Optimization and Validation of a Novel RP-UFLC SPD Method for the Reckoning of Daridorexant in Bulk and Its Pharmaceutical Formulation: Application of Stability Approach

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ABSTRACT

Objective: A comprehensive validated RP-UFLC method was developed to assess the Daridorexant in bulk and pharmaceutical formulations & study of its degradation behaviour.

Methods: The analysis was performed using Shimadzu UFLC system. Chromatographic separation was achieved on Inertsustain C18 column (4.6 × 250 mm, 5 µm) with a mobile phase of methanol and 0.1% OPA (85:15 v/v) at a flow rate of 0.9 mL min^{-1} and detection at 271 nm.

Results: The method showed excellent linearity in the concentration range of 10–60 µg/mL, and consistently exhibits a correlation coefficient (R^2) of 0.999. The LOD and LOQ were found to be 1.006 µg/mL and 3.04 µg/mL, respectively, indicating high sensitivity. To verify the precision and robustness (with a %RSD of <2%), the ICH Q2 (R2) principles were applied. Robustness studies confirmed the reliability of the method over a deliberate change. Degradation behaviour revealed that Daridorexant is relatively stable in all conditions.

Conclusion: The observed degradation levels state the method's specificity and applicability for quality control and stability testing, as they are within acceptable limits.

Keywords: Daridorexant, degradation behaviour, ICH, RP-UFLC

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INTRODUCTION

Daridorexant (DRX)[1-3] is a dual orexin receptor antagonist (DORA)[4,5] that promotes sleep by selectively blocking the binding of wake-promoting neuropeptides orexin-A and orexin-B to their receptors OX1R and OX2R. It is primarily used for the treatment of insomnia[6], helping to improve sleep onset and maintenance without impairing next-day alertness[7]. Chemically, Daridorexant (Figure 1) belongs to the class of diarylacetamide derivatives, exhibiting moderate lipophilicity and good oral bioavailability[8].

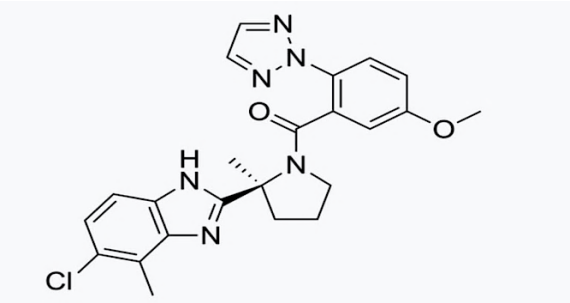

Fig.-1: Chemical Structure of Daridorexant

In recent years, the analysis of Daridorexant in pharmaceutical dosage forms has gained importance due to its therapeutic significance and regulatory requirements[9]. To ensure the quality, efficacy, and

stability of formulations containing Daridorexant[10], it is necessary to develop a reliable analytical method. Among various analytical techniques, Reverse Phase High-Performance Liquid Chromatography is the most preferred due to its high sensitivity, reproducibility, and precision.

A literature review indicated that no approaches were documented as up to my knowledge. The current work sought to develop and validate a stability-indicating RP-HPLC method for determining Daridorexant in bulk and pharmaceutical dose form with high accuracy and precision[11,12]. The new method was validated using the ICH Q2 (R2) analytical method validation criteria[13,14].

MATERIALS AND METHODS

Materials

The study utilized several chemicals and analytical instruments to ensure accurate estimation of drug & its drug product. Daridorexant was obtained as gift sample from Research lab fine chem, Hyderabad, India. The research study utilized Milli-Q water and HPLC-grade methanol sourced from Merck, India. AR grade reagents such as Orthophosphoric acid, hydrochloric acid, sodium hydroxide and hydrogen peroxide were used for preparation of mobile phase and forced degradation studies. Tablet formulation used in the study was purchased from the local pharmacy.

Chromatographic analysis was carried out using a Shimadzu LC-20AD UFLC system equipped with an Inertsustain C18 column (250 mm × 4.6 mm i.d., 5 µm particle size) operating in isocratic mode. Detection was performed using an SPD detector, data acquisition and analysis were performed using LC solution workstation software for UFLC.

Methods

Selection of Mobile phase and diluent

Initially, a variety of mobile phase ratios were tested in order to estimate DRX in bulk and its pharmaceutical preparation. The mobile phase determined to be most appropriate for analysis of DRX was methanol and buffer in a ratio of 85:15 v/v, taking into account system appropriateness parameters such as RT, Tailing factor, number of theoretical plates, and HETP. After removing particulate matter using 0.45µm filter paper, the mobile phase was sonicated to degas it. 0.9 ml/min was the flow rate used for analysis.

Procedure for preparation of mobile phase

To prepare the buffer, 0.1 mL of ortho-phosphoric acid (OPA) was dissolved in 90 mL of Milli-Q water. The pH was subsequently adjusted to 3.0 using 0.1% OPA, and the volume was increased to 100 mL. A 85:15 (v/v) mixture of this methanol and buffer was prepared, filtered, and degassed for accurate and robust routine analysis.

Selection of Diluent

The diluent used to prepare the sample was compatible with the mobile phase and had no discernible effect on the analytes retention or resolution. HPLC grade methanol was employed as a diluent following a number of experiments.

Preparation of Standard Solution

10 mg of DRX was precisely weighed, put into a 10 ml volumetric flask, dissolved in 5 ml of methanol, and sonicated for 10 minutes before the volume was adjusted to 10 ml using methanol. DRX concentration in methanol was 1000µg/ml (stock-A). Aliquots of the stock solution were further diluted with the mobile phase to obtain working standard solutions within the concentration range used for calibration studies.

Sample Solution Preparation

Twenty tablets containing the formulation was weighed and finely powdered. An amount equivalent to 5 mg of the drug was transferred into a volumetric flask containing methanol. The mixture was sonicated for 10 minutes to ensure complete extraction of the drug from the tablet matrix. The solution was filtered using a 0.45 µm membrane filter and diluted appropriately with the mobile phase.

RESULTS AND DISCUSSION

Chromatographic Conditions

Chromatographic separation was performed on a C18 reverse phase Inertsustain column (250 mm × 4.6 mm, 5 µm particle size). The mobile phase consisted of methanol and buffer in the ratio of 85:15 (v/v). The flow rate was maintained at 0.9 mL/min, and the injection volume was 20 µL. Detection was carried out using a SPD UV detector at an optimized wavelength of 271 nm. The column temperature was maintained at 30°C. Prior to analysis, both the mobile phase and sample solutions were filtered through 0.45 µm micropore membrane filters and degassed by sonication. Under these optimized conditions, DRX was eluted at a retention time of 2.90 ± 0.15 mins, producing a sharp and symmetrical peak. The system exhibited excellent baseline stability, reproducibility, and resolution, confirming the suitability of the method for routine analysis.

Method Validation

The suggested approach was validated using the ICH Q2 (R2) guidelines, which include system appropriateness was verified for analytical method validation[13,14].

System Suitability Test

System suitability parameters including theoretical plates, tailing factor, resolution, and retention time were evaluated by injecting the standard solution (DRX) six times to ensure that the chromatographic system was performing properly. Retention time demonstrated excellent reproducibility and higher consistency with a very moderate standard deviation. Theoretical palates and asymmetry factor were within acceptable limits suggesting good column efficiency and peak shape, the results were presented in table 1.

Table 1: Optimized Chromatographic Condition

S. No	Concentration (µg/ml)	Peak Area
1	5	174625
2	10	274275
3	15	379225
4	20	549178
5	25	668418
6	30	776985

Parameters	Observation
Instrument used	Shimadzu LC-20AD HPLC
Mobile Phase	Methanol and 0.1% OPA (85:15)
Column	Intersustain C18 (250 mm x 4.6 mm; 5µm)
Detection Wavelength (nm)	271
Injection volume (µL)	20
Flow Rate (mL/min)	0.9
Runtime (min)	5
Temperature (°C)	Ambient
Mode of separation	Isocratic mode

Specificity

To determine the analyte presence of the components that would be predicted to be present, such as contaminants, degradation products, and matrix components, the specificity of the approach was tested. The drug was assessed in the presence of components expected to be present, and no interfering peaks were identified as depicted in table 2 and fig. 2.

Table-2: Results of System Suitability Studies

S.No.	Parameter	Observation
1	Retention time (min)	2.90 ± 0.15
2	Plate count	15247
3	Tailing factor	0.95
4	%RSD	0.21

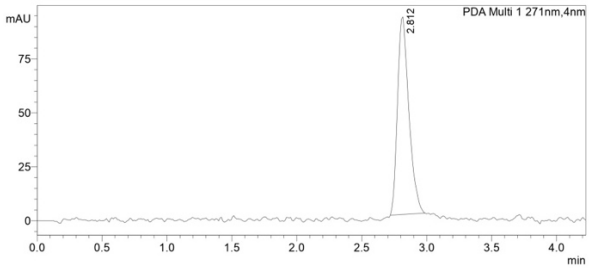

Fig. 2: Chromatogram of DRX

Linearity and Range

After passing system suitability parameter, linearity assessment was conducted by injecting 6 different concentrations of DRX working standard solution in UFLC 5-30 ug/ml respectively. The linearity of the method was assessed through regression analysis with a correlation coefficient (R^2) of 0.9942, indicating excellent linearity between peak area and drug concentration over the specified range. Calibration curve was plotted in figure 3 present's linearity profile and with its values in Table 3.

Table 3: Optimized Chromatographic Condition

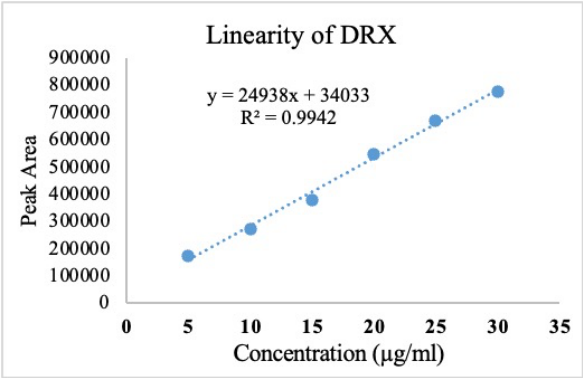

Fig. 2: Calibration Curve of DRX

Precision

Evaluations were conducted utilizing system precision, repeatability, and intermediate precision to meticulously assess the proposed HPLC method's precision in accordance with the criteria outlined in ICH Q2 (R2). The %RSD values were found to be below 2.0%, indicating good precision of the method.

Accuracy:

The correctness of the procedure was determined using the usual addition method in triplicate levels at three

different concentrations. The Known amounts of the reference standard solution were added to the working solution. For every accuracy level and mean %, three shots were administered. The mean % recovery was found within the acceptance limits. The results obtained are reported in Table 4.

Table 4: Recovery Studies

S. No	Spike level (%)	Peak Area	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	% Mean Recovery
1	50	274275	9.99	9.98	99.94	100.58
2		275021		10.01	100.21	
3		278854		10.15	101.61	
4	100	554926	19.98	20.20	101.10	99.76
5		540165		19.12	98.41	
6		547608		19.94	99.77	
7	150	824121	29.9	30.012	100.09	98.89
8		806985		29.38	98.01	
9		811509		29.55	98.56	

Sensitivity

The sensitivity of the proposed method for DRX was determined using the standard deviation of the response and the slope obtained from the calibration curve. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 1.006 µg/mL and 3.04 µg/mL, respectively. The method effectively detects and quantifies the drug within the tested range, indicate the high sensitivity of the developed analytical method.

Robustness

The robustness of the developed RP-UFLC method was evaluated by introducing deliberate, minor variations in analytical parameters to assess the method's reliability under slightly altered conditions. The changes included variations in flow rate (±0.1 mL/min), column temperature (±5°C) and wavelength (±5 nm) relative to the optimized chromatographic. After each modification, parameters such as retention time (RT), tailing factor (TF) and theoretical plates (N) were studied. The results are summarized in Table 5, showing that all variations produced %RSD values below 2%, with negligible shifts in RT and consistent system suitability metrics. This confirms that the method is robust and unaffected by small operational variations and were remain within acceptable limits (%RSD < 2%), confirming the reproducibility of the proposed method.

Table 5: Results showing Robustness study

S.No	Robustness Parameter	Altered Condition	Peak Area ± Std. Deviation (n=3)	% RSD
1	Flow rate (± 0.1 ml/min)	0.8	528085 ± 4623.7	0.87
2		0.9*	515900 ± 7200.6	1.39
3		1.0	573945 ± 5893.5	1.02
4	Column Temperature (± ºC)	30	549500 ± 8058.73	1.46
5		35*	517266 ± 6717.9	1.29
6		40	587568 ± 7416.6	1.26
7	Wavelength (± 5 nm)	266	411419.7 ± 3956.99	0.96
8		271*	516922 ± 6081.2	1.17
9		276	614547.66 ± 8593.28	1.39

Stability studies and forced degradation analysis

The stability studies of DRX, under various stress conditions was performed to assess its degradation behaviour. The forced degradation study was conducted for predetermined time intervals under different stress environments to simulate potential degradation pathways. Acidic and basic hydrolysis were performed by exposing the drug to 0.1 N HCl and 0.1 N NaOH, respectively, at 60°C for 3 h. Oxidative degradation was carried out using 3.0% hydrogen peroxide at room temperature for 3 h. For photolytic degradation, the samples were exposed to ultraviolet (UV) and visible light for 24 h, as recommended by ICH. The drug exhibited controlled degradation under these stress conditions, confirming the stability-indicating nature of the developed RP-UFLC method. The results were presented in table 6.

Table 6: Results of Stress degradation profile

S.No	Stress Conditions	Peak area	% Purity	% Degradation
1	0.1N HCl	562752	97.7	2.3
2	0.1N NaOH	566920	96.98	3.02
3	Oxidative	556835	98.743	1.257
4	Thermal	583231	94.275	5.725
5	Photolytic	606896	90.59	9.41

CONCLUSION

The RP-UFLC method, characterized by its simplicit precision, accuracy and robust enables the reckoning Daridorexant in bulk and its pharmaceutic preparation. The retention time was 2.90±0.15 mi suggest efficient separation with a shorter run time. Tl system accuracy produced %RSD values of <2. indicating remarkable repeatability and consistenc The method demonstrated exceptional accurac achieving mean recovery rates of 99.74%. Tl regression equation obtained from the calibratic curves exhibited outstanding linearity and sensitivit Degradation profile was conducted on the drug & i drug product, and samples remained within permissib limits, demonstrating the method's efficacy indicating stability. The proposed method is efficie and suitable for routine quality control assessments in pharmaceutical companies, as evidenced by the reduced retention time and robust validation metric Furthermore, by minimizing solvent consumptio analysis duration, and ecological footprint, the metho advocates for the tenets of green analytical chemistry

Conflict of Interest

The authors declare that there is no conflict of intere regarding the research work presented in this report.

Consent for Publications

All authors have read and approved the final version this manuscript for publication.

Availability of Data and Material

All data generated or analyzed during this study a included in the manuscript and are available within tl document.

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