Isolation, Identification and Characterization of Degradation Product of Piracetam Using Analytical Techniques

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Abstract: *Piracetam was subjected to the hydrolytic, oxidative, thermal and photolytic stress, according to ICH guideline Q1A (R2). The drug depicted degradation only in basic conditions, while it was stable to other stress conditions (acidic, neutral hydrolysis, thermal and photolytic conditions). A total of one degradation product was formed, which were separated on a BEH column employing an isocratic UPLC method. The degradation product was isolated and characterizes using UPLC-MS/MS, ^{1}H NMR and IR. Initially, a total mass fragmentation pathway of the drug was established with the help of UPLC-MS/TOF studies. Subsequently, the isolated degradation product was subjected to MS/TOF mass studies to achieve their accurate mass, fragment pattern. The MS results helped to ascribe tentative structures to degradation product, which were verified through ^{1}H NMR and IR. Based on the results, a complete degradation pathway for the drug has been proposed.*

Keywords: *Piracetam; Stress testing; Degradation Pathway; UPLC-MS/MS; NMR; IR.*

1. INTRODUCTION

The International Conference on Harmonization (ICH) drug stability evaluation benchmark Q1A (R2) requires that analysis of stability samples should be done through the use of validated stability-indicating analytical methods. It also recommends carrying out of stress testing on this drug substance to determine its inherent stability characteristics and to ensure the suitability of the proposed analytical guidelines [1-7]. The objective of reducing analysis time and maintaining satisfactory efficiency, there has been substantial focus on rapid chromatographic separations. Recently, commercially present an innovative ultra performance liquid chromatography (UPLC) has proven to be one of the most promising developments in the area of rapid chromatographic separations. In the present study, reverse phase chromatographic stability indicating assay method was developed using UPLC for Piracetam bulk drug. Piracetam is a nootropic drug. It shares the same 2-oxo-pyrrolidone base structure with 2-oxo-pyrrolidine carboxylic acid (pyroglutamate). Piracetam is a cyclic derivative of GABA. It is one of the groups of racetams. Piracetam is prescribed by doctors for some conditions, mainly myoclonus, however it is used off-label for a much wider range of applications like aging, alcoholism, alzheimers and senile dementia, depression and anxiety, schizophrenia, closed craniocerebral trauma, protective for breath-holding spells etc. The chemical name of piracetam is 2-oxo-1-pyrrolidineacetamide (Fig. 1) [8-9].
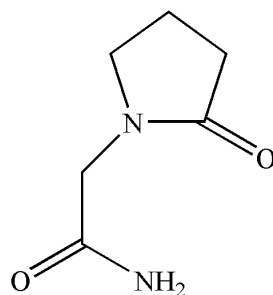
Fig 1. *Structure of Piracetam*

Ultra performance liquid chromatography (UPLC) has been introduced to improve chromatographic performances in terms of efficiency and rapidity, LC has recently evolved in the development of small columns packed with small particles (sub-2 μm) working at elevated pressures (>400 bar). UPLC has been evaluated in terms of practical benefit in speed and efficiency that can be attained compare with current HPLC systems [10-20]. The major objective of the present work is to develop stress degradation studies of Piracetam under different ICH recommended stress conditions, and to establish a validated stability-indicating UPLC method for reducing analysis time and solvents. So far to knowledge there was no method has been reported for UPLC method yet on the development of stability-indicating assay method for this drug.

2. EXPERIMENTAL

2.1. Materials

Piracetam (purity 99.99%) was obtained as a gratis sample from Micro Labs. Limited, Banglore (India). Analytical reagent (AR) Sodium hydroxide and hydrogen peroxide were purchased from S.D. Fine-chem. Hydrochloric acid and acetonitrile (99.8%) was from Merck India (Mumbai). Milli-Q water was produced in the laboratory by Milli-Q water purification system (MA, USA). All other chemicals were of analytical grade.

2.2. Instrumentation

Ultra performance liquid chromatography (UPLC)

UPLC was performed using a Waters Acquity system equipped with binary solvent delivery pump, an auto sampler and PDA detector. The chromatographic separation was performed using a Waters Acquity BEH 150×2.1 mm, 1.7μm, C18 column. The mobile phase containing a mixture of acetonitrile and water in the ratio of 25:75 (v/v) at a flow rate of 0.15 mL/min was used. The detection was obtained at a wavelength of 210 nm. The injection volume was 2 μL; mobile phase was used as a diluent while the column was maintained at 30ºC. Forced degradation studies were carried out with a photo diode array detector. Data were acquired and processed using Empower software.

Quadrupole-Time of Flight-Mass Spectrometry (Q-TOF-MS)

UPLC–MS/TOF studies were carried out on a system in which UPLC (Waters Corporation, Milford, Massachusetts, USA ;) was hyphenated to Q-TOF-MS spectrometer manufactured by Waters Q-TOF Premier (Micromass MS Technologies, Manchester, UK). The accurate mass and composition for the precursor ions and the fragment ions were calculated using the MassLynx V 4.1 software incorporated in the instrument.

Nuclear Magnetic Resonance spectroscopy

NMR was carried out using a Bruker SpectroSpin 400 MHz manufactured Bruker (Canton, Massachusetts, USA). ^{1}H NMR spectra of Degradation product was recorded using dimethyl-sulfoxide (DMSO-d_6) as a dissolving solvent. TMS as an internal standard; chemical shifts are reported as δ/ppm units.

Infra red spectroscopy

The IR spectra of degradation product were obtained using JASCO-FT/IR-470 Plus FTIR spectrophotometer. The sample was prepared with KBr (Merck) which was dried in a hot air oven for 40 minutes before preparing samples.

The pH of the mobile phase was checked on microprocessor water proof pH tester (pH tester 20, eutech instruments, oakton, USA). The overall illumination at the point of placement of samples was 6000 lux, which was tested using a calibrated lux meter (Lutron LX-102 digital light meter, Marcucci S.P.A, vignate, Milan). Thermal stability study was performed in a hot air oven (Oven universal with thermotech thermostat TIC-4000N, S.M. Industries, New Delhi, India).

2.3. Degradation Tests

Stress studies were performed under conditions of dry heat (thermal studies), hydrolysis (acidic, alkaline and neutral), oxidation, and photolysis, as mentioned in ICH Q1A (R2) (1–4). A minimum of four samples were generated for every stress condition, viz., blank solution stored under normal conditions, the blank subjected to stress in the same manner as this drug (Piracetam), a zero time sample containing this drug (which was stored under normal conditions), and this drug solution subjected to stress treatment. Hydrolytic decomposition of Piracetam was conducted at 80 ºC in 5 M HCl, water, and 0.5 M NaOH at a drug concentration of 2 mg/mL until sufficient degradation (~20% of the initial amount) of this drug was achieved. For oxidative stress studies, Piracetam was dissolved at a concentration of 3 mg/mL in 30% H_2O_2 and preserved for two days at room temperature. Photolytic studies of the dry drug and this drug in solution in acetonitrile at a concentration of 2 mg/mL were performed by exposure to sunlight during the daytime (60,000-70,000 lux) for 2 days.

2.4. Development of UPLC Indicative Stability Test and Its Validation

UPLC was performed with a binary solvent delivery pump, an auto sampler and PDA detector of Acquity UPLC system manufactured by Waters Corporation; Milford, Massachusetts, USA; data was acquired and processed using Empower software. The method development and validation of stress testing on piracetam using UPLC/HPLC was recently reported by Sahu K. et al, 2012 [4].

2.5. Isolation and Characterization of Degradation Product

As the drug degraded under alkaline condition to give single degradation product, it has been crucial to isolated and characterized. The amount of 250 mg of the drug was dissolved in 50 mL of 2N NaOH to obtain a 5 mg/mL solution. Drug sample was refluxed at 80^0C for 8 hrs under the stringent monitoring thin layer chromatography. Degradation product was originated (monitored by TLC) appearing that turned prominent and completes the reaction later. The formation of complete degradation product of the piracetam was confirmed by the TLC and the resultant solution was neutralized with HCl acid to give a precipitated product. The product was washed with methanol and dried. Collect the precipitate of degradation product and subjected to UPLC, LC-MS/MS, IR and NMR for structural identification.

The structure elucidation of all the degradation products was achieved with the systematic mass fragmentation, ^{1}H-NMR spectra, FT-IR.

Mass Spectrometry

The accurate mass m/z was found to be is 143.13(Infusion mode ESI-Positive). The same m/z was found in LC-MS/MS ESI-Positive mode.

^{1}H NMR Data

^{1}H NMR, 400 MHz, δ (ppm): 1.237 (t, 2H of CH_2, J=3.08), 2.552 (d, 2H of CH_2, J=3.24), 2.560 (d, 2H of CH_2, J=1.69), 3.862 (d, 2H of CH_2, J=17.6), 6.990 (s, 1H of NH), 7.858 (s, 2H of NH_2), 10.308 (s, 1H of OH). The spectrum of the above given data is shown in supporting material Fig. S1.

FT-IR Data

IR (KBr) ν (cm^{-1}): 1041 (CH bending), 1280 (C-O stretching), 1421 (CH_2 bending), 1681 (CO-NH_2 stretching), 3130 (NH stretching), 3390 (O–H stretching).

3. RESULTS AND DISCUSSION

3.1. UPLC Studies on the Stressed Solutions

The forced-degradation study shows that Piracetam degraded under alkali stress condition. The specificity and selectivity of the method with the samples under these stresses were demonstrated through the evaluation of retention times (R_T), retention factor (k'), resolution, and purity data for all peaks in the chromatograms. Piracetam did not degrade under acid, oxidative, thermal and photolytic stress conditions. In a mixture of solution, only one degradation product was formed. The R_T and k' of this drug and the degradation product are given in Table 1.

Table 1. *Retention times and retention factor of various peaks*

PEAKS	UPLC	
	R_T	k'
Deg01	2.05	1.34
Piracetam	3.56	2.71

R_T = Retention time (minutes)

k' = Retention factor (Expressed as R_T –Void time/Void time)

This drug and degradation products carry the notations Deg01 and Piracetam in accordance with the sequence in which the peaks appeared from left to right on UPLC chromatogram (mixture of stressed sample) (Fig. 2). Ultra-performance liquid chromatography (UPLC) is a new category of separation technique based upon well-established principles of liquid chromatography, which utilizes sub-1.7 μ particles for stationary phase. These particles operate at elevated mobile phase (linear velocities) to affect dramatic increase in resolution, sensitivity and speed of analysis. The
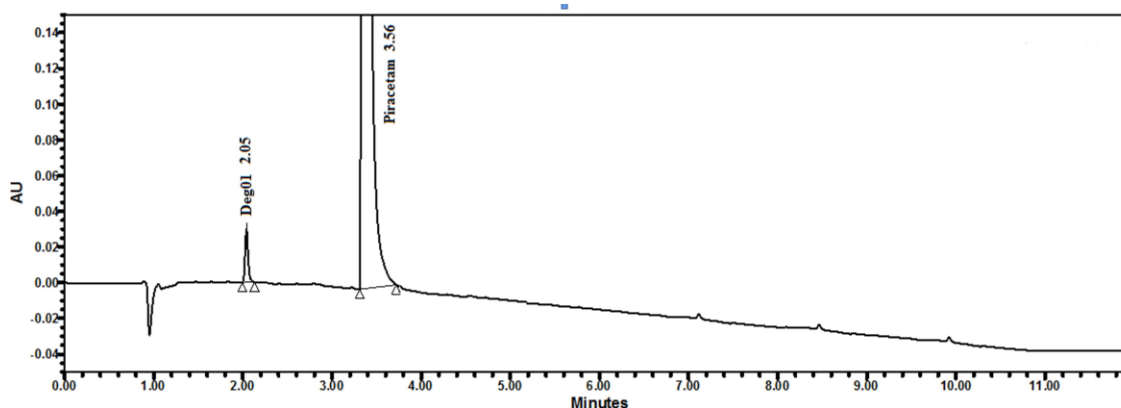

figure 2 is reproduced from the study by Sahu et al., by courtesy of the publishers, Elsevier, B.V.

Fig2. *Chromatogram showing separation of Piracetam and its degradation products in a mixture of stressed samples by UPLC*

3.2. UPLC–MS/MS Studies on Forced Decomposition Samples of Piracetam

The degradation product in UV chromatogram was present in the total-ion chromatogram, recorded by using the method. The mass spectrum of the drug and degradation product is shown in Fig. 03. The observed m/z values for molecular ion peak $[M+H]^+$ and considerable fragments of the drug is 142.8, 125.7, 98.2 and its degradation product is 161.2, 143.7, 84.8. Of these, peak I (Deg01) was found to be degradation product and peak II is of piracetam. According to the m/z values and fragmentation pattern, the structures for degradation products could be proposed. The mass spectrum of degradation product is shown in Fig. 03.
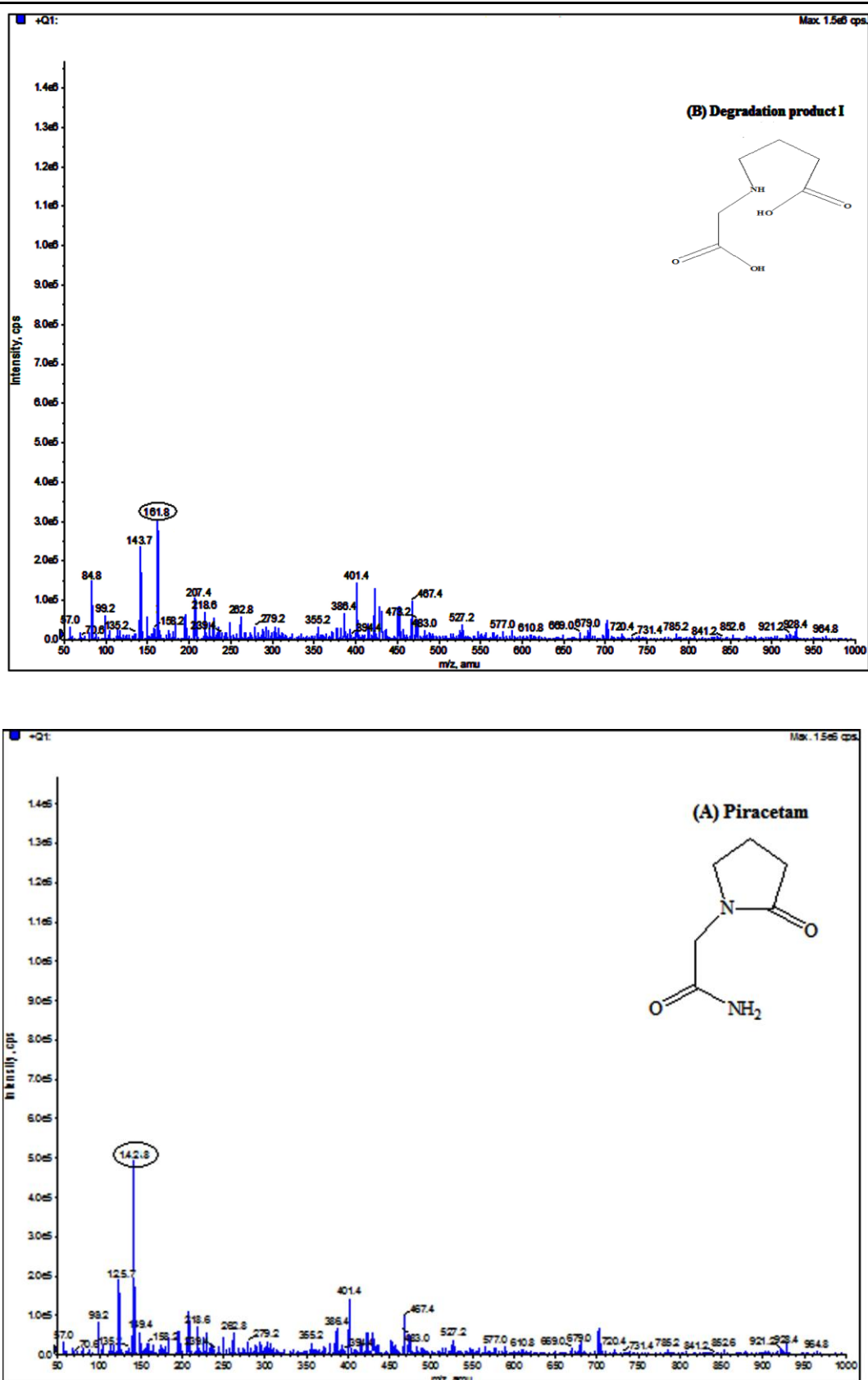
Fig 3. *Mass spectrum of (a) Piracetam and (b) degradation product*

3.3 IR Spectrum

The obtained IR spectrum of degradation product is shown in Fig.04, which also facilitate for the confirmation of degradant i.e., 4-(2-amino-2-oxoethylamino) butanoic acid
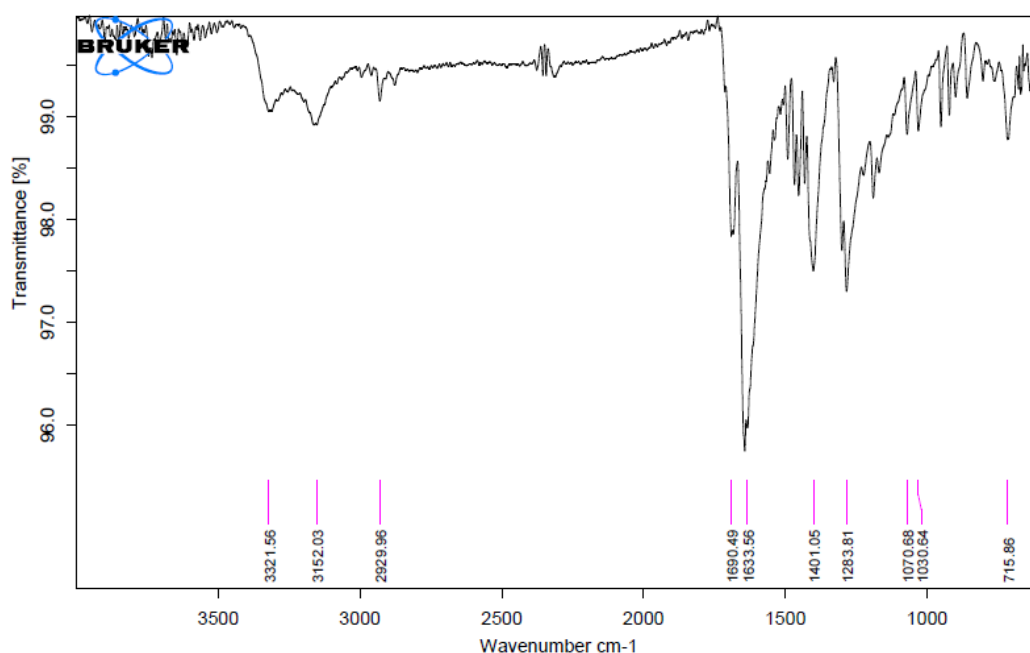
Fig 4. *IR spectra of degradation product*

3.3. Mechanistic Explanation To the Origin of the Degradation Product / Proposed Degradation Pathway of the Drug

The accurate mass of degradation product m/z 161.16 (+ESI) was 18 Da higher than the drug 143.16. This clearly indicated addition of H_2O molecule to the drug. As the application of nitrogen rule and proposed the presence of even nitrogen ie., $C_6H_{12}N_2O_3$ (theoretical mass 160.16) as the most probable molecular formula. Degradation product (DP) is generated from the drug by simple amide hydrolysis in basic condition. Since DP was formed in basic, It was observed that, piracetam undergoes base hydrolysis. The proposed degradation pathway of piracetam is shown in Fig. 05.
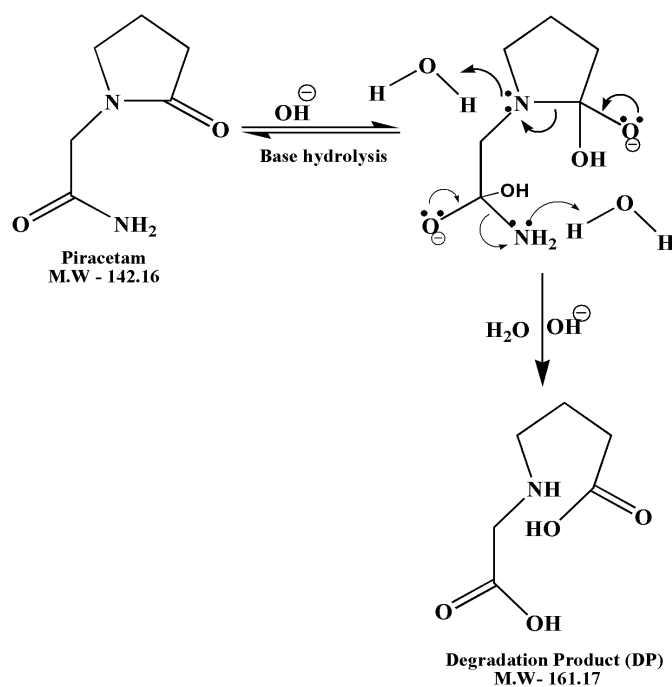

Fig 5. *A proposed pathway/mechanistic approach for the formation of degradation product of piracetam*

4. CONCLUSION

In this study, it was possible to characterize the degradation product using stability-indicating UPLC assay method for piracetam on a BEH column, which could separate the drug and its

degradation products formed under a variety of stress conditions. The drug is only degrading in alkaline conditions confirmed by emerging of single degradation product in a mixture of stress samples. The degradation product is isolated and characterized by IR, NMR and UPLC-MS/MS. The m/z values and fragmentation patterns obtained for the degradation products through UPLC–MS studies helped to confirm the presence of known products and to propose the structures of unknown compounds. The results in totality helped to draw out a more extensive degradation route of the drug. Indirectly, the study highlights the benefit of the use of ICH stress testing approach in the establishment of complete degradation pathways of drugs. It is hoped that this report on stability indicating method and degradation route of Piracetam would be helpful for the multiple generic producers of the drug throughout the world by preserving them from repetition of same studies.

ACKNOWLEDGEMENTS

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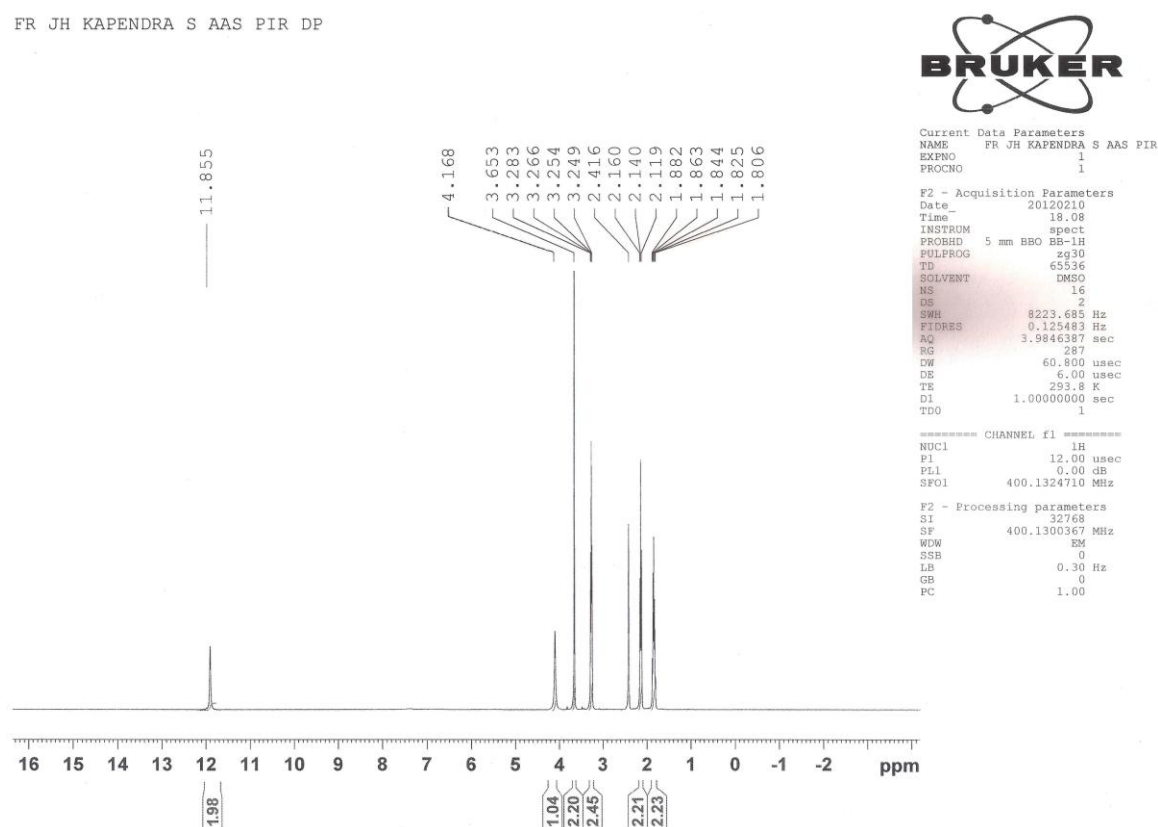
Supporting Material

Figure S1. *^{1}H NMR spectrum of degradation product*

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Mr. Kapendra Sahu received his M. Pharm degree (2009) with distinction from at Rajiv Gandhi Proudyogiki University, Bhopal and submitted his PhD in Pharmaceutical Chemistry (2014) in Jamia Hamdard (Hamdard University), New Delhi. His research has focused on proposed degradation/metabolic pathway, synthetic chemistry, mass spectrometric techniques and drug metabolism. He has >25 original and review articles in international scientific journals.

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