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Development and validation of dissolution test for ritonavir soft gelatin capsules based on in vivo data

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Abstract

The purpose of the study was to develop and validate a dissolution procedure for ritonavir soft gelatin capsules (Norvir®) based on in vivo data. Several conditions such as medium composition, pH, surfactant concentration and rotation speed were evaluated. The method was carried out using the same batch of Norvir® used in a bioequivalence study and the in vivo data were used to select the best dissolution test conditions based on in vitro–in vivo correlation (IVIVC). The dissolution test was validated using a high-performance liquid chromatographic method (HPLC). For this formulation, the best dissolution conditions were achieved using paddle, 900 ml of medium containing water with 0.7% (w/v) of sodium lauryl sulfate at a rotation speed of 25 rpm. Under these conditions a significant linear relationship between fraction of ritonavir absorbed and dissolved was obtained ($R^2 = 0.993$) and a level A IVIVC was established. In the HPLC method a relative standard deviation for intra-day precision was <1.6% and for inter-day precision was <1.4%. Accuracy was from 98.5% to 101.6% over the concentration range required for the dissolution test (4.0–124.0 μg/ml). Both the HPLC method and the dissolution test are validated and could be used to evaluate the dissolution profile of ritonavir soft gelatin capsules.

Keywords: Ritonavir; Dissolution; Validation; In vitro–in vivo correlation

1. Introduction

Drug absorption from a dosage form after oral administration depends on the release of the drug substance from the drug product, the dissolution and/or solubilization of the drug under physiologic conditions, and the permeability of the site of absorption in the gastrointestinal tract. Because of the critical nature of the first two of these steps, in vitro dissolution may be relevant to the prediction of in vivo performance. Based on this general consideration in vitro dissolution tests for immediate release solid oral dosage forms, such as tablets and capsules, are used to assess the lot-to-lot quality of a drug product as well as to guide the development of new drug formulations (FDA, 1997a).

The dissolution procedure should be appropriately discriminating, capable of distinguishing significant changes in a composition or manufacturing process that might be expected to affect in vivo performance (USP 29). The value of dissolution as a quality control tool for predicting in vivo performance of a drug product is significantly enhanced if an in vitro–in vivo correlation (IVIVC) is established (US Pharmacopeial Forum, 2004; FDA, 1997a). IVIVC has been defined as a predictive mathematical model describing the relationship between an in vitro property of a dosage form and an in vivo response (FDA, 1997b).

Ritonavir (ABT-538) is a human immunodeficiency virus (HIV) protease inhibitor indicated for the treatment of autoimmune deficiency syndrome (AIDS) (Sethi, 2002; Raffanti and Hass, 2003). It was approved by the US Food and Drug Administration (FDA) in 1996 (FDA, 2004; Abbott, 2006) and it is one of the most used anti-HIV drugs. Ritonavir is a poorly soluble drug with an oral bioavailability of about 70% being classified as class IV, according to the Biopharmaceutics classification system. This class of drugs are less likely to show IVIVC than class II. However, as the oral bioavailability approaches to 90%, the possibility of development of an IVIVC increases (Amidon et

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al., 1995; Dressman and Reppas, 2000; Lindenberg et al., 2004). This drug was not sufficiently bioavailable in the solid state by the oral rote, requiring the product Norvir® to be formulated as a soft gelatin capsules filled with self-emulsifying drug delivery systems (SEDDS). SEDDS are mixtures of oil, surfactants and solvents used in order to improve the oral absorption of highly lipophilic drug compounds (Bauer et al., 2001; Porter and Charman, 2001; Gursoy and Benita, 2004).

This study describes the development and validation of dissolution test for ritonavir soft gelatin capsules (Norvir®) based on IVIVC. The in vivo data was obtained from a bioequivalence study where a new soft gelatin capsule containing ritonavir was evaluated. The same batch of the reference substance (Norvir® 100 mg, Laboratório Abbott Ltd., São Paulo) used in the bioequivalence study was also used to develop and validate the dissolution test.

2. Materials and methods

2.1. Materials

Ritonavir reference substance and saquinavir internal standard (assigned purity of 99.53% and 99.87%) were kindly donated by Cristália Produtos Químicos Farmacêuticos LTDA (São Paulo, Brazil). Norvir® 100 mg (Abbott) soft gelatin capsules were purchased from the market (batch no. 074672E21). The same batch of Norvir® was used in both in vivo and in vitro studies. Sodium lauryl sulfate (SLS) was obtained from Fisher Scientific (New Jersey, USA). HPLC grade acetonitrile and methanol were from Fischer Scientific (New Jersey, USA) and from Merck (Darmstadt, Germany). Formic acid was from Fischer Scientific (New Jersey, USA). All other reagents and solvents used for the preparation of buffer solutions were analytical grade. Butylated hydroxytoluene, polyoxyl 35 castor oil and oleic acid were from Alfa Química (São Paulo, Brazil). Ultrapure water Milli-Q Plus (Millipore®, Milford, MA, USA) was used for the dissolution medium and throughout the analysis.

2.2. In vivo study

In vivo data was obtained from a bioequivalence study where a new soft gelatin capsule formulation was evaluated. The data presented here are related to the reference formulation (Norvir®). The study was performed on 12 healthy male volunteers (age 20–35 years; weight 60–80 kg) according to a two-period crossover design. The subjects were informed of the purpose, protocol and risks of the study. The clinical study protocol was approved by the local ethics committee. Each subject gave his written consent to participate.

Subjects did not take any other medication or alcohol for at least 14 days prior to and during the entire study. Subjects were randomly divided into two groups and each group received a 100 mg oral dose (one soft gelatin capsule) in each of the two periods, with 200 ml of water after an overnight fast. Fasting was continued for the first 4 h after dosing, and then a light meal was provided. Blood samples were collected just before the drug administration and at 0.33, 0.66, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0 and 15.0 h after dosing. Plasma was separated by centrifugation and stored at −80 °C until assayed.

Table 1
Mass detector conditions

Drug	Parent ion	Daughter ion	Cone (V)	Energy (eV)
Ritonavir	670.6	295.8	30	34
Saquinavir	720.5	569.5	30	34

2.2.1. Plasma assay

Twenty-five microliters of internal standard (saquinavir) and 0.5 ml of phosphate buffer (pH 7.0) were added to 0.5 ml of plasma and treated with a cold mixture of acetonitrile:formic acid (99.95:0.05). The mixture was vortexed for 15 s, sonicated for 10 min and centrifuged at 2000 × *g* for 10 min. An aliquot of 200 μl of the supernatant was transferred to an auto sampler vial and injected in a Shimadzu® (Kyoto, Japan) system using a Shim-pack-RP18 column (5 μm, 4.6 mm × 15 cm) and a mixture of formic acid in water (0.05%, v/v) and acetonitrile (20:80) as mobile phase. The temperature in the auto sampler and in the oven was 10 and 30 °C, respectively. The injections were performed by a Shimadzu SIL-10ADVP automatic injector and the analyses were performed using MassLynx software (Version 3.5). The detection was made using a mass/mass detector (Quattro-LC®, Micromass) equipped with an electrospray ionization interface operating in a positive mode (ESI^+) probe. The spectrometer was programmed in the multiple reaction monitoring (MRM) mode using the transitions shown in Table 1.

The method was validated according to ICH guidelines. The method showed to be specific and linear in the 50–5000 ng/ml range. The R.S.D. for the quality controls (150, 1500 and 4000 ng/ml) were all below 6% and the limit of quantitation presented a R.S.D. of 12%.

2.2.2. Pharmacokinetic analysis

The experimental points in the average plasma concentration versus time curve were fitted with a non linear software (MicroMath Scientist®, v.2.0) using a one compartment open model, according to Eq. (1) and the resulting curve and parameters were used to estimate intermediate plasma concentration data points:

$$C = \frac{FDk_a}{V_d(k_a - k_e)}(e^{-k_e t} - e^{-k_a t}) \quad (1)$$

where C is the plasma concentration at time t; k_e the elimination rate constant; k_a the absorption rate constant; V_d the volume of distribution; D the dose and F is the fraction of the dose absorbed.

The percentage of drug absorbed (FA) versus time was calculated by using Wagner–Nelson method (Shargel and Yu, 2005).

2.3. In vitro dissolution testing

2.3.1. Dissolution study

The development and validation of the dissolution test was performed using a VANKEL® VK 8000 dissolution autosampling station consisting of a VK type bidirectional peristaltic pump, VK 750D digitally controlled heater/circulator, VK

7010 dissolution testing station multi-bath ($n = 8$) with automated sampling manifold. All the dissolution samples were analyzed by HPLC assay. The HPLC system consisted of a Shimadzu (Kyoto, Japan) LC-10ADVP liquid chromatograph, SPD-M10AVP diode array detector, Rheodyne® manual injector, DGU-14A degasser, SCL-10AVP system controller and Class-VP chromatography data system.

2.3.2. Chromatographic conditions

The chromatographic analysis was performed at room temperature, using a Phenomenex® C_{18} reversed phase column (5 μm, 15 cm × 4.6 mm) with a Phenomenex® Universal C_{18} guard column. The mobile phase consisted of a mixture of acetonitrile:water:methanol (53:40:7, v/v/v). The flow-rate was 1.5 ml min^{-1} and the injection volume was 20 μl. The detection of ritonavir was accomplished by ultraviolet absorption at 210 nm.

2.3.3. Solubility determination and sink conditions

The *sink* conditions were determined in different media. HCl 0.1 M, HCl 0.01 M, H_2O + 0.5% sodium lauryl sulfate, phosphate buffer pH 6.0 and acetate buffer pH 4.0 were tested. Vessels ($n = 3$) containing 125 ml of medium were pre-heated to 37 °C before adding an excess of ritonavir (50 mg). The samples were gently rotated. An aliquot (10 ml) was removed from each vessel after 1 and 2 h and filtered. One milliliter of the filtered aliquots were pipetted into 5 ml volumetric flask, neutralized, diluted with mobile phase and injected into the HPLC.

2.3.4. Dissolution test conditions

Dissolution testing of capsules was performed using paddle (USP Apparatus 2), 900 ml dissolution medium pre-heated to 37 ± 0.5 °C. Influence of rotation speed, dissolution medium and concentration of sodium lauryl sulfate was evaluated. Sample aliquots were withdrawn at 15, 30, 45, 60, 90 and 120 min and replaced with an equal volume of the fresh medium to maintain a constant total volume. An auto sampler was used to withdraw aliquots through a 10 μm filter. The use of sinkers was necessary to help center the capsules under the paddle.

2.4. Validation of the dissolution method

The developed dissolution test was validated for specificity, linearity, accuracy and precision according to the US Pharmacopeial Forum, second supplement to USP 29 and ICH guideline (US Pharmacopeial Forum, 2004; USP 29; ICH, 1994, 1996). The chromatographic parameters monitored were peak retention time, capacity factor, tailing factor and theoretical plate number.

2.4.1. Specificity

Specificity was evaluated by preparing samples of placebo. The placebo consisted of all the excipients, sinker and shell capsule without the active ingredient. The excipients were: butylated hydroxytoluene, ethanol, oleic acid and polyoxyl 35 castor oil. Their concentrations in pharmaceutical formulation were based on the literature (Kibbe, 2000) and calculated for a medium weight of content (~939.1 mg) for the capsules. The samples of the placebo were transferred to separate vessels ($n = 3$), filled with 900 ml of dissolution medium at 37 ± 0.5 °C and stirred for 1 h at 150 rpm using paddle (USP Apparatus 2). Aliquots were withdrawn and analyzed by HPLC.

2.4.2. Linearity

A stock solution containing 1 mg/ml of ritonavir was prepared in methanol. The linearity of the method was evaluated in the 4.0–124.0 μg/ml range using stock solution and dissolution medium. The solutions were injected in triplicate every day, during three consecutive days. The linearity was estimated by linear regression analysis.

2.4.3. Accuracy/precision

Accuracy of the method was evaluated by the recovery test of known amounts of ritonavir reference substance added to placebo. Aliquots of 0.72, 4.5 and 9.18 ml of a 10 mg/ml ritonavir standard solution dissolved in methanol was added to vessels containing dissolution medium for a final volume of 900 ml (final concentrations were 8.0, 50.0 and 102.0 μg/ml, of 900 ml respectively), pre-heated at 37 °C and rotated for 1 h at 150 rpm. Aliquots were withdrawn and analyzed by HPLC. Placebo samples were prepared in the same way described in the specificity test.

The same solutions used in the accuracy test were analyzed in order to access the precision of the method. Repeatability (intra-day) and intermediate precision (inter-day) were evaluated based on relative standard deviation (R.S.D.) of the results.

3. Results and discussion

3.1. In vivo study

The data generated in the pharmacokinetic study were used to develop the IVIVC. Fig. 1 shows the mean plasma profile curve after administration of 100 mg oral dose of ritonavir (Norvir®) to 12 healthy volunteers. The study was conducted by the NUCLIMED (Porto Alegre, Brazil).

One data point (0.33 h) showed results below limit of quantification and was not used.

Based on these results, the average plasma concentration versus time profile curve was transformed into percentage of drug absorbed, using the Wagner–Nelson method

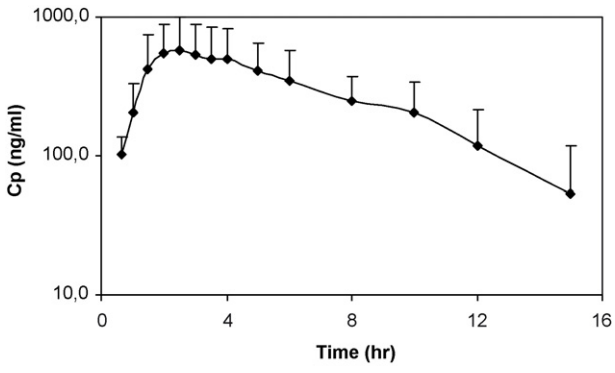

Fig. 1. Average plasma concentration vs. time curve after administration of ritonavir (Norvir®, 100 mg) soft gelatin capsules ($n = 12$).

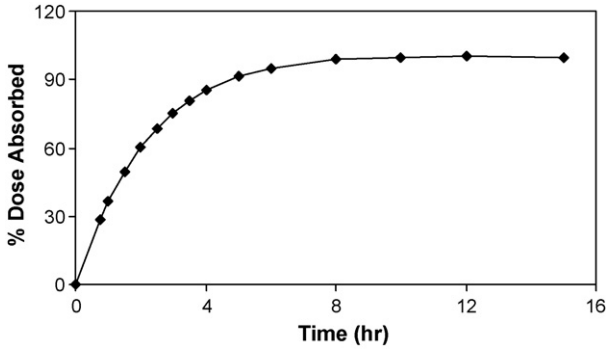

Fig. 2. Percentage of dose absorbed vs. time of ritonavir (Norvir®, 100 mg) soft gelatin capsules using the Wagner–Nelson method.

(Fig. 2). Model-dependent techniques such as Wagner–Nelson and Loo-Riegelman method or model-independent numerical deconvolution are allowed by FDA to calculate absorption profiles (FDA, 1997a).

3.2. Solubility determination and sink conditions

Solubility data were used as the basis for the selection of a dissolution medium for ritonavir. Drug solubility was determined at 37 °C in different media and expressed as percentage of dose dissolved. Based on solubility results for each dissolution medium tested and considering a test volume of 900 ml per vessel, *sink* conditions were satisfied. The term *sink* conditions is defined as the volume of medium at least greater than three times that required to form a saturated solution of drug substance (Rohrs, 2001; US Pharmacopeial Forum, 2004; Brown et al., 2004). According to Table 2, HCl 0.1 M and H_2O + 0.5% SLS were the best media for the ritonavir dissolution test and also ensured *sink* conditions.

3.3. Development of the dissolution test

From pre-selected media, capsules (n = 12) were analyzed using USP Apparatus 2 at 50 rpm, 900 ml dissolution medium at 37 ± 0.5 °C. Sinkers were necessary to help center the capsule under the paddle. Dissolution aliquots were analyzed at several time points (15, 30, 45, 60, 90 and 120 min) to generate dissolution profile in each medium.

Fig. 3 shows the dissolution profiles of Norvir® capsules obtained in HCl 0.1 M and H_2O + 0.5% SLS media. In both media ritonavir showed a fast dissolution rate. More than 80% was dissolved at 15 min in HCl 0.1 M and at 45 min in

Table 2
Percent of ritonavir dissolved in different media

Medium	Dissolved (%)	
	1 h	2 h
HCl 0.1 M	74.2	92.9
HCl 0.01 M	6.20	14.8
4.0 pH buffer	22.1	26.9
6.0 pH buffer	1.61	2.12
H_2O + 0.5% SLS	84.3	99.7

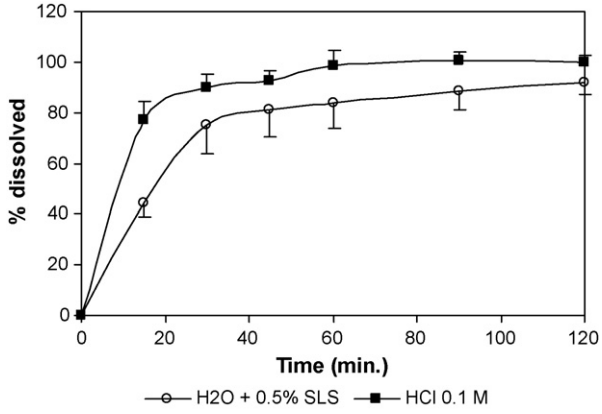

Fig. 3. Mean dissolution profiles of Norvir® capsules (n = 12) in H_2O + 0.5% SLS and HCl 0.1 M as dissolution media using paddle at 50 rpm.

H_2O + 0.5% SLS. However, a very poor correlation was established due to faster in vitro dissolution rate when compared to in vivo absorption rate.

Considering the fast dissolution rate achieved, new rotation speed (25 rpm) was evaluated for each medium. The decrease or increase of the apparatus rotation speed may be justified if the profiles better reflect in vivo performance or the method results in better discrimination (US Pharmacopeial Forum, 2004; USP 29). The results (Fig. 4) show that dissolution rate in HCl 0.1 M medium was greater than H_2O + 0.5% SLS. However, the dissolution profile obtained in H_2O + 0.5% SLS medium at 25 rpm was slower and a better IVIVC was established.

After selecting the dissolution medium, the concentration of the surfactant was adjusted to maximize the sensitivity of the method and to achieve the best IVIVC (Fig. 5). The use of surfactants for the dissolution of sparingly aqueous-soluble drug products is physiologically relevant. A dissolution medium containing surfactant can better simulate the environment of the gastrointestinal tract than a medium containing organic solvents or other non-physiological substance (Shah et al., 1989, 1995).

A plot of the IVIVC was obtained plotting the percentage of drug absorbed versus the percentage of drug dissolved and the line of best fit is shown for each dissolution medium (Fig. 6). The regression analysis of these data demonstrated that the medium

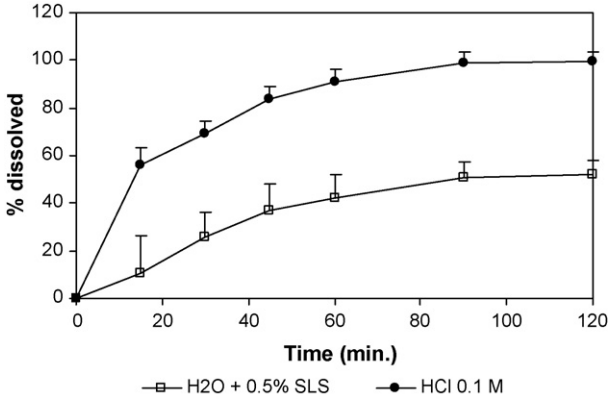

Fig. 4. Mean dissolution profiles of Norvir® capsules (n = 12) in H_2O + 0.5% SLS and HCl 0.1 M as dissolution medium using paddle at 25 rpm.

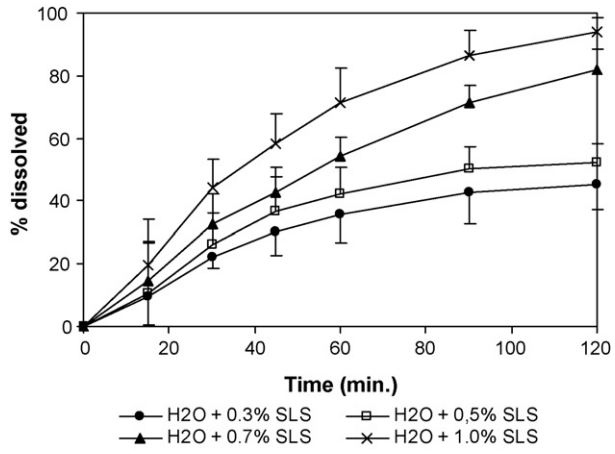

Fig. 5. Mean dissolution profiles of Norvir® capsules ($n = 12$) in different concentration of SLS using paddle at 25 rpm.

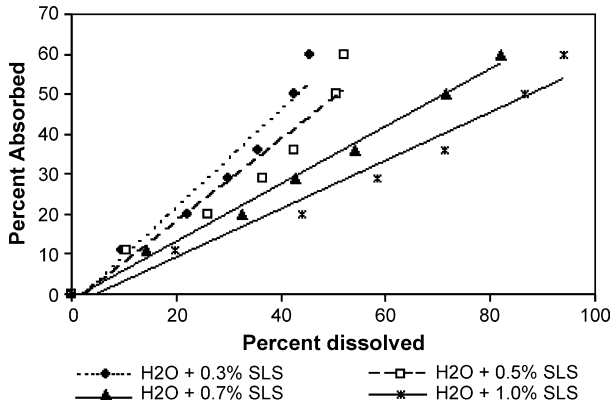

Fig. 6. Plot of mean percentage of dose absorbed vs. mean percentage of dose dissolved for Norvir®. The line of best fit is shown for each dissolution medium.

containing $H_2O + 0.7\%$ SLS and paddle at 25 rpm ($R^2 = 0.993$) was the best conditions for the dissolution test of Norvir® capsules (Table 3) and a level A correlation was established. This level of correlation is the highest category of correlation and represents a point–point relationship between in vitro dissolution and the in vivo input rate of the drug from the dosage form (Uppoor, 2001).

3.4. Validation of dissolution method

3.4.1. Specificity

The specificity of dissolution test was evaluated through the analysis of placebo. The chromatogram obtained through the injection of the placebo solution did not present any other peak in same retention time (4.7 min) of ritonavir (Fig. 7). The chromatographic peak purity tool available in the Class-VP software was used in order to verify the purity. This tool works by analyzing the peak and giving a purity value between 0 and 1. The obtained value was 0.9999, indicating that the analyzed peak was only ritonavir, without interference.

Table 3
Regression analysis[a] for the IVIVC

Medium dissolution	Slope (m)	Intercept (b)	Coefficient of determination (R^2)
$H_2O + 0.3\%$ SLS	1.22	−2.955	0.952
$H_2O + 0.5\%$ SLS	1.03	−2.596	0.935
$H_2O + 0.7\%$ SLS	0.72	−0.994	0.993
$H_2O + 1.0\%$ SLS	0.60	−2.807	0.963

[a] $y = mx + b$.

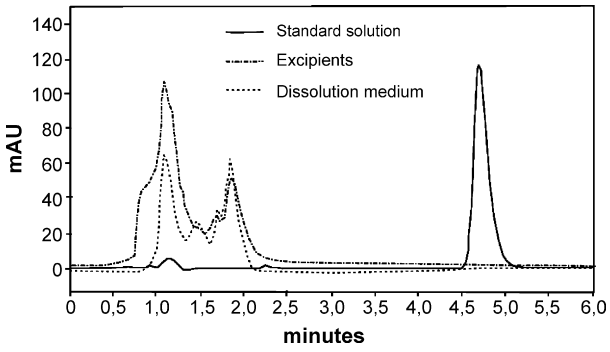

Fig. 7. Specificity test including standard solution, excipients and dissolution medium.

3.4.2. Linearity

The recommended range for the calibration curve is from ±20% below the lowest expected concentration to ±20% above the highest expected concentration of the dissolution test (US Pharmacopeial Forum, 2004; USP 29). Three calibration curves of ritonavir were evaluated (4, 34, 64, 94 and 124 μg/ml), plotting concentrations versus peak area. Linear regression was performed and the equation obtained was $y = 28645.5x + 3018.8$. An average coefficient of determination greater than 0.999 was obtained showing that the method is linear in the above-mentioned range.

3.4.3. Accuracy

Accuracy of the method was evaluated by the recovery test. Percentage recoveries from 95.0% to 105.0% are recommended for the accuracy test (US Pharmacopeial Forum, 2004; USP 29). Recoveries from 98.5 to 101.6 were obtained for the three concentration levels (8.0, 50.0 and 102.0 μg/ml) evaluated. The recovery results (Table 4) showed that the dissolution method was accurate.

Table 4
Accuracy results for ritonavir (% recovery)

Sample	Concentration (%)		
	8 μg/ml	50 μg/ml	102 μg/ml
1	102.5	99.8	98.1
2	101.8	102.0	98.4
3	100.8	102.9	98.3
4	102.9	99.2	98.7
5	100.1	100.7	98.6
6	101.8	101.3	99.2
Average	101.6	101.0	98.5
R.S.D. (%)	1.03	1.36	0.39

Table 5
Intra- and inter-day precision for the ritonavir reference standard solutions

	Concentration (μg/ml)	R.S.D. (%) intra-day	R.S.D. (%) inter-day
Day 1	8.0	0.83	1.03
Day 2	8.0	1.38	
Day 1	50.0	1.59	1.36
Day 2	50.0	1.10	
Day 1	102.0	0.22	0.39
Day 2	102.0	0.30	

3.4.4. Precision

Repeatability was determined by injecting three different solutions of each standard (8.0, 50.0 and 102.0 μg/ml) and the experiment was repeated on a second day to access the intermediate precision. The low R.S.D. values obtained for repeatability and intermediate precision show the good precision of the method (Table 5).

3.4.5. Standard and sample solution stability

Stock solutions of ritonavir reference substance (1.0 mg ml^{-1} and 10.0 ml ml^{-1}) in methanol, kept for at least 3 months at 4 °C, have been shown to be stable. The stability test of sample solution showed that ritonavir was stable in H_2O + 0.7% SLS for at least 12 h (considering the analysis time for routine quality control and dissolution profiles determination) at room temperature. There was no evidence of degradation of ritonavir under these conditions.

4. Conclusions

The value of dissolution test as a quality control tool for predicting in vivo performance of a drug product is significantly enhanced if an IVIVC is established. A meaningful dissolution test for Norvir® soft gelatin capsules was developed using in vivo data. Significant linear level A correlation ($R^2 = 0.993$) between in vitro and in vivo parameters was established. The conditions of the dissolution test selected for Norvir® capsules were 900 ml of medium containing H_2O + 0.7% SLS at 37 °C and USP Apparatus 2 at 25 rpm. The dissolution method was successfully validated according US Pharmacopeial Forum, second supplement to USP 29 and ICH. The HPLC method developed showed to be specific, linear, precise and accurate.

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