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Research Article

SYNTHESIS, ISOLATION AND CHARACTERIZATION OF EFAVIRENZ IN-PROCESS IMPURITY DUE TO THE PRESENCE OF TETRAHYDROFURAN AS SOLVENT

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ABSTRACT

Objectives: The objective of this study is to synthesise and isolate in process impurity of Efavirenz in the presence of Tetrahydrofuran solvent.

Method: In the process related impurity ranging from 0.05% to 0.2% in Efavirenz were detected by a gradient reversed phase high performance liquid chromatography (RP-HPLC).This impurity was isolated from the crude sample of Efavirenz using gradient reversed-phase preparative high performance liquid chromatography.

Results: The unknown impurity of in process impurity of Efavirenz was synthesised and isolated by using the preparative chromatography of purity above 95%. The pure impurity was characterized by using IR, NMR, and MS spectral data and confirm the molecular structure of IUPAC name (4-Chlorobutyl)[4- Chloro-2(4-Cyclopropyl-1,1,1-Trifluro-but-3-yn-2-ol) Phenyl] Carbamate.

Conclusion: The Efavirenz carbamate impurity was synthesized properly by using modern machineries. This is a work for the benefit of the human beings because impurity in the drugs can affect the human body.

Keywords: Efavirenz, Impurities, Spectroscopy, Identification, Characterization and synthesis.

INTRODUCTION

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) specifically used in the treatment of human immunodeficiency virus type-1 (HIV-1) [1].Around 33.4 million people were living with HIV in year 2008 and around 2 million people have died in the same year [2]. Atripla, a fixed dose combination of tenofovir, emtricitabine, and efavirenz, was approved by the Food and Drug Administration (FDA) for the treatment of this disease. Atripla was the first fixed dose formation available in the United States to combine two different classes of antiviral Drugs in a single pill. Many generic products of Atripla are also available, such as Viraday from Cipla and Vonavir from Emcure Ltd. Efavirenz is an anti-HIV drug that reduces the amount of virus in the body. Anti-HIV drugs such as efavirenz slow down damage to the immune system and prevent the occurrence of AIDS-defining illness. Efavirenz was developed by Du Pont Pharma and it was approved for HIV treatment in the United States in 1998 and European licence was granted in May 1999.Efavirenz is marketed by Bristol-Myers Squibb under the trade name Sustiva in the United States, the United Kingdom, Ireland, France, Germany, Italy, and Spain.Generic versions are manufactured in India as Efavir made by Cipla, Estiva made by Genixpharma, Viranz made by Aurobindo and Efferven made by Ranboxy.

Impurity profiling is now receiving important critical attention from regulatory authorities. Different compendia such as the British, the United States and the Brazilian Pharmacopoeies have been specifying limits to allowable levels of impurities present in drug substances or products. Besides, ICH and FDA have published guidelines on residual solvents and impurities in new drug substances and products [3-6].

The control of drug chemical impurities is very difficult issue to the pharmaceutical industries. The contain of very small amount of unwanted chemicals may affect the safety and efficiency of the pharmaceutical products [7]. The monograph was adopted at the Fortieth WHO Expert Committee on Specification for pharmaceutical preparation in October 2005 for addition to the 4th edition of the international pharmacopoeia. Efavirenz is white to slightly pink powder and practically insoluble in water, but freely soluble in methanol. Efavirenz should be kept in a well closed container, protected from light. Chemical name of Efavirenz is (4S)- 6-Chloro-4-(cyclopropylethynyl)-1,4- dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one of CAS No is 154598-52-4. The Molecular formula is $C_{14}H_9ClF_3NO_2$ and the molecular mass is 315.7. The Molecular Structure is given in the figure no-1.

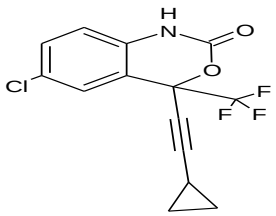

Fig. 1: Molecular structure of Efavirenz API

A literature search revealed that only analytical procedure is available [8-13] for determination of Efavirenz from biological matrices, but nobody has reported synthesis, isolation, and characterization of impurity in the purified form, from Efavirenz API. The present communication involves the isolation, preparation of impurity and characterization by chromatographic and spectroscopic technique.

MATERIALS AND METHODS

Materials and reagents

The raw material of Efavirenz impurity was received from Sitec labs Mumbai, India. The HPLC grade acetonitrile and methanol solvents were obtained from Merck co, Mumbai, India. The HPLC grade Formic acid was obtained from Sigma Aldrich, Mumbai, India. Tetrahydrofuran, Caustic soda laboratory grade reagents were obtained from Merck co, Mumbai.

High performance liquid chromatography (HPLC analytical)

An Agilent HPLC system equipped with 1100 series low pressure quaternary gradient pump along with pulse dampener, Photo diode array detector with auto liquid sampler handling system has been used for the analysis of the sample. A Zorbax SB-CN (Cyanopropyldimethylsilane monolayer) , 4.6mm x15cm x3.5µ column was employed for the testing of reaction mass of Efavirenz impurity. The column eluent was monitored at detection wavelength

250nm. The mobile phase-A was 90 vol. of a 0.05% solution of Trifluroacetic acid and 10 vol of Methanol, mobile phase-B was 10 vol of a 0.05% solution of trifluoroacetic acid and 90 vol of Methanol. After the preparation of both the mobile phase degas and sonicate properly.The gradient procedure was 00 min= 60:40, to16 min= 50:50,16 to 23 min= 35:65, 23 to 28 min = 30:70, 28 to 29 min = 20:80, 29 to31= 20:80, 31 to 32= 60:40, 32 to 40= 60:40. Chromatography was performed at 40 ^{0}c with the flow rate of 1.5 ml/min. Data was recorded by using Chem. station software.

High performance liquid chromatography (Preparative HPLC)

Preparative HPLC is the technique of choice for compound isolation and purification within the pharmaceutical and life science industries. Agilent technologies purification solution from nano gram to gram sample quantities. Agilent 1200 Series purification system with low delay volumes optimized for high recovery and purity, with PDA detector and flow rate is 0.001 to 100 ml/min with max. Pressure 400 bar. A ODS-C18 250mm x 21.2mm x 10µ reverse phase silica column was employed for the separation of Efavirenz in process impurity. Solvent used for the separation was Water (0.1% formic acid): ACN with flow rate of 20 ml/min, with the detection of 250nm.and chromatography was performed at room temperature. The gradient procedure was 00 to 05min = 30:70, 05 to 27min = 10:90, 27 to 28 min = 10:90, 28 to 30 min = 30:70, 30 to 32min= 30:70

Microwave

The use of microwave irradiation in organic synthesis has become increasingly popular within the pharmaceutical and academic arenas, because it is a new enabling technology for drug discovery and development. For the synthesis of the Efavirenz in process impurity used CEM Discover microwave system. It's a system of ISO 9001-2000 approved. This system perform atmospheric (up to 70ml working volume) and pressurized (up to 50 ml working volume) reaction. Use a wide range of vessels as well as standard condensers, addition funnels and stirring options with refluxing capability. CEM reaction tubes are pressure rated to 500 psi and use septa that tolerate multiple piercing for reagent addition or sample withdrawal. Microwave system gave faster reactions with increased yields, improved selectivity, and superior reproducibility. Optimization of the reaction very quickly and in fewer steps gave more time to use creativity to explore the available chemical diversity. The instrument specification, overall dimension is (36.2 cm x 43.7 cm x 22.1 cm), weight = 30lbs, Magnetic frequency= 2450 MHz, Power output= 300 Watts, temperature = -90^{0}C to 300^{0}C.

Mass spectrometry (LC-MS/MS)

The LC–mass spectrometry (MS) and MS-MS studies were carried out on an Ion trap 6320

Series electron spray ion trap spectrometer (Agilent Technologies).The source voltage was kept at3.0 kV. Parameters: nebulizer gas = 30psi; dry gas = 3 L/min; dry temperature= 150 °C; capillary voltage =24500 to 21500 V. Nitrogen was used as both a sheath and auxiliary gas. Mass range was kept at m/z 50–600.The chromatography conditions and mobile phase are column = Zorbax SB-CN (15cmx4.6mm x3.5µm), temperature= 40^{0}C, Wavelength = 250nm, The mobile phase-A was 90 vol. of a 0.05% solution of Trifluroacetic acid and 10 vol of Methanol, mobile phase-B was 10 vol of a 0.05% solution of trifluoroacetic acid and 90 vol of Methanol. After the preparation of both the mobile phase degas and sonicate properly. The gradient procedure was 00 min= 60:40, to16 min= 50:50,16 to 23 min= 35:65, 23 to 28 min = 30:70, 28 to 29 min = 20:80, 29 to31= 20:80, 31 to 32= 60:40, 32 to 40= 60:40.

Nuclear magnetic resonance

The 1H, and 13C nuclear magnetic resonance(NMR) spectroscopy experiment of the impurity was carried out at a frequency of 500 MHz at 25 °C on an NMR spectrometer (Varian, Palo Alto, California). 1H chemical shifts are reported on the δ scale in ppm relative to tetra methyl silane 0.00 and$CDCl_3$ (δ77.00 ppm) and DMSO, D6 (δ=39.50) respectively. ^{1}H experiments were run using a mixing time of 1000ns.

FT-IR Spectroscopy

The IR spectra were recorded in the solid state as KBr dispersion medium using Perkin Elmer spectrum 100 FT-IR spectrophotometer.

Synthesis of impurity

Step-1: In a Microwave glass tube, Efavirenz API (2gm) was charged with Methanol solvent. In that mixture added caustic soda (0.5gm) and water very slowly at 50^{0}C – 55^{0}C and stirred for 40-50 mins. After that expose the reaction mass at 120 ^{0}C at 40-to- 50 watt power in the CEM Discover Microwave for 30 mins interval. After the completion of the reaction, neutralized the mass with dilute hydrochloric acid solution, and get the solid amino alcohol product. Reaction scheme was given in the figure No-2.

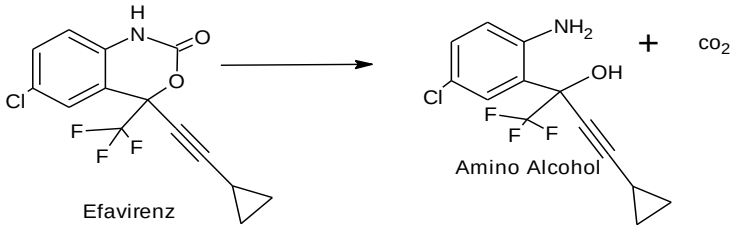

Fig. 2: Step-1 Reaction Scheme.

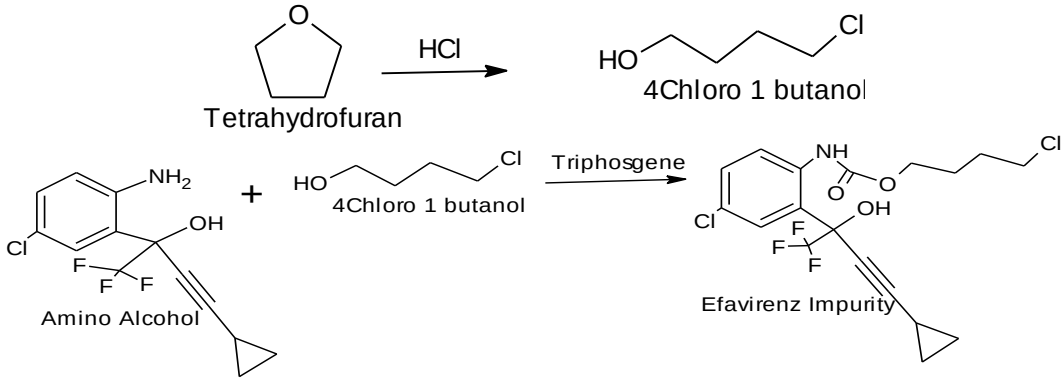

Fig. 3: Step-2 Reaction Scheme

Step-2:Aminoalcohol[(*S*)-5chloro-∞-(cyclopropylethynyl)-2-amino-∞(trifluoromethyl)benzyl methanol] got from step-1, reacts with Triphosgene in presence of Tetrahydrofuran solvent at room temperature gave the Efavirenz API and the impurity (4-Chlorobutyl)[4-chloro-2-(4-Cyclopropyl-1,1,1-trifluoro-but-3-yn-2-ol) phenyl] carbamate, after the degradation of THF with free hydrochloric acid that was generated from Triphosgene and gave 4-Chloro-1-butanol. Reaction scheme were given in Figure No-3.

Reaction mass checked in HPLC and got the required impurity of 41% purity, and checked the reaction mass in LCMS for the confirmation of the required impurity mass. To improve the percentage of the required impurity used preparative chromatography, for the characterization of the impurity.

RESULTS AND DISCUSSION

Detection of impurity by HPLC

Typical analytical HPLC chromatogram of the reaction mass of Efavirenz impurity obtained by using the HPLC method discussed under the heading "High performance Liquid Chromatography (analytical)". The targeted impurity under study is marked as (4-Chlorobutyl)[4-chloro-2-(4-Cyclopropyl-1,1,1-trifluoro-but-3-yn-2-ol) phenyl] carbamate, eluted at retention time of about 21.927 mins. It was given in figure no-4

Isolation of the impurity by prep-HPLC

A simple reverse phase chromatographic system, discussed under the heading, "High performance Liquid Chromatography (preparative) was used for isolation of the impurity. In this chromatographic system, the Efavirenz impurity eluted at about 16.250 mins. The Efavirenz impurity fraction was collected between 15.1min to 17.5min. The impurity fraction was concentrated at room temperature under high vacuum on a Buchii Rotavapour Model R124, the residue was then lyopholized for getting solid impurity. Purity of the impurity was tested in analytical method discussed under the heading, "High Performance Liquid Chromatography" (HPLC). The purity was found to be 95.17 % and it is exhibited in figure no.5, before carrying out spectroscopic experiments.

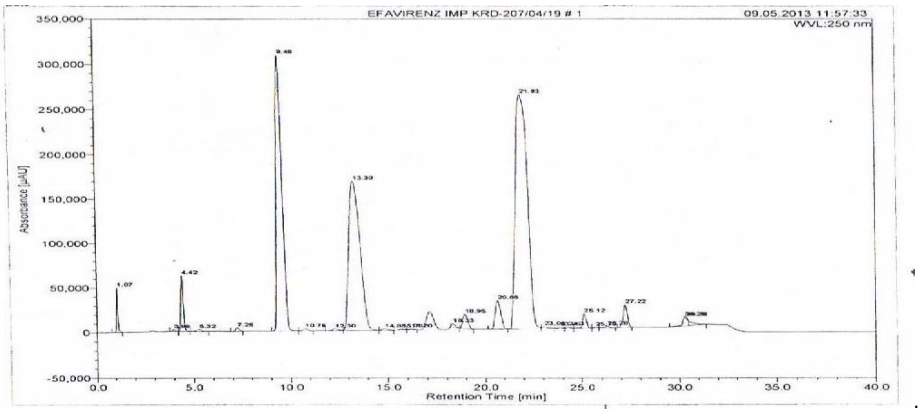

Fig. 4: HPLC Chromatogram of reaction mass

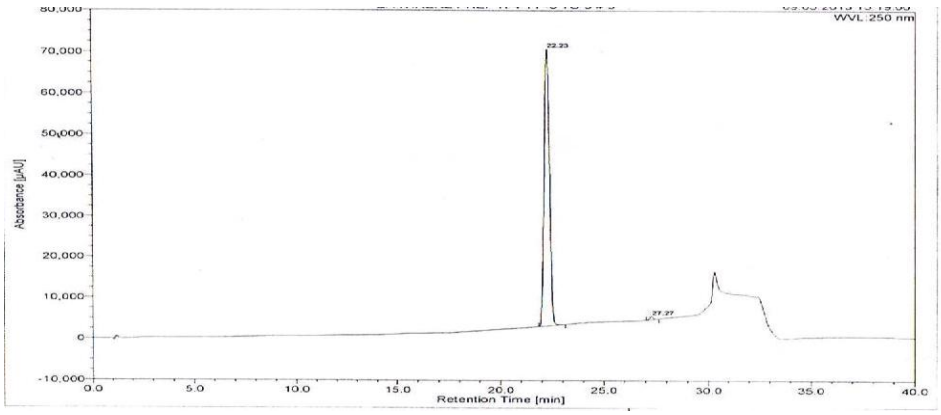

Fig. 5: HPLC Chromatogram of pure Impurity

LC-MS/MS analysis

LC-MS/MS analysis of Efavirenz bulk drug sample and impurity of Efavirenz was performed using the chromatographic system as described under the heading "Mass Spectrometry (LC-MS/MS)".Result of LC-MS/MS analysis revealed that impurity exhibited molecular ion at m/z(M-1)=421.8amu.and it's MS/MS shown 351.6,313.7,217.6 amu.Efavirenz API exhibited molecular ion at m/z(M+1)= 316.0amu, and it's MS/MS shown 242.2, 279.0, 652.9.

Structure elucidation

The Molecular Structure of Efavirenz impurity is as Figure-6. The Molecular Formula is C_{18} $H_{18}Cl_2F_3NO_3$ and its Molecular weight is 424.24amu and its monatomic mass is 423.06 amu.

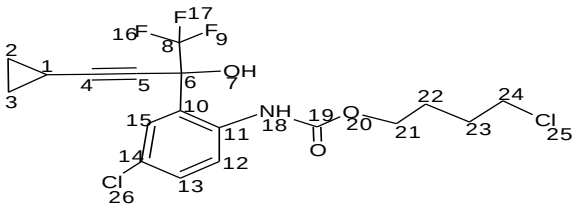

Fig. 6: Molecular Structure of Efavirenz impurity,

The IR spectra recorded in the solid state as KBr dispersion. FT-IR data of Efavirenz API and its impurity exhibited in the figure no-7 and figure no-8.The mass spectra of Efavirenz API and its impurity exhibited in the figure no-9 and figure no-10.The IR spectra and the mass spectral data exhibited in the Table no-1.

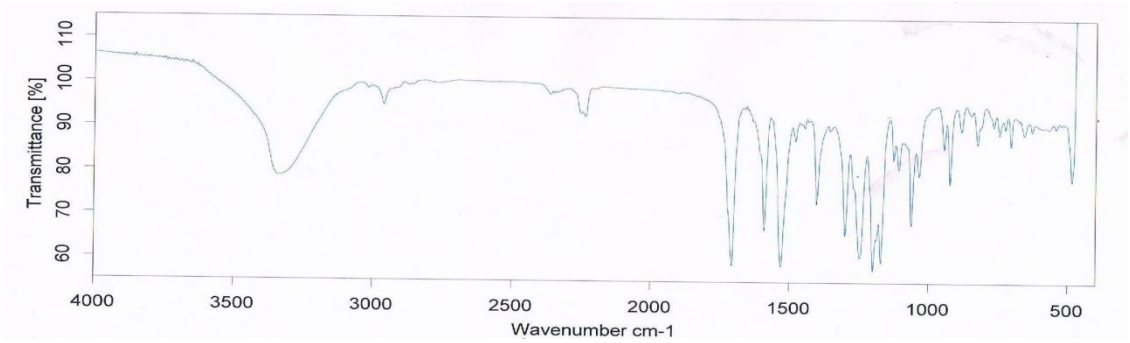

Fig. 7: IR spectra of Efavirenz API.

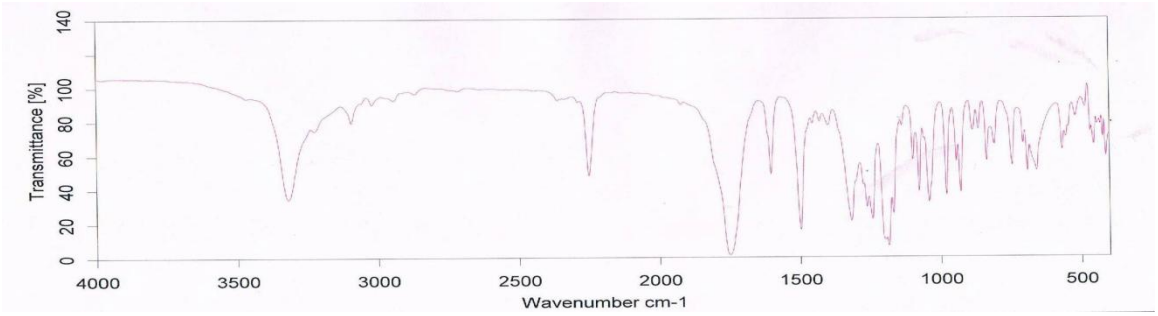

Fig. 8: IR spectra of Efavirenz Imp.

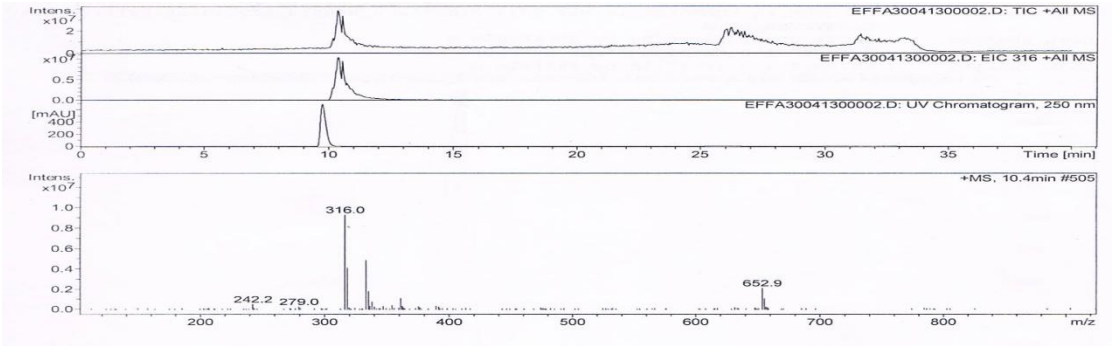

Fig. 9: MS/MS-MS spectra of Efavirenz API

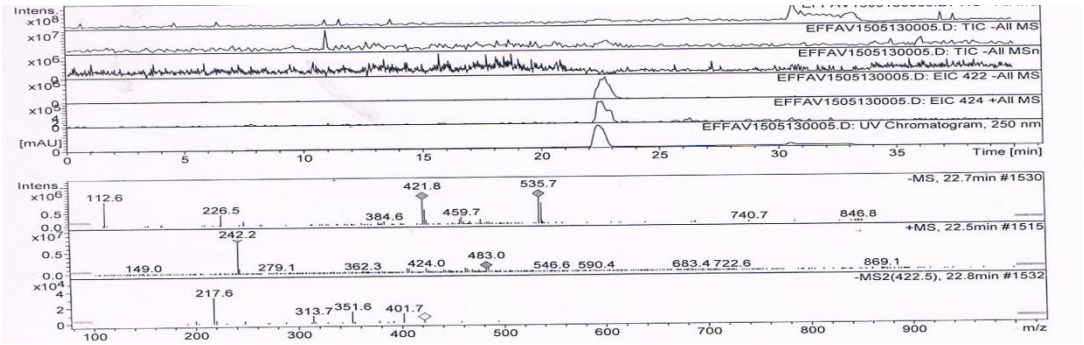

Fig. 10: MS/MS-MS spectra of Efavirenz Impurity

Table 1: IR spectra and MS/MS-MS spectral data of Efavirenz and its Impurity

S. No.	Name of product	IR spectral data	MS,MS/MS data
1	Efavirenz	N-H strong stretch at 3319.6,C=OStretch at 1749.7, HC≡CH at2250.7, C-F at 1319 cm^{-1},1245 cm^{-1}, C-Cl stretch at 1038.3cm^{-1}	m/z 316.0 amu 242.2,279.0, 652.9amu.
2	Efavirenz Impurity. (4-Chlorobutyl)[4-chloro-2-(4-Cyclopropyl-1,1,1-trifluoro-but-3-yn-2-ol)phenyl] carbamate	C-O ether stretch at 1320cm^{-1}, C-Cl alkyl halide at 850 cm^{-1}, O-H stretch at 3350.5cm^{-1},	m/z 421.8 amu. 351.6, 313.7, 217.06 amu.

Efavirenz impurity1H NMR, and 13C NMR spectrum, for structure prediction and detailed assignment shown in table-2 and table-3. And was recorded in DMSO on 500MHz Varian instrument. 1H NMR and 13C NMR spectrum exhibited in the Figure No-11 and Figure No-12 respectively.

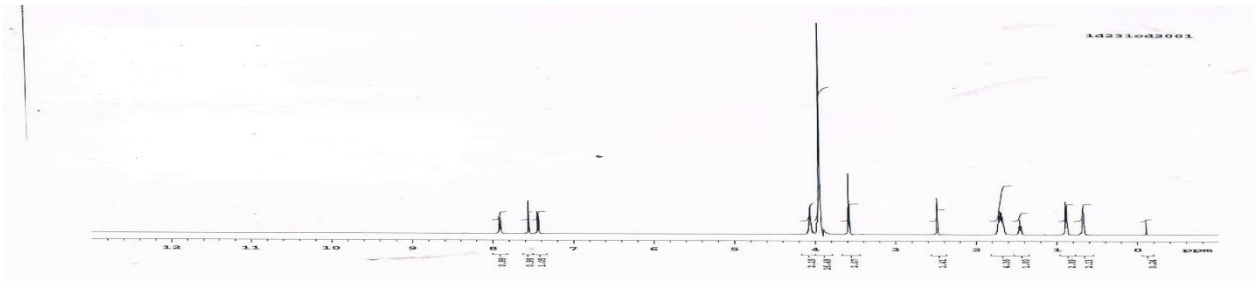

Fig. 11: 1H NMR spectrum of Efavirenz impurity

Table 2: 1H-NMR probable assignment of Efavirenz Impurity

Chemical Shift (ppm)	Number of Protons	Multiplicity	Assignment
0.72	2	Multiplicity	3
0.89	2	Multiplicity	2
1.54	1	Multiplicity	1
1.55-1.8	4	Multiplicity	22,23
3.65	2	Triplet	24
4.09	2	Triplet	21
7.50	1	Doublet	13
7.58	1	Singlet	15
8.00	1	Doublet	12
9.24	2	Singlet	7,18

D_2O Exchange NMR Confirmed the Exchangeable Protons in the molecule.

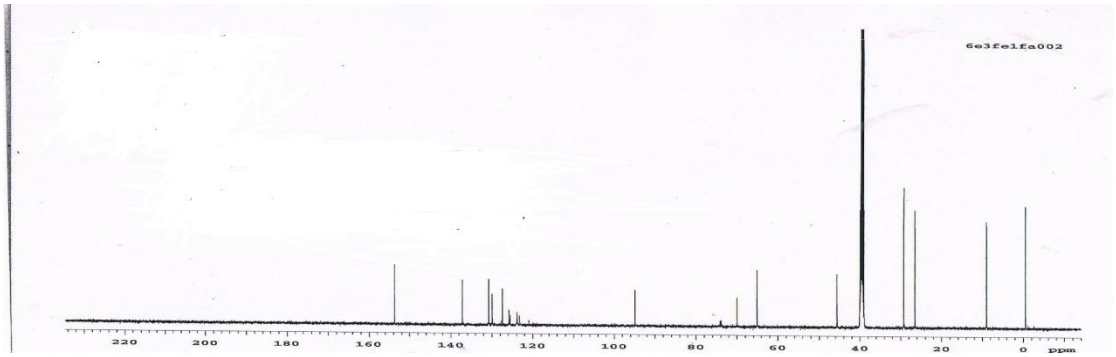

Fig. 12: 13C NMR spectrum of Efavirenz impurity

Table 3: Probable Assignment for the Resonance Bands observed in the ^{13}C NMR spectrum of Efavirenz impurity

Chemical Shift (ppm)	Type of Carbon	Assignment
-0.539	CH	1
8.94	CH_2	2
9.00	CH_2	3
26.54	CH_2	22
29.22	CH_2	23
45.66	CH_2	24
65.06	CH_2	21
69.94	C	5
73.93, 74.18	C	6
94.90	C	4
120.95,123.24,125.52,127.81	C	8
123.89	CH	12
125.82	C	10
127.45	C	14
129.91	CH	15
130.78	CH	13
137.25	C	11
153.80	C	19

CONCLUSION

This research paper describes the synthesis, isolation and structure elucidation of process related impurity of Efavirenz due to the presence of THF as solvent, as reported in USP. The impurity was separated by reverse phase chromatographic technique, by using High performance liquid chromatography (prep-HPLC). The isolated impurity was characterized by using IR, 1H NMR,13C NMR and LC-MS/MS spectroscopic technique. The synthesis of impurity was also discussed in brief.

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