RASĀYAN *J. Chem.*
Vol. 15 | No. 1 |82-95| January - March | 2022
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

STRUCTURE CHARACTERIZATION OF STRESS DEGRADATION PRODUCTS OF ELAGOLIX BY USING HRMS, NMR

S. S. K. Chakravarthy Kotha[1,2], K.V.K. Mohan P[1,2], Raju Doddipalla[1], Dasameswara Rao Kavitapu[1], Chidananda Swamy Rumalla[1], Himabindu Gandham[2,✉], Muralidharan kaliyaperumal[1]

[1]Department of Medicinal Chemistry, Aragen Life Sciences Pvt. Ltd. (Formerly known as GVK Biosciences Pvt Ltd.), 28A, IDA Nacharam, Hyderabad-500076, India.
[2]Department of Engineering Chemistry, Andhra University, Visakhapatnam-530003, India.
✉Corresponding Author: himabinduauce@gmail.com

ABSTRACT

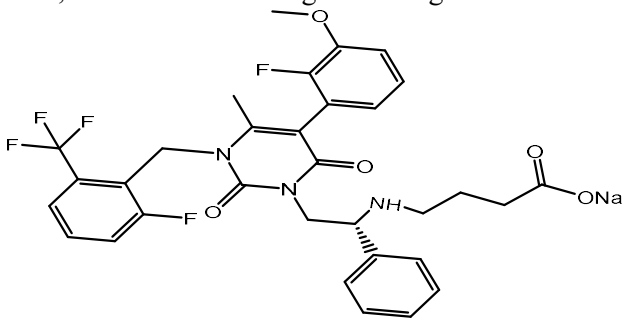
Elagolix Sodium is a recently approved drug used to treat endometriosis in women. Elagolix sodium was exposed to forced degradation (acid, base, neutral, oxidative, thermal, and photolytic) as per International Council for Harmonisation guidelines. Present work describes the structure characterization of Elagolix sodium and its degradants. Three novel degradation products were observed when subjected to oxidative degradation and the drug was stable in other stress conditions. A gradient LC-MS method was developed to separate all the degradants and the Elagolix by using Acquity BEH C18 column with Trifluoroacetic acid and Acetonitrile as mobile phase. Preparative HPLC was used to separate the degradants, further confirmed structurally with HRMS and NMR spectroscopy. The degradation products are novel, not reported earlier.

Keywords: Elagolix, Forced Degradation, Degradation Products, LC-MS, NMR, HRMS.

RASĀYAN *J. Chem.*, Vol. 15, No.1, 2022

INTRODUCTION

Elagolix sodium is commonly called Elagolix (EGX). The chemical formula of Elagolix sodium C32H29F5N3NaO5, Elagolix is used in the form of sodium salt, which is white to off white solid, freely soluble in water. Elagolix melting range is 186-190°C. Elagolix is marketed under Orilissa. Pain associated with endometriosis in women[1-6] can be cured by taking Elagolix orally and it is the first orally active, non-peptide second-generation GnRH antagonist. Elagolix differs from other GnRH antagonist, which is first-generation and peptides. EGX was approved in July 2018 by FDA. EGX is under examination to treat patients suffering from uterine fibroids, prostate cancer in men and heavy menstrual bleeding in women.[7] Two dosage forms 150mg and 200mg are available, once or twice can be taken.[1,7] Prolonged usage of Elagolix leads to side effects. Quality of life has improved by the usage of Elagolix.[8] EGX contains one chiral center, and the structure is given in Fig.-1.



Chemical Formula: $C_{32}H_{29}F_5N_3NaO_5$
Exact Mass: 653.19

Fig.-1: Structure of Elagolix Sodium

http://dx.doi.org/10.31788/RJC.2022.1516527
This work is licensed under a CC BY 4.0 license.

Drug usage can vary from one individual to another depending on the duration and the dosage. Duration of the drug varies from 6 to 24 months. The stability of the drug plays an important role in the drug activity; if the drug is not stable its losses its activity and may form degradation products, finally leading to side effects. ICH has given a set of guidelines to check the stability behavior of the drug; stress degradation is one of the methods to know the drug stability. Few studies reported in the literature are chiral method used to quantify enantiomers of Elagolix[9], Identified Elagolix a potent GNRH antagonist[10], Short study on the clinical efficiency of Elagolix[11], a monograph of pharmacokinetic study and clinical efficacy of Elagolix[12], Combination of Elagolix with other drugs has been clinically assessed[13], Efficacy study of Elagolix in patients with endometriosis.[14] Structural characterization of degradation products have not been reported in any of the literature. The present work deals with the stability of the drug under degradation conditions as per ICH guidelines.[15-18] Three degradation products were identified in oxidative degradation and further purified by preparative, later the structures were confirmed accurately by NMR and HR-MS. Three degradants were marked as DP - 1, 2 and 3, all the DPs are novel, not reported in any of the literature.

EXPERIMENTAL

Chemicals

Elagolix was offered as a gift from a local pharmaceutical company, Hyderabad. Sigma chemicals (ABC, ACN, MeOH), Water (Honeywell). HPLC grade water from Merck Millipore, India. Merck Analytical grade chemicals (HCl, NaOH, H_2O_2, TFA).

Instrumentation

CAD Instrument Details

CAD detector from Thermo coupled with Waters Alliance e2695 HPLC was used to estimate the presence of sodium in Elagolix sodium.

Chromatographic conditions for CAD HPLC

SeQuant ZIC HILIC (4.6x150) mm, 5µm, MP - A: FA (0.1%) in Acetonitrile, MP - B: 30mM Ammonium Acetate + 2mL of formic acid in H2O. Isocratic mode (MP) ratio A (65): B (35)) with 1mL flow, the temperature of column (°C) is 25 and sample dissolved in water.

CAD (Charged Aerosol Detector) **Conditions**

Gas flow rate: 60 psi, Evaporator temperature: 35°C and filter: 1.0 sec. CAD chromatograms are attached in additional data.

Liquid Chromatography-Mass spectrometry (LCMS) Details

Agilent Infinity 1290 LC was used for chromatographic separation. Mass analysis was taken on 6130 Agilent single quadrupole mass spectrometer (SQD) with Electrospray ionization source, data taken in positive ionization mode. Data was acquired using Agilent Open Access software and instrument was controlled by chemstation.

Liquid Chromatography Conditions

EGX and its DPs were separated using UPLC C18 column 2.1x100mm, 1.7µm; 0.05% TFA (solvent A) in water and Acetonitrile (solvent B) as mobile phase (MP), analysis was performed in gradient conditions, Time (min)/% of solvent B: 0/3, 0.4/3, 75/98, 9.6/3, 10/3, temperature (°C) of the column – 35, flow (mL) - 0.45, UV monitored at 215nm. All the DP's and API were freely soluble in acetonitrile and water.

Mass Spectrometry Conditions

Mass data were acquired by using Agilent 6130 SQD detector with ESI, source conditions are drying gas temperature(°C): 300, capillary and fragmentor voltage (V): 4000 and 70, nebulizer (Psi): 40, Drying gas (L/min): 9, nitrogen was used as a drying gas and nebulizing gas. Data were acquired in the mass range from 100-1000 Da.

HR-MS Instrument Details

Orbitrap HRMS was used for analysis. Heated Electrospray Ionization source was used, and the source parameters were Spray (kV) - 3.8, gas temperatures of capillary and Aux are 250 and 400 °C, Sheath and Aux gas is 45 and 14 arbitrary units. Data were acquired in full scan MS using positive ionization mode, mass range: 100-1500 Da. Nitrogen was used as collision gas for mass fragmentation. Normalized collision energy (NCE) improved fragmentation efficiency across mass range. NCE was used between 10-30 eV. Mass accuracy was verified by injecting the reserpine standard before performing the analysis. Excalibur software was used to control the instrument.

Mass Directed Prep HPLC Details

Waters Mass Directed Prep HPLC equipped with 2545 Binary Pump, Waters 2767 Sample Manager and Waters PDA 2998 detector, Waters Acquity QDA mass detector with ESI source was used to isolate the degradants. The whole instrument was controlled by Masslynx software. XSelect CSH Phenyl Hexyl column (19x250mm) 5µm, gradient conditions are A = 5mM ABC (aq), B = ACN, program (%B) 0/20, 2.0/20, 7/80, 13/90, 14/20, 16/20, flow rate: 19 mL/min and data was recorded in maxplot.

NMR Spectroscopy Details

AVANCE NEO 400 NMR of Bruker was used to analyze Elagolix and 3 degradation products. The instrument has equipped with PA-BBO probe with Z-gradient shim, autosampler having a capacity of 60 samples. Probe temperature was 298k with automatic fine-tuning. In proton NMR, TMS as internal reference. ppm is units for Chemical shift, coupling constant is denoted by J, units are Hz. TMS set at zero ppm in proton NMR, a septet was observed at 39.5ppm in carbon NMR for DMSO-D6. Data recorded for 1D (^{1}H and ^{13}C experiments) and 2D (gDQCOSY, gHSQC and gHMBC experiments) NMR spectroscopy.

Supercritical Fluid Chromatography Details

Water Acquity UPC^2 was used to separate the Isomers present in Elagolix (API). UPC^2 consisted of Convergence manager for controlling the Automatic Back Pressure Regulator (ABPR), Acquity Autosampler, Acquity Binary Solvent manager with four channels (pump A was liquid CO2 and pump B was for organic solvents), Column manager consisted of 8 columns with switch-over valve and Acquity PDA. 0.1% Ammonium hydroxide in methanol was used as a makeup solvent for 515 pumps with 0.3 mL/min. Chiralcel OD-3 (4.6*150 mm) 3µm column from Daicel technologies ltd, Japan was used to separate the isomers. The mobile phase consists of A: CO2, B: 0.5% DEA in methanol. Isocratic run with A: B 70:30 ratio, flow (g/min): 3, methanol as a diluent, autosampler temperature was 30°C and automatic back pressure regulator (ABPR) set at 1500 psi. All the modules were controlled by Empower 3 software.

Protonated mass was obtained from the water QDA detector. Mass scan range is 100-1000Da. Capillary Voltage 1.5kV, Cone Voltage - 25V, Source and Probe temperature (°C) 140° and 550, gas flow (L/h) for desolvation and cone are 650 and 50. SFC chromatogram was provided in additional data.

FTIR Details

Functional groups can be identified by using FTIR[19]. Shimadzu IR Affinity-1S was used to run the IR spectrum. Class 1 laser beam was used in IR. Potassium Bromide (KBr) was used as a dispersion medium for making the sample pellet. IR spectrum of EGX and its degradation products were recorded; all the IR data was given in additional data.

CAD Sample Preparation

1mg/mL of both standard and sample solutions were prepared by using HPLC grade water, and the solutions were taken for analysis.

LCMS Sample Preparation

Approximately 0.5 to 1 mg of sample was dissolved in water, acetonitrile.

NMR Sample Preparation
Dimethyl sulphoxide is used for sample preparation; 4-5 mg of sample was used for analysis.

FTIR Sample Preparation
A small portion (100-150 milligram) of KBR was added to a few amounts (1-2 milligram) of the sample, finely grinds the mixture and made the pellet.

Prep Sample Preparation
Sample dissolved in water, acetonitrile and the sample solution is injected on Prep HPLC.

Stress Degradation Studies of EGX
Stress studies were carried out on EGX as specified by the ICH guidelines.[15-18] For acid degradation, 1N HCl, for base degradation 1N NaOH was added to the EGX, both the solutions were divided into two portions, one portion of the solution was kept at RT, the second portion was kept at high temperature (60°C) for 72 hrs, later both solutions were neutralized by using 1N NaOH for an acid solution and 1N HCl for a base solution and taken for further analysis. No degradation was observed with Acetonitrile: water (1:1 v/v) for EGX, kept the solution for 48hrs further taken for analysis. For oxidative degradation, 3% H_2O_2 was added to EGX and kept at room temperature in a dark place for 24hrs. A small portion (100 mg) of a sample was taken into a glass vial for thermal study and the sample was placed in a hot air oven at 120°C for 48hrs, no physical change was observed. For photolytic study, a small portion of EGX was taken in a glass plate and kept under a photo stability chamber for 24hrs; the sample was taken for further analysis.
All the stress degradation samples were collected separately and diluted with acetonitrile-water and used for LCMS analysis. LCMS chromatograms of all the stress conditions are shown in Fig.-2.
Three degradants were formed under oxidative study; the drug was stable in other stress conditions. All the stress condition data of EGX are tabulated in Table-1.

Table-1: Forced Degradation Study Details of Elagolix

S.No.	Stress State	% of API
1	Elagolix	98.56%
2	Acid	97.85%
3	Base	98.09%
4	Oxidation	82.16%, 5.33% of DP-1, 1.48% of DP-2, 5.57% of DP-3
5	Thermal	96.92%
6	Photolytic	97.91%

RESULTS AND DISCUSSION

LCMS Optimization
Initial trials were taken on Acquity BEH C18 column 50x2.1mm, 1.7µm column using FA conditions. Peak shape was not good in formic acid and the resolution was poor between the EGX and the DPs. Optimization was critical with the formic acid mobile phase, later the experiments were carried out by using 0.05% TFA in water and Acetonitrile as a mobile phase by using Acquity BEH C18 2.1x100mm, 1.7µm column. A gradient method was optimized to separate all the peaks present in the mixture, EGX and the DPs had good peak shape with proper ionization in TFA buffer.
Further, all the three degradation products were isolated by using Prep-HPLC, all the Preparative fractions were lyophilized. All the degradants were structurally confirmed by using HRMS and NMR.

Structural Elucidation of DP-1
DP-1 was formed by the forced degradation of EGX with 3% Hydrogen peroxide. HRMS analysis of DP-1 was carried on Thermo Orbitrap MS by using ESI source, the protonated mass of DP-1 is 562.1759 $[M+H]^+$, HRMS data of DP-1 is shown in Fig.-3. HRMS data of DP-1 shows a decrease of 70 Da less compared with EGX, molecular formula is C32H31N3O5F5, 632.2178 $[M+H]^+$. HRMS analysis of DP-1

shows 15.5 ring double bond equivalents (RDBE) whereas EGX shows 16.5 RDBE, there is a difference of C4H6O units less in DP-1 compared with EGX. To confirm the precise structure of DP-1 various NMR experiments like Proton, Carbon, COSY, g-HSQC and g-HMBC were recorded. Table-2 shows the full characterization details of Elagolix (API) and DP-1.

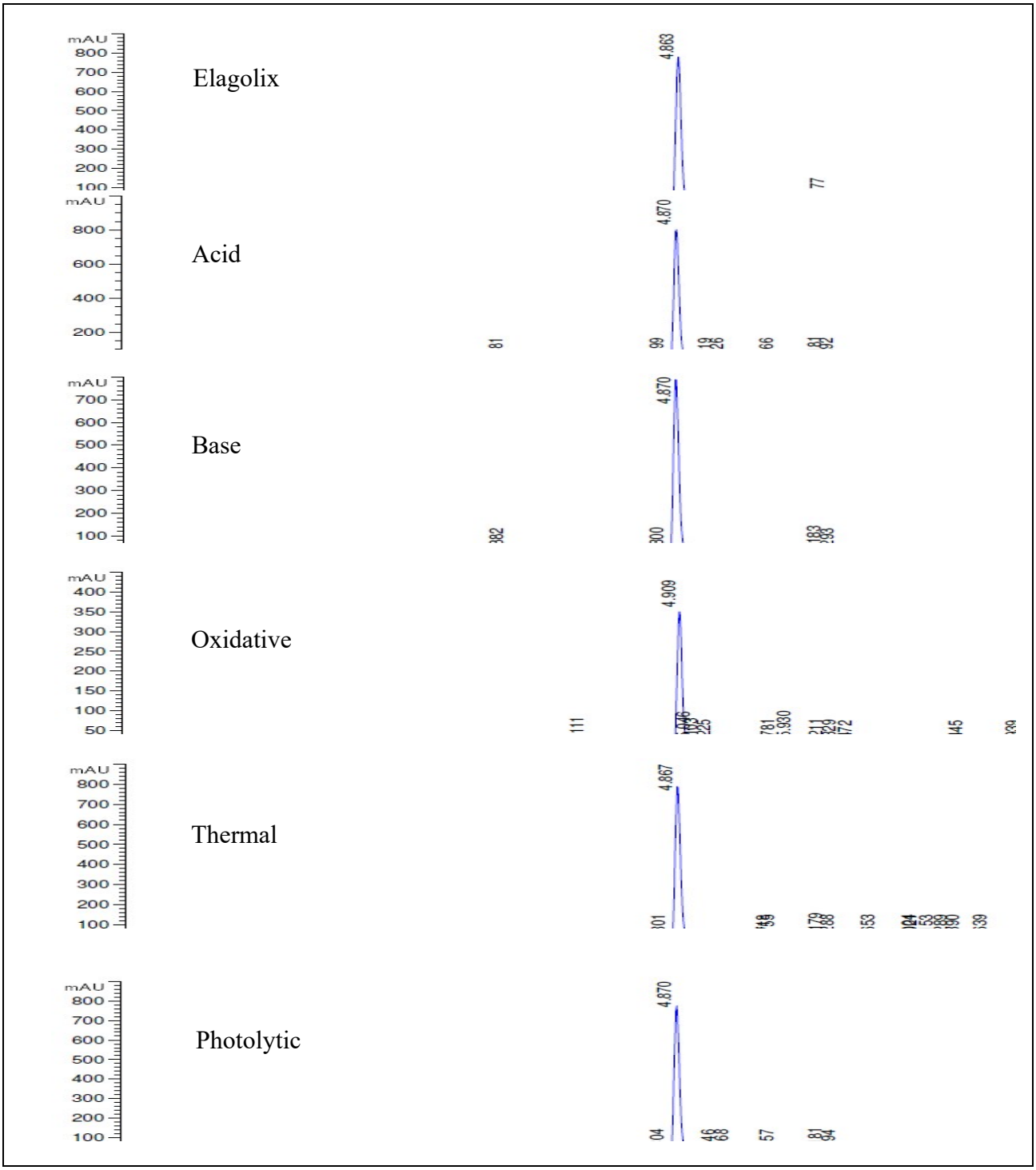


Fig.-2: LCMS Chromatograms of Elagolix, Acid, Base, Oxidative, Thermal and Photolytic.

Table-2: ^{1}H and ^{13}C Labelling of Elagolix, Elagolix DP-1

Elagolix			
S. No. of Atom	Atom Name	ppm Value of Proton and J Value	ppm Value of Carbon
1	-CH	7.64(d,8.0Hz,1H)	122.71
2	-CH	7.54(m,1H)	129.74(d,10H)
3	-CH	7.57(m,1H)	121.21(d,23H)
4	-C	-	159.52&161.98 (d,246Hz)
5	-C	-	128.31
6	-C	-	122.57
7	-C	-	122.23&124.96
8,9,10	-F	-	-
11	-F	-	-
12	-CH2	5.32(geminal doublet,17.2Hz,2 H)	42.75
13	-N	-	-
14	-C	-	151.15
15	-N	-	-
16	-C	-	160.47
17	-C	-	106.86
18	-C	-	150.64
19	-O	-	-
20	-CH3	2.08(s,1H)	17.67
21	-O	-	-
22	-CH2	3.97(m,2H)	46.7
23	-CH	3.89(m,1H)	60.25
24	-NH	Not Observed	-
25	-C	-	141.95
26,30	-CH	7.27(m,2H)	126.9
27,29	-CH	7.21(m,2H)	127.95
28	-CH	7.2(m,1H)	127.02
31	-C	-	122.66
32	-CH	6.58&6.75 (m,2H)	123.63
33	-CH	7.13(m,1H)	123.98
34	-CH	7.16 (m, 1H)	113.36
35	-C	-	147.33
36	-C	-	150.64&148.30 (d,234Hz)
37	-F	-	-
38	-O	-	-
39	-CH3	3.85(s,3H)	55.96
40	-CH2	2.22(m,2H)	47.76
41	-CH2	1.47 (m, 2H)	27.12
42	-CH2	1.77 (m, 2H)	36.12
43	-C	-	176.81
44	-O	-	-
45	-OH	Not observed	-

Elagolix DP-1			
S. No. of Atom	Atom Name	ppm Value of Proton and J Value	ppm Value of Carbon
1	-CH	7.65 (d,7.2Hz, 1H)	122.69
2	-CH	7.57 (m, 1H)	129.84
3	-CH	7.53 (m, 1H)	121.13
4	-C	-	159.57&162.03 (d,246Hz)
5	-C	-	122.55
6	-C	-	122.54
7	-C	-	122.29&125.01 (272Hz)
8,9,10	-F	-	-
11	-F	-	-
12	-CH2	(5.32,geminal d,16.8Hz,2H)	42.86
13	-N	-	-
14	-C	-	151.2
15	-N	-	-
16	-C	-	160.57
17	-C	-	106.88
18	-C	-	150.81
19	-O	-	-
20	-CH3	2.08(S,3H)	17.75
21	-O	-	-
22	-CH2	4.04(m,2H)	43.89
23	-CH	4.19(t,7.2Hz,1H)	63.74
24	-NH	Not observed	-
25	-C	-	140.13
26,30	-CH	7.25(m,2H)	127.55
27,29	-CH	7.26(m,2H)	127.9
28	-CH	7.26(m,1H)	127.12
31	-C	-	122.55
32	-CH	6.58&6.74(m,1H)	123.63
33	-CH	7.15 (m, 1H)	124.1
34	-CH	7.18(m,1H)	113.47
35	-C	-	147.42
36	-C	-	148.39&150.81 (242Hz)
37	-F	-	-
38	-O	-	-
39	-CH3	3.85(s,3H)	56.03
40	-OH	Not observed	-

s denotes singlet, d denote doublet, t denotes triplet, m denote multiplet

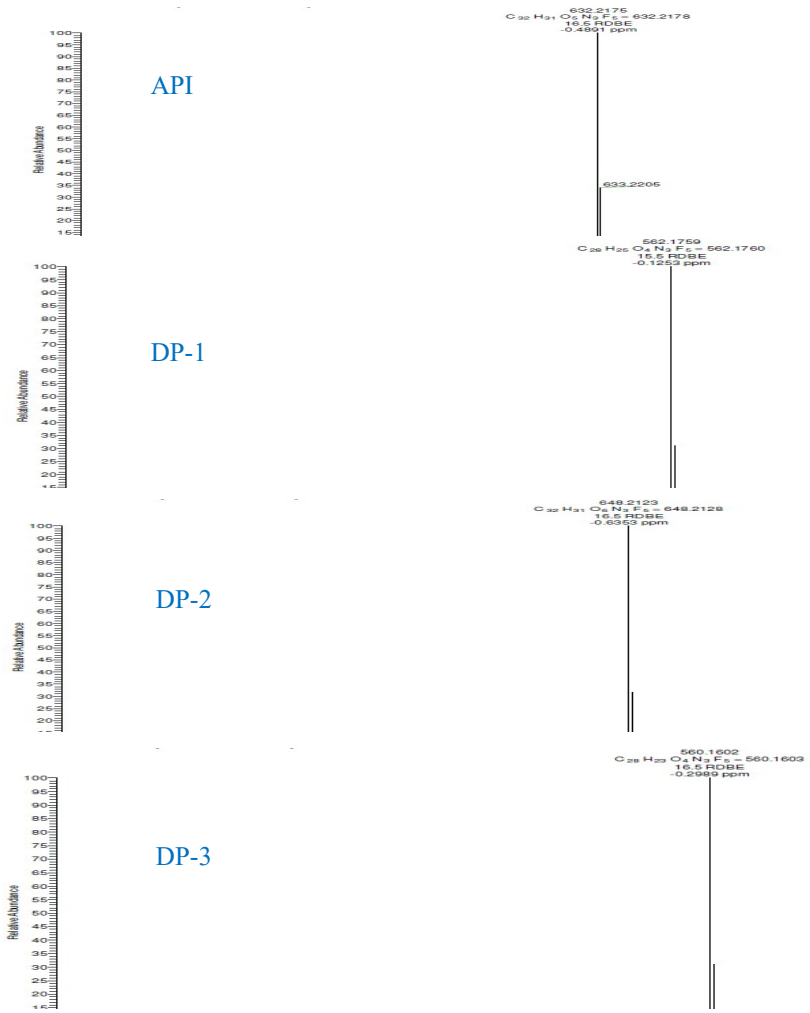


Fig.-3: Elagolix(API), DP-1, DP-2 and DP-3 HR-MS Spectrum

In proton NMR, no protons of butanoic acid chain are observed and in carbon NMR Acid carbonyl carbon is missing, remaining all the correlations match with the API, carbon NMR (Fig.-4a). ^{1}H-^{13}C-gHSQC experiment confirms the presence of one methyl group, two methylene groups and one methine in aliphatic region (Fig.-4b). In ^{1}H-^{13}C-gHMBC experiment all the 2J and 3J correlations are identical with respect to API, here H23 Proton showing connectivity with C22, C25 and C26, C30(2C) carbons, finally the structure has been concluded by eliminating the butanoic acid and the formation of hydroxylamine group at C23 carbon. Fig.-5 shows the DP-1 structure. MR of DP-1 (122 – 126°C). IR absorption frequencies (cm^{-1}) of Elagolix are 3442.03 (O-H), 2947.28 (NH), 1653.02 (C=O), 1316.44 (C-F), 1121.63 (C-O-C) stretching is observed and the absorption frequencies (cm^{-1}) of DP-1 are 1651.09 (C=O), 1467.85 (N-O), 1316.44 (C-F), 1121.63 (C-O-C). Based on the above experiments, the molecular formula of DP-1 is C28H24N3O4F5.

Structural Elucidation of DP-2

HRMS analysis of DP-2 was taken on Thermo Orbitrap instrument with ESI source, and the protonated mass of DP-2 is 648.2123 [M+H]$^{+}$, HRMS data of DP-2 is shown in Fig.-3. HR-MS data shows that there is an increase of 16 mass units with respect to EGX. Both EGX and DP-2 are having 16.5 RDBE this shows that there is no change in the structural rearrangement only an increase of one oxygen atom in the DP-2. The exact structure has been characterized by NMR, details mentioned in Table-3. Both carbon and Proton NMR data of DP-2 is slightly different from EGX (API). By using ^{1}H-^{13}C-gHSQC experiment, protonated carbons in the structure were identified. With the help of ^{1}H-^{13}C-gHMBC experiment the 2J,

3J connectivity's were identified. By comparing the HMBC data of DP-2 with API, no changes in connectivity were observed and finally it was concluded that hydroxylamine group has been formed in the DP-2. Figure-6 shows the DP-2 structure. MR of DP-2 (158 – 162°C). IR absorption frequencies (cm^{-1}) of DP-2 are 346.24 (O-H), 1647.24 (C=O), 1468.82 (N-O), 1317.40 (C-F), 1122.59 (C-O-C). Based on the above experiments, the molecular formula of DP-2 is C32H30N3O6F5.

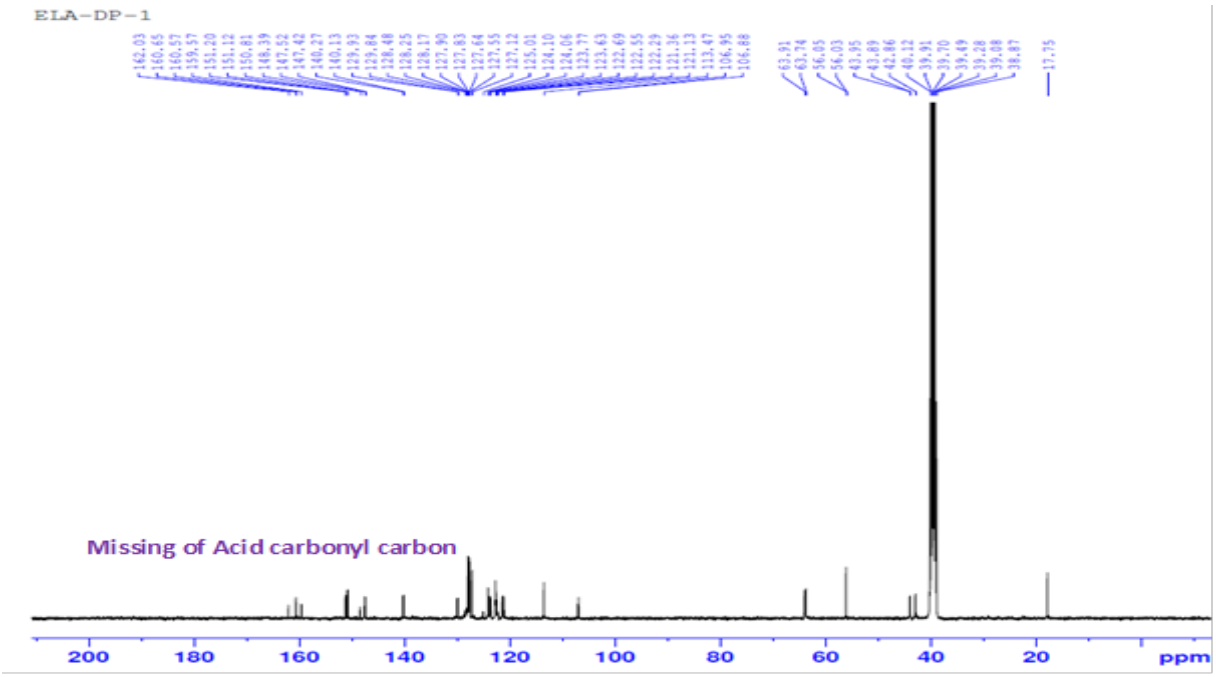


Fig.-4a: Carbon NMR Spectrum of Elagolix DP-1

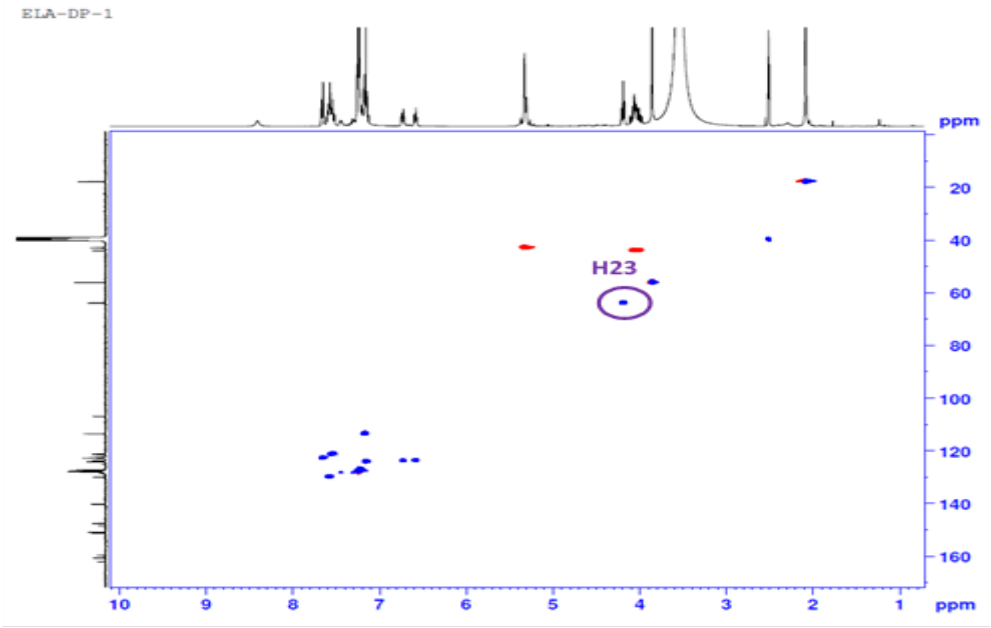
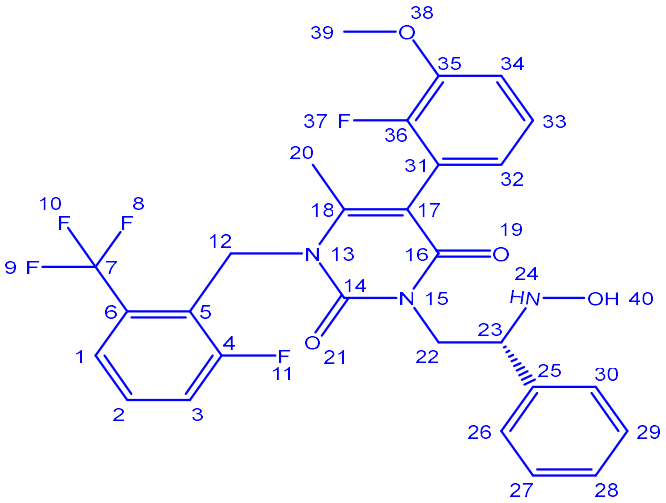
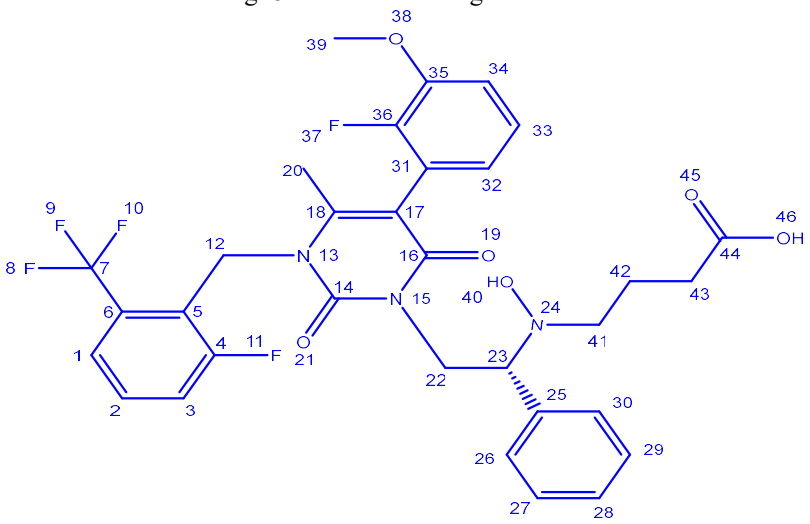


Fig.-4b: gHSQC NMR Spectrum of Elagolix DP-1



Chemical Formula: $C_{28}H_{24}F_5N_3O_4$
Exact Mass: 561.17

Fig.-5: Structure of Elagolix DP-1



Chemical Formula: $C_{32}H_{30}F_5N_3O_6$
Exact Mass: 647.21

Fig.-6: Structure of Elagolix DP-2

Table-3: ^{1}H and ^{13}C Labelling of Elagolix DP-2

Elagolix DP-2			
S. No. of Atom	Atom Name	ppm Value of Proton and J Value	ppm Value of Carbon
1	-CH	7.64 (d, 7.6Hz, 1H)	122.64
2	-CH	7.58 (m, 1H)	129.77
3	-CH	7.52 (m, 1H)	121.07(d,22Hz)
4	-C	-	151.96&161.92(d,246Hz)
5	-C	-	122.51
6	-C	-	122.64
7	-C	-	122.29&124.93(264Hz)
8,9,10	-F	-	-
11	-F	-	-
12	-CH2	5.27(geminal d,17.2Hz,2H)	42.74

13	-N	-	-
14	-C	-	150.97
15	-N	-	-
16	-C	-	160.51
17	-C	-	106.76
18	-C	-	150.48
19	-O	-	-
20	-CH3	2.02(s,3H)	17.62
21	-O	-	-
22	-CH2	4.27(m,2H)	43.53
23	-CH	3.947(t,6.8Hz,1H)	67.57
24	-N	-	-
25	-C	-	136.8
26,30	-CH	7.12(m,2H)	129.04
27,29	-CH	7.17 (m, 2H)	127.5
28	-CH	7.18 (m, 1H)	127.2
31	-C	-	122.64
32	-CH	6.47,6.68(m,1H)	123.52
33	-CH	7.14 (m, 1H)	123.93
34	-CH	7.13 (m, 1H)	113.33
35	-C	-	147.31 (d,11Hz)
36	-C	-	148.29&150.72(d,243Hz)
37	-F	-	-
38	-O	-	-
39	-CH3	3.84(s,3H)	55.94
40	-OH	Not Observed	-
41	-CH2	2.28(m,2H)	56.25
42	-CH2	1.58 (m, 2H)	22.71
43	-CH2	2.08 (m, 2H)	32.07
44	-C	-	175.22
45	-O	-	-
46	-OH	Not observed	-

s denotes singlet, d denote doublet, t denotes triplet, m denote multiplet

Structural Elucidation of DP-3

DP-3 was formed by the forced degradation of EGX with 3% Hydrogen peroxide. HRMS analysis of DP-3 shows a protonated mass of 560.1602 $[M+H]^+$, Fig.-3 shows DP-3 HR-MS data.HR-MS data shows that there is a decrease of 72 mass units when compared with EGX. HRMS analysis shows 16.5 RDBE for both EGX and DP-3. There is a difference of C4H8O units less in DP-3 compared with EGX. To confirm the precise structure of DP-3 various NMR experiments like Proton, Carbon, COSY, HSQC and HMBC were recorded. Full characterization details of Proton and carbon has given in table.4. Proton NMR data of DP-3 was differ from API, observed two methylene groups and one exchangeable proton at 11.3ppm, missing of aliphatic methine and butanoic acid protons. In Carbon NMR one extra aromatic carbon C23 is observed at 154.24 ppm and missing of butanoic acid chain carbons. ^{1}H-^{13}C-gHSQC experiment confirms that only two methylene groups are present in the compound. To check the connectivity in DP-3 ^{1}H-^{13}C-gHMBC experiment were recorded. Here the key correlation is observed at H22 and H26, H30 protons showing connectivity to C23 carbon, and it shows that there is a formation of oxime group by the cleavage of butanoic acid group. Oxime proton and other key points were identified in NMR spectrum as shown in Fig.-7a, 7b and 7C. Fig.-8 shows DP-3 structure. MR of DP-3 (125 – 129°C). IR absorption frequencies (cm^{-1}) of DP-3 are 1708.96 (C=N), 1654.95 (C=O), 1482.32 (N-O), 1317.40 (C-F), 1121.63 (C-O-C). Comparative FTIR Absorption frequencies of Elagolix and three degradants are tabulated in Table-5. Based on the above experiments, the molecular formula of DP-3 is C28H22N3O4F5.

Table-4: ^{1}H and ^{13}C Labelling of Elagolix DP-3

Elagolix DP-3			
S. No. of Atom	Atom Name	ppm Value of Proton and J Value	ppm Value of Carbon
1	-CH	7.60 (d, 7.6Hz, 1H)	122.49
2	-CH	7.54 (m, 1H)	129.56
3	-CH	7.41 (m, 1H)	120.99(d,24Hz)
4	-C	-	159.34&161.81(d,247Hz)
5	-C	-	127.95
6	-C	-	122.35
7	-C	-	122.19&124.84(265Hz)
8,9,10	-F	-	-
11	-F	-	-
12	-CH2	5.22(s,2H)	42.77
13	-N	-	-
14	-C	-	150.39
15	-N	-	-
16	-C	-	160.41
17	-C	-	106.64
18	-C	-	150.94
19	-O	-	-
20	-CH3	2.03(s,3H)	17.65
21	-O	-	-
22	-CH2	5.04(geminal d,16.8Hz,2H)	37.57
23	-C	-	154.24
24	-N	-	-
25	-C	-	133.88
26,30	-CH	7.14(m,2H)	126.68
27,29	-CH	7.24(t,8.0Hz,2H)	127.82
28	-CH	7.35(t,7.6Hz,1H)	128.42
31	-C	-	122.35
32	-CH	6.56(m,1H)	123.48
33	-CH	7.12 (m, 1H)	123.99
34	-CH	7.19 (m, 1H)	113.44
35	-C	-	147.36(d,10Hz)
36	-C	-	148.30&150.73(d,243Hz)
37	-F	-	-
38	-O	-	-
39	-CH3	3.85(s,3H)	55.95
40	-OH	11.3 (Broad signal)	-

s denotes singlet, d denote doublet, t denotes triplet, m denote multiplet

Table-5: Comparative FTIR Absorption Frequencies of Elagolix and Three Degradants

S.No.	Elagolix	DP-1	DP-2	DP-3
1	3442.03 (O-H)	-	3436.24 (O-H)	-
2	-	-	-	1708.96 (C=N)
3	1653.02 (C=O)	1651.09 (C=O)	1647.24 (C=O)	1654.95 (C=O)
4	-	1467.85 (N-O)	1468.82 (N-O)	1482.32 (N-O)
5	1316.44 (C-F)	1316.44 (C-F)	1317.4 (C-F)	1317.4 (C-F)
6	1121.63 (C-O-C)	1121.63 (C-O-C)	1122.59 (C-O-C)	1121.63 (C-O-C)

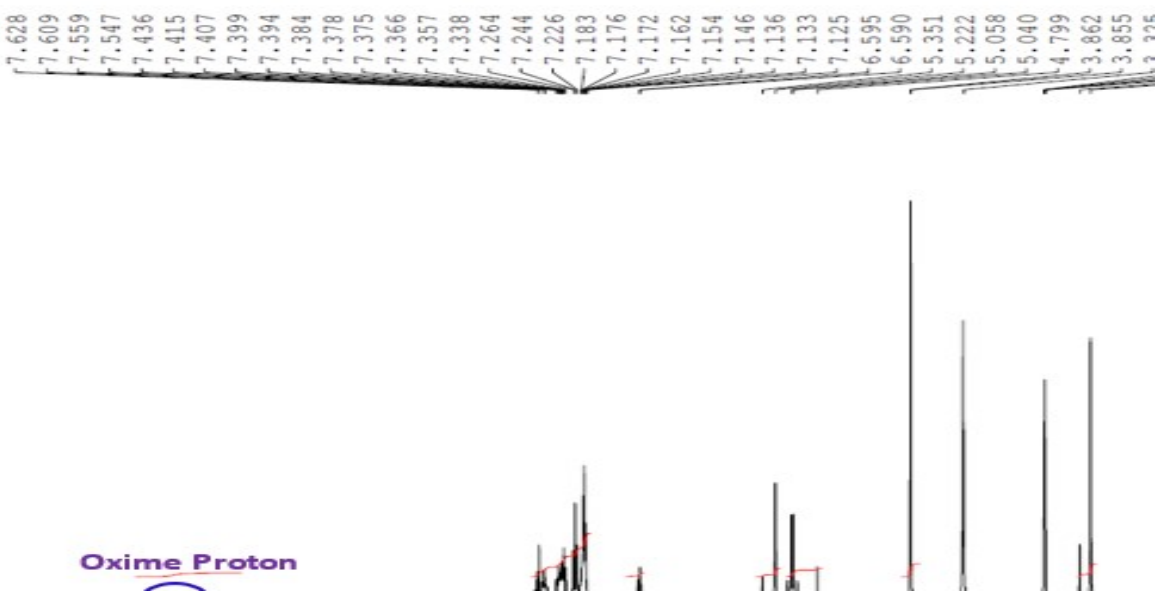


Fig.-7a: Proton NMR Spectrum of Elagolix DP-3

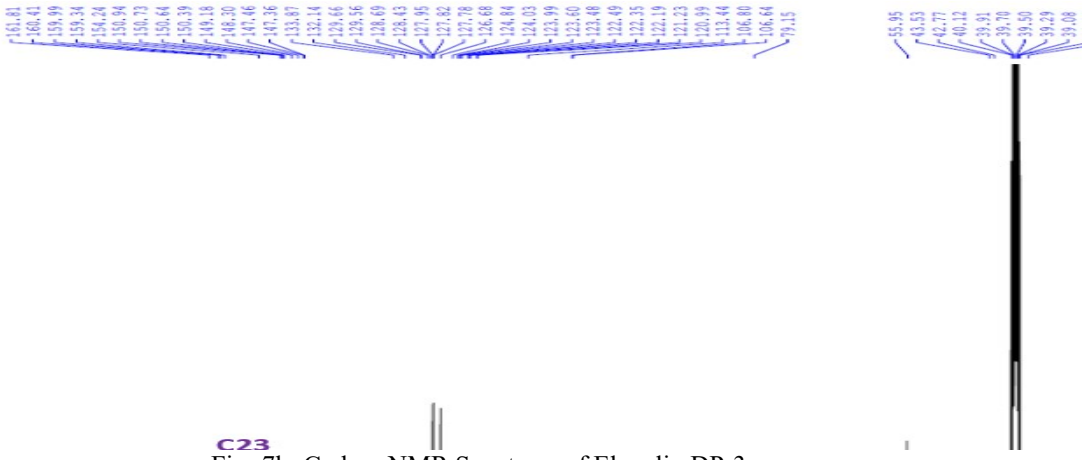


Fig.-7b: Carbon NMR Spectrum of Elagolix DP-3

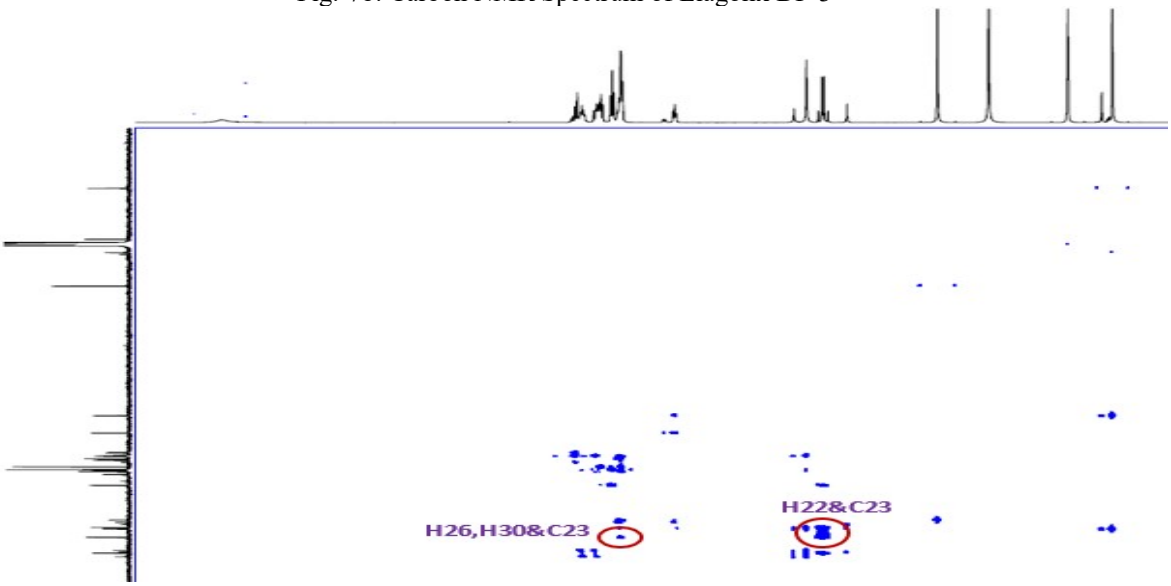
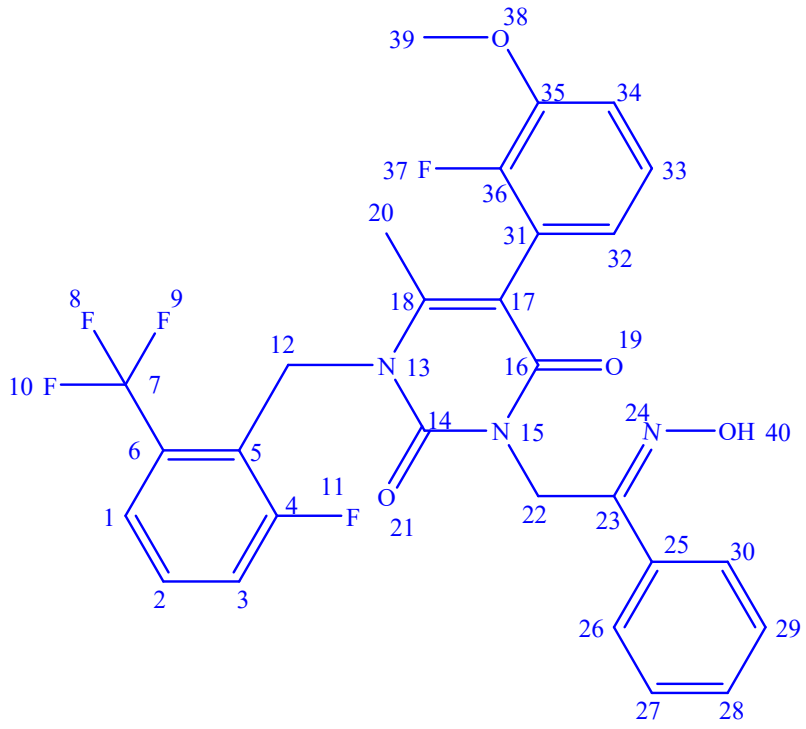


Fig.-7c: HMBC NMR Spectrum of Elagolix DP-3



Chemical Formula: $C_{28}H_{22}F_5N_3O_4$
Exact Mass: 559.15

Fig.-8: Structure of Elagolix DP-3

CONCLUSION

Stress studies of Elagolix were carried out according to ICH. EGX is stable with respect to acidic, basic, neutral, thermal, and photolytic conditions. Oxidative stress condition of EGX gives rise to three Degradation products. All the DPs were well separated in LCMS, further structurally characterized by HRMS and NMR spectroscopy.

ACKNOWLEDGEMENT

The authors are thankful to Aragen Life Sciences Pvt. Ltd. for their support in doing the work.

REFERENCES

1. Orilissa- Elagolix Tablet, Film Coated, *DailyMed*, 5 May 2020, Retrieved 30 May 2020.
2. FL. Tukun, DE. Olberg, PJ. Riss, I. Haraldsen, A. Kaass and J. Klaveness, *Molecules*, **22(12)**, 2188(2017), https://doi.org/10.3390/molecules22122188
3. M. Ezzati and BR. Carr, *Women's Health*, **11(1)**, 19(2015), https://doi.org/10.2217/whe.14.68
4. Orilissa (Elagolix) Health Canada Product Monograph, 4 October 2018, Retrieved 15 February 2019.
5. YN. Lamb, *First Global Approval Drugs*, **78(14)**, 1501(2018), https://doi.org/10.1007/s40265-018-0977-4
6. S. Clemenza, F. Sorbi, I. Noci, T. Capezzuoli, I. Turrini, C. Carriero, N. Buffi, M. Fambrini and F. Petraglia, *Best Practice and Research Clinical Obstetrics and Gynaecology*, **51**, 92(2018), https://doi.org/10.1016/j.bpobgyn.2018.01.021
7. *Adis Insight*: Elagolix.
8. E. Surrey, HS. Taylor, L. Giudice, BA. Lessey, MS. Abrao, DF. Archer, MP. Diamond, NP. Johnson, NB. Watts, JC. Gallagher, JA. Simon, BR. Carr, WP. Dmowski, N. Leyland, SS. Singh, T. Rechberger, SK. Agarwal, WR. Duan, B. Schwefel, JW. Thomas, PM. Peloso, J. Ng, AM. Soliman

and K. Chwalisz, *Obstetrics and Gynecology* **132(1),** 147(2018), https://doi.org/10.1097/AOG.0000000000002675

9. T. Choppari, S. Gunnam, L. N. Chennuru and P. M. Cherla, *International Journal of Pharmaceutical Sciences and Drug Research*, **12(5)**, 517(2020), https://doi.org/10.25004/IJPSDR.2020.120513
10. C. Chen, D. Wu, Z. Guo, Q. Xie, GJ. Reinhart, A. Madan, J. Wen, T. Chen, CQ. Huang, M. Chen, Y. Chen, FC. Tucci, M. Rowbottom, J. Pontillo, YF. Zhu, W. Wade, J. Saunders, H. Bozigian and RS. Struthers, *Journal of Medicinal Chemistry* **51(23)**, 7478(2008), https://doi.org/10.1021/jm8006454
11. M. Neri, GB. Melis, E. Giancane, V. Vallerino, M. Pilloni, B. Piras, A. Loddo, AM. Paoletti and V. Mais, *International Journal of women's Health*, **11**, 535(2019), https://doi.org/10.2147/IJWH.S185023
12. F. Barra , C. Scala, S. Ferrero, *Drugs Today*, **55(4)**, 237(2019), https://doi.org/10.1358/dot.2019.55.4.2930713
13. P. R. Akshanth, J. W. Ng, S. Ahmed Hamed, M. B. Dufek, A. Parikh, C. David Carter, K. Kamradt, N. M. Mostafa and M. Shebley, *The Journal of Clinical Pharmacology*, **60(12)**, 1606(020), https://doi.org/10.1002/jcph.1689
14. B. Carr, MD, L. Giudice, W. P. Dmowski, C. O'Brien, P. Jiang, J. Burke, R. Jimenez, S. Hass, F. Mahesh, C. Kristof , *Journal of Endometriosis and Pelvic Pain Disorders*, **5(3)**, 105(2013), https://doi.org/10.5301/je.5000157
15. ICH (2003) Stability Testing of New Drug Substances and Products Q1A (R2). International Conference on Harmonization, IFMPA, Geneva.
16. TPD (1997) Stability Testing of Existing Drug Substances and Products Directorate Ottawa.
17. S. Yoshioka and V. J. Stella, Chemical Stability of Drug Substances. In Stability of Drugs and Dosage Forms, Eds. Kluwer Academic Publishers, New York, pp. 3–137 (2002).
18. S. B. Kanthale, S. S. Thonte and B. B. Supekar, Development of stability indicating assay method: A Review, https://doi.org/10.13040/IJPSR.0975-8232.10(4).1625-31
19. D. L. Pavia, G. M. Lampman and G. S. Kriz, Eds. Infrared Spectroscopy. In Introduction to Spectroscopy, Brooks/Cole, Thomson Learning, Inc. Washington: United Kingdom, United States, pp. 13–101(2001),

[RJC-6527/2021]