RESEARCH PAPER

Stability Indicating Uv Spectroscopic Method Development And Validation Of Daridorexant In Api And Its Prepared Pharmaceutical Dosage Form

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ABSTRACT:

Objectives: A simple, precise, robust stability-indicating UV spectroscopic method was developed for the determination of Daridorexant (DRX) in bulk and formulation, and to study the Degradation behaviour of the drug as per ICH Guidelines (Q2) (R1) gudielines. **Materials and Methods**: Daridorexant shown its maximum absorbance at 271 nm using 50 % v/v methanol. The calibration curve was drawn over a concentration range of 2-12 µg/ml with a correlation coefficient (r^2) of 0.9991. **Results**: The developed method was validated in accordance with ICH guidelines and shown acceptance for all the parameters. Accuracy was assessed through recovery studies and was found within 98-102%. Degradation studies were performed for the drug and its drug product in all stress conditions, and the amount of drug degraded was calculated. **Conclusion**: The degradation products were monitored and characterized to understand the stability profile of drug. This validated method can be readily applied for routine quality control analysis of Daridorexant in bulk and pharmaceutical dosage forms.

KEYWORDS: Method development, Daridorexant, Degradation behaviour, Stability profile

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1. INTRODUCTION:

Daridorexant (DRX), chemically it is [(2S)-2-(5-chloro-4-methyl-1H-benzimidazol-2-yl)-2-methylpyrrolidine-1-yl]-[5-methoxy-2-(triazol-2-yl) phenyl] methanone (Zala Renuka *et al.,* 2024), is a dual orexin receptor antagonist (DORA) (Stephen *et al*.,202; Clemens *et al*.,2022) used in the treatment of insomnia. It may also be referred to as Daridorexant HCl or Nemorexant Hydrochloride, and is marketed under the brand name Quviviq. Its molecular formula is $C_{23}H_{23}ClN_6O_2$. Chemical structure of Daridorexant shown in Figure 1.

Daridorexant works by targeting the brain's orexin system, which plays a key role in regulating wakefulness. By blocking the effects of orexin, A and B at their receptors—OX1R and OX2R—it helps to suppress wake signals in the brain, making it easier to fall asleep and stay asleep throughout the night.

Developing a stability-indicating method is a crucial step in understanding how stable a drug is over time, both in its pure form and as part of a final product. It plays an important role in meeting regulatory requirements and, more importantly, in ensuring patient

safety (Srinivas Marthal *et al*.,2022; TDA Kumar *et al*.,2024; Malothu Narender *et al*., 2020).

In this research, we focus on designing and validating a new UV spectrophotometric method to accurately measure Daridorexant—both as an active pharmaceutical ingredient (API) and in its prepared dosage form (sunil v. Garad *el al*.,2024; Aggarapu susmitha *et al*.,2024). The method will be carefully validated following ICH guidelines, (Parvateesam *et al.*,2023; Pawar *et al*.,2024) to confirm its accuracy, precision, linearity, and robustness (Rajesh *et al*.,2024; Abdul Talib *et al*., 2019; Prakash *et al*.,2019).

Once validated, this method will be used to test how Daridorexant holds up under different stress conditions, including hydrolysis, oxidation, photolysis, and thermal degradation. These tests will help us to understand the drug's stability profile and identify any potential degradation of products (Hany *et al*.,2020; utkarsha *et al*.,2018).

The results will provide valuable information on the stability profile of daridorexant and contribute to the overall quality control and assurance of the drug product.

II. MATERIALS AND METHODS:

In this study, the drug Daridorexant was sourced from *Idorsia Pharmaceuticals Deutschland GmbH*, located at Marie-Curie-Strasse 8, 79539 Lörrach, Germany, with the sample supplied by *Qualychrome Research Labs*, Hyderabad, India. The dosage form used in our investigation was a laboratory-prepared formulation, containing a labelled dose of 25 mg of Daridorexant.

For the experimental work, methanol and distilled water were used as solvents. To analyze the drug, UV-Visible double beam spectrophotometer (LAB India Pvt. Ltd.) equipped with UV Probe software to record the absorption spectrum was used. Additionally, the IR spectrum of the sample was obtained using a Bruker FTIR spectrometer.

To ensure proper sample preparation for UV-Vis analysis, ultrasonication was utilized to aid the dissolution and dispersion of the drug into the solvent. This technique helps produce a more uniform and reliable solution for accurate spectrophotometric measurements.

III. METHODOLOGY:

Selection of solvent:

To select a suitable solvent for the determination of Daridorexant, Various solvents were used such as Methanol, Methanol (50%v/v), Ethanol, 50%v/v Ethanol, Acetone, and Acetonitrile tested for solubility studies and the drug was freely soluble in Methanol (50%v/v) and was selected as a solvent.

Preparation of standard solution:

10mg of Daridorexant was accurately weighed and transferred into 10ml volumetric flask, make up the volume up to the mark with solvent (50% v/v Methanol), The concentration of the prepared solution is 1000 µg/ml (Stock solution). From the stock solution, respective dilutions were prepared using distilled water.

Preparation of Daridorexant tablet:

25mg of Daridorexant was accurately weighed and transferred into motor and pestle,100mg of microcrystalline cellulose (MCC) and 80mg of mannitol, 10mg of croscarmellose were added to it and blended thoroughly. PVPK30 solution was prepared by dissolving 25mg in 10ml of Isopropyl alcohol and added to the above mixture and granulation has been carried out with sieve no:60, Granules was dried in hot air oven at 45^{o}C for 15-20mins, later cooled under room temperature and added 5mg of talc and magnesium stearate to that, and then compressed the granules with the help of tablet punching machine no.10 punch. Composition of Daridorexant tablets shown in Table 1.

Preparation of sample solution:

Accurately weighed 10 tablets and determined the average weight of each tablet, drug equivalent 10mg was weighed and transfered into 10ml volumetric flask. 10 ml of solvent Methanol (50%v/v) was added and the solution filtered using whattman's filter paper, if necessary, Sonicated for 5 min. The concentration of this solution was 1000µg/ml. Further dilutions were prepared by using Water.

Selection of wavelength:

The 80µl of stock solution was diluted to 10 ml with water to give 8µg/ml. The absorbance of the resulting solution was measured against the respective blank solution (50%v/v Methanol) in the UV region of 200-400nm, which shows maximum absorbance at 271 nm.

IV. VALIDATION OF THE METHOD:

i. Linearity and Range:

To prepare the calibration curve for Daridorexant, six different concentrations ranging from 2 to 12 µg/ml were prepared. For each concentration, the appropriate volume was pipetted into a 10 ml standard volumetric flask, and the volume was made up to the mark using distilled water.

The absorbance of each solution was then measured at 271 nm using a UV spectrophotometer, with 50% methanol (v/v) serving as the blank. Finally, the recorded absorbance values were plotted against the

corresponding concentrations to construct the calibration graph, which helps in determining the linear relationship between concentration and absorbance.

ii. Precision:

Precision of the method was evaluated by analyzing multiple homogeneous samples and expressing the results as % Relative Standard Deviation (% RSD). Both intra-day and inter-day precision studies were performed to assess the consistency of the method over time.

Intra-day:

To evaluate short-term repeatability, 0.08 ml (8 µg/ml) of the stock solution was measured and analyzed six times at different intervals throughout the same day.

Inter-day:

For long-term consistency, the same 0.08 ml (8 µg/ml) sample was analyzed six times across different days. The % RSD was then calculated for both sets of measurements to determine the method's reliability and reproducibility over time.

iii. Assay:

weigh 10 tablets accurately and calculate the average weight per tablet. Using a mortar and pestle, carefully grind the tablets into a fine powder. From this powder, weigh exactly 99.8 mg, which corresponds to the appropriate amount of active ingredient, and transfer it into a 10 ml volumetric flask.

After, add 10 ml of 50% methanol (v/v) to the flask as the solvent. To ensure the drug is fully dissolved and evenly dispersed, sonicate the mixture for 20 minutes.

Once the stock solution is prepared, pipette out 80 µl of this solution—corresponding to a concentration of 8 µg/ml—into another 10 ml volumetric flask. Top it up to the mark with distilled water. Finally, measure the absorbance at 271 nm using a UV spectrophotometer, and repeat the measurement three times to ensure accuracy and consistency.

iv. LOD AND LOQ:

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined using the slope of the calibration curve and the standard deviation obtained from the precision study. These values help define the smallest amount of Daridorexant that can be reliably detected or quantified using the developed method.

v. Robustness:

Robustness is a way to check how reliable a method is when small, intentional changes are made to the testing conditions. It gives an idea about method overall consistency. In this study, the method was tested by slightly varying the wavelength (±3 nm) and the solvent ratio (±5% v/v). After making these changes, the results were evaluated, and the % RSD was calculated to see if the method remained stable and dependable.

vi. Accuracy:

"The accuracy of the proposed method was evaluated through recovery studies performed at three concentration levels (50%, 100%, and 150%) with nine determinations, covering the specified range in replicates and the results are shown in the table 5. The % recovery studies at each level fall within the range of 98.0–102.0%.

vii. Force degradation studies:

Forced degradation studies were carried out to assess the stability of Daridorexant under different stress conditions, including acidic, alkaline, thermal, oxidative, and photolytic environments.

For these studies, an optimized concentration of 8 µg/ml of Daridorexant was prepared from a stock solution. This solution was then treated with 0.1 M HCl for acid hydrolysis, 0.1 N NaOH for alkaline hydrolysis, 3% v/v hydrogen peroxide (H_2O_2) for oxidative degradation. Photolytic degradation, was conducted by exposing the sample solution to UV light in a UV chamber, and the thermal degradation, a separate sample of the same concentration was placed in a hot air oven.

V. RESULTS AND DISCUSSION:

Method Optimization:

The sensitivity of the UV method depends upon the proper selection of wavelength. A sample solution of Daridorexant about 8µg/ml were prepared and checked for the UV absorbance against the blank solution and the λ_{max} for the drug (standard and sample) was observed at 271nm. FTIR spectrum was recorded both for the API and its drug product, which was shown noninterference of excipients as depicted in figure 2 and table 2.

VI. VALIDATION PARAMETER

i. Linearity:

The calibration curve, which plots absorbance against concentration, showed a linear relationship across the range of 2 to 12 µg/ml. The correlation coefficient (r^2) was found to be 0.9991, indicating a very strong linear relationship. This means that the test results were directly proportional to the concentration of the analyte in the sample, confirming the method's reliability for quantitative analysis. Observe the overlay spectrum and calibration curve of Daridorexant in figure 3 & 4. R^2 is 0.9991 which is within the limit (0.9991) and hence the linearity has been passed. Results of linearity shown in Table 3.

ii. Precision:

For a method to be considered precise, the % Relative Standard Deviation (% RSD) of assay results from six replicates should be no more than 2%. In this study, the intra-day precision showed an RSD of 1.151%, while the inter-day precision had an RSD of 1.22%. These values are well within the acceptable limit as mentioned in table 4, confirming that the method is reliable and consistent in its performance over time.

iii. Accuracy:

To assess the accuracy of the method, a standard addition study was carried out using three different concentrations of Daridorexant at 4, 8, and 12 µg/ml. The results showed % recovery values ranging between 98% and 100%, indicating that the method is both accurate and capable of reliably measuring the drug at various concentration levels as presented in table 5.

iv. LOD AND LOQ:

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were studied, helped to establish the smallest amounts of the drug that could be reliably detected and accurately measured by the method and the results were shown in table 6.

v. Robustness:

Robustness studies were conducted by altering the optimised conditions, wavelength and solvent composition were slightly adjusted. The method still produced consistent and reproducible results. This confirms that the method remains reliable under small variations, supporting its robustness and validation and the results are presented in Table 7.

Assay:

The assay was performed by following the same sample preparation procedure outlined in the method validation section. The results showed that the % purity of Daridorexant was within the acceptable range confirming the quality of the formulation indicating that the sample meets quality standards. The % purity of sample was shown in table 8.

Stability Studies:

The drug was tested under various stress conditions, including acidic, basic, oxidative, photolytic, and thermal environments. The drug was found to be stable across all these conditions, indicating the stability and the results were shown in table 9.

VII. CONCLUSION:

The developed UV spectroscopic method proved to be simple, accurate, precise, and robust for analysing daridorexant in both its bulk and tablet formulation. The method demonstrated good specificity, with no interference observed from excipients or other additives present in the commercial product. In the stability studies, noticeable degradation was observed under stress conditions, confirming the method's ability to detect changes in the drug's stability. The statistical analysis of the results further supports that this method is suitable for accurately determining Daridorexant, even in the presence of degradation products. Therefore, the method can be confidently considered a stability-indicating assay and is well-suited for routine use in quality control laboratories within the pharmaceutical industry.

VIII. ACKNOWLEDGEMENT

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IX. ABBREVATIONS:

DRX: Daridorexant; UV: Ultraviolet; ICH: International Council for Harmonisation; LOD: Limit of Detection; LOQ: Limit of Quantification; FTIR: Fourier Transform Infra-Red.

X. CONFLICT OF INTEREST

There is no conflict of interest.

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FIGURES:

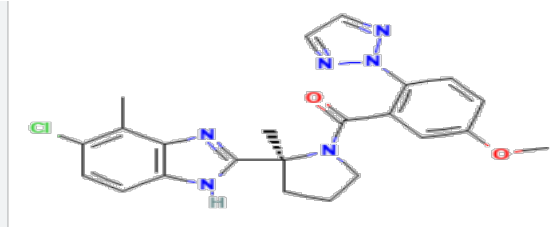

Fig. 1: Chemical Structure of Daridorexant

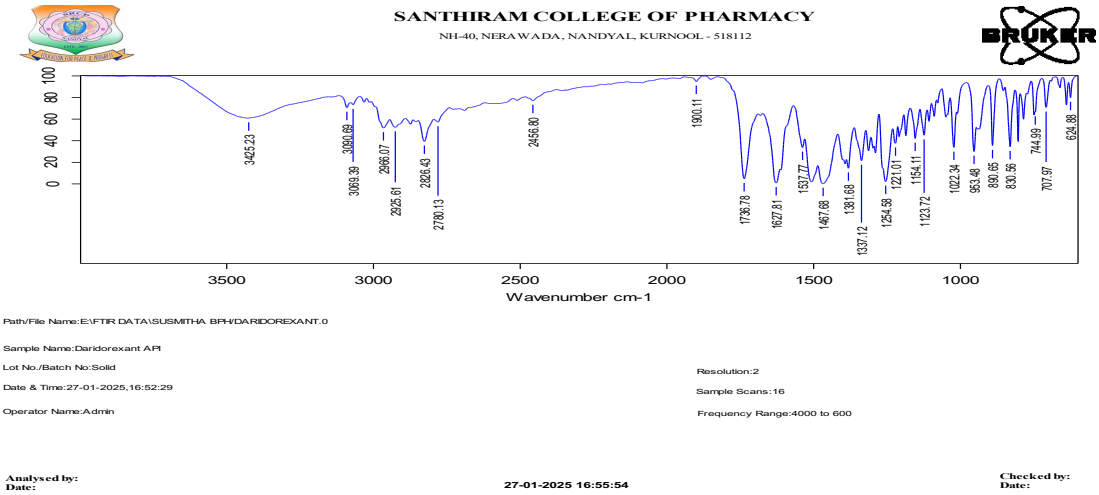

Fig.2: FTIR spectra of Daridorexant API

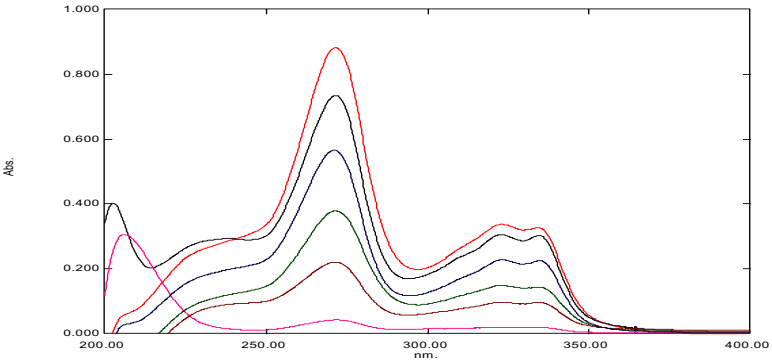

Fig.3: overlay spectrum of Daridorexant at 271nm

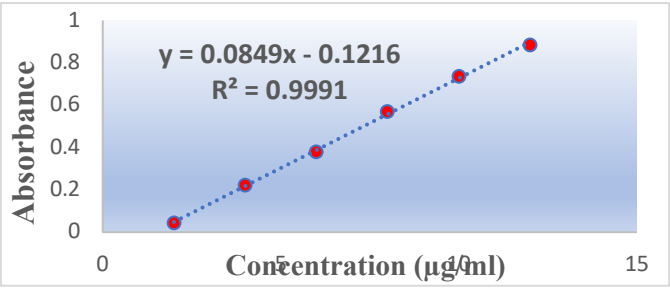

Fig. 4: Calibration curve of Daridorexant

TABLES:

Table No: 1 Composition of Daridorexant tablets

Table: 2

INGREDIENTS	QUANTITY
Daridorexant (API)	25mg
Croscarmellose sodium	10mg
Micro Crystalline Cellulose (MCC)	100mg
Mannitol	80mg
Polyvinyl pyrrolidine K30	25mg in 10ml of isopropyl alcohol
Magnesium stearate	5mg
Talc	5mg

Interpretation of FT-IR Spectra for Daridorexant

Functional group	Wave number (cm^{-1})	Type of vibration
N-H	2826.43	Stretching
C-H	2966.07	Stretching
C=O	1736.78	Stretching
C=C/C=N	1627.81	Stretching
C-Cl	624.88	Stretching
C-H	1900.11	Bending

Table:3 Results of Linearity

S. No	Concentration (µg/ml)	Absorbance
1.	2	0.043
2.	4	0.223
3.	6	0.379
4.	8	0.568
5.	10	0.737
6.	12	0.885

Table:4 Results of Precision

S.NO	INTRADAY	INTERDAY
1.	0.566	0.568
2.	0.564	0.566
3.	0.552	0.552
4.	0.552	0.566
5.	0.551	0.564
6.	0.551	0.554
Mean	0.556	0.561666667
Std.Dev	0.006403	0.006860515
%RSD	1.151641	1.221456684

Table No: 5 Results of Accuracy

S. No	Conc. Level	Absorbance (n=3)	Amount added (mcg/ml)	Amount found (mcg/ml)	% Recovery	Mean % Recovery
1	50%	0.285	4.00	4.014085	100.32	100.79
2		0.286		4.028169	100.67	
3		0.288		4.056338	101.37	

4	100%	0.575	8.00	8.098592	101.20	101.31
5		0.575		8.098592	101.20	
6		0.577		8.126761	101.55	
7	150%	0.838	12.00	11.80282	98.32	98.56
8		0.84		11.83099	98.56	
9		0.842		11.85915	98.79	

Table No: 6 Results of LOD and LOQ

S. No	Parameter	Slope	Std. Dev	Observation (µg/ml)
1	LOD	0.0849	0.008717798	0.325976918
2	LOQ			0.987808844

Table No: 7 Results of robustness

Parameter	Condition	Condition	Absorbance
Wave length (nm) ±4	Low WL	221 nm	0.5929
	Optimized	225nm	0.6507
	High WL	229 nm	0.5363
Mobile phase (ratio) min±10 ratio	Low	70:30	0.6078
	Optimized	80:20	0.6345
	High	90:10	0.6820

Table No: 8 %Assay

SAMPLE	STANDARD (Absorbance)	SAMPLE (Absorbance)
Sample 1	0.568	0.586
Formulation Average weight (Tablet)	249.58mg	
Standard weight	10mg	
Sample weight (Tablet Formulation)	99.8mg	
Label Claim	25mg	
Assay (% Purity)	98.64%	