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Isolation and Characterization of Quetiapine Degradation Products by NMR and HRMS: Development and Validation of Quetiapine by RP-UPLC

KumaraswamyKasani[1, 2], Hima Bindu Gandham[2] ,V.V.S.R.N. Anji Karun Mutha[1, 2], Rudrakshula JD Prasad[1], Muralidharan Kaliyaperumal[1] and Chidananda Swamy Rumalla[1]*

1. Department of Medicinal Chemistry, GVK Biosciences Pvt. Ltd, IDA, Mallapur, Hyderabad, Telangana-500076, **INDIA**
2. Department of Engineering Chemistry, Andhra University, Visakhapatnam-530003, A.P., **INDIA**

Email: chidanand_swamy@yahoo.co.in

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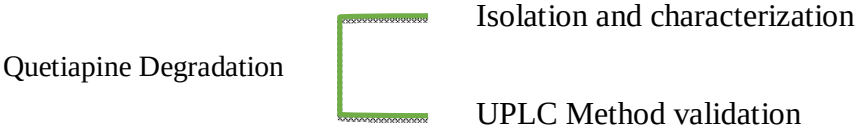
ABSTRACT

Quetiapine fumarate is an atypical antipsychotic drug and it was subjected to stress degradation under acidic, basic and peroxide mediated oxidation. The stress degradation was performed according to ICH guidelinesQ1A (R2), the drug was inert under basic hydrolysis, three degradants (referred as DP-Quet-1, DP-Quet-2, DP-Quet-3) were formed in peroxide mediated hydrolysis and one degradant (referred as DP-Quet-4) was formed in acid hydrolysis. These degradants were initially identified through Liquid Chromatography- Mass Spectrometry and isolated by automated purification system. The structures were established by substantial analysis of High Resolution Mass Spectrometry and 1D, 2D Nuclear Magnetic Resonance Spectroscopy. A stability indicating RP-UPLC method was developed and validated for assay determination of Quetiapine API drug. The Quetiapine RP-UPLC method was validated on Acuity BEH C-18 2.1X100mm, 1.7µm column with shorter runtime of 3 min. The method was validated as per regulatory guidelines in terms of specificity, accuracy, linearity, precision, limit of detection, limit of quantitation and the analysis time is faster than the traditional High performance liquid chromatography.

Graphical Abstract



Keywords: Quetiapine degradation products, HRMS, 1D and 2D NMR, UPLC method validation.

INTRODUCTION

Quetiapine is a dibenzo thiazepine derivative and chemically described as 4-(dibenzo[b,f] [1, 4] thiaze pin-11-yl)-1-(2-(2-hydroxyethoxy)ethyl)piperazin-1-ium. Quetiapine is an atypical antipsychotic drug with a unique receptor-binding profile and it is used in the treatment of schizophrenia or manic

episodes associated with bipolar disorder [**1, 2**]. Complete knowledge of API's stability profile is essential to prevent those risks during manufacturing and storage condition, stress degradation is the process to determine the stability of the drug and formation of degradant components. The aim of the present study is to know the degradation behaviour of Quetiapine under the stress conditions and the stability study was performed as per ICH guidelines and other regulatory authorities [**3-6**].

There are several reports are available Quetiapine stress degradation products characterization on the basis of mass spectrometry, validation [**7-10**] and structures have not been confirmed on the basis of Two- dimensional Nuclear Magnetic Resonance Spectroscopy (2D-NMR).The present study four degradants were identified, the structures have been confirmed by Nuclear Magnetic Resonance Spectroscopy (1D, 2D NMR) and High resolution mass spectrometry. The degradants were identified as DP-Quet-1;11-(4-(2-(2-hydroxyethoxy)ethyl)piperazin-1-yl)dibenzo[b,f][1,4]thiazepine 5,5-di oxide, DP-Quet-2;4-(dibenzo[b,f][1,4]thiazepin-11-yl)-1-(2-(2-hydroxyethoxy)ethyl)piperazine1-oxide, DP-Quet-3;4-(5,5-dioxidodibenzo[b,f][1,4]thiazepin-11-yl)-1-(2-(2-hydroxyethoxy)ethyl) piperazine 1-oxide, DP-Quet-4;(2-((2-aminophenyl)thio)phenyl)(4-(2-(2-hydroxyethoxy) ethyl) piperazin-1-yl)methanone.

Ultra Performance Liquid Chromatography (UPLC) is the alternate for the High-Performance Liquid Chromatography, Ultra Performance Liquid Chromatography technology has been adopted in laboratories around the world. The main advantage of the Ultra Performance Liquid Chromatography system eliminates the significant time and cost. UPLC system flow rate range 0.01mL min^{-1} to 2 mL min^{-1}, back pressure up to 18000 psi and the PDA detector highly sensitive than the HPLC detector. In the present work Ultra Performance Liquid Chromatography technology has been applied to the method validation, assay determination of Quetiapine bulk drug and the analysis time was reduced with good sensitivity and resolution.

MATERIALS AND METHODS

Chemicals and Reagents: Quetiapine was a gifted sample from an API unit in Hyderabad, Chemicals and buffers used for the experiment were HPLC grade formic acid (Rankem), Milli-Q water filtered with 0.25µm filter, Methanol (Rankem), Acetonitrile (Merck), Trifluoroacetic acid (Rankem), Ammonium bicarbonate (Rankem), DMSO-d6(Cambridge isotope limited).

Liquid Chromatography-Q-TOF-High Resolution Mass Spectrometry: Mass accuracy was measured with Q-TOFMicromass instrument and it was coupled with UPLC, Electrospray ES) ionisation source, Micro Chanel Plate Detector (MCP). The experiment conditions were nebulizer gas flow 750 L h^{-1}, cone voltage 25v, capillary voltage 3200v, Micro Chanel Plate Detector voltage 2800v.Leucine Enkephalin (m/z 556.2771) was used to lock mass correction, accurate mass and elemental compositions were calculated with Mass lynx software. The below chromatographic conditions were used for the reaction monitoring. Column: Acuity BEH C-18 2.1 X50mm 1.7 µm, Mobile Phase: 0.075% formic acid in water (A), 0.075% formic acid in acetonitrile. Gradient program (Time % of A) 0/95, 2.5/2, 3.5/2, 4/95flow rate 0.6mLmin^{-1}, Column temp 40°C.

Ultra Performance Liquid Chromatography (UPLC): Ultra Performance Liquid Chromatography coupled with binary solvent manager, PDA detector and column manager were used for the method validation. Acuity UPLC BEH C-18 2.1X100mm 1.7µm column was used for the method validation and Mobile phase-(A)0.07% Trifluoroacetic acid in Acetonitrile,B-0.07% Trifluoroacetic acid in water with gradient program (Time/percentage of A) 0/20,3/98,3.01/20, flow rate 0.3 mL min^{-1}, Column temp 40°C.

Automated Purification System: Reaction mass was isolated with Waters Preparative HPLC coupled with 2545 binary gradient module, automated fraction collector, 2767 sample manager. The chromatographic conditions were used for isolation of degradation products. Column: X select CSH

C-18 19X150mm, 5µm, Mobile phase:A-0.01M ammonium bicarbonate in water, B-Acetonitrile, Gradient Time/% of B 0/20,11/90,11.5/98,13/98,15/20, flow rate 19 mL min^{-1}, detection at 225 nm.

Nuclear Magnetic Resonance Spectroscopy: Quetiapine degradation impurities were recorded on Bruker 500 MHz resolution instrument coupled with auto sampler and data was processed with top spin software, DMSO-d6, deuterium oxide 99.9 ATOM % D solvents were used for the experiments and tetra methyl silane used as internal standard.

Stress methods: The stress degradation was employed to maximize the amount of degradation impurities formed during hydrolytic conditions to generate a sufficient mass of impurity for subsequent NMR and HRMS analysis.1N HCl was used for acid hydrolysis, 1N NaOH was used for base catalyzed hydrolysis and 5% hydrogen peroxide was used for peroxide mediated hydrolysis.

RESULTS AND DISCUSSION

Three major degradants were identified in peroxide hydrolysis and one degradant was identified from acid hydrolysis, the formation degradation products were monitored with LC-MS instrument and the degradation chromatogram was shown in figure 1. The identified degradants were isolated and taken for identification.

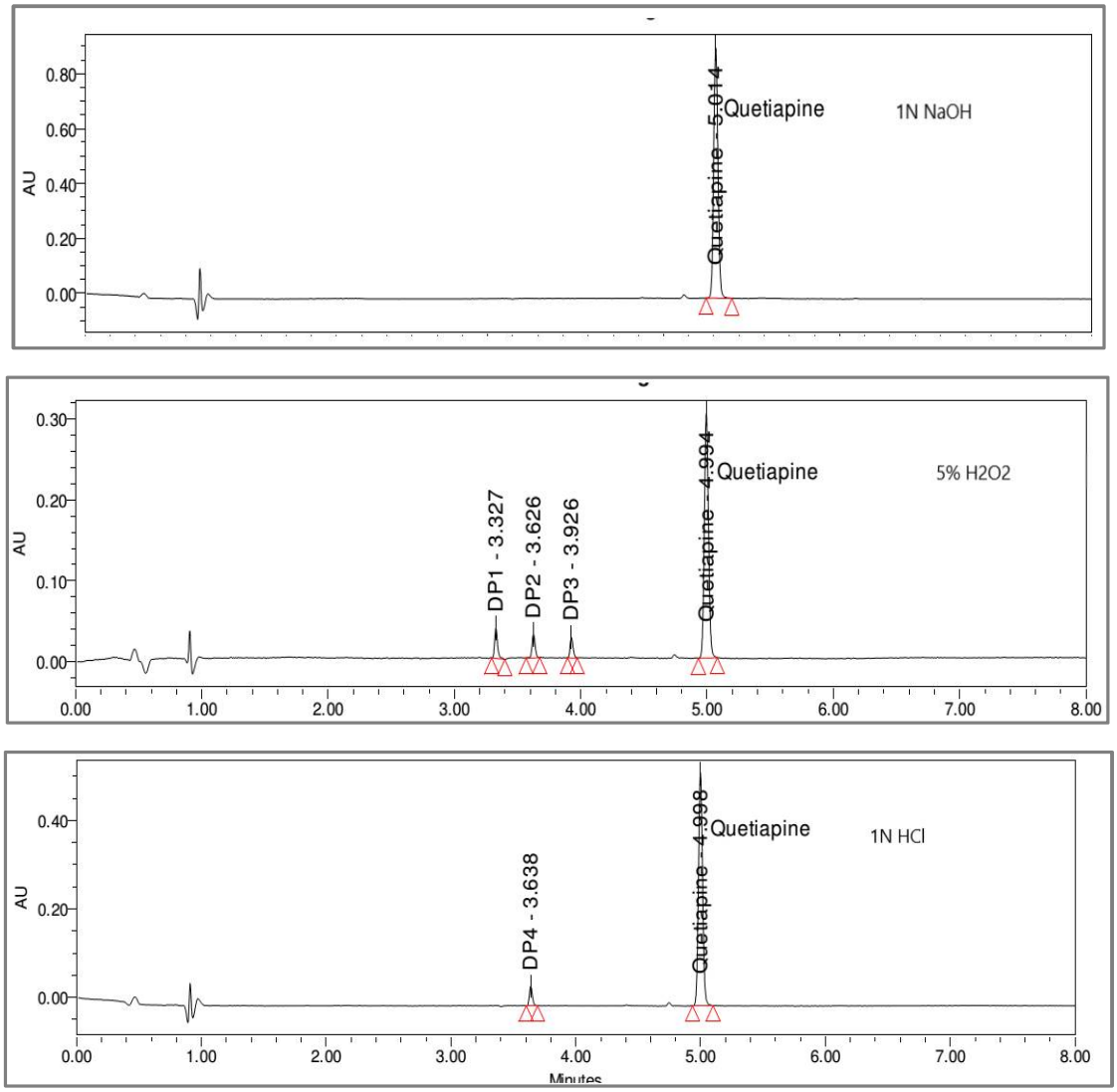


Figure 1. Base, Peroxide and Acid degradation chromatograms of Quetiapine.

Isolation of acid, peroxide degradation products: The degradation products were separated through automated purification system as mentioned method details in section 2.4. Identified degradant products labelled as DP-Quet-1, DP-Quet-2, DP-Quet-3 and DP-Quet-4and the proposed structures are shown in figure 2.

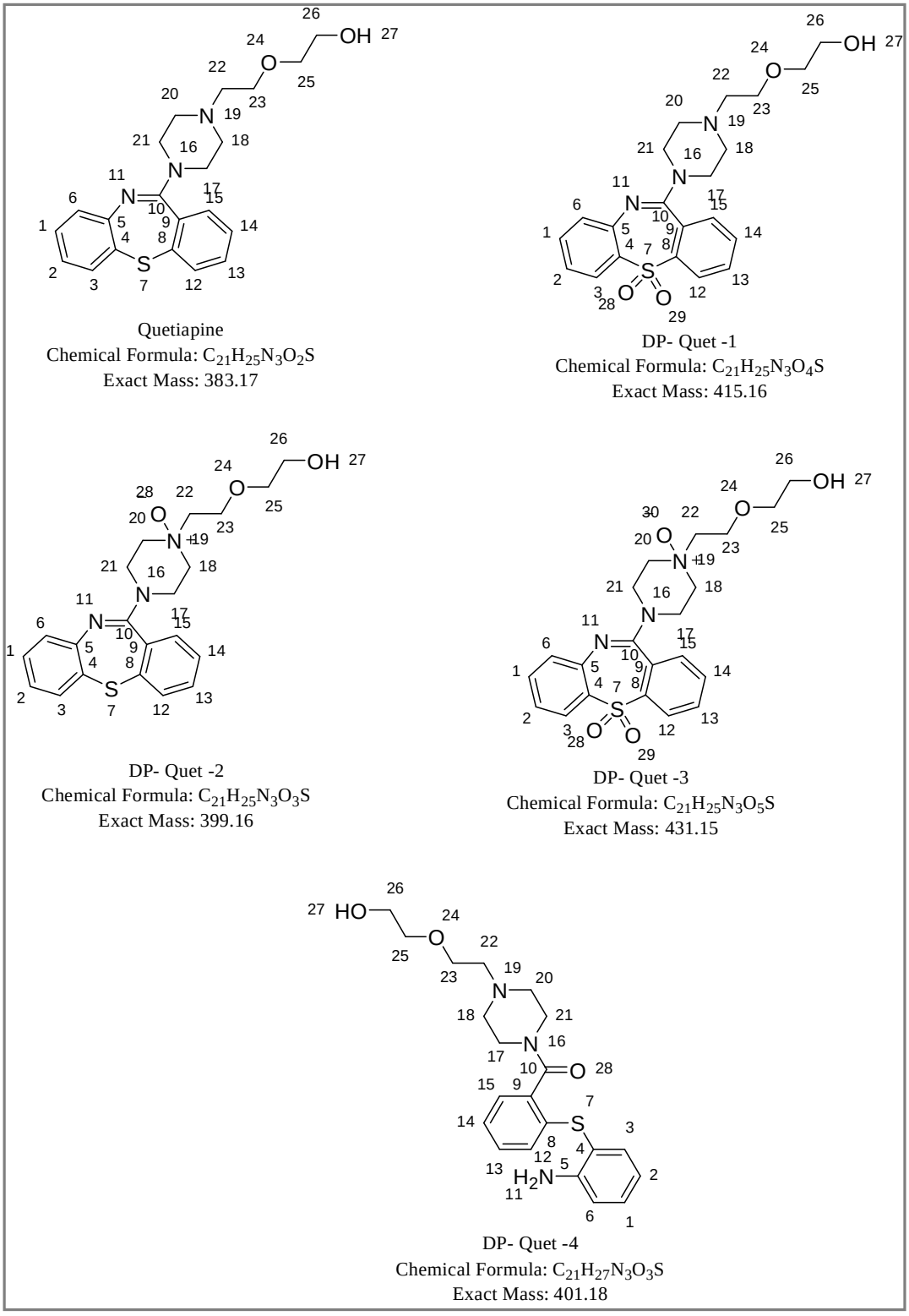


Figure 2. Chemical structures of Quetiapine drug substance and it's degradation products.

Table 1. 1H and 13C Chemical shift values (ppm) of Quetiapine and its degradation Products

Assignment	Quetiapine		DP-Quet -1		DP-Quet -2		DP-Quet -3		DP-Quet -4	
	1H	13C	1H	13C	1H	13C	1H	13C	1H	13C
1	7.18	129.2	7.31	130.5	7.33	130.5	7.56	134.4	7.16	131.1
2	6.89	122.5	7.18	123.4	7.21	123.8	7.18	122.6	6.59	116.6
3	7.37	129	7.44	119.7	7.46	119.8	7.8	125.1	7.32	136.9
4	--	127.2	--	135.7	--	135.7	--	131.9	--	112.2
5	--	148.6	--	142.6	--	142.1	--	145.3	--	150.2
6	6.99	125	6.99	124.7	7.02	124.7	7.18	126.7	6.77	114.9
8	--	138.5	--	147.4	--	147.7	--	142	--	133.4
9	--	133.5	--	123.2	--	121.8	--	124.4	--	136.1
10	--	160	--	156.9	--	156.7	--	158.6	--	167.1
11	--	--	--	--	--	--	--	--	5.39	--
12	7.54	131.9	7.72	119	7.73	119.8	7.99	123.8	6.9	128.1
13	7.43	128.9	7.75	132	7.78	132.2	7.8	132	7.26	129.3
14	7.45	131.2	7.58	130.6	7.58	130.6	7.88	134.6	7.21	126.4
15	7.38	132	7.49	128.4	7.59	128.5	7.78	129.6	7.22	126.1
16	--	--	--	--	--	--	--	--	--	--
17,21	3.43	46.6	3.43	46.6	3.4	42.8	3.4	42.8	3.17,3.66	41.2
18,20	2.47,2.56	52.8	2.47,2.56	52.8	3.07,3.55	63.7	3.1,3.88	63.6	2.42,2.52	57
22	2.52	57.2	2.53	57.1	3.98	63.9	3.96	63.6	2.53	52.7
23	3.54	68.3	3.54	68.2	3.38	69.2	3.32	69.5	3.52	68.3
25	3.41	72.2	3.41	72.2	3.45	72	3.42	72	3.4	72.3
26	3.48	60.2	3.48	60.2	3.5	60.1	3.47	60	3.48	60.2
27	4.61	--	4.61	--	5.1	--	5.1	--	4.61	--

Structure elucidation of DP-Quet-1: DP-Quet-1 had an observed accurate mass of m/z 416.1647 and the protonated elemental composition $C_{21}H_{26}N_3O_4S$ was confirmed by HRMS with below 1 ppm error. The high resolution mass spectrometry data was shown in figure 3.

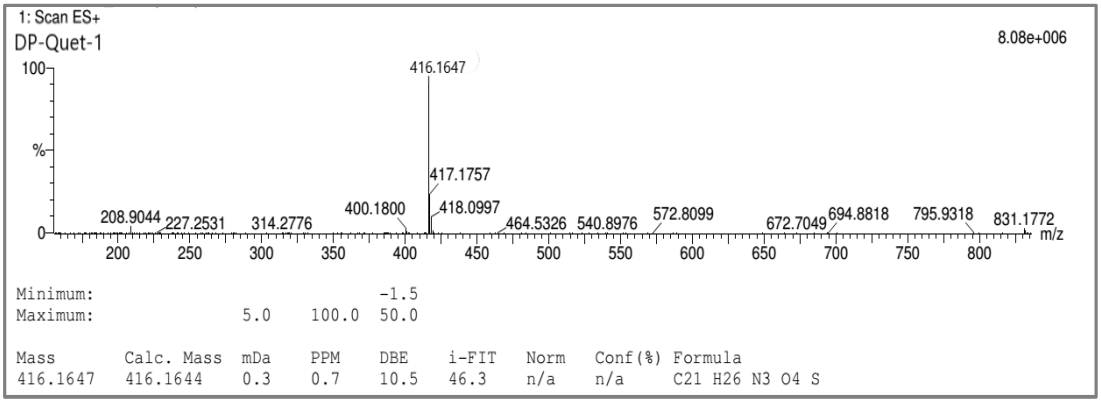


Figure 3. HRMS spectrum of DP-Quet-1.

This m/z 416.1647 data indicating that 32 amu units higher than the Quetiapine (m/z 384.17), it indicates that two oxygen atoms were attacked on drug during peroxide hydrolysis. The challenge is that to identify the position of oxygen atoms on drug substance, it was confirmed by NMR technique. The 1H NMR spectrum revealed that the DP-Quet-1had 17 aliphatic protons, 8 aromatic protons. The ^{13}C NMR spectrum showed 8 aliphatic carbons and 13aromaticcarbons. ^{1}H, ^{13}C chemical shift values for DP-Quet-1 was assigned by using ^{1}H, ^{13}C, HSQC and HMBC Experiments. The assignment of DP-Quet-1 was shown in table 1. The number of protons and carbons observed same as like drug but few protons and few carbons chemical shift values were drastically changed compare to the drug. 3, 12 position proton chemical shift values were moved to downfield 7.44 ppm, 7.72 ppm from 7.37, 7.54 ppm (drug). 3 and 12th position carbon chemical shift values moved to up field 119.7 ppm, 119 ppm from 129, 131.9 ppm (drug). These results supporting those 2 oxygen atoms attacked on 7th position sulphur. DP-Quet-1structure was confirmed as shown in below figure 2.

Structure elucidation of DP-Quet-2: The DP-Quet-2 HRMS data was showed a protonated molecular ion peak at m/z 400.1692 $[M+H]^+$ and molecular formula $C_{21}H_{26}N_3O_3S$ was confirmed by HRMS experiment. The HRMS spectrum of DP-Quet-2 was shown in figure 4.

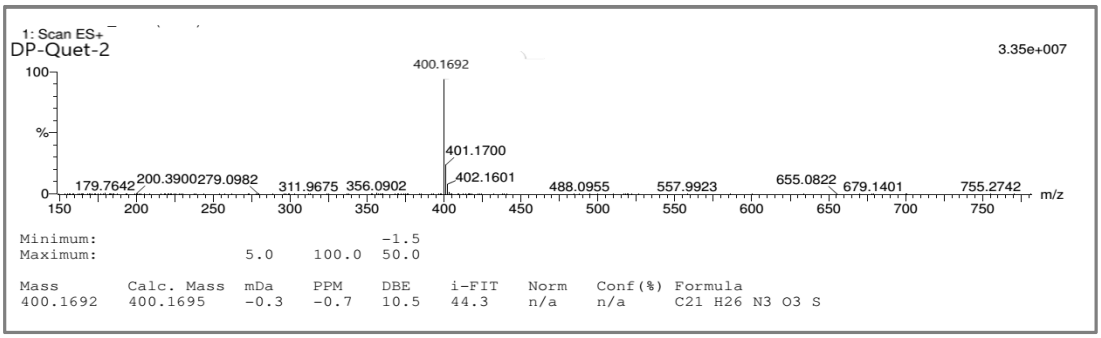


Figure 4. HRMS spectrum of DP-Quet-2.

This mass data indicating that 16 mass units higher than drug (Quetiapine) mass 384.17. The ^{1}HNMR spectrum revealed that the DP-Quet-2 had 17 aliphatic protons, 8 aromatic protons. The ^{13}C NMR spectrum showed 8 aliphatic carbon and 13 aromatic carbons. ^{1}H, ^{13}C chemical shift values for DP-Quet-2 was assigned by using ^{1}H, ^{13}C, HSQC and HMBC Experiments. The assignment of DP-Quet-2 was shown in table 1. A number of protons and carbons observed same as like drug but few protons and few carbons chemical shift values were drastically changed compare to the drug. 18, 20 position proton chemical shift values were moved to downfield 3.07, 3.55 ppm from 2.47, 2.56 ppm (Quetiapine). In same way 22nd position proton chemical shift value also moved to downfield 3.98 ppm from 2.52 ppm (drug). These proton chemical shift value changes indicating that oxygen attacked on 19th position nitrogen. 18, 20 and 22 position carbon chemical shift values were moved to downfield 63.7 ppm and 63.9 ppm from 52.8 ppm and 57.2 ppm (drug). These carbon chemical shift values changes also indicating that oxygen atom were attacked on 19th position nitrogen. DP-Quet-2 structure was confirmed as shown in below figure 2.

Structure elucidation of DP-Quet-3: The High resolution mass spectrometry data showed a protonated molecular ion peak at m/z 432.1590 $[M+H]^+$ corresponding to molecular formula $C_{21}H_{26}N_3O_5S$ and DP-Quet-3 HRMS spectrum was shown in figure 5.

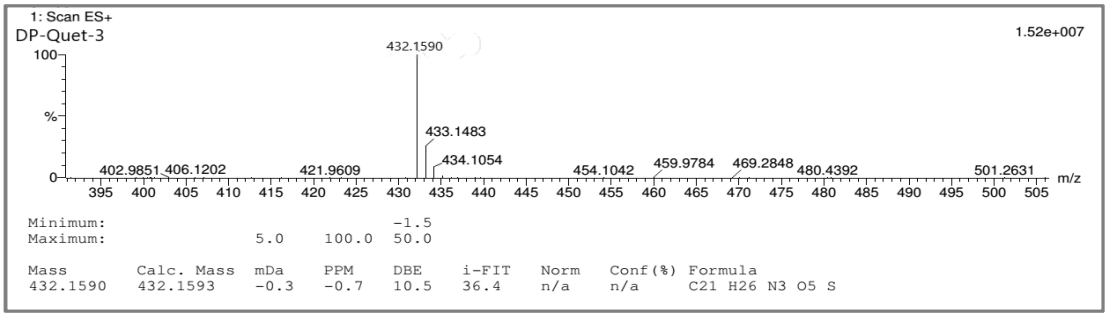


Figure 5. HRMS spectrum of DP-Quet-3.

This mass data indicating that 48 mass units higher than drug (Quetiapine) m/z 384.17. The ^{1}H NMR spectrum revealed that the DP-Quet-3had 17 aliphatic protons, 8 aromatic protons. The ^{13}C NMR spectrum showed 8 aliphatic carbons and 13aromaticcarbons. ^{1}H, ^{13}C chemical shift values for DP-Quet-3 was assigned by using ^{1}H, ^{13}C, HSQC and HMBC Experiments. The assignments of DP-Quet-3 are shown in table 1. A number of protons and carbons observed same as like drug but few protons and few carbons chemical shift values were drastically changed compare to the drug. 18, 20 position proton chemical shift values were moved to downfield 3.1, 3.88 ppm from 2.47, 2.56 ppm(drug). In same way 22nd position proton chemical shift value also moved to downfield 3.96 ppm

from 2.52 ppm (drug). 3, 12 position proton chemical shift values were moved to downfield 7.8 ppm, 7.99 ppm from 7.37, 7.54 ppm (drug). These proton chemical shift value changes indicating that one oxygen attacked on 19th position nitrogen and two oxygen atoms attacked on 7th position sulphur. 18, 20 and 22 position carbon chemical shift values were moved to downfield 63.6 ppm from 52.8 ppm and 57.2 ppm (drug). 3 and 12th position carbon chemical shift values moved to up field 125.1 ppm,123.8 ppm from 129, 131.9 ppm(drug).These carbon chemical shift values changes also indicating that one oxygen atom attacked on 19th position nitrogen and two oxygen atoms attacked on 7th position sulphur. DP-Quet-3 structure was confirmed as shown in below figure 2.

Structure elucidation of DP-Quet -4: DP-Quet-4 had an observed accurate mass of m/z 402.1855, this is an addition of 18.0109 amu to that of Quetiapine (m/z 384.1746).The protonated molecular formula $C_{21}H_{28}N_3O_3S$ was confirmed by HRMS experiment and the HRMS spectrum was shown in figure 6.

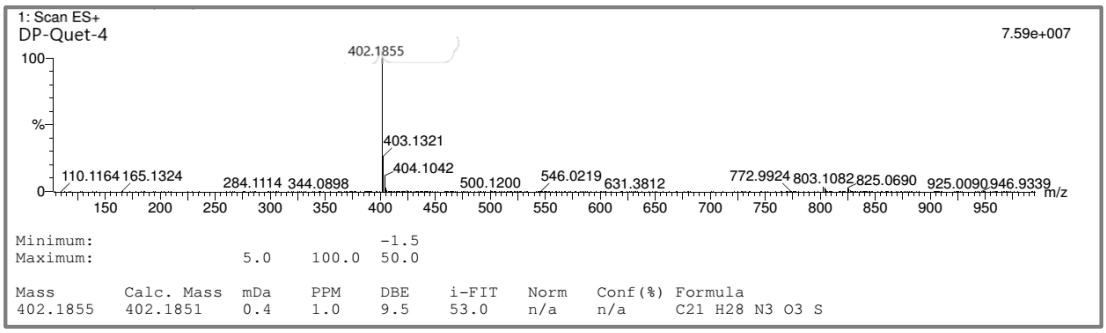


Figure 6. HRMS spectrum of DP-Quet-4.

DP-Quet-4 proton NMR spectra had same protons like drug substance and one amine(-NH2) protons observed at 5.39 ppm. It had same number of carbons as like drug substance in ^{13}C NMR. It had amide carbonyl carbon at 167.1 ppm, 1H NMR and 13C NMR spectrums are shown in figure 7, 8.

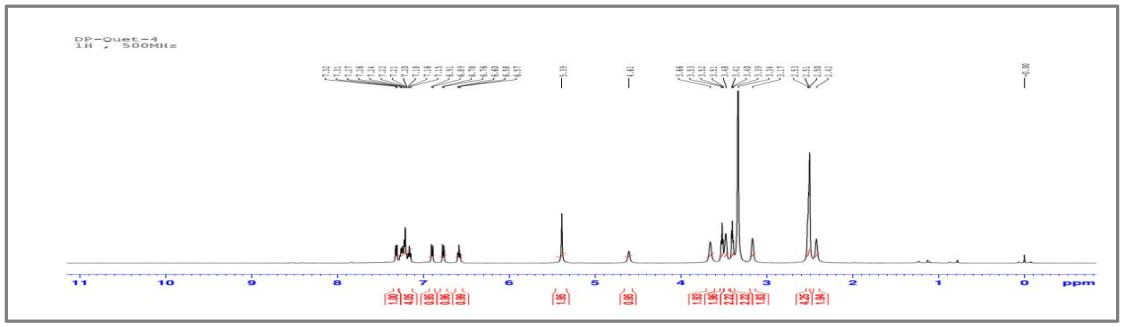


Figure 7. ^{1}H NMR spectrum of DP-Quet-4.

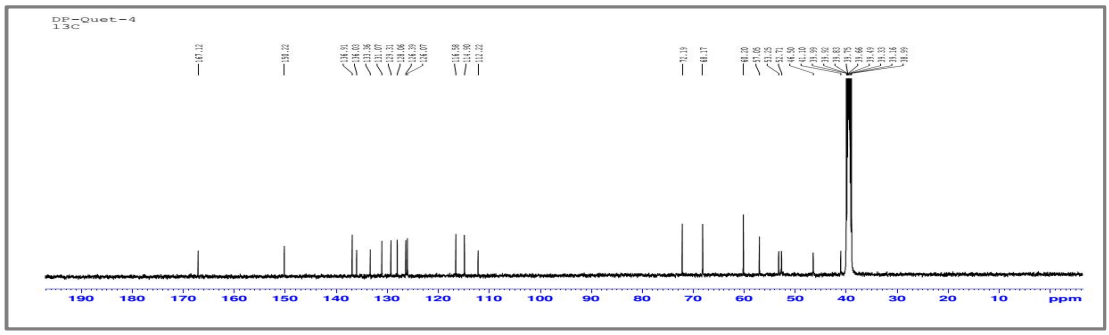


Figure 8. ^{13}C NMR spectrum of DP-Quet-4.

In HMBC Expt, NH_2 (5.39ppm) showed correlation with C-6(114.9ppm) and C-4(112.2 ppm). H-15 (7.22 ppm) and H-17, 21 (3.17 ppm) showed correlation with 10th position carbonyl carbon at 167.1 ppm) as shown in figure 7. These key protons versus carbon correlations in HMBC supporting to structure of DP-Quet-4 as shown in figure 9.

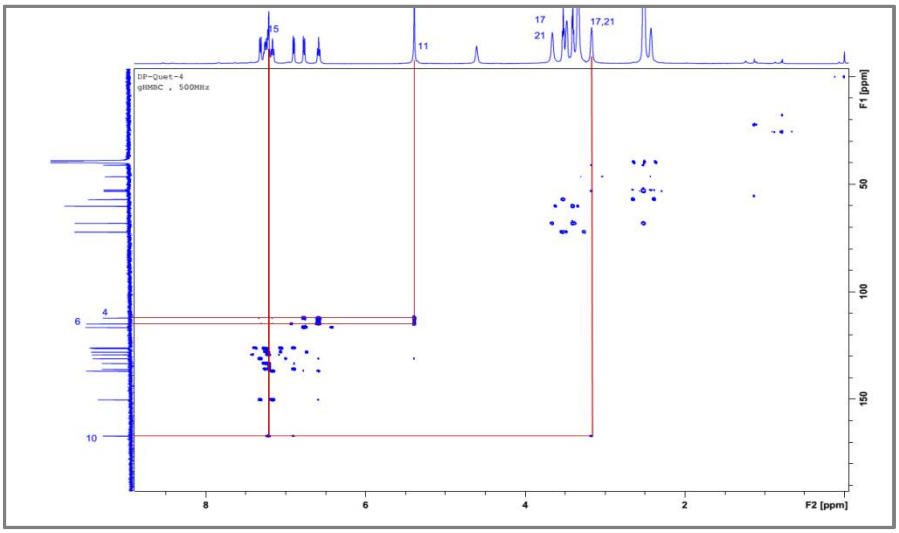
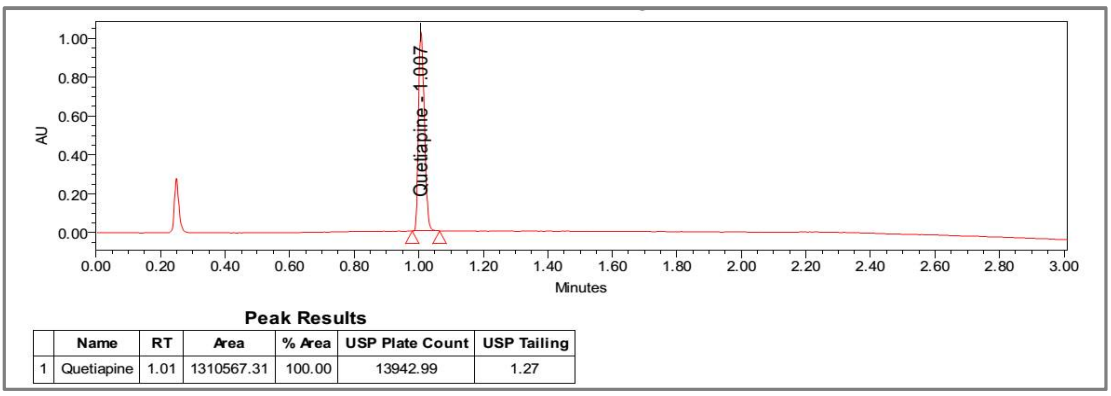


Figure 9. HMBC spectrum of DP-Quet-4.

Method development and validation: UPLC method was developed with 3 minutes run time, Acquity BEH C-18 column was employed for Quetiapine method validation and the chromatographic conditions was mentioned in section2.3. Quetiapine fumarate UPLCmethod was validated as per regulatory guidelines in terms of accuracy, precession (inter, intraday), limit of detection, quantitation and linearity. The precision of the assay method was evaluated by carrying out six independent assays, Linearity was performed with six concentration levels 25%,50%,75%,100%,125%, 150% of the sample. The accuracy of the method was determined by spiking known amount of standard solution in triplicate at levels 50%, 100%, 150% of the sample.

The retention time of the Quetiapine was 1.01 min in UPLC method, Quetiapine standard solution (0.3mg mL^{-1}) was injected in the system for system suitability test and USP Tailing, USP plate count values are 1.27, 13943 and the Quetiapine fumarate UPLC chromatogram was shown in figure 10.



	Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	Quetiapine	1.01	1310567.31	100.00	13942.99	1.27

Figure 10. Quetiapine fumarate UPLC chromatogram.

Method precision (Intraday, inter day) was checked with six repeated concentration preparations and the % of RSD values are less than 1. The limit of detection was 0.02 mg mL^{-1}, signal to noise ratio 17.9 and limit of detection was 0.006 mg mL^{-1}, signal to noise ratio 6.18,Quetiapine linearity was

demonstrated with six concentration levels ranging 0.075-0.450 mg mL^{-1} and the correlation coefficient was greater than 0.999 (Table 2).

Table 2. Validation parameters of Quetiapine

Validation parameter Quetiapine drug	
Intraday method precision(n=6, % of RSD)	0.4
Interday method precision(n=6, % of RSD)	0.6
LOD-LOQ	
Limit of detection (mg mL^{-1})	0.006
Limit of quantification (mg mL^{-1})	0.02
Precision at LOQ (% R.S.D.)	0.7
Linearity	
Calibration range (mg mL^{-1})	0.075-0.450
Calibration points	6
Correlation coefficient	0.999

The accuracy of the method was determined by spiking known amount of Quetiapine standard in known Quetiapine test sample in triplicate at levels 50%, 100%, 150% of the specified limit, recovery of the method was proved, the % of recovery was 103% for the assay of Quetiapine and the results were shown in table 3.

Table 3. Assay recovery of Quetiapine

Level (%)	Amount added (µg mL^{-1})	Amount recovered (µg mL^{-1})	Recovery (%)
50	160.04	166.05	103.75
100	200.1	203.38	101.63
150	300.05	316.18	105.38

Method robustness was checked by changing the chromatographic conditions solvent composition (+- 0.2 mL min^{-1}) pH of the mobile phase (+-0.2), column temperature (+- 5°c) and different systems, there is no significant changes were observed. The mobile phase stability was checked (2,4,7 days) with Quetiapine drug solution and the stability of the Quetiapine drug solution was checked at precise temperature (2-8°C) for the period of 40 days, there was no illustrious changes were observed.

APPLICATION

Quetiapine stress degradation provides degradation pathway, chemical behaviour of the molecule which in helps in the development of formulation and package, UPLC method validation eliminates the significant time and cost.

CONCLUSION

Three major degradation products were identified in Quetiapine during peroxide hydrolysis and one degradation product was formed in acid mediated hydrolysis, these impurities were separated with automated purification technique and characterized by using Nuclear magnetic resonance spectroscopy and High resolution mass spectrometry. The newly developed shorter UPLC method for assay determination of Quetiapine drug substance was found to be capable of giving faster analysis with good resolution and the method was completely validated showing satisfactory data for all tested validation parameters. It is a stability indicating method suitable for faster analysis of Quetiapine bulk drug.

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Conflict of interest: All authors declare that they have no conflict of interest.

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