Optimization of UV Spectrophotometric Method for Estimation of Darunavir in Bulk Drug and Tablet Formulations

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ABSTRACT
A simple and cost effective spectrophotometric method has been developed and subsequent validation of Darunavir in its bulk and formulation with good accuracy and precision. An absorption maximum was determined at 254 nm. Beer’s law was obeyed in the concentration range of 10-35μg/ml with correlation coefficient of 0.998.

Darunavir is a new protease inhibitor, is a retroviral drug used in treatment of (HIV) Type-1 infection. The % assay for Darunavir ranged from 98.4-102.1% in tablet dosage form. Hence the developed method can be used for routine analysis of Darunavir in pharmaceutical formulations.

KEYWORDS: Validation, Simultaneous Estimation, Darunavir, UV Spectrophotometry, Beer’s Law.

Introduction
Darunavir is a protease inhibitor used to treat HIV infection (Fig. 1). Darunavir is an OARAC recommended treatment option for treatment-naïve and treatment-experienced adults and adolescents (Ghosh et al., 2007). It was approved for clinical use in 2006. It is a second-generation protease inhibitor, designed specifically to overcome problems with the older agents in this class. Darunavir was designed to form robust interactions with the protease enzyme from many strains of HIV, including strains from treatment-experienced patients with multiple resistance mutations to protease inhibitors. It is on the WHO’s List of Essential Medicines. Darunavir received attention at the time of its release, as it represents a treatment option for people with drug-resistant HIV. Darunavir is an OARAC recommended treatment option for treatment-naïve and treatment-experienced adults and adolescents. As with other antiretrovirals, darunavir does not cure HIV infection or AIDS, and does not prevent passing HIV to others. Darunavir is a nonpeptidic inhibitor of PR that lodges itself in the active site of PR through a number of hydrogen bonds

In this study, we attempted to optimized a simple UV-spectrophotometric method for analysis of darunavir in bulk and tablet formulations. The work was planned for the selection of solvent and location of λmax of the drug with simultaneous study of Beer’s Lambert’s Law for the determination of UV spectrophotometry for the estimation of Darunavir in the bulk dosage formulations of the marketed drugs (Erwing et al., 1960). Precision and accuracy of the drug were noted at a selected wavelength and its application for the validation protocol was proposed for its formulation was determined (Jenkins., et al. 1986).

Materials and Methods
Darunavir was obtained as gift sample from Noshce Labs Pvt. Ltd, Hyderabad and formulation brand name is PREZISTA (containing 300 mg of darunavir ethanolate). It was purchased from a Hetero pharmacy.

ABBREVIATIONS: RSD: Relative Standard Deviation; SD: Standard Deviation.
All the chemicals used were of analytical grade for UV-Visible spectrometer (UV-1800 Shimadzu). As per the parameters of solubility and observation of well spectrum the following solvents were like Sodium Hydroxide, Hydrochloric acid, Water, selected on the trial bases, which had shown no spectrum, less absorbance, better spectrum, better spectrum respectively (Connors, et al., 1980; Bernard et al., 1985; Kalsi, 1998).

Ten mg of darunavir ethanolate was weighed accurately in the analytical balance and then transferred into 100 mL volumetric flask, dissolved and diluted up to the mark with methanol to obtain (100 μg/mL) solution ranging from 10 μg/mL to 35 μg/mL was taken in 10 mL volumetric flask, dissolved and diluted up to the mark with distilled water to prepare the stock solution at room temperature (Pavia et al., 2006). The standard stock solution of Darunavir was appropriately diluted with water to get the concentration of 10 μg/mL to 35 μg/mL respectively (Sethi, 1996). The solutions were scanned in the range (400 nm to 200 nm) against solvent blank (Beckett and Stenlake, 1997). The absorbance Spectra of drug is depicted in the Fig. 2.

Darunavir has shown maximum absorbance at 263 nm & it was selected for method. Quantitative estimation of Darunavir absorption was determined at the selected wavelength (263 nm) (USP 24, NF19 2000). The results are shown in the Table 1.

Table 1
Absorbance at standard concentration.

<table>
<thead>
<tr>
<th>Concentration range (μg/mL)</th>
<th>Absorbance at 263nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.270</td>
</tr>
<tr>
<td>15</td>
<td>0.386</td>
</tr>
<tr>
<td>20</td>
<td>0.507</td>
</tr>
<tr>
<td>25</td>
<td>0.643</td>
</tr>
<tr>
<td>30</td>
<td>0.761</td>
</tr>
<tr>
<td>35</td>
<td>0.915</td>
</tr>
</tbody>
</table>

Correlation coefficient (r²) 0.9991

Study of Beer Lambert’s Law
The standard stock solution of darunavir was diluted with water to get series of standard concentration from (10-35 μg/mL). Absorbance of each of these solutions was measured at 263 nm using solvent blank (Guzzetta, 2001). The graph plotted as concentration vs. absorbance is depicted in the Fig. 2.

Analysis of Tablet Formulation by Proposed Methods
Ten tablets were weighed to obtain the average tablet weight, which were then powdered. Powder equivalent to 10 mg of darunavir was weighed & transferred to 100 mL volumetric flask & allowed to dissolve in 70 mL methanol (International Conference on Harmonization, 1996). The volume was made up to mark with methanol to get the solution having darunavir 100 μg/mL and the sample solutions were prepared with in the concentration range by using double distilled water.

Validation of the Proposed Method
(a) Accuracy : Use a minimum of three spiking concentration in the excipient solution. Prepare 2 samples of each concentration. Test 6 samples in the triplicate on one run. Measure expected vs. average measured value.

Calculate the % recovery = bias. The total amount of drug was calculated by correcting the absorbance at 263nm. The percent recovery was then calculated by using following formulae (International Conference on Harmonization 1995)

\[ \% \text{ Recovery} = \frac{T - C}{P} \times 100 \]

Where, T = Total drug estimated
C = Amount from pre analyzed tablet powder
P = amount of pure drug added.

(b) Precision (%RSD) : Three dilutions of the sample were prepared of high, medium, low range of concentrations for test triplicates of the each dilutions of the sample in the three different assays for day-to-day, lot-to-lot, technician-to-technician variations (Pankaj et al., 2009)). The average, standard deviation for each point on the curve for each test of individual and % RSD for each point on the curve between the assay run were calculated in the Table 2.

Standard deviation:

\[ S = \frac{\sqrt{\sum(x - x')^2}}{N - 1} \]

Where;

\[ x = \text{observed values} \]
\[ x' = \text{Arithmetic mean} = \frac{\sum x}{N} \]
\[ N = \text{Number of deviations} \]
\[ \% \text{ RSD} = \frac{x}{S.D} \times 100 \]

Table 2
Assay of marketed formulation.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Sample no.</th>
<th>Label claim (in mg)</th>
<th>Amount obtained (in mg)</th>
<th>Assay value (in %)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prezista 1</td>
<td>1</td>
<td>300</td>
<td>295.4</td>
<td>98.4</td>
<td>1.6721 ± 0.77</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>300</td>
<td>305.0</td>
<td>101.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>300</td>
<td>302.8</td>
<td>100.1</td>
<td></td>
</tr>
</tbody>
</table>
(c) **Linearity:** For the dilutions of the sample over the range claimed for the assay the coefficient of correlation R is determined by preparing 6-8 sample dilutions across the claimed range, later test for each dilution in triplicate for 3 runs were conducted. Expected values, actual values, % recoveries for each run were reported. Finally, each set of dilutions as a linear curve and calculate correlation coefficient (r) for each assay was analyzed (Venumadhav, et al., 2010)

\[
 r = \frac{\sum (x - x')(y - y')}{\sqrt{\sum (x - x')^2 \sum (y - y')^2}}
\]

(d) **Limit of Detection (LOD):** It is the lowest amount of the analyte in a sample that can be detected but not necessarily be quantitated as an exact concentration or amount (Balaram, et al. 2011).

\[\text{LOD} = 3.3 \sigma / S\]

(e) **Limit of Quantitation (LOQ):** It is the lowest amount of analyte that can be measured quantitatively in a sample with acceptable accuracy and precision (Balaram, et al., 2011).

\[\text{LOQ} = 10 \sigma / S\]

(f) **Sandell’s Sensitivity:** Sandell’s sensitivity refers to the number of mg of the drug determined converted to colored product, which in a column solution of cross section I cm² shows an absorbance. (Expressed as pg cm⁻²) (Lakshmisiva et al., 2010).

### Results and Discussion

The solubility of Darunavir Ethanolate was determined in a variety of solvents, sample 10 mg was taken in a test tube & various solvents were added to check the solubility. The solvents used were distilled water, sodium hydroxide, Hydrochloricacid, Methanol, Ethanol, Chloroform (Rubeshkumar et al., 2011). Solubility profile of Darunavir ethanolate is given in Table 2. From the solubility studies methanol and water were chosen as solvent (Ramanaih et al., 2012). Taking its availability and the stability condition in account, we have selected the solvent. Drug was dissolved in methanol and was further made to dilutions with water to produce 10 μg/mL. it was scanned in the range of 200 nm-400 nm and it shows constant λ<sub>max</sub> at 263 nm this is shown in the Fig. 3. Stability of the drug checked at the λ<sub>max</sub> absorbance. (ICH, Text on validation of analytical procedures, 1994). The linearity of the drug Darunavir ethanolate was found; its calibration curve was constructed and shown in Fig 3. The optical characteristics such as Beer’s law limit (10-35 μg/mL), Sandell’s sensitivity (0.000026), correlation coefficient (0.9991), slope (0.0258) were calculated and shown in Table 4 & 5. The limit of detection & limit of quantification were determined from the linearity studies (ICH harmonized tripartite Guideline 1994). The limit of detection was found to be 1.22 μg/ml and the limit of quantification was found to be 3.72 μg/ml (Bernard et al., 1985). It has been shown in table 4 & 5.

Percent label claim for Darunavir in tablet analysis was found in the range of 98.46 – 101.2%. Percent Recovery for Darunavir was found in the range of 99.3 – 100.52% with standard deviation well below 2 indicating accuracy of the method. Percent RSD was found to be within limits (<2) (Park et al., 2009).

(a) **Linearity and range:** The study was performed over the series of concentration ranging from 10-35 μg/mL for Darunavir. The graphs of concentration vs. absorbance found to be straight line over the concentration range of these range.

![Spectra of darunavir](image)

(b) **Accuracy:** Accuracy of developed method was determined by recover study at 3 concentration levels by replicate analysis (n=3). Standard drug solutions were added to pre-analyzed sample solution & percentage of total drug content was calculated in the Table 3.

<table>
<thead>
<tr>
<th>Drug sample Concentration (20 μg/mL)</th>
<th>Amount recovered</th>
<th>Percent drug recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 μg/mL</td>
<td>24.9</td>
<td>99.6</td>
</tr>
<tr>
<td>10 μg/mL</td>
<td>30.4</td>
<td>101.3</td>
</tr>
<tr>
<td>15 μg/mL</td>
<td>35.2</td>
<td>100.5</td>
</tr>
</tbody>
</table>

(c) **Precision:** Precision of an analytical method is expressed as SD and %RSD of any measurements. Precision of estimation of Darunavir by proposed method was ascertained by replicate analysis of drug Darunavir. The results are shown in the following Table 4.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Absorbance (20 μg/mL)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.529</td>
<td>0.009602</td>
</tr>
<tr>
<td>2.</td>
<td>0.545</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>0.545</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>0.536</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>0.521</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>0.541</td>
<td></td>
</tr>
</tbody>
</table>
(d) **Limit of detection (LOD) and Limit of quantification (LOQ)**: LOD & LOQ can be determined by the method as per ICH guidelines. The method used in this project is based on standard deviation of the response & the slope of calibration curve (Zarparkar and Bhandari, 2000).

(e) **Sandell’ sensitivity**: Sandell’s sensitivity refers to the number of milligrams of the drug determined, which in a solution of cross section 1 cm² shows an absorbance of 0.000026 (expressed as pg cm⁻²) (Bikshal Babu et al., 2011). The results are presented in the Table 5.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameter</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( \lambda_{\text{max}} ) (nm)</td>
<td>263</td>
</tr>
<tr>
<td>2</td>
<td>Linearity range</td>
<td>10-35 µg/mL</td>
</tr>
<tr>
<td>3</td>
<td>Correlation coefficient ((r^2))</td>
<td>0.9991</td>
</tr>
<tr>
<td>4</td>
<td>Slope</td>
<td>USA</td>
</tr>
<tr>
<td>5</td>
<td>% Relative standard deviation</td>
<td>1.67</td>
</tr>
<tr>
<td>6</td>
<td>Limit of detection</td>
<td>1.22 µg/mL</td>
</tr>
<tr>
<td>7</td>
<td>Limit of quantification</td>
<td>3.72 µg/mL</td>
</tr>
<tr>
<td>8</td>
<td>Sandell’s sensitivity</td>
<td>0.000026</td>
</tr>
</tbody>
</table>

An attempt was made to develop simple and economical methods for the estimation of Darunavir ethanolate by UV method (ICH, Text on validation of Analytical Procedures, 1994). In absorption 263 nm was selected. No effect of dilution was observed on the absorbance of drug at selected wavelength (USP, 2006). The statistical analysis of data obtained for the calibration curve of darunavir ethanolate in pure solution indicated a high level of precision for the proposed method, as evidenced by low relative standard deviation (Bernard et al., 1985). The correlation coefficient was found to be significant. The linearity range was showed straight line passing through origin (International Conference on Harmonization, 1995). The method was validated by accuracy, precision & low values of %RSD. Result of recovery studies also proves the accuracy of the method (Grzegorz and Luc, 2003).

For UV method methanol is selected as a solvent which shows maximum absorbance when compared other solvents like sodium hydroxide and hydrochloric acid. The linearity was obtained for darunavir at 10-35 µg/mL. The precision was confirmed by low values of ± S.D (Standard deviation) and %RSD less than 2% concluded that no changes in validation parameters.

**Conclusions**

The developed UV-spectroscopic derivative spectroscopic and area under curve method gives sensitivity, accurate, precise and economical results for determination of Darunavir Ethanolate in marketed formulation and easily applied for routine analysis. The economical analytical method was developed for darunavir ethonolate. The most striking feature of these methods is its simplicity and rapidity. The developed methods were successfully applied for determination of the drug in commercial formulation.

**References**


USP (2006). The United States Pharmacopoeial Convention, MD, USA.


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