PXRD-12

20-24 May 2013 Institute of Physics
Chinese Academy of Sciences, Beijing, China

Crystal Structure Determination and Rietveld Refinement of Two Anhydrous Rifampicin Polymorphs using High-Resolution Synchrotron X-ray Powder Diffraction

Rafael Cardoso Seiceira

Institute of Drug Technology - Farmanguinhos/Fiocruz

Solid State Laboratory

Rio de Janeiro - Brazil

Beijing - China

(25 May 2013)

Ministério da Saúde

FIOCRUZ
Fundação Oswaldo Cruz

farmanguinhos
Instituto de Tecnologia em Fármacos

This document was presented at PPXRD - Pharmaceutical Powder X-ray Diffraction Symposium

Sponsored by The International Centre for Diffraction Data

PPXRD Website – www.icdd.com/ppxrd

ICDD Website - www.icdd.com

Topics

- Introduction
  - Tuberculosis
  - Tuberculosis Treatment in Brazil
  - Rifampicin
- Objective and Justificative
- Methodology
- Results
- Conclusion
- Farmanguinhos / Fiocruz
- Acknowledgements

Tuberculosis (TB)[1]

- an infectious bacterial disease caused by *Mycobacterium tuberculosis.*
- most commonly affects the lungs, but can attack any part of the body, such as the kidney, spine, and brain.
- It is transmitted from person to person via droplets from the throat and lungs of people with the active respiratory disease.
- The symptoms are: coughing, sometimes with sputum or blood, chest pains, weakness, weight loss, fever and night sweats.
- Tuberculosis is treatable with a six-month course of antibiotics.

[1]World Health Organization.

Tuberculosis (TB)[1]

- About one third of the world's population is infected with tuberculosis (TB) bacteria. Only a small proportion of those infected will become sick with TB.
- People with weakened immune systems have a much greater risk of falling ill from TB.
- In 2011, 8.7 million people fell ill with TB.
- A total of 1.4 million people died from TB in 2011 (including 430 000 people with HIV and 70 000 children) ***But TB is curable and preventable.***
- The number of people falling ill with TB is declining and the TB death rate dropped 41% since 1990. For example, Brazil and China have showed a sustained decline in TB cases over the past 20 years. In this period China, had an 80% decline in deaths.
- About 51 million TB patients have been successfully treated since 1995 worldwide.

[1]World Health Organization.

Tuberculosis Treatment in Brazil

First line anti-TB drugs:	Isoniazid (INH) Rifampicin (RMP) Pyrazinamide (PZA) Ethambutol (EMB)
Second line anti-TB drugs:	Ethionamide Streptomycin

The new scheme consists of tablets with fixed dose combination (FDC):

FARMANGUINHOS

- 2x1 – RMP : INH (150:75)mg , (300:150)mg. ➡ Bioequivalence (300:150)mg
- 4x1 – RMP : INH : PZA : EMB (150:75:400:275)mg. ➡ Transfer Technology International

Advantages of FDC:

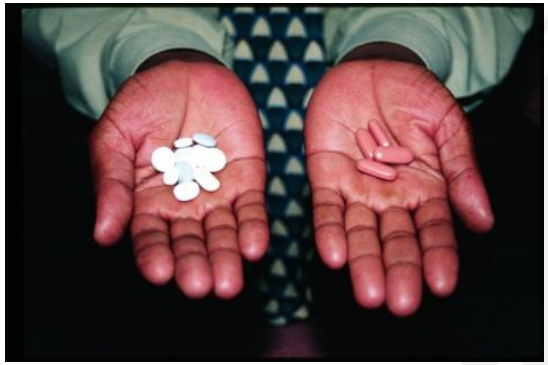


Figure 1 – Advantages of FDC.

- Monotherapy is prevented. Consequently the risk of developing drug resistant strains decreases.

- Prescription and administration are simplified with improved doctor patient relationship and increase adherence.

- Better management, transportation and distribution.

- The risk of incorrect use of rifampicin for other conditions than TB is reduced.

Rifampicin (RNH)

- key component of tuberculosis chemotherapy along with other first line anti-TB drugs
- exhibits polymorphism:
  - ✓exists in two anhydrous polymorphic forms: form I and form II [2]
  - ✓exists as various solvates [3]
  - ✓convert into amorphous form at room temperature or after desolvation [4]
- RNH commercial samples: form I and II pures
  mixture form I and II - with or without amorphous.
- non crystal structure determination.

• *Cambridge Structural Database (CSD);*
• *Articles*

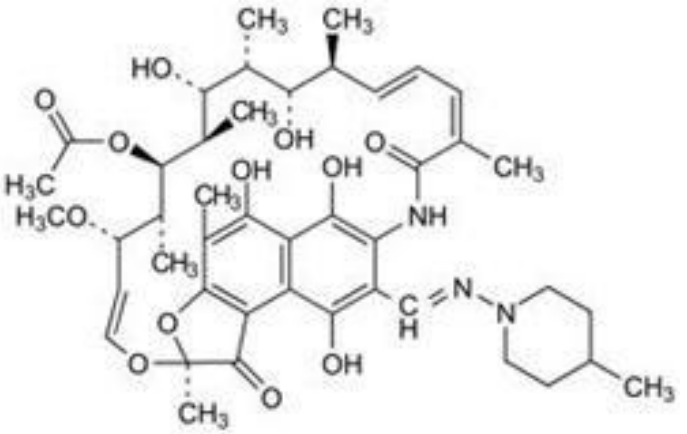


Figure 2 – Chemical structure of rifampicin.

[2] Maher, D., Chaulet, P., Spinaci, S., Harries, A.D., 1997. Treatment of tuberculosis—guidelines for national programmes.World Heath Organization, Geneva, WHO/TB/97/220.
[3] Pelizza, G., Nebuloni, M., Ferrari, P., Gallo, G.G., 1977. Polymorphism of rifampicin. IL Farmaco 32, 471–481.
[4] S.Q. Henwood, W. Liebenberg, L.R. Tiedt, A.P. Lotter, M.M. Villers, 2001. Characterization of the solubility and dissolution properties of several new rifampicin polymorphs, solvates and hydrates. Drug Dev. Ind. Pharm. 27, 1017–1030.

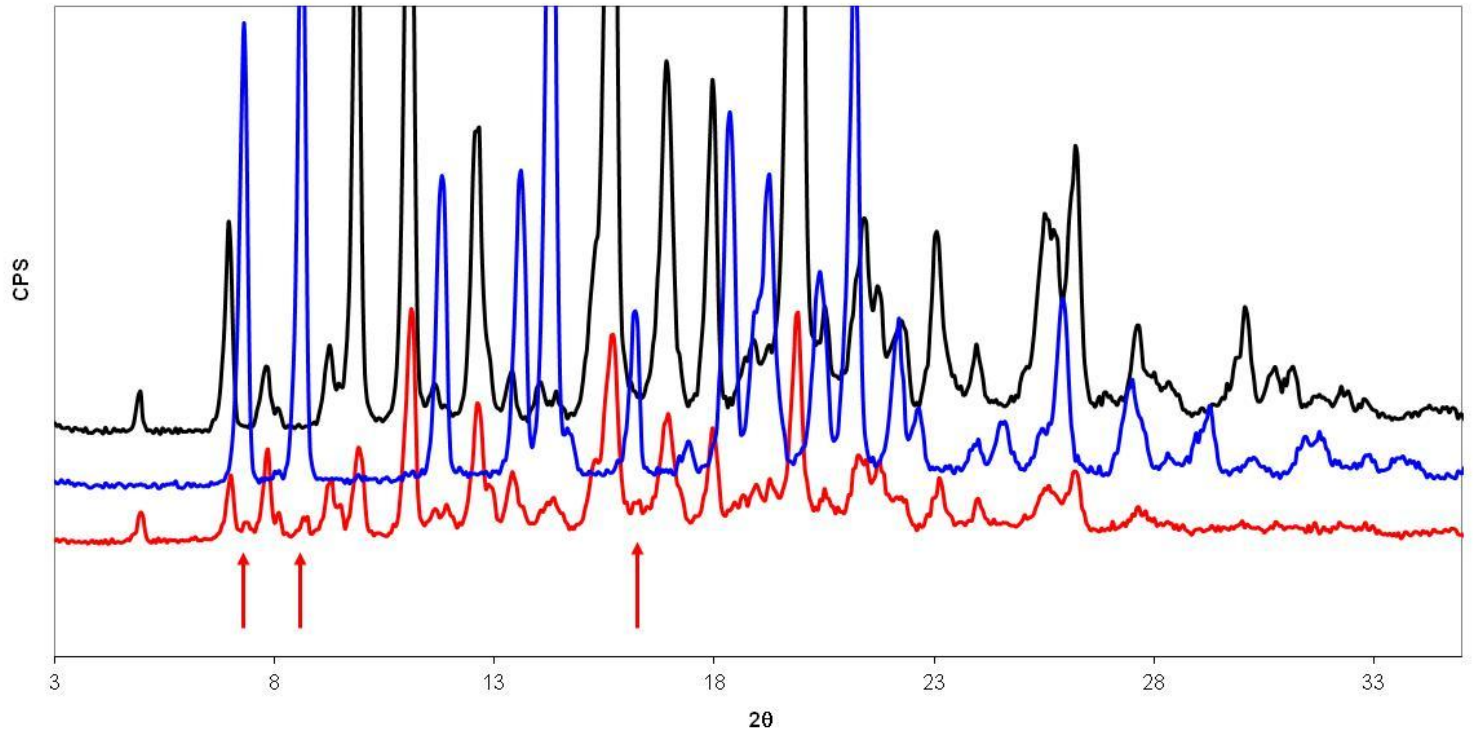
Objective

The objective of the present work was the crystal structure determination of two anhydrous rifampicin polymorphs – form I and II, by means of synchrotron X-ray powder diffraction data and subsequent Rietveld refinements

Justificative

Improving the quality of monitoring polymorphisms in samples of rifampicin and quantification of small amounts of crystalline impurities.

Justificative



How Quantify??

Methodology

Characterization of physical form of rifampicin powder samples.

❑ Thermogravimetric Analysis (TGA)

- TGA / SDTA 851^{e} Mettler Toledo
- Temperature Range: 25 – 1000°C
- Heating Rate: 10°C/min.
- Atmosphere: N_2 (flow rate: 50mL/min)
- Equipment was calibrated with indium and aluminum.

Figure 3 – TGA / SDTA 851^{e} Mettler Toledo.

Methodology

❑ Differential scanning calorimetry (DSC)

- DSC 822^e Mettler Toledo
- Temperature Range: 25 – 300°C
- Heating Rate: 10°C/min.
- Atmosphere: N_2 (flow rate: 80mL/min)
- Equipment was calibrated with indium and zinc.

Figure 4 – DSC 822^e Mettler Toledo.

Methodology

❑ Fourier transformed infrared spectroscopy (FTIR)

- FTIR 660-IR Varian.
- ATR Acessory (ZnSe crystal).
- Range: 4000 – 650 cm^{-1}.
- Resolution: 4 cm^{-1}.

Figure 5 – FTIR 660-IR Varian.

Methodology

❑ X-ray Powder Diffraction (XRPD)

- XRPD D8-Advance Bruker
- CuKα radiation (λ for Kα1 = 1.54060 Å; λ for Kα2 = 1.54438 Å)
- Voltage: 40KV
- Current: 40mA
- Angular range from 3 to 40° 2θ
- Scan speed: 0.02° / second.

Figure 6 – XRPD D8-Advance Bruker.

Results

❑ Thermogravimetric Analysis (TGA)

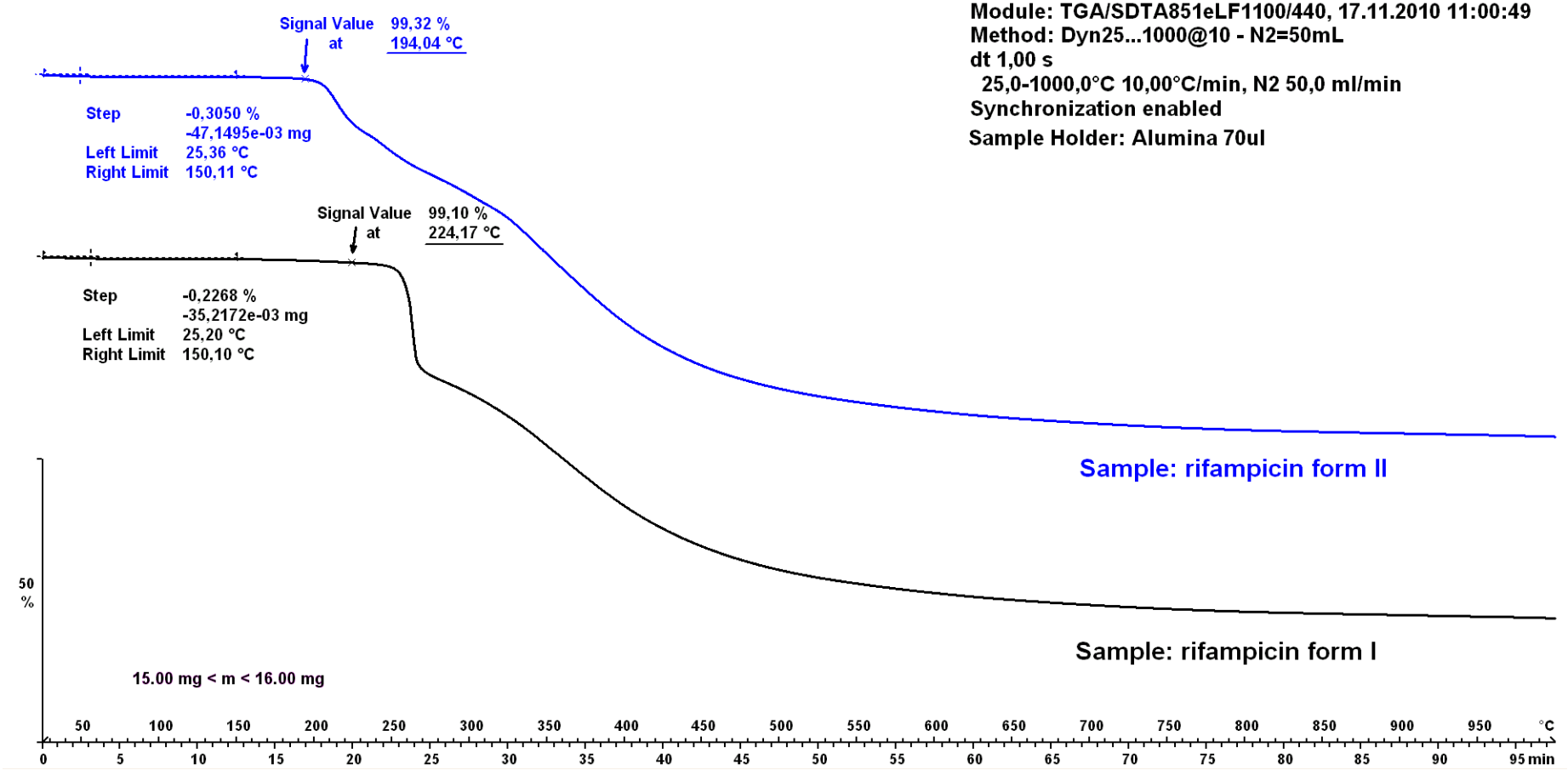


Figure 7 – TGA curves.

Results

❑ Differential scanning calorimetry (DSC)

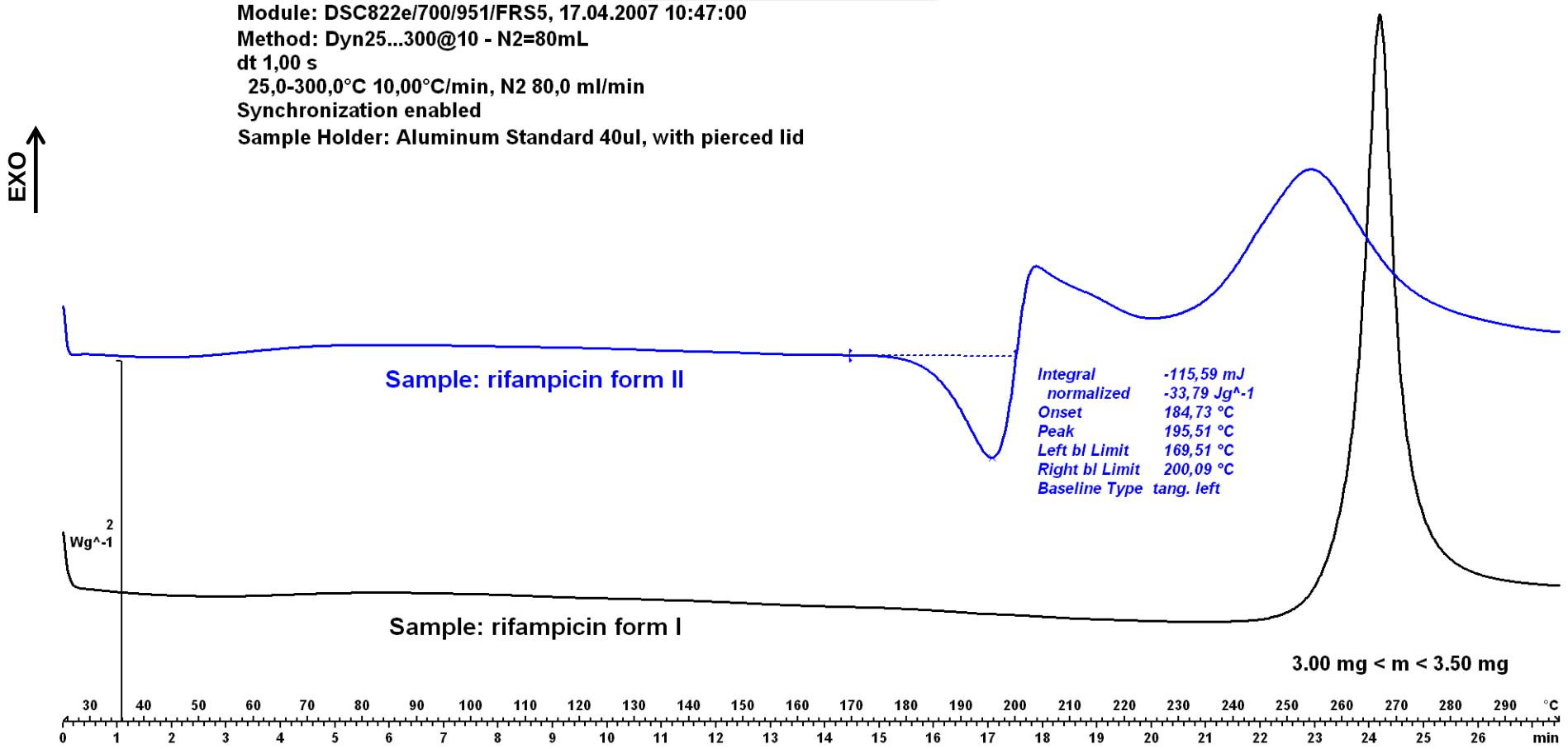


Figure 8 – DSC curves.

Results

❑ **Fourier transformed infrared spectroscopy (FTIR)[5]**

Rifampicin Form I

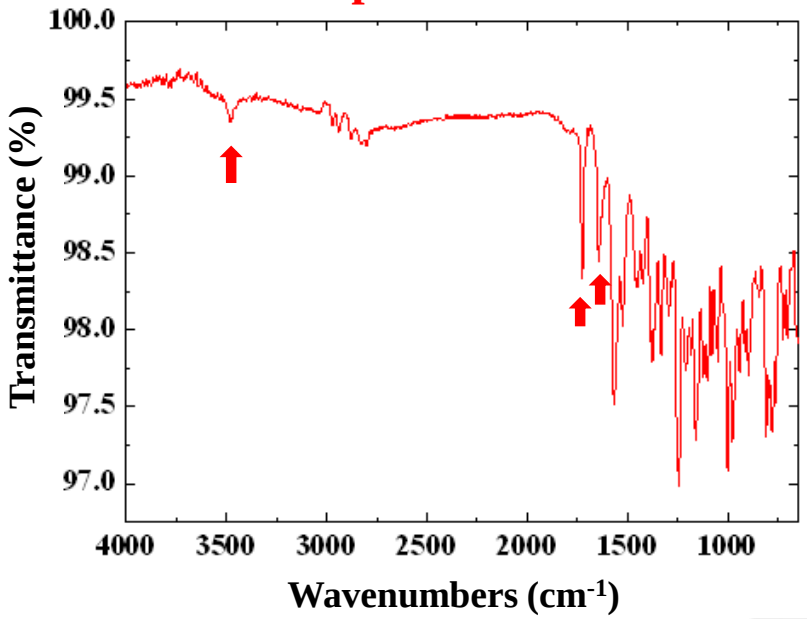


Figura 9: FTIR spectra of rifampicin form I.

- **3480 cm^{-1} (ansa OH);**
- **1722 cm^{-1} (acetyl C=O);**
- **1643 cm^{-1} (furanone C=O).**

Rifampicin Form II

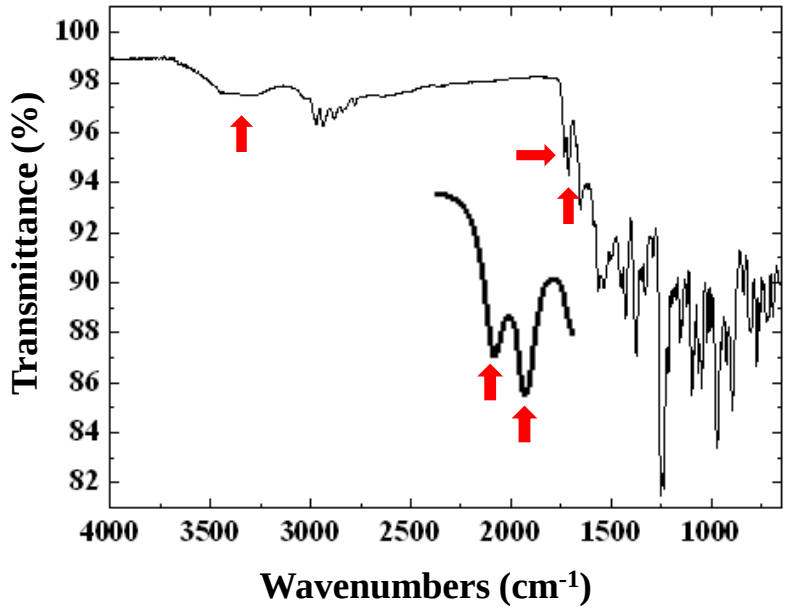


Figura 10: FTIR spectra of rifampicin form II.

- **3356 cm^{-1} (ansa OH);**
- **1732 cm^{-1}(furanone C=O);**
- **1714 cm^{-1} (acetyl C=O).**

[5]*European journal of pharmaceutical sciences*, *22*(2-3), 127–44, 2004.

Results

❑ X-ray Powder Diffraction (XRPD)

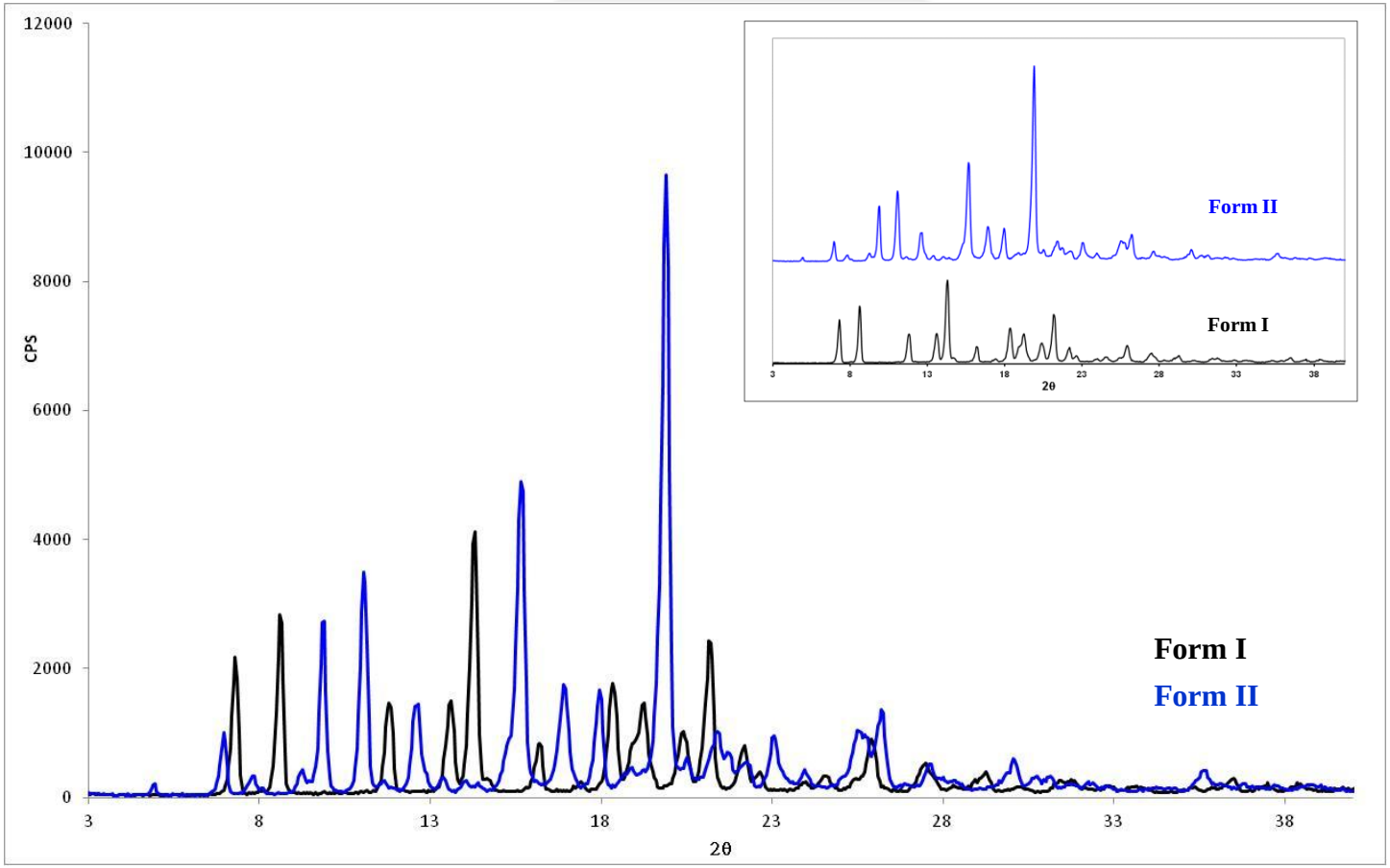


Figure 11 – XRPD .

Methodology

❑ XRPD - Synchrotron Light Laboratory

• The data were collected at the X-ray Powder Diffraction Beamline[6] of the Brazilian Synchrotron Light Laboratory (LNLS, Campinas, Brazil).

• X-rays of λ = 1.23982 Å wavelength were used.

• An *ab initio* algorithm (simulated annealing), implemented in DASH[7] was used for both indexing and for crystal structure determination.

Figure 12 – LNLS, Campinas, Brazil.

[6] F.F. Ferreira, E. Granado, W. Carvalho Jr., S.W. Kycia, D. Bruno, R. Droppa Jr., 2006. X-ray powder diffraction beamline at D10B of LNLS: application to the Ba_2FeReO_6 double perovskite. J. Synchrotron Radiat. 13, 46-53.
[7] W.I.F. David, K. Shankland, J. van de Streek, E. Pidcock, W.D.S. Motherwell, J.C. Cole, 2006. DASH: a program for crystal structure determination from powder diffraction data, J. Appl. Crystallogr., 39, 910.

Synchrotron Light Laboratory (LNLS)

SIRIUS – New Beam Synchrotron Light Laboratory

Figure 13 – LNLS and SIRIUS.

Table 1 – Examples of Beam Synchrotron Light Laboratories.

	LNLS	SIRIUS	Soleil	Diamond	Shanghai
Energy (GeV)	1,37	**3,0**	2,75	3,0	3,5
Diameter (m)	30	**137**	113	179	137
Brightness @ 10 keV*	1	**5,6 x 10^3**	1,6 x 10^3	3,6 x 10^3	2,2 x 10^3
Brightness @ 50 keV*	1	**2,5 x 10^{10}**	1,9 x 10^9	4,4 x 10^9	5,8 x 10^9
Number of dipole lines	24	**20**	32	48	40
Number of straight lines	4	**18**	22	22	18
Nat. Emittance (nm.rad)	100	**1,7**	3,7	2,7	3,9

*Normalized by actual LNLS

Results

❑ Rietveld Refinaments - *Rifampicin Form I*

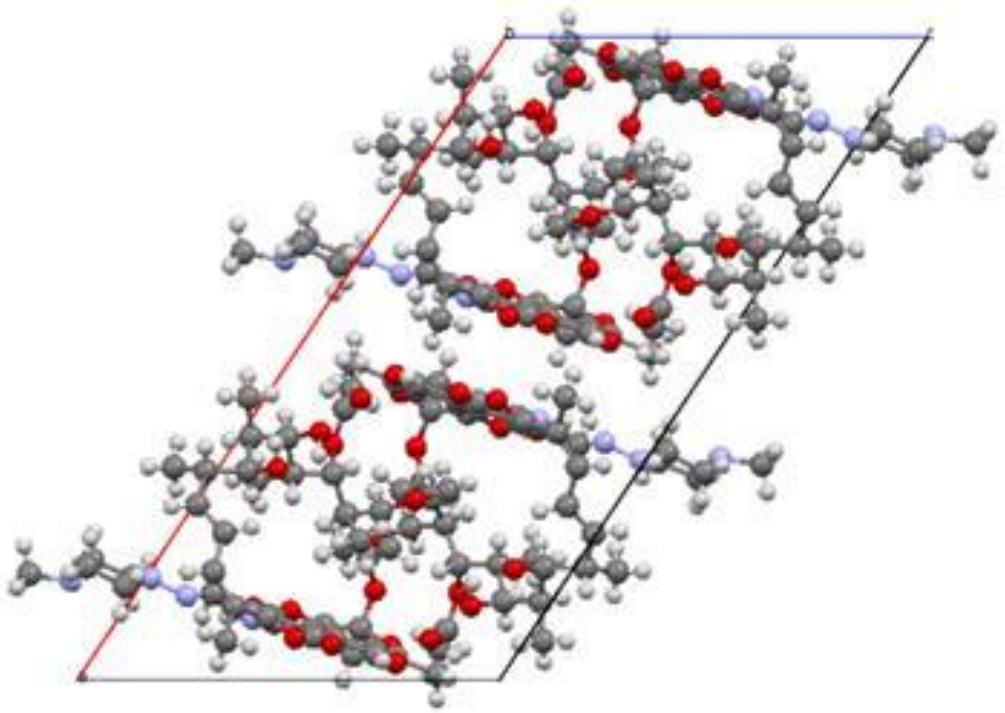


Figure 14 – Crystal packing inside the unit cell of rifampicin form I along the *b*-axis.

Crystallized under a monoclinic crystal system (space group C_2).

Unit cell parameters:

a = 26.0943(2) Å

b = 14.2970(1) Å

c = 14.2794(2) Å

β = 123.6785(5)°, V = 4433.13(7) Å^3

Z = 4, Z' = 1, ρ_{calc} = 1.14544(2) g·cm^{-3}

Results

❑ Rietveld Refinaments - *Rifampicin Form I*

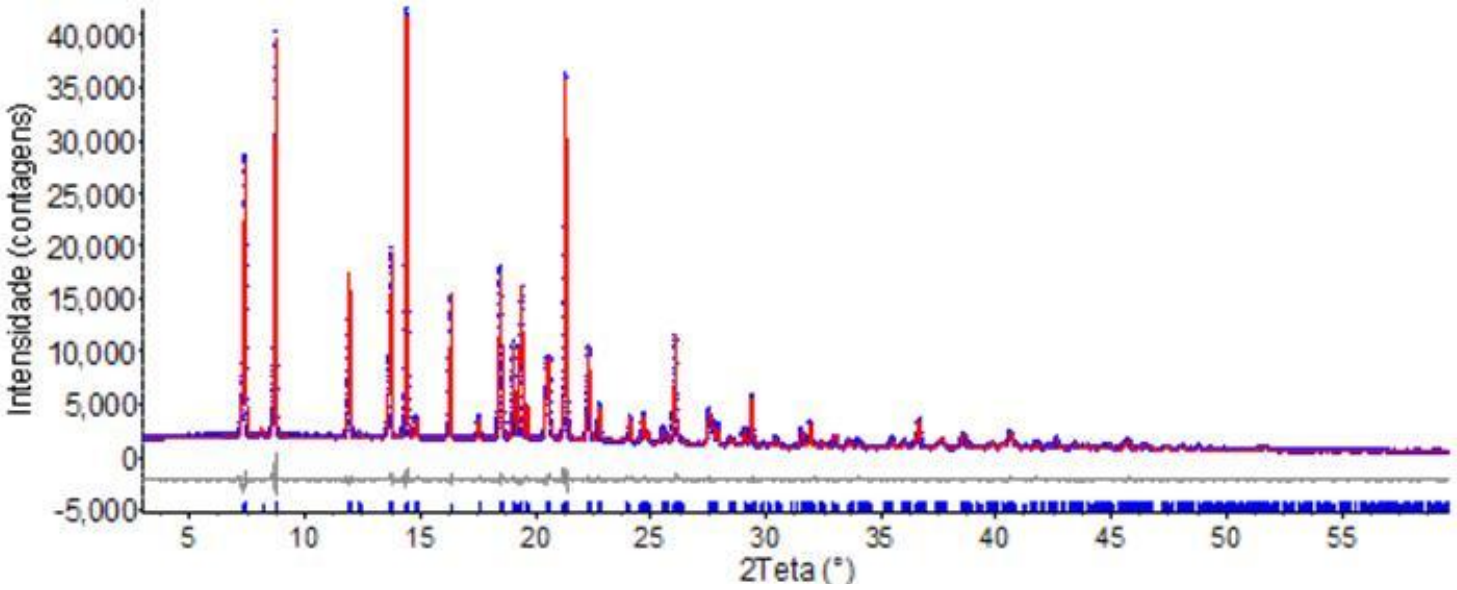


Figure 15 – Rietveld refinament of rifampicin form I.

The goodness-of-fit indicator as well as the R factors were, respectively:

$\chi^2 = 1.79$, $R_{Bragg} = 1.72\%$, $R_{exp} = 2.34\%$, and $R_{wp} = 4.19\%$

Results

❑ Rietveld Refinaments - *Rifampicin Form II*

Crystallized under a monoclinic crystal system (space group $P2_1$)

Unit cell parameters:
a = 17.6014(6) Å
b = 17.7434(12) Å
c = 14.0832(4) Å
β = 91.975(3)°, V = 4395.7(4) Å^3
Z = 4, Z' = 2, ρ_{calc} = 1.15518(10) g·cm^{-3}

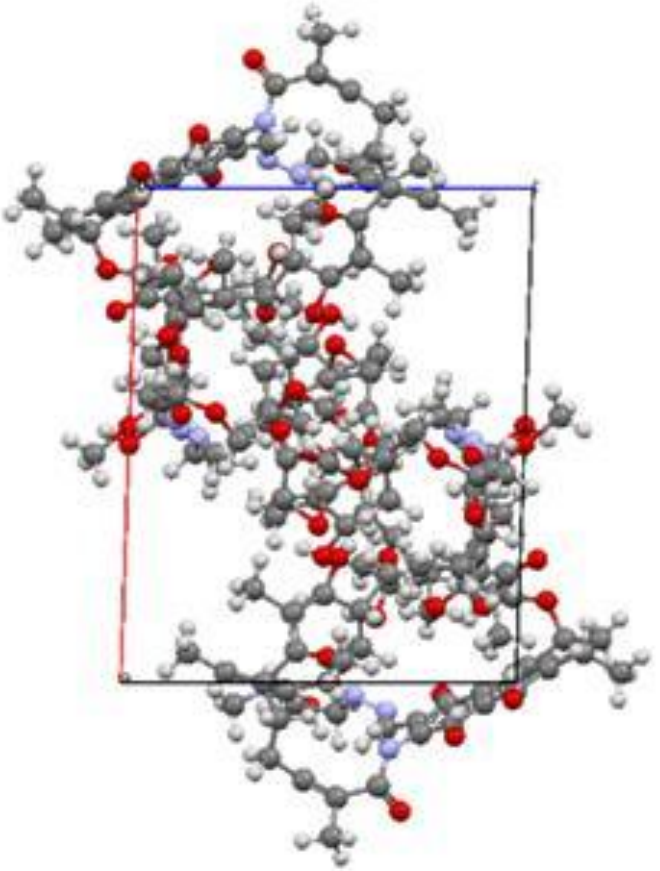


Figure 16 – Crystal packing inside the unit cell of rifampicin form I along the *b*-axis.

Results

❑ Rietveld Refinaments - *Rifampicin Form II*

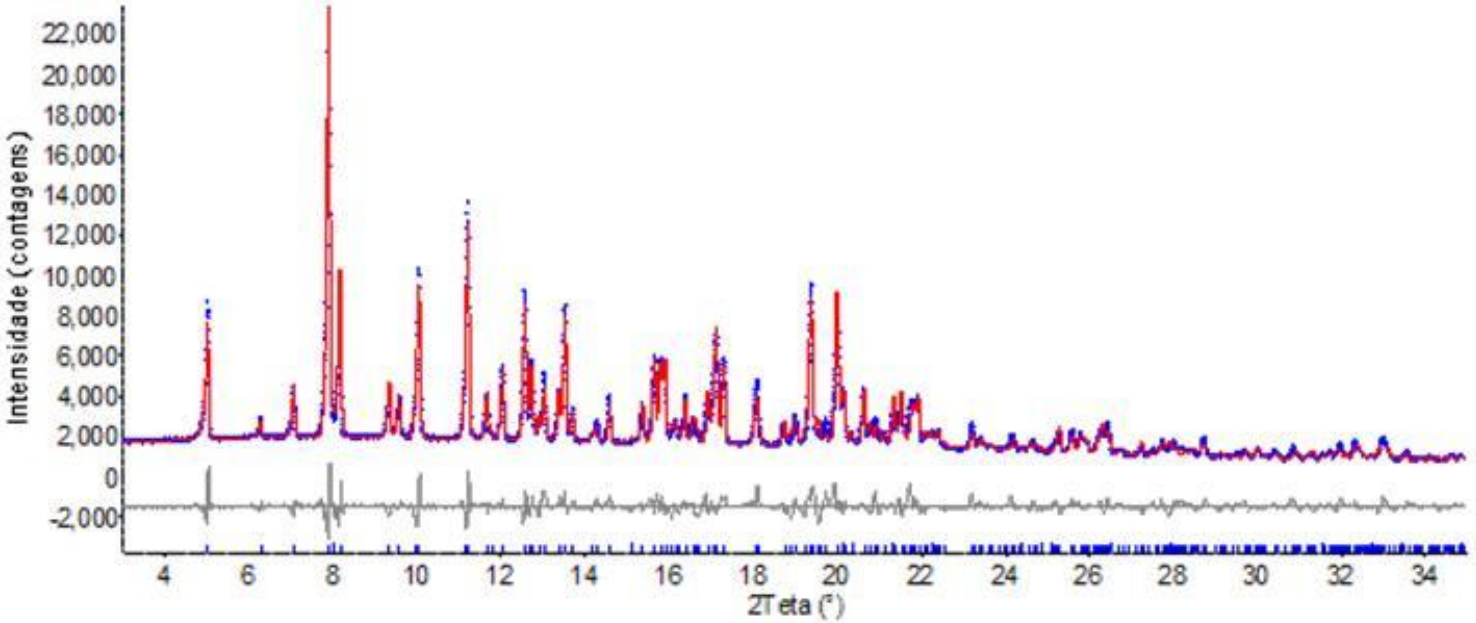


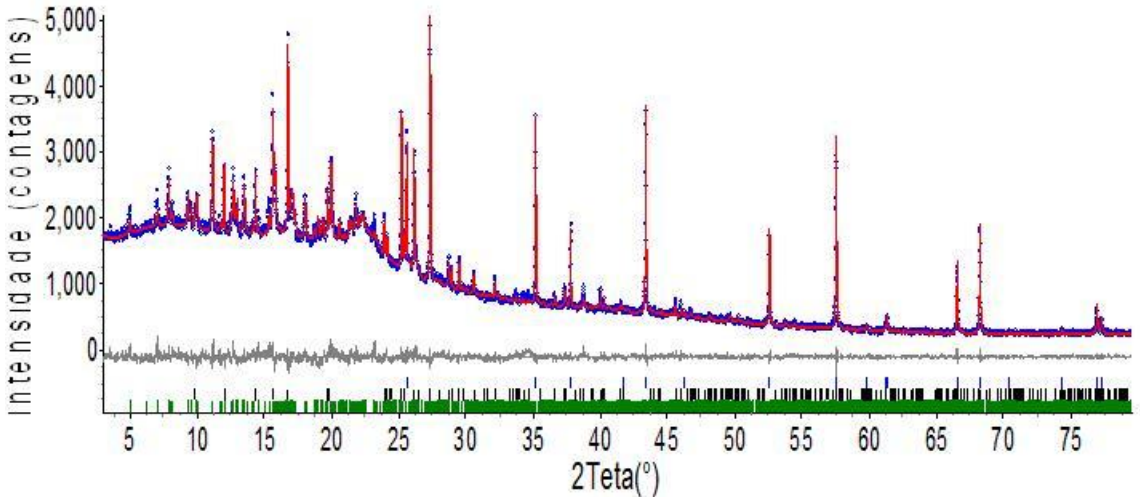
Figure 17 – Rietveld refinament of rifampicin form I.

The goodness-of-fit indicator as well as the R factors were, respectively:

$\chi^2 = 4{,}24$, $R_{Bragg} = 4.09\%$, $R_{exp} = 2.10\%$, and $R_{wp} = 8.89\%$

Quantification of phases in tablets with fixed dose combination (FDC 2x1):

Commercial FDC INH:RMP 1:2 - (75:150)mg:
Internal standard used : Alumina (Al_2O_3) – NIST purity = (99,02 ± 1,11)%



wt% Results:
Al_2O_3 = 10.99%
INH = 17.1%
RMP form II = 22.5%

Amorphous fraction = 55.5%
Crystalline fraction = 44.5%

Figure 18 – Rietveld refinament of FDC INH:RMP (75:150)mg.

The goodness-of-fit indicator as well as the R factors were, respectively:

R_{Bragg} = 0.843% (Al_2O_3), R_{Bragg} = 1.51% (INH), R_{Bragg} = 1.95% (RMP-formII),

R_{exp} = 3.25%, R_{wp} = 3.88% and χ^2 = 1.19

Quantification of phases in tablets with fixed dose combination (FDC 2x1):

Commercial FDC INH:RMP 1:2 – (150:300)mg:
Internal standard used : Alumina (Al_2O_3) – NIST purity = (99.02 ± 1.11)%

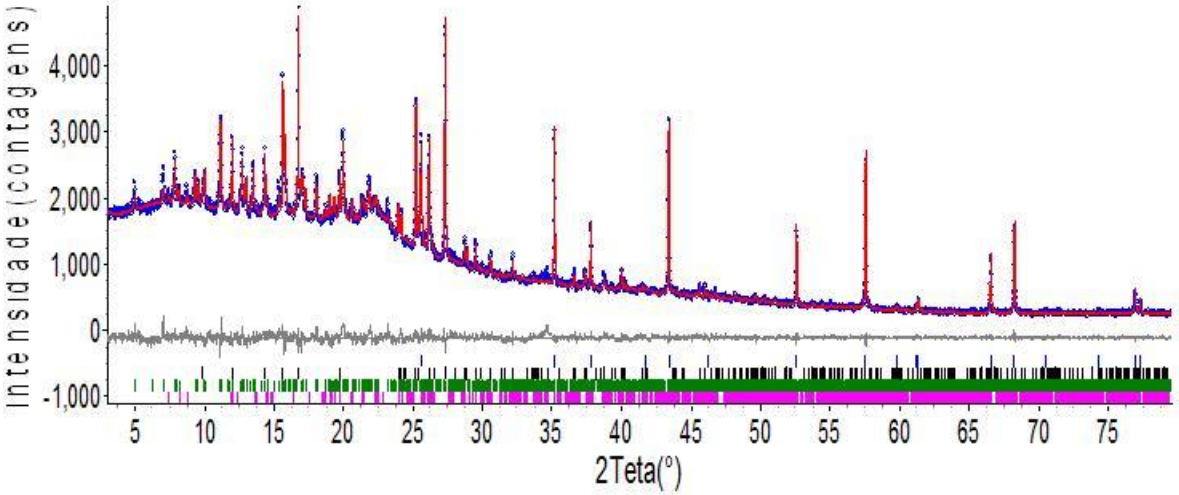


Figure 19 – Rietveld refinament of FDC INH:RMP (150:3000)mg.

wt% Results:
Al_2O_3 = 10.38%
INH = 20.9%
RMP form II = 24.75%
RMP form I = 1.13%

Amorphous fraction = 47.9%
Crystalline fraction = 52.1%

The goodness-of-fit indicator as well as the R factors were, respectively:

R_{Bragg} = 0.751% (Al_2O_3), R_{Bragg} = 1.55% (INH), R_{Bragg} = 2.16% (RMP-formII),

R_{Bragg} = 1.61% (RMP-formI), R_{exp} = 3.25%, R_{wp} = 3.76% and χ^2 = 1.16

Conclusions

1. ***The two anhydrous rifampicin polymorphs – form I and II – were the crystal structure determinated.***

2. ***Ensure better quality control in the analysis of raw materials of rifampicin (quantification of crystalline phases).***

3. ***Identification and Quantification of crystalline phases of rifampicin in commercial products.***

Farmanguinhos / Fiocruz

The Institute of Drug Technology (Farmanguinhos), technical-scientific unit of the Oswaldo Cruz Foundation (Fiocruz), is the largest official pharmaceutical laboratory under the Ministry of Health of Brazil.

Farmanguinhos produces more than a billion pharmaceutical units per year to meet the strategic programs of the Brazilian government and emergency demands in Brazil and abroad.

Among the products developed by Farmanguinhos are the antiretrovirals, antibiotics, antibiotics TB, antimalarials, antihypertensives, and others.

Solid State Laboratory (LEES, in Portuguese)

- **History**

❑ Created since 2001

❑ Solid State Study in works of R&D and routine.

❑ Structure:
- ➢1 X-Ray Powder Diffractometer (XRPD)
- ➢2 Differencial Scanning Calorimeter (DSC)
- ➢1 Thermogravimetric Analyser (TGA)
- ➢1 TGA linked to Infrared Spectrometer (TGA/IR)
- ➢1 Dynamic Mechanical Analyser (DMA)
- ➢1 Hot Stage Microscope (HSM)
- ➢1 Stereo Microscope (SM)
- ➢1 Optical Microscope (OM)
- ➢1 Infrared Microscope (IR/OM)
- ➢2 Particle Size Distribution Analyser by Laser Light Scattering (PSD-LLS)
- ➢1 Dinamic Vapor Sorption (DVS)
- ➢1 Surface Area and Porosity Analyser (SAPA)

❑ Team: 5 Researchers - Altivo Pitaluga Jr
Fernanda Carolina Sousa Fonseca
Janine Boniatti
Rafael Cardoso Seiceira
Sabrina Barros de Carvalho

Acknowledgements

Ministério da Saúde

FIOCRUZ
Fundação Oswaldo Cruz

farmanguinhos
Instituto de Tecnologia em Fármacos

LNLS
Laboratório Nacional
de Luz Síncrotron

Universidade Federal do ABC

LDRX uff

THANK YOU!!!

rcseiceira@far.fiocruz.br

rcseiceira@gmail.com