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Research Article

Development and Validation of a Discriminative Dissolution Method for Atorvastatin Calcium Tablets using *in vivo* Data by LC and UV Methods

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Abstract. A dissolution method to analyze atorvastatin tablets using *in vivo* data for RP and test pilot (PB) was developed and validated. The appropriate conditions were determined after solubility tests using different media, and *sink* conditions were established. The conditions used were equipment paddle at 50 rpm and 900 mL of potassium phosphate buffer pH 6.0 as dissolution medium. *In vivo* release profiles were obtained from the bioequivalence study of RP and the generic candidate PB. The fraction of dose absorbed was calculated using the Loo–Riegelman method. It was necessary to use a scale factor of time similar to 6.0, to associate the values of absorbed fraction and dissolved fraction, obtaining an *in vivo–in vitro* correlation level A. The dissolution method to quantify the amount of drug dissolved was validated using high-performance liquid chromatography and ultraviolet spectrophotometry, and validated according to the USP protocol. The discriminative power of dissolution conditions was assessed using two different pilot batches of atorvastatin tablets (PA and PB) and RP. The dissolution test was validated and may be used as a discriminating method in quality control and in the development of the new formulations.

KEY WORDS: atorvastatin; correlation *in vivo–in vitro*; discriminative method; dissolution; validation.

INTRODUCTION

The association between *in vitro* drug-release profiles and *in vivo* pharmacokinetic data stands as one of the main challenges in biopharmaceutics studies (1). The *in vivo–in vitro* correlation (IVIVC) is described as a relationship between a biological property, or a parameter derived from a biological property produced from a dosage form and a physicochemical property of the same pharmaceutical form (2). This concept allows using an IVIVC as a substitute for pharmacokinetic studies in humans, reducing the number of bioequivalence evaluations during the initial approval process, as well as changes in scales and postregister procedures (3,4). The biological properties most commonly used include pharmacokinetic parameters such as area under the plasma drug concentration *versus* time (AUC) or maximum plasma concentration (C_{max}) obtained after administration of the dosage form. The physicochemical characteristic most used is the *in vitro* dissolution behavior of the product. The relationship between the biological and physicochemical properties may be mathematically expressed in order to obtain an IVIVC (5,6).

Dissolution tests for solid dosage forms should be afford an appropriate discriminatory power, distinguishing significant changes in a composition or manufacturing process that might be expected to affect *in vivo* performance. In the pharmaceutical industry, dissolution testing is a very important tool to guide the development of new formulations and to assess the lot-to-lot quality of a drug product. In biological systems, drug dissolution in an aqueous medium is an important precondition for systemic absorption. The rate at which drugs with poor aqueous solubility dissolve from an intact or disintegrated solid dosage form in the gastrointestinal tract often controls the rate of systemic absorption of the drug (7–9).

IVIVC can be used in drug development, to set dissolution specifications, and to support biowaivers. However, IVIVC cannot be applied to every drug; therefore, it creates challenges concerning the development of relevant dissolution methods and setting meaningful product specifications (10). According to the Biopharmaceutics Classification System, atorvastatin (ATV) is a class II drug (low solubility and high permeability) (11,12). Correlation between *in vivo* results and dissolution tests may be expected for class II drugs because, in this case, the dissolution rate is the primary limiting aspect of absorption (6–8).

A few studies on the dissolution of ATV tablets have been carried out in recent years, as described by Palem *et al.*, Ahjel and Lupulesa, and Narasaiah *et al.* (13–15). However, these studies addressed exclusively the evaluation of the physical and chemical behavior of different formulations, without considering the behavior of the drug *in vivo*. In 2004, the Food and Drug Administration (FDA) published a condition under which the dissolution test can be performed only for quality control of tablets, using USP apparatus 2, at 75 rpm, and 50 mM potassium phosphate buffer pH 6.8 as dissolution medium (16).

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In this context, the aim of the present study was to develop and validate a discriminative dissolution method for ATV tablets to use in laboratory routine, and to demonstrate the release characteristics of the products according to an *in vivo* model. Dissolved fraction data were compared to fraction absorbed data, obtained from a bioequivalence study between the Brazilian reference product (RP) and one pilot lot (PB) generic candidate. High-performance liquid chromatography (HPLC) and ultraviolet (UV) spectrophotometry were used for quantitative drug analysis.

MATERIALS AND METHODS

Materials

The ATV calcium reference substance, with 99.85% purity, two test formulations of ATV calcium tablets, pilot A (PA) and pilot B (PB), placebos of the two respective formulations and bioequivalence study from RP and PB, were kindly supplied by a local pharmaceutical company. The pilot formulations differ in that the level of croscarmellose sodium (a disintegrating agent) is 3% higher in PB, compared to PA. The reference product, (Citalor®) 80 mg tablets (batch no. 0691060), the same lot used in bioequivalence study, was purchased in the domestic market. Acetonitrile and methanol HPLC grade were obtained from TEDIA (Fairfield, USA). Ultrapure water (Millipore®, Milfor, MA, USA) was used for the dissolution medium and throughout the analysis. All other chemicals were of reagent grade.

Quantitative HPLC and UV analysis of ATV Calcium

The literature reviewed does not list any specific analytical method for the dissolution test of ATV tablets. Thus, were developed and validated an HPLC assay method according to the ICH 2005 (17), used in preliminary tests for the dissolution method development.

The HPLC system consisted of a Shimadzu LC model (Kyoto, Japan) composed of a LC-20AT pump, a SPD-M20A photodiode array (PDA) detector, a CBM 20A system controller, a DGU-20A_5 prominence degasser, a column thermostat oven CTO-20AC and an auto sampler SIL-20AC. Data were acquired and processed using LC solution software. Chromatographic analysis was carried out using a RP-18 column (250×4.6 mm; particle size, 5 μm) monitored at 25°C. The mobile phase consisted of a mixture of sodium acetate pH 4.2 buffer:acetonitrile (45:55, *v*/*v*). The flow rate was 1.0 mL/min, and the injection volume was 20 μL. The detection was carried out at 245 nm.

The UV–visible spectrophotometer employed was a double-beam (Shimadzu, Kyoto, Japan), model UV-1800, equipped with 1 cm quartz cells, with a spectral band width (1±0.2 nm), wavelength accuracy of ±0.1 nm. UV Probe software version 2.33 (Shimadzu) was used for instrument control, data acquisition, and analysis.

In vivo Data Treatment for IVIV Correlation

A crossover (2×2) bioequivalence study design was used, in which 61 healthy volunteers received, in each period, the test formulation or the reference formulation. The formulations were administered as a single oral dose followed by blood sampling at least three half-lives after administration. Treatment periods occurred at a minimum interval of seven half-lives (washout period of drug). The pharmacokinetics parameters and their CV, as well as the IC90%, for RP and PB, are shown in Table I.

The *in vivo* data from the bioequivalence study (18) were evaluated by mathematical modeling using the nonlinear regression software Scientist® version 2.0 (MicroMath®). A two-compartment open model and its parameters were used to estimate intermediate plasma concentration data points.

The Loo–Riegelman method was used to calculate the fraction of drug absorbed (FA) in relation to time. The obtained data of fraction absorbed (FA) both for the RP and PB were used together with the values of the dissolved fraction (FD) obtained by the dissolution method proposed, in order to obtain an IVIVC. The obtained data were evaluated by linear regression analysis.

Preliminary *in vitro* Studies

ATV Solubility Determination

ATV *sink* conditions were evaluated in different media, using an excess amount of ATV calcium, bulk material, added to a tube, in triplicate, containing 10 mL of medium test and maintained at 37±0.5°C with magnetic shaking for 24 h. HCl (0.1 M), gastric fluid without enzyme, pH 1.2, citrate buffer pH 3.0, acetate buffer pH 4.0, phosphate buffers pH 5.0, 6.0, and 6.8, and water were tested. After 24 h, all media were centrifuged at 3,000 rpm for 15 min and filtered through a quantitative filter. A 1:5 dilution was made with methanol/water (50:50 *v*/*v*), and the final solutions were filtered to vials with the aid of a 0.45-μm nylon membrane. The final solutions were analyzed by LC method.

Dissolution Instrumentation and Discriminative Conditions Selection

The development and validation of the dissolution test was performed using a Vankel® VK 7010 multi-bath-dissolution testing station (n=8) assembled to the VK 8000 dissolution autosampling station with a bidirectional peristaltic pump. A Digimed potentiometer, model DM-20 (São Paulo, Brazil) was used to determine the pH of all buffer solutions.

All dissolution conditions were performed using 900 mL of different media preheated at 37±0.5°C. Influence of

Table I. Pharmacokinetics Parameters, CV, and IC90% for RP and PB

Parameters	RP	CV(%)	PB	CV(%)
$t_{1/2}$	5.73	49.29	6.16	50.44
t_{max}	1.21	73.88	1.13	67.91
C_{max}	47.505	56.85	38.589	45.5
AUCo-inf	149.282	38.54	141.104	43.11
IC90% C_{max}[a]	111.17:133.95			
IC90% AUCo-$_t$[a]	101.65:116.80			
IC90% AUCo-$_{inf}$[a]	100.81:114.85			

[a] Values in accordance with relation RP and PB

rotation speed, dissolution media composition, and type of apparatus were evaluated. Media aliquots were withdrawn at 5, 10, 15, 20, 30, 45, 60, and 120 min, through a 35-μm filter by the assembled autosampler. All the dissolution samples were analyzed by HPLC and UV methods.

To assess which dissolution media evaluated would provide a better discriminatory capacity, the values of the dissolved and absorbed fraction *versus* time were plotted to graphically reveal the medium, under the conditions of the dissolution test predetermined, which afforded to observe the greatest similarity between the dissolution profiles and the *in vivo* absorption. This would therefore be the medium chosen for the following evaluation steps. Such comparison was performed for both RP and PB under the different test conditions.

Validation of Dissolution Procedure

The dissolution method proposed was validated according to current guidelines (13–19). The parameters evaluated were: specificity, accuracy, linearity, precision, and robustness. Deaeration of the dissolution medium and interference of the filters used were also assessed. Stability studies also were performed.

Specificity

Specificity was evaluated based on the PB placebo with identical qualitative formulation to the RP. The placebo samples were transferred to separate vessels (n=3), with 900 mL of dissolution media at 37±0.5°C and stirred for 90 min at 150 rpm using the USP apparatus 2. Aliquots of these solutions were filtered and analyzed by HPLC and UV methods. The peak purity test performed by PDA was useful to show that the analyte chromatographic peak did not contain more than one substance. UV analysis was performed based on a spectrum obtained by scanning the standard solution and placebo diluted in the dissolution medium from 200 to 400 nm.

Linearity

The linearity of the analytical methods was evaluated in the range of 10.0–175.0 μg/mL for HPLC and 1.0–17.5 μg/mL for UV, using appropriate dissolution medium as second diluent. The solutions were injected in triplicate every day, for three consecutive days in HPLC, and UV readings were obtained concomitantly. Linearity was assessed by regression analysis, which was calculated by the least squares regression method and also by analysis of variance (ANOVA) to assess appropriate fitting.

Accuracy/Precision

Accuracy of the dissolution method was evaluated by the recovery test of known quantities of ATV calcium reference substance added to placebo solution in the dissolution vessels, as doses corresponding to 25, 100, and 125% of the nominal dose of ATV. Therefore, volumes of 1.0, 4.0, and 5.0 mL of a 20-mg/mL solution of ATV reference substance dissolved in methanol were added to vessels containing dissolution medium and placebo, to a final volume of 900 mL, preheated at 37±0.5°C and rotated for 60 min at 50 rpm using the USP apparatus 2. Aliquots were withdrawn, filtered, diluted in dissolution medium when necessary, and analyzed by HPLC and UV. These studies were performed on three different days, and the recovery of the added drug substance was determined.

In order to assess the repeatability of the method, precision was evaluated associated with the accuracy test. Intermediate precision was performed through the execution of the dissolution profile of RP tablets in different days by two analysts. Reference substance solutions were prepared on each day of profile analysis. Repeatability was established based on relative standard deviation (RSD) of the results of accuracy, and intermediate precision was established based on the difference in the mean value between the two dissolution profiles, at each time point.

Medium Deaeration and Filters Evaluation

The medium was deaerated by heating at 42°C followed by vacuum filtration. The dissolution profiles obtained with the deaerated and nondeaerated medium were compared to determine the need for deaeration.

An amount of placebo equivalent to the mean weight of a tablet was transferred to a beaker that was agitated for 1 h at 37±0.5°C and followed by ATV calcium addition to a final concentration of 250 μg/mL. Next, 10-mL aliquots were withdrawn, filtered using a 35-μm filter, diluted in mobile phase to obtain a solution with a final concentration of 100 μg/mL and filtered again in a 0.45-μm membrane before injection. The same procedure was performed with another aliquot of the

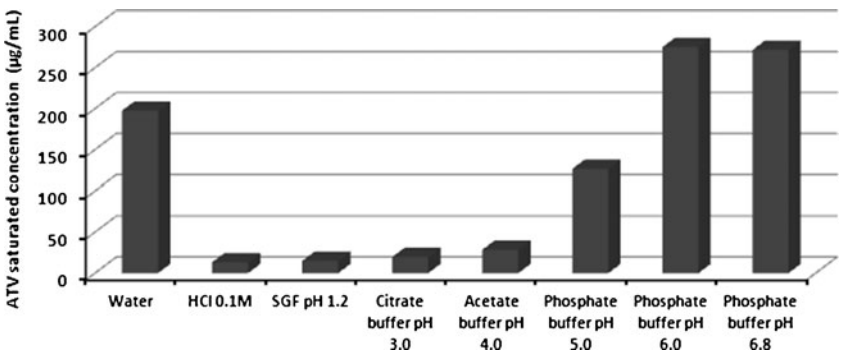

Fig. 1. Solubility of ATV calcium (μg mL^{-1}) in different media at 37°C after 24 h

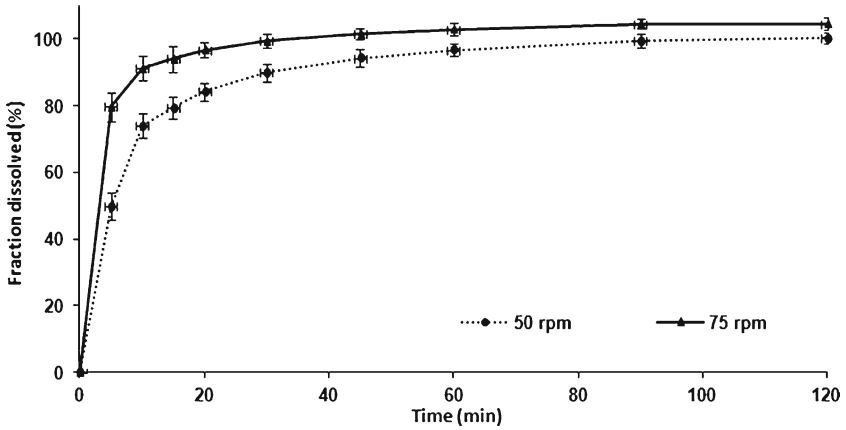

Fig. 2. Dissolution profile of the ATV reference product (n=6), obtained with 900 mL of phosphate buffer pH 6.0, agitation by paddle at speeds of 50 and 75 rpm

same solution, but this was centrifuged for 5 min at 3,000 rpm, not filtered. The solutions were analyzed by LC. For a filter to be acceptable for use, the results of the filtered portions need to be between 98 and 102% of the original concentrations of the unfiltered reference substance solution and of the centrifuged sample solution (2).

Robustness

Robustness of the dissolution method was determined with small modifications in the pH of the selected dissolution media (pH 5.8 and 6.2). The percentages of drug released obtained with the modification on buffer pH were compared

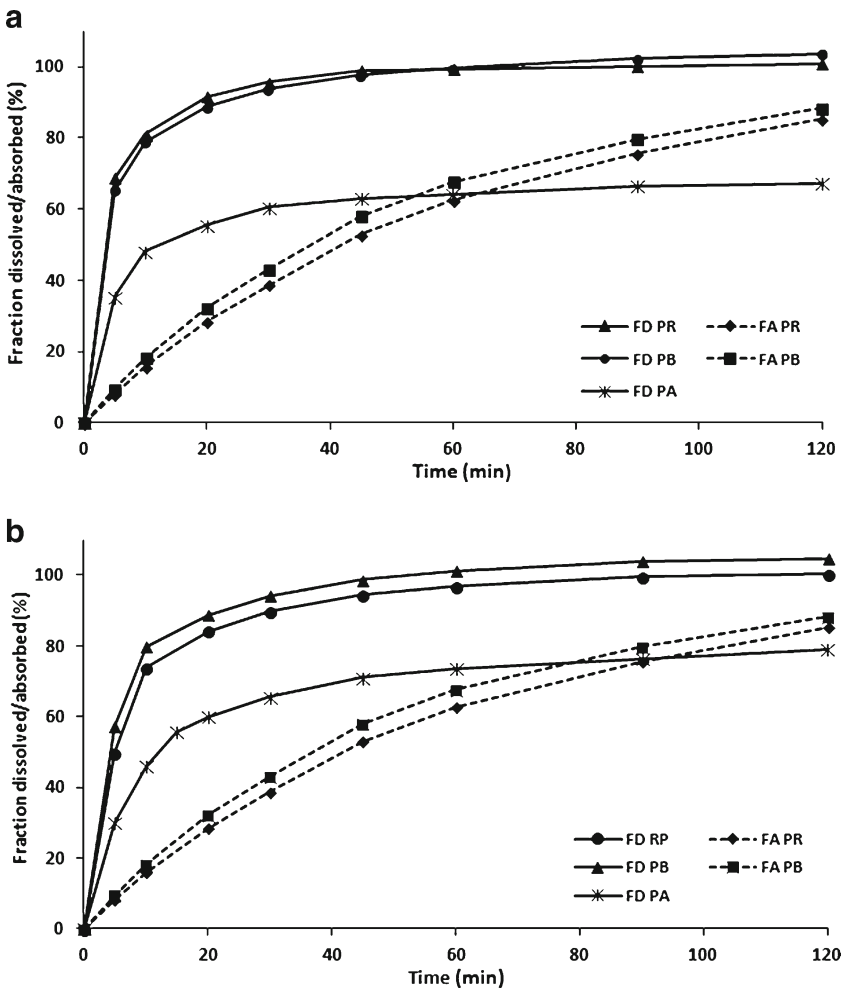

Fig. 3. Dissolution profiles using paddle at 50 rpm, phosphate buffer pH 6.0 (**a**) and 6.8 (**b**), in comparison with the respective absorption profile of the ATV calcium, through which the conditions were defined for more discriminative dissolution method

with the results obtained with buffer pH under the nominal condition (pH 6.0), through Student's *t* test.

Comparison Between HPLC and UV and Between Dissolution Profiles

The applicability of HPLC and UV to assess the ATV release in the dissolution study was compared using the Student's *t* test to demonstrate the equivalence between the methodologies. The values employed were obtained from the intermediate precision, using the amount released in function of time (5, 10, 15, 30, 45, and 60 min) of each individual vessel.

The dissolution profiles obtained for the RP, PA, and PB were compared by the model-independent approach, which includes the difference factor (f_1) and the similarity factor (f_2) (8–20).

RESULTS AND DISCUSSION

ATV Solubility and Sink Conditions

The solubility results (Fig. 1) were used as basis for the selection of dissolution medium for ATV tablets and also for ensuring *sink* conditions. Potassium phosphate buffer pH 6.0 and 6.8 showed *sink* conditions for the major dose of 80 mg. Therefore, these two media were selected for the initial dissolution studies.

Dissolution Conditions Selection

Initially, reference product tablets (n=6) were tested using USP apparatus 1 at 75 and 100 rpm with 900 mL of dissolution medium phosphate buffer pH 6.0 and 6.8 at 37±0.5°C. For the USP apparatus 1, the use of the 40-mesh basket did not supply an adequate transfer of mass from the inside of the apparatus to the medium, and the rotation speed not was suitable, due to the heavy weight of the tablets (around 1,000 mg). Even after increasing the basket rotation speed to 100 rpm, the dissolution of ATV stopped at around 80%.

The results of the profiles obtained were not considered for further evaluation due to the limited release of ATV calcium. Therefore, in an attempt to obtain a complete dissolution of ATV calcium, we tested the paddle USP apparatus 2.

The dissolution experiments using agitation by USP apparatus 2 were performed using 900 mL of buffer pH 6.0, in the agitation speed of 50 and 75 rpm. The profiles obtained showed a high dissolution rate, reaching a rapid release at 75 rpm, especially in the first three collection points, when compared to 50 rpm. In this lower rotation speed, the initial release is slower and somewhat more gradual, but still above the expected values in order to achieve an IVIVC, when compared with the data fraction absorbed (Fig. 2).

In an attempt to slow down the rate of dissolution, we sought to test lower agitation speeds. We used the speeds of 35 and 40 rpm. The results show a decrease in rate of dissolution of the drug in relation to the agitation speeds previously tested, and a gradual increase in the first times; however, these conditions favor the stagnation of the process, and part of the dose remained undissolved.

In order to have complete drug dissolution and obtain a possible IVIVC, we chose to use USP apparatus 2 at 50 rpm,

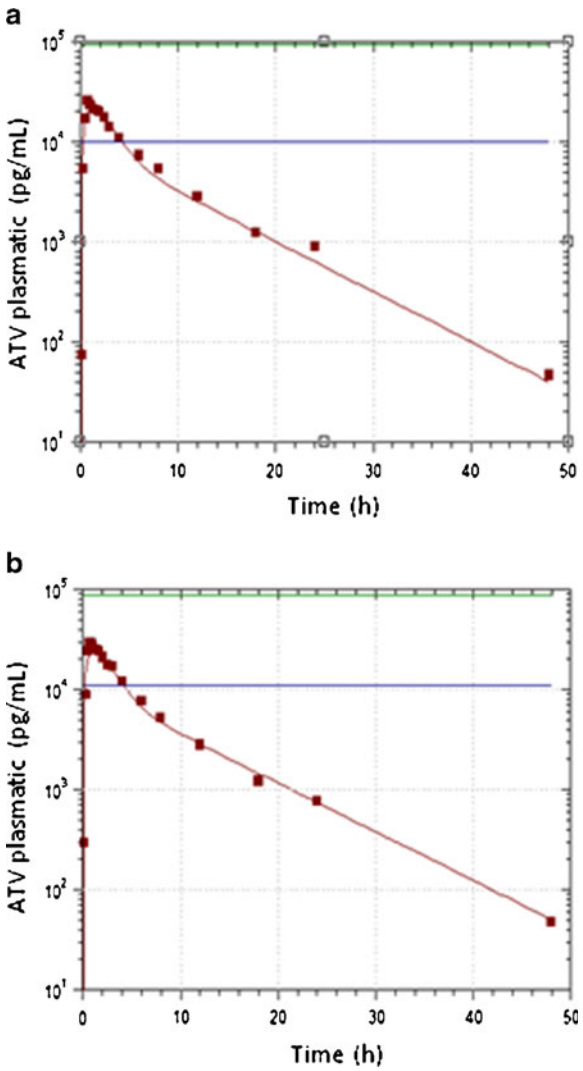

Fig. 4. Average plasma profile of ATV **a** reference product and **b** pilot B modeled to two open compartments by software Science®

Table II. Values of Percentage in Relation to the Time of Dissolution (TD) and Absorption (TA) for the PR and PB Utilized to Establish the Time-Scale Factor

	Reference product		Pilot B	
%	TD (min)	TA (min)	TD (min)	TA (min)
0	0	0	0	0
10	1.1	6.1	1	5
20	2.1	13.4	1.8	11.3
30	3	21.6	2.8	18.3
40	4	30.6	3.7	27.2
50	5	42	4.5	37
60	7,1	56	5.8	48
70	9.3	74	8.2	66
80	15.6	104	13	92
90	30	148	26.5	144

with 900 mL dissolution medium of both pH 6.0 and 6.8 buffers. These conditions were used to estimate the most appropriate dissolution medium discriminatory capability that somehow reflects the *in vivo* absorption. Despite having a dissolution rate higher than of the absorption, these conditions led to complete drug release (PR and PB) at the end of the dissolution profile. The same did not occur with PA, which presents limited dissolution due to the lower concentration of croscarmellose in its composition (Fig. 3).

The discriminative power of a dissolution method is the method's ability to detect changes in the drug product (2). In addition, a method of discriminating dissolution should be considered to reflect the behavior of drug absorption *in vivo*, whereby the dissolution tests *in vitro* conditions mimic the environment of the gastrointestinal tract (TGI) (21). As can be observed in Fig. 3, the potassium phosphate buffer pH 6.0 (A), with $f2=63.74$, represented better the *in vivo* release features of the drug, when compared to phosphate buffer pH 6.8 (B), for which *f2* between RP and PB was 81.19. In spite of the difference in dissolution and absorption speeds, the slight difference of the absorbed fraction between the two formulations can also be observed in the dissolved fraction.

The choice for buffer pH 6.0 was in accordance with the dissolution and absorption profiles of both the PB and RP products. In this medium, employing paddle at 50 rpm, a distinguished dissolution profile between the products was observed, just like the difference in the profiles that describe the absorption phenomena. The same was not observed when buffer pH 6.8 was employed. Therefore, to obtain a discriminative dissolution profiles for ATV calcium tablets the dissolution medium phosphate buffer pH 6.0 stirred using paddle at 50 rpm is proposed as the best alternative.

Complementary to this preliminary phase, the stability results for samples of ATV calcium in the test conditions were in the range 99.59–101.06% in relation to the standard solution freshly prepared. The samples remained stable with values between 100±2.0% of the initial content for a period of 24 h.

In vivo Data Processing aiming at the *in vivo–in vitro* Correlation

ATV calcium *in vivo* data for PB and RP were obtained based on the bioequivalence for a new formulation of ATV calcium. The same batches employed in the *in vivo* study were used in the development of the discriminative method of dissolution and in IVIVC.

The mean plasma profile obtained for ATV in the open two-compartment model is shown in Fig. 4. This model was chosen based on the best mathematical and graphical fit, using the model selection criterion, calculated by the program Scientist® from the mean values of plasma concentration obtained in the bioequivalence study and by the superposition of experimental points with values predicted by the program.

Plasma concentration data *versus* time were transformed into fraction of dose absorbed (FA) using the Loo–Riegelman method. Figure 3a shows the curve of ATV fraction absorbed *versus* time (min) obtained after administration of RP and PB. A faster dissolution profile for both RP and PB tablets were obtained in comparison with FA calculated from *in vivo* bioequivalence study. There is no an appropriate linear relationship between these data, due to their intrinsic difference.

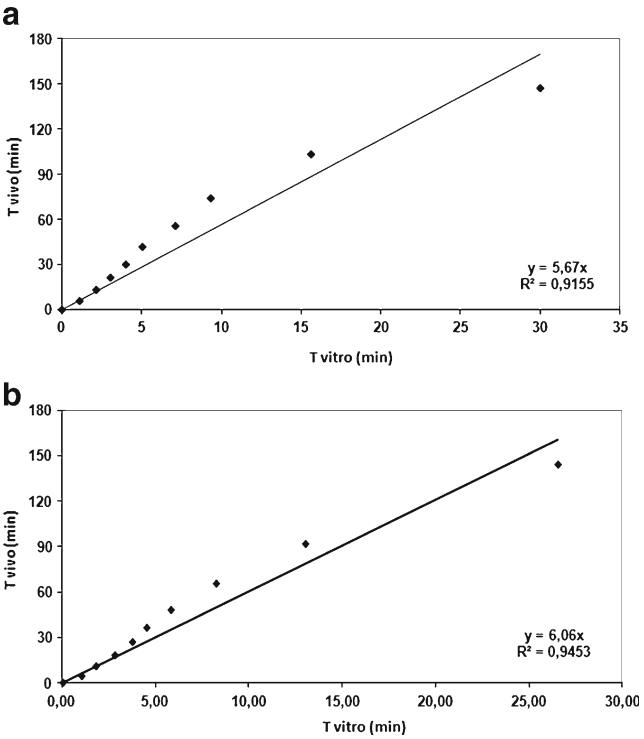

Fig. 5. Linear regression graphic of time necessary to for *in vivo* dissolution *versus* time to *in vitro* dissolution of a certain quantity of ATV calcium: **a** reference product and **b** pilot B

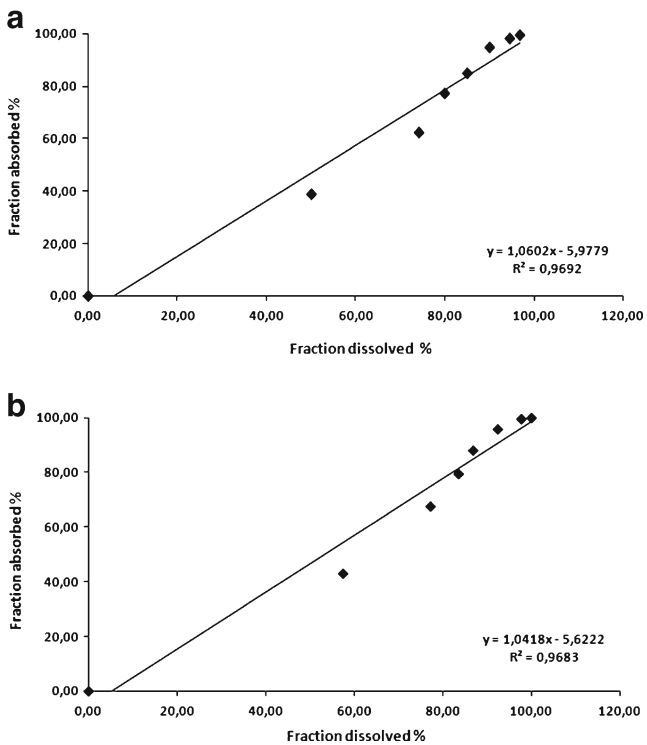

Fig. 6. Average curve of percentage of dose absorbed *versus* the percentage of the dose dissolved for atorvastatin calcium, using the time scale factor: **a** reference product and **b** pilot B

Table III. Analysis of Regression for IVIVC Using Time-Scale Factor

Product	Slope (*a*)	Intercept (*b*)	Correlation coefficient (*r*)
Reference	1.06	−5.98	0.9845
Pilot	1.04	−5.62	0.984

Thus, we applied the so-called time scale factor approach. The scale factor can be used in situations where the *in vitro* dissolution rate is either faster or slower than the rate of absorption *in vivo*. The time-scale factor can be determined by plotting the time required for a certain *in vivo* dissolution *versus* the time required for the same *in vitro* dissolution rate for a dosage drug in the dosage form. After performing a linear regression with adjustment to the origin (zero), the slope of the line is used as scaling factor in time to obtain IVIVC (22).

Therefore, time for certain absorption *in vivo* was plotted *versus* time for the same dissolution *in vitro*, based on the percentage values from 10 to 90%, shown in Table II. Figure 5 represents the correlation between these data, for the RP and PB. The slope values obtained were 5.7 and 6.1, respectively.

Thus, this approach afforded to estimate a scale factor of approximately 6.0. This value was then used to compare the *in vitro* profile with *in vivo* profile scaled for both the ATV calcium PR and PB tablets (Fig. 6). The fraction dissolved in a given time was plotted against the fraction absorbed obtained at a time six times greater. According to the regression analysis data shown in Table III, a linear correlation level A may be constructed for the two tablet brands, using 900 mL of potassium phosphate buffer pH 6.0 and paddle at 50 rpm.

The resulting correlation equations for ATV calcium RP and PB were used to recalculate the predictive FA and percentage internal prediction error, in order to evaluate the accuracy of the proposed model. Every error values revealed the difference found between predicted and calculated fractions absorbed, which would have to be below 10%, as set by the FDA 1997, both for RP and PB. This demonstrates the high precision of dissolution method proposed, which can therefore be used as an alternative to *in vivo* studies (Table IV).

Validation of Dissolution Procedure

Specificity

LC analysis revealed that the placebo formulation had no chromatographic peak, in the same retention time as that of ATV. Peak purity was higher than 0.9999, and it was obtained using a PDA detector. Additionally, the specificity analysis revealed that the UV method did not suffer interference from the excipients at 245 nm. Thus, LC and UV were useful to quantify ATV in the proposed dissolution method.

Linearity

Linearity in the concentration range studied was observed both in HPLC (from 10.0 to 175.0 μg/mL) and UV (1.0–17.5 μg/mL). The determination coefficients were 0.9995 and 0.9999 for HPLC and UV, respectively. Linear regression and the mean equations obtained were $y=46546x-8588.7$ for HPLC and $y=0.0374x+0.083$ for UV employing phosphate buffer pH 6.0. The analysis by ANOVA showed no significant deviation from linearity ($p>0.05$) and the Student's t test was applied to assess the significance of the experimental intercept in the regression equations. The results show that they are not significantly different from the theoretical zero value, since $p>0.05$, for both methods.

Accuracy/Precision

Accuracy of the method was evaluated by the recovery of known amounts of ATV added to the dissolution vessels. For each level of ATV concentration, two determinations were performed on three consecutive days by HPLC and UV. Mean recovery for ATV was (mean% ± RSD) 99.60 ± 1.55, 99.81 ± 1.20, and 99.56 ± 0.93, respectively for each level of the ATV quantity determined by HPLC. Using UV, mean recovery was 99.40 ± 1.82, 101.42 ± 0.94, and 101.82 ± 1.26 for each level of the ATV quantity, indicating adequate accuracy and precision of the dissolution procedure, evidenced by low values of RSD shown in test accuracy, in association with quantitative analysis.

The intermediate precision was determined by comparing two dissolution profiles of reference product. The difference between the mean ($n=6$) values obtained, at each time, for

Table IV. Validation internal of correlation proposed using the correlation equation to obtain the fraction of the dose absorbed (FA) [(A) RP and (B) PB]

	Reference product					Pilot B				
Time (min)	FD	Time (min)×6	FA simulated	FA predicted	Error	FD	Time (min)×6	FA simulated	FA predidected	Error
10	71.05	60	63.55	69.35	9.13	76.15	60	67.60	73.71	9.04
15	80.01	90	76.52	78.85	3.04	83.50	90	80.88	81.37	0.60
20	84.99	120	85.25	84.13	−1.32	86.60	120	88.23	84.60	−4.12
30	89.93	180	94.88	89.37	−5.81	92.20	180	92.81	90.43	−2.56
45	94.39	270	99.85	94.09	-5.76	97.53	270	99.82	95.98	−3.84
60	96.79	360	99.92	96.64	-3,28	99.95	360	99.90	98.50	−1.40
90	99.46	540	99.98	99.47	−0,51	102.78	540	99.96	101.45	1.49

HPLC and UV methods, respectively, demonstrated that all results were below 2.0%, confirming the precision of the method.

Evaluation of the Deaeration of Dissolution Medium and the use of Filters

Dissolved gases present in dissolution media can affect the results in a number of ways. The dissolved gas can significantly change the pH of an unbuffered solution, can interfere with the fluid-flow patterns through bubble formation, and can change the nature of the active ingredient and analytical values (23).

The results of the sample dissolution performed in nondeaerated and deaerated media were not different, and therefore, no interference was observed with the dissolution test.

The filter evaluation is necessary to determine whether it could be used in the dissolution test without drug adsorption and whether it removes insoluble excipients that may otherwise cause high background or turbidity (2). The evaluation of the filters demonstrated that the 35-μm filter and 0.45-μm nylon membrane furnished results within 98–102% of the initial values concentrations of ATV and that they may be used in dissolution tests.

Robustness

Robustness was evaluated through small modifications in buffer solution pH (±0.2 of the nominal value). The statistical analysis performed using the Student's *t* test ($t_{calc} < t_{tab}$ for $\alpha = 0.05$), demonstrated that this level of pH modification did not change the percentage of drug released from the ATV tablets, both by HPLC and UV, showing the robustness of the dissolution method, under this condition.

Evaluation of Dissolution Profiles

The dissolution profiles were compared using the difference and similarity factors. The values obtained were as follows: $f_1 = 6.25$ and $f_2 = 63.74$ for the PB *versus* RP and $f_1 = 49.36$ and $f_2 = 27.04$ for the PA *versus* RP. The results demonstrated that the dissolution profiles obtained showed a very similar behavior between the RP and PB, unlike RP and PA, where the latter has presented a release limited and consequently a distinct release dissolution profile, due to the difference in their formulations, underlining the discriminatory capacity of the method concerning the different formulations tested.

Comparison Between UV and HPLC

The paired Student's *t* test realized to compare the results obtained by the two analytical methods did not detect a significant difference ($p = 0.575$), showing the equivalence between UV and HPLC data for determination of ATV in the dissolution medium.

CONCLUSIONS

The dissolution method developed for ATV calcium tablets was considered discriminative for products with small difference in their composition, using 900 mL of phosphate buffer pH 6.0 and USP apparatus 2 at 50 rpm. A level A *in vivo–in vitro* correlation was established for ATV tablets with the aid of a time scale factor 6.0. The conditions were successfully validated according to USP 34, 2011 and ICH, 2005, showing that the experimental conditions were biorelevant. The dissolution method was also discriminative for pilot products tested, demonstrating changes in the different formulations. Results show that the *in vitro* dissolution method was accurate, precise, linear and specific. No significant difference was observed between the UV and HPLC, assuring that both can be used to evaluate the release profile of ATV tablets.

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