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STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF CARIPRAZINE HYDROCHLORIDE IN HUMAN PLASMA

Mohini Shelke, Rahul Godge*, Tejas Sahane, Onkar Pawar, Sujata Kasar

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ABSTRACT
Objective: The objective of the study is to create and validate the easy, dependable, accurate, sensitive, and selective RP-HPLC method for estimating Cariprazine HCl in human plasma. **Methodology:** The sample was prepared using the protein precipitation extraction method. The chromatographic separation was performed with an AGILENT C18 column (250mm x 4.6ID) as the stationary phase and a mobile phase consisting of a 75:25 v/v solution of Methanol and 0.1% Orthophosphoric acid at a flow rate of 0.7 ml/min. The DAD detector was used to carry out the detection at 253 nm. Cariprazine HCl had a reduced retention duration of 2.46 minutes. **Results & Discussion:** The calibration curve had a correlation coefficient of 0.998 and was linear over the concentration range of 1–5µg/ml. The method's accuracy was shown at levels between 80%, 100%, and 120% of the specification limit. The developed method exhibited excellent precision, with interday precision ranging from 0.07% to 1.77% and intraday precision from 0.03% to 0.26%. It was discovered that the recovery of Cariprazine HCl was within the 98% range. Cariprazine HCl was discovered to have a Limit of Detection (LOD) of 0.053µg/ml, and the Limit of Quantification was found to be 0.160µg/ml. **Conclusion:** The solution was injected in duplicate, and the % RSD was measured. The results indicate that the proposed method can be effectively utilized for the routine analysis of Cariprazine HCl in human plasma. The forced degradation studies indicate that the drug is susceptible to Hydrolytic and Photolytic degradation.

INTRODUCTION

Research on bioavailability and bioequivalence, the quantitative assessment of drugs and their metabolites, drug development, clinical, pharmacokinetics, and basic biomedical and pharmaceutical sciences investigations rely on methods for measuring medications in biological fluid [1]. Cariprazine HCl has the chemical formula $C_{21}H_{33}Cl_3N_4O$. Cariprazine is a derivative of piperazine and an atypical antipsychotic drug that was initially created in Hungary [2,3]. Its initial worldwide approval was in the US in September 2015 and was afterward given Health Canada's approval in April 2022. Currently, bipolar I disorder's manic or mixed episodes, depressive periods, and

*Department of Pharmaceutical Quality Assurance, Pravara Rural College of Pharmacy Pravaranagar, Tal-Rahata, District-Ahmednagar, Maharashtra, India

***For Correspondence:** **rahulgodge@gmail.com**

schizophrenia are all treated with it. Cariprazine HCl is available as a capsule formulation for oral use and is sold under Vraylar and Reagila [3]. A literature review found that very few analytical techniques for measuring specific concentrations of Cariprazine hydrochloride have been documented. Ultraviolet Spectroscopy [4], Reverse Phase High-Performance Liquid Chromatography [5], and LC-MS/MS/QT [6]. However, conventional analytical techniques cannot ascertain the drug concentration in human plasma.

The goals of developing and validating bioanalytical methods include making sure that analytes are quantified accurately and consistently in biological samples; determining the specificity, precision, accuracy, and robustness of the technique; adhering to regulatory requirements; and producing repeatable results for toxic kinetic and pharmacokinetic studies. Drug estimation is measured using the bioanalytical technique; an analytical method for bioanalysis needs to be developed and validated to achieve this.

To our knowledge, papers must describe techniques designed to measure this analyte in human plasma. Bioanalytical approaches aid in evaluating medication absorption rate and extent and comparing various formulations by precisely measuring drug levels in plasma. Furthermore, they prove that innovative and generic medications are bioequivalent. The suggested approach must yield a straightforward, precise, accurate, and affordable bioanalytical method for RP-HPLC-based measurement of Cariprazine HCl in human plasma.

MATERIAL AND METHODS

Materials

Pure Cariprazine HCl was received as a gift sample from Swapna Roop Drugs and Pharmaceuticals in Aurangabad, India. Fisher Scientific in India provided the HPLC grade ortho-phosphoric acid and methanol. We purchased acetonitrile HPLC grade from SD Fine Chem. Ltd in Mumbai, India. P.M.T. Blood Bank, located in Loni, India, provided the human plasma.

Instruments:

AGILENT liquid chromatography was used for the analysis, with an Agilent DAD detector at 253 nm, an Autosampler, and a pump series 1100. Substances were separated using a C18 column (Agilent column, 250 x 4.6 mm, 5µm) within a reverse phase system.

Extraction of Plasma

Extraction of human plasma by protein precipitation method to separate all blood components from the plasma. Take 15ml of human plasma and add 45ml acetonitrile. (Extraction solvent as 1:3 proportion) transfer into the Eppendorf tube. Then, all the sample was mixed for 5 min and reciprocated the sample at 3000 rpm at a suitable time vortex, and centrifuge liquids were collected. Further, the supernatant liquids were collected from the Eppendorf tube, which is free from other blood components, and refrigerated the plasma sample until the analysis was performed. Reconstitute with the diluent and inject into the HPLC [7-8].

Cariprazine HCl Standard Stock Solution

Accurately weigh and transfer 10 mg of Cariprazine HCl into a 100 ml clean and dry volumetric flask. It was diluted with 75 ml of methanol and 5 ml of human plasma, then made up the volume up to 100 ml with methanol. The mixture was then vertically shaken for 30 minutes and centrifuged for one hour at 5,000 rpm. After that, membrane filters were used to filter it and obtain a clear organic solution. It provides 100µg/ml standard Cariprazine HCl Stock solution [8-9].

Working standard solutions

Serial dilutions were made from standard stock solution by pipetting out 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, and 0.5 ml of Cariprazine HCl. They dissolved them in 10 ml of volumetric flask using mobile phase as a diluent, giving a final 1-5µg/ml concentration.

Method Validation:

1. Linearity

A calibration graph was constructed using calibration standards generated through plasma containing Cariprazine HCl at varying concentrations. The X-axis represents plasma concentration, ranging from 1 to 5µg/ml, while the Y-axis corresponds to peak area.

2. Accuracy

The method's recovery was ascertained by incorporating a known quantity of the medication into the reference concentration. Three concentrations of Cariprazine Hydrochloride standard were used for the recovery: 80%, 100%, and 120%.

3. Precision

In evaluating precision for intraday and interday analyses, 2µg/ml concentrations, 3µg/ml, and 4µg/ml were scrutinized. A standard solution was injected at varying intervals to assess intraday precision.

4. Limit of detection (LOD)

The calibration plot's slope and LOD were calculated using the standard deviation approach, applying the equation 3.3 x σ/S. The measured value was 0.053µg/ml. Because of the low concentration observed, the technique is adequately sensitive.

5. Limit of quantification (LOQ)

The calibration plot's slope and LOD were calculated using the standard deviation method, and 10 x σ/S, respectively 0.160µg/ml, were discovered to be. The drug can be estimated at a deficient concentration because less analyte was discovered.

6. Robustness

The robustness of the procedure was examined by significantly altering the chromatographic conditions. Under these altered chromatographic conditions, the standard solutions were injected. A deviation of ±1% in the ratio of the mobile phase. One percent variation in the mobile phase flow rate and ± 1% variation in the wavelength of UV spectroscopy. The peak symmetry, retention time, and separation factor were calculated under these modified conditions.

7. System Suitability Standards:

Five injections of a working standard solution containing Cariprazine HCl at a concentration of 100µg/ml were injected and analyzed under optimized chromatographic conditions like mobile phase consisting of a 75:25 v/v solution of Methanol and 0.1% Orthophosphoric acid, flow rate of 0.7 ml/min and detection at 253 nm using DAD detector. This was conducted to assess the consistency of results concerning the relative standard deviation (RSD), which should consistently remain below 2% as per ICH guidelines. Furthermore, various system suitability parameters were examined and assessed, such as retention time, theoretical plates, and tailing factor [11-15].

RESULTS

Method Development:

Selection of wavelength:

A 20 µg/ml Cariprazine HCl solution was scanned between the UV spectrum range 200 nm to 400 nm. It was observed that Cariprazine HCl shows maximum absorption at 253 nm, which is chosen as a detection wavelength. The drug exhibited good absorption at this wavelength, as shown in Figure 1.

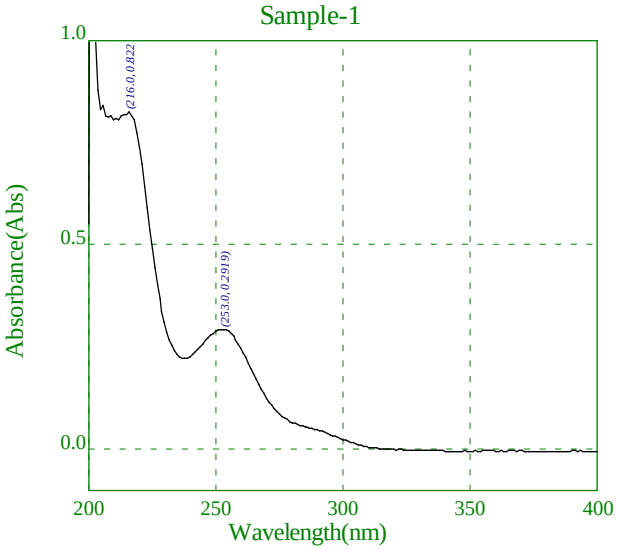

Fig.1 Overlain Spectrum of Cariprazine HCl

Chromatographic Optimization

An RP-HPLC method was designed specifically for cariprazine HCl to facilitate routine analysis in biological fluids. The drug's polarity played a role in choosing its mobile phase. Following several trials, Table 1 displays the final chromatographic condition chosen. Along with having ideal peak characteristics, the peaks were symmetrical and well-resolved. Figure 2 displays a typical chromatogram of Cariprazine HCl.

Table 1. Chromatographic state of Cariprazine HCl

Specifications	State of Chromatography
Mobile Phase	Methanol: 0.1% OPA (75:25v/v)
Stationary Phase	C18 (Agilent) 4.6 x 250 mm Particle size: 5 µm
Flow Rate	0.7 ml/min
Retention Time	2.461min
Run Time	10 min
Detection Wavelength	253 nm
Theoretical Plate	4609
Volume of Injection	20 µl

Chromatographic separation of Cariprazine HCl in biological fluid:

Under fixed chromatographic conditions, CariprazineHCL gives a straight baseline, and the peak was symmetrical, giving a

retention time of 2.46 minutes. Figure 2 shows a typical chromatogram of Cariprazine HCl in human plasma.

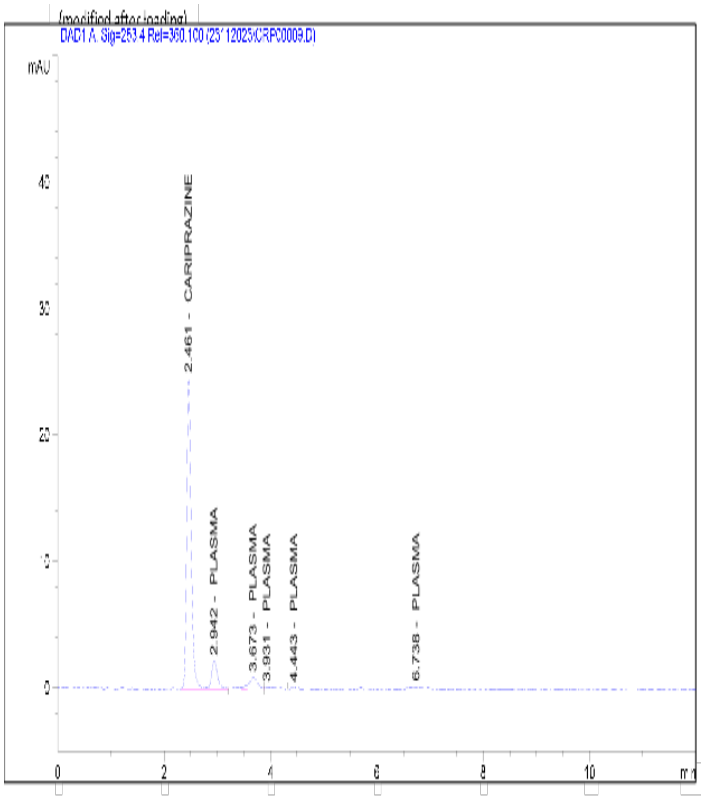

Figure 2: Chromatogram of Cariprazine HCl in Human Plasma.

Assay Preparation:

Capsule Solution Preparation:

Accurately weigh out 10 mg of Cariprazine HCl powder from the Capsule and dissolve it in 95 ml methanol and 5ml of human plasma in a 100 volumetric flask, shake vertically for 30 min, then centrifuge for 1 hour at 5000 rpm. After that, membrane filters were used to filter it and obtain an organic solution. This provides **stock II** solutions of 100µg/ml. From stock solution-II, pipette out 0.3 ml and dilute it to 10 ml using mobile phase to give the concentration of 3µg/ml. The assay outcomes are displayed in Table 2.

Table 2: Assay findings for the Cariprazine HCl formulation (Carinia 1.5 mg Capsule)

Drug	Cariprazine HCl
Concentration	3 ug/ml
Label Claim	1.5 mg
Amount Found	3.03
% RSD	0.068
% Assay	101.08

Method Validation:

1. **Linearity:**

Calibration plots were generated by graphing peak area against the respective concentrations. Linearity was confirmed through serial dilutions of the sample, measuring concentrations incrementally at 1, 2, 3, 4, and 5 µg/ml. Refer to Figure 3 for findings.

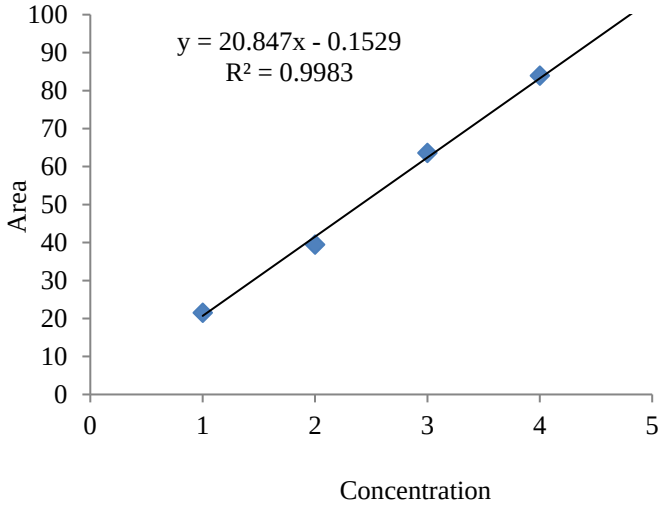

Figure 3: Calibration Curve for Cariprazine Hydrochloride

2. **Accuracy and Recovery Study:**

The developed method demonstrated high accuracy with 80%, 100%, and 120% recovery concentrations. After that, each dilution was injected two times (n=2). The data obtained falls within acceptable limits, confirming the method's accuracy. Table 3 shows the results of the accuracy studies. The test was passed with specification RSD < 2 %.

Table 3: Accuracy studies

% Level	Concentration (µg/ml)	Area	Amount Recovered (µg/ml)	% Recovery	Mean% recovery
80	8	198678.68	8.04	100.5	101.06
	8	198778.21	8.13	101.62	
100	10	222123.5	10.02	100.2	100
	10	221991.5	9.98	99.8	
120	12	242686.8	11.92	99.33	98.87
	12	242006.4	11.81	98.41	

3. Precision

Precision studies were carried out by Intraday and Interday studies, where plasma concentrations of 2µg/ml, 3µg/ml, and 4µg/ml were individually injected into HPLC. Each dilution was injected two times (n=2). Mean peak areas were calculated for each concentration, and precision %RSD values were determined from these measurements. The results of Intraday and Interday studies are shown in Table 4.

Table 4. Intraday and Interday precision

Concentration (µg/ml)	Intraday Precision		Interday Precision	
	Mean Area ± SD	% RSD	Mean Area ± SD	% RSD
2	2234.21 ± 0.21	0.23	2247.9 ± 0.24	0.26
3	3343.39 ± 0.68	0.52	3491.11 ± 0.72	0.59
4	4468.24 ± 0.99	0.39	4491.44 ± 1.1	0.45

4. Robustness

Robustness studies were carried out by changing the flow rate, mobile phase, and wavelength, which shows that the method is robust with fewer deviations. Results are shown in Figure 5.

Table 5. Robust Analysis of Cariprazine HCl

Factors that Vary	Area of Peak	SD	% RSD
Standard	63.57	0.58	0.91
Flow Rate + 0.1ml/min	54.84	0.36	0.65
Flow Rate - 0.1ml/min	74.09	0.29	0.39
Mobile Phase Ratio 74:26 (v/v)	62.1	1.16	1.87
Mobile Phase Ratio 76:24 (v/v)	61.03	0.82	1.35
Wavelength +1nm	50.64	0.03	0.07
Wavelength – 1nm	56.7	0.09	0.16

5. System suitability standards

System suitability parameters were studied with tailing factor, retention time, and theoretical plates, which gave results within acceptance criteria. The results of the system suitability studies are shown in Table 6.

6. Forced Degradation

Studies on forced degradation were conducted to evaluate the suggested method's stability and specificity. The percentage of drug deterioration in solution was estimated after standard and degraded samples were inserted within the chromatography system using various conditions such as acid, alkali, oxidative, photolytic, and Neutral degradation. Outcomes are displayed in Table 7.

Table 6: System suitability study

Parameters	Cariprazine HCl	Acceptance Criteria
Tailing factor	0.74	< 2
Retention time	2.46	> 2
Theoretical plates	4609	> 2000

Table 7. Findings from the study on Forced Degradation

Conditions of Stress	Area	% Recovery	% Actual degradation
Acid	1181	0.00	100
Alkali	1211	0.00	100
Oxidative	1184	0.00	100
Hydrolytic	60.87	95.75	4.25
Photolytic	59.33	93.32	6.68

I. Acid degradation:

0.3ml of the Cariprazine HCl was added in 10 ml of volumetric flask and mixed with 5 ml of 0.1N HCL and 5 ml of mobile phase to obtain stock solutions 3µg/ml, which was kept at 35 °C for 24 hours. After 24 hours, the sample was neutralized with 0.1 N NaOH before injection. The results are displayed in Figure 4A

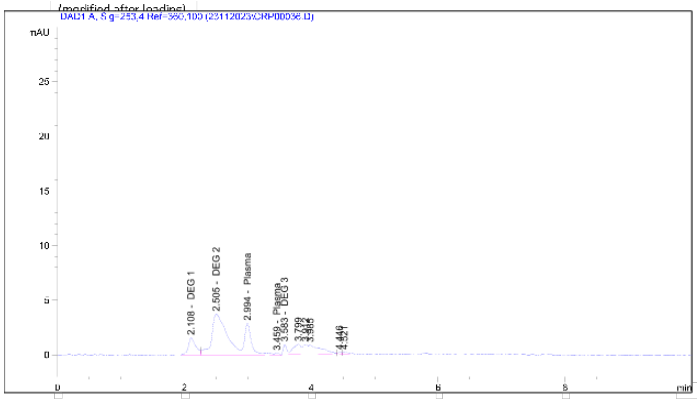

Figure 4A Acidic Degradation

II. Alkali degradation:

0.3ml of the Cariprazine HCl was added in 10 ml of volumetric flask and mixed with 5 ml of 0.1N NaOH and 5 ml of mobile phase to obtain stock solutions 3µg/ml, which was kept at 35 °C for 24 hours. After 24 hours, the sample was neutralized with 0.1 N HCL before injection. The results are displayed in Figure 4B.

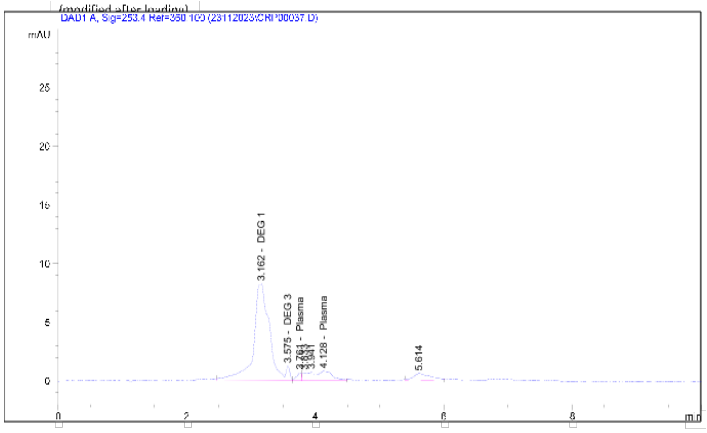

Figure 4B. Basic Degradation

III. Oxidative degradation:

To obtain 3µg/ml, 5 ml of 0.1N Hydrogen Peroxide was combined with 0.3 ml of the Cariprazine HCl stock solution. The mobile phase was then added to the mixture to dilute it and The mixture was maintained at 35°C for 24 hours. To evaluate the sample's stability, Cariprazine HCl solutions were introduced to the system, and the chromatograms were then recorded. Figure 4C represents the findings.

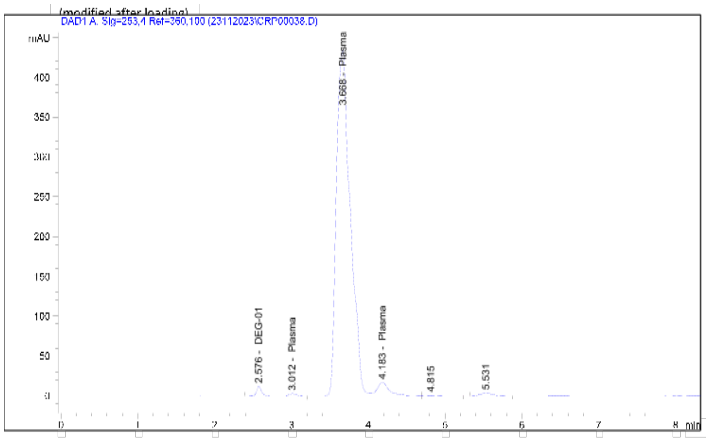

Figure 4C Oxidative Degradation

IV. Photolytic degradation

A 50mg sample was exposed to sunlight for 24 hours, and the results were used to examine the impact of UV radiation on the stability of Cariprazine hydrochloride. Before analysis, methanol was added to the stressed sample and a 0.45µm membrane was used for filter. Figure 4D displays the results.

V. Hydrolytic degradation

To investigate stress testing in neutral conditions, a 24-hour reflux of the Cariprazine HCl in water at 60°C was conducted. To assess if the material was stable enough for the HPLC analysis, the resulting solution was diluted to create an 8µg/ml solution, which was then injected. Chromatograms were then recorded. The results are displayed in Figure 4E

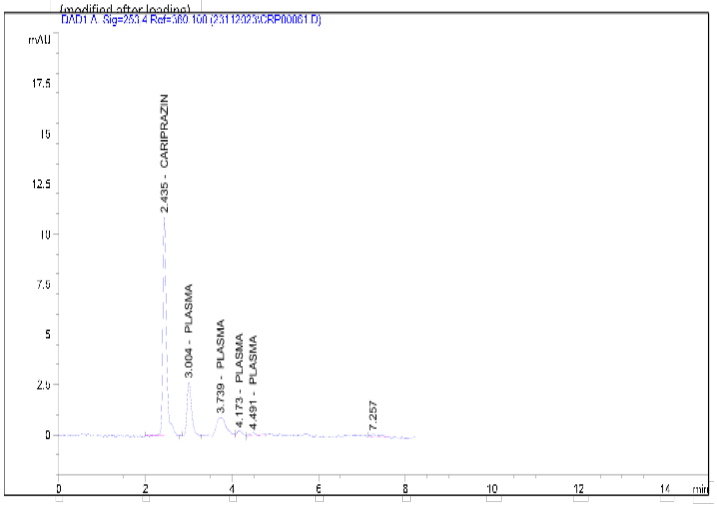

Figure 4D Photolytic Degradation

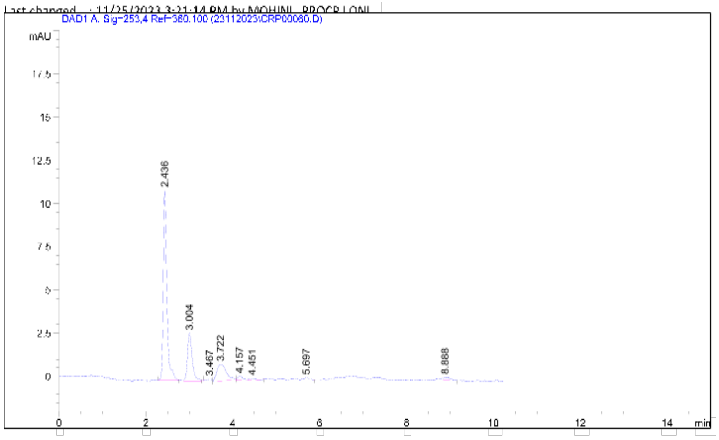

Figure 4E Hydrolytic Degradation

The method assessed how Cariprazine HCl degraded under various stress conditions in compliance with ICH guidelines. According to tests on forced deterioration, the medication is vulnerable to hydrolytic and photolytic degradation. Degradation was reported to be 4.25 % for hydrolytic and 6.68% for photolytic.

DISCUSSION

The developed RP-HPLC method in human plasma using protein precipitation extraction for sample preparation was found to be a very simple, reliable, precise, accurate, sensitive, and selective analytical method for the estimation of Cariprazine HCl. The drug's polarity played a role in choosing its mobile phase. Following several trials, the final chromatographic condition with mobile phase ratio 75:25v/v combination of Methanol: 0.1% OPA was utilized and chosen; along with having ideal peak characteristics, good resolution, and less

retention time, the peaks were symmetrical and well-resolved. It was discovered that the Cariprazine HCl retention time was 2.46 minutes. The Cariprazine HCl limit of detection was 0.053µg/ml. The method sensitivity is evident from the limit of quantification values, which stand at 0.160 µg/ml. The method assessed how Cariprazine HCl degraded under various stress conditions in compliance with ICH guidelines. The present study found difficulties during the forced degradation study of Cariprazine HCl under Acidic, Basic, and Oxidative stress conditions. Due to plasma Under these stress conditions, the drug degraded up to 100%, and the % recovery was found to be 0%. So, the forced degradation studies indicate that the drug is susceptible to Hydrolytic and Photolytic degradation. The method is suitable for routine quantitative analysis in pharmaceutical dosage forms.

CONCLUSION

This study has shown that the simple, dependable, accurate, sensitive, and selective RP-HPLC technique for determining Cariprazine HCl content in human plasma was developed and validated using the protein precipitation extraction method. The approach is compassionate, even with a 20 µl injection volume. The procedure was verified in accordance with USFDA regulations, and every parameter satisfied the requirements for approval. The method assessed how Cariprazine HCl degraded under various stress conditions in compliance with ICH guidelines. Based on representative chromatograms for forced degradation experiments, it can be concluded that the method is appropriate for routinely quantifying Cariprazine HCl in human plasma. Additionally, the stability-indicating method can be used for routine drug analysis in bulk form. Due to technological advancements and the growing need for precise and sensitive analytical approaches, Bioanalytical procedures will remain vital for comprehending biological systems, finding biomarkers, and guaranteeing the efficacy and safety of medications and other items. Bioanalytical methods are invaluable for comprehending biological systems, identifying illnesses, ensuring product safety, and expanding scientific research and healthcare. These procedures are crucial for researching biological processes.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTION

All authors, including Rahul Godge and Mohini Shelke, contributed to the experimental part. Rahul Godge, Mohini Shelkeand Sujata Kasar performed sample preparation and analysis of results. Tejas Sahane and Onkar Pawar prepared the draft of the manuscript, and all authors commented on the prepared manuscript draft. All authors equally reviewed the final manuscript.

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