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DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING RP-HPLC METHOD FOR DETERMINATION OF CARIPRAZINE IN BULK DRUG

Pooja Tiwari* and Purnima D. Hamrapurkar

Department of Pharmaceutical Analysis, Prin. K. M. Kundnani College of Pharmacy, Plot no. 23, Jote Joy Building, Rambhau Salgaonkar Road, Cuffe Parade, Mumbai - 400005, Maharashtra, India.

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***Corresponding Author**
Pooja Tiwari
Department of
Pharmaceutical Analysis ,
Prin. K. M. Kundnani
College of Pharmacy, Plot
no. 23, Jote Joy Building,
Rambhau Salgaonkar Road,
Cuffe Parade, Mumbai -
400005, Maharashtra, India.

ABSTRACT

A simple, specific, accurate and stability-indicating reversed phase high performance liquid chromatographic method was developed for the determination of Cariprazine, using a C-18 Inertsil ODS-3 (250mm×4.6mm×5µm) column and a mobile phase composed of 0.05M Ammonium Acetate Buffer : CAN (50:50,v/v), pH of buffer is 4.8 adjusted with acetic acid. The retention times of Cariprazine was found to be 5.1 min. The developed method was validated as per International Conference on Harmonization guidelines for the parameters of accuracy, precision, specificity, linearity, solution stability and system suitability. Linearity was established for Cariprazine in the range of 1-3µg/ml. The percentage recoveries of Cariprazine was found to be in the range of 98.26 - 100.47%. Cariprazine was subjected to acid, alkali hydrolysis, oxidation, thermal and photolytic degradation. The degradation studies indicated, Cariprazine to be susceptible to acid hydrolysis, base hydrolysis, oxidation and thermal degradation. The degradation products of Cariprazine in acidic, basic, oxidative and thermal conditions were well resolved from the pure drug with significant differences in the retention time values. This method can be successfully employed for quantitative analysis of Cariprazine in bulk drugs.

KEYWORDS: Cariprazine, Stability indicating method, Degradation products, Validation.

INTRODUCTION[1]

Cariprazine is an Atypical antipsychotic which is indicated in the treatment of schizophrenia and bipolar disorder. It acts as D_2 and D_3 receptor partial agonist, with high selectivity towards the D_3 receptor. It is also preferably useful as an add-on therapy in major depressive disorder.

Chemically it is N'-[trans-4-[2-[4-(2,3-Dichlorophenyl)-1-piperazinyl]ethyl]cyclohexyl]-N,N-dimethylurea (Fig no. 1). The molecular formula is $C_{21}H_{32}Cl_2N_4O$. It has a molecular weight 427.411 g/mol.

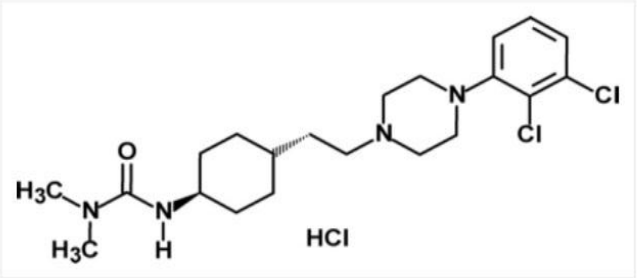

Fig No 1: Chemical structure of the drug.

Literature survey reveals that no analytical HPLC methods are available for the estimation of Cariprazine. Hence an attempt has been made to develop and validate chromatographic method for determination of Cariprazine from bulk drug using High Performance Liquid Chromatography (HPLC). Attempt has also been made to indicate that the method developed and validated is stability indicating method.

MATERIAL AND METHODS

Chemicals and Reagents: Cariprazine was procured from Cipla pharmaceuticals Ltd. as a gift sample. HPLC grade water was procured from J. K. Labs Mumbai and other reagents such as Acetonitrile, Methanol, IPA was procured from Merck chemicals cooperation Ltd. Mumbai India.

Equipment: The estimation of Cariprazine was carried using JASCO HPLC system with Jasco MD-2018 plus Intelligent PDA Detector using ChromNAV software as an integrator, Analytical Balance (Mettler Toledo), pH meter (Lab India) and a sonicator (Spectralab) The column used for separation of Cariprazine is INERTSIL ODS-3 (250 mm×4.6 mm×5 um).

Preparation of Standard stock solution: 10 mg of Cariprazine was accurately weighed and transferred to a 10 ml volumetric flask, added 4 mL of diluent, sonicated to dissolve and volume was made up to the markwith the diluent (1000µg/ml) (Solution A). 1ml of this solution A was diluted to 10ml with diluent (100µg/ml) (Solution B). Further, 1ml of this solution B was diluted to 10ml with diluent (10µg/ml) (Solution C). 2mL of solution C was diluted to 10mL with diluent to final concentration of 2µg/ml.

Preparation of mobile phase: 0.798gm of ammonium acetate was weighed accurately and transferred in 200ml beaker. 200ml of water was added, sonicated and the pH was adjusted to 4.8 with glacial acetic acid. Mixed the buffer and acetonitrile in the ratio of 50:50 (v/v) and sonicated for 15 minutes.

Preparation of Diluent: Mixed Acetonitrile and Water in the ratio of 50:50 (v/v) and sonicated for 15 minutes.

INITIAL METHOD DEVELOPMENT

Choice of Wavelength: 10mg of Cariprazine was weighed accurately and transferred to 10ml volumetric flask and the volume was made up with diluent to give a concentration of 1000µg/ml (Solution A). Solution A was further diluted with diluent to get the concentration of 10µg/ml. Uv spectrum of a solution having a concentration of 10µg/ml was recorded using diluent as blank. It showed absorbance at a wavelength of 217nm, so it was selected λmax for Cariprazine.

Choice of Column: The selection of the column is given in Table No 1.

Table No 1: Experimental trials for choice of columns.

Column	**Observation**	**Inference**
C_8	Poor retention of the analyte	Broad and poor peak shape
C_{18}	Improved retention of analyte	Improved peak shape

Choice of Mobile phase: The selection of mobile phase is given in Table No 2.

Table No 2: Experimental trials for selection of mobile phase.

Mobile phase	**Observation**	**Inference**
Methanol:Water	High baseline noise	Interfernce at the Retention time of the analyte
ACN:Water	No precision in the Retention time of the analyte. Broad and poor peak shape	Use of buffer required

Validation of the optimized method

Specificity: The specificity was carried out by injection a blank solution (diluent) and no interfering peak at the Retention time of the analyte was seen.

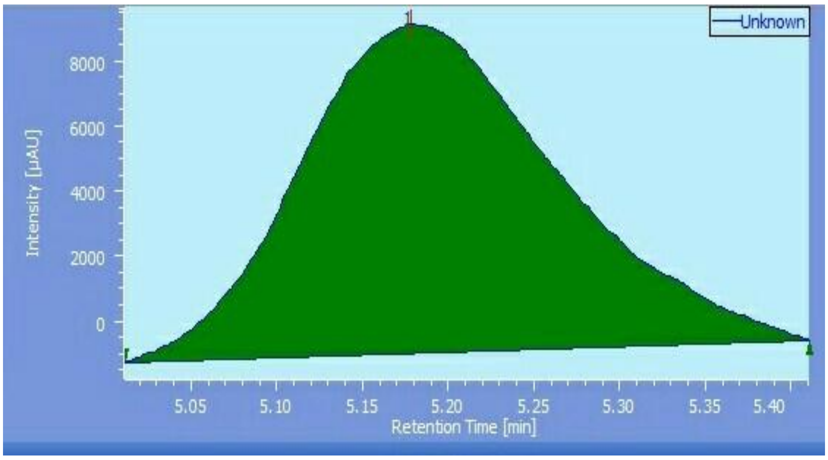

Fig No 2: Chromatogram of Blank.

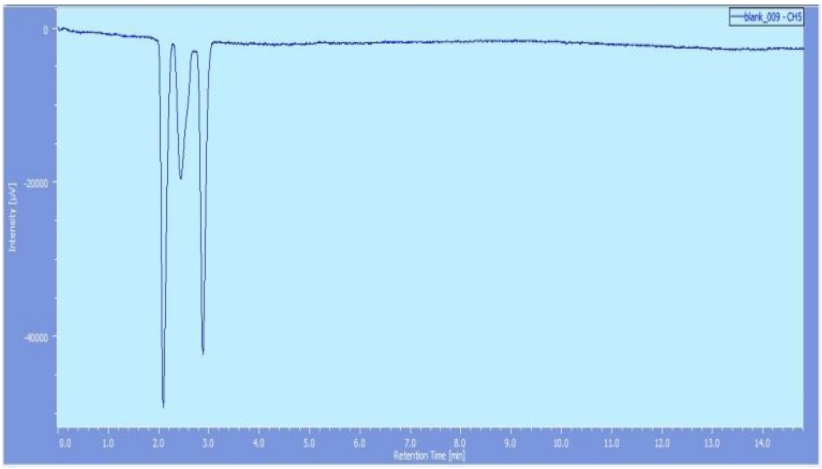

Fig No 3: Chromatogram of Standard.

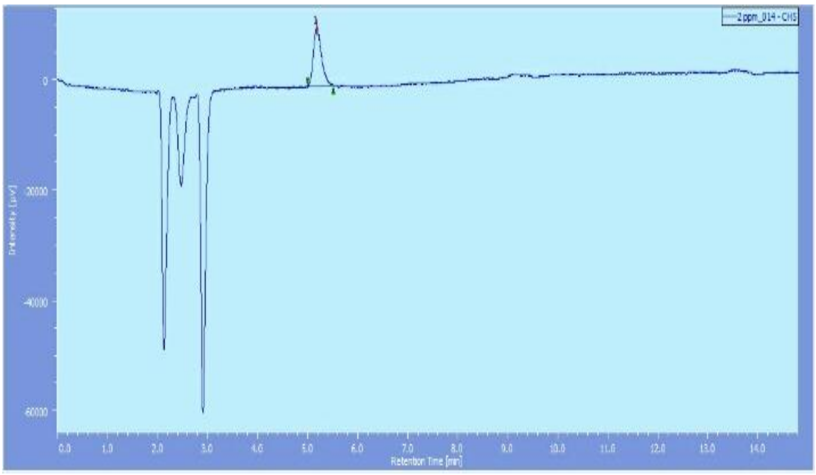

Fig No 4: Chromatogram for peak purity.

System suitability: System suitability test was carried out to verify that the analytical system is working properly to give accurate and precise results. Standard solution (2.0 µg/ml of Cariprazine) was injected six times, and the chromatograms were recorded.

Table No 3: System suitability data for Cariprazine.

Sr.No	System suitability parameters	Observations	Acceptance criteria
1	Cariprazine standard solution	2.0µg/ml	----
2	Area	119245	----
3	Retention time	5.178	----
4	NTP	5228	NLT 2000
5	Asymetry factor	1.302	NMT 2.0

Linearity: The linearity was studied over the concentration range of 1.0 µg/ml to 3.0µg/ml by injecting the standard solutions of the Cariprazine and injecting six times into the HPLC system by keeping the injection volume constant.The peaks were plotted against the concentrations to obtain the linearity graphs and the correlation coefficient was determined.

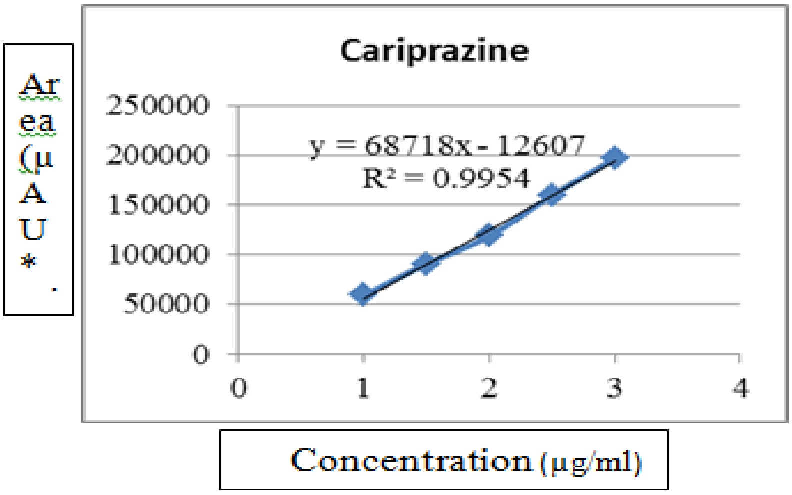

Fig. No. 5: Callibration curve for Cariprazine.

Precision: The precision studies were carried out for the developed analytical method by injecting six replicate injections of the concentration 1.0µg/ml, 2.0µg/ml, and 3.0µg/ml (50%, 100% and 150% of the working level). Intra and Interday precision studies were carried out by estimating the corresponding responses for the solutions of above three concentrations levels on the same day and on a different day respectively.

Accuracy: % Recovery study was performed using a minimum of 3 concentration levels, each in triplicate determinations. It was carried out by spiking blank concentrations of 50%, 100% and 150% of working level (1.0µg/ml, 2.0µg/ml, and 3.0µg/ml) and obtaining the percent recovery by putting the values of the areas of the peak obtained in the calibration curve to obtain the values of the concentration injected.

Sensitivity: The Limit of Detection (LOD) and Limit of Quantification (LOQ) for the developed method were determined by progressively injecting low concentrations of the Standard solution of Cariprazine using the developed HPLC method. This was carried out until a signal to noise ratio of NLT 3:1 and NLT 10:1 is obtained for LOD and LOQ respectively.

Solution stability

The solution stability studies was evaluated for three different concentrations (low, medium and high) i.e. 1.00µg/ml, 2.00µg/ml and 3.00µg/ml and six replicate each which were stored for 3 days at refrigerated temperature of 10ºC - 15ºC. The sample analysis was performed at initial time zero and after those 6, 9, 24, 48 and 72 hours.

Table No 4: Statistical data of Validation.

Sr.No	Validation Parameters	Cariprazine
1	Sytem suitability	NTP-5228 %RSD-0.71% Asymetry factor-1.302
2	Specificity	No interference was found at the Rt of the analyte
3	Linearity	R^2=0.9954 (Linear) y = 68718x - 12607
4	Accuracy	99.37%
5	Interday precision(%RSD)	0.89%
6	Intraday precision(%RSD)	0.71%
7	LOD	0.2µg/ml
7	LOQ	0.7µg/ml
8	Solution Stability(%RSD)	0.81%

Force Degradation: To prove the stability indicating nature of the method, forced degradation studies were carried out by exposing the stock solution of the drug to the following conditions:

1. Acid hydrolysis.
2. Base hydrolysis.
3. Oxidation degradation.
4. Thermal degradation.
5. Photolytic degradation.

Acid Hydrolysis: 2ml of the solution A (1000µg/ml) was pipetted out in a 10ml volumetric flask. The solution was treated with three different concentrations of HCL i.e 0.1N, 0.5N and 1.0N separately and then neutralized with the same Molar concentrations of Sodium Hydroxide solution i.e 0.1N, 0.5N and 1.0N separately and the volumes were made up with the diluents (mobile phase) to give a solution of 200µg/ml. Finally, the solution was loaded into HPLC and the corresponding chromatogram was recorded.

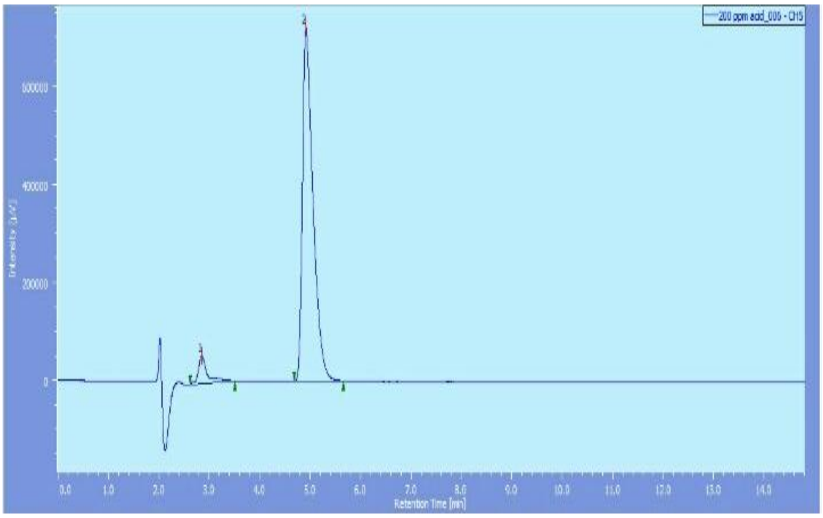

Fig No 6: Chromatogram of Acid hydrolysis.

Base Hydrolysis: 2ml of the solution A(1000µg/ml) was pipetted out in a 10ml volumetric flask. The solution was treated with 2ml of 0.1N Sodium hydroxide for 10minutes. This solution was then neutralized with 2ml of 0.1N Hydrochloric acid and the volume was made up with the diluent(mobile phase) to give a solution of 200µg/ml. Finally, the solution was loaded into HPLC and the corresponding chromatogram was recorded.

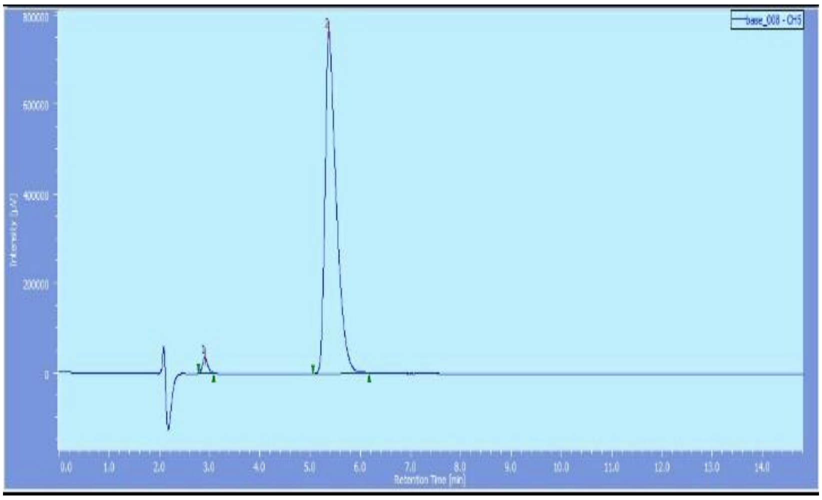

Fig No 7: Chromatogram of Base hydrolysis.

Oxidative degradation: 2ml of the solution A (1000µg/ml) was pipetted out in a 10ml volumetric flask. The solution was treated with 1ml of 3% H_2O_2 solution kept as such at room temperature for 2 hours. The volume was made up with the diluents (mobile phase) to give a solution of 200µg/ml. Finally, the solution was loaded into HPLC and the corresponding chromatogram was recorded.

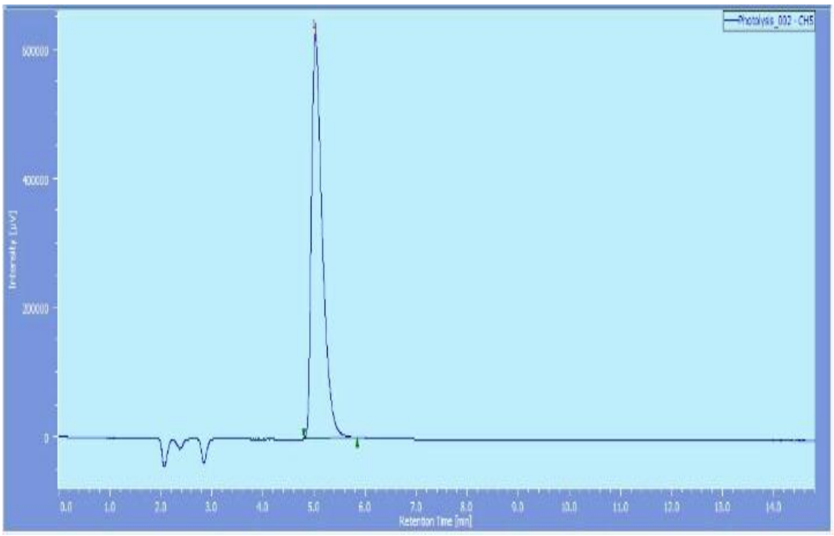

Fig No 8: Chromatogram of Oxidative degradation.

Thermal Degradation: 2ml of the solution A (1000µg/ml) was pipetted out in a 10ml volumetric flask. The solution was kept in water bath at 80°C for 2 hours. The volume was made up with the diluents (mobile phase) to give a solution of 200µg/ml. Finally, the solution was loaded into HPLC and the corresponding chromatogram was recorded.

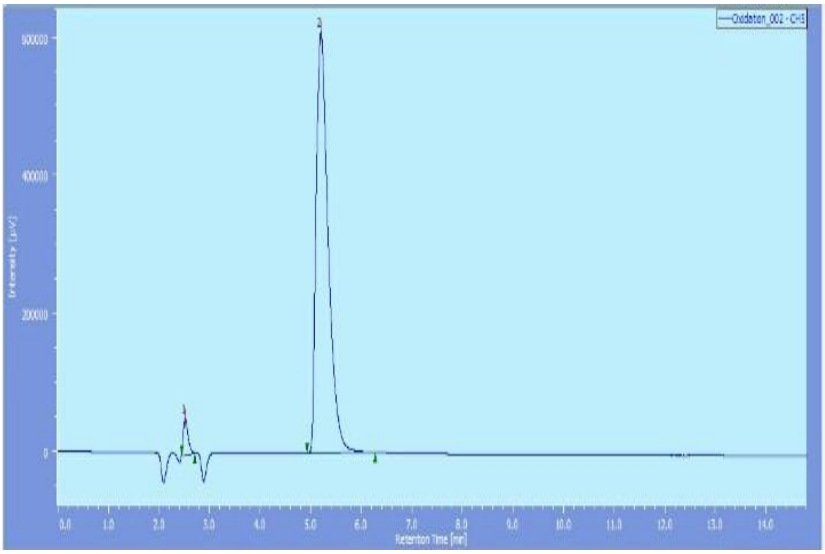

Fig No 9: Chromatogram of Thermal hydrolysis.

Photolytic Degradation: 2ml of the solution A(1000µg/ml) was pipetted out in a 10ml volumetric flask The solution was kept in sunlight for 2 hours. The volume was made up with the diluent(mobile phase) to give a solution of 200µg/ml. Finally, the solution was loaded into HPLC and the corresponding chromatogram was recorded.

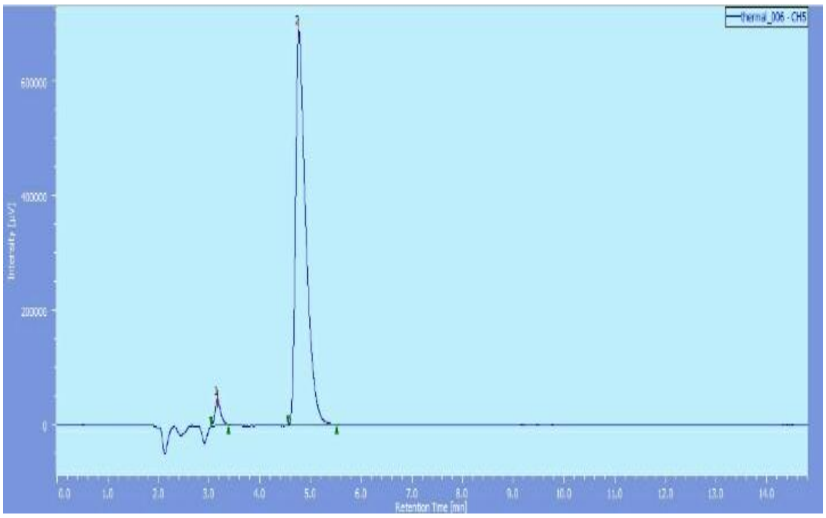

Fig No 10:- Chromatogram of Photolytic degradation.

Table No 5: Results for Forced degradation studies.

Sr.No	**Degradation Condition**	**Retention time for degradation products(min)**	**Cariprazine Percent degradation**
1	Acid Hydrolysis	2.7	6.6%
2	Base Hydrolysis	3	2.11%
3	Oxidative Degradation	2.4	3.3%
4	Photolytic Degradation	--	No Degradation
5	Thermal Degradation	3.3	3.6%
6	Total Degradation		15.61%

RESULT AND DISCUSSION

Selection of the column and mobile phase: The drug is basic in nature. It contains cyclohexane ring and heterocyclic rings containing nitrogen. So reverse phase chromatography was found to be best choice for its analysis. The effectiveness of C_8 and C_{18} Columns were evaluated and it was found that C_{18} Column showed better retention of the drug. The method development was initiated using methanol as organic modifier but was found to produce baseline noise leading to interference of the analyte causing broad and peak asymetry. Hence the organic modifier was replaced with ACN which showed no baseline noise which intereferd with analyte peak. Ammonium acetate, formic acid were added to adjust the mobile phase pH to have a stable retention time but these buffers were not able to provide a stable retention time for the analyte peak hence sodium acetate was used which provided stable retention time and a good peak shape with minimum asymetryfactor. As the drug is a base a basic pH woud led to its more retention on the column leading to a longer run time, hence the pH was adjusted to a acidic pH two units below the pka of the drug such that it would get eluted at a suitable retention time which provides a suitable run time for routine analysis of the drug.

Forced Degradation studies: The forced degradation studies indicate that the drug is susceptible to base hydrolysis and oxidative degradation. The degradation observed for base hydrolysis was found to be 9.39% and for oxidative degradation was found to be 7.7%. The representative chromatograms for forced degradation studies reveal that the method is a stability indicating method and can be used for the routine analysis of drug in bulk form.

CONCLUSION

The RP-HPLC method developed for determination of Cariprazine is linear, precise, accurate, rapid, sensitive and specific as evident from the validation results. The developed method is also stability indicating and can be successfully applied for the routine analysis of drug in bulk form.

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Conflict of interest: Nil.

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