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Characterization of Paliperidone Palmitate Impurities Identification, Isolation and Structural Characterization of Process Related Impurities of Paliperidone Palmitate

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ABSTRACT

Paliperidone palmitate is a antipsychotic agent which is used for treatment of Schizophrenia and Schizoaffective disorders. Paliperidone palmitate is a long-acting injectable formulation of paliperidone palmitoyl ester. As per the regulatory authorities it is important to provide the complete information about the drug and the related impurities which are generated during the drug manufacturing process. To identify the paliperidone palmitate process related impurities here we are using reverse phase liquid chromatography coupled with mass spectrometry technique. Four impurities were separated by using X Select CSH C18 (150mm x3.0mm, 2.5µm) column in a gradient mixture of 0.05% TFA in water and 0.05% TFA in acetonitrile. Further the impurities were isolated by using preparative HPLC. Impurities were identified by using HRMS and structurally characterized by one dimensional (1D) (proton ^{1}H, Carbon ^{13}C) and two dimensional (2D) (COSY, HSQC, HMBC) NMR spectroscopy. By using this highly advanced and sophisticated technique it is easier to characterize the structural elucidation of four impurities of Paliperidone palmitate.

High Lights

- LC-MS method was optimized and developed to determine the impurities present in Paliperidone palmitate.
- Characterization of impurities was not reported in any literature.
- In present paper all the four impurities were identified and characterized by using various high-end analytical techniques such as HRMS, NMR, and FT-IR.

Keywords: Paliperidone palmitate, process impurities, identification by LCMS, Isolation by Preparative HPLC, Characterization by HRMS and NMR.

INTRODUCTION

Paliperidone works as an antagonist at serotonin 5-HT_{2A} and dopamine D2 receptors. Paliperidone also blocks alpha 1-adrenergic receptors and slightly less histaminergic and alpha 2-adrenergic receptors. Paliperidone (as Invega) was approved by the Food and Drug Administration (FDA) for the treatment of schizophrenia in 2006 and schizoaffective disorder in 2009. It is used as schizophrenia and schizoaffective disorder [**1**]. Paliperidone palmitate is commercially available as Invega Sustenna and Invega Trinza. Paliperidone palmitate formulation was approved by the FDA under the brand name Invega Trinza. Paliperidone palmitate long-acting injection compared to risperidone for schizophrenia [**2**]. Invega Trinza (paliperidone palmitate) a 3-month injection, is an atypical antipsychotic indicated for the treatment of schizophrenia in patients after they have been adequately treated with Invega Trinza (1-month paliperidone palmitate) for at least four months.

Paliperidone should be used with caution in patients with known cardiovascular disease, cerebrovascular disease, or conditions that would predispose patients to hypotension and in antipsychotic-naive patients. Paliperidone is contraindicated in patients with a known hypersensitivity to either paliperidone, risperidone, or to any excipients of the formulation. Paliperidone palmitate is not recommended to treat elderly patients with dementia related psychosis. Adverse effects of this drug are tachycardia, head ache, insomania, hyperprolactinaemia [**3**] and sexual dysfunction. Other common side effects are cough, extrapyramidal side effects, orthostatic hypotension, weight gain, anxiety and constipation [**4, 5**].

Paliperidone chemical name is 3-[2-[4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl]ethyl]- 9-hydroxy-2-methyl-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-4-one. Physical description is white to off white colour solid and melting range is 184.9-191°C. Paliperidone is commercially available in palmitate ester form.

Some of literature reports reveal the Paliperidone palmitate synthetic process [**6**]. Paliperidone is combining with palmitic acid in presence of di-chloro methane (DCM), tri ethyl amine (TEA) and DMAP at 25-30°C for 2 h, then filtered with suitable filtration kit. Paliperidone palmitate is formed. Physical description is white to off white colour solid and melting range is 115.8-118.8°C. Paliperidone palmitate synthetic route was represented in figure 1. Paliperidone and Paliperidone palmitate structures were represented in figure 6.

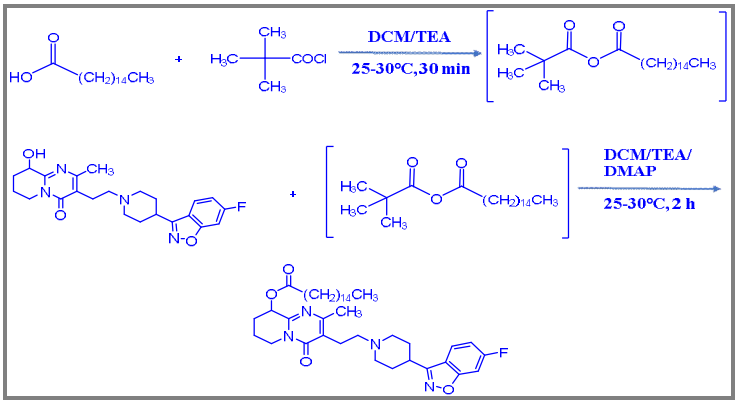


Figure 1. Synthetic route of Paliperidone palmitate.

Palmitic acid chemically called as hexadecanoic acid. It is most commonly used saturated fatty acid and found in animals, microorganisms and plants. Commercially available Palmitic acid quality is not as per process requirements. Actually, palmitic acid is isolated by extraction from palm oil as per reported literature [**7**]. Palmitic acid (C-16) having higher (C-17 and C-18) and lower (C-12, C-14 and C-15) homologues as per literature. It was observed that palmitic acid having higher and lower

homologues produce same level of higher and lower esters in Paliperidone palmitate ester due to same chemical properties [**6**]. It is also very difficult to reduce from drug product during crystallization due to similar properties. Chemical structures of impurities were represented in figure 6.

Previous reports reveal the stable methods [**8-10**] to quantify the Paliperidone and anhydro Paliperidone [**11**]. Some of methods were reported by HPLC ESI MS method [**12-14**] and GC method [**15**] and bio samples analysis methods [**16-18**]. The main objective of this research work is to Identify, Isolate and characterize process related impurities of Paliperidone Palmitate.

MATERIALS AND METHODS

Chemicals and Reagents: Solvents and buffers used for this analysis is formic acid (HPLC grade), Trifluoroacetic acid (HPLC grade), Chloroform (HPLC grade), Ammonium bicarbonate from Sigma Aldrich, HPLC grade methanol and acetonitrile were procured from Merck, India. Dimethyl sulfoxide-d6 (DMSO-D6) solvent (Cambridge Isotope Laboratories, Inc.; D, 99.9% + 0.03% V/V TMS as internal reference standard). The purified water utilized during analysis was obtained from Milli-Q plus purification system (Millipore, Amsterdam, Netherlands).

Ultra Performance Liquid Chromatography-Mass Spectrometry (LCMS): Liquid chromatography separation was performed on Agilent 1290 infinity with Diode array detector (DAD). Method development was performed to obtain a good peak shape and good separation of all the analytes.

Method development Trial number-1:

Chromatographic conditions: BEH C18 50 mm x2.1 mm, 1.7µm column; mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in acetonitrile; column oven temperature: 35°C; flow rate 0.6ml/min; gradient program: mobile phase B 0min 3%, 0.4 min 3%, 7.5 min 98%, 9.5 min 98%, 9.6 min 3% and 10 min 3%. Method development trial-1 overlay chromatogram representation was represented in figure 2(a).

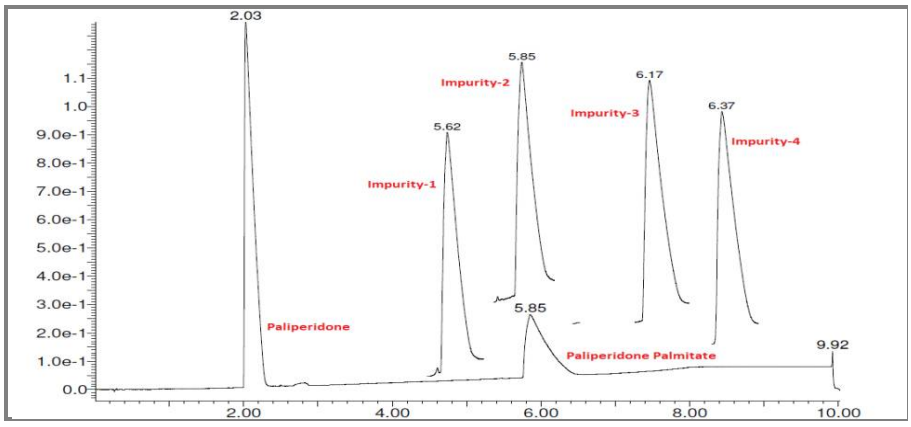


Figure 2(a). Method development trial-1 overlay chromatogram representation.

Observation: Retention time of Paliperidone is 2.03 min; Paliperidone palmitate is 5.85 min, impurity-1 is 5.62 min; impurity-2 is 5.85min, impurity-3 is 6.17 min, impurity-4 is 6.37 min.

Paliperidone was well separated with other analytes but impurity-2 and Paliperidone palmitate were eluted at same retention time. Impurity-1, 3 and 4 were well separated.

Method development Trial number-2:

Chromatographic conditions: BEH C18 50 mm x 2.1 mm, 1.7 µm column; mobile phase A: 0.1% formic acid in water; mobile phase B: 0.1% formic acid in acetonitrile; column oven temperature:

35°C; flow rate 0.6ml/min; gradient program: mobile phase B 0min 3%, 0.4min 3%, 8.0min 98%, 9.5 min 98%, 9.6 min 3% and 10 min 3%. Method development trial-2 overlay chromatogram representation was represented in figure 2(b).

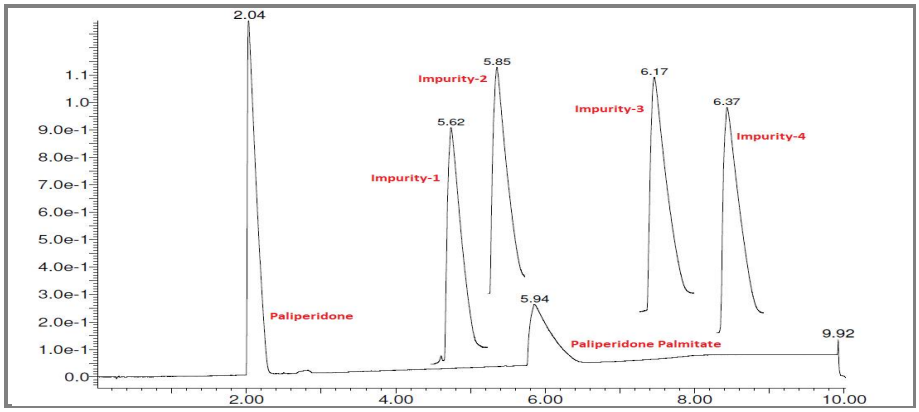


Figure 2(b). Method development trial-2 overlay chromatogram representation.

Observation: Retention time of Paliperidone is 2.04 min, Paliperidone palmitate is 5.94 min, impurity-1 is 5.62 min, impurity-2 is 5.85 min, impurity-3 is 6.17 min, impurity-4 is 6.37 min.

All analytes were separated and impurity-2 and paliperidone palmitate is not much seperated. Different chromatographic conditions were tried and finally the below method is suitable to separate all the analytes with good peak shape.

Column used for this analysis is X Select CSH C18, 3.0mm × 150 mm, 2.5µ; from Waters India limited, Mobile phase consisting of A: 0.05% Trifluoroacetic acid (Aq), B: Acetonitrile. The gradient started from 3% (B) and hold for 1 min and it reaches to 98% (B) in 8.0 min and then hold to 98% (B) till 11.0 minutes and then stabilized to 3% (B) in 1 min. Sample dissolved in small amount of chloroform and acetonitrile as diluent in the complete study. The detection wavelength was monitored at 238 nm which is the lambda maximum for all the components present in the analysis.

The mass instrument used for this study is Agilent 6130 single quadrupole mass spectrometer operated in dual polarity - positive and negative with electrospray ionization source (ESI). Full scan mode 100-1500 Daltons (Da) was used for the MS optimization. The capillary voltage and Drying gas temperature were set for this study is 3.0 kV and 350°C respectively. The fragmentor voltage was set at 70 V. The drying gas flow was set at 12 L/min. The nebulizer pressure was set at 35 psig. The LC instrument and the mass spectrometer were controlled by using chemstation openlab software. The injection volume was 1.0 µL with autosampler temperature maintained at 10°C, column temperature was maintained at 35°C and the chromatographic runtime of 12.0 min was used.

High Resolution Mass Spectrometry: Samples were analysed on Thermo Q Exactive orbitrap MS with ESI ion source; instrument parameters (source) are: Spray Voltage: 3500 V; Aux gas heater Temperature: 440°C; Capillary Temperature: 270°C. Sheath gas flow rate: 53; Aux gas flow rate: 14; Sweep gas flow rate: 3. Reserpine (monoisotopic mass: 608.2734 Da) was used to check the accuracy of the Mass system. Dionex ultimate 3000 LC was used, Mass data was acquired by using Xcalibur software.

Mass mediated Preparative HPLC: Mass mediated preparative HPLC equipped with waters pump module 2545, Waters PDA detector module 2998, sample manager module 2767, Waters 3100 single quadrupole mass detector were used. Masslynx software was used to control the instrument. Waters X Select CSH C18 column with dimensions 250 mm x 19 mm, 5 µm was used to separate the impurities. Mass spectrometry source parameters: Capillary voltage was maintained at 3.5 kV, Source

temperature 120°C, Desolvation temperature at 350°C, Desolvation gas flow was set at 650 L h^{-1}, Cone gas flow was set at 50 L h^{-1}. 0.1% Trifluoroacetic acid (Aq) and acetonitrile (30: 70, v/v) was used as a makeup solvent with makeup flow of 0.3 mL min^{-1} to the mass detector and 1:1000 splitting ratio was maintained for the proper ionization.

High Performance Liquid Chromatography: Waters 2695 alliance modules containing 2996 photo diode array detector with Empower 3 chromatographic software.

UV spectral absorbance: All four impurities, Paliperidone, Paliperidone Palmitate were analysed and evaluated the UV nanometre absorbance values. First maximum absorbance was observed at 236 to 239 nm and second maximum absorbance was at 276-279 nm. Figure 3 represented all the analytes UV spectral absorbance values.

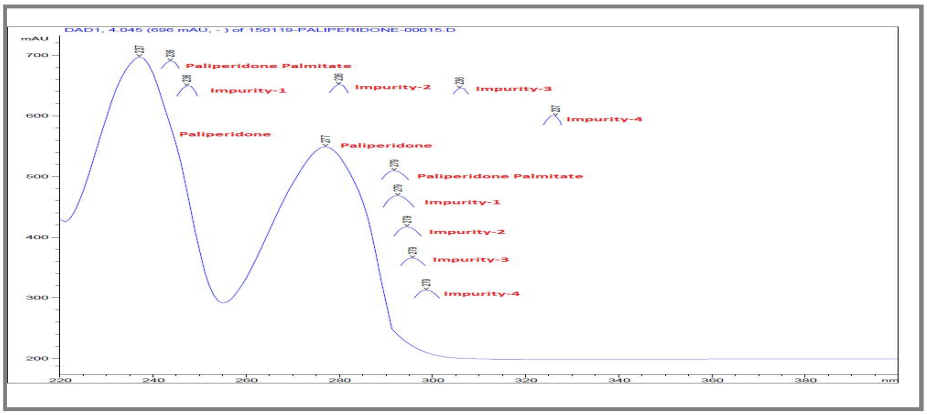


Figure 3. UV spectral absorbance values for all analytes.

Nuclear Magnetic Resonance Spectroscopy: NMR analysis of Paliperidone Palmitate and its isolated impurities were taken on Agilent MR400MHz NMR instrument equipped with 5mm ONE NMR probe with Z- gradient shim system which has the sensitivities of 480:1 and 225:1 for ^{1}H and ^{13}C nuclei respectively and also equipped with auto sampler with 100 samples hold capacity. All the NMR analysis has been performed at 298K probe temperature with fine automatic tuning and matching for the frequency of respective nuclei. ^{1}H NMR spectra were referenced to tetra methyl Silane (TMS) singlet at zero(0) ppm and referenced DMSO-D6 septet at 39.5 ppm in carbon NMR. Following are the key parameters used for NMR analysis.

One dimensional (1D) analysis: ^{1}H NMR data acquired and processed with following parameters like spectral width (SW) =17.95 ppm, relaxation delay time (D1)=1 sec, number of scans (NT)=16, number of data points (NP)=64k, 90° pulse width (PW90)=7.4 µsec, acquisition time (AT)=4.0 sec, operating spectrometer frequency (SF) =399.63MHz and line broadening (LB)=0.5Hz. ^{13}C NMR data acquired and processed with following parameters like spectra width (SW)=248.8 ppm, relaxation delay (D1)=3 sec, number of scans (NT)=4000, data points (NP)=64k, 90° Pulse width (PW90)=7.6 µsec, acquisition time (AT)=1.31sec line broadening (LB) =2.0Hz spectrometer frequency (SF) =100.48MHz parameters.

Two dimensional (2D) analysis: Homonuclear ^{1}H-^{1}H gDQCOSY experiment has been performed to know the proton-proton correlations with following parameters (SW)=18.00 ppm in both F1 and F2 projections, relaxation delay time (D1)=1sec, number of scans (NT)=16, number of data points (NP)=1078(F2) and 400(F1), dummy scans (SS)=32. 1H-13C gHSQC was done to know the ^{1}J correlations between proton-carbon with (SW)=18.00ppm(F1) and (SW1)=240.0 ppm(F2), relaxation delay time (D1)=1sec, number of scans (NT)=16, number of data points (NP)=1078(F2) and 400(F1), dummy scans (SS)=32 and gHMBC has been performed to reveal the exact structure of impurities

with (SW)=18.00 ppm(F1) and (SW1) = 240.0 ppm(F2), relaxation delay time (D1)=1sec, number of scans (NT) =16, number of data points (NP)=1078(F2) and 400(F1), dummy scans (SS)=32 parameters. 10 mg of sample has been dissolved in Deuterated DMSO-D6 (Cambridge Isotope Laboratories, Inc.; D, 99.9% + 0.03% V/V TMS as internal reference standard) solvent.

FT-IR spectroscopy: Fourier transform infrared spectroscopy (FT-IR) was used to obtain the information about the presence of various functional groups like alcohol, ketone present in the impurities [**19**]. Perkin Elmer spectrum 100 model was used with KBr as dispersion medium to make the sample pellets.

Sample preparation

LCMS analysis*:* Around 0.5 mg of sample was dissolved in chloroform and dilute with acetonitrile and analysed as per the conditions mentioned above.

NMR analysis: Around 10.0 mg of sample was dissolved in deuterated DMSO-D6 solvent (Cambridge Isotope Laboratories, Inc.; D, 99.9% + 0.03% V/V TMS as internal reference standard) and analysed as per the conditions mentioned above.

FTIR analysis: Around 2-3 mg of sample was mixed with 5 mg of dried KBr and pellet was prepared for the complete FTIR analysis.

Preparation of crude Sample for Purification: Sample was dissolved in 5-10 mL of mixture of solvents like chloroform, acetonitrile, water to make a clear solution and the resultant solution was used for separating the impurities in prep HPLC.

RESULTS AND DISCUSSION

Paliperidone Palmitate was analysed to confirm the purity of the product by using LCMS. The impurity masses were identified by using mass spectrometer as per the parameters mentioned above. Preparative HPLC method was developed for impurities isolation. The four impurities were isolated, identified and characterized by LCMS, HRMS, NMR (1D and 2D) and FT-IR and other physical properties were evaluated.

Identification of impurities: LCMS method was used to evaluate the impurity profile in Paliperidone palmitate. All four impurities and Paliperidone Palmitate were well separated in LCMS. Crude LCMS report of Paliperidone palmitate were represented in figure 4(a). All masses corresponds to each impurity were represented in the report. Table 1 shows the peak retention times and LC area percentages of all the four impurities along with Paliperidone Palmitate.

Table 1. Peak retention time of each analyte with RRT and Area%

Peak name	RT(min)	RRT	Area %
Paliperidone palmitate	7.61	--	94.49
Impurity-1	7.23	0.95	0.96
Impurity-2	7.43	0.97	1.17
Impurity-3	7.79	1.02	1.22
Impurity-4	7.95	1.04	1.51

Isolation of impurities of Paliperidone palmitate: The impurities are having adequate percentage to purify in Prep HPLC. Purification was carried out by using Waters XSelect CSH C18 column with dimensions 250 mm x 19 mm, 5 µm and mobile phase A (0.1% Trifluoroacetic acid in water); mobile phase B (0.1% TFA in acetonitrile); gradient program: mobile phase B at 0min 15%, 2min 15%,

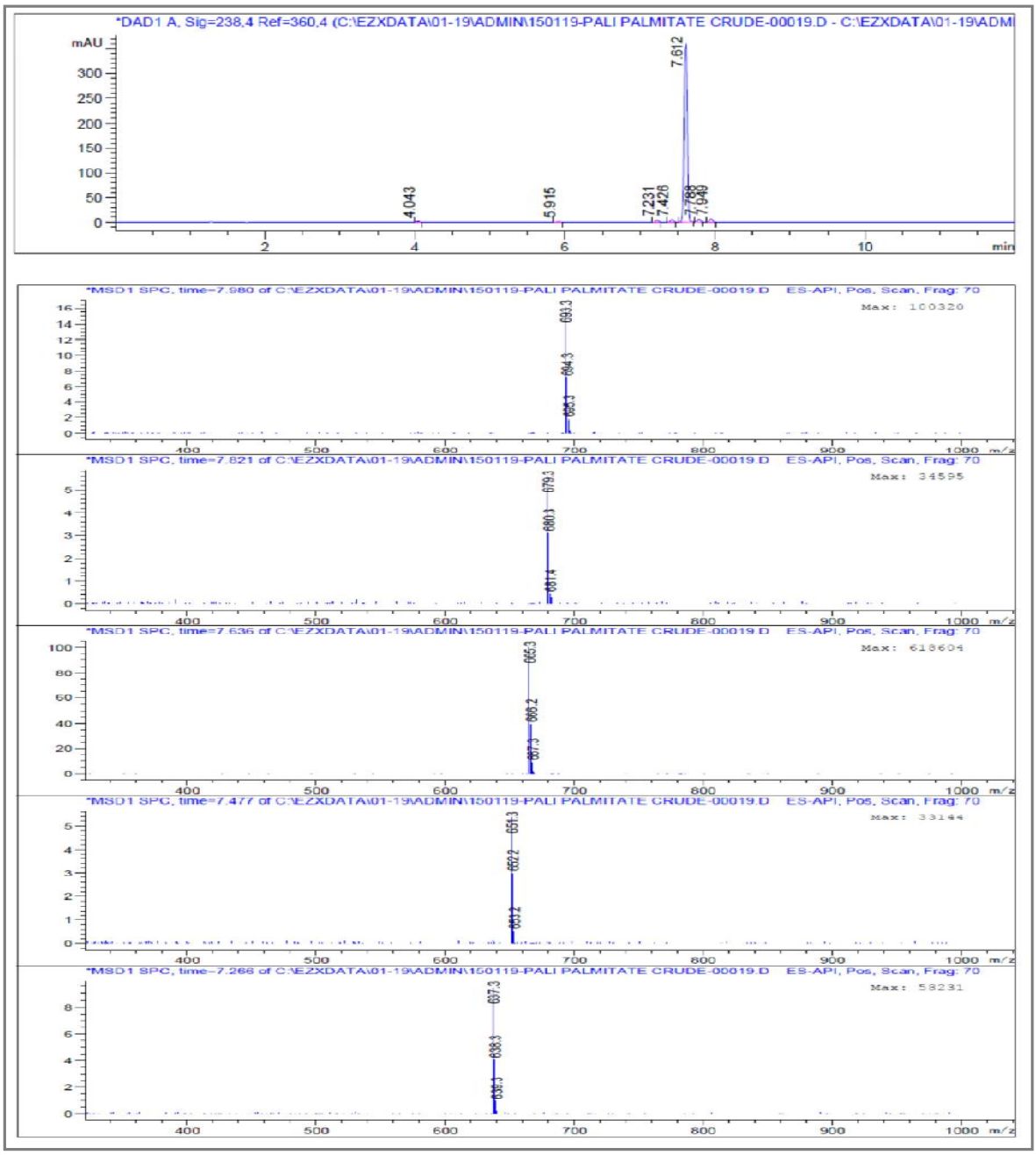


Figure 4(a). Crude LCMS report of Paliperidone palmitate.

10min 50%, 15 min 90%, 20 min 90%, 21 min 98%, 23 min 98%, 23.1min 15%, 26 min 15%. Crude sample solutions were injected in consecutive injections and the fractions had been collected on the basis of mass threshold parameters of total ion chromatogram. The fractions corresponding to molecular weight of Impurity1-637.4 (M+H), Impurity2-651.4 (M+H), Impurity3-679.4 (M+H), Impurity4-693.4 (M+H) and paliperidone palmitate -665.4 (M+H) collected separately and lyophilized to get free solid. LCMS analysis were taken to all the impurities and Paliperidone Palmitate, LCMS reports were represented in figure 4(b), 4(c), 4(d), 4(e) and 4(f). Labeled chromatogram was represented in figure 4(g).

Characterization of Paliperidone, Paliperidone palmitate and its impurities: Paliperidone, Paliperidone palmitate and all four impurities were characterized by using advanced analytical instruments like NMR, HRMS, FT-IR and other physical properties were evaluated.

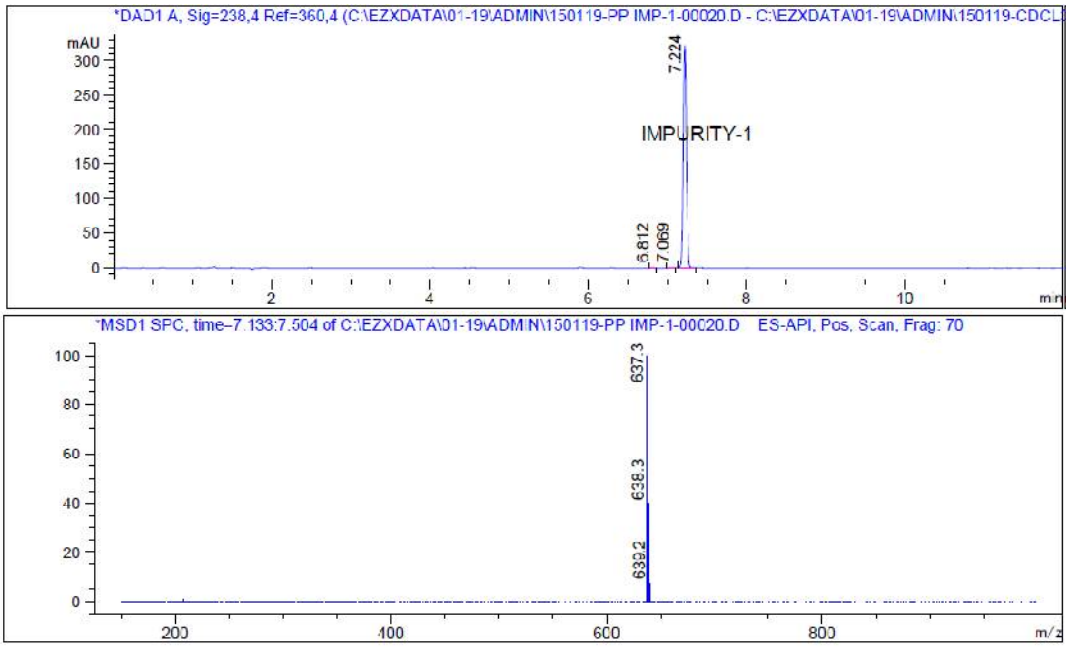


Figure 4(b). LCMS report of Impurity-1.

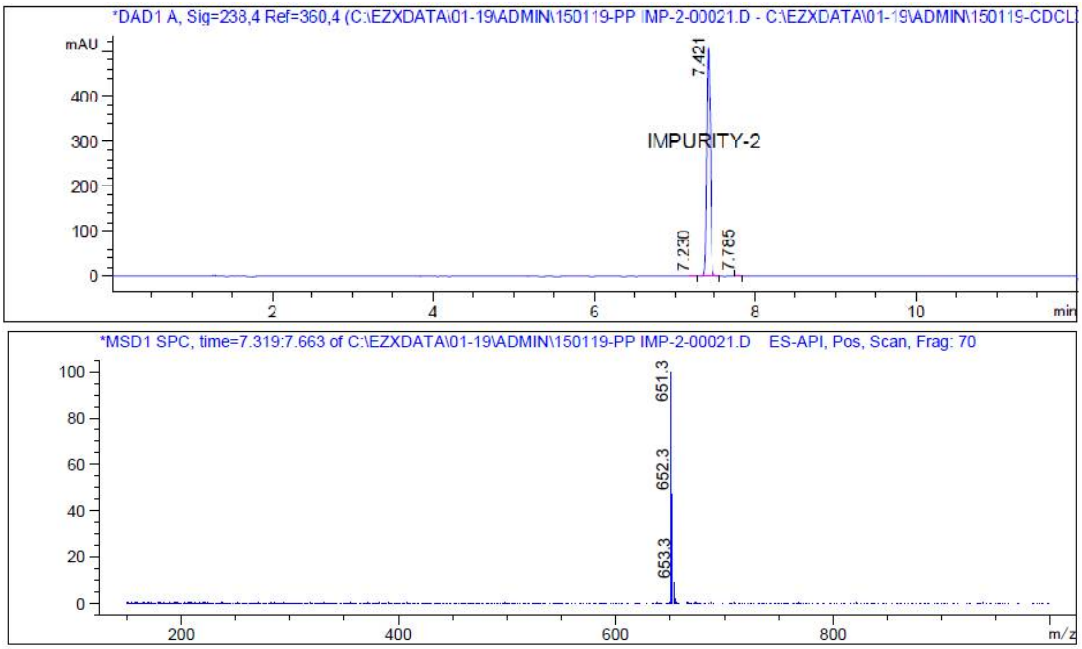
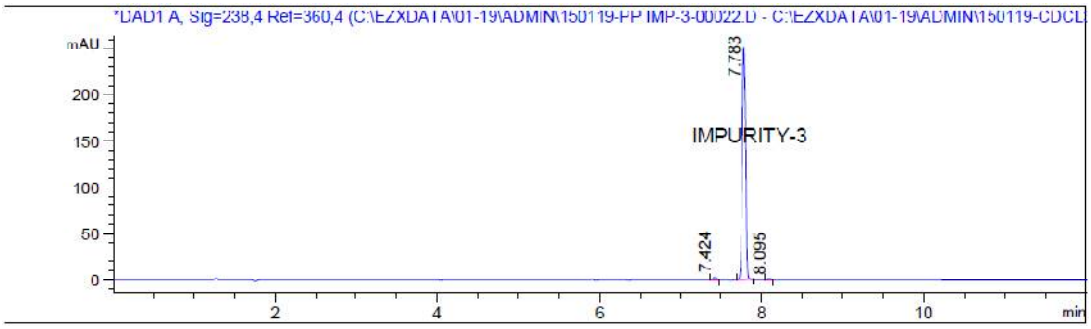


Figure 4(c). LCMS report of Impurity-2.



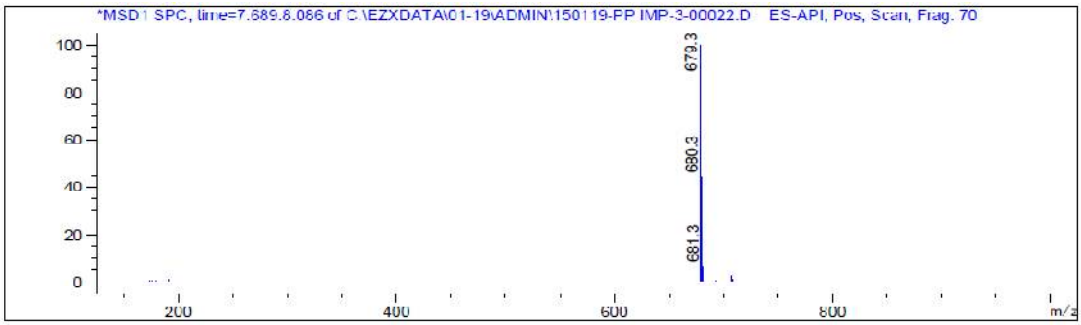


Figure.4(d). LCMS report of Impurity-3.

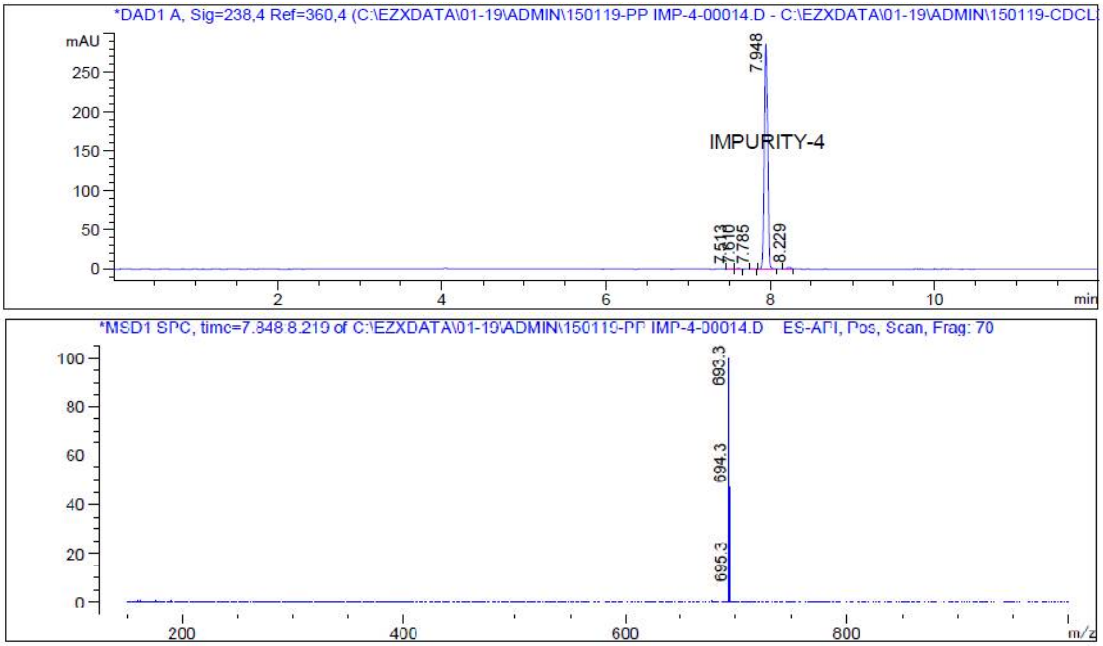


Figure.4(e). LCMS report of Impurity-4.

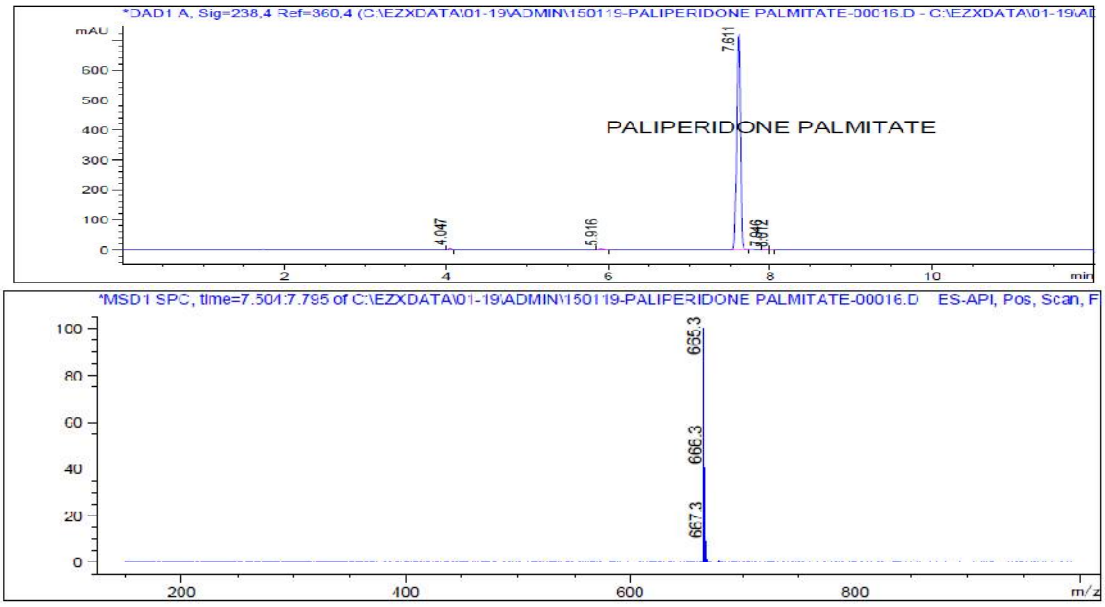


Figure 4(f). LCMS report of Paliperidone Palmitate.

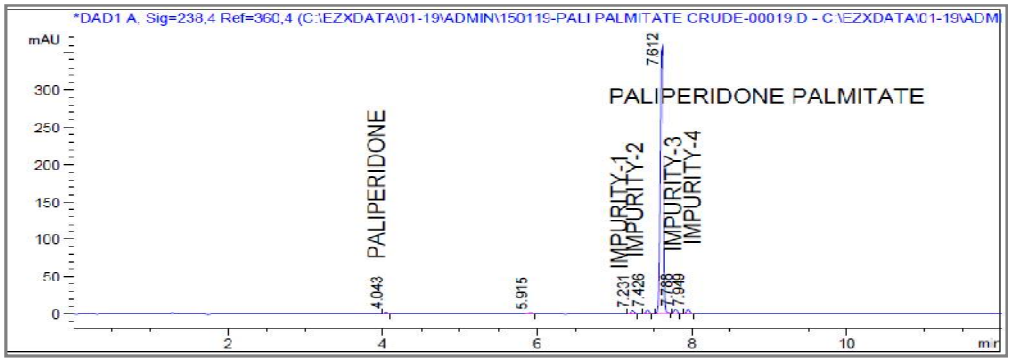


Figure 4(g). Labeled chromatogram.

Characterization of Paliperidone: Characterization was started from High Resolution Mass Spectrometry (HRMS), Orbitrap was used to measure the exact mass ESI MS $[M+H]^+$. Paliperidone was recorded with HRMS to obtain the exact mass, Orbitrap was used to measure the exact mass of Paliperidone which was 427.2135(1.2288 ppm error) for the calculated molecular formula of $C_{23}H_{27}FN_4O_3$ as shown in figure 5(a). The obtained exact mass and the theoretical calculated molecular formula correlated with Paliperidone. To further elucidate the structure of Paliperidone by using spectroscopic techniques. Structure of Paliperidone was confirmed with spectroscopy techniques like ^1H NMR, ^{13}C, HMBC, HSQC and FT-IR. NMR chemical shifts of Paliperidone were compared with the values of Paliperidone Palmitate and four Impurities which were shown the values in table 2(A), 2(B), 2(C). The precise structure of Paliperidone was further confirmed by NMR studies and its full characterization details has been given in table 2(A). From FT-IR, major frequencies of all the four impurities, Paliperidone and Paliperidone Palmitate are tabulated in the below table 3. Proton NMR spectra of Paliperidone were shown in figure 7(a). Structure of Paliperidone was shown in figure 6 and melting range of Paliperidone is 184.9-191°C.

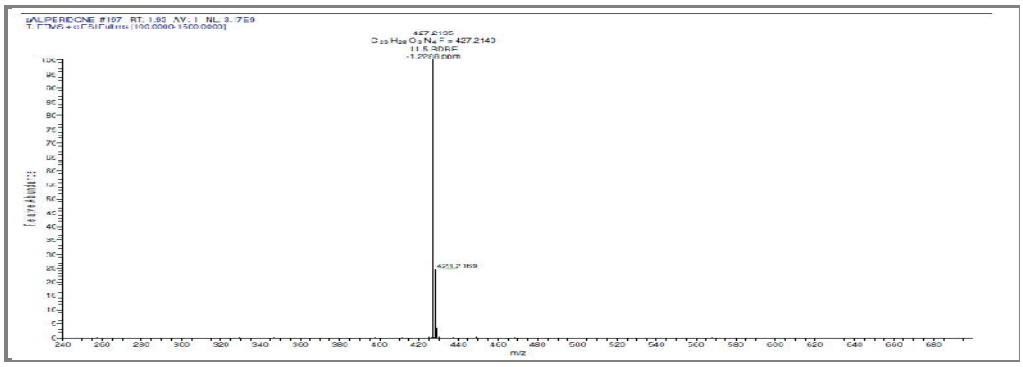


Figure 5(a). HRMS report of Paliperidone.

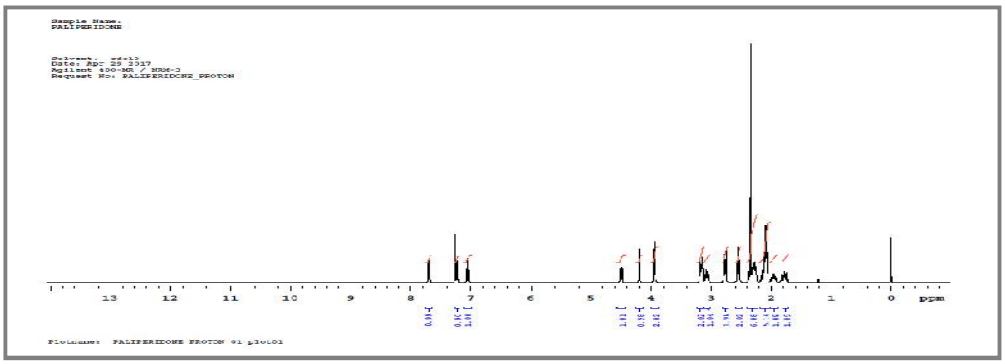


Figure.7(a). NMR spectra of Paliperidone.

Table 2 (A). Comparative NMR assignments for Paliperidone and Paliperidone Palmitate

Paliperidone				Paliperidone palimitate			
Atom No	Type of Atom	1H Chemical Shift (PPM) Coupling Const(J)	13C Chemical Shift (PPM) Coupling Const(J)	Atom N	Type of Atom	1H Chemical Shift (PPM) Coupling Const(J)	13C Chemical Shift (PPM) Coupling Const(J)
1	CH	7.05(td, 8.80, 2.0Hz, 1H)	112.12-12.38(d, 25.3Hz)	1	CH	7.05(td, 8.80, 2.0Hz, 1H)	112.13-112.38(d, 25.3Hz)
2	C	-	162.82-65.32(d, 249.5Hz)	2	C	-	162.84-165.34(d, 249.5Hz)
3	CH	7.22(dd, 8.4, 2.4Hz, 1H)	97.25-97.52(d, 26.7Hz)	3	CH	7.24(dd, 8.4, 2.4Hz, 1H)	97.27-97.54(d, 26.7Hz)
4	C	-	163.81-63.94(d, 13.4Hz)	4	C	-	163.83-163.97(d, 13.4Hz)
5	C	-	117.34	5	C	-	117.35
6	CH	7.70(dd, 8.8, 5.2Hz, 1H)	122.52-22.63(d, 11.2Hz)	6	CH	7.70(dd, 8.8, 5.2Hz, 1H)	122.55-122.66(d, 10.5Hz)
7	O	-	-	7	O	-	-
8	N	-	-	8	N	-	-
9	C	-	161.1	9	C	-	161.12
10	F	-	-	10	F	-	-
11	CH	3.07(m, 1H)	34.62	11	CH	3.07(m, 1H)	34.66
12	CH2	2.10(m, 2H)	30.6	12	CH2	2.09(m, 2H)	30.62
13	CH2	2.27, 3.17(m, 2H)	53.41	13	CH2	2.27, 3.16(m, 2H)	53.42
14	N	-	-	14	N	-	-
15	CH2	2.27, 3.17(m, 2H)	53.41	15	CH2	2.27, 3.16(m, 2H)	53.42
16	CH2	2.10(m, 2H)	30.6	16	CH2	2.09(m, 2H)	30.62
17	CH2	2.55(t, 7.2Hz, 2H)	56.6	17	CH2	2.55(t, 6.8Hz, 2H)	56.53
18	CH2	2.77(t, 7.2Hz, 2H)	23.9	18	CH2	2.77(t, 6.8Hz, 2H)	23.96
19	C	-	120.53	19	C	-	121.31
20	C	-	162	20	C	-	162.15
21	N	-	-	21	N	-	-
22	C	-	157.36	22	C	-	152.39
23	N	-	-	23	N	-	-
24	C	-	157.73	24	C	-	158.69
25	CH3	2.34(s, 3H)	21.15	25	CH3	2.32(s, 3H)	21.39
26	CH2	3.96(t, 7.2Hz, 2H)	42.28	26	CH2	3.87, 4.06(m, 2H)	42.25
27	CH2	1.93, 2.12(m, 2H)	18.5	27	CH2	2.01, 2.07(m, 2H)	18.04
28	CH2	1.76, 2.31(m, 2H)	26.94	28	CH2	2.09(m, 2H)	25.61
29	CH	4.50(dd, 10.0, 6.0Hz, 1H)	67.05	29	CH	5.76(t, 5.2Hz, 1H)	68.62
30	O	-	-	30	O	-	-
31	OH	4.19(s, 1H)	-	31	O	-	-
				32	C	-	172.59
				33	CH2	2.38(t, 7.6Hz, H)	34.38
				34	O	-	-
				35	CH2	1.70(m, 2H)	24.98
				36	CH2	1.36(m, 2H)	29.06
				37	CH2	1.25(m, 2H)	29.25
				38	CH2	1.25(m, 2H)	29.46
				39	CH2	1.25(m, 2H)	29.64
				40	CH2	1.25(m, 2H)	29.64
				41	CH2	1.25(m, 2H)	29.64
				42	CH2	1.25(m, 2H)	29.67
				43	CH2	1.25(m, 2H)	29.67
				44	CH2	1.25(m, 2H)	29.6
				45	CH2	1.25(m, 2H)	29.34
				46	CH2	1.33(m, 2H)	31.91
				47	CH2	1.34(m, 2H)	22.67
				48	CH3	0.87(t, 6.8Hz, 3H)	14.8

S=singlet; d=doublet; m=multiplet; dd=doublet of doublet; td=triplet of doublet;

Characterization of Paliperidone Palmitate: Paliperidone Palmitate was recorded with HRMS to obtain the exact mass, Orbitrap was used to measure the exact mass of Paliperidone Palmitate which was 665.4431(0.9118 ppm error) for the calculated molecular formula of $C_{39}H_{57}FN_4O_4$ as shown in figure 5(b). The obtained exact mass and the theoretical calculated molecular formula correlated

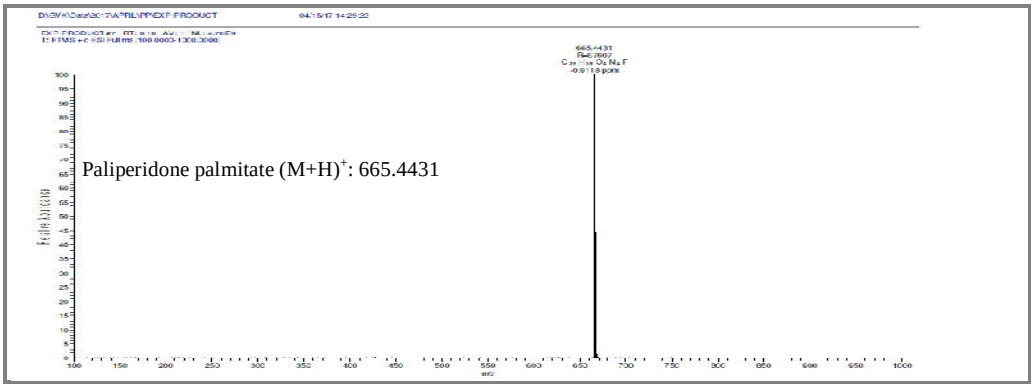


Figure 5(b). HRMS report of Paliperidone palmitate.

with Paliperidone Palmitate. To further elucidate the structure of Paliperidone Palmitate by using spectroscopic techniques. Structure of Paliperidone Palmitate was confirmed with spectroscopy techniques like ^{1}H NMR, ^{13}C, HMBC, HSQC and FT-IR. NMR chemical shifts of Paliperidone Palmitate were compared with the values of Impurities which were shown the values in table 2(A). The precise structure of Paliperidone Palmitate was further confirmed by NMR studies and its full characterization details has been given in table 2(A). In proton NMR ester chain showing 31 protons (14 CH2 and 1 Methyl proton and these were confirmed by HSQC experiment. HMBC experiment confirms H35 (1.70ppm), H33 (2.38ppm), H29 (5.76ppm) protons showing connectivity to C32 carbon (172.59ppm). From FT-IR, major frequencies of all the four impurities and Paliperidone Palmitate are tabulated in the below Table.3 Proton NMR spectra of Paliperidone Palmitate were shown in figure 7(b). Structure of Paliperidone Palmitate were shown in figure 6 and melting range of Paliperidone Palmitate is 115.8 - 118.8°C.

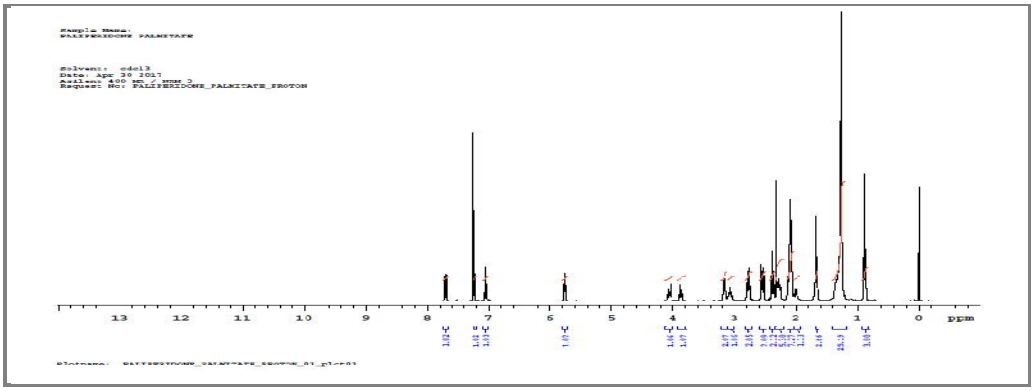


Figure 7(b). NMR spectra of Paliperidone Palmitate

Characterization of Impurity-1: Impurity characterization was started from High Resolution Mass Spectrometry (HRMS), the impurity-1 and was recorded with HRMS to obtain the exact mass, Orbitrap was used to measure the exact mass ESI MS $[M+H]^+$ of the impurity which was 637.4116(1.2563 ppm error) for the calculated molecular formula of $C_{37}H_{53}FN_4O_4$ as shown in figure 5(c). The obtained exact mass and the theoretical calculated molecular formula correlated with Impurity-1. To further elucidate the structure of Impurity-1 by using spectroscopic techniques. Structure of Impurity-1 was confirmed with spectroscopy techniques like ^{1}H NMR, ^{13}C, HMBC, HSQC and FT-IR. NMR chemical shifts of Impurity-1 were compared with the values of Paliperidone Palmitate (API) were shown the values in table 2(B). The precise structure of Impurity-1 was further confirmed by NMR studies and its full characterization details has been given in table 2(B). In proton NMR ester chain showing 27 protons (12 CH2 and 1 Methyl proton and these were confirmed by HSQC experiment. HMBC experiment confirms H35 (1.67ppm), H33 (2.38ppm), H29 (5.76ppm) protons showing connectivity to C32 carbon (172.47ppm). From FT-IR, major frequencies of all the

four impurities and the API are tabulated in the below table 3. Proton NMR spectra of Impurity-1 were shown in figure 7(c). Assigned structure of Impurity-1 was shown in figure 6 and melting range of Impurity-1 is 114.6 - 118.1 °C.

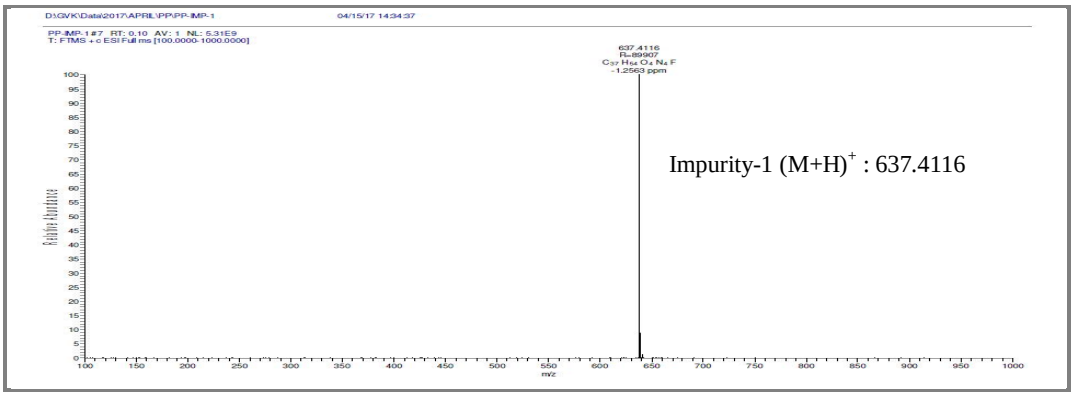


Figure 5(c). HRMS report of Impurity-1.

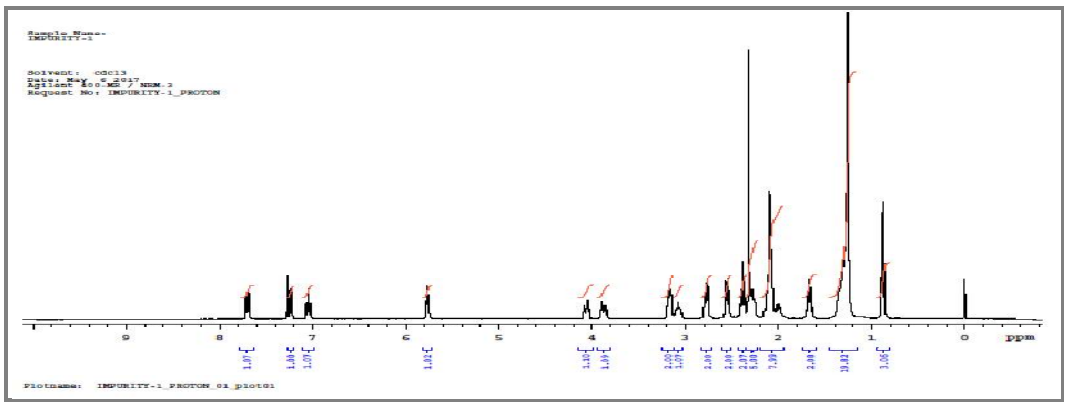


Figure 7(c). NMR spectra of Impurity-1

Characterization of Impurity-2: The impurity-2 and was recorded with HRMS to obtain the exact mass, Orbitrap was used to measure the exact mass ESI MS $[M+H]^+$ of the impurity which was 651.4271(1.4551 ppm error) for the calculated molecular formula of $C_{38}H_{55}FN_4O_4$ as shown in figure 5(d). The obtained exact mass and the theoretical calculated molecular formula correlated with Impurity-2. To further elucidate the structure of Impurity-2 by using spectroscopic techniques. Structure of Impurity-2 was confirmed with spectroscopy techniques like ^1H NMR, ^{13}C, HMBC, HSQC and FT-IR. NMR chemical shifts of Impurity-2 were compared with the values of Paliperidone Palmitate (API) were shown the values in table 2(B). The precise structure of Impurity-2 was further confirmed by NMR studies and its full characterization details have been given in table 2(B). In proton NMR ester chain showing 29 protons (13 CH2 and 1 Methyl proton and these were confirmed by HSQC experiment. HMBC experiment confirms H35 (1.67ppm), H33 (2.38ppm), H29 (5.76ppm) protons showing connectivity to C32 carbon (172.49ppm). From FT-IR, major frequencies of all the four impurities and the API are tabulated in the below table 3 Proton NMR spectra of Impurity-2 were shown in figure 7(d). Assigned structure of Impurity-2 was shown in figure 6 and melting range of Impurity-2 is 121.2–124.9°C.

Characterization of Impurity-3: The impurity-3 and was recorded with HRMS to obtain the exact mass, Orbitrap was used to measure the exact mass ESI MS $[M+H]^+$ of the impurity which was 679.4584(1.2892 ppm error) for the calculated molecular formula of $C_{40}H_{59}FN_4O_4$ as shown in figure 5(e). The obtained exact mass and the theoretical calculated molecular formula correlated with Impurity-3. To further elucidate the structure of Impurity-3 by using spectroscopic techniques.

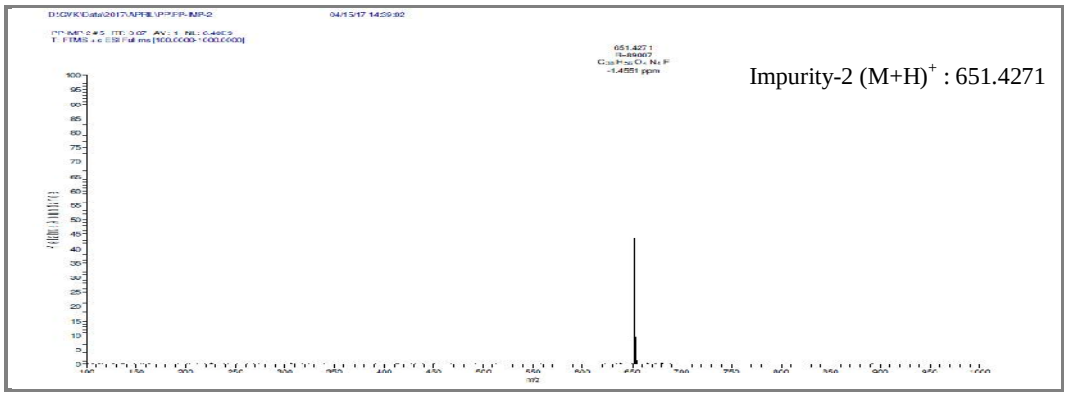


Figure. 5(d) HRMS report of Impurity-2.

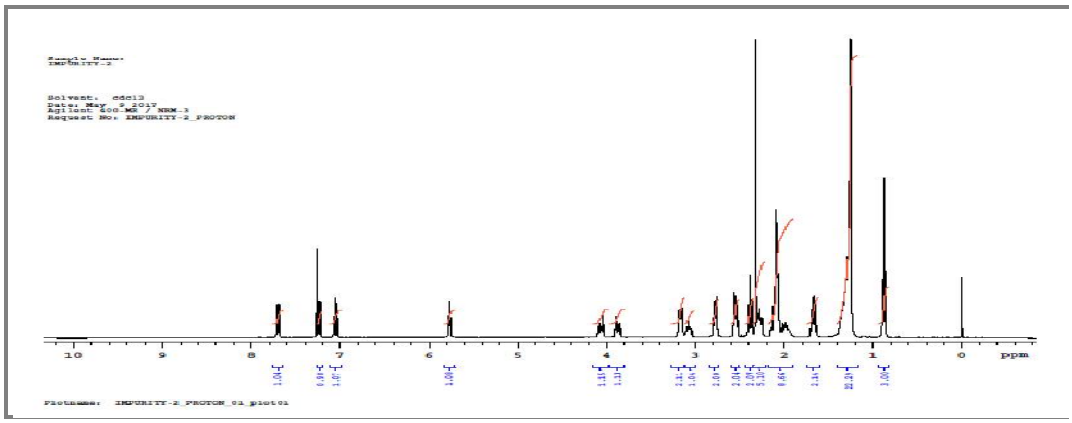


Figure 7(d). NMR spectra of Impurity-2.

Structure of Impurity-3 was confirmed with spectroscopy techniques like ^{1}H NMR, ^{13}C, HMBC, HSQC and FT-IR. NMR chemical shifts of Impurity-3 were compared with the values of Paliperidone Palmitate (API) were shown the values in table 2(C). The precise structure of Impurity-3 was further confirmed by NMR studies and its full characterization details has been given in table 2(C). In proton NMR ester chain showing 33 protons (15 CH2 and 1 Methyl proton and these were confirmed by HSQC experiment. HMBC experiment confirms H35 (1.66ppm), H33 (2.38ppm), H29 (5.77ppm) protons showing connectivity to C32 carbon (172.52ppm). From FT-IR, major frequencies of all the four impurities and the API are tabulated in the below table 3 Proton NMR spectra of Impurity-3 was shown in figure 7(e). Assigned structure of Impurity-3 were shown in figure 6 and melting range of Impurity-2 is 120.5–125.4°C.

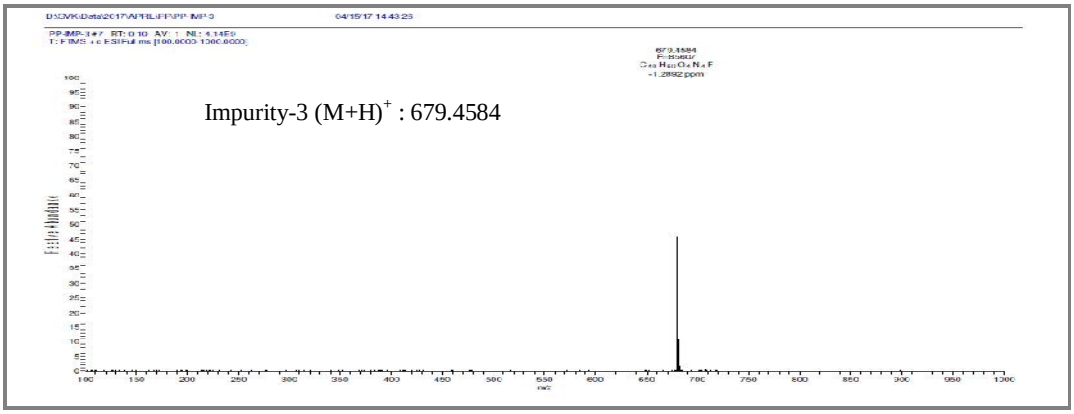


Figure 5(e). HRMS report of Impurity-3.

Table 2(B). Comparative NMR assignments for Impurity-1 and Impurity-2

Impurity-1				Impurity-2			
Atom No	**Type of Atom**	**1H Chemical Shift (PPM) Coupling Const(J)**	**13C Chemical Shift (PPM) Coupling Const(J)**	**Atom No**	**Type of Atom**	**1H Chemical Shift (PPM) Coupling Const(J)**	**13C Chemical Shift (PPM) Coupling Const(J)**
1	CH	7.05(td, 8.80, 2.0Hz, 1H)	112.11-12.36(d, 25.0Hz)	1	CH	7.05(td, 8.40, 1.6Hz, 1H)	112.11-12.37(d, 25.0Hz)
2	C	-	162.80-65.30(d, 249.7Hz)	2	C	-	162.81-65.31(d, 249.7Hz)
3	CH	7.24(dd, 8.0, 2.0Hz, 1H)	97.23-97.49(d, 26.5Hz)	3	CH	7.24(dd, 8.4, 2.0Hz, 1H)	97.25-97.51(d, 26.5Hz)
4	C	-	163.78-63.92(d, 13.7Hz)	4	C	-	163.81-63.94(d, 13.7Hz)
5	C	-	117.3	5	C	-	117.33
6	CH	7.70(dd, 8.8, 4.8Hz, 1H)	122.54-22.65(d, 10.6Hz)	6	CH	7.70(dd, 8.4, 5.2Hz, 1H)	122.53-22.64(d, 10.7Hz)
7	O	-	-	7	O	-	-
8	N	-	-	8	N	-	-
9	C	-	161.07	9	C	-	161.1
10	F	-	-	10	F	-	-
11	CH	3.06(m, 1H)	34.58	11	CH	3.06(m, 1H)	34.63
12	CH2	2.09(m, 2H)	30.53	12	CH2	2.09(m, 2H)	30.59
13	CH2	2.28, 3.15 (m, 2H)	53.35	13	CH2	2.28, 3.15 (m, 2H)	53.4
14	N	-	-	14	N	-	-
15	CH2	2.28, 3.15 (m, 2H)	53.35	15	CH2	2.28, 3.15 (m, 2H)	53.4
16	CH2	2.09(m, 2H)	30.53	16	CH2	2.09(m, 2H)	30.59
17	CH2	2.54(t, 7.2Hz, 2H)	56.45	17	CH2	2.54(t, 7.2Hz, 2H)	56.51
18	CH2	2.77(t, 7.2Hz, 2H)	23.9	18	CH2	2.77(t, 7.2Hz, 2H)	23.93
19	C	-	121.23	19	C	-	121.28
20	C	-	162.11	20	C	-	162.12
21	N	-	-	21	N	-	-
22	C	-	152.38	22	C	-	152.37
23	N	-	-	23	N	-	-
24	C	-	158.67	24	C	-	158.67
25	CH3	2.32(s, 3H)	21.34	25	CH3	2.32(s, 3H)	21.38
26	CH2	3.87, 4.06(m, 2H)	42.23	26	CH2	3.88, 4.07(m, 2H)	42.23
27	CH2	2.01, 2.08(m, 2H)	17.99	27	CH2	2.01, 2.08(m, 2H)	18.02
28	CH2	2.09(m, 2H)	25.57	28	CH2	2.09(m, 2H)	25.59
29	CH	5.76(t, 5.6Hz, 1H)	68.57	29	CH	5.76(t, 5.6Hz, 1H)	68.6
30	O	-	-	30	O	-	-
31	O	-	-	31	O	-	-
32	C	-	172.47	32	C	-	172.49
33	CH2	2.38(t, 7.6Hz, 2H)	34.34	33	CH2	2.38(t,7.6Hz, 2H)	34.36
34	O	-	-	34	O	-	-
35	CH2	1.67(m, 2H)	24.94	35	CH2	1.67(m, 2H)	24.96
36	CH2	1.34(m, 2H)	29.01	36	CH2	1.34(m, 2H)	29.04
37	CH2	1.25(m, 2H)	29.21	37	CH2	1.25(m, 2H)	29.23
38	CH2	1.25(m, 2H)	29.42	38	CH2	1.25(m, 2H)	29.44
39	CH2	1.25(m, 2H)	29.59	39	CH2	1.25(m, 2H)	29.61
40	CH2	1.25(m, 2H)	29.59	40	CH2	1.25(m, 2H)	29.61
41	CH2	1.25(m, 2H)	29.59	41	CH2	1.25(m, 2H)	29.61
42	CH2	1.25(m, 2H)	29.55	42	CH2	1.25(m, 2H)	29.61
43	CH2	1.25(m, 2H)	29.29	43	CH2	1.25(m, 2H)	29.58
44	CH2	1.33(m, 2H)	31.86	44	CH2	1.25(m, 2H)	29.32
45	CH2	1.33(m, 2H)	22.62	45	CH2	1.33(m, 2H)	31.88
46	CH2	0.87(t, 6.8Hz, 3H)	14.05	46	CH2	1.33(m, 2H)	22.65
				47	CH2	0.87(t, 6.8Hz, 3H)	14.06

S=singlet; d=doublet; m=multiplet; dd=doublet of doublet; td=triplet of doublet;

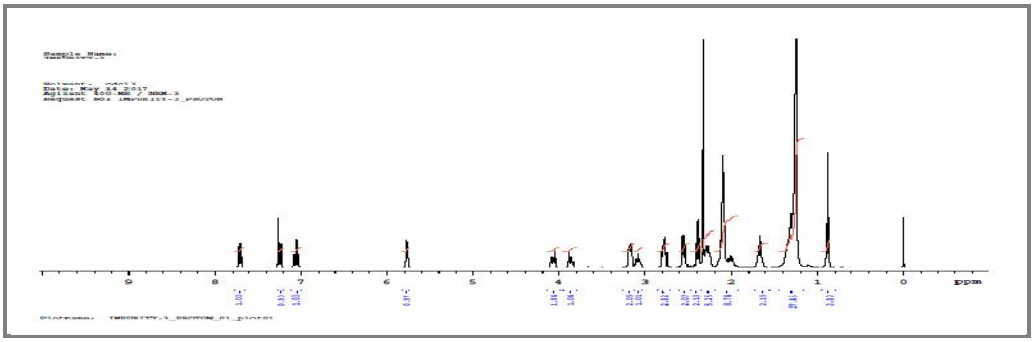


Figure.7(e). NMR spectra of Impurity-3.

Characterization of Impurity-4: The impurity-4 and was recorded with HRMS to obtain the exact mass, Orbitrap was used to measure the exact mass ESI MS $[M+H]^+$ of the impurity which was 693.4741(1.2993 ppm error) for the calculated molecular formula of $C_{41}H_{61}FN_4O_4$ as shown in figure 5(f). The obtained exact mass and the theoretical calculated molecular formula correlated with Impurity-4. To further elucidate the structure of Impurity-4 by using spectroscopic techniques. Structure of Impurity-4 was confirmed with spectroscopy techniques like ^1H NMR, ^{13}C, HMBC, HSQC and FT-IR. NMR chemical shifts of Impurity-4 were compared with the values of Paliperidone Palmitate (API) was shown the values in table 2(C). The precise structure of Impurity-4 was further confirmed by NMR studies and its full characterization details have been given in table 2(C). In proton NMR ester chain showing 35 protons (16 CH2 and 1 Methyl proton and these were confirmed

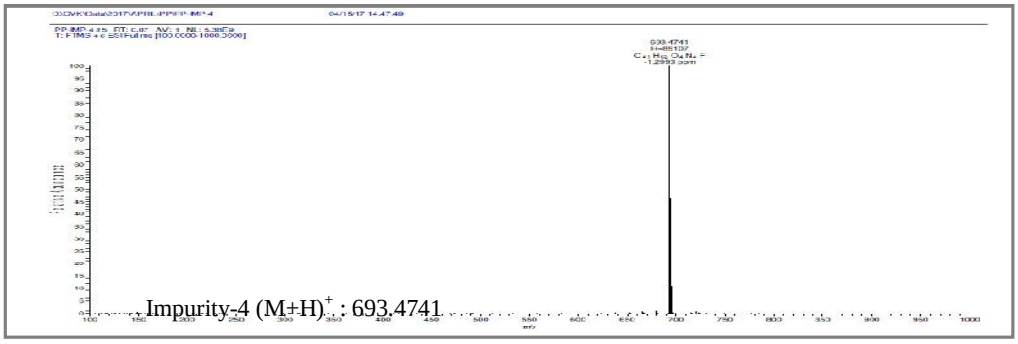


Figure 5(f). HRMS report of Impurity-4.

by HSQC experiment. HMBC experiment confirms H35 (1.68ppm), H33 (2.38ppm), H29 (5.76ppm) protons showing connectivity to C32 carbon (172.48ppm). From FT-IR, major frequencies of all the four impurities and the API are tabulated in the below table 3. Proton NMR spectra of Impurity-4 were shown in figure 7(f). Assigned structure of Impurity-4 was shown in figure 6 and melting range of Impurity-2 is 115.5-119.0°C.

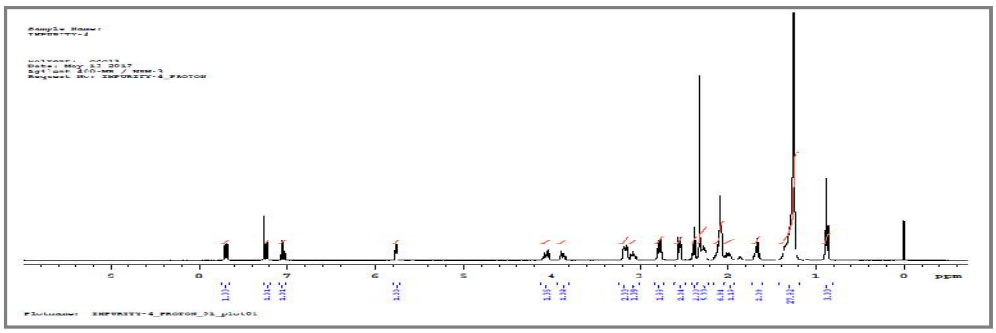
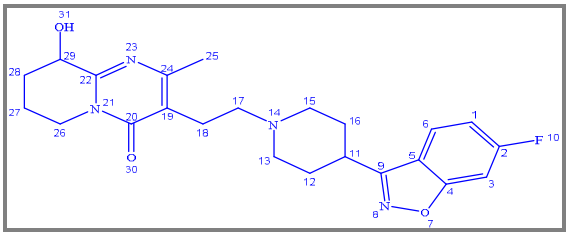


Figure.7(f): NMR spectra of Impurity-4.

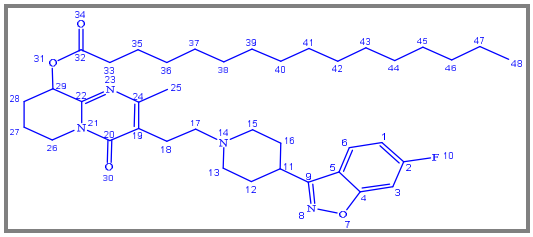
Table 2(C). Comparative NMR assignments for Impurity-3 and Impurity-4

Impurity-3				Impurity-4			
Atom No	Type of Atom	1H Chemical Shift (PPM) Coupling Const(J)	13C Chemical Shift (PPM) Coupling Const(J)	Atom No	Type of Atom	1H Chemical Shift (PPM) Coupling onst(J)	13C Chemical Shift (PPM) Coupling Const(J)
1	CH	7.05(td, 8.80, 2.0Hz, 1H)	112.12-112.37(d, 25.3Hz)	1	CH	7.05(td, 8.80, 2.4Hz, 1H)	112.11-112.36(d, 25.0Hz)
2	C	-	162.77-165.25(d, 248.8Hz)	2	C	-	162.81-165.31(d, 249.7Hz)
3	CH	7.25(dd, 8.4, 2.0Hz, 1H)	97.25-97.51(d, 26.7Hz)	3	CH	7.24(dd, 8.4, 2.4Hz, 1H)	97.25-97.51(d, 26.5Hz)
4	C	-	163.75-163.88(d, 12.6Hz)	4	C	-	163.81-163.94(d, 13.7Hz)
5	C	-	117.26	5	C	-	117.34
6	CH	7.70(dd, 8.8, 4.8Hz, 1H)	122.53-122.64(d, 11.2Hz)	6	CH	7.70(dd, 8.4, 4.8Hz, 1H)	122.53-122.64(d, 10.6Hz)
7	O	-	-	7	O	-	-
8	N	-	-	8	N	-	-
9	C	-	161.08	9	C	-	161.11
10	F	-	-	10	F	-	-
11	CH	3.08(m, 1H)	34.59	11	CH	3.07(m, 1H)	34.64
12	CH2	2.09(m, 2H)	30.53	12	CH2	2.09(m, 2H)	30.61
13	CH2	2.29, 3.17(m, 2H)	53.37	13	CH2	2.27, 3.16(m, 2H)	53.37
14	N	-	-	14	N	-	-
15	CH2	2.29, 3.17(m, 2H)	53.37	15	CH2	2.27, 3.16(m, 2H)	53.37
16	CH2	2.09(m, 2H)	30.53	16	CH2	2.09(m, 2H)	30.61
17	CH2	2.54(t, 7.2Hz, 2H)	56.46	17	CH2	2.54(t, 7.2Hz, 2H)	56.52
18	CH2	2.77(t, 7.2Hz, 2H)	23.84	18	CH2	2.76(t, 7.2Hz, 2H)	23.94
19	C	-	121.22	19	C	-	121.29
20	C	-	162.11	20	C	-	162.12
21	N	-	-	21	N	-	-
22	C	-	152.34	22	C	-	152.36
23	N	-	-	23	N	-	-
24	C	-	158.69	24	C	-	158.66
25	CH3	2.32(s, 3H)	21.38	25	CH3	2.32(s, 3H)	21.38
26	CH2	3.86, 4.06(m, 2H)	42.28	26	CH2	3.85, 4.06(m, 2H)	42.23
27	CH2	2.01, 2.09(m, 2H)	17.94	27	CH2	2.01, 2.08(m, 2H)	18.03
28	CH2	2.09(m, 2H)	25.53	28	CH2	2.09(m, 2H)	25.59
29	CH	5.77(t, 5.6Hz, 1H)	68.58	29	CH	5.76(t, 6.0Hz, 1H)	68.61
30	O	-	-	30	O	-	-
31	O	-	-	31	O	-	-
32	C	-	172.52	32	C	-	172.48
33	CH2	2.38(t, 7.6Hz, 2H)	34.34	33	CH2	2.38(t, 7.6Hz, 2H)	34.36
34	O	-	-	34	O	-	-
35	CH2	1.66(m, 2H)	24.94	35	CH2	1.68(m, 2H)	24.96
36	CH2	1.34(m, 2H)	29.03	36	CH2	1.34(m, 2H)	29.04
37	CH2	1.25(m, 2H)	29.24	37	CH2	1.25(m, 2H)	29.24
38	CH2	1.25(m, 2H)	29.44	38	CH2	1.25(m, 2H)	29.45
39	CH2	1.25(m, 2H)	29.59	39	CH2	1.25(m, 2H)	29.66
40	CH2	1.25(m, 2H)	29.66	40	CH2	1.25(m, 2H)	29.66
41	CH2	1.25(m, 2H)	29.66	41	CH2	1.25(m, 2H)	29.66
42	CH2	1.25(m, 2H)	29.66	42	CH2	1.25(m, 2H)	29.66
43	CH2	1.25(m, 2H)	29.66	43	CH2	1.25(m, 2H)	29.66
44	CH2	1.25(m, 2H)	29.66	44	CH2	1.25(m, 2H)	29.66
45	CH2	1.25(m, 2H)	29.66	45	CH2	1.25(m, 2H)	29.66
46	CH2	1.25(m, 2H)	29.58	46	CH2	1.25(m, 2H)	29.58
47	CH2	1.33(m, 2H)	31.88	47	CH2	1.25(m, 2H)	29.32
48	CH2	1.33(m, 2H)	22.65	48	CH2	1.33(m, 2H)	31.89
49	CH3	0.87(t, 6.8Hz, 3H)	14.1	49	CH2	1.33(m, 2H)	22.65
				50	CH3	0.87(t, 7.2Hz, 3H)	14.07

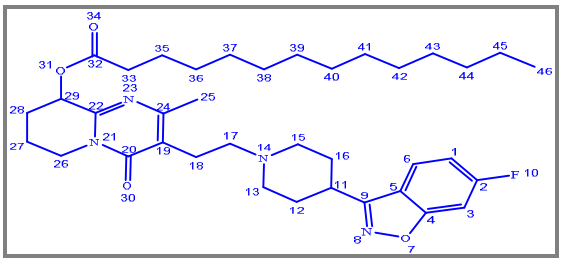
S=singlet; d=doublet; m=multiplet; dd=doublet of doublet; td=triplet of doublet;



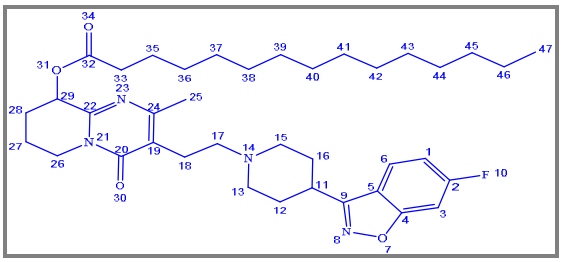
Chemical Formula $C_{23}H_{27}FN_4O_3$ Exact Mass 426.21
Paliperidone.



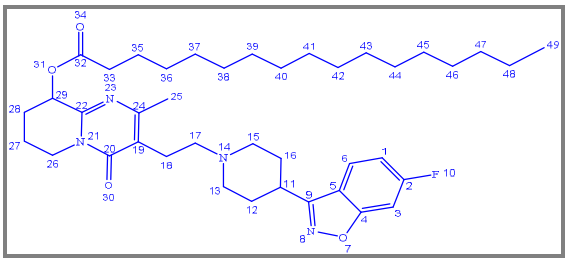
Chemical Formula $C_{39}H_{57}FN_4O_4$ Exact Mass 664.44
Paliperidone Palitate.



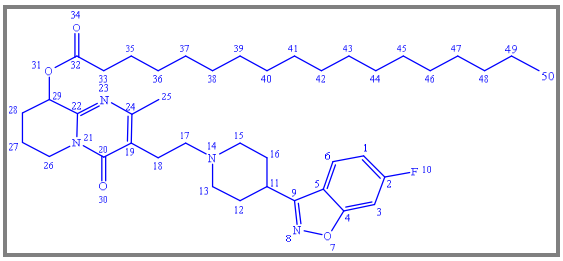
Chemical Formula $C_{37}H_{53}FN_4O_4$ Exact Mass 636.41
Impurity-1



Chemical Formula $C_{38}H_{55}FN_4O_4$ Exact Mass 650.42
Impurity-2



Chemical Formula $C_{40}H_{59}FN_4O_4$ Exact Mass 678.45
Impurity-3.



Chemical Formula $C_{41}H_{61}FN_4O_4$ Exact Mass 692.47
Impurity-4.

Figure 6. Chemical structures and position numbering of Paliperidone, Paliperidone Palmitate, Impurity-1, Impurity-2, Impurity-3 and Impurity-4.

Table 3. Comparative FTIR Absorption frenquencies of Paliperidone, Paliperidone Palmitate and four impurities

S.No.	Paliperidone	Paliperidone palmitate	Impurity-1	Impurity-2	Impurity-3	Impurity-4
1	3452	3452	3451.5	3450.6	3453.6	3452
2	3233.9	3088.3	3088.5	3088	3088	3088.6
3	3088.6	2920.5	2921.1	2920.8	2920.3	2921
4	2921	2850.1	2850.4	2850.3	2850.2	2850.1
5	2850.1	2797.9	2797.9	2797.8	2797.9	2797.9
6	2797.9	2759.6	2759.6	2759.6	2759.6	2759.9
7	2759.9	2667	2666.9	2667	2667.1	2666.9
8	2666.9	1736.9	1737.1	1736.5	1736.4	1737.3
9	2084.3	1651.4	1651.7	1651.8	1651.6	1651.2
10	1897.8	1614.5	1614.8	1614.5	1614.6	1615
11	1737.3	1540.5	1540.5	1539.9	1539.9	1540.2
12	1651.2	1515.2	1515.1	1515.1	1515.2	1515.2

Over all information from NMR: 1D, 2D NMR studies has been conducted for paliperidone, paliperidone palmitate and its four homologous series of impurities. It has been noticed that the chemical shift of chiral proton is seen at 4.5ppm in paliperidone and is shifted to down field i.e. 5.76 ppm in paliperidone palmitate, however there is no much shift observed for chiral carbon in carbon

NMR (experimental observation is only 1.5 ppm down field shift). The main difference was found in proton NMR of paliperidone palmitate compound and its impurities is at 1.25 ppm signal and its integration, same has been observed in carbon NMR at 29 ppm. Complete structure characterization data of all six compounds has been captured in the table 2(A), 2(B) and 2(C) fluorine couplings of proton and carbon were also calculated and tabulated, chemical names were represented below.
Chemical names of Paliperidone palmitate and impurities were mentioned below:

Paliperidone Palmitate: (9RS)-3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-4-oxo-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-9yl hexadecanote.

Impurity-1: (9RS)-3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-4-oxo-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-9yl butadecanoate.

Impurity-2: (9RS)-3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-4-oxo-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-9yl pentadecanoate.

Impurity-3: (9RS)-3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-4-oxo-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-9yl heptadecanoate.

Impurity-4: (9RS)-3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-4-oxo-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-9yl octadecanoate.

APPLICATION

A step by step systematic procedure is designed to identify and characterize the impurities of Paliperidone Palmitate by using LCMS, HRMS, FTIR and NMR.

CONCLUSION

Paliperidone palmitate is having great potential to cure schizophrenia, schizoaffective disorder and paliperidone palmitate may have advantages over other currently available long-acting injections. Paliperidone is stable in palmitate ester form and medical use is also established. These four impurities were evaluated by using LCMS and isolated by using preparative HPLC, Characterization was confirmed by using High Resolution Mass Spectrometry, ^{1}H, ^{13}C, 2D NMR, and FT-IR, Physical properties were evaluated. In this approach we can easily identify and characterize the unknown impurities.

Conflicts of interest: There are no conflicts to declare.

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