cdot}HSO_4$ (CLP) (Fig. 1) is an US-FDA approved novel pharmaceutical substance for the reduction of atherosclerotic events. CLP acts by selective and irreversible inhibition of ADP-induced platelet aggregation and is used to treat blood clots in coronary artery, peripheral vascular and cerebrovascular disease. The drug is available on the market for administration in oral form.

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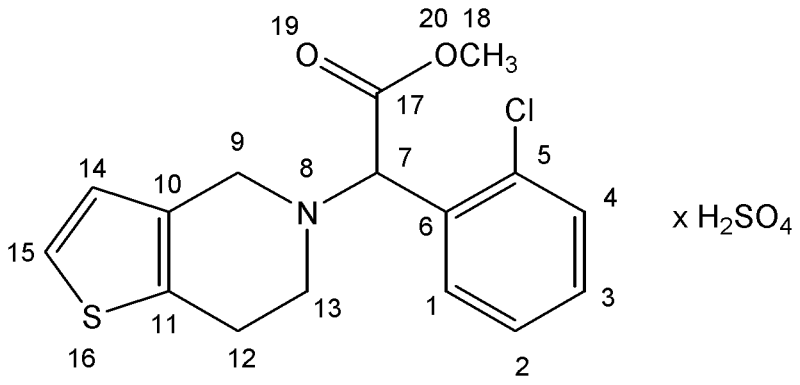

Figure 1. Structure of clopidogrel bisulphate.

Of the six known polymorphs of CLP, only two crystalline forms (Form I and Form II) have therapeutic activity and are in commercial use [1].

Polymorphism is a frequent phenomenon in pharmaceutical formulations [2,3], and different drug polymorphs exhibit different physical and chemical properties. As such, the various polymorphic forms of a drug can possess biopharmaceutcal and pharmacokinetic properties [4, 5]. Because of these differences in activity, quantification of the polymorphs present in specific pharmaceutical formulations is necessary.

Solid drug formulations are also associated with specific regulatory and intellectual property issues [6]. At the present time, regulatory authorities require pharmaceutical companies to investigate and control polymorphism in drug substances to ensure product quality, safety, and performance [7]. In particular, manufacturers must declare that their product and its active pharmaceutical ingredients (APIs) do not undergo solid phase transformations which could affect quality of the product within its shelf life. Thus, stability relationships between different solid forms of the drug substance and storage conditions have to be established in order to avoid phase transitions. Gaining such information requires suitable solid state analytical methods, in order to differentiate between polymorphic forms and solvates of the drug substance, and often, methods for quantifying these solid forms.

Form II of clopidogrel bisulphate is patented, as well as several solvate and amorphous forms of the substance [8], but Form I has now become available for generic development. Because clopidogrel bisulphate polymorphs comprise an enantiotropic system, and Form II is the thermodynamically more stable at room temperature [1,9], there is potential for the transformation of Form I into Form II, both in the production stage and during storage [10,11]. This fact requires a suitable analytical technique for the detection and quantification of the stable form within the metastable form. The X-ray powder diffraction (XRPD) analysis is a powerful tool that is widely applied for this purpose, and mixtures of polymorphs are ideal subjects for XRPD analysis.

In the present work we describe the application of XRPD analysis as a technique for: (i) identification and quantification of the forms of CLP present in both the API and tablets, (ii) investigation of Form stability after 12 months of storage, (iii) investigation of the possibility of phase transitions occurring over a temperature range spanning from room temperature to the melting point, both in tablets and pure CLP.

2. Experimental

2.1. Methods

Pure polymorph I (CLP Form I) of clopidogrel bisulphate, excipients and two tablet samples were generously provided by Hemofarm A.D. (Vršac, Serbia). Tablets marked as L-3044/P/No.1 (Sample 1) and L-3044/P/No.2 (Sample 2) were stored at 30°C and 65 % relative humidity (RH) for 12 months.

2.2. X-ray powder diffraction

The XRPD analysis was conducted in the standard Bragg-Brentano θ-2θ geometry using a Seifert MZ IV goniometer at room temperature. Standard sample holders (22 mm diameter) were carefully filled with the powder samples. The XRPD patterns in the range 2θ of 6° and 40° were recorded using CuKa radiation ($\lambda = 1.5406$ Å) and the following measurement conditions: tube tension of 30 kV, tube current of 30 mA, step-scan mode with a step size of 0.02° and counting time of 15 s/step.

Diffraction studies were also carried out at 5 different higher temperatures, on both tablet and pure CLP Form I samples. Before collecting X-ray data, samples were allowed to rest for 30 minutes at 40°C, 50°C, 60°C, 80°C and a temperature close to the melting point (150°C for tablet samples and 160°C for pure CLP Form I), respectively. Samples were investigated by X-ray diffraction in transmission geometry using a conventional powder diffractometer (Seifert V-14, CuK_α radiation at 1.5406 Å) with an automated high-temperature kit Paar HTK-10.

2.3. Polarizing optical microscopy

Structural studies using polarizing optical microscopy (POM) were performed on a Carl Zeiss Jena polarizing microscope equipped with a hot-stage for controlled heating and cooling of the sample.

3. Results and discussion

Fig. 2 shows the XRPD patterns obtained for CLP Form I. Profile fitting and subsequent peak finding was performed using the pseudo-Voight function [12] implemented in X-fit software [13]. In Form I, the three highest intensity peaks were observed at 23.26°, 25.61° and 20.64° 2θ, while Form II displayed high intensity peaks at 21.69°, 23.0° and 18.48° 2θ (in agreement with database values). At the lower 2θ angle, Form I showed specific peaks at 9.29°, 10.97°, 11.62°, 13.92°, 14.43° and 14.90° while unique peaks were present at 8.78°, 9.62°, 12.28°, 12.90°, 13.04° and 13.62° for Form II.

3.1. Identification of polymorph(s) in tested tablets

The powder obtained from the tablets was analyzed by XRPD. Although the excipient peaks (Fig. 2) complicated the XRPD patterns of the tested samples, identification of CLP peaks was possible in all cases. Two tested samples were found to contain Form I (Fig. 2). Unique peaks related to Form I were unambiguously identified, and no peaks due to Form II were observed (Fig. 2). In addition, the absence of both most prominent reflections in the angle region $2\theta = 12-14°$ and the most intense peak of Form II positioned at $2\theta = 21.69°$ indicating that Form II is not present in the tablet preparations.

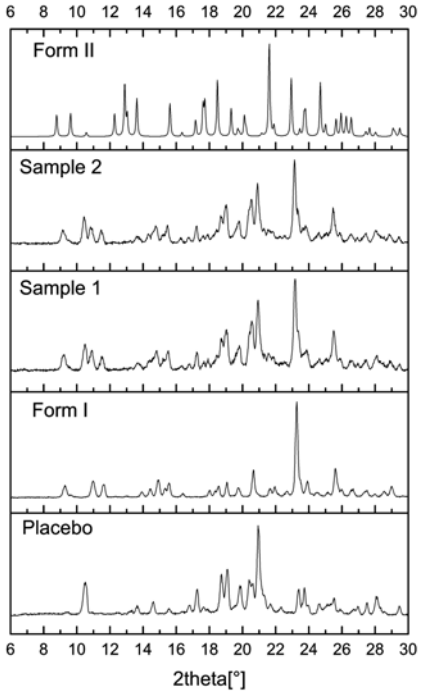

Figure 2. Diffractograms for the Placebo, CLP Form I, Sample 1 (L-3044/P/No.1), Sample 2 (L-3044/P/No.2) and CLP Form II, from the bottom to the top.

The position of crystalline reflections (2θ) for tablets remained the same within tolerance of $\pm 0.02°$, regardless of the intensity of the background scattering. Increased background scattering was attributed to the presence of excipients in the tablets. However, in all cases, comparison revealed that the peak positions were in accordance with the USP limits. The USP general chapter on X-ray diffraction states that identity is established if the scattering angles of the ten strongest reflections obtained for an analyte agree within $\pm 0.20°$ with that of reference material [14]. In all formulations, relative intensities of the Form I peaks decreased. The intensity of the diffraction peaks can be affected by dilution with excipients in the dosage form and/or by the effect of preferred orientation. Accurate quantification using XRPD relies on the absence of orientation effects and requires particle size uniformity. The differences in the particle size distribution of APIs and excipients are a significant problem. Moreover, the differences in the mass absorption coefficients between the drug substance and excipients may result in severe deviations from linearity during quantification [15]. Sophisticated methods of background subtraction and pattern-fitting by the Rietveld method may help in the quantification of APIs in dosage forms using XRPD [16].

3.2. XRPD at 40°C, 50°C, 60°C, 80°C and temperature close to the melting point

In Fig. 3 (a), (b) and (c) are presented typical diffraction spectra for Form I of clopidogrel bisulphate and both samples, collected at a temperature range spanning from room temperature to a temperature near the sample melting point, in order to rule out the possibility of phase transitions.

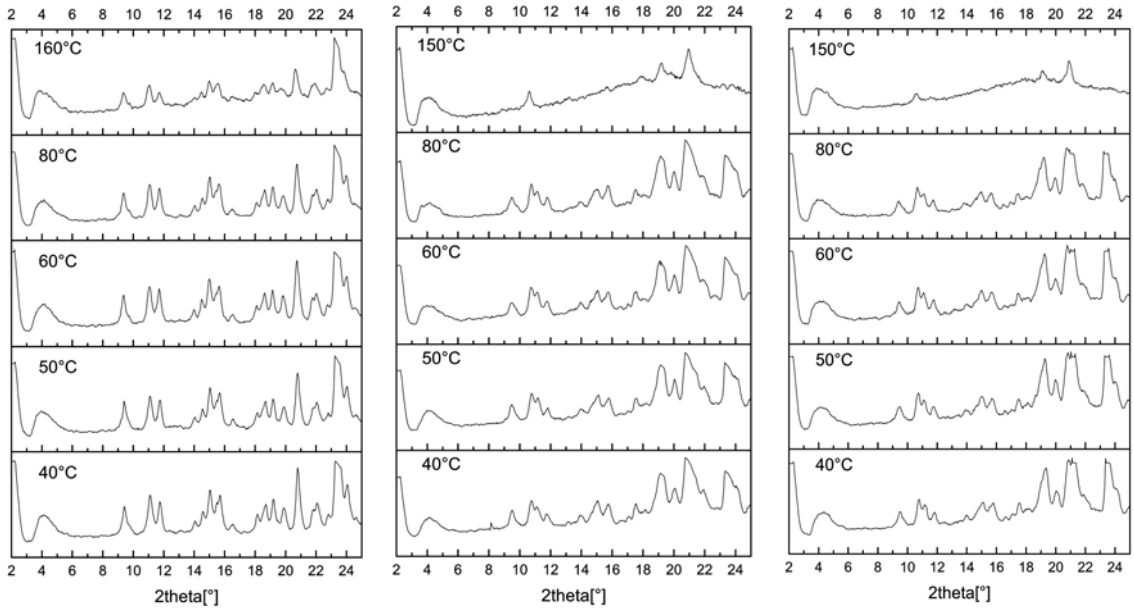

Figure 3. Diffractograms of (a) pure CLP Form I. (b) Sample 1 and (c) Sample 2 at 40°C, 50°C, 60°C, 80°C and temperature close to the melting point.

The X-ray diffractometry measurements did not register any phase transition in any of the samples at 40°C, 50°C, 60°C and 80°C. However, broadening and an increase in the intensity of the diffuse amorphous halo was observed with all samples at temperatures close to the melting point. In the case of pure CLP Form I, some low-intensity peaks disappeared, while the others diminished in intensity due to the decreased crystallinity near the melting temperature. For the two tablet samples, which are multi-component mixtures, only the three most prominent excipient peaks remained at 150°C, namely peaks at 10.49°, 19.06° and 20.95° 2θ, while peaks corresponding to Form I of CLP (which were visible at lower temperatures) had disappeared.

3.3. Polarizing optical microscopy

Both tablet samples and Form I of CLP were slowly heated up to 80°C, with additional 60 minutes rest at 40°C, 50°C and 60°C. Simultaneously, the samples were observed for changes in their texture. No visible changes were noted in comparison with their texture at room temperature, providing evidence that Form I of clopidogrel bisulphate and the two tablet samples are stable up to 80°C.

This method was used to determine the melting points of both samples. Pure clopidogrel bisulphate melts at 173°C. Due to the fact that the drug preparation is a multiphase mixture, melting starts at 154°C and the last remaining solid phase melts at 210°C.

4. Conclusion

Characterization of different polymorphic forms of active pharmaceutical ingredients and pharmaceutical dosage forms as well, is necessary in pharmaceutical industry. Forms I and II of CLP can be easily distinguished by XRPD, which is preferable method for API and tablets testing. Moreover, XRPD is often used during stability studies, i.e. to test the quality of tablets during the validity period. We conclude that the condition 30°C/65%

RH did not change the phase composition of the sample materials. Neither of the tested samples, which were conditioned for 12 months, were found to contain CLP Form II.

Acknowledgments

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