VALIDATED VISIBLE SPECTROPHOTOMETRIC METHOD FOR
ESTIMATION OF DARUNAVIR IN BULK AND PHARMACEUTICAL
DOSSAGE FORM USING 1, 2 NAPHTHOQUINONE 4-SULPHONATE
REAGENT

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ABSTRACT

The aim of the present study is to develop a new simple, precise Spectrophotometric method for estimation of Darunavir using 1, 2 Naphthoquinone 4-sulphonate in presence of an alkaline medium. Analysis was performed on Shimadzu UV-1800 UV–Visible spectrophotometer with spectral bandwidth of 1nm and using a pair of 10mm matched glass cells. The method is based on the formation of bright orange color complex formed by reaction of Naphthoquinone sulphonate with Darunavir. The nucleophilic substitution proceeds quantitatively in alkaline medium (0.01N NaOH) with an absorption maximum at 460nm. The calibration curve is linear over concentration range of 10-80µg/ml and the regression equation obtained was Y=0.014x+0.012 with regression coefficient $R^2=0.9997$ and the Sandell’s sensitivity was found to be 0.09µg/ml. Accuracy and precision studies performed were found to be within limits.

Key words: Darunavir (DRN), Spectrophotometric, 1, 2 Naphthoquinone 4-sulphonate (NQS), Pharmaceutical dosage form.

INTRODUCTION

Darunavir (fig. 1) is chemically (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl N-[(2S,3R)-3-hydroxy-4-[N-(2-methylpropyl)(4-aminobenzene)sulfonamido]-1-phenylbutan-2-yl] carbamate. It is white to off-white powder that is very slightly soluble in water and soluble in methanol [1, 2]. DRN is generally co-administered along with Ritonavir (100mg) [3].
Darunavir is an inhibitor of Dimerisation and the catalytic activity of the HIV-1 protease. It selectively inhibits the cleavage of HIV encoded Gag-Pol polyproteins in the virus infected cells, thereby preventing the formation of infectious virus particles [4].

Literature survey reveals that there are reports describing the determination of Darunavir in Plasma using liquid chromatography coupled with Tandem Mass Spectroscopy [5], HPTLC method for determination of drug in rat plasma [6], few RP-HPLC methods [7, 8], and few Spectrophotometric methods [9,10] and one Visible method using NQS in presence of buffer [11]. So, the focus of present study is to develop and validate a rapid, stable, accurate, precise and economic Visible Spectrophotometric method using simple alkaline solution (NaOH) for estimation of DRN in tablet dosage form.

![Structure of Darunavir](image)

**Figure 1: Structure of Darunavir**

**MATERIALS AND METHODS**

**Apparatus**

Shimadzu UV-1800 UV-Visible spectrophotometer was employed with spectral bandwidth of 1nm attached to computer loaded with shimadzu UV PC software (UV Probe) version 2.31 and using a pair of 10mm matched glass cells. Analytical balance Systronics, pH meter Systronics 802 and water bath Kemi were used for the study.

**Chemicals and Reagents**

Naphthoquinone 4-sulphonate reagent AR grade obtained from Qualigens. NaOH AR grade and Methanol AR grade were obtained from Merck India and triple distilled water was used for entire study.

**Drug samples**

Pharmaceutical grade Darunavir (99.75%) was procured from Hetero Pharma Ltd. Hyderabad, India. Commercial dosage form DARUVIR tablets were manufactured by CIPLA ltd, Mumbai, India, each tablet contains 300mg dosage were purchased from local pharmacy.
Method development

Preparation of reagent solutions

Preparation of NQS solution (0.5% w/v)
Prepared by dissolving 0.5gm of NQS in 100ml volumetric flask containing small amount of water, shake well and made up the final volume with water. The solution is always prepared freshly and protected from sunlight.

NaOH solution (0.04% w/v, 0.01N)
Prepared by weighing accurately 0.04 g of anhydrous sodium hydroxide and dissolved in 100 ml of distilled water.

Preparation of standard stock solution
A stock solution of Darunavir was prepared by accurately weighing 100 mg of pure drug and transfer into 100 ml volumetric flask and dissolved in10 ml methanol and final volume made to 100 ml with methanol (1mg/ml).

Preparation of Working Standard Solution
From stock solution 10ml was further diluted to 100ml with 70% methanol to get the concentration 100µg/ml.

Optimization of method
The objective is to optimize the assay method for colorimetric estimation of Darunavir. Based on the literature survey made and methods given in official pharmacopoeias, trails were carried out using various chromogenic reagents. Finally, on using 1, 2 Naphthoquinone 4-sulphonate reagent in presence of base NaOH showed a stable color complex with good linearity. The trials were performed by changing the volume ratio of NaOH and 1, 2 Naphthoquinone 4-sulphonate reagent and water. Trails were also proceeded by changing the normality’s of base and %w/v of NQS reagent. Finally the method was optimized with each 1ml concentration of 0.5%w/v NQS and 0.01N NaOH.

Determination of λmax
From the above working standard solution, 5ml was pipette out into a 10ml volumetric flask. Then added 1ml of 0.01N sodium hydroxide solution and 1ml of (0.5%) NQS reagent and the volume was made up with distilled water left at room temperature for 15min. Then the bright orange colored complex solution formed was scanned in UV-VIS Spectrophotometer in the
range 800-400nm against reagent blank prepared in the same manner without analyte and the wavelength corresponding to maximum color intensity ($\lambda_{\text{max}}$) was found to be at 460nm (fig. 2).

![Figure 2: $\lambda_{\text{max}}$. Spectrum of DRN with NQS](image)

**Construction of Calibration Curve**

Aliquots of standard solution containing 10-80 µg/ml were prepared by transferring 1ml, 2, 3, 4, 5, 6, 7; 8 ml of working standard solution into a series of 10ml volumetric flask and 1ml of 0.01N NaOH was added to each flask followed by addition of 1ml of 0.5% NQS solution. And left at room temperature for 15min. The optical density of formed orange colored complex was measured at 460nm against corresponding reagent blank. Regression equation was calculated and $R^2=0.999$. The amount of Darunavir in sample was estimated from corresponding Regression equation shown in fig. 3.

![Figure 3: Calibration curve of Darunavir by Visible method](image)

**Estimation of Pharmaceutical Formulation**

An accurately weighed portion of powder equivalent to 10mg of Twenty tablets of DARUVIR (containing 300mg of DRN) were weighed and was transferred to 10ml standard volumetric flask containing 5ml of methanol, shake well and final volume was made with
remaining quantity of methanol to obtain solution of DRN (1000µg/ml). The mixture was then filtered through Whatman 41 filter paper. Further dilution was made with 70% methanol to obtain required concentration 100 µg/ml. From this solution transfer 3ml of test solution into the 10ml volumetric flask and follow according to the procedures described earlier for method development to get a concentration 30 µg/ml. The obtained color intensity was measured at 460nm and the amount was estimated using corresponding regression equation. The results of formulation were shown in table 1.

Method validation
The methods were validated according to ICH guidelines to study linearity, accuracy and precision [12, 13].

Linearity
In order to find out the linearity range of the proposed visible method, studies were carried out by plotting optical density of analyte against their respective concentrations. A good linear relationship (R^2=0.999) was observed between the concentrations of Darunavir and the corresponding optical density. The regression equation was found to be y=0.012x+0.014. (Where y is the optical density and x is the concentration of Darunavir). The slope, intercept and the correlation coefficient of the drug were shown in table 2.

Precision
The precision of an analytical method express the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of an analytical method is usually expressed as the variance, standard deviation or coefficient of variance of a series of measurements.

Precision can be performed in 3 different ways
System Precision (Reproducibility) was achieved by injecting the same concentration 6 times to the system, Method precision (Repeatability) was achieved by repeating the same procedure 6 times on the same day. In Intermediate precision, inter-day and inter-day studies were carried out. System precision and method precision were carried out for the present study. Test concentration of 30µg/ml was prepared and its color intensity was measured against the reagent blank. % RSD was calculated and found to be within limits. The results obtained were tabulated below in 3 and 4.
ACCURACY
Accuracy is expressed as the closeness of the results from standard samples to that of the actual known amounts. To determine the accuracy of the proposed method, recovery studies were carried out at three different spiking levels 80%, 100%, 120% by adding different amounts (24mg, 30mg, and 36 mg) of bulk sample to the pre-analyzed formulation. The solutions were suitably diluted in the range and then each of the dilution was observed six times. The percentage recovery of the drug was calculated. The results were shown in the Table 5.

Optimization of variables

i, Effect of reagent concentration (NQS)
The effect of NQS reagent concentration over DRN was studied using different volumes of NQS ranging from 0.1-0.6% w/v. The reaction was observed with every NQS concentration. But the concentration of 0.5%w/v NQS brought up the exact beer limits. As the concentration NQS increases there is an increase in absorbance, but after 0.5% NQS concentration there was a gradual decrease in linearity.

ii, Effect of different bases on DRN
To generate the nucleophilic reaction alkaline medium was necessary. Different inorganic bases were brought up for study, NaOH, NaHCO₃, KOH; Na₂CO₃. All were prepared in the concentration range from 0.01N-0.1N. Best results were obtained in case of 0.01N NaOH solution. Where in case of other bases either produced no color difference between blank and test or produced inaccurate results or upon dilution produced precipitate. The optimum concentration 0.01N NaOH was selected, where as 0.1N NaOH concentration produced intense color reaction with high absorption results.

iii, Effect of time and temperature
The effect of time and temperature on reaction mixture was carried out. The reaction mixture was analysed for every 15min and found that the color complex was stable for 4 hours. Also the color complex was subjected to different temperatures from 30⁰C-60⁰C. The complex showed no change in optical density when heated up to 60⁰C, where upon further increase in temperature showed negative effects.
iv. Job ratio method

Jobs method was employed where aqueous solutions of Darunavir and NQS concentrations were prepared. Series of 10ml portions of master solutions of DRN and NQS were made up comprising different complimentary proportions. Each 10ml volumetric flask contains 1ml drug solution, 1ml alkali and increased concentration of reagent from 0ml to 8ml and final volume was made up with water and optical density was measured for each concentration. At one particular concentration, there will be no increase in optical density value. Under the optimum conditions, the stoichiometry of reaction between DRN and NQS investigated by jobs method was found to be 1:1, where accurate and repeated results were obtained and graph was shown below in Fig. 4.

![Darunavir curve by Job ratio method](image)

**Figure 4: Darunavir curve by Job ratio method**

**RESULTS AND DISCUSSION**

Different analytical methods were performed with various reagents, finally a stable color complex was obtained by using 0.5% w/v NQS in presence of alkaline medium NaOH (0.01N), which showed the maximum color intensity at 460nm and obeyed linearity in the range from 10-80µg/ml. Regression equation was calculated from least square regression method of analysis. This equation was used for the estimation of formulation. The assay method carried out showed that dosage form is in good agreement with the label claim amount found (99.66%). Proposed method was validated as per ICH guidelines. The color intensity of complex formed showed stability for 4 hours. This reagent is economic and can be used for routine analysis. The developed method was found to be precise as the %RSD values for method precision and system precision were found to be less than 2%. Accurate recoveries 98.6 to 101.25 of the drug were obtained at each added concentration, which indicates that the method was accurate. Sandell’s sensitivity was found at 0.09µg/ml.
Table 1: Results of Marketed formulation analysis

<table>
<thead>
<tr>
<th>Formulation Name</th>
<th>Labelled amount in mg</th>
<th>Assay concentration (µg/ml)</th>
<th>Amount found(%)Mean* (n=5)</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>DARUVIR</td>
<td>300</td>
<td>30</td>
<td>99.66</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Table 2: Optical characteristics of Darunavir

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wave length (λ) (nm)</td>
<td>460</td>
</tr>
<tr>
<td>Beer’s law limits (µg/ml)</td>
<td>10-80</td>
</tr>
<tr>
<td>Regression equation (Y = mx+c)</td>
<td>0.014x+0.012</td>
</tr>
<tr>
<td>Slope</td>
<td>0.012</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.014</td>
</tr>
<tr>
<td>Correlation coefficient (R^2)</td>
<td>0.999</td>
</tr>
<tr>
<td>Color stability</td>
<td>4hrs</td>
</tr>
<tr>
<td>Sandell’s sensitivity (µg/ml)</td>
<td>0.09</td>
</tr>
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</table>

Table 3: System Precision data of Darunavir

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Optical Density (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.39</td>
</tr>
<tr>
<td>30</td>
<td>0.38</td>
</tr>
<tr>
<td>30</td>
<td>0.36</td>
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<tr>
<td>30</td>
<td>0.38</td>
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<tr>
<td>30</td>
<td>0.37</td>
</tr>
<tr>
<td>30</td>
<td>0.39</td>
</tr>
<tr>
<td>%RSD=0.03</td>
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Table 4: Method Precision data of Darunavir

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Optical Density (n=6)</th>
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</thead>
<tbody>
<tr>
<td>30</td>
<td>0.39</td>
</tr>
<tr>
<td>30</td>
<td>0.36</td>
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<tr>
<td>30</td>
<td>0.4</td>
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<td>30</td>
<td>0.43</td>
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<td>30</td>
<td>0.38</td>
</tr>
<tr>
<td>30</td>
<td>0.42</td>
</tr>
<tr>
<td>%RSD=0.089</td>
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</table>
Table 5: Recovery data of Darunavir

<table>
<thead>
<tr>
<th>Concentration taken (pre analysis) µg/ml</th>
<th>Recovery level(%) / spiked level</th>
<th>Added amount (µg/ml)</th>
<th>Mean Amount found (µg/ml) (n=6)</th>
<th>Mean % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>30µg/ml</td>
<td>80%</td>
<td>24</td>
<td>54.3</td>
<td>101.25</td>
</tr>
<tr>
<td>30µg/ml</td>
<td>100%</td>
<td>30</td>
<td>59.6</td>
<td>98.6</td>
</tr>
<tr>
<td>30µg/ml</td>
<td>120%</td>
<td>36</td>
<td>65.7</td>
<td>99.1</td>
</tr>
</tbody>
</table>

CONCLUSION

In this method the selected drug showed good linearity, sample recovery was within limits and the assay of the tablet was in good agreement with the label claim and suggested non-interference of formulation excipients in the estimation. Mobile phase and solvents are simple to prepare and economical, reliable and less time consuming. So it can be deduced that the simple, precise, accurate, specific, economical and short proposed method was found to be most useful for analysis purpose.

ACKNOWLEDGMENT

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REFERENCE


