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Crystalline structure of the marketed form of Rifampicin: a case of conformational and charge transfer polymorphism

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ABSTRACT

Rifampicin is a semi-synthetic drug derived from rifamycin B, and currently integrates the fixed dose combination tablet formulations used in the treatment of tuberculosis. It is also used in the leprosy polychemotherapy and prophylaxis, which are diseases classified as neglected according to the World Health Organization. Rifampicin is a polymorphic drug and its desirable polymorphic form is labeled as II, being the main goal of this study the elucidation of its crystalline structure. Polymorph II is characterized by two molecules with different conformations in the asymmetric unit and the following lattice parameters: a = 14.0760 (10) Å, b = 17.5450 (10) Å, c = 17.5270 (10) Å, β = 92.15°. Differently to the previously reported structures, a charge transference from the hydroxyl group of the naphthoquinone of one conformer to the nitrogen of the piperazine group of the second conformer was observed. The relevance of the knowledge of this crystalline structure, which is the preferred polymorph for pharmaceutical formulations, was evidenced by analyzing raw materials with polymorphic mixtures. Thus, the results presented in this contribution close an old information gap allowing the complete solid-state characterization of rifampicin.

1. Introduction

Rifampicin (RIF) (Fig. 1), 2,7-(epoxypentadeca [1,11,13] trienimino) naphtho [2,1-*b*]furan-1,11(2H)-dione,5,6,9,17,19,21-hexahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-8-[*N*-(4-methyl-1-piperazinyl)formimidoyl]-2,7-acetate, is a semi-synthetic drug derived from rifamycin B, which is characterized by containing an aliphatic chain bridging two non-adjacent positions of an aromatic nucleus. RIF is described as a reddish brown crystalline powder [1] or as orange-red to red-brown crystals. It is slightly soluble in water, acetonitrile, methanol and ethanol [2].

RIF currently integrates the fixed dose combination tablet formulations used in the treatment of tuberculosis, and it is also used in the leprosy polychemotherapy [3,4] and prophylaxis [5] which are diseases classified as neglected according to the World Health Organization (WHO) [6–9]. A search in the Cambridge Structural Database (CSD) [10] brings seven crystalline forms of this drug, being one polymorph [1] (form I), two structures related to the pentahydrate form [11,12] and four solvates [13,14]. Taking into consideration that there are several solid forms of this drug, it was necessary to establish and standardize the most appropriate form to be used in pharmaceutical formulations, once different polymorphic forms can present different physicochemical properties as solubility and stability. Furthermore, it was already reported that the RIF polymorphs exhibit variable bioavailability [6]. It is known that polymorph I is the most stable form of RIF, however polymorph II, which is the metastable, is the form commonly marketed and, therefore, used on the pharmaceutical production [15]. Related to polymorph II, solubility and stability varies according to the pH, based on the concomitant presence of basic (phenol) and acid (amines) functional groups, presenting pKa of 1.7 and 7.9 related to the 4-hydroxyl group and nitrogen of the piperazine group, respectively.

The present study was conducted in order to elucidate the crystalline structure of the polymorph II of RIF by Single X-ray

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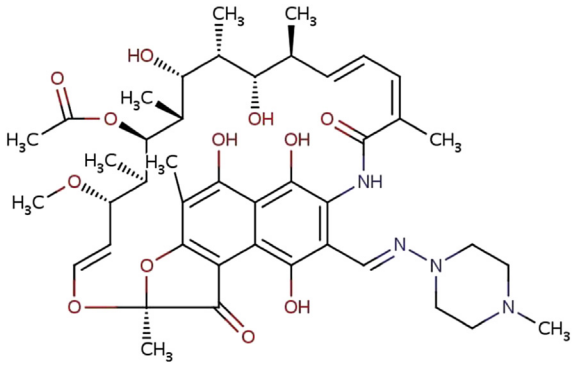

Fig. 1. - Molecular structure of rifampicin.

diffraction. The relevance of this result was proved by analyzing raw materials using X-Ray powder diffraction (XRPD). The conformational polymorphism of RIF was discussed with the support of a Hirshfeld's surface analysis, and the BFDH algorithm was applied to predict the crystal morphology of form II.

2. Materials and methods

2.1. Materials

Crystals of the polymorph II of RIF were selected from the raw material batch produced by NOVARTIS and gently provided by the Núcleo de Pesquisas em Alimentos e Medicamentos (NUPLAM, Federal University of Rio Grande of Norte).

2.2. X-ray powder diffraction

X-ray powder diffractograms were obtained using a D8 Advanced Bruker AXS, equipped with a theta/theta goniometer, operating in the Bragg-Brentano geometry with a fixed specimen holder, Cu Kα (0.15419 nm) radiation source and a LynxEye detector. The voltage and electric current applied were 40 kV and 40 mA, respectively. The opening of the slit used for the beam incident on the sample was 0.6 mm. The sample was scanned within the scan range of $2\theta = 5°–35°$ continuous scan, in a step scan mode (0.01 step size and 5 s) at room temperature.

2.3. Single crystal X–ray structure determination

Single-crystal X–ray diffraction data collection (ϕ scans and ω scans with κ offsets) were performed on an Enraf–Nonius Kappa–CCD diffractometer (95 mm CCD camera on κ–goniostat) using graphite–monochromated MoKα radiation (0.71073 Å) at 100 K. The software COLLECT [16] and Denzo–Scalepack package of softwares [17] were applied for acquisition, indexing, integration and scaling of Bragg reflections. The final cell parameters were obtained using all reflections. No absorption correction was applied. The structure was solved using Olex2 [18], with the ShelXT [19] structure solution program using Direct Methods and refined with the ShelXL [20] refinement package using Least Squares minimization.

The programs MERCURY (version 3.6) [21] and ORTEP–3 [22] were used to prepare the crystallographic information file (CIF) and artwork representations for publication. The CIF of the structure Rifampicin form II was deposited at the Cambridge Structural Data Base under the code 1023165.

3. Results and discussion

Polymorph II of RIF crystallizes in the monoclinic $P2_1$ space group, with the following lattice parameters: a = 14.0760 (10) Å, b = 17.5450 (10) Å, c = 17.5270 (10) Å, β = 92.15°. The crystallographic data is presented in Table 1. The asymmetric unit contains two ionic species of rifampicin (Z' = 2). On the other hand, the crystalline structure of the polymorph I belongs to the monoclinic space group C2 and has just one molecule per asymmetric unit. It was determined from x-ray powder diffraction measurements by Ibiapino and coworkers [1]. Regarding to morphology and crystalline habit, polymorph II present rod crystals, similar to the one that was reported in a previous work [6].

As stated, the asymmetric unit presents two ionic species with different conformations (Fig. 2). Rifampicin is a complex molecule, characterized by various groups as phenol, ester, acetyl, furanonic, amide groups and *ansa* OH. Both ionic species (Conformers A and B) are not centrosymmetric and not superimposable, as it is possible to infer from the overlap of the conformers (Fig. 3).

Comparing these conformers with the one observed in form I, it is possible to observe that the *ansa* chain is highly prone to conformational changes due to its high flexibility and seems to be the most prominent evidence in favor of the conformational polymorphism of RIF. A large set of intramolecular interactions, shown in Table 2, stabilize each one of the conformers. Special attention needs to be deposited in the piperazine ring, since it plays a very important role in the intermolecular interactions. In all the conformers, this ring adopts a chair conformation, but it is flipped up and down of the naphthoquinone plane in form II (conformer B) and form I, whereas it is perpendicular to this plane in the conformer A of form II.

Another very important feature of the polymorph II is that one of the piperazine nitrogen atoms (N4B) is protonated in conformer B. Non-neutral RIF conformers were previously observed in the pentahydrated form and some solvates, but in all these cases, the charged molecules are due to a zwitterionic conformation [12]. That is not the case of the polymorph II of RIF, where a charge transfer between the two conformers is observed. The proton attached to piperazine N4B–C43BH_3 moiety of conformer A is transferred from one of the hydroxyl group of the naphthoquinone chromophore of conformer B. In this way, both conformers of the asymmetric unit are non-neutral with opposite charges.

The crystal packing of the polymorph II is determined by several intermolecular hydrogen bonds listed in Table 2. Conformers A and B form a helicoidally chain alternating along the b-axis (Fig. 4). The corresponding *ansa* chains of both conformers are linked by the O9B–H9B···O8A. On the other hand, the protonated nitrogen of the piperazine ring of conformer B exhibit a bifurcated hydrogen bond connecting to the *ansa* chain and naphthoquinone groups of the conformer A (N4B–H4B···O1A and N4B–H4B···O7A, respectively). In addition, the form B piperazine ring is also connected to the conformer B *ansa* chain through the C40B–H40B···O7A bond. The proton donor of conformer A is mainly involved in an intramolecular interaction (O1A-H1A···O4A), but it also participate in weak hydrogen bonds with the piperazine ring (C41B–H41A···O4A) and *ansa* chain (C29B–H29B···O4A) of different conformer B ionic species.

Based on the determined structure, the BFDH algorithm [23–25] implemented in Mercury [21] was applied to predict the crystal morphology of form II, which was compared with those of form I [1] and the pentahydrated form [12] in Fig. 5. Ibiapino et al. [1] have observed a good agreement between the crystalline habit of form I and the predicted morphology. The same conclusion can be raised by comparing the expected crystalline habit of form II with the one observed in a raw material of this polymorph. It is also interesting