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Research Article

Analyzing Daridorexant Using RP-HPLC Method: Development And Validation

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ABSTRACT

A rapid and precise reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed for the estimation of Daridorexant in tablet formulations. The separation was achieved using an Inertsil - ODS C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase composed of Methanol: Acetonitrile (90:10). The flow rate was set at 1.0 ml/min, and detection occurred at 254 nm. The retention time for Daridorexant was 3.444 minutes. The method was validated for linearity, accuracy, and precision, with linearity observed in the range of 10-70 µg/ml. The percentage recovery for Daridorexant fell within the range of 100.18 ± 0.043. This validated method provides a stability-indicating approach for quality control and clinical studies.

INTRODUCTION

High Performance Liquid Chromatography (HPLC) is a powerful analytical technique widely used in pharmaceutical research, quality control, and clinical studies. It enables the separation, identification, and quantification of individual components within complex mixtures. In HPLC, a mobile phase (typically a solvent or solvent mixture) is forced through a separation column containing small porous particles of a stationary phase. The sample components interact with the stationary phase to varying degrees, resulting in their separation. Detection occurs after leaving the column, and the resulting chromatogram provides valuable information about the composition and concentration of the analytes. The development of HPLC, particularly the transition from low-pressure glass columns to high-pressure metal columns, revolutionized analytical chemistry. This improved form of column liquid chromatography allows precise determination of compounds, including pharmaceuticals like Daridorexant. In this study, we present a validated RP-HPLC method for estimating Daridorexant in tablet

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formulations. The method demonstrates excellent linearity, accuracy, and precision, making it suitable for quality control and clinical applications.

MATERIALS AND METHODS

Stock Solution Preparation:

A 100 mg working standard of Daridorexant was dissolved in 100 ml volumetric flask (V.F.) using methanol. The solution was sonicated for 30 minutes, resulting in a 1000 ppm (parts per million) concentration.

Further Dilution (Trials Solution):

From the stock solution, 10 ml was transferred to a 100 ml V.F. and diluted with methanol up to the mark. The resulting solution had a concentration of 100 ppm.

Selection of Wavelength:

A standard solution of Daridorexant was scanned in a UV spectrophotometer between 200 nm and 400 nm using diluents as a blank. The maximum absorption wavelength (λ max) for Daridorexant was found to be 254 nm.

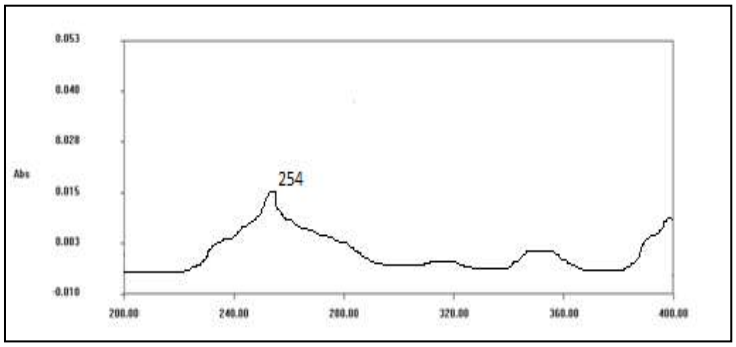

Figure 1 UV Spectrum of Daridorexant showing λmax at 254nm.

Development of HPLC Method:

The objective of this study was to enhance the assay technique for simultaneous quantification of Daridorexant. Based on literature surveys, optimization was achieved through the following trials.

Validation of Developed Method

Method validation is a critical process that ensures the suitability and reliability of an analytical method for its intended purpose. Through well-documented experimental studies, validation demonstrates that the performance characteristics of the method meet the necessary requirements. In the pharmaceutical industry, where accurate and consistent results are paramount, method validation plays a pivotal role. The validation process involves assessing various parameters to verify the method's robustness and accuracy. These parameters include Specificity, Linearity, Precision, Accuracy, Limit of Detection (LOD), And Limit of Quantification (LOQ). By adhering to guidelines from the International Conference on Harmonization (ICH).

Results And Discussions

Optimized Method for Daridorexant Estimation

1. **Mobile Phase Preparation:**

- Prepare the mobile phase by mixing Methanol and Acetonitrile in a volumetric ratio of 90:10 (V/V).
- Sonicate the mobile phase for 30 minutes to ensure thorough mixing.
- Filter the mobile phase through a 0.45-micron filter paper to remove any particulate matter.

2. **Stock Solution Preparation:**

- Weigh 100 mg of Daridorexant working standard and dissolve it in a 100 ml volumetric flask using methanol.
- Sonicate the solution for 30 minutes to achieve complete dissolution. This results in a 1000 ppm (parts per million) solution of Daridorexant.

3. **Further Dilution (Optimized Method Solutions):**

- Transfer 4 ml of the stock solution to a 100 ml volumetric flask.
- Add methanol to the mark and sonicate the solution for 10 minutes.
- The resulting solution has a concentration of 40 ppm.

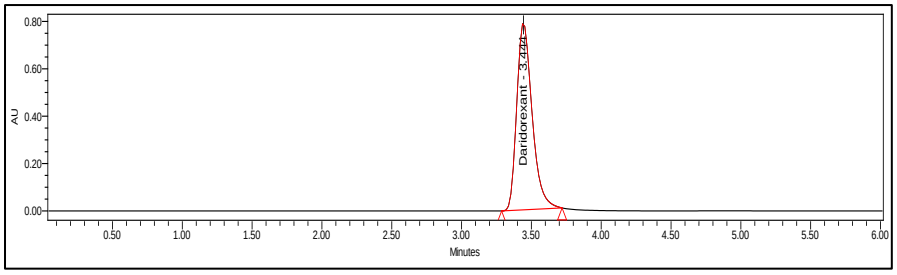

Figure 2 Standard chromatogram at Rt of 3.444 for standard

System Suitability:

A standard solution of Daridorexant, prepared according to the test method, was injected five times into the HPLC system. System suitability parameters were evaluated from standard chromatograms by calculating the relative standard deviation (% RSD) for Daridorexant retention times and peak areas.

Validation Stock Solution Preparation:

We prepared a 1000 ppm (parts per million) stock solution of Daridorexant by dissolving 100 mg of the working standard in a 100 ml volumetric flask using methanol. The solution was sonicated for 30 minutes.

Table 1 System Suitability Parameters

Parameters	Method
Stationary phase (column)	Inertsil -ODS C_{18}(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol: Acetonitrile (90:10)
Flow rate (ml/min)	1.0 ml/min.
Run time (minutes)	6 min.
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	254 nm.
Drug RT (min)	3.444 min.

Table 2 Results for ensuring System Suitability

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	3.444	1115623.12	11035	1.012
2	3.446	1115684.35	11042	1.016
3	3.442	1115601.99	11054	1.023
4	3.441	1115674.56	11038	1.014
5	3.442	1115688.56	11045	1.019
6	3.446	1115684.35	11042	1.016
Mean	3.443	1115654.51	11042	1.016
SD	0.002124	39.5337	-------	
% RSD	0.058088	0.00352	-------	

Specificity:

To evaluate specificity, both standard and sample solutions were prepared according to the test method and subsequently injected into the chromatographic system. This step ensures that the method can accurately distinguish the analyte of interest (Daridorexant) from any potential interfering substances or impurities.

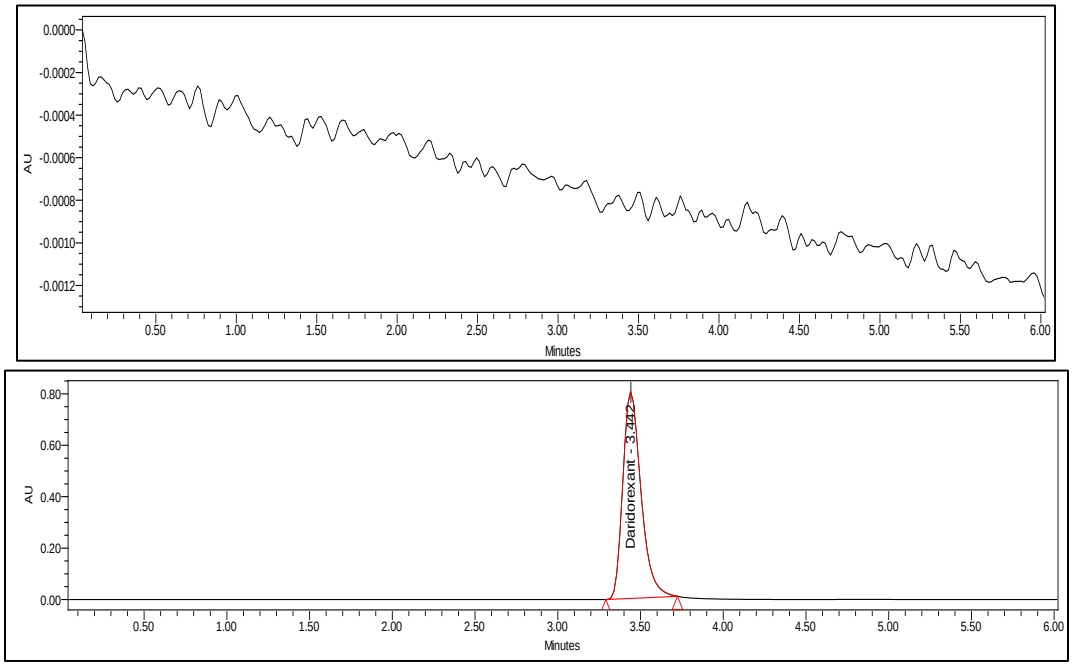

Figure 3 Chromatogram of Blank & Standard of Daridorexant

Linearity:

To evaluate linearity, a series of solutions were prepared using the Daridorexant working standard. Concentration levels ranged from 20 ppm to 70 ppm (parts per million) of the target concentration. Absorbances of these solutions were recorded at 248 nm. Calibration curve was plotted, absorbance vs concentration.

Table 3 Linearity Data

Concentration (ppm)	Average Area
0	0
20	557827.45
30	836741.48
40	1115655.45
50	1381456.65
60	1673482.32
70	1952396.25

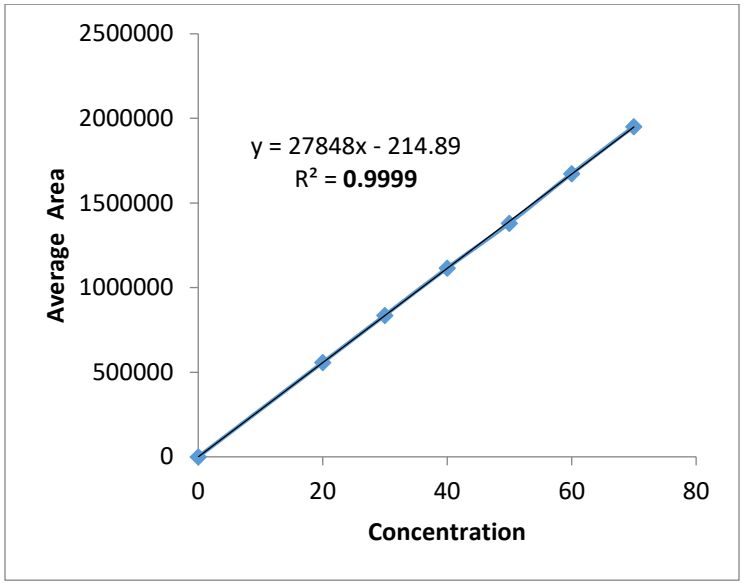

Figure 4 Linearity Chart

Precision: Repeatability:

System Precision:

A standard solution, prepared according to the test method, was injected six times into the chromatographic system.

Method Precision:

Six individual sample preparations were made following the test method, and each solution was injected separately.

The observed results demonstrate that the test method exhibits precision. Refer to Table 4 and 5 for details on system precision and method precision.

Intermediate Precision (Analyst-to-Analyst Variability):

A study was conducted involving two analysts following the same test method. (Table 6)

These assessments ensure the reliability and consistency of our HPLC analysis.

Table 4 Data for System precision

	Injection	Peak Areas	%Assay
Concentration 40ppm	1	1115589.45	100.16
	2	1115601.05	100.17
	3	1115596.58	100.16
	4	1115608.89	100.17
	5	1115582.65	100.16
	6	1115608.89	100.17
Statistical Analysis	**Mean**	1115595.72	100.16
	SD	10.1579	0.00091
	% RSD	0.00091	0.00091

Table 5 Data of Method precision

	Injection	Peak Areas of Daridorexant	%Assay
Concentration 40ppm	1	1115568.87	100.16
	2	1115590.63	100.16
	3	1115579.42	100.16
	4	1115601.55	100.17
	5	1115595.45	100.16
	6	1115610.62	100.17
	Mean	1115591.09	100.16

Statistical Analysis	**SD**	15.09947	0.00135
	% RSD	0.001353	0.00135

Table 6 Data of Intermediate precision

	Injection	**Peak Areas of Daridorexant**	**%Assay**
Concentration 40ppm	1	1115593.56	100.16
	2	1115568.95	100.16
	3	1115599.15	100.17
	4	1115602.08	100.17
	5	1115618.45	100.17
	6	1115622.12	100.17
Statistical Analysis	**Mean**	1115600.71	100.17
	SD	19.16885	0.001720
	% RSD	0.001718	0.001717

Accuracy:

An accuracy study was conducted to evaluate the reliability of the method. The drug assay was performed in triplicate, following the test method. Equivalent amounts of Daridorexant were added to each volumetric flask at three spike levels: 50%, 100%, and 150% of the labeled amount. The average percentage recovery of Daridorexant was then calculated. This assessment ensures that the method provides accurate results for quantifying Daridorexant.

$$\%\text{Recovery} = \frac{\text{Amount found}}{\text{Amount added}} \times 100$$

Concentration % of spiked level	**Area**	**Amount added (ppm)**	**Amount found (ppm)**	**% Recovery**	**Statistical Analysis of % Recovery**	
50% Sample 1	557853.51	20	20.03	100.19		
50% Sample 2	557823.48	20	20.03	100.19	**MEAN**	100.19
50% Sample 3	557860.56	20	20.04	100.19	**%RSD**	0.00352
100 % Sample 1	1115601.96	40	40.06	100.16		
100 % Sample 2	1115580.45	40	40.06	100.17	**MEAN**	100.17
100% Sample 3	1115620.56	40	40.06	100.17	**%RSD**	0.00179
150% Sample 1	1673484.25	60	60.10	100.16		
150% Sample 2	1673482.12	60	60.10	100.16	**MEAN**	100.16
150% Sample 3	1673476.85	60	60.10	100.16	**%RSD**	0.00022

Ruggedness:

We conducted a study to assess the variability between different HPLC systems under similar conditions but at different times. Six samples were prepared and analyzed following the test method. By comparing the results obtained from two distinct HPLC systems, we demonstrate that the assay test method is robust in handling system-to-system variability.

Table 7 Data on System Variability (Ruggedness)

Sr. No.	**Peak area**	**Assay % of Daridorexant**
1	1115642.78	100.17
2	1115586.45	100.16

3	1115621.32	100.17
4	1115601.74	100.17
5	1115595.42	100.16
6	1115611.11	100.17
Mean	1115609.80	100.17
%RSD	0.00181	0.00180

Robustness:

Effect of Variation of Flow Rate:

We conducted a study to assess the impact of flow rate variation on the HPLC system. Standard solutions, prepared according to the test method, were injected into the system using flow rates of 1.0 ml/min and 1.2 ml/min. The evaluation of system suitability parameters revealed that both flow rates fell within acceptable limits. Notably, Daridorexant was effectively separated from other peaks, and its retention times closely matched those obtained using the mobile phase at a flow rate of 1.0 ml/min.

Table 8 Data on Robustness

	Std Area	**Tailing factor**		**Std Area**	**Tailing factor**		**Std Area**	**Tailing factor**
Flow 0.8 ml	1108456.25	1.111	**Flow 1.0 ml**	1115647.14	1.115	**Flow 1.2 ml**	1123864.24	1.128
	1108444.66	1.115		1115632.32	1.117		1123888.46	1.130
	1108471.52	1.117		1115639.64	1.115		1123878.23	1.129
	1108462.59	1.121		1115621.35	1.116		1123845.16	1.129
	1108473.19	1.123		1115611.54	1.117		1123854.54	1.128
Avg	1108461.64	1.117	**Avg**	1115630.39	1.116	**Avg**	1123866.12	1.129
SD	11.71848	0.0047	**SD**	14.2029	0.001	**SD**	17.4834	0.0008
%RSD	0.00105	0.4273	**%RSD**	0.00127	0.0896	**%RSD**	0.00155	0.0741

LOD And LOQ (Limit of Detection and Limit of Quantitation):

From the linearity plot the LOD and LOQ are calculated:

$$\text{LOD} = \frac{3.3\ \sigma}{S} = \frac{3.3 \times 39.5337}{27848} = 0.00146$$

$$\text{LOQ} = \frac{10\ \sigma}{S} = \frac{3.3 \times 39.5337}{27848} = 0.0141$$

Analysis of Marketed Sample:

Market sample analysis was conducted using the formulation of Daridorexant, marketed under the brand name “Quviviq” by Idorsia Pharmaceuticals.

Table 9 Data for Market sample

Injection	**Peak Areas of Daridorexant**	**%Assay**
1	1115504.27	100.16
2	1115537.01	100.16
3	1115372.84	100.15
4	1115081.54	100.12
5	1115275.64	100.14
6	1115537.02	100.16
Mean	1115354.26	100.146
SD	184.9345	0.016733
% RSD	0.01658	0.016709

SUMMARY AND CONCLUSION

In the development of our analytical approach, we investigated several critical parameters. First, we determined that the maximum absorbance of Daridorexant occurred at 254 nm, providing an optimal wavelength for detection. Next, we set the injection volume to 20 µl, resulting in well-defined peak areas. The choice of the Inertsil C18 column yielded favorable peak shapes. Considering flow rate optimization, we settled on 1.0 ml/min due to good peak area, retention time, and resolution. Regarding the mobile phase composition, we explored various ratios. Ultimately, we selected a Methanol: Acetonitrile (90:10) mixture, which exhibited symmetrical peaks and high resolution. This mobile phase was employed in our planned research. Our system and procedure underwent accuracy assessment, demonstrating precision well within the desired range. Linearity investigations revealed excellent correlation coefficients and curve fitting. Across a concentration range of 20-70 ppm, our analytical approach exhibited linearity for both Daridorexant and the reference compound. Additionally, robustness and ruggedness tests were successfully passed, with relative standard deviations showing excellent consistency. These findings ensure the reliability and suitability of our HPLC method for quantifying Daridorexant in real-world samples.

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