Atenolol characterization in solid formulations available in Brazilian market

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Atenolol is the most used drug in Brazil to hypertension treatment. Two crystal structures are known for this molecule: a racemic form (R,S)-atenolol and a pure form S-atenolol. The racemic form is found in commercial tablets. X-ray powder diffraction (XRD) is an adequate tool to study crystalline structures including drugs. Using the Rietveld Method with XRD data it is possible to quantify the crystalline structures existing in the raw material. Other methods like Le Bail and Pawley can be used to the profile fit and phases identification. For this work we analyzed three tablets of atenolol, two generics and the reference (materials were purchased from a drugstore at the city of Araraquara). These tablets were analyzed by Rietveld, Le Bail and Pawley methods. All tablets exhibited the racemic mixture API (R,S)-atenolol. Some crystalline excipients could be characterized: magnesium carbonate hydrate, lactose monohydrate and talc. The conclusion is that the three methods can be efficiently used to characterize the three atenolol tablets.

Key words: Atenolol, X-ray powder diffraction, Rietveld Method, Le Bail Method, Pawley Method

I. INTRODUCTION

Atenolol, $C_{14}H_{22}N_2O_3$, belongs to a class of medicines named β-blockers and is used in the hypertension treatment and irregular heartbeat control, to prevent heart attack.

According to the Secretary of Health of Brazil (Brasil, 2011), there are approximately 600 million of hypertensive patients in the world and 50% of future diseases can occur because of hypertension. In Brazil, atenolol belongs to the National List of Essentials Drugs, in tablet form, because of its importance and it is available for free, since February 2011. Drugs prescribed for the treatment of hypertension and diabetes, together, were responsible for the 34% of the deaths in Brazil in 2009 (Marques, 2011).

Atenolol exhibits two known crystalline structures: (*R*,*S*)-atenolol (de Castro, 2007) and *S*-atenolol (de Castro *et al.*, 2007). It is known that the two molecules *R* and *S* exhibit different biological activities (de Castro *et al.*, 2007). Atenolol is produced as a racemic mixture of two enantiomers (*R* and *S*). According to Pearson *et al.* (1989) the (-)-atenolol is the responsible for the antihypertensive action. Lima *et al.* (1997) reported that the β-blockers can bond to β receptors, and that S (-) enantiomers are the main responsibles for the β-blocker activity.

It is well accepted that solid characterization can be done using several techniques like thermal analysis (DSC and TG), spectroscopic (IR, Raman, NMR) and X-ray powder diffraction (XRPD). Among them, XRPD is the most appropriate to investigate crystalline structures.

The Rietveld Method (RM) (Rietveld, 1969) is used to investigate crystalline structures, being possible to quantify phases and to extract microstructural information as crystallite size (medium) and micro deformation. In order to employ the RM we need to know the structural model. But if the crystalline structures are not identified, other methods like Le Bail (LBM) (Le Bail *et al.*, 1988) and Pawley (PM) (Pawley, 1981) can be used. There are cases in which even the partial crystal structure is not known. For cases like that, XRPD may be used for phase identification, but when the amount of some polymorph is low, this can be a non trivial problem to overcome. Resolution is also a problem for cases with great overlap of polymorphs peaks, even when the amount of polymorphs is significant.

Considering that the steps in the solid medicaments processing can lead to variations in crystal structure of the pharmaceutical raw material, differences in it is physicochemical properties may occur, and XRPD is a good tool for identifying these changes in the crystalline pharmaceutical products.

However, only the Rietveld method not always in some cases is not enough for the full characterization of the API polymorphs present in drugs, if some of the crystal structure is unknown. For these cases, the PM and/or LBM can be used. In this work, we compare the tree methods (RM, PM and LBM) in the analyses of atenolol API in tablets available in the Brazilian market.

II. EXPERIMENTAL

Three commercial tablets of atenolol from different laboratories were purchased in Brazilian drugstores: the reference of 25mg (R) and two generic of 25mg (G-01 and G-02).
The tablets were coating removed and then ground and sieved (200mesh).

X-ray powder diffraction data, from 2 to 40° (2θ) with $\Delta 2\theta = 0.02°$, were recorded at room temperature using a Rigaku RINT2000 copper (Cu$K\square$) rotating anode diffractometer operating at 42 kV and 120 mA and the powder was softly pressed in a flat-plate sample holder. A receiving slit of 0.3 mm, divergence slit of 0.25 mm and 2.5° divergence Soller slit were used. Curved graphite monochromator in the diffracted beam were used. The refinements were performed using TOPAS Academic v.4.1 software. (Coelho, 2007)

The refinements were performed in three ways, depicted below.

RM: Rietveld method. The atenolol and excipients crystalline form was adjusted through the Rietveld method. For the compounds with unknown crystal structures, their peaks were fitted individually.

LBM: Le Bail method. The atenolol powder patter was fitted through the Le Bail method. The excipients were refined through the Rietveld method. For the compounds with unknown crystal structures, their peaks were fitted individually.

PM: Pawley method. The atenolol powder patter was fitted through the Pawley method. The excipients were refined through the Rietveld method. For the compounds with unknown crystal structures, their peaks were fitted individually.

III. RESULTS AND DISCUSSION

The analyzed tablets have their inactive ingredients, according to the medicament, listed in the Table I, of which hypromellose, microcrystalline cellulose and starch are amorphous, and lactose monohydrate, magnesium carbonate hydrate, magnesium stearate and talc are crystalline (Akao and Iwai, 1977; Fries *et al.*, 1971; Rayner and Brown, 1973; Wada and Matsubara, 1994). In refinements performed by LBM, PM and RM, parameters like background, asymmetry, anisotropy and specimen displacement were adjusted. Background was adjusted using 10 coefficients in the Chebyschev polynomial.

Table I. Excipients that are contained in each tablet (samples R, G-01 and G-02).

	R	G-01	G-02
Croscarmellose sodium			X
Gelatin	X		
Hypromellose		X	
Lactose monoydrate			X
Magnesium carbonate hydrate	X	X	X
Magnesium stearate	X	X	X
Microcrystalline cellulose			X
Silicon dioxide			X
Sodium lauryl sulfate	X	X	X
Sodium starch glycolate	X		
Starch	X	X	X
Stearic acid		X	
Talc			X

The space group symmetry and lattice dimensions of the identified phases employed in Rietveld refinements are listed in Table II. In the cases of magnesium stearate and sodium lauryl sulfate, their crystal structures were not found in the literature. For these two cases, powder diffraction data were collected in our laboratory using raw materials which were donated by the pharmaceutical industry. The powder patterns for them are in agreement with that found in the literature (Swaminathan and Kildsig, 2001; Wada and Matsubara, 1994) and (Gendre *et al.*, 2012) respectively.

Table II. Partial crystal structure data used in the refinements.

	Space group	V (Å^3)	a (Å)	b (Å)	c (Å)	α (º)	β (º)	γ (º)	Reference
(R,S)-atenolol	C 2/c	2974.75	55.83(3)	5.559(3)	9.734(2)	90	100.042(6)	90	(de Castro, 2007)
Magnesium carbonate hydrate	$P2_1/c$	690.118	10.105(5)	8.954(2)	8.378(4)	90	114.44(5)	90	(Akao, 1977)
Lactose monohydrate	$P2_1$	782.287	7.982(2)	21.562(3)	4.824(1)	90	109.57(3)	90	(Fries, 1971)
Talc	C1	454.471	5.293(2)	9.179(3)	9.469(3)	90.57(3)	98.91(3)	90.03(3)	(Rayner, 1973)

Rietveld Method

The Rietveld plot for tablet R is shown in Figure 1. The figure illustrates the calculated pattern of (R,S)-atenolol (black line), representing 21.5(4) wt% in the sample. This tablet also presents 78.5(4) wt% of magnesium carbonate hydrate. The peak in 2θ = 5.5º can be attributed to the most intense peak of the magnesium stearate, and was individually adjusted.

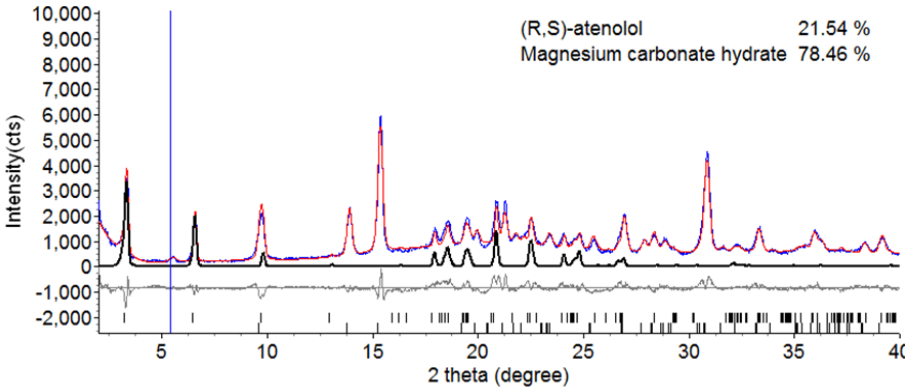

Figure 1. Rietveld plot for reference tablet with structures of (R,S)-atenolol and magnesium carbonate hydrate. The peak in 2θ = 5.5º was attributed to magnesium stearate.

The Rietveld plots for the generic drugs G-01 and G-02 are shown in Figure 2 and Figure 3 respectively. The result for the generic drug G-01 is similar to the reference R, with 77.7(3) wt% of magnesium carbonate hydrate and 22.3(3) wt% of (R,S)-atenolol.

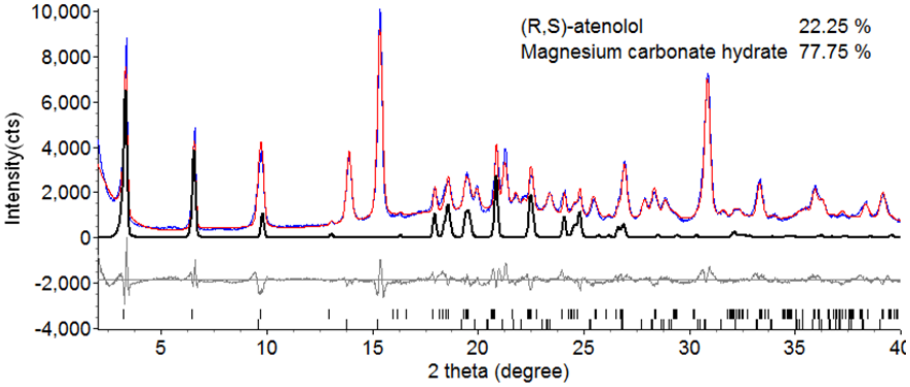

Figure 2. Rietveld plot for sample G-01, with structures of (R,S)-atenolol and magnesium carbonate hydrate.

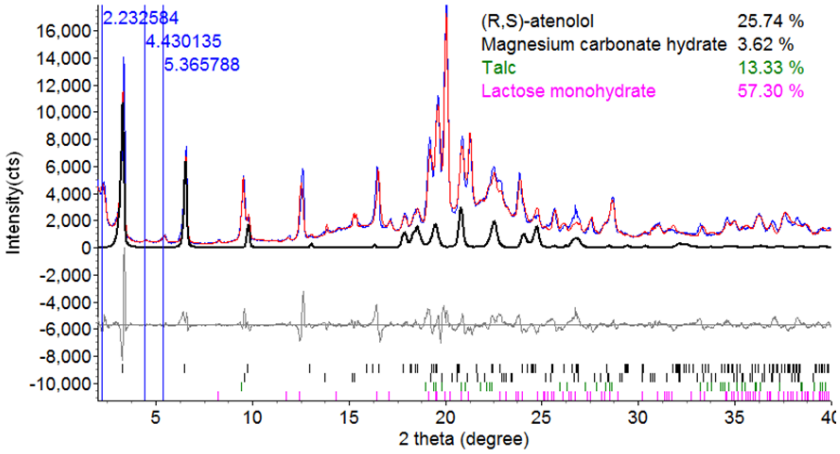

Figure 3. Rietveld plot for sample G-02, with the structures of (R,S)-atenolol, magnesium carbonate hydrate, lactose monohydrate and talc. The peaks in 2θ = 2.2 and 4.4º were attributed to sodium lauryl sulfate and the peak in 2θ = 5.4º was attributed to magnesium stearate.

The drug G-02 presents the crystalline phases API (R,S)-atenolol (26.5(6) wt%), magnesium carbonate hydrate (3.8(2) wt%), talc (14.4(5) wt%) and lactose monohydrate (55.3(8) wt%). For this case, tree peaks at 2θ = 2.23; 4.43 and 5.37º were observed and they do not correspond to the crystalline phases cited above. The first two peaks can assigned to sodium lauryl sulfate and the last one can be assigned to magnesium stearate.

Le Bail and Pawley

In the Le Bail and Pawley refinements, for all samples, the partial atenolol crystal structure was used. For the excipients the RM was applied.

In the LB refinements we used the cell parameters and space groups listed in Table II.

Figure 4 illustrates the Le Bail fit for the sample R. In black bold is the (R,S)-atenolol pattern obtained. The calculated pattern in the Figure 4 is not quite different of that obtained with the Rietveld method (Figure 1). Some small differences are visible for the relative peak heights of atenolol, like that around 10° and 19.5° (2θ), but that does not invalidate the use of the Le Bail method for the phase identification. The magnesium carbonate hydrate was fitted using the Rietveld method. As in the case performed by RM, the peak at 2θ=5.5º was assigned to the magnesium stearate phase.

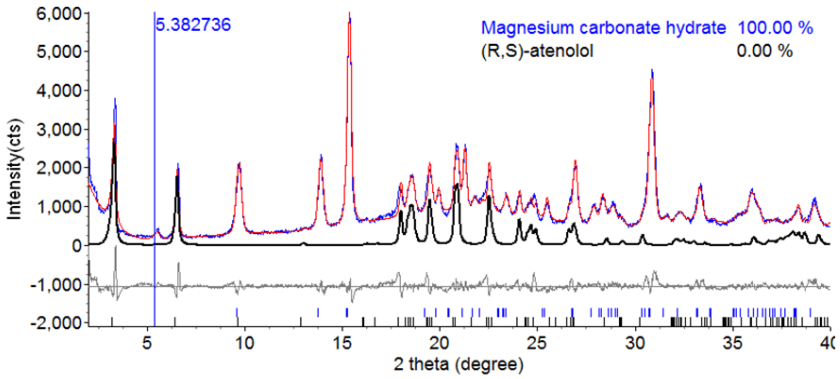

Figure 4. Le Bail plot of the sample R. The peak in 2θ= 5.5º was attributed to magnesium stearate.

Figure 5 shows the LB fit to the data of sample G-01, where it is possible to observe that the atenolol LB pattern (black bold) is in good agreement with that obtained in the RM refinement.

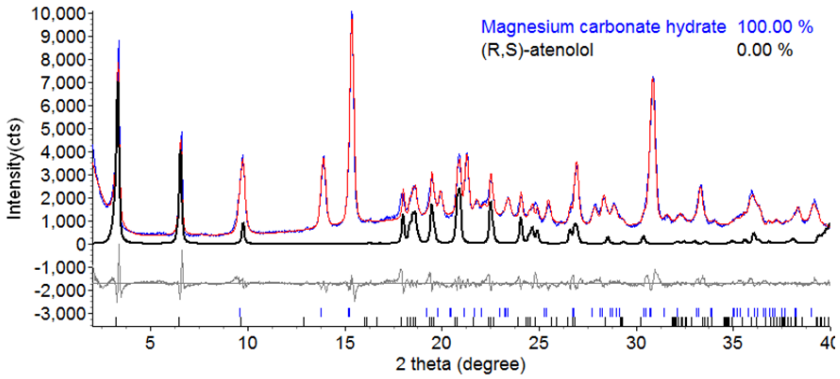

Figure 5. Le Bail plot of the sample G-01.

Also for sample G-02 (Figure 6) one can observe that the atenolol LB pattern is in good visual agreement to that obtained using RM.

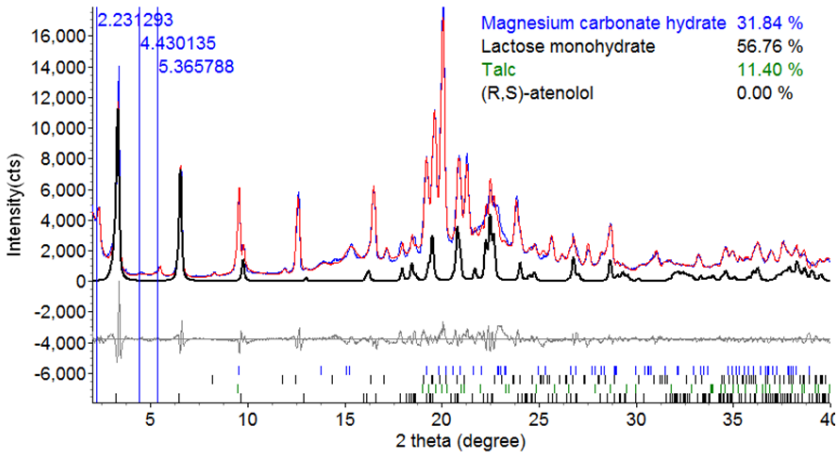

Figure 6. Le Bail plot of the sample G-02, with (R,S)-atenolol and the excipients magnesium carbonate hydrate, lactose monohydrate and talc. The peaks in 2θ = 2.2 and 4.4º were attributed to sodium lauryl sulfate and the peak in 2θ = 5.4º was attributed to magnesium stearate.

The Figures 7, 8 and 9 show the Pawley fits to the data of the samples R, G-01 and G-02 respectively. In these cases, too, the atenolol powder pattern could be adequately fitted through the Pawley method providing similar results of the Rietveld Method.

The refinements agreement indexes are in Table III.

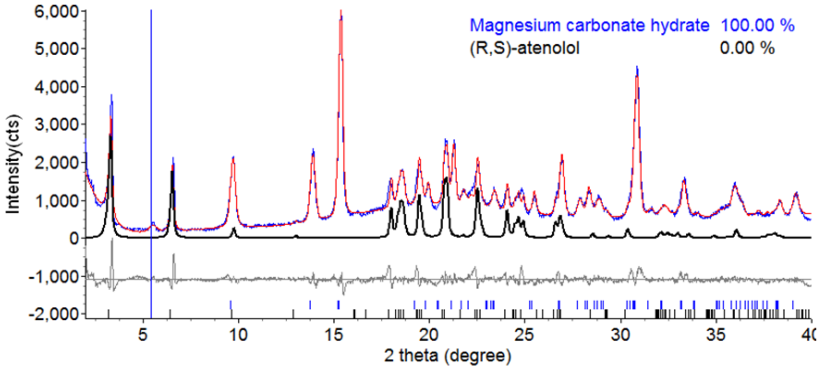

Figure 7. Pawley plot of the sample R. The peak in 2θ=5,5º was attributed to magnesium stearate.

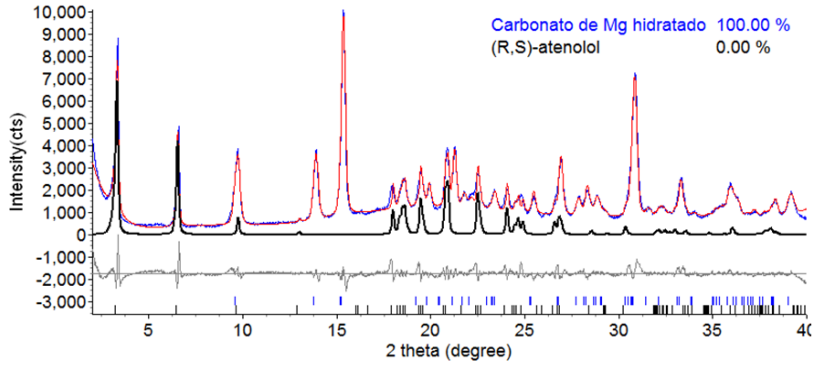

Figure 8. Pawley plot of the sample G-01.

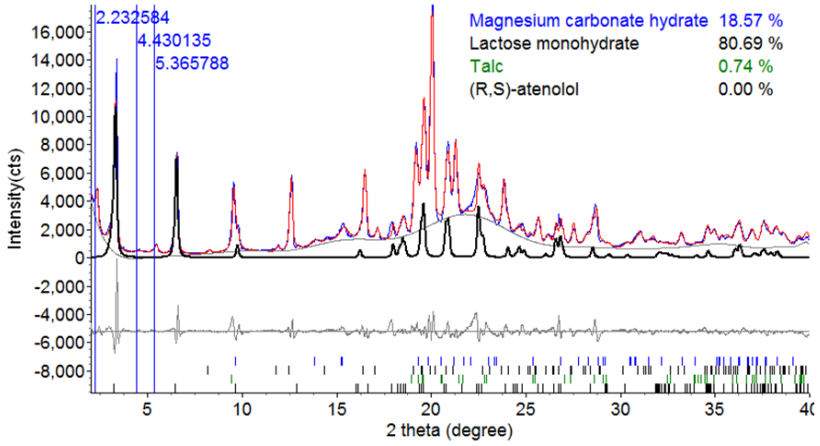

Figure 9. Pawley plot of the sample G-02, with (R,S)-atenolol and excipients magnesium carbonate hydrate, lactose monohydrate and talc. The peaks in 2θ = 2.2 and 4.4º were attributed to sodium lauryl sulfate and the peak in 2θ = 5.4º was attributed to magnesium stearate.

Table III. Refinements Agreement indexes. Codes: ATL (atenolol), LacM (Lactose monohydrate), MCH (Magnesium carbonate hydrate) and Talc (talc).

	R			G-01			G-02		
	Rietveld	Le Bail	Pawley	Rietveld	Le Bail	Pawley	Rietveld	Le Bail	Pawley
R_{wp} (%)	10.894	9.606	9.341	10.010	8.781	9.897	13.208	9.619	10.369
R_{exp} (%)	3.344	3.342	3.221	2.661	2.656	2.553	2.169	2.182	2.097
χ^2	3.256	2.875	2.909	3.761	3.306	3.876	6.090	4.408	4.945
% ATL	21.5(4)			22.3(3)			25.7 (6)		
R_{Bragg} ATL	7.768			4.119			6.072		
% MCH	78.5(4)	-	-	77.7(3)	-	-	3.6(2)	31 (1)	18(4)
R_{Bragg} MCH	4.910	3.025	2.855	4.348	3.071	3.182	6.567	1.844	1.796
% LacM							57.3(6)	56(1)	80(5)
R_{Bragg} LacM							4.725	2.887	2.745
% Talc							13.3(4)	11.4(3)	1(4)
R_{Bragg} Talc							3.998	3.234	6.096

Conclusion

The results showed that all tablets contain the (R,S)- atenolol form, which was identified and the structure was refined using the collected powder patterns through the three refinement methods. The reference tablet presented the excipient magnesium carbonate hydrate and one peak that was attributed to the existence of the magnesium stearate phase. For one of the generic tablets studied (G01) only the characteristic powder patterns of the active principle and the excipient magnesium carbonate hydrate were observed. The other generic tablet G02 exhibits the existence of the lactose monohydrate, talc and three peaks attributed to the excipients magnesium stearate and sodium lauryl sulfate.

We can conclude that the three methods used (RM, LBM and PM) were efficient for the identification of the active principle form presented in atenolol tablets analyzed. It is not accurate to quantify phases using LBM and PM, but they are a rapid and sometimes satisfactory alternative to identify different pharmaceutical compounds from their characteristic powder diffraction peaks.

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