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Development and validation of an RP-UPLC method for the estimation of Cariprazine Hydrochloride in bulk drug and pharmaceutical dosage form

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Abstract
Cariprazine hydrochloride, an atypical antipsychotic used in the management of schizophrenia, bipolar I disorder, and as adjunctive therapy for major depressive disorder, lacked a simple, validated UPLC method for its routine estimation in both bulk drug and pharmaceutical dosage form. The present study aimed to develop and validate a UPLC method for Cariprazine hydrochloride in accordance with ICH Q2(R1) guidelines. Chromatographic separation was achieved under optimized UPLC conditions, giving a sharp, symmetric Cariprazine hydrochloride peak at a retention time of 3.523 min, with 7431 theoretical plates and a tailing factor of 1.09. The method was linear over 10–60 µg/mL, with a regression equation of y = 2537.14x + 48889.53 and a correlation coefficient (r^2) of 0.99988. The LOD and LOQ, calculated from the linearity regression, were found to be 0.765 µg/mL and 2.319 µg/mL respectively, confirming adequate sensitivity. The developed UPLC method was validated for precision (system %RSD 0.71%, method %RSD 0.88%), accuracy (recovery 98.33–99.55%), ruggedness (%RSD 0.71%), and robustness (percentage change 0.38–0.60% under deliberate variation of mobile phase and flow rate), with all parameters within ICH-acceptable limits. The validated UPLC method was successfully applied to the estimation of Cariprazine hydrochloride in bulk drug (99.88% purity) and the marketed capsule formulation Caripriza (99.91% of the 3 mg label claim), confirming its applicability across both matrices. The developed UPLC method is simple, rapid, sensitive, accurate, precise, rugged, and robust, and represents a useful addition to existing analytical methodology for the routine quality control of Cariprazine hydrochloride.

Keywords: Cariprazine hydrochloride, method development, method validation and assay

Introduction

Cariprazine is an atypical antipsychotic agent chemically described as N'-[trans-4-[2-[4-(2,3-dichlorophenyl)-1-piperazinyl] ethyl] cyclohexyl]-N, N-dimethylurea, with the molecular formula C21H32Cl2N4O and a molecular mass of 427.41 g/mol. Pharmacologically, cariprazine acts as a partial agonist at dopamine D3 and D2 receptors, with a notably strong preference for the D3 receptor subtype, and also shows partial agonist activity at serotonin 5-HT1A receptors and antagonist activity at serotonin 5-HT2B and 5-HT2A receptors. It is marketed under the brand names Vraylar (USA) and Reagila (Europe), and as Cariquel, Carilift, Ciprazet, Caripriza, Carinia, and Carispec in India. Cariprazine is indicated for the treatment of schizophrenia in adults and adolescents, for acute manic or mixed episodes and depressive episodes associated with Bipolar I Disorder, and as an adjunctive treatment for Major Depressive Disorder. It is available as oral capsules in strengths of 1.5 mg, 3 mg, 4.5 mg, and 6 mg.

A literature survey revealed that a small number of analytical methods have been reported for the estimation of cariprazine, including a UV spectrophotometric method and reverse-phase HPLC methods for the drug substance and its impurities, developed on conventional HPLC platforms with run times in the range of 8–20 minutes. To the best of the available literature survey, no ultra performance liquid chromatography (UPLC) method has yet been reported for the simultaneous estimation of cariprazine in bulk drug substance and pharmaceutical dosage form. Since UPLC employs sub-2-micrometre particle column technology, it offers considerably higher resolution, sensitivity, and speed of analysis compared with conventional HPLC, making it well suited for high-throughput quality control testing. The present study was therefore undertaken to develop and validate a simple, rapid, sensitive, and economical RP-UPLC method for the estimation of cariprazine in bulk drug substance and in its marketed capsule formulation, in accordance with ICH Q2(R1) guidelines. Figure 01 depicts the chemical structure of cariprazine.

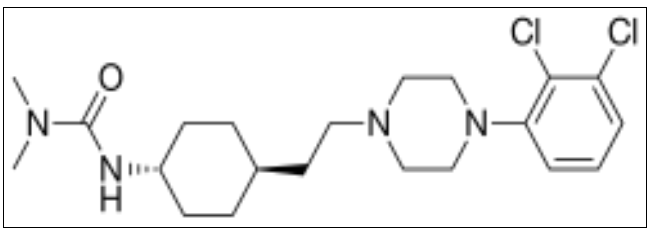

Fig 1: The Chemical Structure of Cariprazine

Mateials and Methods

Chemicals and Reagents

The working standard drug Cariprazine (99% purity) was obtained as a gift sample from Aurobindo Pharma Limited. The marketed pharmaceutical formulation, Cariprazine capsules 3 mg (label claim), was procured from a local pharmacy. HPLC-grade acetonitrile, methanol, and water, along with potassium dihydrogen phosphate (KH2PO4) and orthophosphoric acid (OPA), were procured from Merck Chemicals Private Limited, Mumbai.

Preparation of 0.01 M Phosphate Buffer (pH 4.5)

A 1000 mL volumetric flask was filled with precisely weighed 1.36 g of potassium dihydrogen phosphate, which was then dissolved in approximately 800 mL of HPLC-grade water. The mixture was thoroughly dissolved by sonication and stirring. Orthophosphoric acid was used to adjust the solution pH to 4.5 ± 0.05, after which the volume was made up with HPLC-grade water and thoroughly mixed. The buffer was filtered through a 0.22 µm membrane filter before use.

Preparation of Mobile Phase

Acetonitrile and phosphate buffer (pH 4.5) were combined in a 60:40 (v/v) ratio. Before use, the mobile phase was sonicated for 15 minutes to eliminate dissolved gases and filtered through a 0.22 µm membrane filter.

Preparation of Standard Drug Solution

Cariprazine (10 mg), accurately weighed, was transferred to a 10 mL volumetric flask and completely dissolved in approximately 7 mL of acetonitrile (diluent) using an ultrasonic bath. The volume was adjusted with the same diluent and filtered through a 0.22 µm membrane filter to obtain a standard stock solution of 1000 µg/mL. This stock solution was serially diluted using the mobile phase as diluent to prepare working standard solutions of the required concentrations for method development and validation.

Preparation of Formulation (Sample) Solution

Twenty capsules of the commercial cariprazine formulation (3 mg label claim) were weighed to determine the average fill weight. A quantity of powder equivalent to 10 mg of cariprazine was accurately weighed into a 10 mL volumetric flask. Approximately 7 mL of acetonitrile was added, and the mixture was sonicated for 15 minutes to ensure total drug extraction from the capsule matrix. After cooling to room temperature, the volume was adjusted with the same diluent to yield a 1000 µg/mL sample stock solution, which was filtered through a 0.22 µm membrane filter, discarding the first few millilitres of filtrate. Appropriate aliquots of the filtered sample stock solution were further diluted with mobile phase to obtain the working sample solution concentration required for assay and validation studies.

Method Development

Selection of Wavelength: Using the PDA detector, a solution of cariprazine was scanned between 200 and 400 nm to determine an appropriate detection wavelength. The wavelength corresponding to maximal absorbance was selected for method development and validation.

Optimization Trials: To obtain a symmetric peak shape, adequate resolution, and a short run time, a series of trials was conducted by systematically varying the column, mobile phase composition and ratio, and flow rate, as summarised in Table 01.

Table 1: Optimization Trials for RP-UPLC Method Development of Cariprazine (Wavelength 235 nm, Injection Volume 2 µL, Ambient Temperature, Run Time 6 min, for all trials)

Trial	Mobile Phase	Column	Flow Rate	Observation
1	Acetonitrile: Water (70:30 v/v)	ACQUITY BEH C18 (2.1 × 100 mm, 1.7 µm)	0.3 mL/min	Broadening of peak
2	Acetonitrile: Phosphate buffer pH 4.5 (50:50 v/v)	ACQUITY BEH C18 (2.1 × 100 mm, 1.7 µm)	0.3 mL/min	Split peaks
3	Acetonitrile: Methanol (60:40 v/v)	ACQUITY BEH C18 (2.1 × 100 mm, 1.7 µm)	0.3 mL/min	Unknown peak
4	Acetonitrile: Phosphate buffer pH 4.5 (60:40 v/v)	ACQUITY HSS C18 (2.1 × 50 mm, 1.7 µm)	0.3 mL/min	Asymmetric peak with broadening
5	Acetonitrile: Phosphate buffer pH 4.5 (55:45 v/v)	ACQUITY HSS C18 (2.1 × 50 mm, 1.7 µm)	0.3 mL/min	Symmetric peak (optimized)

Trial 5 was selected as the optimal chromatographic condition, as it produced a sharp, symmetric, and well-resolved peak. The finalized optimized chromatographic conditions are summarised in Table 02.

Table 2: Optimized Chromatographic Conditions

Parameter	Condition
Mobile Phase	Acetonitrile: Phosphate buffer pH 4.5 (60:40 v/v)
Column	ACQUITY HSS C18 (2.1 × 50 mm, 1.7 µm)
Flow Rate	0.3 mL/min
Wavelength	235 nm
Injection Volume	2 µL
Temperature	Ambient
Run time	6 min

Method Validation

In accordance with ICH Q2(R1) requirements, the developed RP-UPLC method was validated with respect to system suitability, specificity, LOD and LOQ, linearity, precision, accuracy, ruggedness, and robustness. Duplicate injections of standard and sample solutions were used throughout the validation.

System Suitability: Six replicate injections of the standard solution were analysed under the optimized conditions, and peak area, retention time, theoretical plates, and USP tailing factor were assessed. The acceptance criteria used were: theoretical plates > 2000, USP tailing factor < 2, and peak area %RSD < 2.

Specificity: Specificity was evaluated by injecting the diluent (blank) and placebo (excipient blend without drug) and confirming the absence of interfering peaks at the retention time of cariprazine.

LOD and LOQ: Standard solutions of progressively decreasing concentration were prepared by serial dilution, and the signal-to-noise (S/N) ratio was recorded for each. The LOD was taken as the concentration giving an S/N ratio of approximately 3, and the LOQ as the concentration giving an S/N ratio of approximately 10; both were additionally calculated from the residual standard deviation and slope of the linearity regression (LOD = 3.3σ/S; LOQ = 10σ/S).

Linearity: The standard stock solution was diluted to obtain six concentration levels, and the corresponding peak area was recorded for each under the optimized conditions. A calibration curve of peak area versus concentration was plotted, and the regression equation and correlation coefficient (r^2) were calculated.

Precision: Precision was assessed as system precision (repeatability, six replicate injections of the 100% working standard solution on the same day) and method precision (intraday and inter-day precision, with duplicate injections examined at three different times on the same day and on three consecutive days respectively), and was expressed as %RSD using the formula: %RSD = (Standard Deviation / Mean) × 100.

Accuracy: Accuracy was determined by the standard addition (spiking) method. The pre-analysed sample formulation was spiked with known amounts of cariprazine working standard at 50%, 100%, and 150% of the label claim, and the percentage recovery at each level was calculated as: % Recovery = (Amount found / Amount added) × 100.

Ruggedness: Ruggedness (intermediate precision) was assessed by having a second analyst independently analyse the standard solution at 100% working concentration under the same optimized chromatographic conditions, and comparing the resulting peak areas and %RSD.

Robustness: Robustness was assessed by making small, deliberate changes to the chromatographic conditions — flow rate (± 0.02 mL/min) and mobile phase organic-component ratio (± 5%) — while holding all other parameters constant, and evaluating the impact of each variation on assay results and system suitability parameters.

Assay: The validated method was applied to determine the percentage purity of the bulk drug substance and the percentage label claim of the marketed capsule formulation, using the formulae: % Purity (Bulk Drug) = (Test peak area / Reference standard peak area) × (Reference standard concentration / Test concentration) × (Reference standard purity / 100) × 100; and % Assay (Dosage Form) = (Sample peak area / Standard peak area) × (Standard concentration / Sample concentration) × (Label claim / 100) × 100.

Statistical Analysis: Mean, standard deviation (SD), and percentage relative standard deviation (%RSD) were computed for all replicate determinations (n = 3 or n = 6, as applicable) using: Mean ($\bar{y}$) = Σx_i / n; SD = $\sqrt{[\Sigma (x_i - \bar{y})^2 / (n - 1)]}$; %RSD = (SD / $\bar{y}$) × 100. Least-squares linear regression was used to determine the slope, intercept, and correlation coefficient (r^2) of the calibration curve.

Results and Discussion

Selection of Wavelength

The PDA detector was used to scan a reference solution of cariprazine across the UV spectrum. The wavelength of highest absorbance, 235 nm, was chosen for method development and validation (Figure 02).

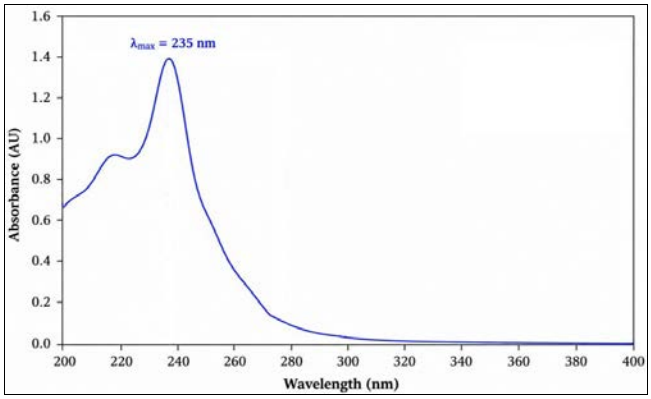

Fig 2: UV Spectra of Cariprazine (λmax = 235 nm)

Optimized Chromatogram

The mobile phase composition, column, and flow rate were varied across five trials (Table 01). Trial 5 was selected as the optimal condition, producing a symmetric, well-resolved peak. Under the optimized chromatographic conditions, cariprazine eluted at a retention time of 3.523 minutes with 7431 theoretical plates and a tailing factor of 1.09, demonstrating a sharp, symmetric peak suitable for quantitative analysis (Figure 03, Table 03).

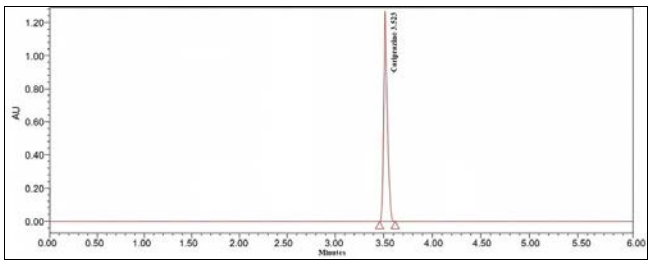

Fig 3: Optimized Chromatogram of Cariprazine

Table 3: Results for Optimized Chromatogram

S.NO	Drug	Retention Time (Min)	Theoretical Plates	Tailing Factor
1	Cariprazine	3.523	7431	1.09

Method Validation

System Suitability

A mean retention time of 3.5223 minutes (%RSD 0.06%) and a mean peak area of 125,951.3 (%RSD 0.74%) were obtained from six replicate injections of the reference solution. Both values are well below the ICH-acceptable limit of 2% RSD, confirming that the chromatographic system was suitable for the intended analysis (Table 04).

Table 4: Results for System Suitability

S.NO	Injection	Retention Time (Min)	Peak Area
1	Injection-1	3.520	124264
2	Injection-2	3.524	126574
3	Injection-3	3.521	126039
4	Injection-4	3.523	125756
5	Injection-5	3.525	126978
6	Injection-6	3.521	126097
Mean		3.522333	125951.3
STD		0.001966	933.3715
%RSD		0.06	0.74

Specificity

No interference from the diluent (blank) or the placebo (excipient blend) was observed at the retention time of cariprazine, confirming the specificity of the method.

LOD and LOQ

The LOD and LOQ, calculated from the residual standard deviation and slope of the linearity regression, were found to be 0.765 µg/mL and 2.319 µg/mL respectively, confirming the sensitivity of the method for detecting and quantifying cariprazine at low concentrations.

Linearity

Linearity was demonstrated over the concentration range of 10–60 µg/mL, with a regression equation of y = 2537.14x + 48889.53 and a correlation coefficient (r^2) of 0.99988, meeting the ICH Q2(R1) acceptance criterion of $r^2 > 0.999$ (Figure 04).

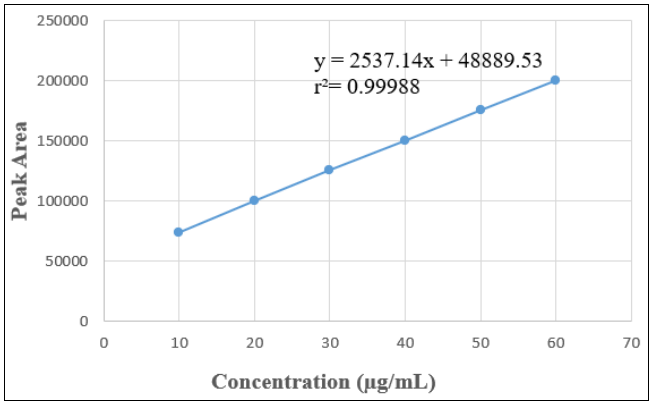

Fig 4: Linearity Graph for Cariprazine

Precision

The developed method demonstrated accurate and repeatable performance, with peak area %RSD of 0.71% for system precision and 0.88% for method precision, both well within the ICH-acceptable range of < 2% (Table 05).

Table 5: Results for System and Method Precision

Parameter	Mean Retention Time (Min)	Mean Peak Area	%RSD (Peak Area)
System Precision (n=6)	3.5225	125532	0.71
Method Precision (n=6)	3.522667	125911.7	0.88

Accuracy

The accuracy of the method was confirmed by percentage recovery of cariprazine, which ranged from 98.33% to 99.55% across the 50%, 100%, and 150% spiking levels, with %RSD values of 0.45%, 0.33%, and 0.56% respectively — within the acceptance criteria of 98–102% recovery and < 2% RSD (Table 06).

Table 6: Results for Accuracy (Recovery Study)

Recovery Level	Amount Found (µg/mL, mean)	% Recovery (mean)	%RSD
50%	2.23	99.11	0.45
100%	2.96	98.66	0.33
150%	3.71	99.02	0.56

Ruggedness (Intermediate Precision)

Analysis by a second analyst produced a peak area %RSD of 0.70%, well under the acceptable limit of < 2%, confirming that the method is robust to analyst-to-analyst variation (Table 07).

Table 7: Results for Ruggedness

Parameter	Mean Retention Time (Min)	Mean Peak Area	%RSD (Peak Area)
Ruggedness (n=6, second analyst)	3.5215	124948.5	0.70

Robustness

Under small, deliberate changes to the mobile phase ratio and flow rate, the percentage change in cariprazine peak area ranged from 0.38% to 0.60%, well below the permissible limit of < 2%, demonstrating that the method is resilient to minor variations in chromatographic conditions (Table 08).

Table 8: Results for Robustness

S. No	Condition	Retention Time (Min)	Peak Area	% Change
1	Standard	3.523	125648	—
2	Mobile phase +5% ACN (65:35)	3.521	124895	0.59
3	Mobile phase −5% ACN (55:45)	3.524	126257	0.48
4	Flow Rate 0.32 mL/min	3.522	125167	0.38
5	Flow Rate 0.28 mL/min	3.525	126243	0.47

Assay

The validated RP-UPLC method was applied for the quantitative estimation of cariprazine in both bulk drug substance and the marketed capsule formulation. The percentage purity of the bulk drug substance was found to be 99.88%, and the percentage assay of the marketed formulation (Caripriza, 3 mg label claim) was found to be 99.91% of the label claim, confirming the method's suitability for estimating cariprazine in both matrices (Table 09).

Table 9: Results for Assay – Bulk Drug and Formulation

S. No	Sample	Brand	Label Claim	Peak Area	Amount Found	% Assay
1	Bulk Drug (API)	—	—	125498	—	99.88
2	Formulation	Caripriza	3 mg	125389	2.997 mg	99.91

Conclusion

A simple, rapid, sensitive, and economical RP-UPLC method was successfully developed and validated for the

estimation of cariprazine in bulk drug substance and pharmaceutical dosage form. The method demonstrated excellent linearity, precision, accuracy, ruggedness, and robustness, in full compliance with ICH Q2(R1) guidelines. With a total run time of only 6 minutes, the method offers a considerable improvement in analysis speed over previously reported RP-HPLC methods for cariprazine, while retaining high resolution and sensitivity. The proposed RP-UPLC method can therefore be reliably employed for the routine quality control analysis of cariprazine in bulk drug substance and in commercial capsule formulations.

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