

Development and validation of a dissolution method for ritonavir soft gelatin capsules using UV-derivative spectroscopy

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Abstract

The aim of this work was to develop and validate a UV-derivative spectroscopic assay (UV-D) to evaluate the dissolution profile of ritonavir softgel capsules. The analytical performance characteristics of the proposed UV-D method were evaluated comparing with previously reported HPLC method. The linear dynamic range was 5.0-100.0 µg ml⁻¹ with a correlation coefficient (r) > 0.999, the mean intra- and inter-assay relative standard deviation were lower than 3.3 and 2.8, respectively, and the mean recoveries were between 95.1-104.7%. The assay data obtained for LC and spectroscopic method showed non-significant difference at the confidence level of 95% (P= 0.05). The dissolution profiles obtained were compared using difference factor (f1) and similarity factor (f2) and demonstrated that the profiles obtained were similar (f1=12.4 / f2= 59.0). The UV-D assay developed is usefull to evaluate the dissolution profile of RTV soft gelatin capsules and for analysis routine, being an alternative to the LC method, being faster, simpler and using fewer solvents.

Keywords: Ritonavir; Dissolution; UV-derivative spectroscopy.

Introduction

Human immunodeficiency virus (HIV) is a retrovirus that can lead to acquired immunodeficiency syndrome (AIDS). HIV protease is essential for viral infectivity and cleaves the viral polyprotein (gag-pol) into active viral enzymes (reverse transcriptase, protease and integrase) and structural proteins. Protease inhibitors act by binding reversibly to the active site of HIV protease. This prevents the protease from cleaving the viral polypeptide precursor and blocks viral maturation. Cells incubated in the presence of HIV protease inhibitors produce viral particles that are immature and noninfectious [1,2].

Ritonavir (RTV, ABT-538, Fig. 1) is a peptidomimetic inhibitor of both HIV-1 and HIV-2 proteases. Protease inhibitors are widely used in the management of HIV infection. RTV is a potent pharmacological booster administered at low dosages (100–200 mg/day) and is used to increase serum concentrations of other protease inhibitors and to improve their pharmacokinetic profiles, which allows for once- or twice-daily dosing of these agents [3,4]. The drug has been effectively used for over twenty years, and is available in pharmaceutical forms as soft gelatin capsules and oral solution [5].

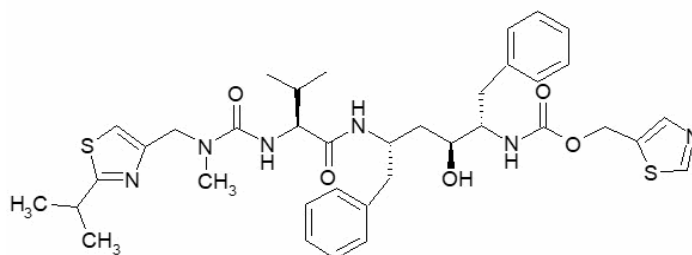


Figure 1: Molecular structure of ritonavir.

RTV is a poorly soluble drug with an oral bioavailability of approximately 70% and, because of its low aqueous solubility, Norvir® is formulated as a soft gelatin capsule in a self-emulsifying drug delivery system (SEDDS). SEDDS are isotropic mixtures of oil, surfactants, and solvents used to enhance the oral bioavailability of highly lipophilic drugs. Thus, for drugs that exhibit dissolution rate-limited absorption, these systems may improve the rate and extent of absorption and result in more reproducible blood-time profiles. This is a potential strategy to deliver new drug molecules that exhibit poor aqueous solubility and enhance their bioavailability [6,7]

There are several reports in the literature describing the quantitative analysis of RTV, and most of the methods used include high-performance liquid chromatography (HPLC) to quantify the drug alone and in combination with other anti-HIV agents in plasma. HPLC is a widely exploited technique that is mainly employed by quality control laboratories in the pharmaceutical industry, as this method is used for the separation and subsequent dosing of most drugs in official compendia such as the 2018

United States Pharmacopoeia (USP). Although USP 41[8] provides a monograph for this drug, ultraviolet (UV)-derivative spectroscopy is a technique that provides a characteristic spectrum profile, which facilitates the characterization of the compound and eliminates curve deformation caused by the presence of pharmaceutical excipients and products of degradation in the pharmaceutical form. Because of its properties and characteristics, this method presents itself as a very useful tool in the analysis of associated drugs in tablets, capsules, and creams, among others, without the need for preliminary separation. Therefore, the method in question is an alternative that is inexpensive and efficient, when compared to HPLC.

Dissolution tests are important to evaluate drug release of solid and semisolid dosage forms. *In vitro* dissolution tests for immediate-release solid oral dosage forms, such as tablets and capsules, are used to assess batch-to-batch quality of a drug product as well as to guide the development of new drug formulations and ensure continuing product quality and performance after certain changes (formulation, manufacturing process, site of manufacture, and scale-up of the manufacturing process [9]). Thus, the aim of this study was to develop a UV-derivative (UV-D) spectroscopic assay to evaluate the dissolution profile of RTV soft gelatin capsules in routine analysis. The method developed was validated by assessing its linearity, specificity, accuracy, and precision according to the USP 41 and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines. The new UV-D method was statistically compared with the recently developed HPLC method based on *in vitro-in vivo* correlation (IVIVC, [10]).

Materials and Methods

Materials

RTV reference substance (assigned purity of 99.53%) was 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 the reference substance (Norvir® 100 mg, Laboratório Abbot Ltda., São Paulo, Brazil) used in the bioequivalence study was also used to develop and validate the UV-D method (Rossi et al., 2007). Sodium lauryl sulfate (SLS) and HPLC-grade methanol were obtained from Fisher Scientific (New Jersey, USA). Butylated hydroxytoluene, polyoxyl 35 castor oil, and oleic acid were obtained from Alfa Química (São Paulo, Brazil). Ultra-pure water (Milli-Q Plus, Millipore®, Milford, MA, USA) was used as the dissolution medium and throughout the analysis.

Dissolution study

Validation of the dissolution test was performed using a VANKEL® VK 8000 dissolution auto-sampling station consisting of a VK type bidirectional peristaltic pump, VK 750D digitally controlled heater/circulator, and VK 7010 dissolution testing station multi-bath ($n = 8$) with automated sampling manifold. All dissolution samples were analyzed via UV-derivative assay. A double-beam UV-visible spectrophotometer (SHIMADZU, Japan) model UV-1601 PC was used for spectroscopic measurements. The software employed was UVPC personal spectroscopy software, version 3.9. For all solutions tested, the second derivative spectra (UV-D²) was recorded over the range 280-250 nm against solvent in 1-cm quartz cells, fixing $\Delta\lambda$ 4 nm, and scaling factor 100. The amplitude values of D² were measured at 260.0 nm for RTV (zero crossing of excipients).

Dissolution studies of RTV capsules

Dissolution testing of RTV capsules ($n = 12$) was performed using 900 mL dissolution medium containing H₂O and 0.7% SLS at 37 °C $\pm$ 0.5 and USP Apparatus 2 (paddle) at 25 rpm [10]. Sample aliquots were withdrawn at 15, 30, 45, 60, 90, and 120 min and replaced with an equal volume of fresh medium to maintain a constant total volume. An auto-sampler was used to withdraw aliquots through a 10- μ m filter. The use of sinkers was necessary to help center the capsules under the paddle. The dissolution samples were analyzed via both UV-D and HPLC methods.

Comparison of in vitro dissolution profiles

The dissolution profiles were compared using a model-independent method, where the two profiles are compared only at the observed time points. The model-independent method includes the difference factor (f_1) and the similarity factor (f_2). The f_1 factor measures the percentage error between two curves over all time points (Eq. 1):

$$f_1 = \{[\sum_{t=1}^n |R_t - T_t|] / [\sum_{t=1}^n R_t]\} \times 100 \quad (1)$$

where n is the number of time points, and R_t and T_t are the percentages of reference and test product dissolved at each time point, respectively. The percentage error is zero when the test and drug reference profiles are identical and increases proportionally with the dissimilarity between the two dissolution profiles.

f_2 is a logarithmic transformation of the sum of squared error of differences between the test and the reference products over all time points (Eq. 2):

$$f_2 = 50 \times \log \{ [1 + (1/n) \sum_{t=1}^n (R_t - T_t)^2]^{-0.5} \times 100 \} \quad (2)$$

This factor is 100 when the test and reference profiles are identical and tends to 0 as the similarity decreases. According to the Food and Drug Administration (FDA), two dissolution profiles are declared similar if f_1 is between 0 and 15 and if f_2 is between 50 and 100 [11].

Preparation of standard and sample solutions

RTV chemical reference substance (CRS) was accurately weighed and dissolved in a volumetric flask with methanol to generate stock standard solutions containing 1 mg.mL⁻¹ and 10 mg.mL⁻¹ analyte. Twenty Norvir® soft gelatin capsules (100 mg RTV) were separately weighed and their contents were washed out with methanol, collected in a 100-mL volumetric flask, diluted to this volume with the same solvent, and finally mixed and filtered through a 0.45-µm nylon filter. Working solutions of the reference substance and sample were appropriate diluted with dissolution medium before UV-D² measurement.

Validation of the dissolution method

The dissolution method was validated according to USP 41 and ICH guidelines using a D² spectroscopic method and compared with the reported method [8,10].

Interference was evaluated by preparing placebo samples. The placebo consisted of all excipients, sinker, and shell capsule without the active ingredient. The inactive ingredients were butylated hydroxytoluene, ethanol, oleic acid, and polyoxyl 35 castor oil. Their concentrations in pharmaceutical formulation were based on the literature [12] and calculated for the average weight of content of the capsules (approximately 939.1 mg). Placebo samples were transferred to separate vessels (n = 3), filled with 900 mL dissolution medium at 37 °C ± 0.5, and stirred for 1 h at 150 rpm using a paddle (USP Apparatus 2). Aliquots were withdrawn and analyzed via UV-D² spectroscopy.

The linearity of the method was tested by analyzing five different RTV concentrations over a range of 5.0-100.0 µg.mL⁻¹ (n = 3) using stock solutions (1 mg.mL⁻¹) diluted with dissolution medium. Linearity was estimated via linear regression analysis.

Accuracy (recovery) of the method was tested by adding known amounts of RTV reference substance to the placebo solutions. Aliquots of 0.72, 4.5, and 9.0 mL of a 10 mg.mL⁻¹ RTV standard solution dissolved in methanol were added to vessels containing dissolution medium to obtain a final volume of 900 mL (final concentrations were 8.0 µg.mL⁻¹, 50.0 µg.mL⁻¹, and 100.0 µg.mL⁻¹,

respectively), pre-heated at $37\text{ }^{\circ}\text{C} \pm 0.5$, and mixed for 1 h at 150 rpm. Aliquots were withdrawn and analyzed. Placebo samples were prepared in the same way described in the specificity test.

The solutions used in the accuracy test were analyzed here again in order to assess the precision of the method. Repeatability (intra-day) and intermediate precision (inter-day) were evaluated based on relative standard deviation (RSD) of the results.

Results and Discussion

The usual analytical techniques used in dissolution tests are UV spectroscopy and HPLC. UV spectroscopy is used whenever possible as results can be obtained faster, the analysis is simpler, and fewer solvents are usually used. Other methods such as HPLC are used when there is significant interference from excipients or among drugs in the formulation. But even in this case, the use of UV-D must be considered. It is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands. UV-D has been used in the analysis of many pharmaceutical formulations, and has proven particularly useful in eliminating interference from excipients and co-formulated drugs [13, 14, 15, 16].

Analytical performance characteristics of the proposed UV-D method, including specificity, linearity, precision, and recovery, were evaluated and compared with that of the HPLC method described by Rossi et al [10].

Validation of the dissolution method

Specificity

Standard solutions of RTV ($50.0\text{ }\mu\text{g}\cdot\text{mL}^{-1}$), samples ($50.0\text{ }\mu\text{g}\cdot\text{mL}^{-1}$), and placebo samples were evaluated over the range of 300-200 nm in zero-derivative orders (Fig. 2). Capsule excipients showed the highest absorption in the studied interval, and therefore the largest interference with the original RTV spectrum. A standard solution ($50.0\text{ }\mu\text{g}\cdot\text{mL}^{-1}$) and placebo sample were subjected to first-to-fourth-order derivatives. The spectra showed that the absorbance at 260.0 nm was exclusively due to RTV as the influence of the sample placebo was not observed at this wavelength, as shown in Fig. 3. This approach of quantitative determination is called the “zero-crossing technique”. This technique exploits signal crossing through the abscissa for a given component in a mixture to assign the absorbance value to remaining components. The results showed that second-derivative measurements are feasible for the analysis of RTV without interference from placebo constituents.

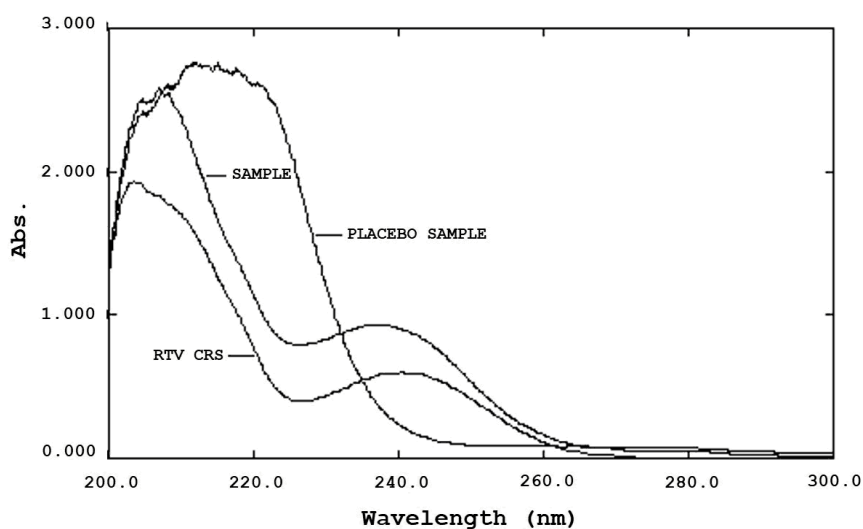


Figure 2: Zero-order spectra of ritonavir standard (RTV CRS), sample (capsule) and placebo sample in dissolution medium.

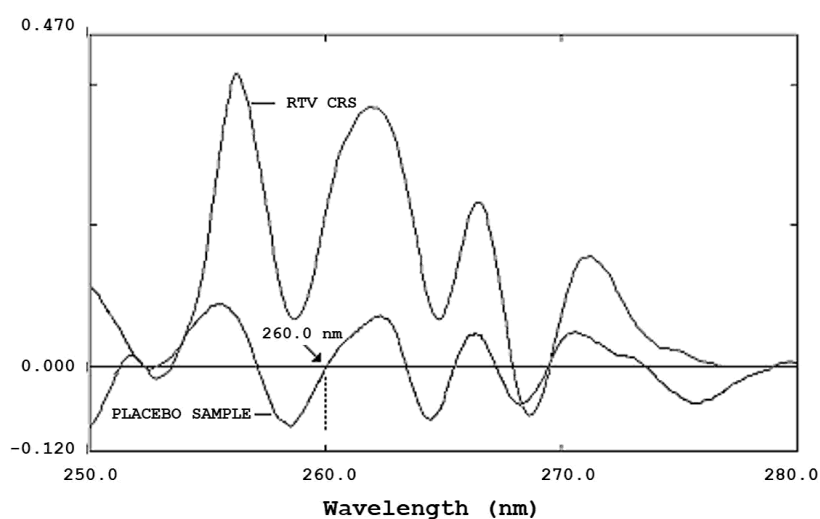


Figure 3: Second-order spectra of ritonavir standard ($50.0 \mu\text{g.mL}^{-1}$) and placebo sample in dissolution medium.

Linearity

According to USP 41 [8], linearity and range are established by preparing solutions of the drug, ranging in concentration from $\pm 20\%$ below the lowest expected concentration to $\pm 20\%$ above the highest expected concentration of the dissolution test. To assess linearity, three standard curves of RTV were evaluated ($5, 25, 50, 75$, and $100 \mu\text{g.mL}^{-1}$), plotting concentrations versus absorbance.

The method showed good linearity in the 5.0-100.0 $\mu\text{g}\cdot\text{mL}^{-1}$ range. The correlation coefficient (r) was greater than 0.999, indicating good correlation between concentration and absorbance. The data were validated by means of analysis of variance, which demonstrated significant linear regression and no significant deviation from linearity ($p < 0.05$). The overlay of zero-order and second-order derivative spectra of RTV standard solutions showed that the measured amplitude was proportional only to the concentration of the assayed compound (Fig. 4).

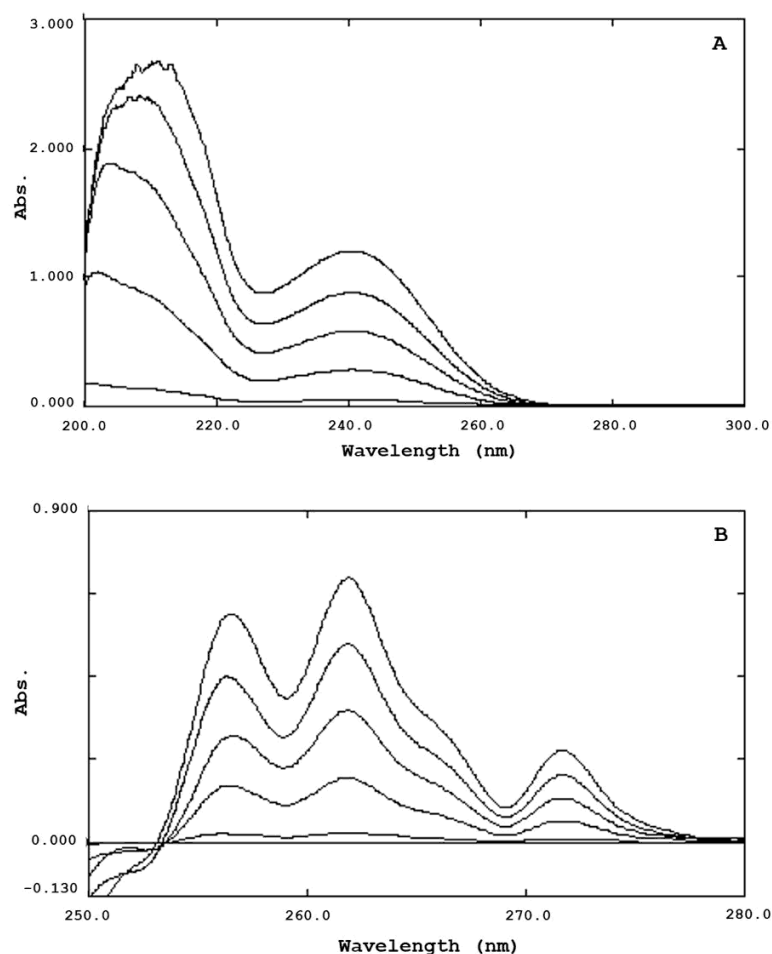


Figure 4: Overlay of zero-order spectra (A) and second-order spectra (B) for ritonavir standard solutions (5.0 – 100.0 $\mu\text{g}\cdot\text{mL}^{-1}$) in dissolution medium.

Precision and accuracy

The precision of this analytical method was determined by assaying three different solutions of each standard (8.0 $\mu\text{g}\cdot\text{mL}^{-1}$, 50.0 $\mu\text{g}\cdot\text{mL}^{-1}$, and 100.0 $\mu\text{g}\cdot\text{mL}^{-1}$) in triplicate, and the experiment was repeated on a different day to assess the intermediate precision. The results of repeatability and intermediate precision experiments were expressed in terms of RSD from mean intra-day and inter-day

assays and are shown in Table 1. The RSD values obtained for repeatability and intermediate precision were lower than 3.3 and 2.8, respectively, indicating good precision of the method.

Table 1: Repeatability and intermediate precision data for ritonavir

Day	Concentration ($\mu\text{g}\cdot\text{mL}^{-1}$)	R.S.D. (%)	R.S.D. (%)
		intra-day	inter-day
Day 1	8.0	2.29	2.8
Day 2	8.0	3.29	
Day 1	50.0	3.11	2.5
Day 2	50.0	1.83	
Day 1	100.0	1.71	2.2
Day 2	100.0	2.92	

Accuracy was calculated as the percentage recovery by assaying the known added amount of RTV reference substance in the placebo. The accuracy of the method ranged from 95.1 to 104.7% at the three concentrations tested (8.0, 50.0, and 100.0 $\mu\text{g}\cdot\text{mL}^{-1}$), indicating that the dissolution test was accurate (Table 2).

Table 2. Accuracy of assay for ritonavir (% recovery)

Sample	Recovery (%)		
	8 $\mu\text{g}\cdot\text{mL}^{-1}$	50 $\mu\text{g}\cdot\text{mL}^{-1}$	100 $\mu\text{g}\cdot\text{mL}^{-1}$
1	99.4	102.0	96.7
2	96.6	97.6	99.7
3	95.1	103.7	99.5
4	102.6	100.9	96.2
5	96.1	103.6	101.5
6	99.2	104.7	101.0
Average	98.2	102.1	99.1
R.S.D. (%)	2.8	2.5	2.2

Comparison of *in vitro* dissolution profiles

Twelve Norvir[®] capsules were subjected to a dissolution test and analyzed via both the developed UV-D assay and the HPLC method (Fig. 5). Student's t-test was used to compare both methods. The results showed non-significant differences at the confidence level of 95% ($p = 0.05$) with the t statistic being smaller than the critical value, suggesting that both methods are equivalent.

Dissolution profiles obtained using HPLC and UV-D methods were compared (12 capsules each) using a model-independent approach. The difference factor ($f_1 = 12.4$) and similarity factor ($f_2 = 59.0$) demonstrated that the profiles obtained were similar.

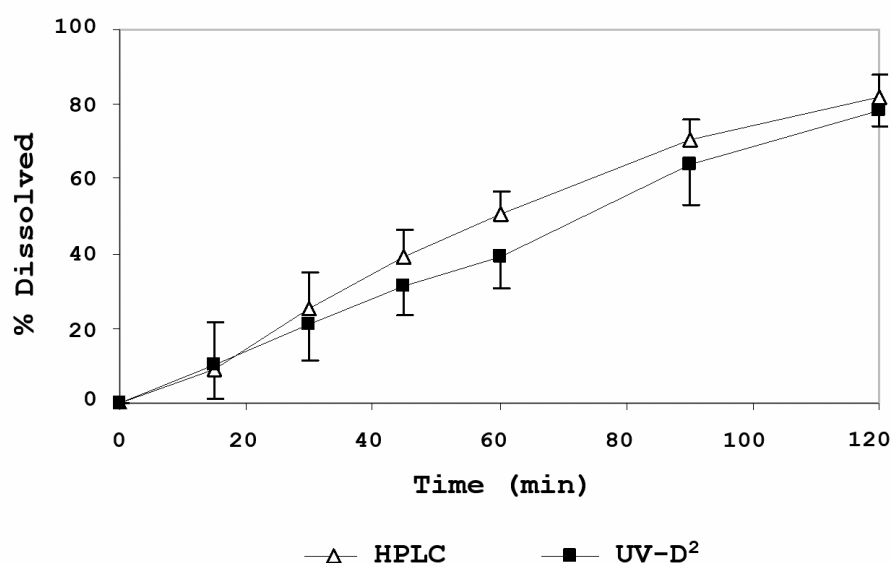


Figure 5: Mean dissolution profiles of Norvir[®] capsule (n=12) obtained by UV-derivative and LC method.

Conclusion

The importance of RTV in the clinical treatment of AIDS justifies the need for analytical procedures assuring the quality of its pharmaceutical dosage form. The proposed UV-D method for the quantitative determination of RTV in capsules offers selectivity without interference of the excipients when compared with the conventional UV spectroscopic method, as well as simplicity, accuracy, and precision. It presents an alternative to the HPLC method as statistical analysis by Student's t-test suggests that the two methods are equivalent. The proposed method could be used to evaluate the dissolution profile of RTV soft gelatin capsules.

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Conflict of interest: The authors declare that they have no conflict interest.

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