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Development and Validation of First Derivative Spectrophotometric Method for Quantification of Darunavir in Tablets

Josilene Chaves Ruela Corrêa[1*], Cristina Helena dos Reis Serra[2] and Hérida Regina Nunes Salgado[1]

[1]Drugs and Pharmaceuticals Quality Control Laboratory, School of Pharmaceutical Sciences, Univ Estadual Paulista, Rod. Araraquara-Jaú, km 1, 14801-902; Araraquara, Brazil.
[2]School of Pharmaceutical Sciences, University of São Paulo, São Paulo-SP, Brazil.

Authors' contributions

All authors have contributed equally for this article. All authors have read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Darunavir is widely used in HIV/AIDS therapy. It is a HIV protease inhibitor that has excellent efficacy against the virus. The aim of this study is to develop and validate an analytical method fast and free of interferences for determination of darunavir ethanolate as raw material and tablet dosage form.
Methodology: As the formulation excipients show high interference in darunavir determination by a direct UV absorption measurement a derivative spectrophotometry was applied. A selective, easy and fast method was achieved employing simple and cheap instrumentation by using first-order derivative spectrophotometry.
Results: The first-derivation of spectrum of the drug measured between 200 and 400 nm allowed identification of the analyte and showed absence of placebo interference. The assay was based on the absorbance at 276nm. The linear concentration range was established from 11 to 21 µg/mL. The intra-day and inter-day precision expressed as RSD was 0.06% and 3.75% respectively with mean recovery of 99.84%.
Conclusion: The proposed analytical method is able to quantify darunavir as raw material and tablets and can be used routinely by any laboratory applying a spectrophotometer

**Corresponding author: Email: josilenechavescorrea@gmail.com;*

with a derivative accessory. The great difference of the method proposed here is that it proves to be free of placebo interferences as well as simple, fast and low cost.

Keywords: HIV/AIDS; darunavir; pharmaceutical dosage form; analytical validation; derivative spectrophotometry.

1. INTRODUCTION

Darunavir is a HIV protease inhibitor and represents a great advantage in HIV/AIDS treatment since it has excellent efficacy against the virus [1].

Many studies have showed that darunavir has significantly improved virological and immunological outcomes compared with others PIs and it is generally well tolerated. Darunavir shows low bioavailability, around of 37%, but when it is administered in combination with ritonavir, a potent inhibitor of CYP3A4, and food the bioavailability is increased to 82%. The most important route of metabolism of darunavir is by cytochrome P450 (CYP450) enzymes, mainly CYP3A4, therefore, compounds that alter CYP3A4 activity and expression might influence darunavir concentrations [1].

Darunavir (Fig. 1) is marketed in ethanolate form under the brand name of Prezista™ and it is available in different dose strength such as 75, 150, 300, 400 and 600 mg. It is also is available as an oral suspension in which the darunavir dose is 100 mg/mL. The dosage form used in this study was Prezista ™ 300 mg darunavir tablets. This dosage has the following composition: 325.24 mg of darunavir ethanolate (equivalent to 300 mg of darunavir) and the inactive ingredients (crospovidone, magnesium stearate, Opadry ™ II White (partially hydrolyzed polyvinyl alcohol, titanium dioxide, macrogol and talc) and Prosolv ™ (microcrystalline cellulose and colloidal silicon dioxide) [2].

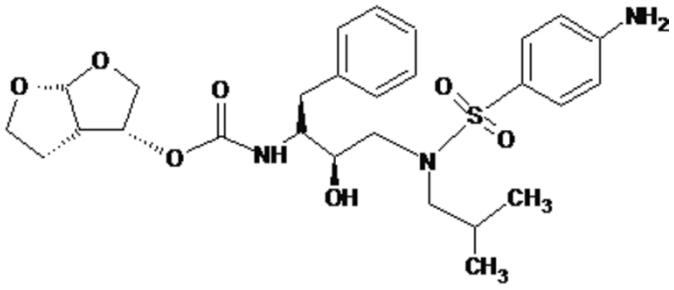

Fig. 1. Chemical structure of darunavir

To analyze the pharmaceutical active ingredient in a dosage form can be a challenge depending on the inactive ingredients used and the techniques and apparatus available. The interference of inactive ingredients over the measurements is something very common and in many cases the derivative spectrophotometry is a tool often applied to develop a fast and selective analytical method.

The aim of this work was to develop and validate a selective first derivative spectrophotometric method to determine darunavir ethanolate in raw material and tablets.

Rocha & Teixeira describes that the analytical applications of derivative spectrophotometry are based on the fact that the derived measurements are always proportional to the concentration of the analyte, which is shown by the differentiation of the Lambert-Beer law. Using spectral derivation it is possible to observe increased selectivity; sensitivity and improved detection limit [3].

As it was reported by Corrêa and coworkers in a review paper, there are some studies applying high performance liquid chromatography (HPLC) with ultraviolet detector or mass spectrometer for determination of darunavir in biological samples and tablets [4-9]. Patel and coworkers developed a HPLC method for the estimation of darunavir in tablet dosage form using a C18 column and the effluents were monitored at 267 nm [9]. Mane and colleagues also have developed a HPLC method for darunavir in tablets applying a C18 column and an UV detector at 264 nm [10]. A quantitative high performance thin layer chromatography (HPTLC) method for determination of darunavir was developed and validated by Patel and coworkers [7] and by Ramesh and coworkers [8]. The first authors used the wavelength 267 nm to detect the drug [11] and the second authors used reflectance scanning at 262 nm and mass spectra, $[M + Na]^+$, m/z 569.80, to detect the drug [12]. A spectrophotometry method had already been used to determine darunavir in tablets; it was developed by Reddy and Ramireddy [9]. The inconvenience of this method is the dependence of a previous reaction.

To determine darunavir in the presence of degradation products some analytical methods have been developed using HPLC with UV [2] and mass detection [14] Darunavir is not described in any pharmacopeia or official compendium. The method described here uses first-derivative spectrophotometry. It shows to be more specific than direct absorption measurement and faster than HPLC assay. The great difference of the method proposed here is that it proves to be free of placebo interferences as well as simple, fast and low cost.

2. METHODOLOGY

2.1 Apparatus and Reagents

A UV-Visible spectrophotometer (UV 1800, Shimadzu, Japan) and the software UV Probe was used for the assay. Hydrochloric acid, sodium acetate, potassium phosphate sodium hydroxide, and acetic acid (reagent grade) were used. Sample and standard solutions were filtered through a quantitative paper filter (2 µ, JProlab, Brazil).

Darunavir chemical reference (assigned purity of 98%) was obtained from Sequoia Research (Pangbourne, United Kingdom), darunavir raw material was extracted from Prezista™ tablets by ethanol extraction using an adaptation of the method described by Berginc and coworkers [15]. The purity of extracted darunavir was confirmed by HPLC analysis at 267 nm and by infrared spectroscopy. The darunavir peak surface represented 96.6% of the total chromatogram surface area and by infrared spectroscopy darunavir standard and extracted sample have showed the same characteristic peaks. It was standardized against darunavir chemical reference which contained 94% of darunavir. Darunavir ethanolate tablets 300 mg (Prezista™) were purchased from local market. The placebo of darunavir tablets was obtained as result of the extraction from Prezista™. The details of this extraction was given in a previous publication [2].

2.2 Standard Solutions Preparation

Darunavir standard stock solution was prepared by dissolving 6.5 mg of standard accurately weighed in 200 mL volumetric flask. Darunavir was solubilized with 40 mL methanol and 100 mL water. It was kept in ultrasonic bath for 10min, and the volume was making up to volume with water. Further diluting to volume with water six final concentrations were obtained: 11, 13, 15, 17, 19 and 21 µg/mL.

2.3 Sample Preparation

To prepare the sample solution, 20 darunavir tablets were weighed, powdered and its powder mixed making a pool. A quantity equivalent to 6.5 mg of darunavir was transferred into a 200 mL volumetric flask with 40 mL methanol and 100 mL water, kept in ultrasonic bath for 10 minutes, and the remaining volume was made up with water. The working concentration, 15 µg/mL, was achieved by dilution using water to fill the volumetric flask. Placebo solution was prepared at the same procedure.

2.4 Instrumentation

The spectrophotometer was programmed to generate the zero order and first order derivative scan between 200 and 400 nm, using its medium scan speed to acquire spectra signals, the sampling interval was 0.2, slit width was 1.0, *delta lambda* was 2.0 and scaling factor was 1.0. Smoothing was not used. The quantitation involved measurement of amplitude of the zero order peaks at 267nm and first-derivative valley at 276 nm.

3. RESULTS AND DISCUSSION

3.1 Analytical Method Development

The selectivity of the derivative method is one of the most important characteristics compared to zero order spectrophotometry and it shows to be effective in darunavir case which suffers placebo interference but using the first order derivative method the interference has disappeared.

The procedure performed to reach a selective spectrophotometric method was conducted using darunavir raw material, tablets and placebo. Sample solutions were prepared in different solutions as hydrochloric acid 0.1 M, acetate buffer (pH 4.5), phosphate buffer (pH 6.8) and water. The darunavir absorption was measured at the range of 200 – 400 nm and the derivation of the absorption curve was calculated by the software (Figs. 2 and 3). The percentage of placebo's interference was calculated using the zero order and the first order derivation of the absorption curve. As the order of derivative has to be carefully selected, since there is usually an increased noise level with increasing derivation order, the first order was tried first. The next order was not tried since good results were found using the first order derivation.

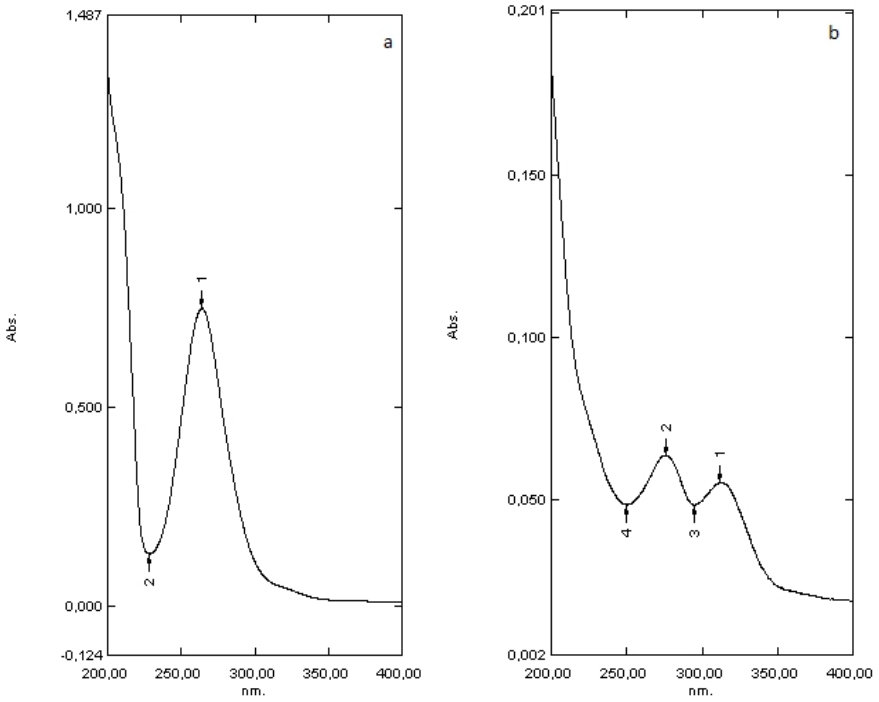

Fig. 2. Zero order UV absorption spectrum of (a) darunavir tablets, and (b) placebo at 15 µg/mL.

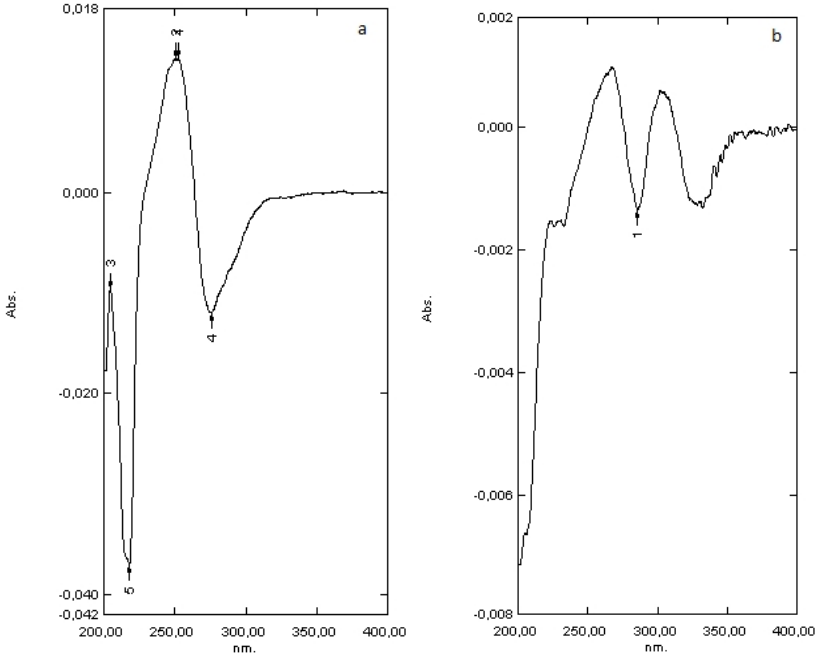

Fig. 3. First order derivative UV spectrum of (a) darunavir tablets, and (b) placebo at 15 µg/mL.

Table 1. Placebo interference at darunavir determination

Media	Interference (%)	
	Zero order (267 nm)	First order (276 nm)
Water (20 % of MeOH)	7.92	0
Hydrochloric acid 0.1 M	5.70	0
Acetate buffer, pH 4.5 (20 % of MeOH)	3.25	0
Phosphate buffer, pH 6.8 (20 % of MeOH)	1.35	0

All samples were evaluated by first derivative order in four different solution media. The table 1 shows that the interference of placebo disappears when the first order is applied. The wavelength used was 276 nm because, as shown by figure 2, it is an intense, better characterized valley. The increased selectivity observed in the derivative spectroscopy is based on the relationship between the amplitude of the derivative absorbance and the wavelength which is inversely proportional to the bandwidth of the ordinary spectrum [16].

Darunavir was determined in tablets in triplicate using the different solubilization media and the most precise result was found when the aqueous solution of methanol was applied. The aqueous solution of methanol (20 % of methanol) was chosen to solubilization of the samples.

3.2 Method Validation

The specificity of the method was evaluated by comparison of absorbance spectra of darunavir raw material, tablets and placebo samples. The absence of interfering compounds was verified by comparison of the first-derivative spectrum of placebo, raw material and product samples. The method was validated in agreement with ICH guide lines [17].

After parameters of the method were established the method was validated. The linearity was assessed with concentrations 11 to 21 µg/mL as an effort to work within the range where the linearity is usually observed (0.2 to 0.8 absorbance units, AU). Thus the selected working concentration was 15 µg/mL. The linearity was evaluated by the least square regression method. The method showed good linearity over the concentration range. The coefficient of determination was equal to 0.997 and the regression equation was $y = 0.017x - 0.0039$ (*y,* amplitude of first-derivative spectrum; *x,* concentration µg/mL) (Table 2). The validity of the method was verified by means of the analysis of variance (ANOVA). According to ANOVA there is linear regression in the tested concentration range (Fcalculated > Fcritical; $P = 0.05$) and there is no deviation from linearity (Fcalculated < Fcritical; $P = 0.05$).

The samples were analyzed in triplicate in all concentration range at the same day (within-day precision) and also at low, medium (working) and high concentration on different days (between-day precision). The method showed good precision with relative standard deviation (RSD) of 0.06 and 3.75, respectively for within-day and between day assays (Table 2).

The accuracy of the method was determined by the mean recovery. Placebo samples were spiked with darunavir raw material and solutions at the six concentrations of linear range

were performed in triplicate. The mean result was 99.84% (Tables 2 and 3) indicating an agreement between the true value and the value found.

The limits of detection and quantification were determined based on the standard deviation of the response and the slope, based on the calibration curve. This way to calculate the limits is recommended and explained by the ICH guideline [17]. They were calculated as 3 σ/S and 10 σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation. The limits of detection and quantification were 0.09 and 0.30 µg/mL, respectively (Table 2).

Table 2. Validation parameters

Parameters	Results
Useful concentration, µg/mL	15
Analytical curve	0.0038X – 0.0017
Determination coefficient (r^2)	0.997
R.S.D. within-day (%)	0.06
R.S.D. between-day (%)	3.75
Recovery (%)	99.84
RSD of recovery (%)	1.52
Detection limit[a], µg/mL	0.09
Quantification limit[b], µg/mL	0.30

[a] 3 σ/S; [b] 10 σ/S.

Table 3. Recovery data

Added amount (µg/mL)	Recovery (%)
11.0	98.79
13.0	98.54
15.0	100.25
17.0	99.28
19.0	100.09
21.0	102.08

To evaluate the robustness of the proposed method low and deliberate changes were made on the time of ultrasonic bath. Sample and standard solutions were submitted during nine, ten (standardized condition) and eleven minutes. The interference of these changes over assay test was monitored (Table 4).

Table 4. Robustness data

Time at ultrasonic bath (min)	Assay (%)
9.0	97.68
10.0	96.64
11.0	96.13

A method is said to be robust when the alterations produce no significant changes in the results. The results for the robustness test show values considerably low and not significant in routine analyses.

4. CONCLUSION

This study describes a useful, rapid and reliable method for darunavir assay. The proposed method was used successfully to determine darunavir raw material and tablets. The results of the validation studies show that the spectrophotometric method is selective, accurate and has significant linearity and precision without any interference from the excipient.

The new UV derivative method allows a rapid and economical quantitation of darunavir raw material and tablets without any time-consuming sample preparation. Moreover, the spectrophotometric methods involve simpler and cheaper instrumentation compared to other instrumental techniques, as for instance, chromatographic methods. It also does not depend on previous reaction for the measurements of drug concentration as a spectrophotometric method previously published and cited in this paper. The method proposed can be easily applied in routine practice by any laboratory possessing a spectrophotometer with a derivative accessory.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

The authors do not have any direct financial relation with the commercial identities mentioned in this paper. There is no conflict of interest for any of the authors.

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