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Synthesis, Isolation, and Characterization of Isomeric Impurity of Dutasteride.

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Abstract*: In the process development of dutasteride in the laboratory analysis showed some impurity peaks in HPLC ranging from 0.05 to 0.1%. The same samples were analyzed by LCMS method and identified peak at m/z 508 (desmethyl dutasteride), 530 (dihydro dutasteride) and 528 (isomer of dutasteride). Among these impurities a simple and rapid preparative high-performance liquid chromatography (HPLC) method has been developed to isolate and characterized 17alpha-N-[2,5-bis(trifluoromethyl) phenyl] carbamoyl-4-aza-5alpha-androst-1-ene-3-one (alpha-isomer of dutasteride). Different isolation technique conducted and finally Isomeric impurity of dutasteride separated and Characterized by using Preparative HPLC,MS,[1]H and [13]C NMR spectroscopy.*

Keywords: *Dutasteride, Impurities,Synthesis,Isolation,Characterization,flashchromatography, preparative hplc, mass spectra, nmr.*

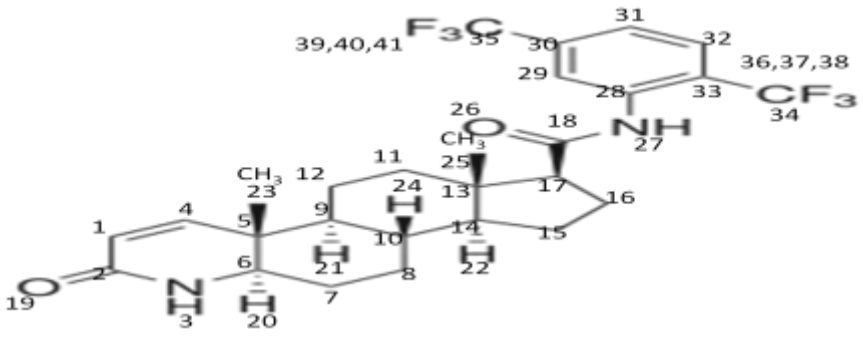
I. Introduction

Dutasteride was a dual inhibitor of 5α-reductase, which was developed by Glaxo-Smith- Kline Company and approved by Food and Drug administration (FDA). For marketing in USA in June 2003. It was suitable to prevent and to treat benign prostatic hyperplasia (BPH). Because dutasteride has lesser side effect and can effectively decrease prostatic volume, reduce the possibility of urinary retention, and hence prevent the risk related with operation and bio-process effect of BPH. The drug has been widely used in clinical treatment of benign prostatic hyperplasia [(1, 2)].

One of the usual and frequent male aged diseases was benign prostatic hyperplasia (BPH). According to statistic, the incidence of BPH among age males of elder than 50 was 30%-- 50%, with age of 70-90 was 70%. and males elder than 80 was even greater than 90%. Therefore BPH was an important research topic for many scholars in china and abroad [(3)].The therapy of BPH has been attracted wide attention in the medical society.

Steroid 5α-reductase type 1 and type 2 are expressed tissue specifically in human [(4)] and are responsible for local production of the more potent androgen dihydrotestosterone (DHT) from testosterone (t). DHT is believed to play an important role in the pathology of benign prostatic hyperplasia (BPH) [(5)], acne [(6)], hirsutism [(7)], and male pattern baldness [(8)].Dutasteride is currently available in the market as a drug for benign prostatic hyperplasia under the brand name of AVODART.

Dutasteride is a white to pale yellow power with the chemical name (5α, 17β)-N-(2,5 bis(trifluoromethyl) phenyl)-3-oxo-4-azaandrost-1-ene-17-carboxamide. It's CAS No is 164656-23-9.The Chemical formula is $C_{27}H_{30}F_6N_2O_2$ and the molecular weight of Dutasteride is 528.53 gm/mol. The molecular structure of Dutesteride is as below:



Physicochemical properties: Dutasteride is a white to pale yellow powder with a melting point of 242 to 250°C. It is soluble in ethanol (44 mg/mL), methanol (64 mg/mL) and Polyethylene glycol 400 (3 mg/mL), but it is insoluble in water.

There are several process impurities/related substances associated with the manufacture of Dutasteride. Different process related impurities have been observed with various synthetic route and/ or manufacturing process. Three known impurities have been mentioned in international pharmacopoeia. All these impurities are never present together while we are preparing bulk drug or formulation.

The international pharmacopoeia specifies that individual impurity is not more than 0.1% and total impurity is not more than 0.7%. During the process development of dutasteride in the laboratory analysis showed some impurity peaks in HPLC ranging from 0.05 to 0.1%. A comprehensive study has been carried out to isolate or to prepare and characterization of this impurity. To the best of our knowledge of regulatory authorities [(9)] all the impurity which are at the level of >0.1% must be identified and characterized.

A literature search revealed that only analytical procedure is available but nobody has reported synthesis, isolation, and characterization of impurity in the purified starting from 3-oxo-4-aza- 5α- androstane-17β carboxylic acid [(10)]. The present communication involves the isolation or preparation of impurity and characterization by chromatographic and spectroscopic technique.

II. Experimental Section

2.1. Materials and reagents :

The raw material of Dutasteride was received from Sitec labs Mumbai, India. The HPLC grade acetonitrile and methanol solvents were obtained from Merck co, Mumbai,India. The preparative grade ethyl acetate and hexane were obtained from Merck co, Mumbai, India.

2.2. High performance liquid chromatography (HPLC):

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. An Inertsil C-18, 4.6mm x 25cm x5µ column was employed for the separation of Isomer of Dutasteride. The column eluent was monitored at detection wavelength 215nm. The isocratic gradient pattern employed for the separation of isomeric impurity of Dutasteride was as ACN: Water = 55: 45. Chromatography was performed at room temperature using a flow rate of 1.0 ml min^{-1}. Data was recorded by using Chem station software.

2.3. 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 Zodiac 250mm x 20mm x 10µ silica column was employed for the separation of isomeric impurities of Dutasteride. Solvent used for the separation was Hexane: Ethyl acetate = 15: 85, with flow rate of 10 ml/min, with the detection of 254nm.

2.4.Flash Chromatography:

Flash chromatography known as medium pressure chromatography, was differed from the conventional technique. The compound of interest should have a TLC Rf of = 0.15 to 0.20 in the solvent system I choose. Binary solvent system with one solvent having a higher polarity of the eluent. Higher polarity of solvent increases rate of elution for all compounds. Common binary solvent systems in order of increased polarity are dichlomethane/hexane, ether/hexane, hexane/ethyl acetate and dichloromethane/methanol. For the separation of dutasteride isomeric impurity used Teledyne ISCO combi-flash, solvent used Hexane/ Ethyl acetate and column used 24gm silica of 40µ size. Detection wavelength was 254nm with the flow of 10ml/min.

2.5. 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 as described under the heading "HPLC" earlier. The flow rate was maintained at 1 mL/min.

2.6. 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 and 13C 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) in 13C NMR, respectively. A D_2O exchange experiment was performed to confirm the exchangeable protons. ^{1}H and ^{13}C experiments were run using a mixing time of 1000ns.

2.7. FT-IR spectroscopy:

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

III. Synthesis of Impurity

Synthesis of 17∞ –N[2,5 bis(trifluoro methyl) phenyl] carbamoyl-4- aza- 5∞ –androst- 1- ene- 3-one (∞-isomer of dutasteride).

A solution of 3-oxo-4-aza-5∞-androstane-17β- carboxylic acid, was heated in toluene at azeotropic reflux for 40 min. To the resulting solution was cooled to 25to 30 ^{0}c, pyridine was added under nitrogen atmosphere, followed by thionylchloride for 30 min. The reaction mass was stirred for 2.5 hrs at 30 ^{0}c Ammonia gas was passed into the reaction mass for 6hrs. The obtained solid was filtered and dried.

The above obtained solid taken in xylene, added with potassium carbonate and heated for 2hrs, then cooled to 30^{0} c. Copper powder and [2, 5 bis(trifluoromethyl) iodo benzene were added and the resulting mixture was heated to 140 ^{0}c with vigorous stirring for the period of 55hrs. After cooling the reaction mass to 55^{0}c, filtered out the copper powder and the organic layer was distilled out under vacuum and the solid obtained was the mixture of ∞ and β isomer of dutasteride. This solid was taken for preparative isolation for purification of ∞- isomer of dutasteride for characterization.

IV. Results and Discussion

4.1. Detection of impurity by HPLC :

Typical analytical HPLC chromatogram of dutasteride bulk drug and it's isomer obtained by using the HPLC method discussed under the heading "High performance Liquide Chromatography (analytical)" is shown in figure 1. The targeted impurity under study are marked as ∞-isomer impurity eluted at retention time of about 27.683mins and the dutasteride eluted at retention time of about 30.033 mins. respectively.

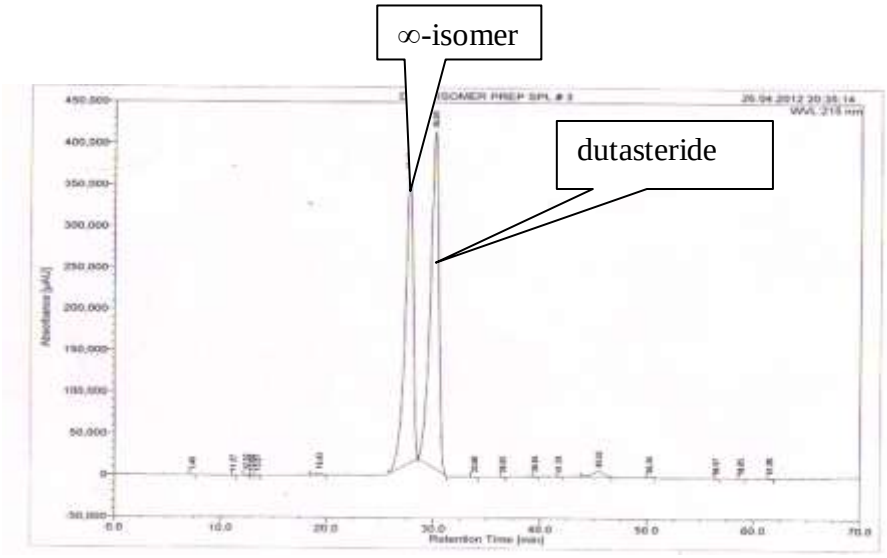


Figure-1

4.2. Isolation of the impurity by Flash Chromatography:

A simple normal phase chromatographic system, discussed under the heading "Flash Chromatography" was used for increasing the percentage of the impurity, it does not give the pure material for characterization.

4.3. Isolation of the impurity by prep-HPLC:

A simple normal phase chromatographic system, discussed under the heading, "High performance Liquid Chromatography (preparative) was used for isolation of the impurity. In this chromatographic system, dutasteride eluted at about 21.623 mins whereas the isomer impurity eluted at about 17.597 mins

respectively.The isomer impurity fraction was collected between 17.592min to 20.0min(Figure-2). The impurity fraction was concentrated at room temperature under high vacuume on a Buchii Rotavapour Model R124. The concentrated volume take in acetonitrile solvent and put it in the Lyopholizer for getting solid isomer 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 99.119% before carrying out spectroscopic experiments.

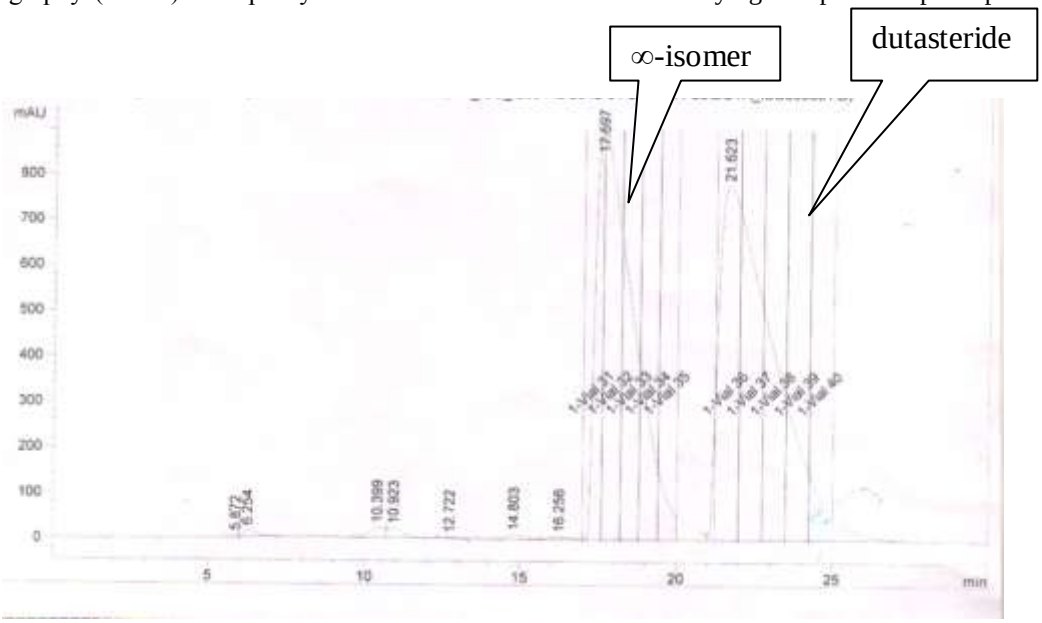


Figure-2

The isolated impurity was obtained 99.119% pure and showed in the figure-3. The purity was obtained by the method discussed under the heading," High performance liquid chromatography (HPLC)".

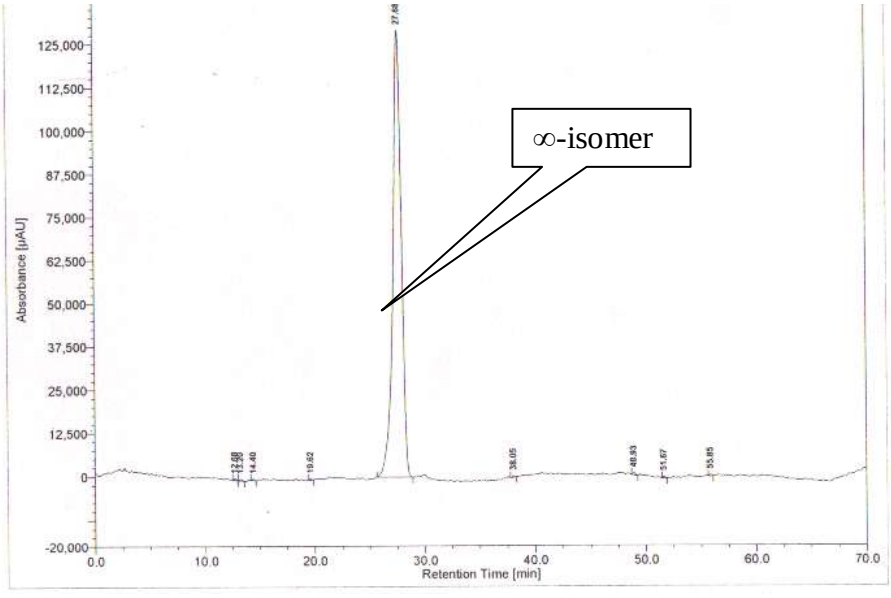


Figure-3

4.4. LC-MS/MS analysis:

LC-MS/MS analysis of dutasteride bulk drug sample and isomer impurity of dutasteride 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 (isomer of dutasteride) exhibited molecular ion at m/z(M+1)=529.4 and (M-1)=527.1amu.(Figure-4) and dutasteride API exhibited molecular ion at m/z(M+1)=529.4amu, and (M-1)=527.1(Figure-5).Figure-5 exhibited the molecular masses of dutasteride API and its impurity. Based on this fact it was assumed that the impurity was similar to that of dutasteride which might be a isomer.

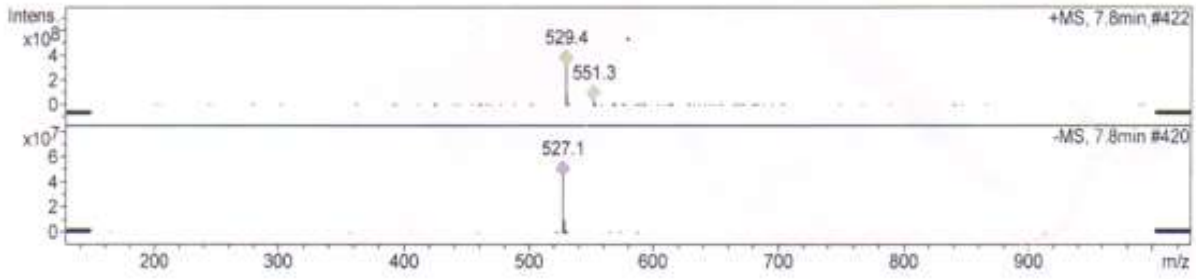


Figure-4

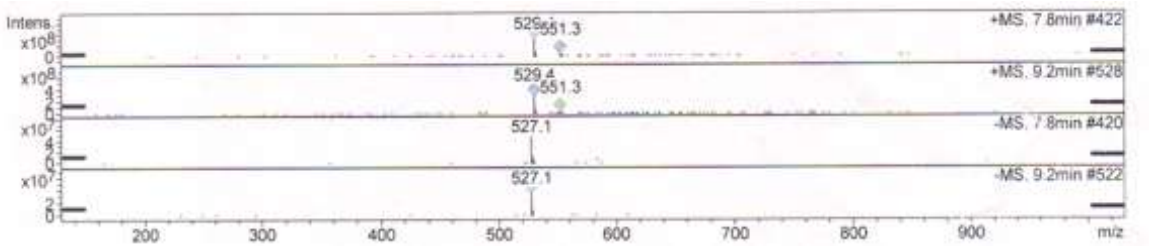


Figure-5

V. Structure Elucidation

(comparision study of dutasteride and isomer impurity)

The IR spctra recorded in the solid state as KBr dispersion.IR spectrum at 1683 & 1607 cm^{-1} clearly indicate the presence of two carbonyl groups. IR spectrum of dutasteride and impurity exhibited in Figure-6 and Figure-7 respectively.

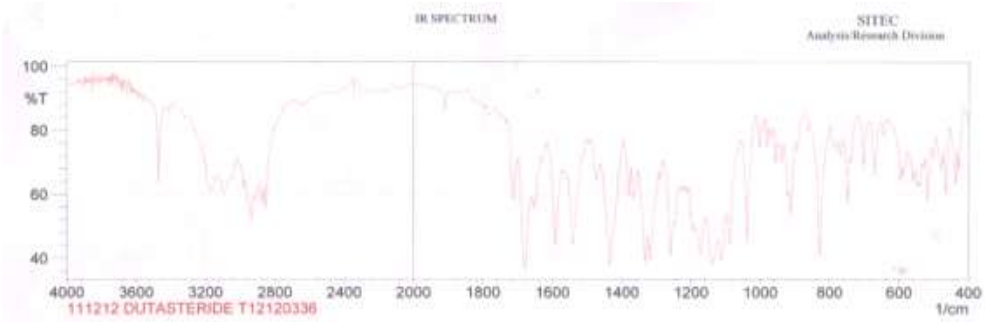


Figure-6

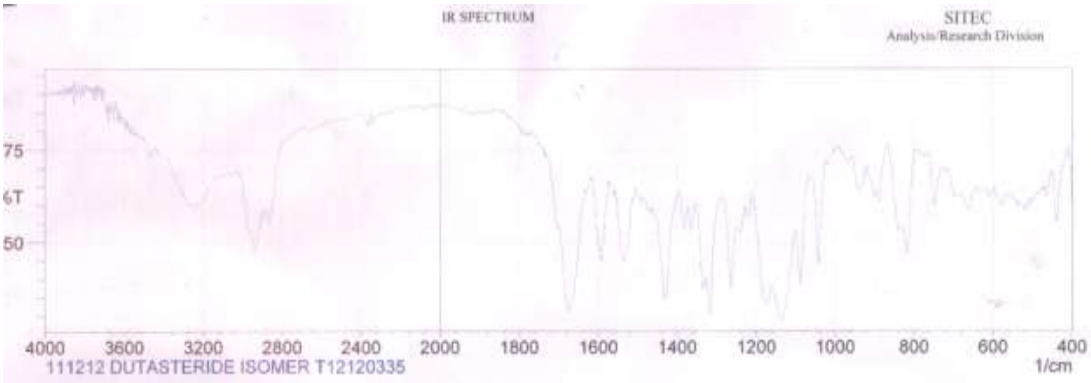


Figure-7

The spectral data of this impurity was compared with that of dutasteride spectral data.

LC-MS analysis exhibited molecular ion for this impurity at m/z=529.4(M=1) and it was same as the mass shown for dutasteride API, hence it was an isomer. The 1H NMR spectrum of dutasteride and ∞-dutasteride exhibited a sharp change in 17th position proton and the 13c NMR of the both showed a sharp shift in the 17th

position carbon. 1H NMR and the 13c NMR of dutasteride exhibited in Figure-8 and Figure-9, and1H NMR and 13c NMR of isomer impurity exhibited in Figure-10 and Figure-11 respectively.Dutastride and ∞-dutasterde 1H NMR and 13c NMR comparison shown in table-1 and table-2.

Table-1, 1H NMR spectral data of dutasteride and process related impurity.

	Dutasteride		Impurity	
position	Shift/1H/ppm	assignment	Shift/1H/ppm	Assignment
27	1H- 9.35(s)	NH	1H- 9.35(s)	NH
32	1H-7.95(m)	Aromatic-H	1H-7.95(m)	Aromatic-H
31	1H-8.90(m)		1H-8.90(m)	
29	1H-8.85(m)		1H-8.85(m)	
28	1H-7.35(m)		1H-7.35(m)	
4	1H-6.80(s)	Olifenic-H	1H-6.80(s)	Olifenic-H
1	1H-5.60(s)	Olifenic-H	1H-5.60(s)	Olifenic-H
3	1H-3.6(s)	NH	1H-3.6(s)	NH
20	1H-3.4(d)	H	1H-3.4(d)	H
17	1H-2.55(d)	β-H	1H-2.7(d)	∞-H
7	2H-2.49(t)	H	2H-2.49(t)	H
16	1H-2.05(m)	H	1H-2.05(m)	H
11	2H-1.7(m)	H	2H-1.7(m)	H
15,12,24,8,21	8H-0.9-1.5(m)	H	8H-0.9-1.5(m)	H
23	3H-0.85(s)	Methyl	3H-0.85(s)	Methyl
25	3H-0.6(s)	Methyl	3H-0.6(s)	Methyl

Table-2:13c NMR spectral data of dutasteride and process related impurity.

Position	13c-of dutasteride/ppm	13c- of Isomer impurity/ppm
18	172.38	175.03
2	165.126	165.20
4	150.43	150.51
28	136.99	136.58/136.59
30	133.07/132.81	132.91/132.65
35	127.96/127.91	128.06/128.02
32	127.83/127.73	127.09/126.86
1	127.87	125.58
33	126.37/126.34	123.90/124.24
34	124.21/123.90	123.05
31	123.10/123.01	122.63
29	122.04/121.79	121.72/122.06
6	59.06	59.02
14	55.41	53.81
17	55.26	50.06
11	40.01	34.97
13	44.28	45.603
9	47.07	46.83
10	34.82	33.83
5	36.94	40.11
8	29.02	29.55
7	25.04	25.11
15	24.009	25.54
12	20.59	20.59
23	13.31	19.92
25	11.8	11.82

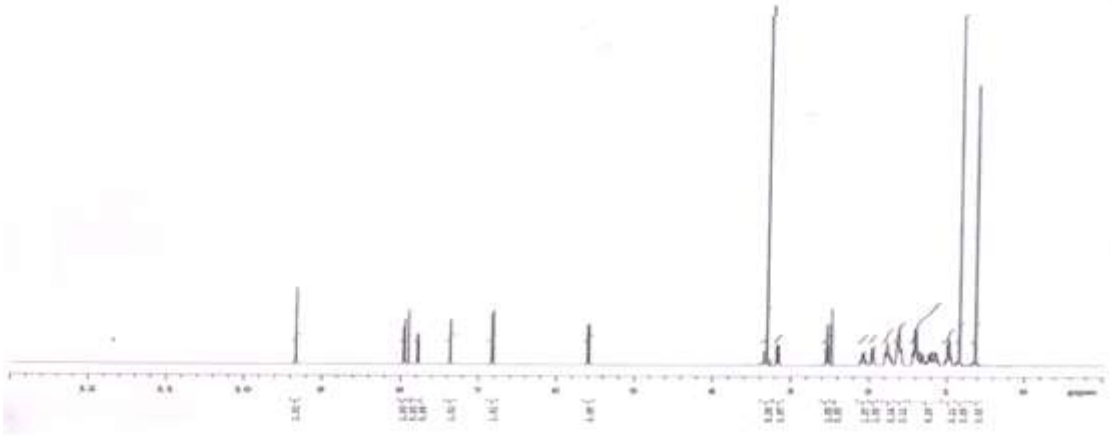


Figure-8

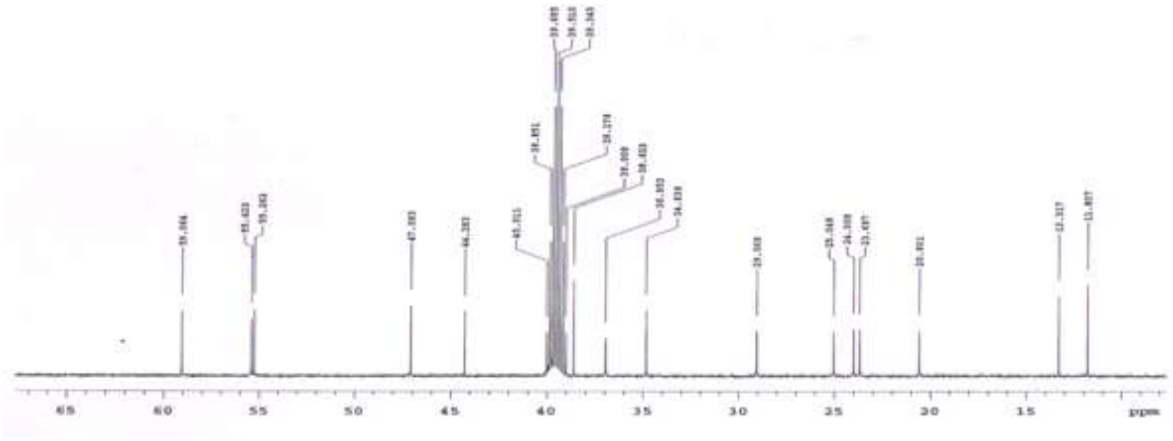


Figure-9

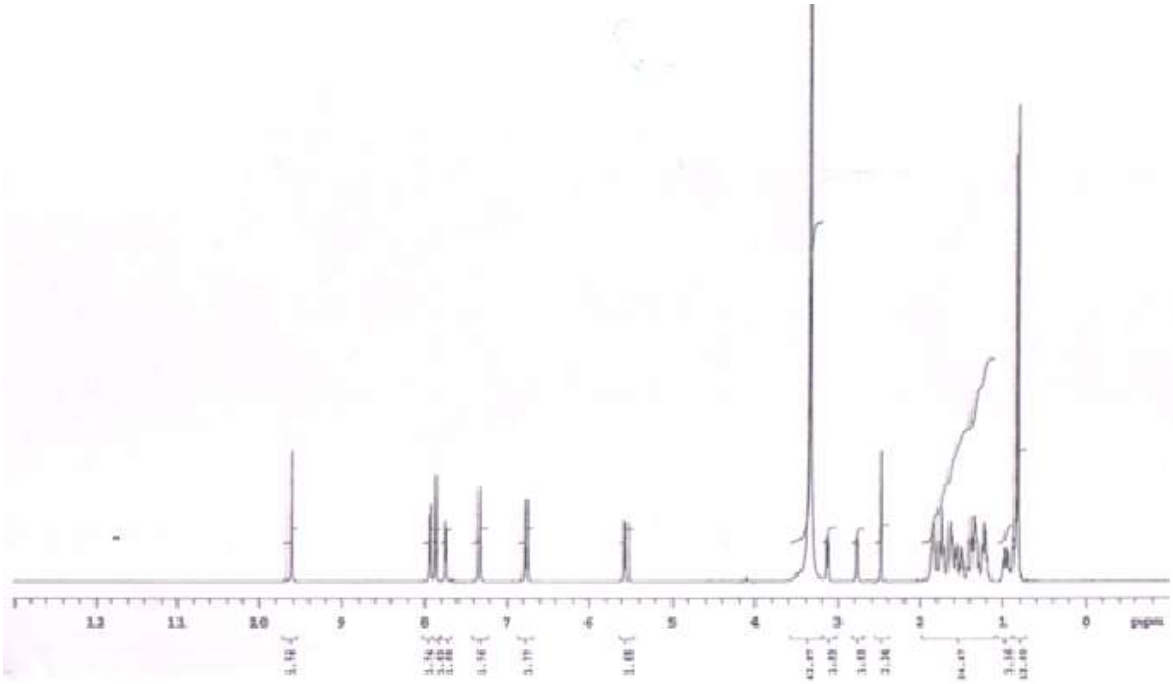


Figure-10

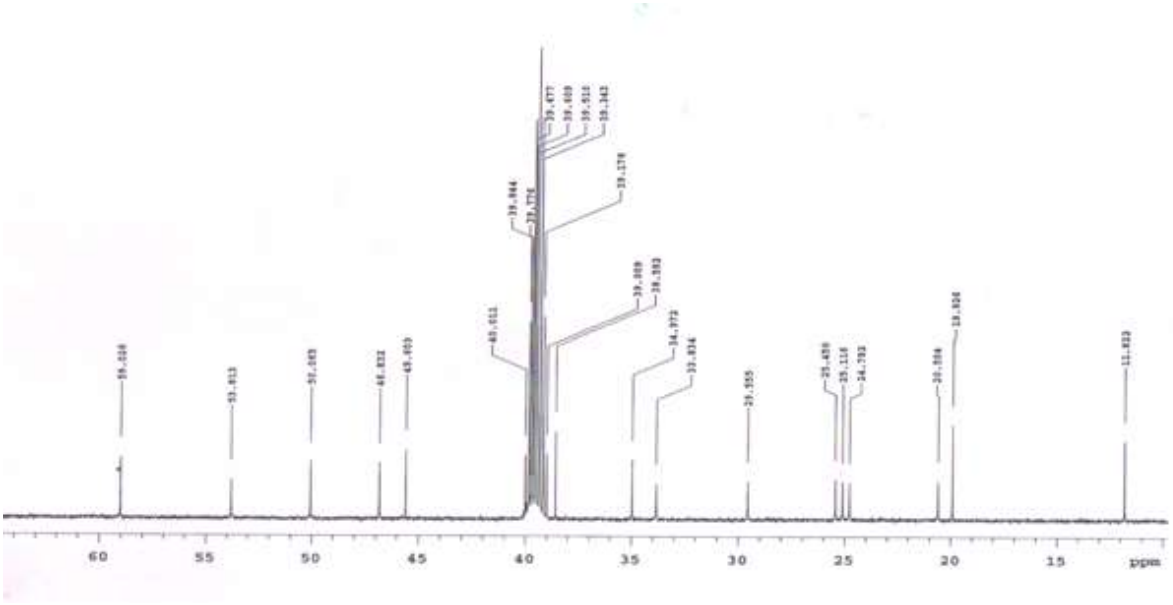


Figure-11

VI. Conclusion

This research paper describes the synthesis, isolation and structure elucidation of process related isomer impurity of dutasteride. The impurity was separated by normal 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 spectroscopic technique. The synthesis of impurity was also discussed in brief.

Acknowledgment

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