DEVELOPMENT AND VALIDATION OF A SIMPLE UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF DARUNAVIR ETHANOLATE BOTH IN BULK AND MARKETED DOSAGE FORMULATIONS

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ABSTRACT
A simple, rapid, specific and cost effective spectrophotometric method has been developed using a solvent composed of Methanol: Acetonitrile: Water (60:30:10) to determine the Darunavir Ethanolate (DRV) content in bulk and pharmaceutical dosage formulations. A pre-determined $\lambda_{\text{max}}$ of 267nm, it was proved linear and obeys beer’s law in the concentration range of 2 to 12µg/ml, and exhibited good correlation coefficient ($R^2=0.991$). The validity of the described procedure was assessed. Statistical analysis of the result has been carried out revealing high accuracy and good precision. The proposed method was successfully applied to the determination of DRV in pharmaceutical formulations without any interference from common excipients. This method was successfully applied to the determination of DRV content marketed brand the results were in good agreement with the label claims. The obtained results proved the method can be employed for the routine analysis of DRV in bulk as well as in the commercial formulations.

KEYWORDS: Darunavir Ethanolate; UV spectrophotometry; Validation

INTRODUCTION
Darunavir Ethanolate (DRV) is an inhibitor of the human immunodeficiency virus (HIV-1) protease. It selectively inhibits the cleavage of HIV-1 encoded GagPol polyproteins in
infected cells, thereby preventing the formation of mature virus particles. Development of a spectrophotometric method is based on the knowledge of the chromatographic process. A good method development strategy require only as many experimental runs as are necessary to achieve the desired final result. The scope of developing and validating analytical method is to ensure a suitable method for a particular analyte more specific, accurate and precise the main objective for that is to improve the condition and parameter, which should be followed in the development and validation.\cite{1,2,3,4}

In this study, efforts were made to develop a simple, easy and economic UV spectrophotometric method using diluents composed Methanol: Acetonitrile: Water (60:30:10) for the determination of darunavir ethanolate in the raw materials as well as in the marketed dosage formulations. The developed method was optimized and validated as per the guidelines of International Conference on Harmonization (ICH) and demonstrated excellent specificity, linearity, precision and accuracy for DRV. The chemical structure of DRV is shown in Figure 1.

![Fig 1- Chemical structure of Darunavir Ethanolate (DRV)](image)

Literature survey reveals that, some study about HPLC determination of darunavir ethanolate has published. The aim of this study is to develop a fast, simple, reliable, selective, sensitive and inexpensive UV spectrophotometric method for the determination of DRV in bulk drug and commercial pharmaceutical formulations as tablet and its validation.

**EXPERIMENTAL (MATERIALS AND METHODS)**

**Instruments**

A Shimadzu UV–visible spectrophotometer (UV1800, Shimadzu Corporation, Kyoto, Japan) was used for all absorbance measurements with matched quartz cells.
Materials
All chemicals and reagents were of analytical grade. DRV in the form of powder with certificate of analysis was provided by Alkem Research Centre, Mumbai. Pharmaceutical grade excipients were obtained from Pharmaceutical Technology Lab. of Maharashtra.

Preparation of Diluent
Prepared by mixing analytical grade Methanol: Acetonitrile: Water in the ratio of 60:30:10 and then sonicate for 5min.

Determination of wavelength of maximum absorption
A standard stock solution was prepared by dissolving DRV in diluent to make final concentration 10µg/ml. The \( \lambda_{max} \) was found by UV spectrum of DRV in the range of 200–400 nm and it was found to be 267 nm using diluent as blank.

Linearity and range
For linearity study, six solutions at different concentrations (2, 4 6, 8, 10, and 12 µg/ml) were prepared using six different aliquots of DRV, and the obtained data were used for the linearity calibration plot.

Intra-day precision (repeatability) and inter-day precision study (intermediate precision)
Standard stock solution (10µg/ml) of DRV was prepared by diluent. Make the three concentration of (2, 6, and 12 µg/ml), by using diluent. Take absorbance at 267nm. Calculate the % RSD.

Accuracy study
This study was carried out using the stock solution (10µg/ml). Take three concentrations 2 µg/ml, 6µg/ml, and 12µg/ml. And take six reading of these concentrations. Calculate the % RSD of the concentration.

Application of the proposed procedure for the determination in tablets
The proposed procedure was adopted for determination of DRV in tablets in following manner. The marketed tablet formulations of DRV were used for the purpose of analysis. Twenty tablets were weighed and average weight was calculated, crushed to fine powder. The powder equivalent to 10mg of DRV was transferred in 100 ml volumetric flask and dissolved in diluents and sonicate for 5 minutes. The volume was made to get final
concentration 100μg/ml. the solution was then filtered through whatmann filter paper (no. 41). This solution was used as stock solution.

The working solution of drug 10μg/ml was prepared from standard stock solution. The absorbance of working solution was measured and amount of DRV was calculated from calibration curve. The readings were taken in six times and the same procedure was repeated with another marketed tablet formulation.

All the marketed tablet formulations contain excipients which are added along with active pharmaceutical ingredient. These substances may cause some interference during estimation of active pharmaceutical ingredient. A recovery study was carried out on marketed formulations and the result obtained showed that, there was no interference from excipients. From the result of recovery study it can be claimed that, the method can be used for estimation of DRV in tablet dosage forms. The results obtained shown in Table 4.

RESULTS AND DISCUSSION
Statistical evaluation of analysis and recovery study was carried out. The data obtained from the proposed method showed accuracy of method. The values of relative standard deviation were satisfactorily low. The percentage recovery of 99% to 101% was indicative of accuracy of method.

Method validation
The method was validated with respect to linearity and range, accuracy and precision, limit of detection, specificity, accuracy, precision and robustness.

Linearity and range
The calibration curve (Fig 2) obtained was evaluated by its correlation coefficient. The absorbance of the samples in the concentration range of 2 to 12 μg/ml (Table 1 & 2) was linear with a correlation coefficient (R2) 0.991.

These solutions obeyed Beer-Lambert’s low in the above concentration
Table 1: Data for calibration curve

<table>
<thead>
<tr>
<th>Concentration μg/ml</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.344</td>
</tr>
<tr>
<td>4</td>
<td>0.576</td>
</tr>
<tr>
<td>6</td>
<td>0.742</td>
</tr>
<tr>
<td>8</td>
<td>0.973</td>
</tr>
<tr>
<td>10</td>
<td>1.098</td>
</tr>
<tr>
<td>12</td>
<td>1.399</td>
</tr>
</tbody>
</table>

Table 2: Linear regression data for calibration curve

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>2-12 μg/ml</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.991</td>
</tr>
<tr>
<td>Slope</td>
<td>0.101</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.148</td>
</tr>
</tbody>
</table>

Accuracy and precision

Accuracy and precision was investigated by analyzing three concentrations of DRV (i.e. 80, 100 and 120% of 300mg DRV tablet) in three independent replicates on the same day (Intra-day accuracy and precision) and on three consecutive days (Inter-day accuracy and precision). The data evaluated was summarized in Table 3.

The intra-day and inter-day precision study of the developed method confirmed adequate sample stability and method reliability. The relative standard deviation (RSD values and also the low RSD values obtained from the analysis of the pharmaceutical formulations (Table 3) indicates good intermediate precision of method.

To validate prediction ability of suggested method, different concentrations of DRV samples were prepared and analyzed. The results were satisfactory. Using standard addition technique, the method was further validated. The standard addition technique was carried out by adding excipients with the addition of DRV at 80%, 120% and 180%, respectively in sample solution. The percent recoveries of the three concentrations were found to be close to 100%, indicative of high accuracy. The high percent recoveries indicate no interference from ingredients and excipients that might be present in different formulations. The data evaluated was summarized in Table 4.
Table 3: Results for determination of intra-day and inter-day precision

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Intraday precision* (RSD %)</th>
<th>Inter-day precision* (RSD %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet 1</td>
<td>0.493</td>
<td>0.895</td>
</tr>
<tr>
<td>Tablet 2</td>
<td>0.694</td>
<td>0.589</td>
</tr>
<tr>
<td>Tablet 3</td>
<td>0.448</td>
<td>0.758</td>
</tr>
</tbody>
</table>

*Percentage RSD of six samples

Table 4: Results of recovery and precision

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Amount added (%)</th>
<th>Recovery* (% ± S.D)</th>
<th>RSD* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet - 1</td>
<td>80</td>
<td>79.438 ± 0.687</td>
<td>0.685</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>99.448 ± 0.875</td>
<td>0.880</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>120.462 ± 0.432</td>
<td>0.358</td>
</tr>
<tr>
<td>Tablet - 1</td>
<td>80</td>
<td>79.658 ± 0.726</td>
<td>0.912</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>99.267 ± 0.772</td>
<td>0.779</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>119.803 ± 0.908</td>
<td>0.758</td>
</tr>
</tbody>
</table>

*Percentage RSD of six samples

Robustness

Repeatability is based on the results of the method operating over short time interval under same conditions. The low RSD values of intra-day precision (Table 3), recovery (Table 4), and pharmaceutical preparations (Table 1 & 2) showed high repeatability.

Specificity in the presence of excipients degradation product

The specificity of the analytical method was proved by comparing the spectra of degradation product of sample solution with that of accuracy sample (Fig. 2).

![Fig 2- UV Spectra for specificity study](image-url)
CONCLUSION
The proposed UV method is simple, accurate, precise, specific and highly sensitive; developed and validated for the determination of darunavir ethanolate in bulk and tablet dosage form. The method is economical rapid and do not require any sophisticated apparatus in contrast to chromatographic methods. Hence, the proposed method can be successfully used for routine quality control analysis of drug in marketed preparations.

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REFERENCES


