Research Article

# Development and Validation of Darlolutamide in Pharmaceutical Dosage Form by RP-HPLC

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**ABSTRACT**

A simple, rapid, economical, precise and accurate RP-HPLC method for Darolutamide in its Pharmaceutical Dosage Form has been developed. The separation was achieved by Hemochrome Intsil C18 (250 mm x 4.6 mm, 5µ) column and 0.1% Trifluoroacetic Acid: Acetonitrile (60:40) as mobile phase, at a flow rate of 1 mL/min. Detection was carried out at 285 nm. Retention time of Darolutamide was found to be 8.82 min. The method has been validated as per ICH guidelines linearity, accuracy and precision. Linearity observed for Darolutamide 25-150 µg/mL. Developed method was found to be accurate, precise and rapid for estimation of Darolutamide in its Dosage Form.

**Keywords:** Method development and Validation; Darolutamide; RP-HPLC.

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## INTRODUCTION

Darolutamide, sold under the brand name Nubeqa, is an antiandrogen medication which is used in the treatment of non-metastatic castration-resistant prostate cancer in men.[1-2] It is specifically approved to treat non-metastatic castration-resistant prostate cancer (nmCRPC) in conjunction with surgical or medical castration.[2] The medication is taken by mouth twice per day with food. [2]

Side effects of darolutamide added to castration may include fatigue, asthenia, pain in the arms and legs, and rash. Darolutamide is a nonsteroidal antiandrogen (NSAA), and acts as a selective antagonist of the androgen receptor (AR). [3-4] It has been referred to as a second- or third-generation NSAA.[5-6]

Darolutamide is chemically N-[(2S)-1-[3-(3-chloro-4-cyanophenyl)pyrazol-1-yl]propan-2-yl]-5-(1-hydroxy ethyl)-1H-pyrazole-3-carboxamide. May be a formulation containing associate degree androgenic hormone receptor (AR) antagonist with potential antineoplastic activity. Darolutamide binds to ARs in target tissues; later, inhibiting androgen-induced receptor activation and facilitating the formation of inactive complexes that can't translocate to the nucleus. This prevents binding to and transcription of AR-responsive genes that regulate glandular cancer cell proliferation. This ultimately results in associate degree inhibition of growth in AR-expressing glandular cancer cells. [7]

Darolutamide may be a nonsteroidal androgenic hormone receptor antagonist for the treatment of castrate-resistant, non-metastatic glandular cancer (nmCRPC). This condition happens within the majority of patients with advanced glandular cancer WHO are treated with androgenic hormone receptor antagonists. although previous treatment for glandular cancer has been no-hit for these patients, the cancer eventually progresses to become immune to existing therapies. [7]

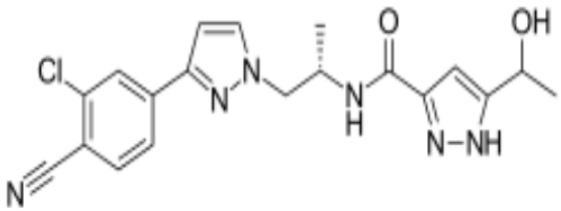


**Figure 1:** Chemical structure of Darolutamide.

## MATERIALS AND METHODS

### Chemicals and Reagents:

Darolutamide (Nubeqa) reference standard having defined potency of 99.4 % (on anhydrous basis) and Darolutamide tablets (300 mg) were obtained from the Central Drugs Testing Laboratory, Mumbai. Methanol and Acetonitrile of HPLC grade obtained from Merck life science Pvt. Ltd. were used. Ultra-purified HPLC grade distilled water from Milli-Q® system (Millipore, Milford, MA, USA) water purification

unit was used. High flow nylon membrane filter (0.45 µm) was purchased from Axiva Sichem Pvt. Ltd.

**Instrumentation:**

PerkinElmer UV/VIS Spectrophotometer having PerkinElmer UV WinLab ES software/version 6.0.4.0738 was used for all spectrophotometric measurements. The chromatographic separation was achieved on Thermo HPLC system consisting of Quaternary Gradient pump LC-20AD, a total-volume injection type autosmpler SIL-20A HT, a dual-wavelength UV/VIS absorbance detector SPD-20A and a column oven CTO-20AC operated using a software LC LabSolutions/version 1.23 SP1. All weighings were carried out using Sartorius Analytical Balance. [8]

**Selection of wavelength:**

10 mg of Darolutamide standard was weighed accurately and transferred to 100.0 ml volumetric flask and volume was made up to the mark with methanol (1000 µg/ml). The aliquot portion of the standard stock solution of Darolutamide was diluted appropriately with Methanol so as to obtain a solution of 10 µg/ml concentration. The above solution was scanned in the range of 400.0 nm to 200.0 nm using UV/Vis spectrophotometer and Methanol used as blank. Darolutamide showed maximum absorbance at 285.0 nm as shown in Fig. 2. Hence, the same wavelength was selected for the analysis of the Darolutamide.

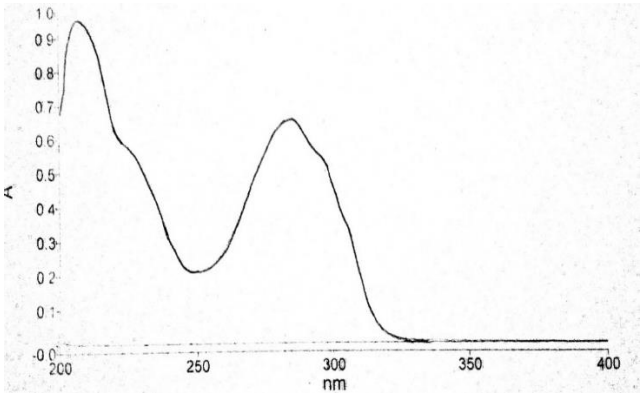


**Figure 2:** UV Spectra of Darolutamide

**Preparation of Buffer for Mobile Phase:**

0.1% Trifluoroacetic Acid transferred to 1000 ml mobile phase bottle, added 1000 ml water and sonicated for few minutes using an ultra sonicator. Further, vacuum filtered through 0.45 µm high flow nylon membrane filter.

**Selection of Diluent:**

Considering the chemical nature of Darolutamide, Methanol used as first diluent and further diluations made in Mobile Phase for all standard and sample preparations.

**Preparation of Mobile Phase:**

0.1% Trifluoroacetic Acid and Acetonitrile in the ratio of 60:40 % v/v was used as a mobile phase for the present study. The mobile phase was sonicated and degassed using an ultra sonicator.

**Preparation of standard solution:**

A standard solution of concentration 100 µg/ml of Darolutamide was prepared using a diluent.

**Analysis of Marketed Formulation:**

Twenty tablets of (NUBEQA) Darolutamide (300 mg) were weighed and their average weight was determined. The tablets were then crushed to fine powder using mortar and pestle and powder equivalent to 100 mg of Darolutamide was weighed and transferred to 100.0 ml volumetric flask and dissolved in sufficient quantity of diluent. The contents were sonicated for 10 minutes and the final volume was made up to the mark with diluent. Further dilutions were made to get 100µg/ml of sample solution.

10 µl volumes of standard and sample solutions of Darolutamide in triplicates were injected into the HPLC system for performing assay on the above tablets. Mean, SD, and % RSD of sample peak areas and % assay were calculated and reported. The results are depicted in Table 9 and the chromatogram of sample (100 µg/ml) solution of Darolutamide is shown in Fig. 5.

**Method Optimization:**

Considering the chemical nature of the molecule, base deactivated (BDS) column was the first choice for the retention of the drug. Different mobile phase systems containing water and organic solvents such as Acetonitrile, Methanol in different ratios were tried using different makes of HPLC columns.

Initial trials on HPLC were carried out on Inert sustain C18 (4.6 mm X 125 mm, 5 µm column, with mobile phase 25 mM Potassium dihydrogen phosphate (pH 3.5): Acetonitrile in a ratio of 70:30 % v/v at a flow rate of 1 ml/min. However, poor peak shapes with long retention times were observed with above trials.

Further trials were conducted on Hemochrome Intsil C18 (250 mm x 4..6 mm, 5µ) column using different buffer systems. Finally, good peak shape and acceptable system suitability parameters were found with mobile phase comprising of 0.1% Trifluoroacetic Acid with Acetonitrile in the ratio of 60:40 % v/v on Hemochrome Intsil C18 (250 mm x 4.6 mm, 5µ) column.

## RESULT AND DISCUSSIONS

Validation of developed RP-HPLC method was done for parameters such as linearity, precision, accuracy and recovery, LOD, LOQ, and robustness as per ICH guidelines[9].

**Linearity:**

Linearity studies on Darolutamide standard solutions were performed in the concentration range of 25-150 µg/ml. Linearity of Darolutamide was found to be linear with Correlation coefficient ($r^2$) value as 0.9991 and regression equation was found to be y=2290.6x-2749.6**,** having the slope 2290.6 and y-intercept 2749.6. The linearity data is shown in Table 2. The graph of peak areas obtained verses respective concentrations was plotted in terms of slope,

intercept, and correlation coefficient value as shown in Fig. 6.

**Table 1:** System suitability studies of Darolutamide

| Standard | Area | Retention time |
|---|---|---|
| 1 | 235549 | 8.82 |
| 2 | 235948 | 8.81 |
| 3 | 236063 | 8.80 |
| 4 | 235838 | 8.80 |
| 5 | 236490 | 8.80 |
| 6 | 235934 | 8.80 |
| **Mean** | 235970.333 | 8.81 |
| **SD** | 344.205316 | 0.008367 |
| **% RSD** | 0.15 | 0.10 |
| **Limit** | NMT 2.0 % | NMT 1.0 % |

*SD= Standard Deviation; %RSD= Percentage relative standard deviation.; NMT= Not more than.*

**Table 2:** Linearity data of Darolutamide

| Linearity level | Concentration (µg/ml) | Peak Area |
|---|---|---|
| 1 | 25 | 57539 |
| 2 | 50 | 117834 |
| 3 | 75 | 176422.5 |
| 4 | 100 | 231781 |
| 5 | 125 | 293663.5 |
| 6 | 150 | 341825.5 |

**Precision:**

***System Precision:***

Six replicate injections of a standard solution of Darolutamide (100 µg/ml) were injected into HPLC while performing system precision studies. The mean, SD, and % RSD of peak areas of six replicate injections were calculated and reported and the results are shown in Table 3.

**Table 3:** System Precision (Standard)

| Injection no. | Area at 285 nm | Limit |
|---|---|---|
| 1 | 235549 | NMT 2.0 % |
| 2 | 235948 | |
| 3 | 236063 | |
| 4 | 235838 | |
| 5 | 236490 | |
| 6 | 235934 | |
| **Mean** | 235970.333 | |
| **SD** | 344.205316 | |
| **% RSD** | 0.15 | |

*SD= Standard Deviation; %RSD= Percentage relative standard deviation; NMT= Not more than.*

***Method Precision (Assay Repeatability):***

Six replicate injections of standard solution of Darolutamide (100 µg/ml) and six sample solutions of Darolutamide (100 µg/ml) in triplicates were injected into the HPLC system. Mean, SD, and % RSD of % Assay were calculated and reported. The mean assay percentage results of Darolutamide sample solutions are shown in Table 4.

**Table 4:** Method Precision (Sample)

| Sample no. | % Assay | Limit |
|---|---|---|
| 1 | 100.03 | NMT 2.0 % |
| 2 | 98.44 | |
| 3 | 98.59 | |
| 4 | 98.36 | |
| 5 | 97.98 | |
| 6 | 99.84 | |
| **Mean** | 98.87 | |
| **SD** | 0.775 | |
| **% RSD** | 0.78 | |

*SD= Standard Deviation; %RSD= Percentage relative standard deviation.; NMT= Not more than.*

***Intermediate Precision:***

This was performed on two different days. Six replicates of standard solution of Darolutamide (100 µg/ml) and six sample solutions of Darolutamide (100 µg/ml) in triplicates were injected into the HPLC system. Mean, SD, and % RSD of % Assay were calculated and reported for the same. The results are summarized in Table 5.

**Table 5:** Intermediate Precision/ Interday Precision

| Sample no. | Day 1 | Day 2 | Limit |
|---|---|---|---|
| 1 | 100.03 | 99.5 | NMT 2.0 % |
| 2 | 98.44 | 99.3 | |
| 3 | 98.59 | 99.4 | |
| **Mean** | 99.02 | 99.40 | |
| **SD** | 0.88 | 0.07 | |
| **% RSD** | 0.886 | 0.075 | |

*SD= Standard Deviation.; %RSD= Percentage relative standard deviation.; NMT= Not more than.*

**Accuracy and Recovery (Standard addition method):**

Accuracy is the closeness of test results obtained by a particular method to the true value. Recovery studies were done by standard addition method by adding known amount of standard solution to the preanalyzed formulation at three different levels (110 %, 120 %, 130%). At each level three determinations were performed and mean % recovery was calculated and reported. Results for accuracy studies at various concentration levels are shown in Table 6.

**Table 6:** Accuracy studies of Darolutamide

| % level | STD spiked (µg/ml) | Amount recovered (mg) | % amount recovered | % recovery | Mean % recovery |
|---|---|---|---|---|---|
| 100 | 0 | 307.84 | 102.6 | 102.6 | 101.81 |
| 110 | 2 | 334.23 | 111.4 | 101.3 | |
| 120 | 4 | 361.28 | 120.4 | 100.4 | |
| 130 | 6 | 401.12 | 133.7 | 102.9 | |

**LOD and LOQ:**

Following formulae were used to estimate Limit of detection (LOD) and limit of quantification (LOQ) of Darolutamide from calibration curve method:

LOD = 3.3 × ∝/s

LOQ = 10 × ∝/s

Where ∝ denotes the standard deviation of regression line response and s denotes the slope obtained from the calibration curve. Solutions of desired concentrations for LOD and LOQ were prepared and injected.

The sensitivity of measurement of Darolutamide by the current method was estimated in terms of Limit of Detection and Limit of Quantitation. The results are summarized in Table 7.

**Table 7:** LOD and LOQ data of Darolutamide

| Injection no. | Area at 285 nm |
|---|---|
| 1 | 235549 |
| 2 | 235948 |
| 3 | 236063 |
| 4 | 235838 |
| 5 | 236490 |
| 6 | 235934 |
| **Mean** | 235970.333 |
| **SD** | 344.205316 |
| **% RSD** | 0.15 |
| **Regression equation** | y=2290.6x-2749.6 |
| **Slope (S)** | 2290.6 |
| **LOD=3.3σ/S (µg/ml)** | 0.49 |
| **LOQ=10σ/S (µg/ml)** | 1.50 |

*SD= Standard Deviation.; %RSD= Percentage relative standard deviation.; LOD= Limit of Detection.; LOQ= Limit of Quantitation.*

**Robustness:**

The Robustness of the method was performed by changing flow rate (± 2 %), Mobile Phase composition (± 2 %) and wavelength (± 2 nm). Under different chromatographic conditions, three sample solutions of Darolutamide were prepared and injected in triplicates along with six replicate injections of a standard solution of Darolutamide. Mean, SD, and % RSD of % estimation were calculated and reported for the same. The results are shown in Table 8 and no significant deviation was found in the results.[10]

**METHOD VALIDATION**

The method was found to have good linearity over the concentration range of 25-150 µg/ml with a correlation coefficient of 0.9991.

For system precision, the % RSD for peak areas of Darolutamide standard solution was found to be 0.15 and the mean assay percentage results of Darolutamide sample solutions were found to be within limits and % RSD was found to be 0.78, hence the method was found to be precise. The % RSD values of intermediate precision and assay were found to be within the acceptance limits.

The method was found to be accurate as the mean percent recovery of Darolutamide sample solutions was found to be 101.81 % which was within limit i.e. between 98 %-102 %. The LOD and LOQ values were found to be 0.49 µg/ml and 1.50 µg/ml respectively for Darolutamide. Lower values for LOD and LOQ demonstrate that the method developed is accurate and precise as it can detect and quantify the analyte at very low concentration.

Reproducible results were obtained which proves the method to be robust. % RSD of % assay during changes in method parameters was less than 2.0 % and the results were not adversely affected by these changes. High percent recovery values and very low SD and % RSD values confirm that the current developed method is suitable for routine analysis of Darolutamide in its pharmaceutical dosage form.

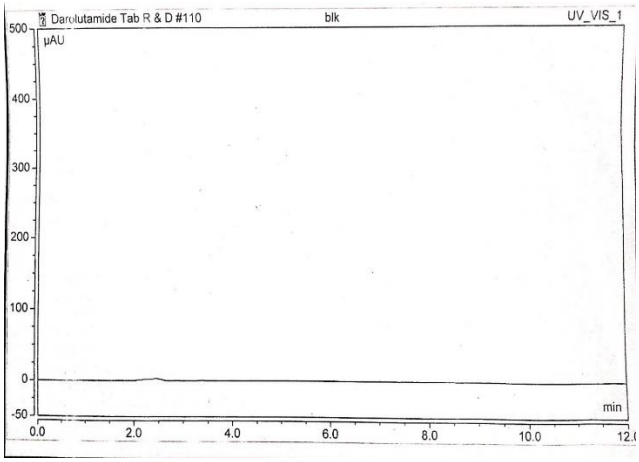


**Figure 3:** Chromatogram of Blank solution

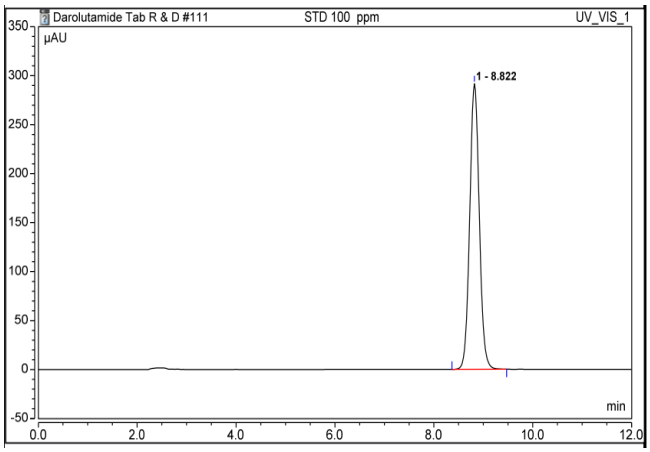


**Figure 4:** Chromatogram of Standard solution of Darolutamide

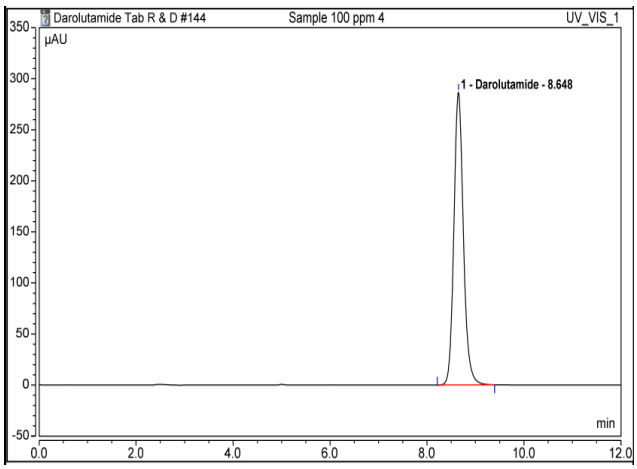


**Figure 5:** Chromatogram of Sample solution of Darolutamide

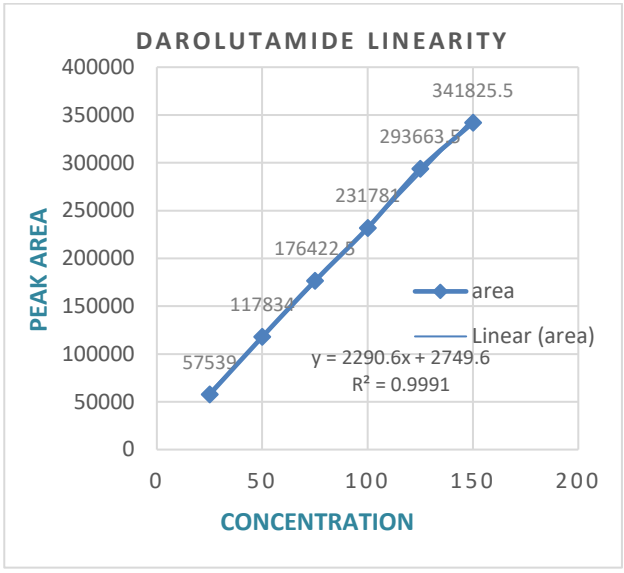


**Figure 6:** Linearity graph of Darolutamide

## CONCLUSION

The proposed RP-HPLC method was successfully validated for parameters such as linearity, precision, accuracy, and recovery, LOD, LOQ, and robustness as per ICH guidelines. The method was found to be simple, rapid, economical, accurate, and precise. All validation parameters were within the acceptance limits. This method uses simple reagents along with minimum preparation procedures. Hence this method can be used for routine analysis and quality control of Darolutamide in tablet dosage form in pharmaceutical industry.

**Table 8:** Robustness studies of Darolutamide

| Parameter | Change in parameter (±) | % Estimation | Mean | SD | % RSD | Limit |
|---|---|---|---|---|---|---|
| Wavelength (± 2 nm) | 283 | 102.88 | 102.92 | 0.04 | 0.04 | NMT 2.0 % |
| | 285 | 102.96 | | | | |
| | 287 | 102.92 | | | | |
| Temperature (± 2 %) | 30° | 103.04 | 103.0 | 0.02 | 0.02 | |
| | 35° | 103.01 | | | | |
| Flow rate (± 2 %) | 0.98 | 103.22 | 103.03 | 0.17 | 0.17 | |
| | 1.00 | 102.97 | | | | |
| | 1.02 | 102.89 | | | | |

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