

## Validação do método de análise quantitativa de fases por DRX em IFA: caso Tibolona

## Validation of the method of quantitative phase analysis by XRD in API: case of Tibolone

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**Resumo:** Neste trabalho foram investigados diferentes modelos estruturais e microestruturais aplicados na análise de dados de difração de raios-X de misturas de fases polimórficas da Tibolona com concentrações conhecidas. Os dados de raios-X obtidos em diferentes instrumentos de difração foram analisados usando os mesmos modelos de análise via método de Rietveld. Os resultados da análise quantitativa das concentrações de fases mostram que, independentemente do instrumento utilizado, os modelos de cálculos seguem a mesma sistemática nos valores de erros finais. A estratégia para escolha do modelo de análise que leva ao menor erro de medição é apresentada neste trabalho.

**Palavras-chave:** fármacos, polimorfismo, análise quantitativa, difração de raios-X.

**Abstract:** In this study different structural and microstructural models applied to X-ray analysis of powder diffraction data of polymorphic mixtures of known concentrations of Tibolone were investigated. The X-ray data obtained in different diffraction instruments were analysed via Rietveld method using the same analytical models. The results of quantitative phase analysis show that regardless of the instrument used, the values of the calculated concentrations follow the same systematics with respect to the final errors. The strategy to select a specific analytical model that leads to lower measurement errors is here presented.

**Keywords:** drugs, polymorphism, quantitative analyses, X-ray diffraction.

## 1. INTRODUCTION

The quantitative phase analysis by X-ray diffraction of organic solids which compose active pharmaceutical ingredients (API) is susceptible to many factors (instrumental, knowledge of materials properties, and method of data treatment) that influence the final result. The Rietveld method is accepted as the most accurate approach to extract from X-ray data the values of concentrations of phases in phase mixtures [1]. However, the choice of structural and microstructural model in this method of analysis becomes a critical factor from the point of view of its contribution to the errors of calculated concentrations. This situation represents a serious problem for the pharmaceutical industry and regulatory organizations in production and distribution of safe and efficient solid drugs.

This study investigates, in a systematic manner, different assumptions about structural and microstructural properties of phases of Tibolone during the data modelling. Tibolone is a synthetic steroid used to relieve hypo-estrogenic symptoms and protect against bone loss in post-menopausal women [2]. The general and specific constraints for the reliable quantitative phase analyses were established on the base of multiple experimental data and variable concentrations of phases of Tibolone.

## 2. EXPERIMENTAL

### 2.1. Sample preparation

Three standard mixtures of known concentrations, referred hereafter as M1, M2 e M3, were prepared using Tibolone pure Form I (commercial) and Form II (prepared according to Araujo, *et.al.* [2]). The concentrations of the mixtures are shown in the Table 1. An analytical balance with calibrated accuracy of 0.01 mg was used to establish the real phase proportions in the studied samples. The overall mass of each prepared sample was

approximately 1g. The mixtures were placed in eppendorfs and stirred in vibratory mixer mill for 30 minutes. Table 1 presents the acceptable lower limits (M1, preferably M2) concentrations for a crystalline pure Form I [3], and the expected guess for a detection limit of Form II in Tibolone

**Table 1.** Sample identification and phase composition.

Tibolone (API)	Nominal [F1-F2] (%)	Real [F1-F2] (%)
M1	90-10	90.09-9.91
M2	95-5	94.99-5.01
M3	99-1	98.73-1.27

F1= Tibolone Form I and F2 = Tibolone Form II

### 2.2. X-ray data collection

The instruments used for X-ray data collections, their basic configurations, and sample preparation aspects are listed in the Table 2.

**Table 2.** Instruments and sample preparation types used in X-ray experiments.

Instruments	Geometry	Tube	Detector	Powder Sample
1. X'Pert Pro Panalytical	Reflection, Bragg-Brentano	Cu K $\alpha$	PiXcel 1-D	Mixed and pressed *Mixed, grinded and pressed
2. D8 Focus Bruker	Reflection, Bragg-Brentano	Co K $\alpha$	Scintillator 0-D	Mixed and pressed
3. Agilent Technologies	Transmission, Debye Scherrer	Cu K $\alpha$	CCD 2-D	Mixed powder in capillary

Labs: 1 - NanoBusiness; 2 -DIMAT-LADES; 3 - DIMAV

### 2.3. Structural and Microstructural models

Eight models of Form I and Form II of Tibolone were employed to calculate the concentrations of these phases in the samples. Different constraints for structural and/or microstructural properties of phases distinguish these models (see Table 3). Model 1 assumes the fixed structural parameters for Form I and Form II of Tibolone taken from data base of Cambridge Crystallography Data Centre (No.: Form I 1126814 and Form II 1126815), and isotropic crystallite size peaks

broadening (Voigt function) for diffraction peak shape fitting. Subsequent models include other refinement options, as shown in Table 3.

**Table 3.** Description of models.

Structural and Microstructural Models	Model Constraints							
	1	2	3	4	5	6	7	8
1. Isotropic crystallite size broadening	green	green	green	green	green	green	green*	green
2. Lattice Parameters	red	green	green	green	green	green	green	green
3. Texture (spherical harmonics)	red	red	green	green	green	green	green	green
4. Hydrogen atoms inserted in Form I	red	red	red	green	green	green	green	green
5. Isotropic atomic displacement parameter, $B_{eq}$	red	red	red	red	green	green	green	red
6. Atomic coordinates (constrained to maximum displacement of 0.0016)	red	red	red	red	red	green#	green	green
7. Anisotropic crystallite size broadening	red	red	red	red	red	red	green#	red
8. Anisotropic atomic displacement parameters, $U_{ij}$	red	red	red	red	red	red	red	green

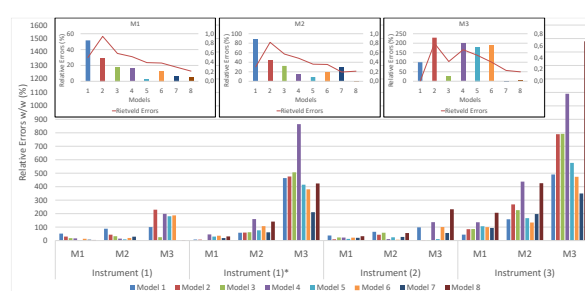
green = applied or variable; red = not applied or not variable  
\* applied only to Form II; # applied only to Form I

### 3. RESULTS AND DISCUSSION

Figure 1a shows the selected diffraction patterns of samples M1, M2 and M3 obtained on Instrument 1. Figure 1b shows the experimental XRD pattern of M1 sample and the one calculated from the Rietveld refinement of model 8. The use of the latter model results in the excellent agreement between calculated and real concentrations of phases in the sample. Figure 2 summarises the relative errors of the calculated concentrations of Form II for all samples (Table 1) and experimental conditions (Table 2) used in this study. As one can expect, the values of relative errors increase with decreasing concentration of Form II. This is a general trend which can vary for a particular structural and microstructural model. One can also note that the results obtained on instruments 1 and 2 have markedly better agreement with the real values of concentrations than the ones of instrument 1\* and 3. The main distinction between these sets of conditions is the  $2\theta$  resolution of diffraction peaks. This resolution is worse in case of instruments 3 and 1\* due to a low angular resolution of CCD detector and

additional crystallite size peak broadening induced by grinding, respectively.

The inset of Figure 2 compares the values of real relative errors (the relative differences between the real and calculated concentrations) and standard deviations for concentrations of Form II obtained from Rietveld refinement of a particular model. The calculated and real errors are comparable for models that result in  $R_{wp}$ -factor below 12 and GoF below 2 (see Figure 3).



**Figure 2.** Relative errors in concentration.

A sophistication of the structural and microstructural model considerably reduces the fit indexes (Figure 3) and approaches the real and calculated values of concentrations and respective errors. The considerations of the effect of texture (model 3), of additional molecular weight due H atoms (model 4) and of atomic displacement parameter, ADPs, (model 5) were the most impacting on a convergence of calculated values of concentrations to the real ones. Further models sophistications, such as refinements of atomic coordinates (model 6), of anisotropic crystallite size broadening (model 7) and of anisotropic atomic displacement parameters (model 8), resulted in nearly constant calculated errors despite of better fit indexes.

### 4. CONCLUSIONS

The obtained result showed that a good resolution of X-ray diffraction data is a necessary condition for reliable quantitative analysis of polymorphism in Tibolone. The Rietveld method of analysis of diffraction patterns gives reliable estimations of

concentrations of Form II with respective errors for concentrations above 5 wt%. The modelling of microstructural (texture) and structural (H atoms, ADPs) features was required to achieve this result. Additional models sophistications, such as consideration of anisotropy of crystallite size broadening and anisotropy of ADPs may be necessary to obtain reliable results in case of lower concentrations of Form II.

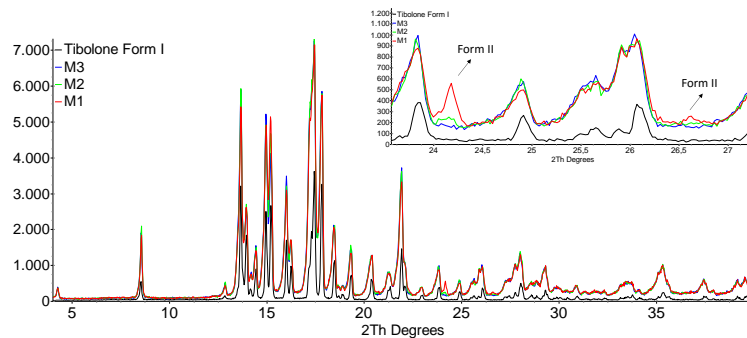
### Acknowledgments

The e-Diffraction Lab from NanoBusiness Company is grateful to the agencies CNPq,

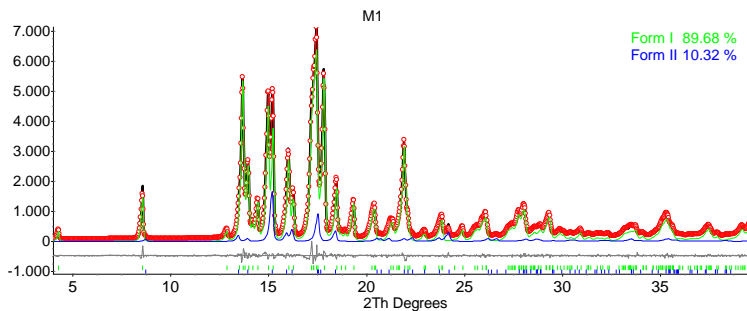
FAPERJ and to INMETRO for the collaboration in this research.

### 5. REFERENCES

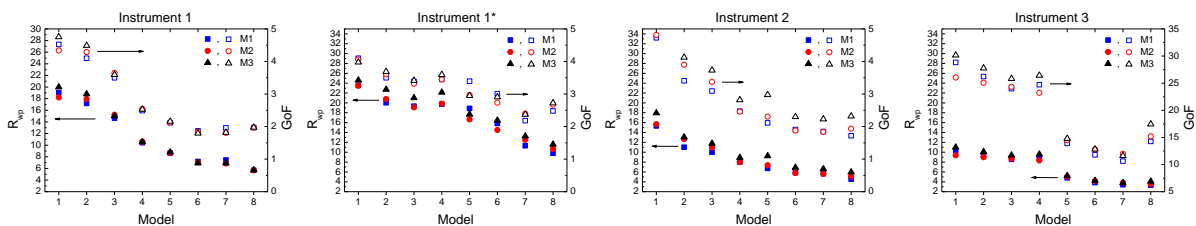
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**Figure 1a.** Diffraction patterns of M1, M2 and M3 samples. Inset emphasises the 2θ regions of two not overlapped peaks of Form I and Form II.



**Figure 1b.** Experimental (black curve), calculated (red curve) and difference (Grey) from Rietveld refinement (model 8) XRD patterns of the sample M1. Form I (Green curve) and Form II (Blue curve).



**Figure 3.**  $R_{wp}$  and  $GoF$  indexes ( $R_{wp} = \sqrt{\sum W_m(Y_{o,m} - Y_{c,m})^2 / \sum W_m Y_{o,m}^2}$ ;  $GOF = chi2 = R_{wp}/R_{exp}$ ).