X-ray diffraction applied to the characterization of the fixed dose combination tablets for the treatment of tuberculosis.

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***Abstract*— The powder x-ray diffraction (PXRD) can be used in pharmaceutical studies for characterization of medications, identification and quantification of polymorphs. Thus, this study aimed to verify the potential of the XRD technique to evaluate fixed-dose combination (FDC) tablets for the treatment of tuberculosis, as well as, the individual active pharmaceutical ingredients (APIs). In order to ensure the efficacy, safety and quality of a drug, it is needed the knowledge of the solid state properties of APIs used in the formulation. The investigated FDC tables were composed of rifampicin (RIF), isoniazid (ISO), pyrazinamide (PYR) and ethambutol (ETA). The PXRD technique was used to characterize and identify the different samples studied. Comparing different lots of the FDC, it was possible to differentiate each API, as well as the different polymorphs in the reference sample of the rifampicin.**

***Index Terms*—Characterization; FDC; X-ray Diffraction**

I. INTRODUCTION

The development of a pharmaceutical form involves several steps aiming at the creation of a physical system that contains the APIs and excipients, satisfying the quality requirements that ensure its effectiveness and safety.

The solid form is the most widely used for drugs delivering because of the convenience and stability, reliability in the dosage, protection, and better acceptance by the patient[1].

The APIs and their delivering methods are primarily responsible for the speed and the extent to which the drug becomes available for absorption.

Although the chemical purity is acceptable, national and imported APIs, which are used as raw materials in the manufacture of medicines, often exhibit differences in solid state characteristics (polymorphism, particle size, crystal habits etc.). Characteristics that may affect the stability or availability of the solid form of the drug should be monitored and controlled, therefore the physical characterization of solids has become an extremely important area in pharmaceutical industry[2].

The solid state is characterized by having a defined three-dimensional structure with a defined volume. The solids can be distinguished as crystalline and amorphous. Depending on whether or not having a regular structure in the arrangement of the molecules that form them[3]. In amorphous solids, the molecules do not exhibit a long-range order in arrangement of atoms, while in the crystalline solids, the atoms are arranged over long atomic distances forming a three-dimensional structure which is called crystalline network. As a result, the crystal has a structure characterized by regular repetition of components having properties of homogeneous, symmetric and anisotropic systems.[4]

Differences in the solid state can affect the physical and chemical properties and interfere with therapeutic properties and manufacturing of drugs.[5]

The characterization of solid state properties of a drug using appropriate analytical techniques is thus an essential prerequisite for the development of solid pharmaceutical forms.[6]

In this sense, regulatory agencies such as FDA (Food and Drug Administration) and ANVISA (Surveillance Brazilian National Agency) have established specific regulatory requirements for assessing the characteristics in the solid states of drugs.[7]

Different analytical techniques have been employed in order to obtain a complete characterization of drugs in the solid state including: X-ray Diffraction, Thermal Analysis, and Spectroscopic and Microscopic Techniques.[8]

The PXRD technique is very important for the monitoring of the crystalline form of a drug during the various stages of development, as any phase shift due to polymorphic interconversions, desolvation of solvates, hydrate formation and changes in the degree of crystallinity may change the solubility, the physical and chemical stability of the drug. These differences may modify the behavior of the molecule as the organic medium, which may directly affect the biodistribution

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and therefore its efficiency.[10]

The fixed-dose combination tablets are recommended by the World Health Organization and the International Union Against Tuberculosis and Lung Disease as an additional measure in order to expand treatment adherence, reduce dropout rates, and achieve the disease control.[11-13]

The proposal of the FDC tablets was to include in the same pharmaceutical form the four drugs used in the treatment regimen for tuberculosis: rifampicin - 150mg, isoniazid - 75mg, pyrazinamide - 400mg, ethambutol - 275mg. Therefore, many advantages were observed over the drugs used alone (monotherapy). Among them, there is the reduction in prescribing errors and the amount of ingested pills, a fact that favors the patient's adherence to treatment.

Although the FDC tablets are recommended by the World Health Organization and the International Union against Tuberculosis and Lung Disease for tuberculosis, the properties of the solid state of each of their assets, as well as, the FDC tablet need to be better elucidated in order to discern alternatives to improve their therapeutic action.

Therefore, this study aimed to verify the potential of the PXRD technique to evaluate different batches of FDC tablets and their APIs, characterizing them as a way to establish quality parameters for those tablets and assist in technological improvement of medicines based on the studied drugs.

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II. Experimental

A. *Materials and Methods*

Three different batches of the fixed-dose tablets were evaluated: (BATCH 1 - BNAH280553), (BATCH 2 - BNA300230) and (BATCH 3 - BNA300244), which were obtained from LUPIN LTD – India. Besides FDC tablets, the following active pharmaceutical ingredients were also analyzed by X-ray powder diffraction: RIFAMPICIN (USP reference, batch 1 and batch 2), Isoniazid (USP reference, batch 1 and batch 2), pyrazinamide (USP reference, batch 1 , lot 2), ethambutol (USP reference, lot 1, lot 2).

The FDC tablets were reduced to a fine powder with the aid of a mortar and pestle. After standardization of the grain size to obtain crystal uniformity of the solid, the samples were subjected to the diffraction tests, where they have been characterized by crystallographic technique (PXRD) to identify each APIs and to evaluate properties such as polymorphism.

The X-ray diffractograms of the samples were obtained by the powder method in a conventional instrument (D8 Advanced by Bruker AXS) with radiation CuKα. The accelerating voltage was 40 kV and the current used was 40 mA. The measurements of the samples of the tablets and the respective APIs were performed using Bragg Brentano geometry. All measurements were performed in a range between 3 and 35 degrees, with a 0.020º increment and 2 º.min^{-1} speed.

B. *Results and Discussion*

The recorded PXRD patterns for the investigated samples of RIF (USP reference, batches 1 and 2) were presented in figure 1, where they are compared to those of the pure polymorphs I and II. According to these results it was possible to observe that the USP reference standard for RIF is a mixture of the polymorphs I and II. The same conclusion was raised for Batch 1, which is also a mixture of the two polymorphs. On the other hand, Batch 2 contains only the polymorph II.

In the case of the FDC formulations (3 batches), it was possible to identify all components using PXRD, as it is shown in Figure 2. In addition, our results confirm that in these tablets, RIF is formulated mainly using the polymorphs II. Due to that, special care must be taken if the USP reference standard is used in the quality control. As it was proved in Figure 1, despite the chemical purity, it contains a polymorph mixture which not only could lead to erroneous structural identifications but also to differences in other physicochemical properties, such as solubility.

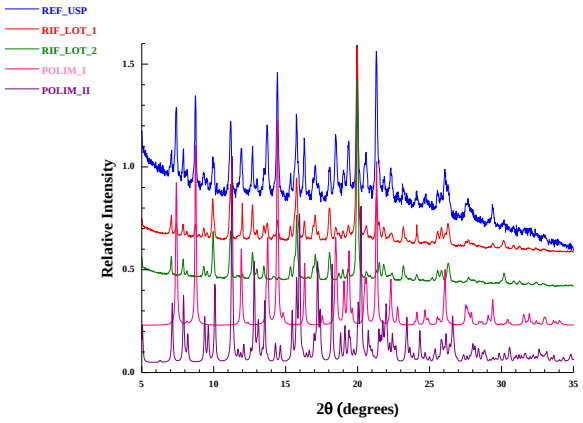

Figure 1: X-ray diffraction spectrum of the REF USP RIF, Batch 1, Batch 2 and polymorphs I and II.

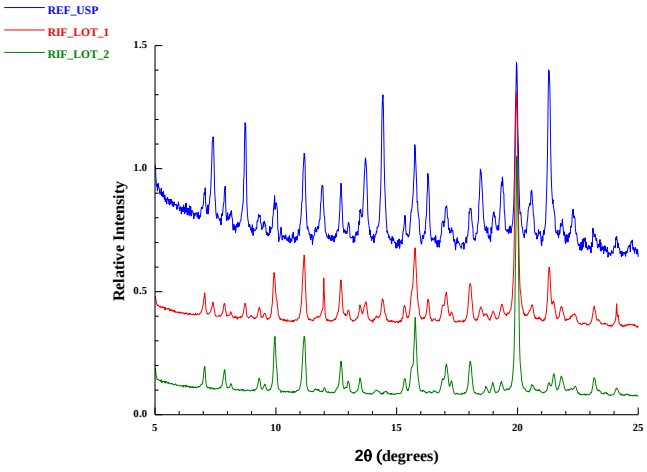

Figure 2: X-ray diffraction spectrum of the REF USP RIF, Batch 1 and Batch 2.

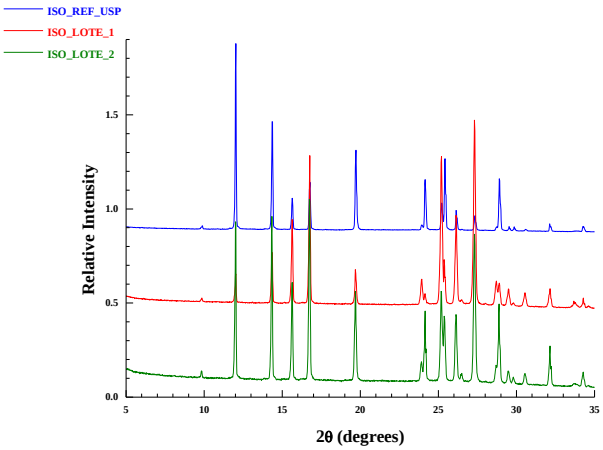

Figure 3: X-ray diffraction spectrum of the REF USP ISO, Batch 1 and Batch 2.

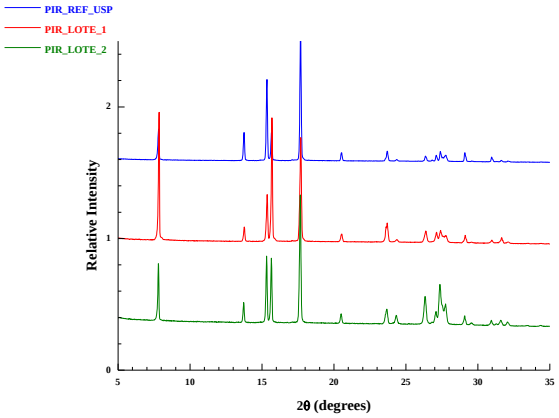

Figure 4: X-ray diffraction spectrum of the REF USP PYR, Batch 1 and Batch 2.

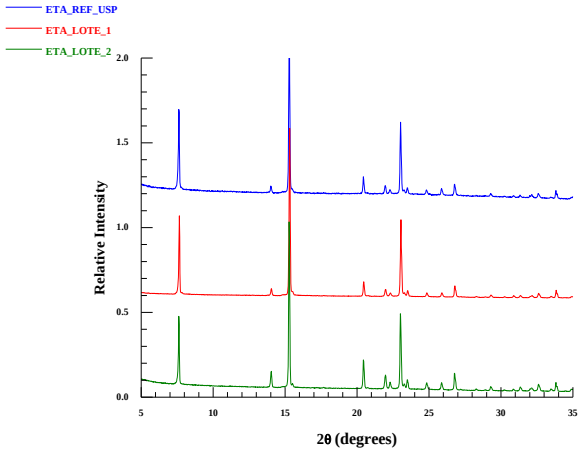

Figure 5: X-ray diffraction spectrum of the REF USP ETA, Batch 1 and Batch 2.

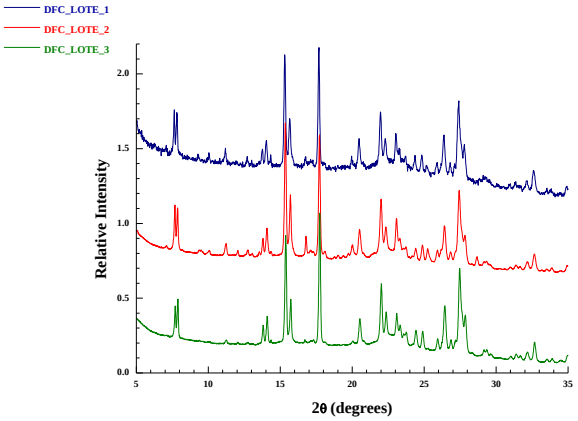

Figure 6: X-ray diffraction spectrum of the different Baths formulation of the FDC tablet.

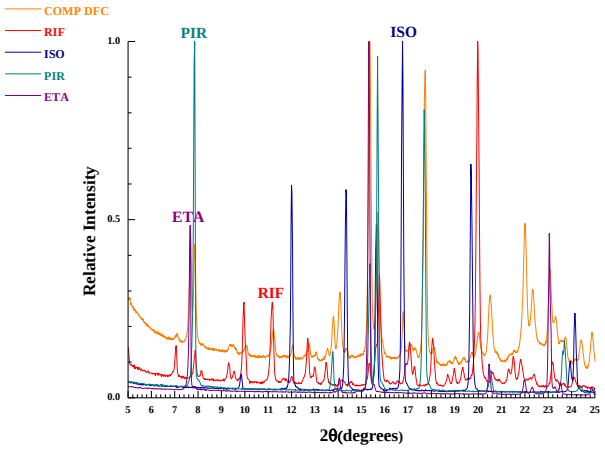

Figure 7: X-ray diffraction spectrum of the da formulation of the FDC tablet, RIF, ISO, PYR, ETA.

III. Conclusion

Considering the recorded experimental results and in the light of the objectives outlined in the design of this study, it can be concluded that powder X-Ray diffraction has been proved to be very useful to characterize the fixed-dose tablets and the different crystalline forms of the APIs. This technique has provided information that identified each of the ingredients in three different batches of FDC tablets, and confirmed the existence of different crystal forms (polymorph I and polymorph II) in all analyzed batches of rifampicin.

This research is still in progress and it will be complemented with a thorough characterization of both tablets and raw materials using other analytical techniques (Infrared and Raman spectroscopies, differential scanning calorimetry and thermogravimetry).

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