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Identification, Isolation and Structural Characterization of an impurity in varenicline tartrate drug product.

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ABSTRACT: An unknown impurity was observed during the stability studies and under oxidative stress condition of varenicline tartrate tablet samples. The impurity was enriched and isolated using preparative HPLC and characterized by using techniques such as ESI-LC-HRMS,1D NMR,2D NMR. Structure of impurity was further confirmed by single crystal analysis. On the basis of spectral data analysis, the impurity is identified as hydroxy derivative of varenicline. The current work is a discussion of the evidences leading to the structure of impurity related to varenicline.

KEYWORDS:-Varenicline tartrate; unknown impurity; ESI-LC-HRMS; NMR; Single crystal XRD.

I. INTRODUCTION

Varenicline tartrate, 7,8,9,10-tetrahydro-6,10-methano-6H-pyrazino[2,3-h][3]benzazepine (2R,3R) - 2,3-dihydroxy butanedioate, is a high affinity partial agonist for α4β2 nicotinic acetylcholine receptors used in the treatment of smoking cessation. [1,2].During the course of the dosage form development, it was found that there was significant drug degradation in varenicline tablets under accelerated stress testing i.e. 40°C/75%RH. Due to its high potency, varenicline dosage forms require high dilution with excipients and as a result the reactivity of varenicline with excipients is profound [3]. Varenicline drug substance and drug product were exposed to thermally induced oxidative forced degradation condition involving 3% peroxide stress reagent for 1 hour at room temperature. Further, varenicline drug product was also placed at 40°C/75% RH for 6 months. The degradant observed in stress condition using 3% peroxide solution in water and stability condition (40°C/75% RH for 6 months) was same as confirmed by RRT and UV spectral data comparison.

The safety of a drug product is dependent not only on the toxicological properties of the active drug substance, but also on the impurities that it may contain. As per International Conference on Harmonization (ICH) guidelines for impurities in new drug substances, reporting threshold is 0.05% and identification threshold is 0.1% or 1.0 mg per day intake (whichever is lower) for maximum daily dose ≤2 g/day. Considering the daily dose of varenicline, this unknown impurity was found to be above identification limit. Hence the task of identifying and subsequently characterizing the impurity was carried out. [4,5]. In the continuing research; efforts on the impurity profiling, herein, a comprehensive study undertaken has been described to identify and characterize the unknown impurity in Varenicline tablets. To the best of our knowledge, the identification, separation and structural elucidation of this unknown impurity by HRMS, 1D and 2D-NMR and single-crystal X-ray diffraction analysis, and the plausible prospects of the formation of varenicline related impurity with alcoholic (-OH) group substituted on the middle fused aromatic ring system are first detailed in this paper. In this research work an LCMS compatible method was used to separate and identify the impurity on HRMS instrument. The impurity was subsequently isolated by preparatory set up and characterized using different characterization techniques.

II. EXPERIMENTATION

Trifluoroacetic acid (AR grade from SD fine chemicals, India), CDCl3 (deuterated chloroform, Euriso-top), Acetonitrile (HPLC grade, Merck, India), ethyl acetate and Methanol (HPLC grade, Merck, India) were purchased. Ultra-pure water was used which was procured from Millipore MilliQ plus water purification system.

High performance reversed phase liquid chromatography:

The samples were subjected to stress condition using 3% peroxide solution in water. In the related substance (RS) method, used for the separation of impurities; an unknown peak was observed in the peroxide degradation stress samples

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with area of around 1.5%. Hence it was decided to pursue complete structural characterization of impurity.

Isolation of impurity by PREP-HPLC:

For impurity enrichment, samples were kept in 3% peroxide stress degradation for 24 hours maintaining temperature of 80 °C in water bath. The impurity was isolated on prep system procured from Shimadzu (LC-20AP). The column used was YMC Actus triart C18 (250*20) mm, 5µM. Column flow was kept at 16.0 mL/min. Mobile phase consisted of 0.1% TFA in water as aqueous mobile phase and Methanol: THF as organic phase in a ratio of (70:30). Gradient run was given to separate out the impurity. The time program employed was as follows: Time/%B: 0.01/0, 50.00/10, 60.00/10, 60.01/100, 62.00/100, 62.01/0, 66.00/0. After lyophilisation, pure impurity was isolated and characterized by NMR, MS and single crystal studies.

Mass spectrometry and elemental composition:

Electrospray ionization experiments were performed using a Q Exactive plus Orbitrap (Thermo scientific-model) high resolution mass spectrometry instrument (HRMS) equipped with Xcalibur software 4.2.28.14. The positive and negative electrospray data were obtained by varying the values of parameters critical for ionizing the compound. Capillary voltage was changed from 3.5 to 4.5 kV, collision energy was varied from 10 to 80 eV, auxiliary gas heater temp was monitored from 300- 350 °C, while the collision energy of 30 eV was kept for obtaining daughter ions of the impurity.

NMR spectroscopy:

The NMR experiments were performed on Bruker 400 MHz Avance III HD instrument with 5mm PABBO BB/19F-1H/D Z-GRD Z108618 probe using $CDCl_3$ as solvent at 30°C. The data was processed using Topspin 3.2 software. ^{1}H, ^{13}C and 2D NMR experiments such as correlation spectroscopy (COSY), heteronuclear multiple bond correlation spectroscopy (HMBC), heteronuclear multiple quantum coherence (HMQC) were performed to ascertain the exact position of substitution of alcoholic (-OH) group on varenicline moiety.

Single crystal XRD study:

The data was collected using Bruker D8 Quest X-ray Diffractometer with Mo Kα radiation at room temperature 293 °K and the unit cell dimensions were determined. Further data integration led to the identification of 3D structure of the molecule. The varenicline related impurity was crystallized using slow evaporation method. The crystals were grown using the mixture of Ethyl acetate and Methanol as solvent in a 5 mL glass beaker. The ratio of weight/volume was taken to dissolve 5 mg of compound by adding the mixture of solvents to get a clear solution inside the 5 mL beaker. The beaker was closed with parafilm and solvent was left to evaporate.

After a few weeks, tiny crystals were formed at the bottom of the beaker under the optical microscope. Further incubation at room temperature led to crystals of the size of diffraction quality. By picking one single crystal from the beaker on to the crystal mounting loop, diffraction experiment was performed. With multiple trials of the crystals, a good quality diffracting crystal was identified for data collection.

III. RESULTS AND DISCUSSION

MS and Elemental composition data:

The chemical formula for impurity under examination is $C_{13}H_{13}N_3O$ with an exact mass of 227.1058. The chemical formula of protonated molecule of impurity is $C_{13}H_{14}N_3O$, while the theoretical exact mass for the protonated form is 228.1136. The observed m/z value for the impurity is 228.1128 (Figure 3). Mass accuracy was observed to be -3.50 ppm which suggested that the chemical formula of impurity is $C_{13}H_{13}N_3O$. The MS/MS fragmentation done on HRMS system is shown in figure 1. The fragments obtained correspond to the m/z values 155.0599, 159.0551, 181.0758, 185.0707, 199.0863, 211.0863. The structures of the fragments obtained with respect to the molecular ions are represented in figure 2.

NMR data:

Results of chemical shift values of varenicline and varenicline related impurity have been tabulated in table 1 and table 2 respectively. The structure of impurity along with the numbering of atoms is depicted in figure 11. The protons at C-3 and C-6 in Varenicline are symmetrical through the axis of symmetry and hence both are showing a combined singlet peak of two protons at 8.006 ppm. The ^{1}H NMR spectra of the impurity suggested that the chemical shift (δ) values of impurity (table 2) were similar to those of varenicline (table 1), except at central fused benzene ring where the NMR signal corresponding to the proton at C-3 is missing in case of impurity under consideration. This shows the

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absence of proton at C-3 whereas proton at C-6 gives a singlet peak at 7.518 ppm. Carbon atom at C-3 is seen at 151.922 ppm in ^{13}C NMR and HMQC spectrum of impurity. The axis of symmetry is lost in the case of impurity due to substitution at C-3.

The COSY spectrum of impurity shows that proton at C-8 and C-9 are correlating with each other while proton at C-6 is not correlating with any proton (Figure 4). Protons at C-11 correlate with protons at C-12a and C-16b, protons at C-15 correlate with proton at C-16b and C-14a, protons at C-12b and C-14b correlate their respective geminal protons C-12a and C-14a (Figure 5). Keeping into perspective protons at position C-16, the COSY spectrum of impurity shows proton at C-16b is correlating with proton at C-16a by geminal coupling whileproton at C-16b is correlating with protons at C-11 and C-15 by vicinal coupling. Here, symbols a and b denote the protons attached to a particular carbon in a different chemical environment.

The HMQC spectrum of impurity confirms the protons attached to respective carbon atoms at positions C-6, C-8 and C-9 (Figure 6). Further, the positions of C-11, C-12, C-14, C-15, C16 have been established with their respective protons observed in the HMQC spectrum of upfield region (Figure 7). The carbon atom at C-3 does not possess a proton and hence there is no carbon-proton correlation for the C-3 carbon as displayed in the HMQC spectrum of impurity (Figure 6). The HMBC spectrum of impurity shows proton at C-8 giving correlation with carbons at C-4 and C-9, while proton at C-9 is giving correlation with carbon at C-8 and C-5 indicating these groups are in vicinity. Proton at C-6 is showing correlation with C-1 and C-5. (Figure 8). Carbon at C-3 is correlating with proton at C-14b and C-16a by long range coupling in the HMBC spectrum of impurity (Figure 9).

Single Crystal XRD data

The data collection was performed and the crystal structure was determined. The data was refined to a reliability index of 6%. Single crystals of CHNO indicates the Oak Ridge Thermal-Ellipsoid Plot. A suitable crystal was selected and mounted on a Bruker IuS Diffractometer. The crystal was kept at a temperature of 273.15 °K during data collection. Using Olex2, the structure was solved with the olex2.solve structure solution program using Charge Flipping and refined with the SHELXL refinement package using Least Squares minimization.

The magnetic susceptibility tensor of the biaxial crystal in orthorhombic, monoclinic, and triclinic systems has three different principal values, $\chi 1$, $\chi 2$, and $\chi 3$, and the corresponding principal axes. For the orthorhombic system, the crystallographic and magnetic axis coincided. For the monoclinic system, the b axis coincided with one of the magnetic axis. For the triclinic system, there is no general relationship between the crystallographic and magnetic axes. Owing to their biaxial magnetic nature, biaxial crystals can be aligned biaxially (or three-dimensionally) if an appropriate dynamic magnetic field (DMF) is applied. Among the biaxial crystals, most orthorhombic crystals exhibit only one orientation under DMF, because the crystallographic and magnetic susceptibility axes coincide. On the other hand, the monoclinic and triclinic crystals exhibit two or four orientations (twin orientations) that have the same magnetic energy.

Hydroxy varenicline impurity has been determined from the SCXRD to be monoclinic having the space group P21/c, and the lattice parameters were a/A° = 7.513(15), b/A° = 9.9974(18), c/A° = 13.648(3) Å, α/° = 90, β/° = 94.981(8) ◦ and γ/° = 90 respectively. The R1 and wR2 values were 0.0868 and 0.1881. Bond length obtained between carbon at position C-3 and OH group is 1.324 °A. This bond length is characteristic of an oxygen atom attached to carbon by a single bond in the middle fused ring system of hydroxy varenicline impurity.

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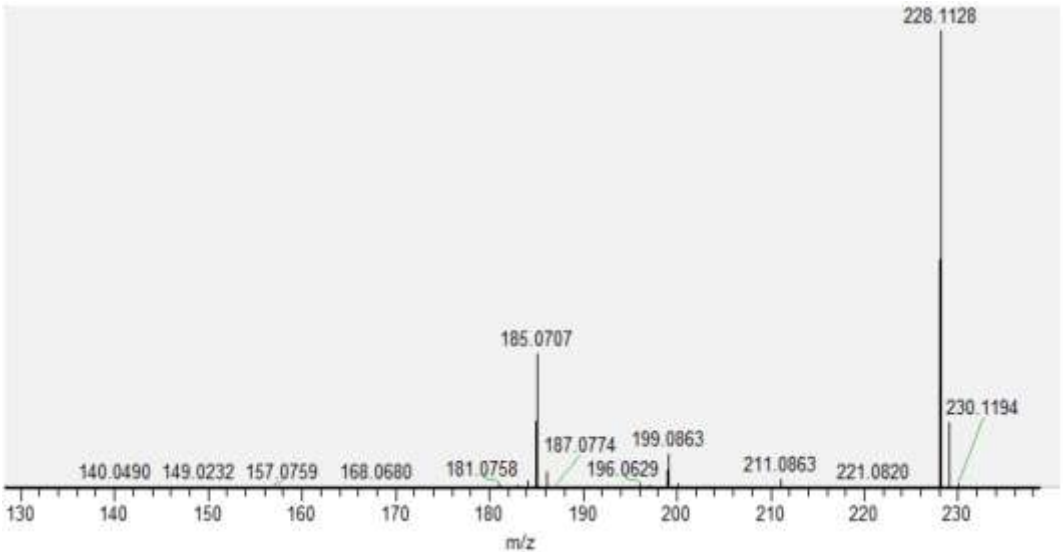

Figure 1 MS/MS spectrum of hydroxy Varenicline impurity

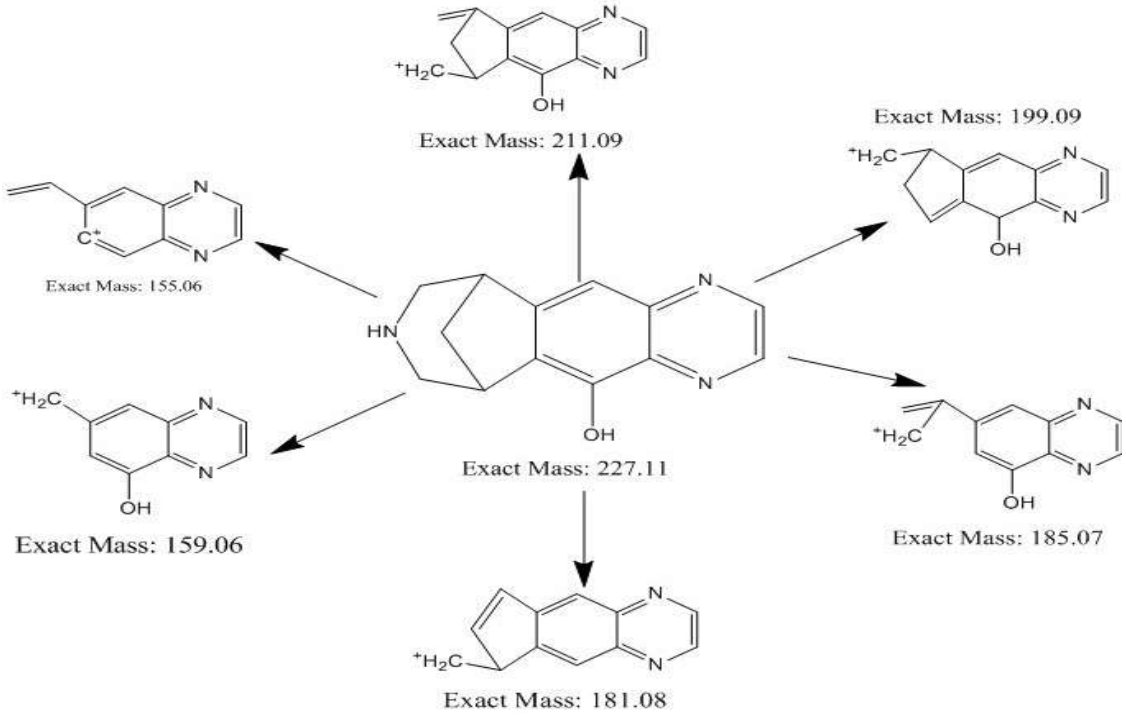

Figure 2 Fragments of Hydroxy Varenicline Impurity

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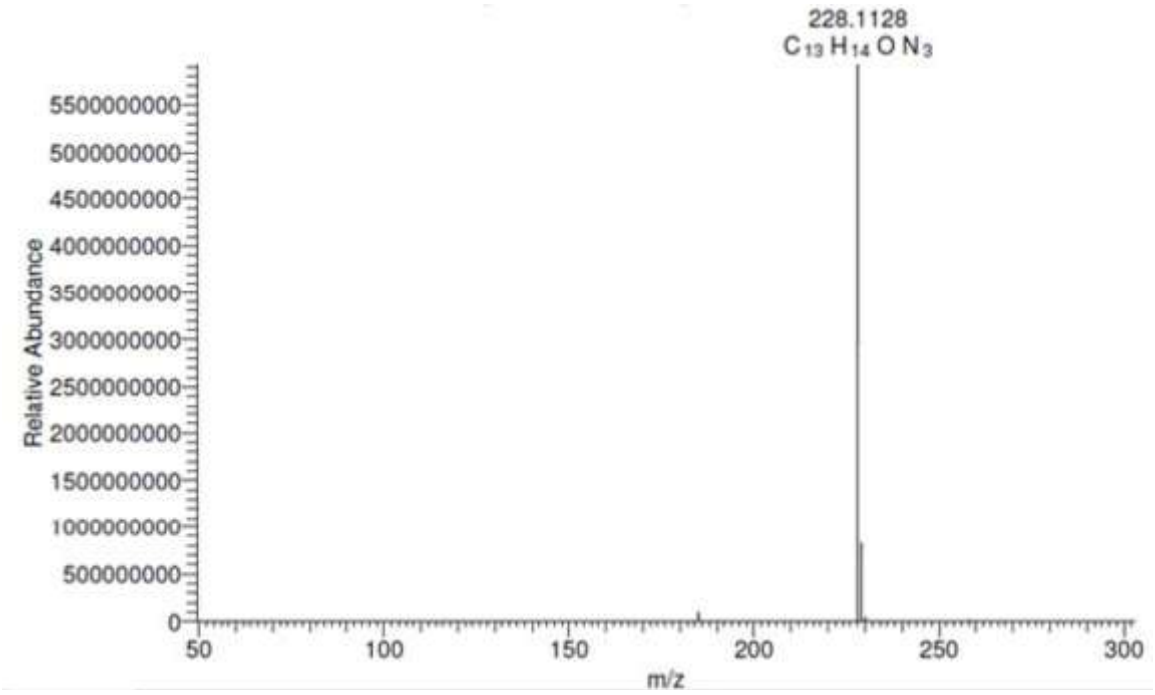

Figure 3 Mass spectrum of Hydroxy Varenicline Impurity

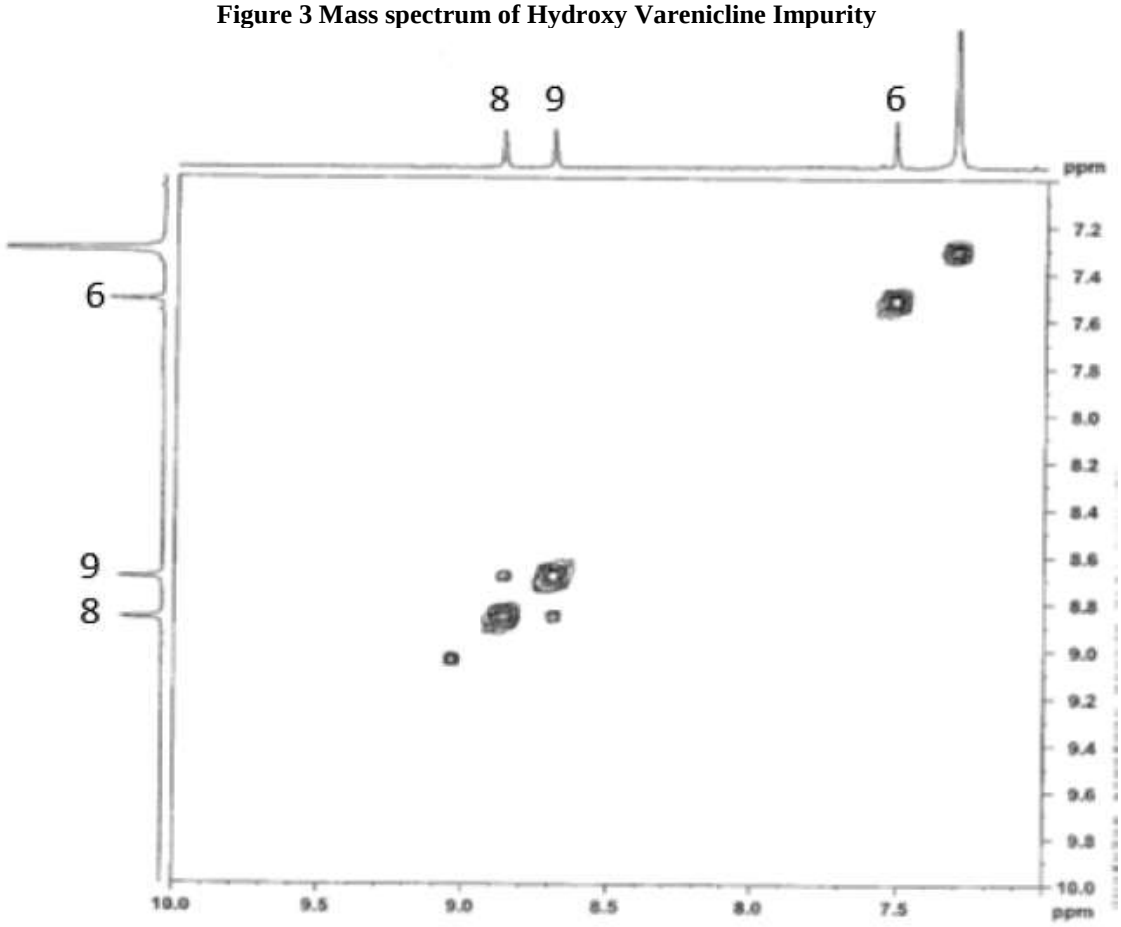

Figure 4 COSY Spectrum of Hydroxy Varenicline Impurity

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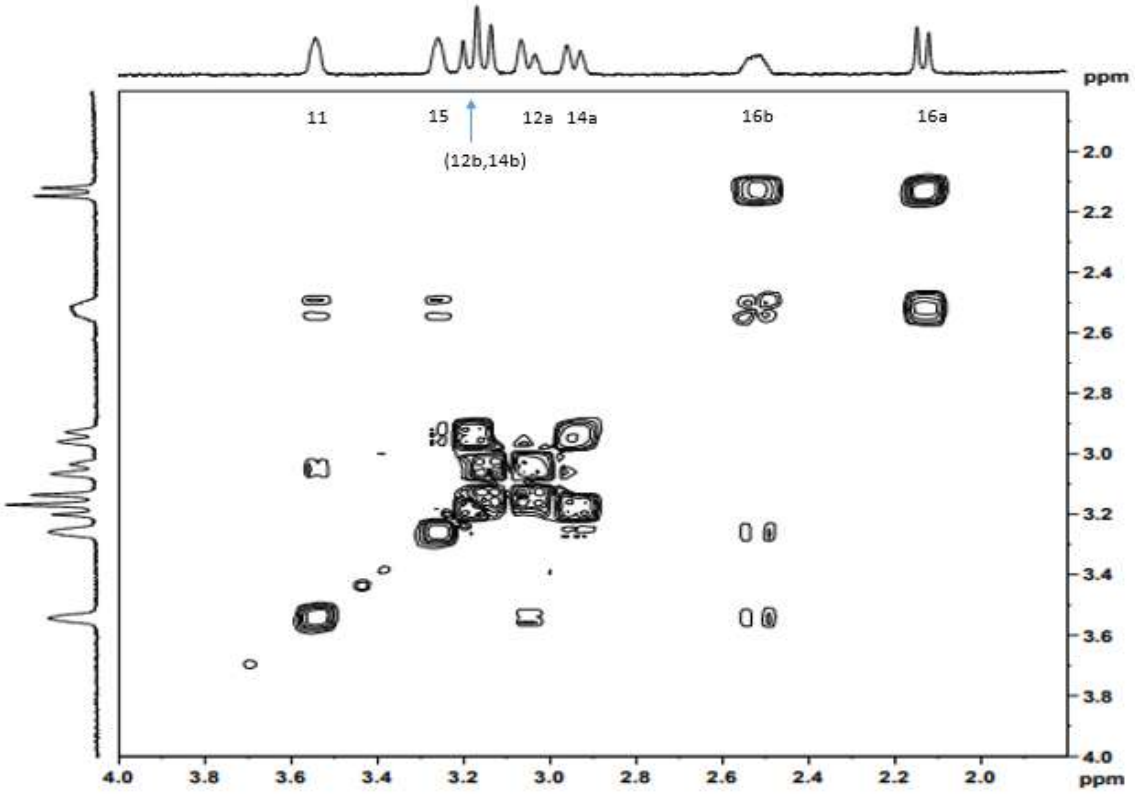

Figure 5 COSY Spectrum of Hydroxy Varenicline Impurity (Upfield region)

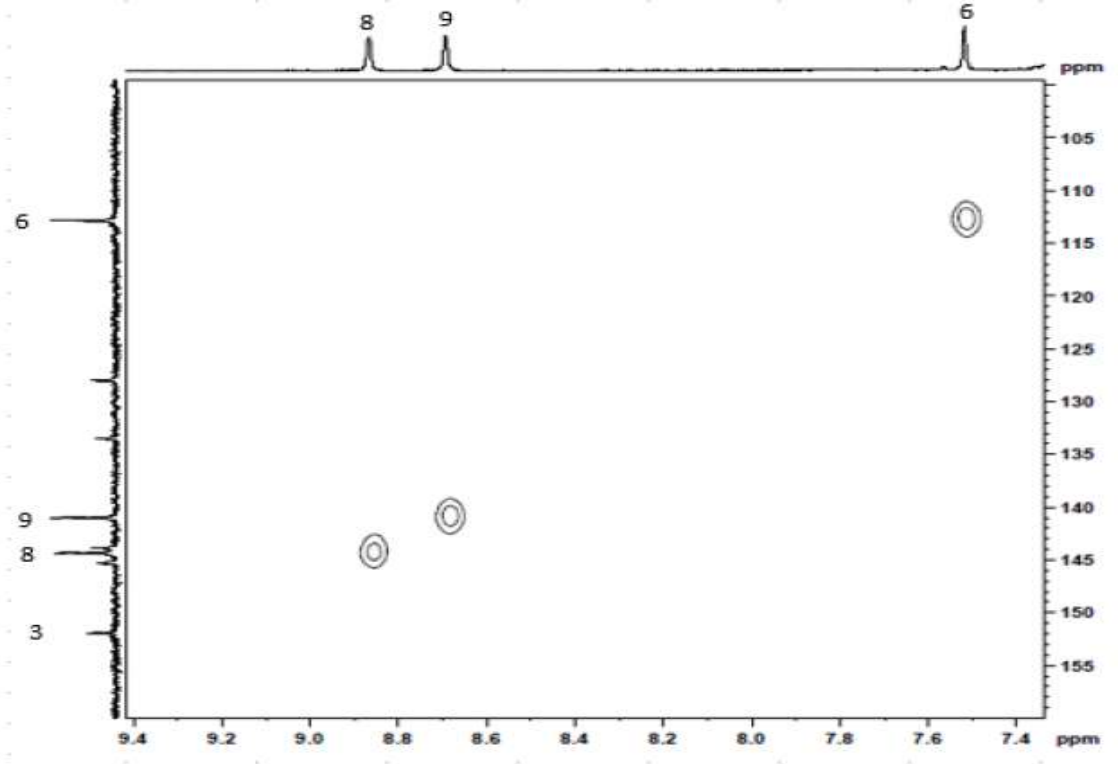

Figure 6 HMQC Spectrum of Hydroxy Varenicline Impurity (downfield region)

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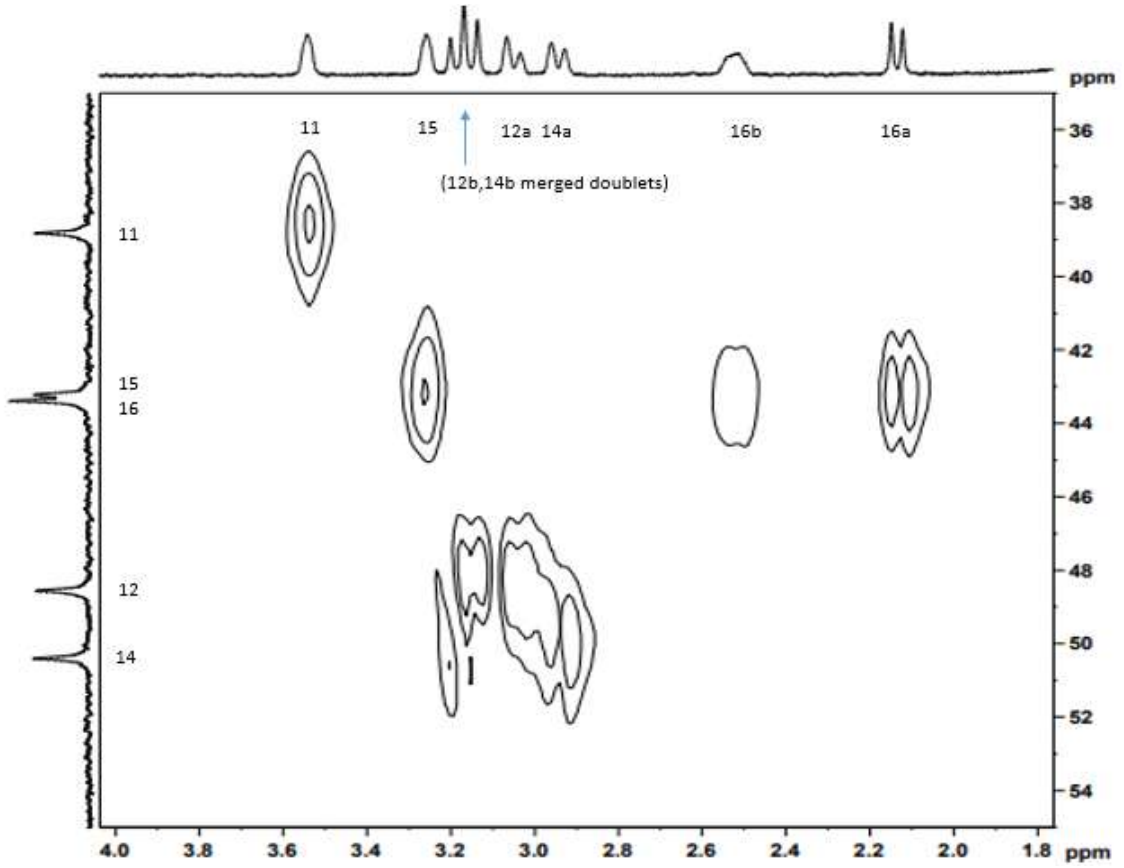

Figure 7 HMQC Spectrum of Hydroxy Varenicline Impurity (upfield region)

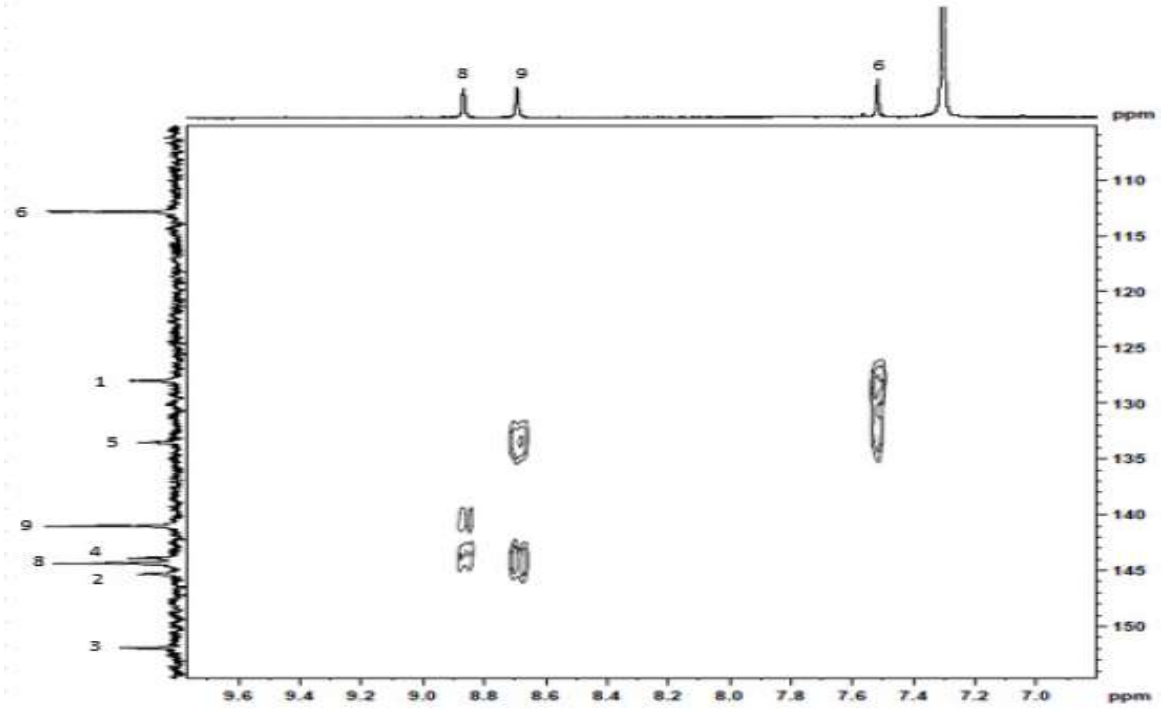

Figure 8 HMBC Spectrum of Hydroxy Varenicline Impurity (Downfield region)

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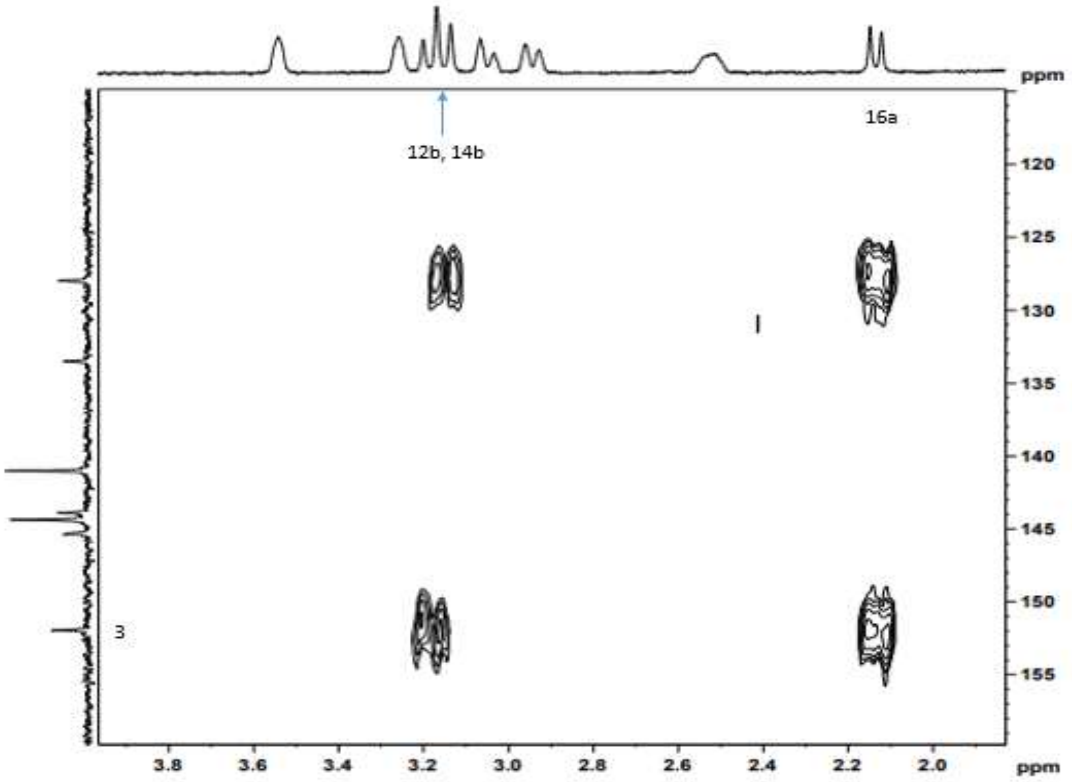

Figure 9 HMBC Spectrum of Hydroxy Varenicline Impurity (upfield region)

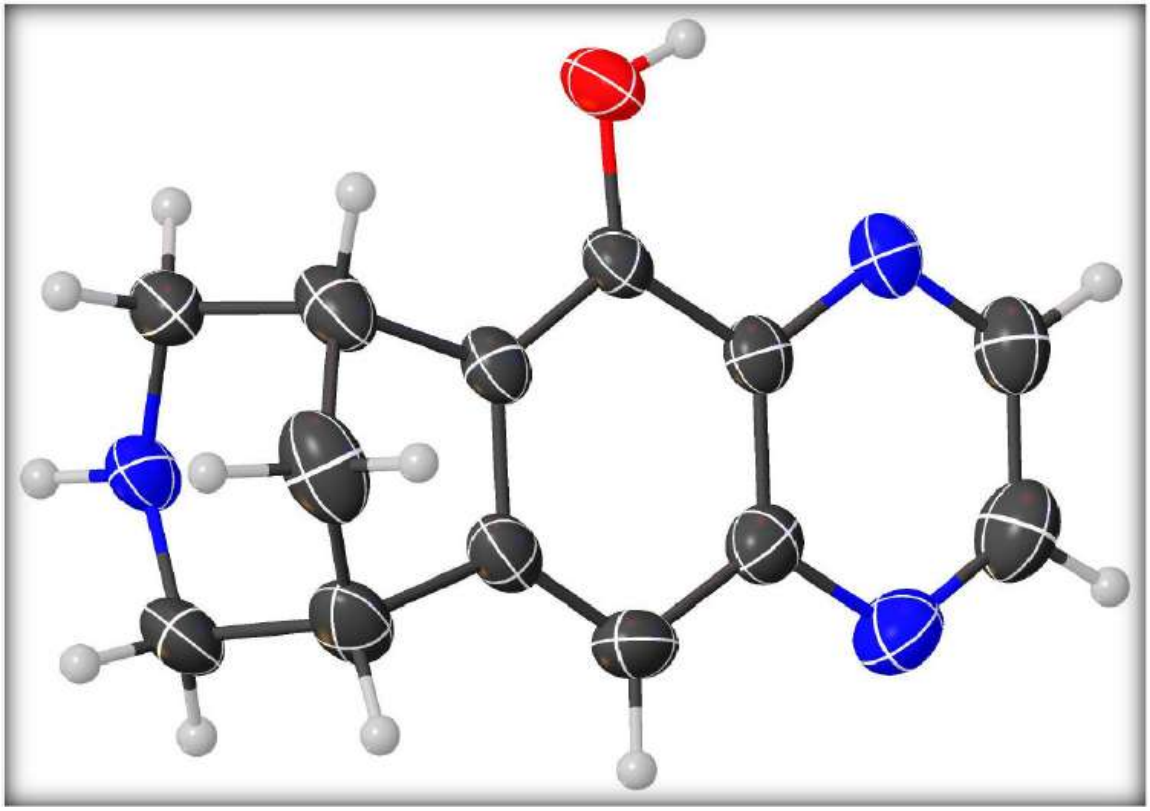

Figure 10: Single crystal of CHNO by Oak Ridge Thermal-Ellipsoid Plot

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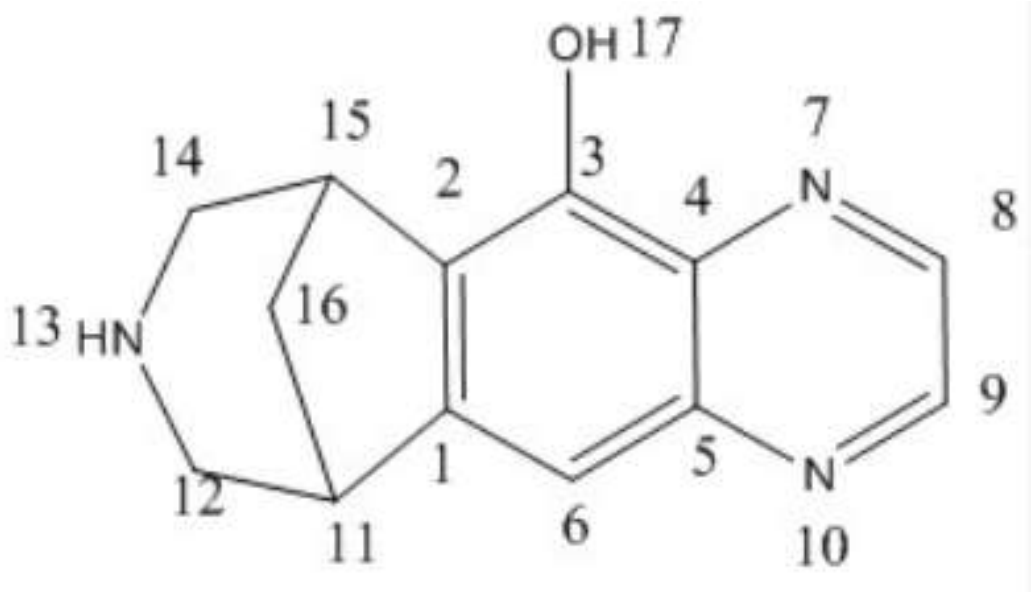

Figure 11: Structure of varenicline related impurity (3-hydroxy varenicline)

Table 1 Spectral assignments of varenicline

Varenicline			
S.No.	Position of proton	δ (ppm)	^{1}H Assignments
1	3, 6	8.006	s,2H
2	8, 9	8.917	s,1H
3	12a, 14a	3.342	d,2H
4	12b, 14b	3.139	d,2H
5	11, 15	3.526	bs, 2H
6	16a	2.186	d
7	16b	2.354	m,1H

Table 2 Spectral assignments of impurity (Hydroxy varenicline)

Hydroxy varenicline impurity					
S. No.	Position of proton	δ (ppm)	^{1}H Assignments	Position of Carbon	δ (ppm)
1	6	7.518	s,1H	11	38.808
2	8	8.695	s,1H	15	43.213
3	9	8.868	s,1H	16	43.379
4	11	3.544	m,1H	12	48.549

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5	12a	3.067	d,1H	14	50.399
6	14a	2.96	d,1H	6	112.83
7	12b, 14b	3.169	dd (merged), 2H	1	127.991
8	15	3.258	bs, 1H	5	133.506
9	16a	2.149	bs, 1H	9	140.998
10	16b	2.531	d	4	143.862
11			m,1H	8	144.354
12				2	145.341
13				3	151.922

IV. CONCLUSION:

The structure of impurity was studied using MS, NMR and single crystal techniques. The data obtained from each of these techniques corroborate to the hydroxy varenicline impurity with structure as shown in Figure 11.

V. ACKNOWLEDGEMENTS:

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