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Identification, separation and mass spectral characterization of degradants in Cariprazine HCl by LC-MS/MS/QTOF

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Abstract: The Present work aims at the determination of degradation products by a developing an efficient method which was achieved by utilizing Waters C18 150x4.6mm, 3.35-micron column with flow rate of 1mL/min in an innovative, simple, and an accurate high-performance liquid chromatographic method. The mobile phase of methanol and 0.1% orthophosphoric acid in proportion of 50:50 (% v/v) ratio was employed at a wavelength of 216 nm. The recommended approach demonstrated good linearity in the Cariprazine HCl concentration range of 5 µg/mL to 75 µg/mL. Studies on precision and recovery have shown accuracy rates ranging from 98 to 102%. All robustness conditions have an RSD of less than 2%. Solvent stability is maintained over 24 hours even when subjected to high stress levels. According to ICH guidelines, method validation is performed, and the parameters investigated include precision and accuracy as well as specificity and stability along with robustness, linearity, the limit of detection and the limit of quantification as well as the limit of detection. The ultimate composition of degradation products was determined using Tandem Mass Spectrometry with Liquid Chromatography and all the degradant are formed at various stress conditions were successfully characterized and the possible pathways were demonstrated.

Keywords: Degradant; ICH Guidelines; LCMS; RP-HPLC; cariprazine HCl. © 2022 ACG Publications. All rights reserved.

1. Introduction

The Pharmaceutical technology that has progressed in the formulation elements of medicine, analytical technique has become increasingly important in the evaluation of successfully generated formulations. Individual compound identification and quantification of their degradation products in complicated organic mixtures is a difficult problem. The significant implications for a wide range of medications are very crucial in determination of quality attributes and the shelf life of the drug.

Cariprazine Known as Vraylar in the US and Reagila in Europe, is an atypical antipsychotic [1-2] that is used to treat schizophrenia [3-4], bipolar mania, and bipolar depression [5]. D3 and D2 are receptor partial agonists, having a high affinity for the D3 receptor, are the primary modes of action. Schizophrenia and mania [6] akathisia were the subjects of successful Phase III trials published in early 2012, while bipolar disorder I, depression was the subject of a successful Phase II trial published in later 2015 [7-8]. It might also be used in conjunction with other treatments for patients suffering from severe depressive illness. It is used for the treatment of bipolar I disorder, schizophrenia, and manic or mixed episodes. Its side effects might develop as early as the first day of treatment.

Akathisia [9-10] and sleeplessness [11-12] are the most common adverse effects of Cariprazine. Although Cariprazine does not affect prolactin levels, it does not enhance the QT interval on an electrocardiogram like many other antipsychotics. Short-term clinical studies showed additional pyramidal effects such as dizziness, nausea [13], vomiting, anxiety, and constipation [14]. This

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phenomenon was described as "not significantly different from that reported in patients treated with placebo [15]" however, the prevalence of movement abnormalities was labelled as "quite high" in a second study [16-17].

The Prerequisite for analytical test procedure is that it should be a validated stability indicating method. The validation was carried out by the ICH guidelines of Q1A(R2) – Stability test guidelines; [18] which condemns the establishment of stability characteristics by stress stability studies. Accordingly, the aim of present study is to establish the stability of Cariprazine HCl (CPZ) and to Identify, Separate the degradant by Stability –indicating assay method through stress studies at a variety of ICH recommended test conditions.

The chemical name of CPZ is (3-(4-(2-(4-(2,3-dichlorophenyl) piperazin-1-yl)ethyl)cyclohexyl)-1,1-dimethyl urea. CPZ is a novel, second generation, antipsychotic agent which was approved by the FDA in September 2015 [patent ref] developed by Gedeon Richter Ltd; for the treatment of schizophrenia & bipolar disorders. Figure 1 shows the chemical structure of Cariprazine HCl.[19]

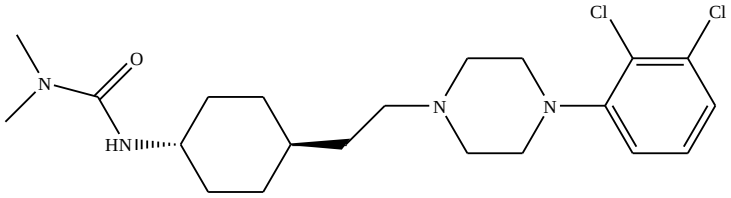


Figure 1. The Chemical structure of Cariprazine

A thorough survey of literature search of CPZ had revealed that there was only one available article [20] for the determination of Cariprazine HCl by RP-HPLC.

According to ICH guidelines, degradation product identification and characterization must be based on formal stability studies. In order to identify and characterize degradation products, conventional procedures (e.g., column chromatography) or hyphenated techniques (e.g., LC–MS, LC–nuclear magnetic resonance (NMR)) can be utilized. These approaches can give a better understanding of the structure of impurities, thereby expanding the knowledge space of possible structural alarms for genotoxicity and allowing for tighter management of such impurities. It should be highlighted that for those contaminants generated during formal shelf-life stability tests and over the qualification threshold limit, structural analysis of degradation products is required. Hence it was a very challenge to develop a new validated HPLC method for separation and characterization of obtained degradant by mass spectral analysis using LC-MS/MS/QTOF which proves to be sensitive and effective in successful separation of degradant to determine their pathway of degradation.[21]

Hence it was a very challenge to develop a new validated HPLC method for separation and characterization of obtained degradant by mass spectral analysis using LC-MS/MS/QTOF.

2. Materials and Methods

2.1. Chemicals and Reagents

CPZ was obtained as a gift sample from Aurobindo laboratories Ltd. Hyderabad, India. The reagents required like Acetonitrile, Methanol, Orthophosphoric acid were HPLC grade procured from Merck. HPLC grade water (milli -Q or equivalent) were purchased from Merck. All the weighing in the preparations were done with the help of Mettler Toledo (ME-204) electronic precision balance. The degassing of required solvents and mobile phase were achieved with the use of unichrome ultrasonicator. All the solvents, stock solutions, buffers, working standards and degradant were stored with the help of the Thermofischer Scientific refrigerator.

2.2. Instrumentation

The instrument employed throughout the analysis of Cariprazine was carried out using Waters, alliance e 2695 HPLC provided with high speed autosampler, column oven, degasser and coupled to the SCIEX QTRAP 5500 mass spectrometer equipped with triple quadrupole mass spectrometer. The data acquisition was under the control of class empower – 2 software.

2.3. Optimized LC and Mass Parameters

The prior trails in a process of achievement of an optimized method with different combinations of the mobile phases and other parameters which are clearly depicted through the chromatograms present in the supporting document. The approach was initially experimented with various mobile phases in different compositions based on their solubility and pH values.

The separations were performed on a reverse phase waters Symmetry C18(150 mm x 4.6 mm) column with the particle size of 3.5 micron, maintained at ambient temperature. The separation was achieved with (ACN: Buffer (50: 50) as mobile phase with 0.1% OPA buffer. The mobile phase was filtered using the 0.45-micron nylon membrane and degassed by ultrasonicator before injecting the sample for analysis. The flow rate of mobile phase was maintained at 0.1 mL/min. The injection volume of 20 microliter was optimized for analysis. The typical operating source conditions for MS scan of CZP in positive mode were optimized as follows. The scan mode was MRM. The ion spray voltage was set at 5500v and source temperature 500 °C.

2.4. Preparation of Buffer

Transfer 1 mL of orthophosphoric acid into 1000 mL volumetric flask and then dissolved in 500 mL of HPLC grade water. It was sonicated for 30 minutes and the volume was finally made up to 1000mL. This solution was filtered through 0.45 µ nylon syringe filter thus the buffer was prepared.

2.5. Preparation of Mobile Phase

For the preparation of mobile phase, mix methanol and 0.1% orthophosphoric acid in proportion of 50:50 (% v/v) ratio. The resultant mixture was sonicated for 15 min and was filtered through the 0.45 µ nylon syringe filter. The mobile phase was used as diluent in further procedures.

2.6. Preparation of Standard Solution

Transfer accurately weighed 5 mg of drug into a 10mL volumetric flask and diluted with diluent. Further dilute 1mL of the above solution with 10 mL diluent for the preparation of standard solution.

2.*7. Wavelength Optimization*

Cariprazine HCl solution was scanned across the range of 200-400 nm using a PDA detector and the highest absorbance of Cariprazine HCl was found to be at 216 nm. As a result, the technique validation was carried out at 216 nm.

2.8. Method Validation

The analytical approach was verified according to ICH Q2 (R1) criteria for characteristics such as the LOD, the LQ, forced degradation, and stability in order to assure system compatibility, specificity, and accuracy.

2.8.1. Specificity

If the analyte can be analyzed without any interference from other components (impurities, degradant or excipients), then the test is considered specific. It was confirmed comparing the chromatograms of Cariprazine HCl with blank.

2.8.2. System Suitability

System appropriateness and performance were evaluated using a series of tests. These parameters are all found to be within permissible limits for the USP system: plate count, tailing, and % RSD.

2.8.3. Accuracy

To measure the accuracy of a method, one has to look at how closely the test results, it produces match up to the real value. There were three distinct concentrations tested in the recovery experiments. Three injections were administered at each level, and the quantity of drug present, the % recovery, and the associated standard deviation were all determined. Each level was completed three times.

2.8.4. Precision

Analytical precision is measured by how closely individual test findings agree. Multiple samples of a homogenous sample were analyzed. The repeatability, intra-day, and inter-day fluctuations were used to evaluate the accuracy of the current approach. Analysis of samples taken at various times of the day and on several days served to verify this parameter.

2.8.5. Linearity

Methodological linearity is defined as the capacity of an analytical procedure to provide findings that are directly proportional to the analyte concentrations within a predetermined range. Six sets of standard solutions were used to measure linearity. The peak area vs concentration of the standard solution was plotted on the calibration curve, and the regression equation was derived. The slope, intercept, and correlation coefficient were all calculated using the least squares approach.

2.8.6. Degradation Effects and Its Characterization

Cariprazine HCl was degraded in part due to the various forced degradation settings. Using forced deterioration, the method's appropriateness of the deteriorated drug was shown in a test. Preventive measures can be taken during formulation to minimize the danger of drug instability thanks to the studies, which also provide information on drug instability. PDA detectors may be used to determine the peak's purity. LC-MS/MS identified and described five distinct DPs (DP1-DP5). It was discovered that the drug is unstable under certain circumstances, and researchers used this knowledge to take precautions during formulation to prevent this.

2.8.6.1. Acid Degradation

1 mL of stock solution was transferred to a volumetric flask of 10 mL, and 1 mL of 1N HCl was added and left for 15 minutes. Add 1 mL of 1N NaOH after 15 minutes and dilute to the desired strength. Inject the solution into the HPLC system after it has been filtered via a syringe filter. The degradation products were discovered using LC-MS.

2.8.6.2. Alkali Degradation

For 15 minutes, 1 mL of 1N NaOH was added to 1 mL of the standard stock solution in a volumetric flask with a capacity of 10 mL. Make up to the diluent mark after 15 minutes by adding 1 mL of 1N HCl. Inject the solution into the HPLC system after it has been filtered via a syringe filter. The degradation products were discovered using LC-MS.

2.8.6.3. Peroxide Degradation

One mL of the standard stock solution was transferred to a 10mL volumetric flask, one mL of 30% hydrogen peroxide was added, and the flask was heated for 30 minutes at 60°C before the diluent was added. Inject the solution into the HPLC system after it has been filtered via a syringe filter. The degradation products were discovered using LC-MS.

2.8.6.4. Reduction Degradation

Add 1 mL of a 30- percent sodium bicarbonate solution to 1 mL of the standard stock solution, heat for 15 minutes at 60°C, then cool and then make up with diluent. Inject the solution into the HPLC system after it has been filtered via a syringe filter. The degradation products were discovered using LC-MS.

2.8.6.5. Thermal Degradation

Exposed to 105°C for three hours, the Cariprazine standard was tested. A 10mL volumetric flask was used to transfer 5 mg of material. Sonicate to dissolve, then dilute to the desired volume with 5 mL of diluent. For 60 minutes, this solution is boiled in an RB flask at 60 degrees Celsius. After that remain the flask at room temperature after that add diluent to further dilute 1mL to 10mL. Cariprazine HCl does not degrade in thermal degradation condition.

2.8.6.6. Photo Degradation

For the UV degradation procedure, the standard solution was exposed to sunlight for 12 hours before being refluxed at 60°C for 30 minutes. The substance was separated using HPLC. The degradation products were discovered using LC-MS.

2.8.6.7. Hydrolysis degradation

A volumetric flask containing 10 mL of HPLC water and 1 mL of standard stock solution was transferred, heated for 30 minutes at 60°C, and then cooled to make up with a diluent. Inject the solution into the HPLC system after it has been filtered via a syringe filter. In hydrolysis breakdown conditions, Cariprazine HCl is unaffected.

2.8.7. Robustness

Reliability during typical use may be judged by an analytical procedure's capacity to withstand tiny but purposeful changes to method parameters, and this is known as robustness. As a part of the robustness testing, the HPLC system was injected with a standard solution and underwent chromatographic conditions such as reduced flow rate (0.2 mL/min) and reduced organic content (10 percent). The effects of the adjusted parameters were evaluated in order to derive the separation factor, retention duration, and peak asymmetry.

2.8.8 Stability

The HPLC analytical solution was prepared and injected every 0 to 24 hours, depending on the instrument usage and injection sequence.

3. Results and Discussion

3.1. Method Development

Analytical methods for Cariprazine HCl quantification were found to be accurate, precise, and cost-effective, according to the study's aim. Isocratic development processes were used to evaluate acidic buffers, methanol, and acetonitrile. The results were encouraging stationary phases, including phenyl, amino and C_4, C_{18}, C_8, phenyl were utilized. Each trial's step plate count and retention duration

were also tweaked to optimize outcomes. Symmetry C_{18} column, a moving phase of 0.1% orthophosphoric acid and methanol was used, as was an UV detector calibrated for 216 nm. The whole concert lasted little over six minutes. Table 1 shows the chromatographic settings of different trials and the figures from 2-6 shows, the chromatograms obtained by using the table 1 conditions. The optimized chromatographic conditions were shown in Table 2.

Table 1. Chromatographic conditions of different trials

S. No	Mobile phase	Column	Flow rate	Observation
1	Acetonitrile + Water (80:20)	Waters X-Bridge C18	1ml/min	Peak is not splitted properly
2	Acetonitrile + Water (60:40)	Inertsil ODS	1ml/min	Here two peaks are observed
3	Acetonitrile + 0.1%OPA (60:40)	Inertsil ODS	1ml/min	Plate count is not within the limit
4	Methanol + 0.1% OPA (80:20)	Waters Symmetry C18	1ml/min	Less retention time is observed
5	Methanol + 0.1% OPA (50:50)	Waters Symmetry C18	1ml/min	This method is suitable for validation

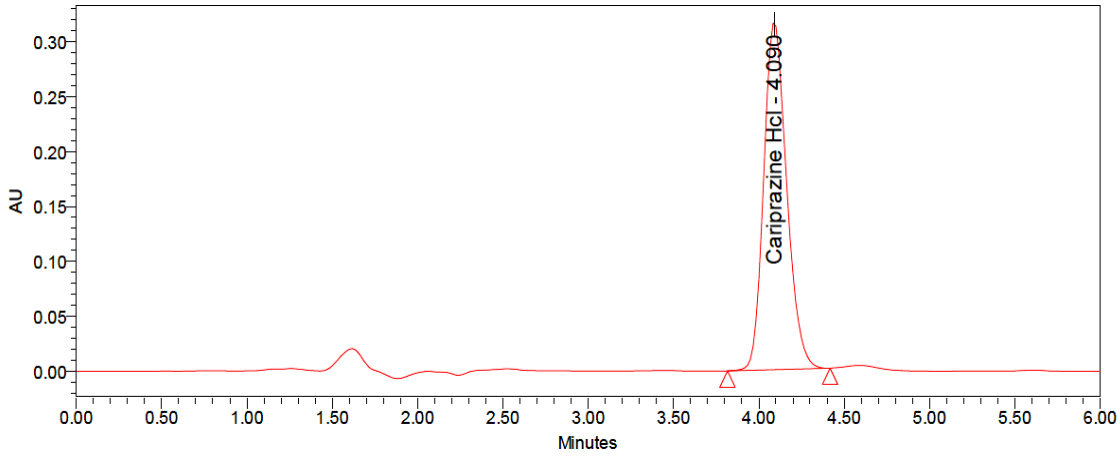


Figure 2. HPLC Chromatogram of caripazine optimized conditions

In Figure 2 the peak didnot splitted properly using acetonitrile and water as mobile phase and an X-bridge column. In figure 3 an unknown peak was formed and the baseline was not sufficient using acetonitrile and water as mobile phase and inertsil ODS column. In figure 4 peak shape was not good and the plate count was not within the limit by using acetonitrile and 0.1% OPA as mobile phase and inertsil ODS column. In figure 5 less retention time was observed using methanol and 0.1% OPA as mobile phase and the symmetry C18 column. In the figure 6 peak shape, plate count, tailing factor found to be within the limit using methanol and 0.1% OPA as mobile phase and symmetry C-18 column.

Table 2. Optimized HPLC method conditions

S.No	Parameter	Conditions
1	Column	Symmetry C_{18} 150 mm x 4.6mm, 3.5µ
2	Flow rate	1.0 mL/min
3	Wave length	216 nm
4	Injection volume	20 µL
5	Run time	6 min
6	Mobile phase	0.1% OPA + Methanol (50:50)

3.1.1. Specificity

The API and was accurately weighed and injected into the HPLC apparatus in accordance with the test procedure. Cariprazine HCl did not interfere with the chromatograms of blank solution. Figure 7 depicts a typical specificity chromatogram. Analysis of the chromatograms of Cariprazine HCl and blank solution showed no interference.

3.1.2. System Suitability

The HPLC system was tested for system suitability parameters using the reference solution and confirmed to be within limits. Standard peak areas were used to compute the relative standard deviation (RSD) %. The RSD % of comparable injections was found to be 1.35, tailing was found to be 1.2 and plate count was 4294 indicates all the results were within the acceptable range. Table 3 shows the findings, while figure 8 shows the suitability chromatogram.

Table 3. Results of system precision

S. No	System suitability parameter	Acceptance criteria	Cariprazine HCl
1	% RSD	NMT 2.0	1.35
2	USP Tailing	NMT 2.0	1.20
3	USP Plate count	NLT 3000	4294

3.1.3. Linearity

The linearity concentration of Cariprazine was produced between 5 and 75 micrograms per milli litre. Y=63400x+28236 was discovered to be the regression equation, and the correlation coefficient is 0.999 indicates the linearity results were found to be within the limit. Figure 9 depicted the linearity calibration plot, and the findings were shown in table 4.

Table 4. The **r**esults of linearity

S. No.	Cariprazine HCl	
	Conc. (µg/mL)	Area
Linearity-1	5.00	371255
Linearity-2	12.50	826268
Linearity-3	25.00	1703314
Linearity-4	37.50	2350807
Linearity-5	50.00	3059642
Linearity-6	62.50	4068593
Linearity-7	75.00	4805591
Slope		63400.30
Intercept		28236.13
CC		0.9991

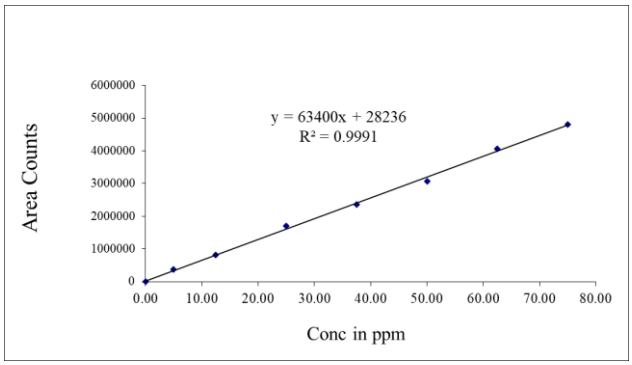


Figure 3. The linearity calibration plot

3.1.4. Robustness

Although the flow rate (0.2 mL) and organic solvent (10 percent) are little different in robustness, the RSD does not vary much (percent). By increasing the flow of 0.2 mL means at a flow of 1.2 mL/min, the %RSD was found to be 0.06, by decreasing the flow of 0.2 mL means at a flow of 0.8 mL/min, the f% RSD was found to be 0.15. By increasing the organic of 10% means at the mobile phase ratio of 55: 45, the %RSD was found to be 0.32 and by decreasing the organic of 10% means at the mobile phase ratio of 45: 55, the %RSD was found to be 0.26. All these results indicate the %RSD was found to be within the limit. Table 4 shows the outcomes of the investigation.

Table 4. Results of the robustness

S.No	Parameter name	Cariprazine HCl % RSD
1	Flow (0.8mL/min)	0.15
2	Flow (1.2mL/min)	0.06
3	Organic solvent (+10%) (55:45)	0.32
4	Organic solvent (-10%) (45:55)	0.26

3.1.5. Stability

The stability of Cariprazine HCl was tested over the course of a 24-hour period at room temperature. In terms of purity, there isn't much of a difference. Table 5 displays the results of the investigation.

Table 5. Results of stability

S.No	Stability	Purity of Cariprazine HCl in RT	Purity of Cariprazine HCl in 2-8°C
1	Initial	100	0.00
2	6 h	99.8	-0.20
3	12 h	99.8	-0.20
4	18 h	99.1	-0.90
5	24 h	98.1	-1.90

3.1.6. Precision

Every stage of the injection and testing procedure, from standard preparation of final results, was closely monitored to ensure precision. In order to assess the repeatability of the test, the percent relative standard deviation was determined from a minimum of six measurements.

In intermediate precision six replicates of a standard solution containing Cariprazine HCl (50µg/ml) were analysed on a different day. Peak areas were calculated which were used to calculate %RSD

values. The present method was found to be precise as the RSD values were less than 2% and also the percentage assay values were close to be 100%.

Table 4. Intraday precision results

S No	Method Precision Cariprazine HCl		Intermediate Precision Cariprazine HCl	
	Area	% Assay	Area	% Assay
1	2948264	99.0	2938262	98.7
2	2949491	99.1	2936513	98.1
3	2937437	98.5	2947542	98.8
4	2944641	98.9	2954684	99.1
5	2931517	98.5	2931509	98.5
6	2946124	98.8	2946118	98.8
%RSD (n=6)	0.24	0.26	0.29	0.34

3.1.8. Accuracy

Testing the correctness of the data was done using recovery studies at three different doses (50 percent, 100 percent and 150 percent). Researchers developed Cariprazine APIs with strengths ranging from 25 to 75 µg/mL. It was determined that the healing rate was between 98% and 100%. The accuracy data were shown in Table 6.

Table 6. Results of accuracy of Cariprazine HCl

S. No.	% Level	% Recovery	Avg % Recovery
1	50	98.0	98.5
2		98.4	
3		99.2	
4	100	99.2	99.1
5		99.0	
6		99.2	
7	150	98.3	98.2
8		98.0	
9		98.3	

3.2. Degradation Effects and Its Characterization

3.2.1. Acid Degradation

When Cariprazine HCl was decomposed in acid, it produced two products at 5.948 minutes (DP-1) and 11.639 minutes (DP-2) and these degradation products were discovered using LC-MS.

3.2.2. Alkali Degradation

When Cariprazine HCl was decomposed in alkali, it produces two degradation products. Degradation products having retention times of 5.919 minutes (DP-1) and 11.653 minutes (DP-2), were discovered using LC-MS analysis.

3.2.3. Peroxide Degradation

When peroxide is used to decompose Cariprazine HCl, just one product is formed with the retention time of 6.624 minutes (DP-3), LC-MS detected the degradation product.

3.2.4. Reduction Degradation

When Cariprazine HCl is reduced, there is just one breakdown product. A degradation product with retention duration of 7.859 minutes (DP-4) was discovered using LC-MS.

3.2.5. Thermal Degradation

Cariprazine HCl does not degrade in thermal degradation condition.

3.2.6. Photo Degradation

When exposed to sunshine, Cariprazine HCl degrades to a single degradation product. LC-MS identified a degradation product with retention duration of 5.088 minutes (DP-5).

3.2.7 Hydrolysis degradation

In hydrolysis breakdown conditions, Cariprazine HCl is unaffected.

Table 9 shows the findings of forced degradation studies of obtained degradation products created under various stress settings.

Table 9. Forced degradation results of Cariprazine HCl

Degradation Condition	% of Purity	% of Degradation	Purity Angle	Purity Threshold	DPs formed
Unstressed Degradation	100	0	0.165	0.458	-
Acid Degradation	75	25	0.093	0.301	DP-1, DP-2
Alkali Degradation	75.7	24.3	0.088	0.296	DP-1, DP-2
Peroxide Degradation	73.5	26.5	0.165	0.313	DP-3
Reduction Degradation	76.3	23.7	0.165	0.313	DP-4
Thermal Degradation	96.1	3.9	0.165	0.313	-
UV Degradation	79	21	0.104	0.288	DP-5
Hydrolysis Degradation	98.3	1.7	0.99	0.277	-

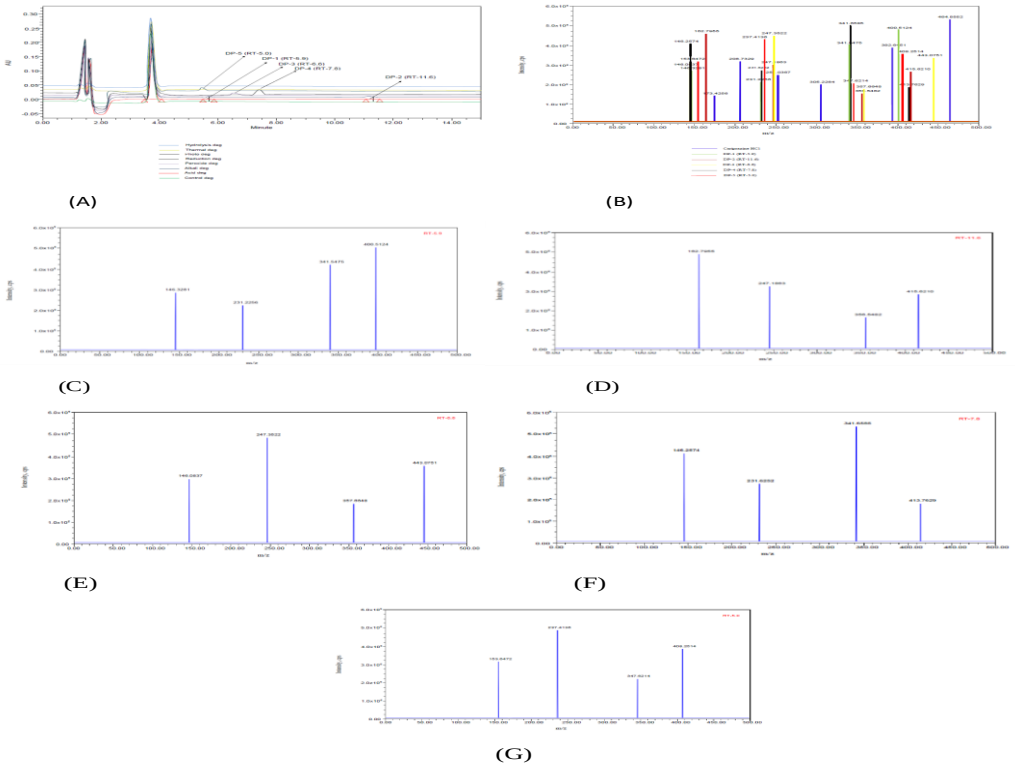


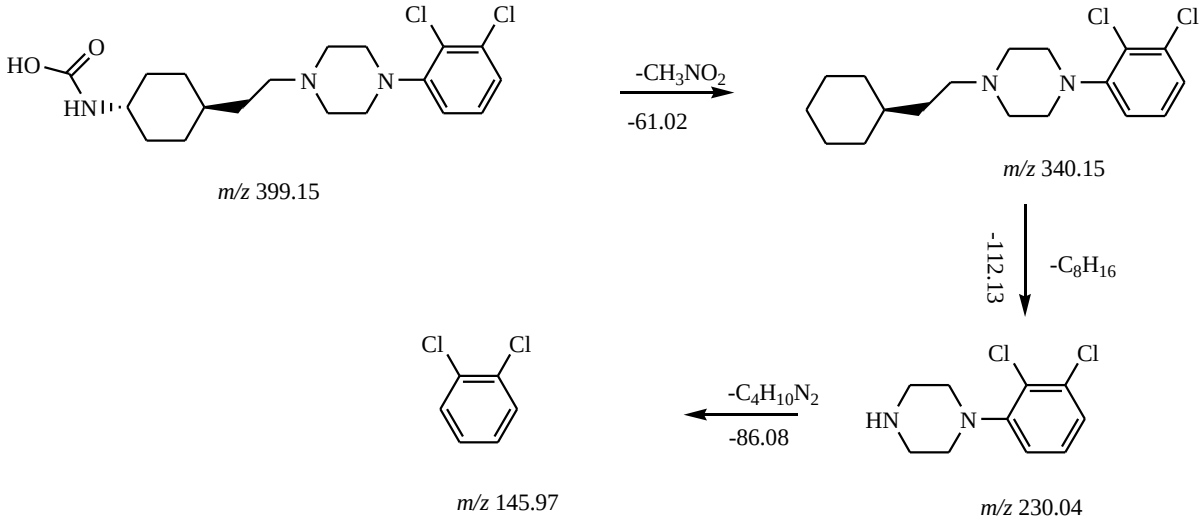
Figure 4. (A) Overlay chromatogram of degradant of Cariprazine (B) An Overlay mass spectrum of degradant of Cariprazine (C) Mass spectra of DP-1 at RT-5.9 (D) Mass spectra of DP-2 at

RT- 11.6 (E) Mass spectra of DP-3 at RT-6.6 (F) Mass spectra of DP-4 at RT-7.8 (G)Mass spectra of DP5 at RT-5.0

3.3. Characterization of DPs by LC-MS/MS

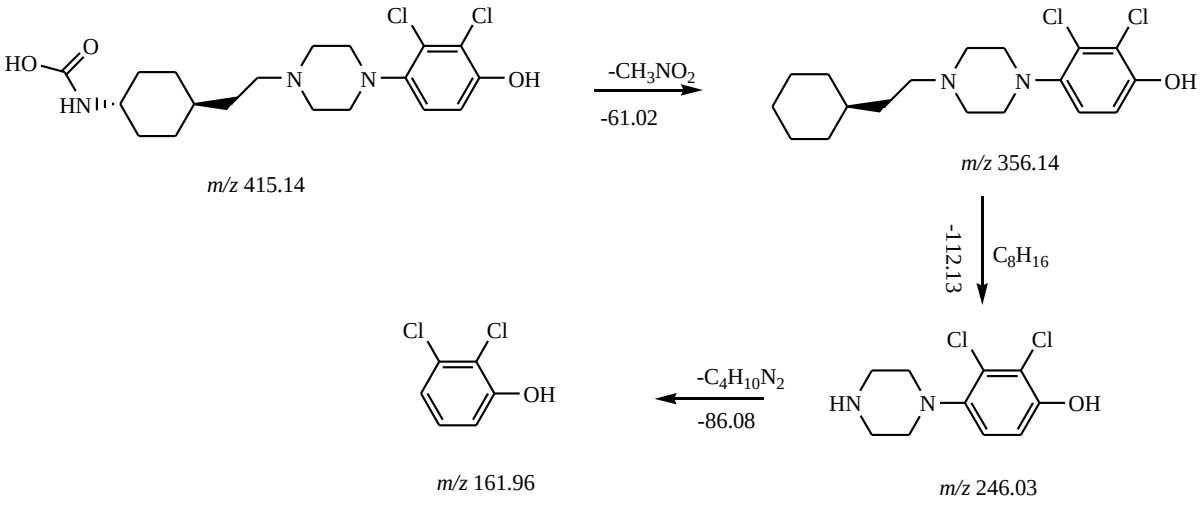
The characterization of the degradant formed in the stress studies were clearly explained in each condition separately and the overlay chromatogram and mass spectrum in Figure 4A to 4G had clearly depicted the formation of DP I to V.

The fragmentation process of 5.9 RT (DP1) of *m/z* 400 under acid and alkali degradation conditions is shown on Scheme 1. With respect to the loss of C_8H_{16} and CH_3NO_2, as well as the reduction in the amount of CH_3NO_2, the spectrum shows numerous product ions at *m/z* 341, 231 and 146, respectively (the loss of $C_4H_{10}N_2$). The suggested approach has been validated using MS/MS studies and precise mass measurements.



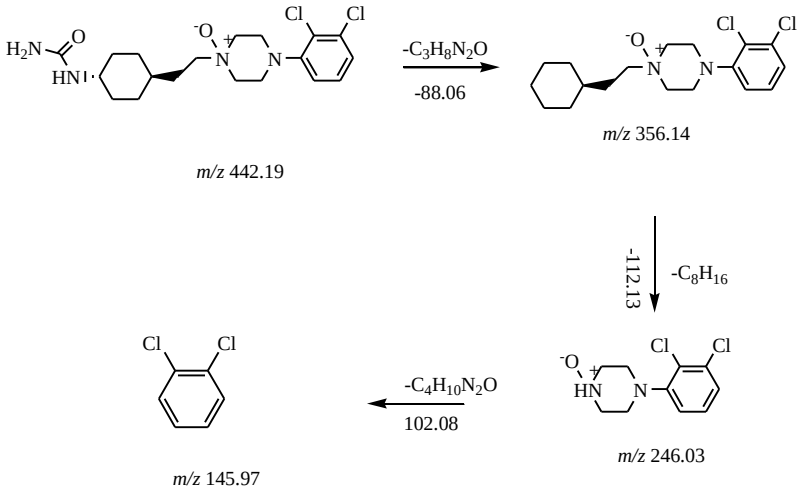
Scheme 1. Proposed fragmentation pathway of degradant at 5.9 RT (DP1)

Acid, alkali degradation product 11.6 RT (DP2)of *m/z* 415 fragmentation pathway is shown in Scheme 2 (below). In the spectra, there are several product ions at *m/z* 356 (loss of CH_3NO_2), *m/z* 247 (loss of C_8H_{16}), and *m/z*-162 (loss of CH_3NO_2) (loss of $C_4H_{10}N_2$). The suggested approach has been validated using MS/MS studies and precise mass measurements.



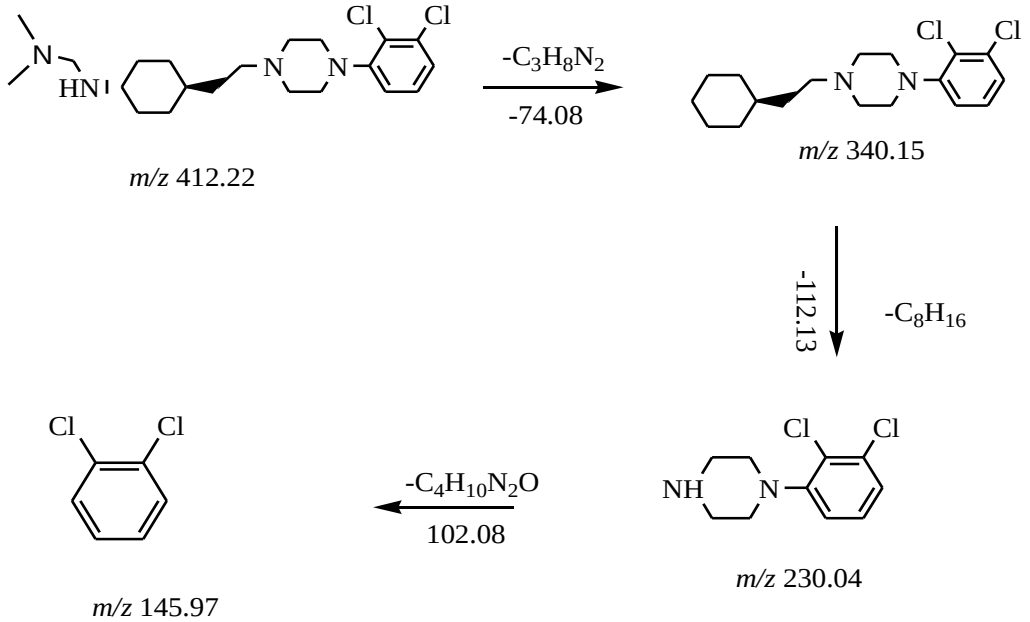
Scheme 2. Proposed fragmentation pathway of degradant at 11.6 RT (DP2)

The degradation product 6.6 RT *m/z* 443 (DP3) fragmentation pathway is shown in Scheme 3 which is formed when subjected to peroxide degradation. It is evident that $C_3H_8N_2O$ is lost at *m/z* 357, *m/z* 247, and *m/z* 146 in the spectrum (loss of $C_4H_{10}N_2O$). The suggested approach has been validated by MS/MS studies and precise mass measurements.



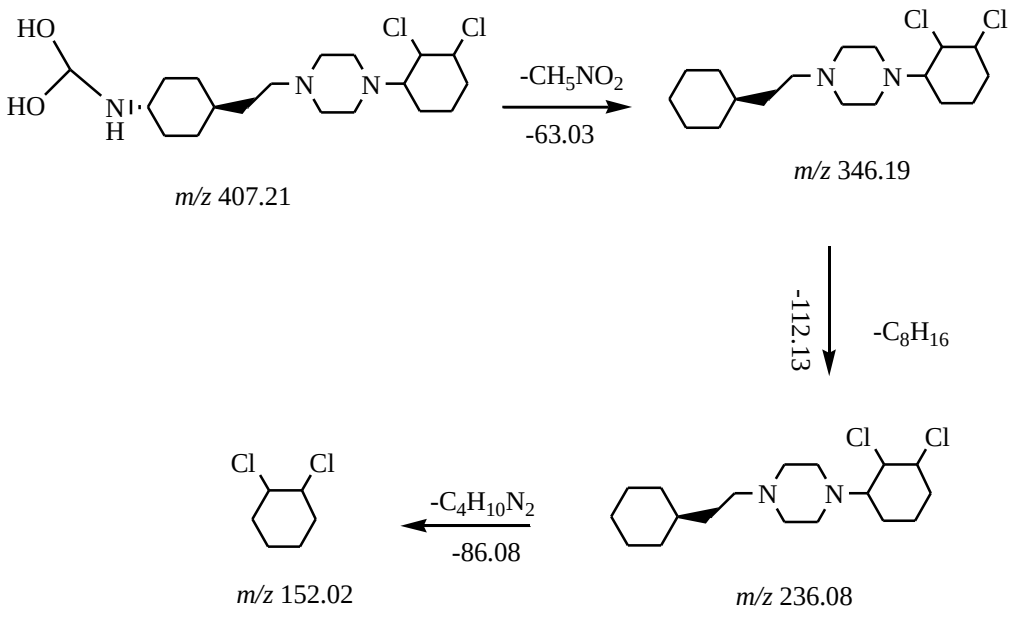
Scheme 3. Proposed fragmentation pathway pf degradant at 6.6 RT (DP3)

The reduction degeneration state considered as a model, Scheme 4 depicts the fragmentation process of the degradation product 7.8 RT (DP4) of *m/z* 413. When $C_3H_{10}N_2$ is lost, the spectrum shows plenty of product ions at *m/z* 341, *m/z* 231 (loss of C_8H_{16}), and *m/z* 146 (loss of C_8H_{16}) (loss of $C_4H_{10}N_2$). The suggested approach has been validated using MS/MS studies and precise mass measurements.



Scheme 4. Proposed fragmentation pathway pf degradant at 7.8 RT (DP4)

Depicts the photolytic degeneration-induced fragmentation of degradation product 5.0 RT (DP5) of *m/z* 408. Product ions at *m/z* 347 (loss of CH_5NO_2), *m/z* 237 (loss of C_8H_{16}) and *m/z* 153 (loss of C_8H_{16}) are prominent in the spectrum (loss of $C_4H_{10}N_2$). The suggested approach has been validated using MS/MS studies and precise mass measurements.



Scheme 5. Proposed fragmentation pathway of degradant at 5.0 RT ((DP5)

4. Conclusion

In this study, a novel, rapid, an economical, sensitive and readily available HPLC method was developed for the simultaneous estimation of Cariprazine HCl along with the degradant formed during the stress- studies which were identified and separated and further characterized using MS/MS ToF. This was the first reported method as per literature survey till date. Validation of all parameters such as linearity, accuracy, specificity, robustness, method precision was carried out and found to be within the appropriate limits. RSD values of all the required parameters were found to be less than 2%, which means that the method is valid and the results derived are in fair agreement. The preferred method could therefore easily be applied to the quantification of degradant and further can be tested for the toxicity studies of the degradant that were successfully characterized.

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Supporting Information

Supporting information accompanies this paper on https://acgpubs.org/journal/journal-of-chemical-metrology

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