



A comprehensive forced degradation studies of Cariprazine hydrochloride using LC-HRMS/MS and *in silico* toxicity predictions of its degradation products

Mohit Jain^{*}, Shahnawaz Khan

Department of Chemistry, JK Lakshmipat University, Jaipur, Rajasthan 302026, India

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ABSTRACT

Cariprazine hydrochloride is a second-generation antipsychotic drug and has been approved by the US Food and Drug Administration and the European Medicines Agency. Herein, we have reported a stability-indicating assay method for the Cariprazine hydrochloride and characterization of its major degradation products using LC-HRMS/MS. The drug substance Cariprazine hydrochloride was subjected to acid, base, oxidation, thermal and photolytic stress degradation. A total of five novel degradation products (DP-1 to DP-5) of Cariprazine hydrochloride drug substance were formed under various acid, base and oxidative conditions. *In silico* toxicity of the degradation products was evaluated using Leadscape prediction platform. We successfully separated Cariprazine Hydrochloride peak from degradation products (DP-1 to DP-5) by using gradient elution on an Inertsil C18 column (150 × 4.6 mm, 5 μm). The drug substance was found to be labile to acidic, alkaline hydrolytic, and oxidative conditions and stable to photolytic and thermal stress conditions. The degradation pathways were delineated by explaining the putative mechanism of degradation in various conditions. The stability-indicating assay method was validated according to the ICH guideline and hence can be used for routine quality control and stability study analysis of Cariprazine hydrochloride in pharmaceutical industries and research laboratories.

1. Introduction

Cariprazine hydrochloride (CAR), 3-[4-[2-[4-(2,3-dichlorophenyl)piperazin-1-yl]ethyl] cyclohexyl]–1,1-dimethylurea hydrochloride, is one of the second generation antipsychotic drug. It is widely administered for the treatment of schizophrenia, bipolar mania and bipolar depression. It acts as a D2 and D3 partial agonist receptor, however it is more selective toward the D3 receptor [1–6]. It was developed by Actavis, and is approved by the United States Food and Drug Administration (US FDA) on 17 September 2015. It is marketed as Vraylar in the United States and Reagila in Europe as tablets for oral administration.

Forced degradation of a drug molecule using chemical and environmental conditions is a widely used process to evaluate the stability of a drug molecule under the domain of ICH Q1A,B [7,8]. It assists in identifying potential degradation products and reveals information about possible degradation pathways. The presence of such degradation products in the drug substance may significantly reduce the therapeutic efficacy of a drug. Also, these impurities can contribute to overall toxicity which may ultimately lead to safety as well as regulatory

concern about the drug product. Hence, the characterization of degradation products is imperative to understand the susceptibility of the drug substance to degradation [9,10]. Potential toxicity due to degradants in the drug products can be estimated by *in silico* software such as Leadscape, Multicase, Nexus etc.

Advanced spectroscopic and hyphenated technologies enable fast and unambiguous structural characterization of degradation products. High-performance liquid chromatography (HPLC), Liquid chromatography-high resolution tandem mass spectrometry (LC-HRMS/MS) and Nuclear magnetic resonance (NMR) are the most widely used technique for characterization of the DPs. LC-HRMS/MS is one of the most efficient tools currently used for quantification as well as structural characterization of analytes at trace levels in a variety of biological matrices [11–13]. Further, the application of orbitrap, Time of flight (TOF) mass analyzers have paved a significant stride in qualitative analysis of DPs in revealing their structures. Understanding of changes in the structure of the drug molecules during the forced degradation is highly useful in the elucidation of the degradation mechanism [14–22]. The degradation profile also finds suitable storage conditions for the

^{*} Correspondence to: JK Lakshmipat University, India.

E-mail address: 1008mohit@gmail.com (M. Jain).

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corresponding drug molecule.

A comprehensive literature survey revealed few LC-HRMS/MS method has been reported for the quantification and characterization of Cariprazine hydrochloride and its active metabolites [23,24]. An HPLC method was developed and validated by Pooja *et. al.* for the determination of Cariprazine hydrochloride in bulk drugs [25]. However, to the best of our knowledge, no reported literature is available for the characterization of degradation products of Cariprazine hydrochloride and the *in silico* studies. The forced degradation of Cariprazine is quite vital for the optimization and monitoring of the manufacturing process of Cariprazine hydrochloride, quality control and to propose suitable storage conditions. In this present study, we have identified and characterized five DPs in Cariprazine hydrochloride and further assessed their toxicity through *in silico* evaluation. The optimized assay method was validated and can be used for routine testing in quality control laboratories.

2. Experimental

2.1. Materials and reagents

Cariprazine hydrochloride API (99.4%) was received from local a pharmaceutical company in mumbai india. Ammonium formate AR grade (Sigma-Aldrich, St. Louis, MO, USA), Formic acid (98%) AR grade (Sigma-Aldrich, St. Louis, MO, USA), Acetonitrile HPLC grade (Merck Life Sciences, Worli, Mumbai, India) and Methanol HPLC grade (Merck Life Sciences, Worli, Mumbai, India). The lab grade reagents, 3-chloroperoxybenzoic acid (MCPBA), Azobisisobutyronitrile (AIBN), potassium peroxymonosulfate (Oxone) and hydrochloric acid (HCl) from Spectrochem Pvt Ltd. (Bangalore, India), 30% hydrogen peroxide (H₂O₂) and sodium hydroxide (NaOH) from Sigma-Aldrich (Bangalore, India), Milli-Q water (Millipore, Bedford, MA, USA) was used throughout the experiment.

2.2. Forced degradation study

All stress sample solutions of Cariprazine hydrochloride were prepared at 400 µg/mL in a mixture of acetonitrile-water (50:50, v/v). The test samples were sonicated well and filtered through 0.22 µm membrane filters before the analysis. The summary of the degradation behavior of Cariprazine hydrochloride under different stress degradation conditions are shown in Table 1.

2.3. HPLC method

Cariprazine assay analysis was performed on a Waters Alliance e2695 HPLC system with photodiode array detector 2998 liquid chromatography using a C18 column (Inertsil ODS 3 V 5 µm, 150 mm × 4.6 mm). The chromatographic system was operated using gradient elution

Table 1
Summary of degradation behavior of CAR.

Degradation study	Degradation Conditions	DP's formed
Acid hydrolysis	5 N HCl at 80 °C for 72 h	DP-1
Base hydrolysis	5 N NaOH at 80 °C for 72 h	DP-1
Oxidative degradation	• 30%hydrogen peroxide at room temperature for 48 h	DP-2 & DP-3
	• 1.0% MCPBA at room temperature for 1 h	DP-3
	• 1% AIBN at 60 °C for 4 h	DP-1, DP-2, DP-3,
	• 5% solution of potassium peroxymonosulfate for 2 h	DP-4 & DP-5
Photolytic Degradation	UV-light and visible light of not less than 200 Wh/m ² and 1.2 million lux hr	No degradation
Thermal Degradation	90 °C for 5 days	No degradation

at ambient temperature and UV detection at 220 nm. Mobile phase A consisted of 0.1% formic acid solution and mobile phase B consisted of 0.1% formic acid solution and acetonitrile (20:80, v/v) with a timed gradient mode: 0–3 min: 15% B; 3 – 15 min: 15–70% B; 15–20 min: 70–90% B; 20–20.1 min: 90 – 15% B; 20.1–25 min: 15% B, respectively with the flow rate of 1.0 mL/min. The injection volume was 10 µL and the sample concentration was 400 µg/mL, prepared in the diluent.

2.4. LC-MS method

LC-HRMS/MS studies were performed on Vanquish UHPLC(Thermo, San Jose, USA) instrument coupled with Q Exactive Plus Orbitrap High-resolution mass spectrometry(Thermo, San Jose, USA) and equipped with electrospray ionization (ESI) source. The data acquisition was done using Xcalibur software. The following instrument settings were applied during analysis: sheath gas at 35 arb, auxiliary gas at 10 arb, spray voltage at + 3.2 kV in positive mode, the capillary temperature at 320 °C, auxiliary gas heater temperature at 350 °C, s-lens RF level at 60%.

2.5. In silico prediction of Cariprazine hydrochloride and its DPs

Toxicity of DPs was predicted and compared with Cariprazine hydrochloride with Leadscape® model Applier 3.1.0–40 computational platform. This software package works in bacterial mutagenicity models based on the experimental data from the Ames test. The models cover both expert-rule and statistical methodologies for complete ICH M7 compliance. The software deduced the toxic potentiality of DPs by incorporating different aspects of the chemical entity and developed a comparative statistical analogy with molecules having known toxicity [26].

3. Results and discussion

To develop a stability-indicating assay method, chromatographic conditions were optimized to achieve the separation of drug from DPs. Initial systematic trials were taken to improve the peak shape and resolution between Cariprazine hydrochloride and DPs. To start a method development, it is generally important to know the physicochemical properties of the molecule like pKa, solubility, absorptivity and wavelength maxima of the drug. pKa value of the drug is important as most of the changes in retention occur at pH values within ± 1.5 units of the pKa value. As Cariprazine hydrochloride is a mid-polar, basic molecule having a pKa value of 7.9 so, the initial trial was taken in 0.1% formic acid as a mobile phase. Different types of C18 columns and gradients were evaluated. The effect of mobile phase B composition shows good separations between Cariprazine hydrochloride and DPs as shown graphically in Fig.S1. Finally, after several optimization trials, a good symmetrical peak and resolution were observed on Inertsil ODS 3 V(150 × 4.6 mm, 5 µm) column. The column was thermo-stated at 25 °C to avoid any shift in retention time. The gradient elution program was employed with a mobile phase-A containing 0.1% formic acid solution and mobile phase-B containing 0.1% formic acid solution and acetonitrile (20:80, v/v). The gradient program was set as follows: 0–3 min: 15% B; 3 – 15 min: 15–70% B; 15–20 min: 70–90% B; 20–20.1 min: 90 – 15% B; 20.1–25 min: 15% B, with the flow rate of 1.0 mL/min. All the chromatograms were processed and analyzed at the wavelength of 220 nm. The same method was transferred for the characterization of degradation products by LC-ESI-HR-MSⁿ studies, by optimizing all the MS parameters as discussed in Section 2.3.

3.1. Degradation behaviour of Cariprazine hydrochloride

The degradations of Cariprazine hydrochloride were performed in acidic, basic, oxidative, thermal and photolytic conditions. It was found to degrade in acidic, basic and oxidative conditions, while stable in photolytic as well as thermal conditions. A total of five DPs were

observed from the drug, in which DP-1 was formed in acidic and basic degradation. DP-1 to DP-5 were formed in oxidative conditions. The overlay of HPLC chromatograms of all the degradation samples is given in Fig. 1. The drug was found stable in initial hydrolytic degradation condition at 2 N NaOH and 2 N HCl at 80 °C for 24 hrs. After increasing the strength and time interval of acid and base, DP-1 was formed in 5 N NaOH and 5 N HCl at 80 °C for 72 h. Oxidative degradations were carried out using hydrogen peroxide, 3-chloroperoxybenzoic acid, azobisisobutyronitrile and potassium peroxydisulfate to study the degradation behavior of the drug substance under the influence of different oxidative reagents [27–29]. The results are graphically represented in Fig.S2. In 30% Hydrogen peroxide at room temperature for 48 h, we found major degradation DP-2 and minor DP-3, whereas treated with 1% 3-chloroperoxybenzoic acid at room temperature for 1 h, we found DP-2 as a major degradation and the same observation was found in 1% azobisisobutyronitrile at 60 °C for 4 h and the overlay chromatogram as shown in Fig.S3. But while treating with a 5% solution of potassium peroxydisulfate for 2 h, it resulted in five major DP-1 to DP-5 impurities. The mass balance results are shown in Table S1.

3.2. Mass fragmentation behaviour of Cariprazine hydrochloride and its DPs

The MS/MS spectra of Cariprazine hydrochloride and its DPs were recorded and the mass fragmentation pathways have been established as shown in Scheme 1 to Scheme 6. The mass fragmentation behavior of Cariprazine hydrochloride was delineated and the structures of the fragment ions were characterized based on accurate mass measurement. Additionally, the respective fragmentation behaviors of DPs were

compared to that of Cariprazine hydrochloride, which enabled us to identify the structural modification during degradation. The High-resolution mass spectrometric data of Cariprazine hydrochloride and its DPs were summarized in Table S2.

3.3. LC-HRMS/MS of Cariprazine hydrochloride

The elemental composition and fragmentation pathway of the drug was established by using LC-HRMS/MS. The protonated Cariprazine hydrochloride showed a peak at RT 15.6 min with m/z 427.2021 $[M+H]^+$ and a dichlorine isotopic pattern in the mass spectrum (Fig. S4a). The fragmentation spectrum of drug showed product ion at m/z 382.1441, m/z 339.1385, m/z 243.0452, m/z 231.0449, m/z 195.1490, m/z 188.0026, m/z 89.0709 and 72.0444. The first daughter ion began with the loss of C_2H_7N (dimethylamine) from m/z 427.2021, forming a fragment ion at m/z 382.1441 in the MS/MS spectrum, which on the subsequent loss of CHNO (isocyanic acid) led to fragment at m/z 339.1385. The fragment ion at m/z 243.0452 was formed due to the loss of C_7H_{12} from m/z 339.1385. The fragment ion at m/z 231.0449 could be formed in three ways i.e. loss of $C_8H_{14}Cl_2N$ from m/z 427.2021, loss of $C_9H_{13}NO$ from m/z 382.1441 or loss of C_8H_{12} from m/z 339.1385. The fragment ion at 188.0026 was formed after the elimination of C_3H_5N from m/z 243.0452. As there was no chlorine isotopic pattern in the fragment of m/z 195.1490, it could be due to the loss of $C_8H_7Cl_2N$ from m/z 382.1441. The fragment ion at m/z 89.0709 was formed as a result of the loss of $C_{18}H_{24}Cl_2N_2$ from m/z 427.2021. The fragment ion at m/z 72.0444 was formed due to the loss of NH_3 from m/z 89.0709. The fragmentation pathway of Cariprazine hydrochloride is illustrated in Scheme 1.

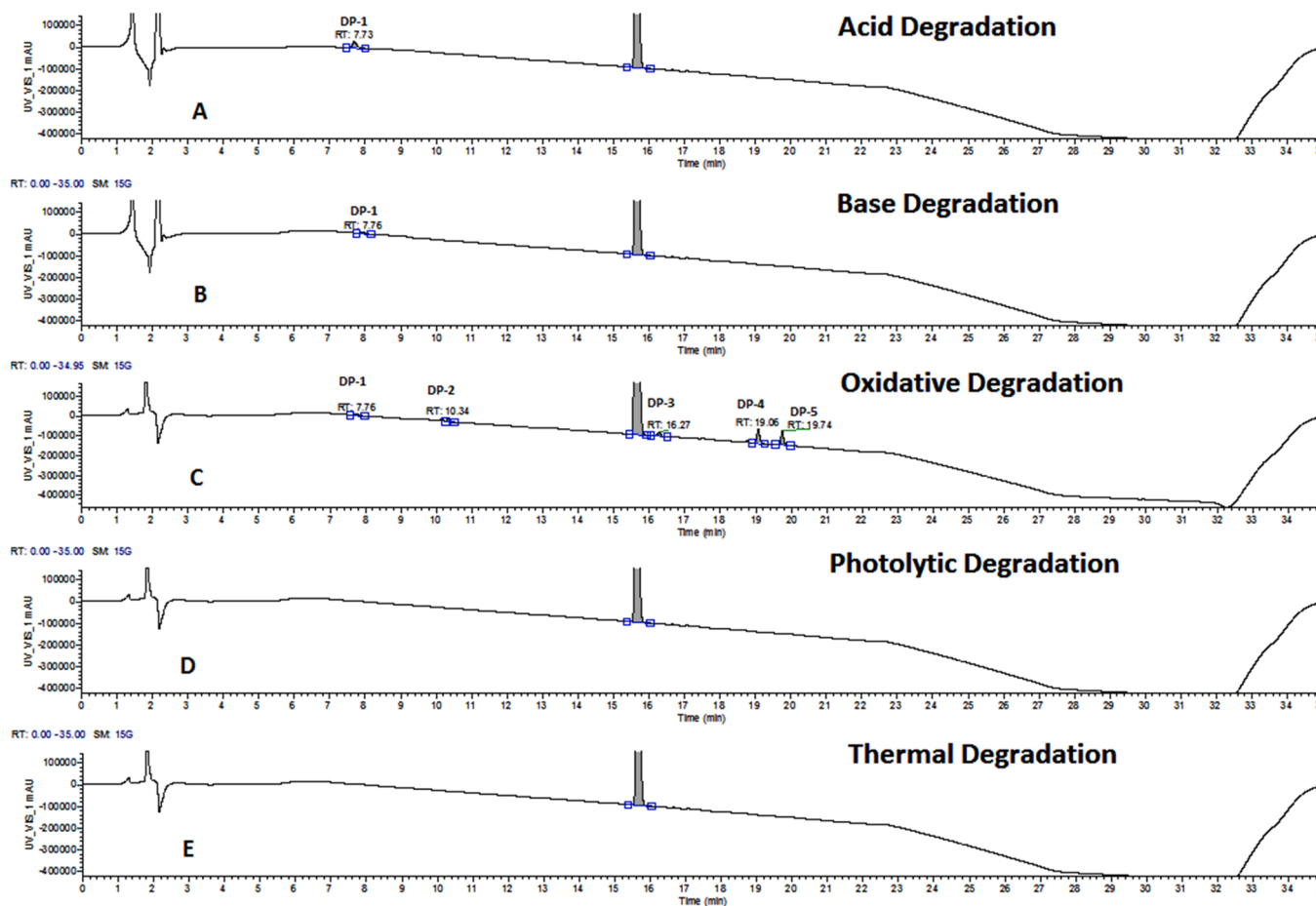
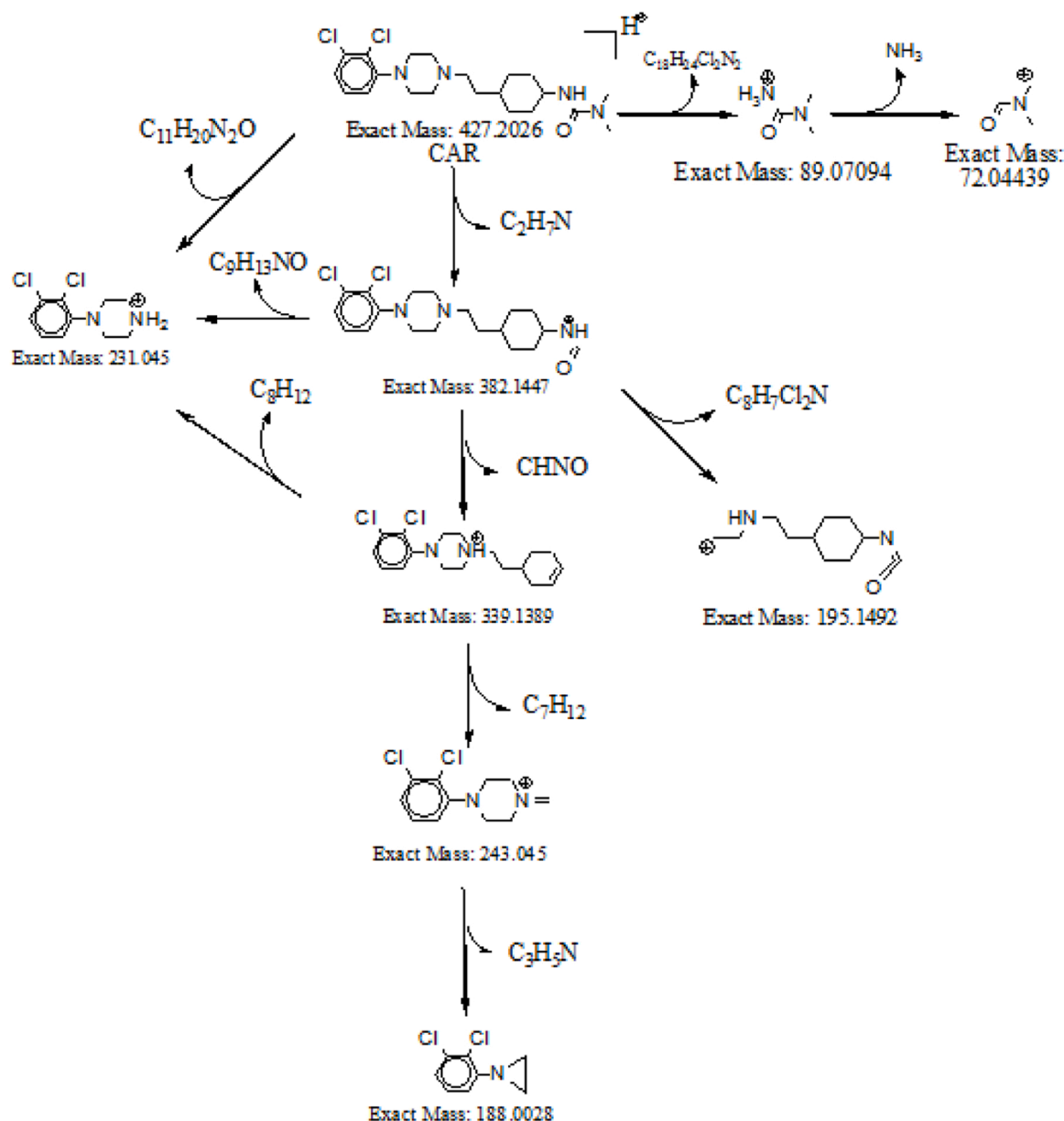


Fig. 1. Overlay Chromatogram of forced degradation samples a) 2 N HCl at 80 °C for 24 hrs, b) 2 N NaOH at 80 °C for 24 hrs, c) 5% solution of potassium peroxydisulfate for 2 h, d) 1.2 million lux hrs and 200 Wh/m², e) 90 °C for 5 days.



Scheme 1. Proposed mass fragmentation behavior of CAR.

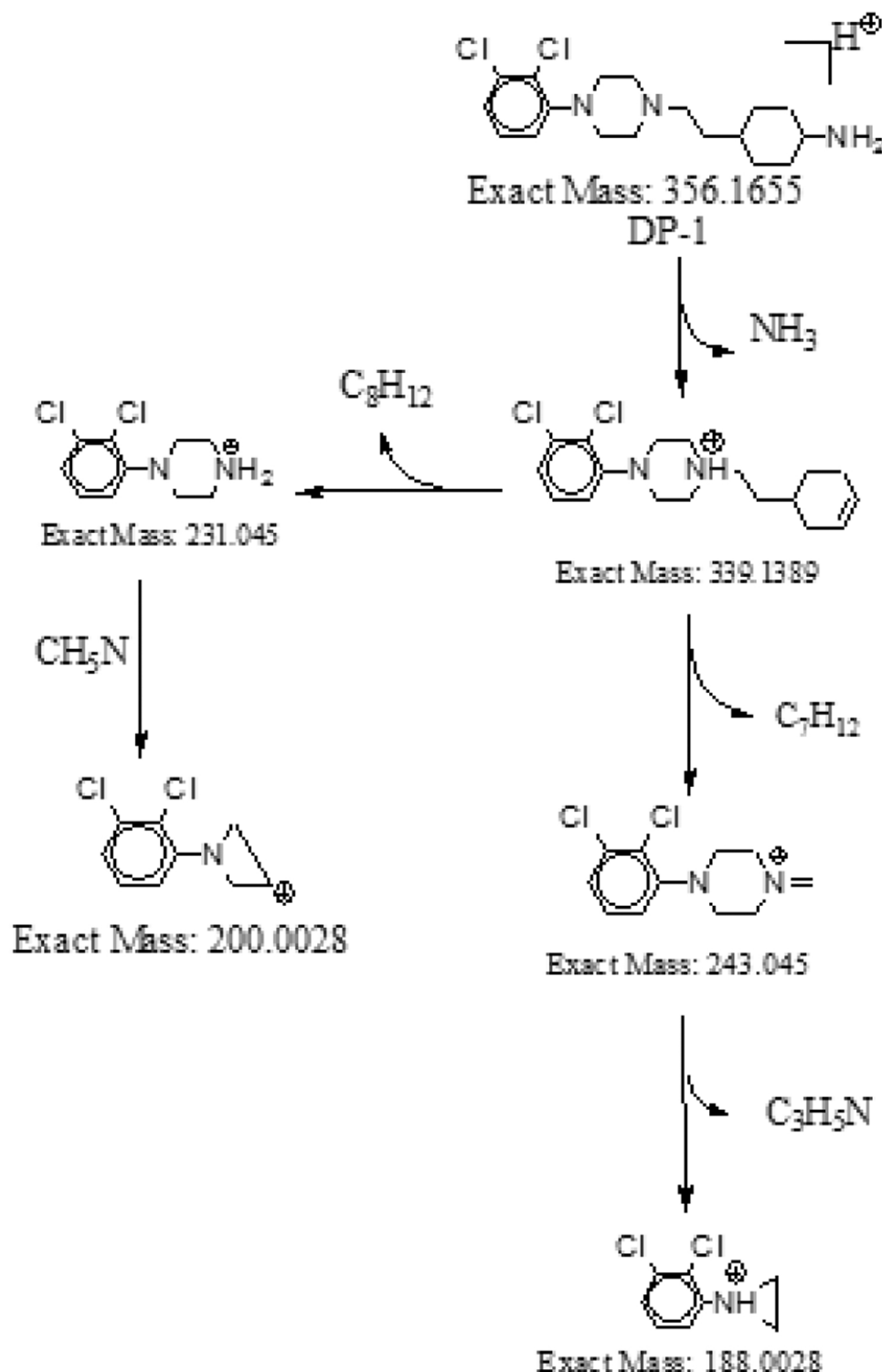
3.4. DP-1

DP-1 was obtained due to the hydrolysis of Cariprazine hydrochloride in acid, base and oxidative condition with the protonated peak at RT 7.73 min with an m/z 356.1651. The fragmentation spectrum of DP-1 showed product ion at m/z 339.1385, m/z 243.0448, m/z 231.0448 and m/z 188.0028 which were common fragments ion in Cariprazine hydrochloride (Fig. S4b). Based on the above data DP-1 was identified as 4-(2-(4-(2,3-dichlorophenyl)piperazin-1-yl)ethyl) cyclohexanamine, with a chemical formula of $C_{18}H_{28}Cl_2N_3$ and mass error of -1.12 ppm. The fragmentation pathway of DP-1 is illustrated in Scheme 2.

3.5. DP-2

DP-2 was formed in oxidative stress degradation with the peak at RT 10.34 min and protonated m/z 459.1920. There is a difference of 31.9899 Da between the drug and the DP-2 structure, indicating the existence of two oxygen atoms in the DP-2 structure. It was also fragmented to generate product ions at m/z 442.1891, m/z 423.1707, m/z

378.1129, m/z 335.1071, m/z 241.0291, m/z 226.0056, m/z 201.0105, m/z 188.0026, m/z 89.0709, and m/z 72.0444 (Fig. S4c). The daughter ion m/z 442.1891 was generated by the loss of OH from m/z 459.1920 in the MS/MS spectrum. In the MS/MS spectrum of m/z 459.1920, it can be seen that N-oxide loses the OH radical rather rapidly, giving rise to $[M+H-OH]^{++}$ ions. The loss of 2 H_2O from m/z 459.1920 resulted in the formation of the fragment ion m/z 423.1707, indicating that DP-2 is a Di N-oxide breakdown product generated by N-oxidation on both nitrogens of the piperazine ring of Cariprazine hydrochloride [30–33]. Furthermore, m/z 378.1129 (Loss of C_2H_7N from 423), m/z 335.1071 (Loss of CHNO from 378), m/z 241.0291 (Loss of C_2H_7N from 423), m/z 335.1071 (Loss of CHNO from 378), m/z 241.0291 (Loss of C_7H_{10} from 335). The loss of the C_3H_3N , C_2H_2N radical and methyl radical resulted in the formation of the three fragments m/z 226.0056, m/z 201.0105 and m/z 188.0026 respectively, from the m/z 241.029. The fragment ions m/z 89.0709 and m/z 72.0444, both of which were common in Cariprazine hydrochloride, indicated that the dimethylurea functional group is intact. Based on the above data DP-2 is identified as 1-(2,3-dichlorophenyl)-4-(2-((dimethylcarbamoyl)amino) cyclohexyl)



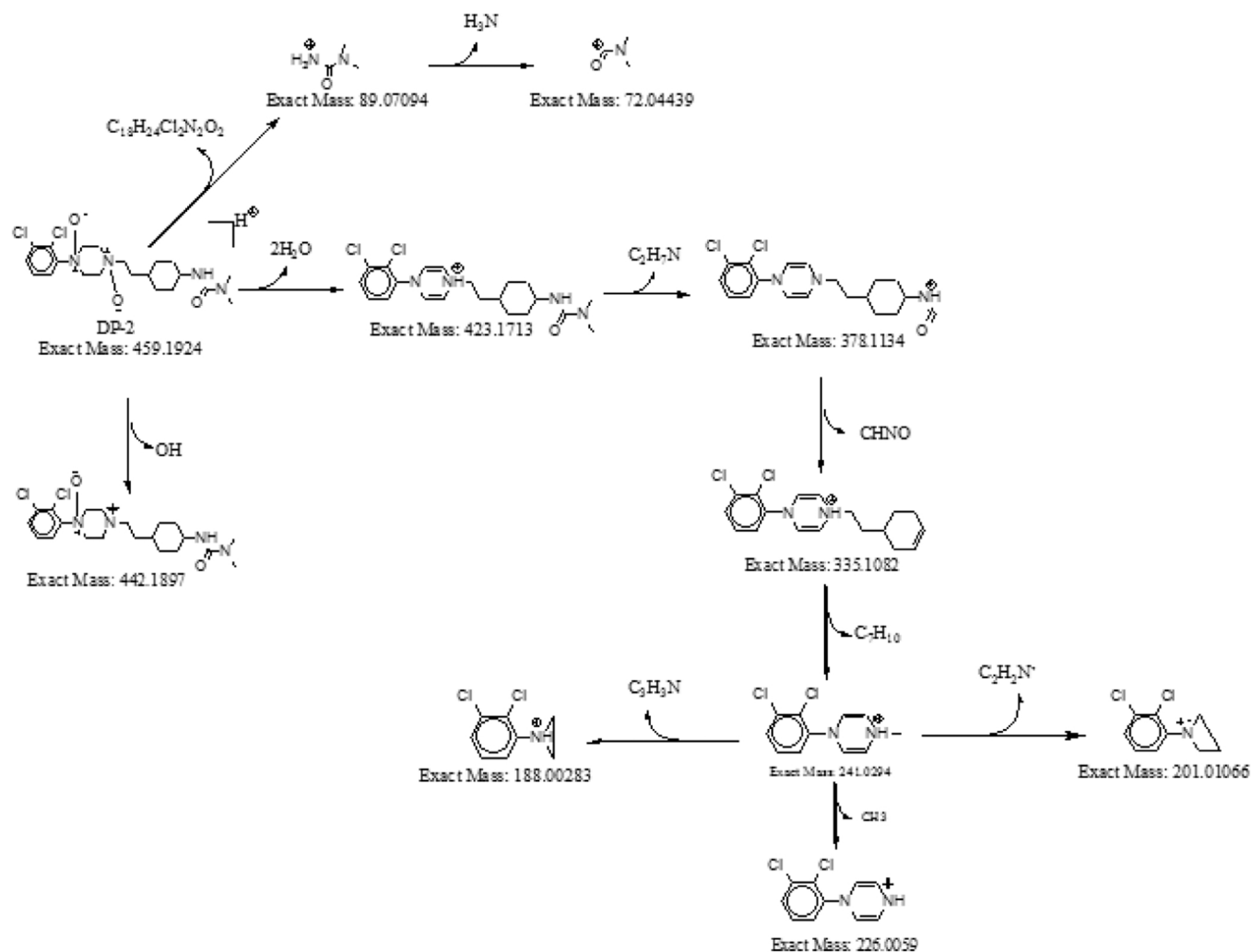
Scheme 2. Proposed mass fragmentation behavior of DP-1.

ethyl) piperazine-1,4-dium-1,4-bis(olate), with a chemical formula of $\text{C}_{21}\text{H}_{32}\text{Cl}_2\text{N}_4\text{O}_3$ with a mass error of -0.87 ppm. The fragmentation pathway of DP-2 is illustrated in [Scheme 3](#).

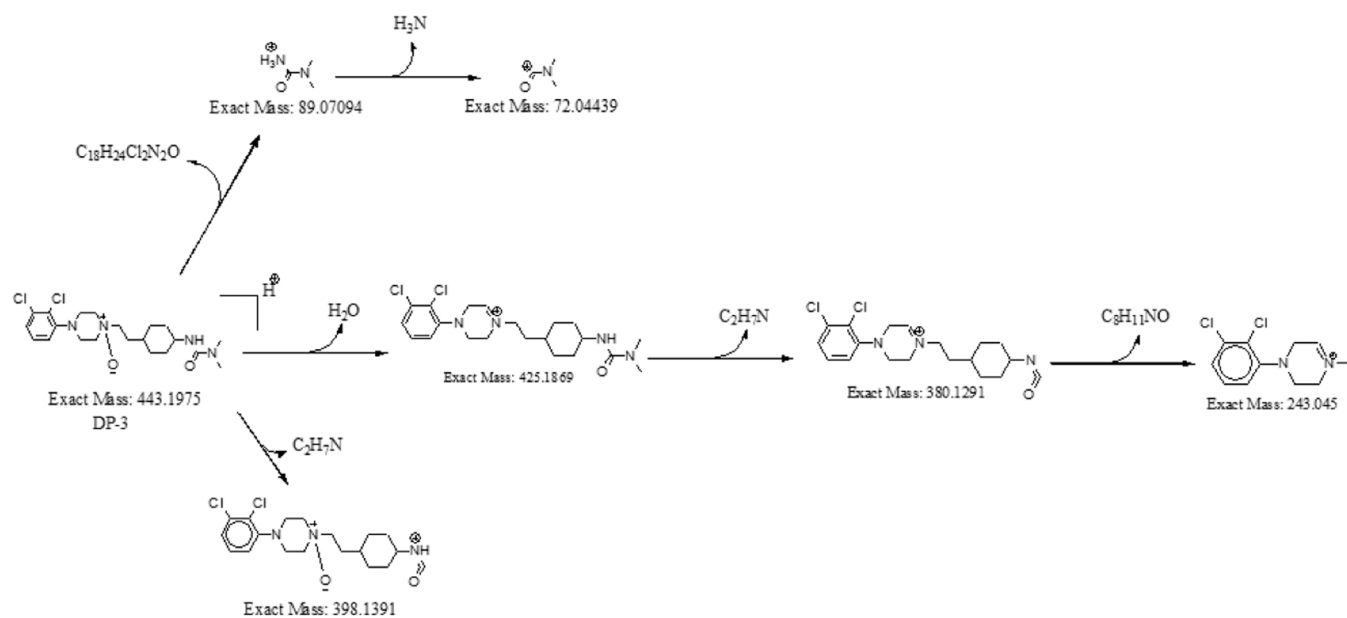
3.6. DP-3

DP-3 was formed in oxidative stress degradation with a peak at RT 16.3 min and protonated m/z 443.1972. It was observed that 15.9951 Da has a higher molecular mass than the drug, implying that

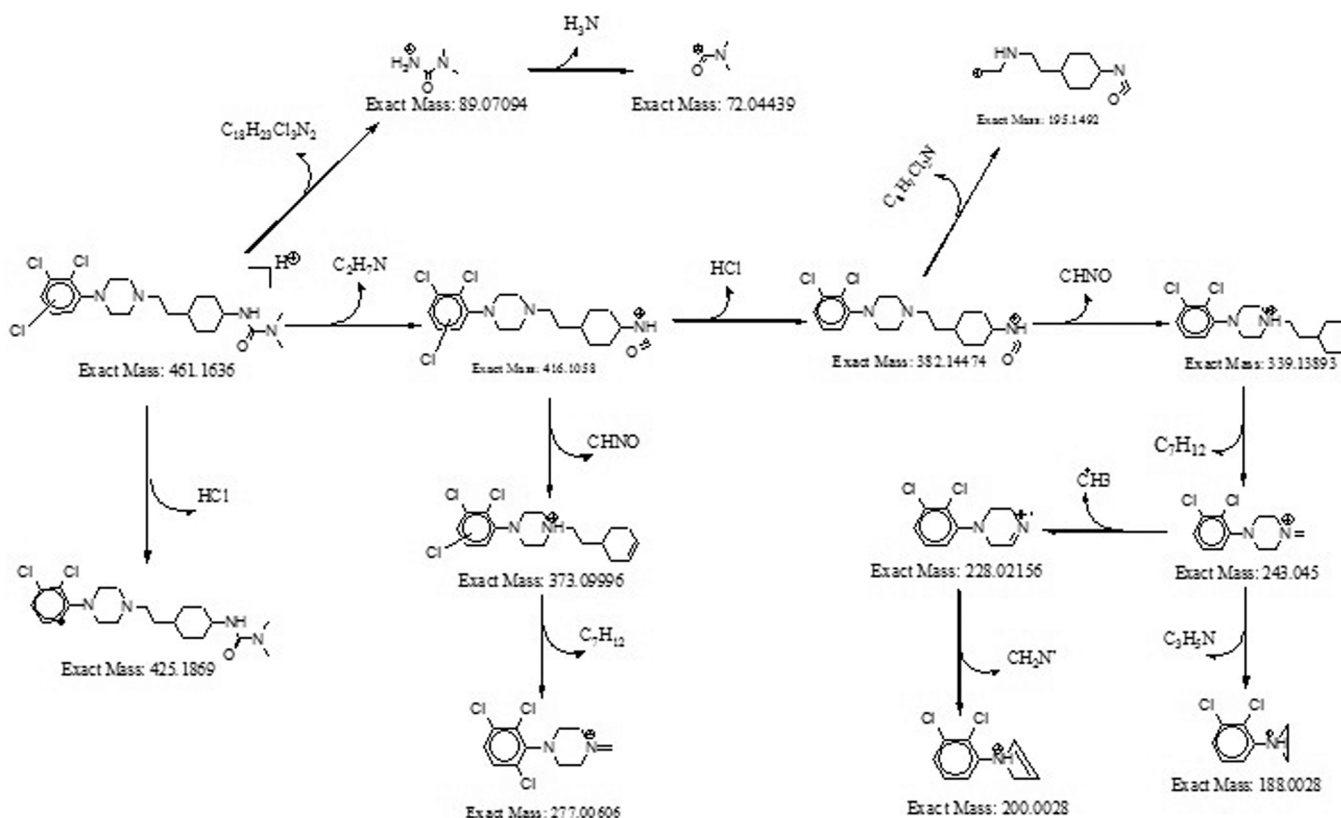
oxygen atoms are present in the DP-3 structure. The fragmentation spectrum of DP-3 showed product ion at m/z 425.1863, m/z 398.1396, m/z 380.1289, m/z 243.0448 m/z 89.0709, and m/z 72.0443 (Fig.S4d). The m/z 443.1972 began with the three fragmentation of m/z 425.1863, m/z 398.1391 and m/z 89.0709 due to the loss of H_2O (water), $\text{C}_2\text{H}_7\text{N}$ (dimethylamine) and $\text{C}_{18}\text{H}_{24}\text{Cl}_2\text{N}_2\text{O}$ respectively. While the second fragment was formed by the loss of $\text{C}_2\text{H}_7\text{N}$ (dimethylamine) at m/z 380.1289 from m/z 425.1863 and m/z 243.0448 (loss of $\text{C}_8\text{H}_{11}\text{NO}$ from m/z 380). The fragment ions m/z 89.0709 and m/z 72.0443 both of



Scheme 3. Proposed mass fragmentation behavior of DP-2.



Scheme 4. Proposed mass fragmentation behavior of DP-3.



Scheme 5. Proposed mass fragmentation behavior of DP-4.

which are common in Cariprazine hydrochloride, indicated that the dimethylurea functional group is intact. Based on the above data DP-3 is identified as 4-(2,3-dichlorophenyl)-1-(2-{4-[(dimethyl carbamoyl)amino]cyclohexyl}ethyl)piperazin-1-ium-1-olate with a chemical formula of $C_{21}H_{32}Cl_2N_4O_2$ with a mass error of -0.68 ppm. The fragmentation pathway of DP-3 is illustrated in Scheme 4.

3.7. DP-4

DP-4 was formed in oxidative stress degradation with a peak at RT 19.0 min having protonated m/z 461.1629 and a characteristic trichloro isotopic pattern. It was observed that DP-4 has 33.9608 Da more mass than the Cariprazine hydrochloride, which indicates the presence of chlorine atoms in the DP-4 structure. To know the position of the chlorine in Cariprazine hydrochloride, fragmentation were performed and critically assessed product ions at m/z 425.1863, m/z 416.1054, m/z 382.1441, m/z 373.0994, m/z 339.1383, m/z 277.0058, m/z 243.0446, m/z 228.0212, m/z 200.0026, m/z 195.1490, m/z 188.0025, m/z 89.0708 and m/z 72.0443 (Fig.S4e). The first began with the formation of two fragments of m/z 425.1863 and m/z 416.1054 from m/z 461.1629 due to the loss of $Cl^\cdot$ (chlorine radical) and C_2H_7N dimethylamine, respectively [33]. Trichloro isotopic pattern was observed in m/z 416.1629, which was fragmented into two fragments of m/z 382.1441 and m/z 373.0984, formed due to the loss of HCl (Hydrochloride) and CHNO isocyanate, respectively. Whereas, m/z 373.0984 fragmented into m/z 277.0058 with the loss of C_7H_{12} and having a trichloro isotopic pattern, which indicated that the dimethyl urea functional group lacks a chlorine atom. Further, fragments ion m/z 382.1441, m/z 339.1383, m/z 243.0446, m/z 195.1490, m/z 188.0025, m/z 89.0708, m/z 72.0443 are also common in Cariprazine hydrochloride and indicated that the chlorine atom is attached to the dichlorobenzene ring. Based on the above data, DP-4 is identified as 3-(4-(2-(4-(2,3,6-trichlorophenyl)piperazin-1-yl)ethyl)cyclohexyl)-1,1-dimethylurea, with chemical

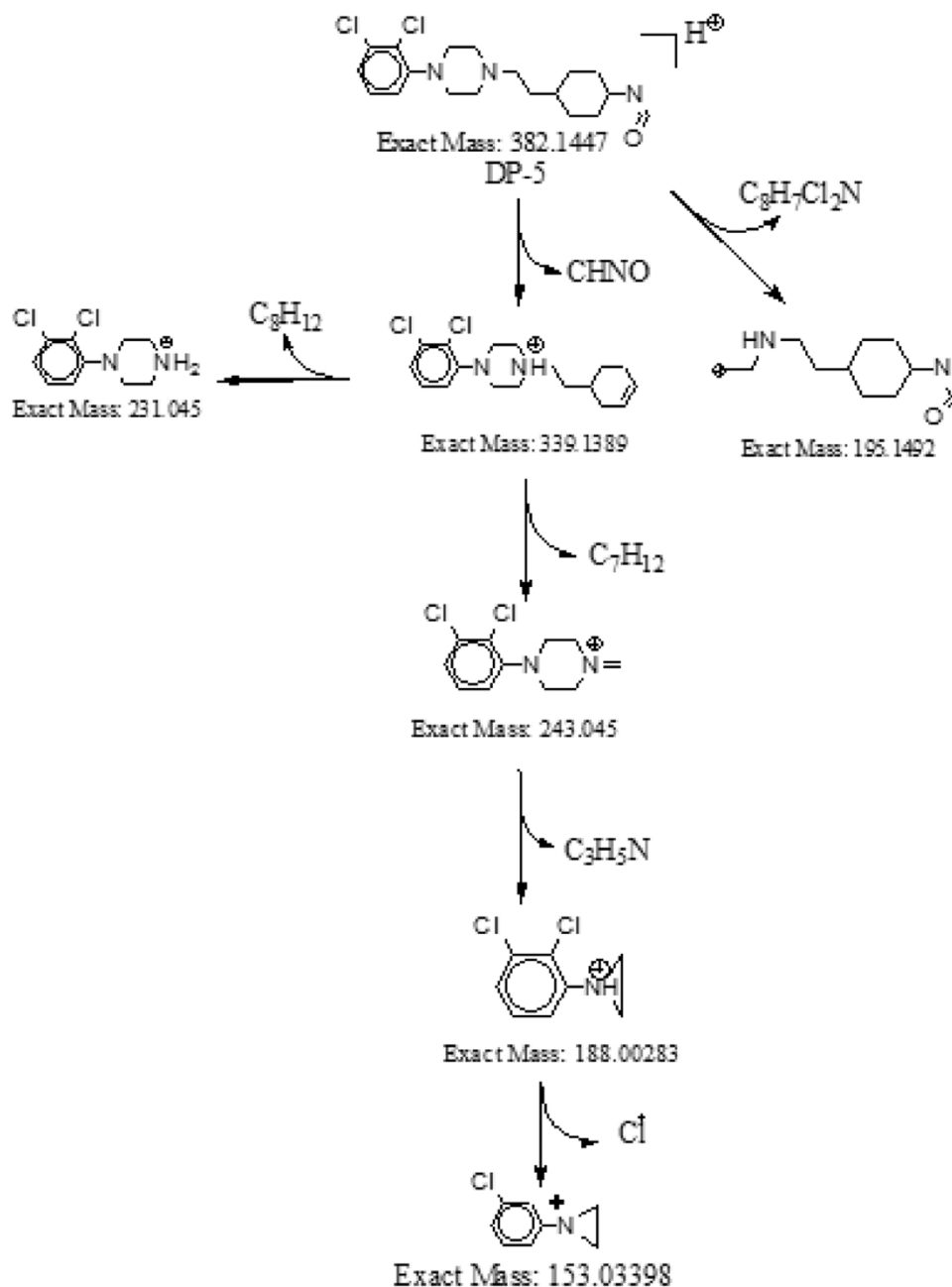
formula of $C_{21}H_{31}Cl_3N_4O$ with a mass error of -1.52 ppm. The fragmentation pathway of DP-4 is illustrated in Scheme 5.

3.8. DP-5

DP-5 was formed in oxidative stress degradation with a peak at RT 19.7 min having a protonated m/z 382.1443. The mass difference of 45.0578 Da compare to the drug indicates DP-5 was formed by the loss of the dimethylamine group from the drug. Further, it was fragmented to give product ions at m/z 339.1385, m/z 243.0448, m/z 231.0447, m/z 195.1490 and m/z 188.0027 which are common fragments ion in Cariprazine hydrochloride (Fig. S4f). Based on the above data DP-5 is identified as 1-(2,3-dichlorophenyl)-4-(2-(4-isocyanatocyclohexyl)ethyl)piperazine, with a chemical formula of $C_{19}H_{25}Cl_2N_3O$ with a mass error of -1.05 ppm. The fragmentation pathway of DP-5 is illustrated in Scheme 6.

3.9. Degradation pathway of the drug

The outcome of stress study result shows that oxone was oxidising the Hydrochloride (HCl) salt of Cariprazine hydrochloride to chlorine ($Cl^\cdot$) radical which substituted the hydrogen atom of aromatic ring via aromatic radical substitution reaction to give DP-4 [34]. Under the same degradation conditions, Cariprazine hydrochloride underwent deprotonation at amine (NH) group of urea to form crossponding isocyanate species with elimination of dimethylamine [35]. This species generated an amine product (DP-1) via formation of unstable carbamic acid with water. Further, degradation of urea functionality of compound like Cariprazine hydrochloride to corresponding amine (DP-1) under acidic/basic condition [36] is also known. As tertiary amine moieties are prone to oxidation to form N-oxide [30]. Oxone mediated oxidation of tertiary amine center of piperazine ring in Cariprazine hydrochloride to mono-oxidized DP-3 was found. The further oxidation of other N-center



Scheme 6. Proposed mass fragmentation behavior of DP-5.

of DP-3 with the oxone lead to dioxidized DP-2. The plausible mechanism of DP-1 to DP-5 is shown in Fig. S5.

3.10. Method validation

The proposed SIAM (stability-indicating assay method) was validated as per ICH guidelines [37] in terms of selectivity, linearity, accuracy, precision, robustness and solution stability. The specificity of the method has been determined by the forced degradation of Cariprazine hydrochloride. The peak purity evaluation was carried out by using a PDA detector and confirmed that the Cariprazine hydrochloride peak was homogenous and pure in all the analyzed stress samples, thus demonstrating the specificity of the method. Linearity of the method was evaluated at five concentration levels from 80% to 120% of Cariprazine hydrochloride concentrations. The linearity was satisfactorily demonstrated with a five point calibration graph between 80% and 120% of the

Cariprazine hydrochloride concentration. The slope, intercept and regression coefficient values were determined by the least squares linear regression analysis. The accuracy of the method was evaluated in triplicate using three concentration levels at 80%, 100% and 120% i.e. ($\pm 20\%$ specification level). The % recovery and % RSD for Cariprazine hydrochloride were calculated. The precision of the method was verified by repeatability and intermediate precision. Repeatability was checked by injecting six individual preparations of Cariprazine hydrochloride sample solution at 100% concentrations. The % RSD of an area of Cariprazine hydrochloride was calculated. The intermediate precision of the method was also evaluated using different analyst and performing the analysis on different days. Solution stability of the sample solution was established by analyzing the sample solution at different time intervals at room temperature (25°C). The robustness of the method was studied with deliberate alteration in the flow rate of the mobile phase and column temperature. The optimized flow rate of the mobile phase was

1.0 mL/min and the same was altered by ± 0.2 units i.e. from 0.8 mL/min to 1.2 mL/min. The effect of column oven temperature on the resolution was studied at 23 °C and 27 °C instead of room temperature (25 °C). Method validation results are summarized in Table S3.

3.11. In silico toxicity evaluation

Leadscope software was used in the present investigation for an evaluation of the toxicity of the impurities and active pharmaceutical ingredients. Table S4 lists the toxicity predictions for the degradation products DP-1 to DP-5. The software predicted the negative values of mutagenicity for these structures. Thus, these five DPs were expected not to have any mutagenicity.

4. Conclusion

Forced degradation is an important part of the drug development process as it provides critical information about the degradation chemistry of drug substances. In this context, the present study presents five novel degradation products of Cariprazine hydrochloride and their identification by LC-HRMS/MS. Acidic and basic conditions furnished amine degradation product of urea functionality (DP-1). N-oxidative impurities are formed (DP-2 & DP-3) with oxidizing agents 3-chloroperoxybenzoic acid, hydrogen peroxide and azobisisobutyronitrile. Interestingly, Oxone furnished not only different N-oxides, halogenated impurities of Cariprazine hydrochloride but also isocyanate and amine-based impurities (DP-1 to DP-5). Further, the toxicity of Cariprazine hydrochloride and its DPs were predicted by Leadscope software, which indicated that all the DPs were non-mutagenic. The HPLC method was also validated according to current ICH guidelines and is evidently selective, linear, precise, accurate and robust within the validated range. This method can be used conveniently in pharmaceutical analysis for stability monitoring and routine quality control samples.

CRedit authorship contribution statement

Mohit Jain: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, **Shahnawaz Khan:** Conceptualization, Investigation, Project administration, Supervision, Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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The authors like to express their gratitude to IIT SAIF, Bombay, for conducting HRMS analysis.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jpba.2022.114955](https://doi.org/10.1016/j.jpba.2022.114955).

References

- [1] Leslie Citrome, Cariprazine for the treatment of schizophrenia: a review of this dopamine D3-preferring D3/D2 receptor partial agonist, *Clin. Schizophr. Relat. Psychoses* 10 (2) (2016) 109–119.
- [2] R.H. Campbell, M. Diduch, K.N. Gardner, C. Thomas, Review of cariprazine in management of psychiatric illness, *Ment. Health Clin.* 7 (5) (2018) 221–229.
- [3] A. Fagioli, J.A. Alcalá, T. Aubel, et al., Treating schizophrenia with cariprazine: from clinical research to clinical practice. Real world experiences and recommendations from an International Panel, *Ann. Gen. Psychiatry* 19 (2020) 55.
- [4] Williea Earley, Suresha Durgam, Kaifenga Lu, István Laszlovsky, Marcc Debelles, John Kane, M.B Safety and tolerability of cariprazine in patients with acute exacerbation of schizophrenia, *Int. Clin. Psychopharmacol.* 32 (6) (2017) 319–328.
- [5] Highlights of prescribing information for VRAYLAR™ (cariprazine) capsules. https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/2043701bl.pdf.
- [6] Leslie, Citrome (Clinical Professor of Psychiatry & Behavioral Sciences) Cariprazine: chemistry, pharmacodynamics, pharmacokinetics, and metabolism, clinical efficacy, safety, and tolerability, *Expert Opin. Drug Metab. Toxicol.* 9 (2) (2013) 193–206.
- [7] ICH guideline, Q1A (R2) Stability Testing of New Drug Substances and Products, 21 International Conference on Harmonisation, IFPMA, Geneva, Switzerland, 2000.
- [8] ICH guideline, Q1B Photostability Testing of New Drug Substances and Products, 19 International Conference on Harmonisation, IFPMA, Geneva, Switzerland, 1996.
- [9] P. Sengupta, B. Chatterjee, R.K. Tekade, Current regulatory requirements and practical approaches for stability analysis of pharmaceutical products: a comprehensive review, *Int. J. Pharm.* 543 (1–2) (2018) 328–344.
- [10] M. Narayanan, et al., Critical practical aspects in the application of liquid chromatography–mass spectrometric studies for the characterization of impurities and degradation products, *J. Pharm. Biomed. Anal.* (2013), <https://doi.org/10.1016/j.jpba.2013.04.027>.
- [11] M.W. Attwa, A.A. Kadi, H. Alrabiah, H.W. Darwish, LC–MS/MS reveals the formation of iminium and quinone methide reactive intermediates in entrectinib metabolism: in vivo and in vitro metabolic investigation, *J. Pharm. Biomed. Anal.* 160 (2018) 19–30.
- [12] M.W. Attwa, A.A. Kadi, H.W. Darwish, Belizatinib: Novel reactive intermediates and bioactivation pathways characterized by LC–MS/MS, *J. Pharm. Biomed. Anal.* 171 (2019) 132–147.
- [13] M.W. Attwa, A.A. Kadi, H.W. Darwish, H. Alrabiah, LC-HRMS/MS reveals the formation of reactive ortho-quinone and iminium intermediates in saracatinib metabolism: phase I metabolic profiling, *Clin. Chim. Acta* 482 (2018) 84–94.
- [14] Sahu A.K., Goswami A., Kate A.S., Sengupta P., Identification and structural characterization of potential degraded impurities of ribociclib by time of flight–tandem mass spectrometry, and their toxicity prediction, *J. Pharm. Biomed. Anal.*, doi: <https://doi.org/10.1016/j.jpba.2021.113933>.
- [15] M. Narayanan, T. Handa, P. Sharma, S. Jhaja, P.K. Muthe, P.K. Dappili, R.P. Shah, S. Singh, Critical practical aspects in the application of liquid chromatography–mass spectrometric studies for the characterization of impurities and degradation products, *J. Pharm. Biomed. Anal.* 87 (2014) 191–217.
- [16] M.K. Sharma, R.P. Shah, P. Sengupta, Amalgamation of stress degradation and metabolite profiling in rat urine and feces for characterization of oxidative metabolites of flibanserin using UHPLC–Q–TOF–MS/MS, H/D exchange and NMR technique, *J. Chromatogr. B* 1139 (2020), 121993.
- [17] R.A. Zubarev, A. Makarov, Orbitrap mass spectrometry, *Anal. Chem.* 85 (11) (2013) 5288–5296, doi: [10.1021/ac4001223](https://doi.org/10.1021/ac4001223).
- [18] VakamullaMalati, AnumalaRaghupati Reddy, K. Mukkanti, M.V. Suryanarayana, A novel reverse phase stability indicating RP-UPLC method for the quantitative determination of fifteen related substances in Ranolazine drug substance and drug product, *Talanta*, <https://doi.org/10.1016/j.talanta.2012.03.067>.
- [19] Matheus Wagner Lago, MarianeLago Friedrich, Gabrielle Dinecklopp, ThiagoBelarmino de Souza, Paola de Azevedo Mello and Andréa Inês Horn Adams, Capillary zone electrophoresis method to assay tipranavir capsules and identification of oxidation product and organic impurity by quadrupole-time of flight mass spectrometry, *Talanta*, <https://doi.org/10.1016/j.talanta.2018.01.012>.
- [20] Marcelo DonadelMalesuik, HeloisaMazotti Lopes Gonçalves, Cássia Virginia Garcia, Márcia Rodrigues Trein, Nance Beyer Nardi, Elfrides Eva SchermanSchapoval, Martin Steppe, Identification, characterization and cytotoxicity in vitro assay of nitazoxanide major degradation product, *Talanta* Volume 93 (2012). Pages 206–211, ISSN 0039-9140, <https://doi.org/10.1016/j.talanta.2012.02.014>.
- [21] Prakash Niguram&Abhijeet S. Kate, Structural characterization of forced degradation products of empagliflozin by high resolution mass spectrometry, *Journal of Liquid Chromatography & Related Technologies*, 42:13–14, 417–428, DOI: [10.1080/10826076.2019.1625368](https://doi.org/10.1080/10826076.2019.1625368).
- [22] Shruti Chopra, Ann Van Schepdael, Jos Hoogmartens, Erwin Adams, Characterization of impurities in tylosin using dual liquid chromatography combined with ion trap mass spectrometry, *Talanta* Volume 106 (2013) 29–38. ISSN 0039-9140. <https://doi.org/10.1016/j.talanta.2012.12.008>.
- [23] G. Mészáros, Éva Ágai-Csongor Pásztor, Margit Kapás, Sensitive LC-HRMS/MS methods for the quantification of RGH-188 and its active metabolites, desmethyl- and didesmethyl-RGH-188 in human plasma and urine, *J. Pharm. Biomed. Anal.* 48 (2008) 388–397, <https://doi.org/10.1016/j.jpba.2007.12.016>.
- [24] Kiss B., Némethy Z., Fazekas K., Kurkó D., Gyertyán I., Sághy K., Laszlovsky I., Farkas B., Kirschner N., Bolf-Terjéki E., Balázs O., Lendvai B., Preclinical pharmacodynamic and pharmacokinetic characterization of the major metabolites of cariprazine, *Drug Des. Devel. Ther.* 2019, 13, 3229–3248. <https://doi.org/10.2147/DDDT.S188760>.
- [25] Pooja Tiwari, Purnima D. Hamrapurkar, Development and validation of a stability indicating RP-HPLC method for determination of cariprazine in bulk drug, *World J. Pharm. Res.* 9 (4) (2020), <https://doi.org/10.20959/wjpr20204-16991>.

- [26] Catrin Hasselgren, Joel Bercu, Alex Cayley, Kevin Cross, Susanne Glowienke, Naomi Kruhlak, Wolfgang Muster, John Nicolette, M. Vijayaraj Reddy, Roustem Saiakhov, Krista Dobo, Management of pharmaceutical ICH M7 (Q)SAR predictions – the impact of model updates, *Regul. Toxicol. Pharmacol.* Volume 118 (2020), 104807. ISSN 0273-2300, <https://doi.org/10.1016/j.yrtph.2020.104807>.
- [27] D. Shandilya, R. Israni, P. Joseph, A. Kumar, M. Pillai, Identification of oxidative degradation products of lansoprazole by using high resolution mass spectrometry spectral data, *Int. J. Anal. Mass Spectrom. Chromatogr.* 5 (2017) 57–69, doi: 10.4236/ijamsc.2017.53004.
- [28] Vivek Dhiman, Ankit Balhara, Saranjit Singh, Shristy Tiwari, Samanthula Gananadhamu, M.V.N. Kumar Talluri, Characterization of stress degradation products of nintedanib by UPLC, UHPLC-Q-TOF/MS/MS and NMR: Evidence of a degradation product with a structure alert for mutagenicity, *Journal of Pharmaceutical and Biomedical Analysis*, Volume 199, 2021, 114037, ISSN 0731-7085. <https://doi.org/10.1016/j.jpba.2021.114037>.
- [29] Dhiman V, Patil K, Velip L, Talluri MVNK, Gananadhamu S, Comprehensive degradation profiling and influence of different oxidizing reagents on tinoridine hydrochloride: Structural characterization of its degradation products using HPLC and HRMS, *Rapid Commun Mass Spectrom.* 36 (1) (2022), e9210 doi:10.1002/rcm.921.
- [30] Pradipbhai D. Kalariya, M.V.N. Kumar Talluri, Prinesh N. Patel, R. Srinivas, Identification of hydrolytic and isomeric N-oxide degradants of vilazodone by on line LC-ESI-MS/MS and APCI-MS, *J. Pharm. Biomed. Anal.* Volume 102 (5) (2015) 353–365.
- [31] Krishna, Murali & Rao, Sumathi & Venugopal, N. (2017). Identification of Degradation Impurities in Aripiprazole Oral Solution Using LC-MS and Development of Validated Stability Indicating Method for Assay and Content of Two Preservatives by RP-HPLC. *Journal of Liquid Chromatography & Related Technologies.* 40. 10.1080/10826076.2017.1357572.
- [32] M.M. Thummar, P.N. Patel, B.S. Kushwah, G. Samanthula, Application of UHPLC method for separation and characterization of major photolytic degradation products of trazodone by LC-MS and NMR, *New J. Chem.* (2018). DOI: 10.1039/C8NJ03545H.
- [33] Marie-Josèphe Galmier, Bernadette Bouchon, Jean-Claude Madelmont, Fabrice Mercier, Frédéric Pilotaz, Claire Lartigue, Identification of degradation products of diclofenac by electrospray ion trap mass spectrometry, *J. Pharm. Biomed. Anal.* Volume 38 (Issue 4) (2005). Pages 790-796, ISSN 0731-7085, <https://doi.org/10.1016/j.jpba.2005.02.016>.
- [34] Saji Thomas, Saroj Kumar Paul, Sanjeev Shandilya, Ashutosh Agarwal, Nitesh Saxena, Arun Kumar Awasthi, Hari babu Matta, Dharam Vir and Chandra S. Mathela, Identification and structural elucidation of two process impurities and stress degradants in darifenacin hydrobromide active pharmaceutical ingredient by LC-ESI/MSn, *Analyst*, 2012, 137, 3571–3582.
- [35] Stefano Salvestrini, Paola Di Cerbo, Sante Capasso, Kinetics and mechanism of hydrolysis of phenylureas, *J. Chem. Soc. Perkin Trans. 2* (2002) 1889–1893.
- [36] Fred S. Tanaka, Ronald G. Wien, Eugene R. Mansager, Photolytic demethylation of monuron and demethylmonuron in aqueous solution, *Pestic. Sci.* 13 (3) (1982) 287–294, <https://doi.org/10.1002/ps.2780130310>.
- [37] IHT Guidelines. Validation of Analytical Procedures: Text and Methodology. Geneva, Switzerland: International Conference on Harmonization Q2 (R1), 2005; pp 11–12.