Asian Journal of Chemistry; Vol. 26, No. 14 (2014), 4375-4380

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16603

Impurities in Finasteride: Identification, Synthesis, Characterization and Control of Potential Carry-Over Impurities from Reagents Used for the Process

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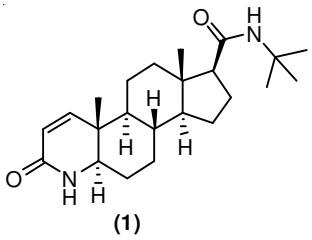
Received: 29 October 2013; *Accepted*: 31 December 2013; *Published online*: 5 July 2014; AJC-15479

An assessment of the impurity profile of finasteride and possible carry-over related substances likely to arise during the synthesis of finasteride is described in this article. Impurities in reaction mass were monitored by HPLC, potential impurities isolated with preparative HPLC and structures were substantiated by ^{1}H NMR, MS and MS-MS. Impurities RRT's were established by HPLC co-injection. Based on the spectral data structure of impurity I and impurity II were characterized as cyclohexyl and phenyl analog of finasteride.

Keywords: Synthesis, Preparative isolation, Liquid chromatography, Mass spectrometry, NMR, MS, Characterization.

INTRODUCTION

Finasteride[1] (**1**; Fig. 1) is a synthetic 4-azasteroid compound and a specific inhibitor of Type II 5α-reductase wherein 5α-reductase is an intracellular enzyme that converts the androgen testosterone into 5α-dihydrotestosterone (DHT). The mechanism of action of finasteride is based on its preferential inhibition of Type II 5α-reductase through the formation of a stable complex with the enzyme. Inhibition of Type II 5α-reductase blocks the peripheral conversion of testosterone to DHT, resulting in significant decreases in serum and tissue DHT concentrations[2]. Finasteride (1) is sold as Propecia and Proscar by Merck & Co, the former is commonly used for the treatment of male pattern baldness[3] and the latter for prostatic hyperplasia. FDA approved oral administration of finasteride is only five mg per day. However, the presence of a trace level related substance can influence the efficacy and safety of the final product. It is mandatory to identify, characterize and control any single maximum impurities in an API, if present above the accepted limits of 0.1%[4]. To achieve this, understanding of the overall manufacturing process, quality assessment of raw materials and reagents used in the process, detection, identification and control of impurities at the source is critical for delivering finasteride of high quality. In this article, we wish to report two carry-over impurities likely to arise during the synthesis of finasteride (**1**). In connection with our continuing interest in azasteroids, we identified, synthesized and established a control in specification of DCC where carry-over of starting material in DCC resulted undesired related substances in finasteride (**1**).



(1)

Fig. 1. Structure of finasteride

EXPERIMENTAL

The crude sample of finasteride was obtained from Dr. Reddy's Laboratories Ltd, Hyderabad, India. The unknown impurities were isolated by preparative HPLC. The HPLC grade acetonitrile, tetrahydrofuran, methanol and formic acid were obtained from the Merck Co., Mumbai, India. The water used for preparing mobile phase was purified using a Millipore Milli-Q+ purification system.

HPLC (analytical): A Waters Model Alliance 2690-separation module equipped with a Waters 996-photo diode array detector was used for the studies. The analysis was carried out on Novapak, C-18 columns, 250 × 4.6 mm *i.e.*, 4 µM particle size with a mobile phase consisting of solvent A (water), solvent B (tetrahydrofuran) and solvent C (acetonitrile) (8:1:1). Sample was prepared at 1 mg/mL concentration in acetonitrile and water (1:1, v/v). The injection load was 15 µL. The eluents were detected at 210 nm at a flow rate of 1.8 mL min^{-1}. The column temperature was maintained at 55 °C. The data was recorded using Waters Millennium software.

HPLC (preparative): A Waters preparative HPLC system equipped with W600 quaternary solvent delivery module and Delta prep 2487 dual wavelength UV detector was used. Data was processed through Waters Empower software. A Reprosil C_{18} column (250 × 20 mm, 7 µm particle size) was used for preparative work. An isocratic method was used with the mobile phases such as 0.1 % Formic Acid (Aqueous) and methanol with 60:40 ratio. The flow rate was kept at 20 mL/min and the column eluent was monitored at 210 nm at ambient temperature.

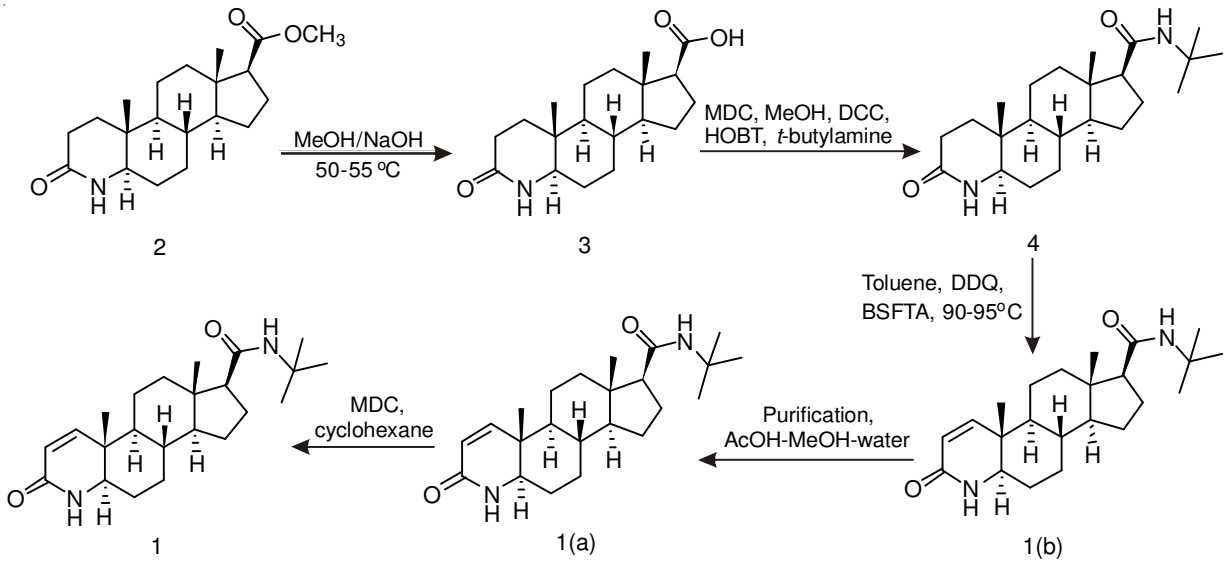
LC-MS and LC-MS/MS: Analytical LC conditions described in Fig. 2 were applied for the LC-MS analysis. Electrospray ionization (ESI) and tandem mass spectrometry experiments were performed using a triple quadrupole mass spectrometer (PE Sciex model API 3000). The positive and negative electrospray data were obtained by switching the capillary voltage between +5000 and -4500V, respectively. The electrospray ionization (ESI) was performed by using collision potential (30 V) and nitrogen gas was used to assist nebulisation in the collision cell for MS-MS studies.

Ultra performance liquid chromatography (UPLC)-TOF MS: Finasteride and its isolated impurities were directly infused into the UPLC-TOF-MS instrument. Leucine Encephalin was used as Lock spray for accurate mass measure. The mass spectra of impurities were recorded on LCT premier XE, Waters Micromass Mass Spectrometer.

NMR spectroscopy: NMR experiments were performed on Varian spectrometer operating at 500 MHz in $CDCl_3$ at 25 °C. The ^1H chemical shift values were reported on the δ scale in ppm, relative to TMS (δ = 0.00) and the ^{13}C chemical shift values were reported relative to $CDCl_3$ (δ = 77.00 ppm) as internal standards. Standard pulse sequences provided by Varian were used for distortion less enhancement by polarization transfer (DEPT), gradient double quantum filtered correlation spectroscopy (gDQCOSY), gradient hetero nuclear single quantum coherence spectroscopy (gHSQC), gradient hetronuclear multibond coherence spectroscopy (gHMBC) (J = 8.0 Hz) experiments. Nuclear Overhauser Effect Spectroscopy (NOESY) experiment was run using a mixing time of 600 ms.

Reported (**Scheme-I**) finasteride (Fig. 1) manufacturing process starts with the hydrolysis of **2** with MeOH-NaOH to give **3**. Amidation of **3** in presence of N,N'-Dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBT) with *t*-butylamine gave **4**. Ring-A oxidation in presence of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and N,O-*bis*(trimethylsilyl)trifluoroacetamide (BSFTA) in toluene gave crude finasteride (**1b**). Purification of crude product with acetic acid, methanol and water gave pure **1**(**a**). Re-crystallization of **1**(**a**) in MDC-cyclohexane gave finasteride (**1**) of desired polymorphic form. Two unknown impurities were observed in the HPLC chromatogram of crude **1**(**b**) at about 1.6 and 1.7 RRT's with respect to the parent peak to the extent of 0.1 % along with known impurities of finasteride, impurity-A (**4**) and impurity-C[6] (over oxidized impurity).

The process was robust and met predefined quality and quantity criteria, however product 1(b) has to be purified again for removal of unknown impurities **I**[7] and **II**[8]. No doubt an additional purification step effectively washes out the impurities



Scheme-I: Finasteride (**1**) manufacturing process[5]

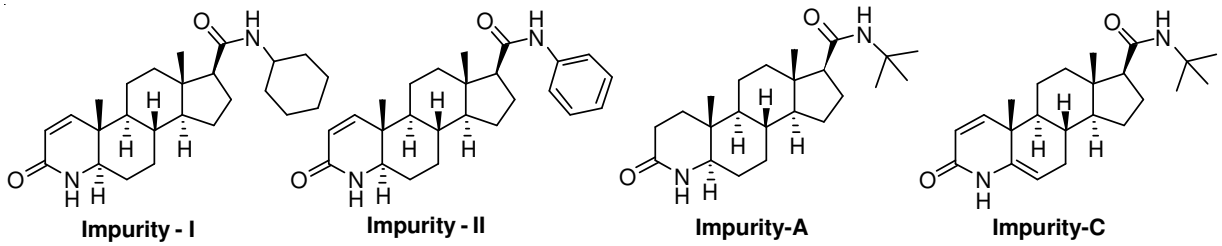
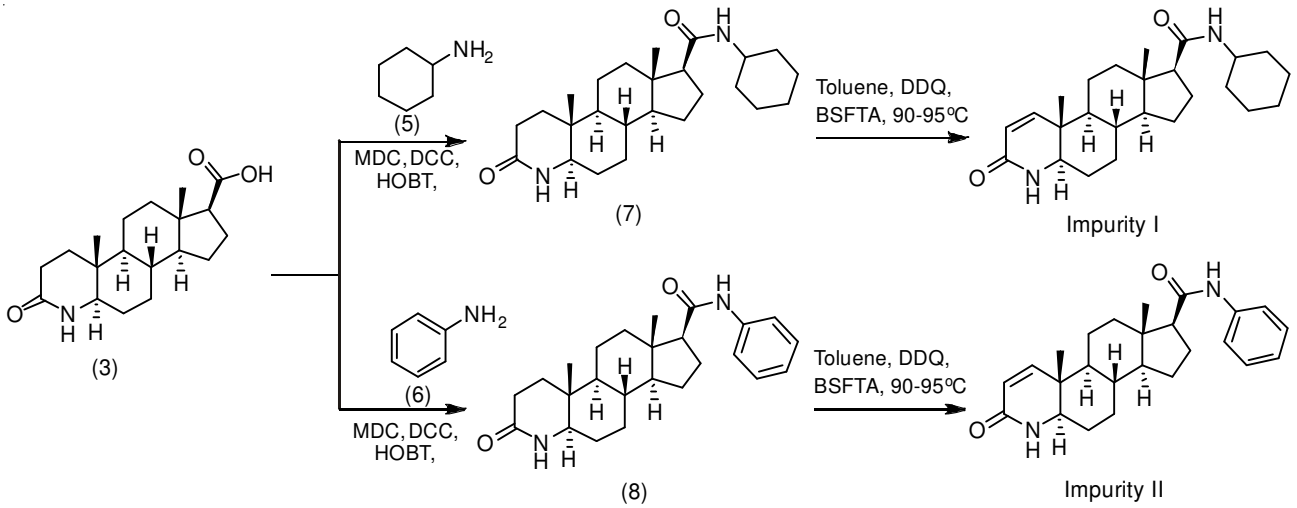


Fig. 2. Impurities of finasteride (**1**)



Scheme-II: Synthesis of Finasteride impurities

from the product but the use of an additional purification step affects the overall yield as well as an additional unit operation on a large-scale manufacturing results in poor utilizations of assets. Impurity profile of a drug substance is critical for its safety assessment and manufacturing process. Since the levels of unknown impurities in crude finasteride were above the acceptable limits, a comprehensive study was carried out by isolation, characterization and synthesis of these impurities with the pathway of their formation.

Amidation of **3** in presence of N,N'-dicyclohexyl-carbodiimide and Hydroxybenzotriazole with amine (**5** & **6**) gave amide (**7** & **8**). Oxidation of Ring A in **7** and **8** in presence of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone and N,O-*bis*(trimethylsilyl)trifluoroacetamide in toluene gave impurity **I** and **II**.

RESULTS AND DISCUSSION

Detection of impurity by HPLC: The crude finasteride was analyzed by HPLC method described in Fig. 3. The analysis showed presence of two unknown peaks (Fig. 3) at about 1.6 RRT (impurity-I) and 1.7 RRT (impurity-II).

Isolation of impurity by preparative HPLC: An isocratic method as discussed in experimental section was used for the isolation of these impurities. The fractions of impurities were collected and the organic layer was evaporated by rotavapor under high vacuum at 45 °C. The remaining aqueous solution was kept for lyophilization. Finally the impurities were isolated as fluffy solids.

Structure elucidation of impurity-I: In the positive mass spectrum, the protonated, $[M + H]^+$ ion was detected at *m/z* 399.4 (Fig. 4). The molecular ion of impurity-I was found to be at *m/z* 398. The mass difference between finasteride and impurity-I was 26 amu. The positive HR-MS spectrum showed protonated molecular ion peak at *m/z* 399.3012 corresponding to molecular formula $C_{25}H_{39}N_2O_2$ $[M^+ + H]$. The molecular formulae of finasteride ($C_{23}H_{36}N_2O_2$) and impurity-I were compared and found that, the impurity-I has addition of two carbons and two hydrogen atoms. Further, this can be attributed to one double bond equivalence.

The MS/MS data of impurity-I was compared with finasteride. The impurity-I has also showed two major fragments at *m/z* 331 and 317 (Fig. 5). The common fragment at 317 confirms that the change has taken place on aliphatic amide group.

The ^{1}H NMR (500 MHz, $CDCl_3$) spectrum (Fig. 6) of impurity-I showed two additional protons in aliphatic region

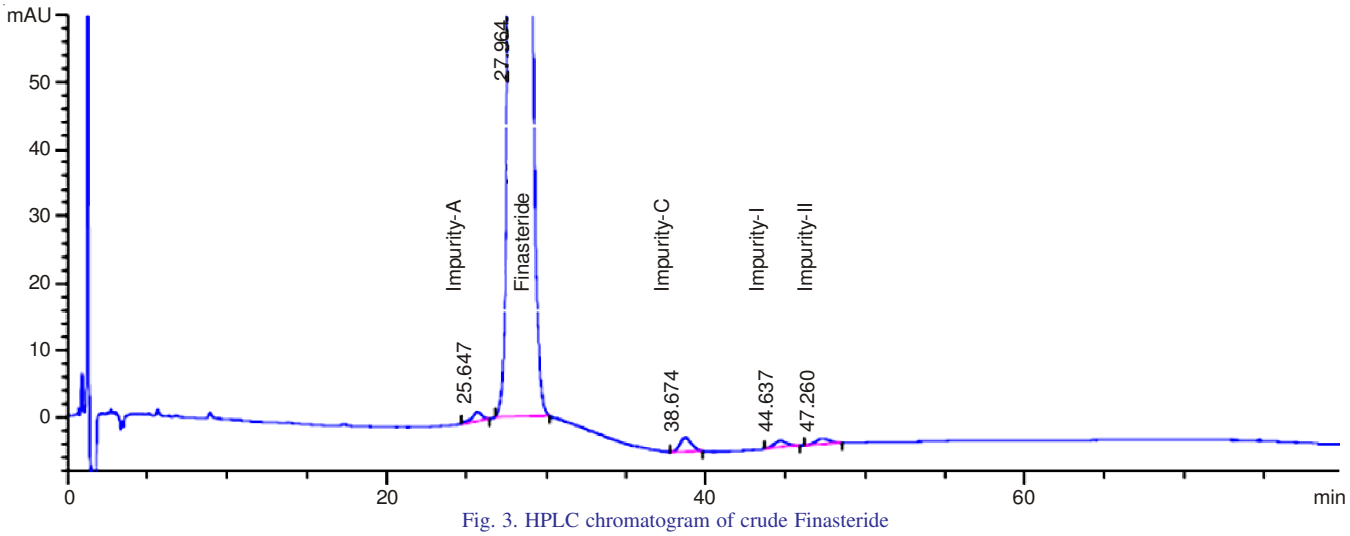


Fig. 3. HPLC chromatogram of crude Finasteride

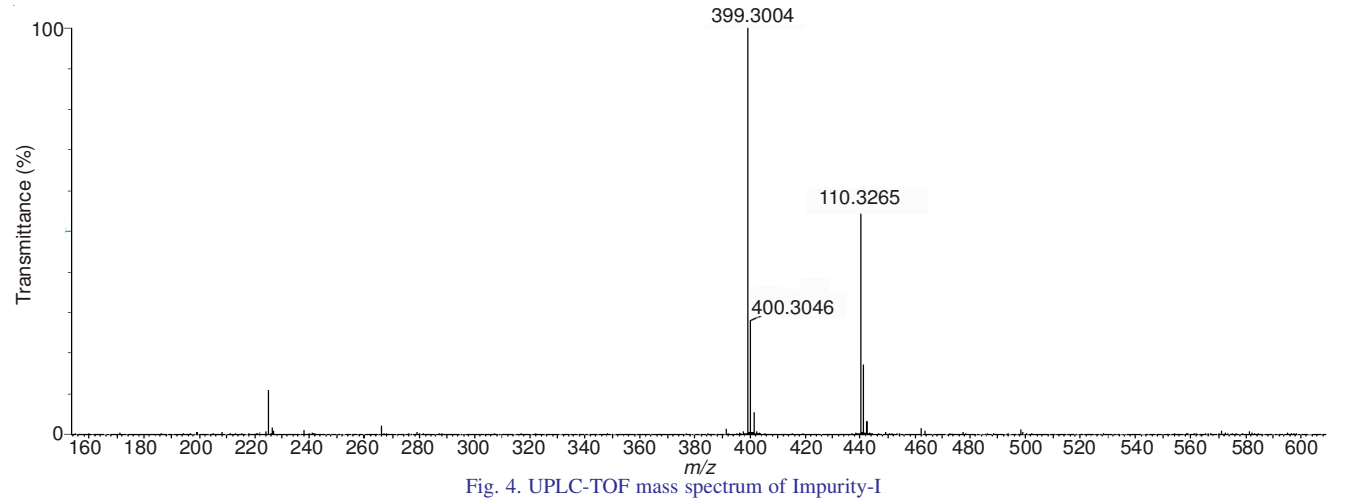


Fig. 4. UPLC-TOF mass spectrum of Impurity-I

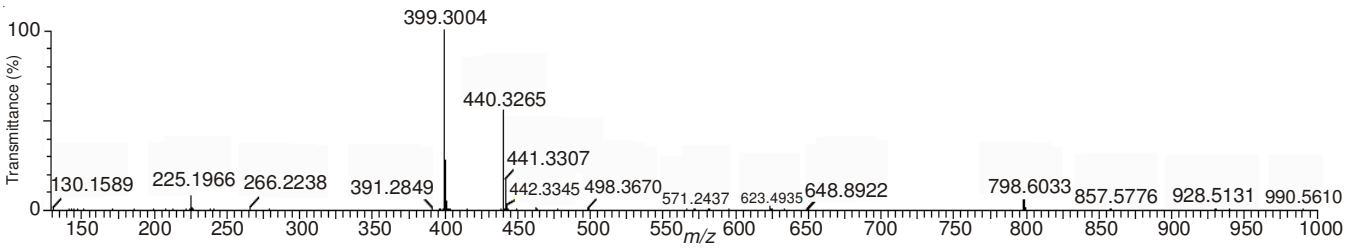


Fig. 5. MS/MS spectrum of Impurity-I

when compared with finasteride. It was observed that the signal at δ 1.40 ppm corresponding to *t*-butyl moiety was absent. This observation was further supported by ^{13}C NMR spectrum in $CDCl_3$ showed absence of carbon signals at 50.6 and 28.7 ppm of finasteride. The HSQC spectrum showed one methine and three methylene signals. It was observed that two of the three methylene groups correspond to two carbons each. The COSY spectrum showed correlations between the amide proton at 5.16 ppm and a methane proton at 3.82 ppm, which further showed correlations with the additional methylene signals. The NMR observations show that the additional group corresponds to a cyclohexyl moiety.

These observations from NMR and MS data confirmed that the structure of impurity-I as *t*-butyl group of finasteride substituted by cyclohexyl group.

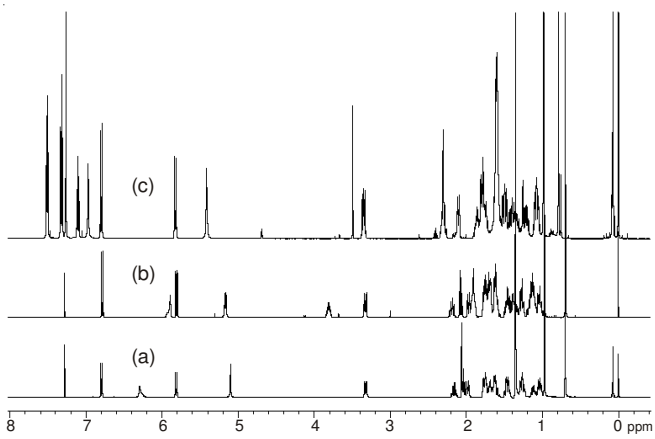


Fig. 6. Overlay of ^{1}H NMR data: (a) Finasteride, (b) impurity-I and (C) impurity-II

Structure elucidation of impurity-II: In the positive mass spectrum, the protonated, $[M + H]^+$ molecular ion peak was detected at *m/z* 393.4 (Fig.7). The molecular ion peak of impurity-II was found to be at *m/z* 392. The mass difference between finasteride and impurity-II was 20 amu. The positive HR-MS spectrum showed protonated molecular ion at *m/z* 393.2537 corresponding to molecular formula $C_{25}H_{33}N_2O_2$ $[M^+ + H]$. The molecular formulae of finasteride ($C_{23}H_{36}N_2O_2$) and impurity-II were compared and found that the impurity-II has two carbons more and less by four hydrogen atoms. Further, this can be attributed to four additional double bond equivalences. The MS/MS data of impurity-II was compared with finasteride. Finasteride gave two major fragments at *m/z* 317 and 305. The impurity-II showed one major fragment at *m/z* 325 (Fig. 8). The fragment of finasteride at *m/z* 305 showed 20 amu differences with the fragment observed for impurity-II at *m/z* 325. This showed the relation between these two fragments.

The ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of impurity-II showed five protons in aromatic region corresponding to one phenyl moiety and the absence of protons corresponding to *t*-butyl moiety, which was observed at δ 1.40 ppm in finasteride. This could be due to the substitution of *t*-butyl moiety by phenyl group. The amide proton at δ 6.96 ppm showed long range ^{1}H ^{13}C correlations (HMBC) with two quaternary carbon signals at δ 170.60 ppm and δ 137.91 ppm. The signal at δ 170.60 ppm corresponds to the carbonyl carbon and the signal at δ 137.91 ppm corresponds to the aromatic carbon. The remaining NMR signal assignments were comparable with those of finasteride. These observations from NMR and MS data (Figs. 8 and 9) confirmed that the structure of impurity-II as *t*-butyl group of finasteride substituted by phenyl group.

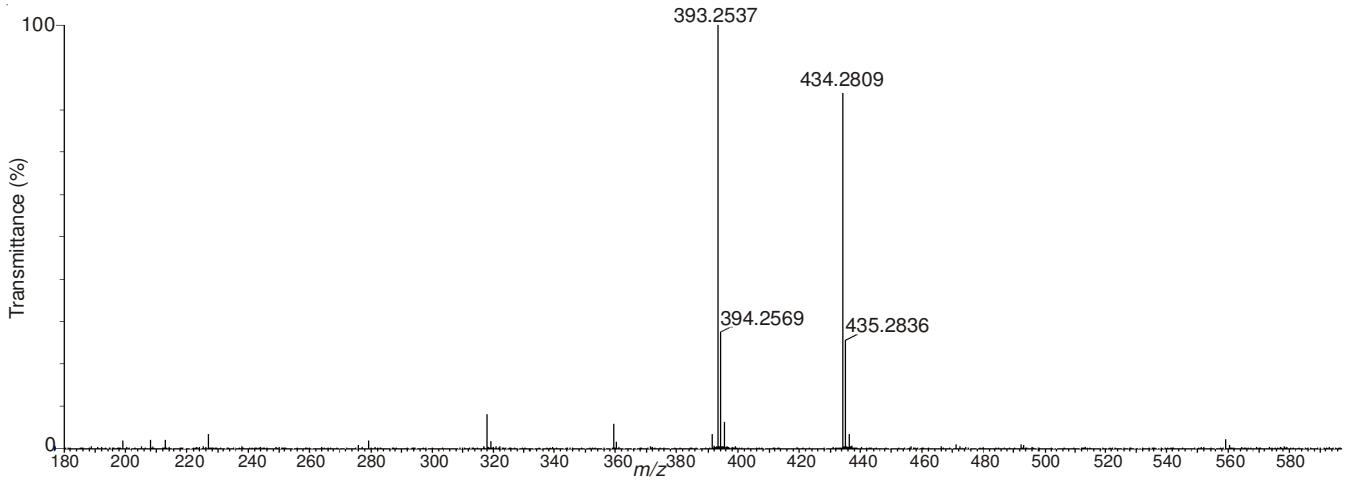


Fig. 7. UPLC-TOF mass spectrum of Impurity-II

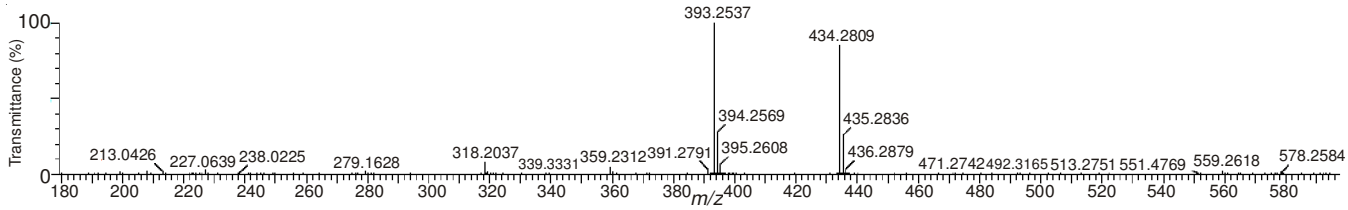


Fig. 8. MS/MS spectrum of Impurity-II

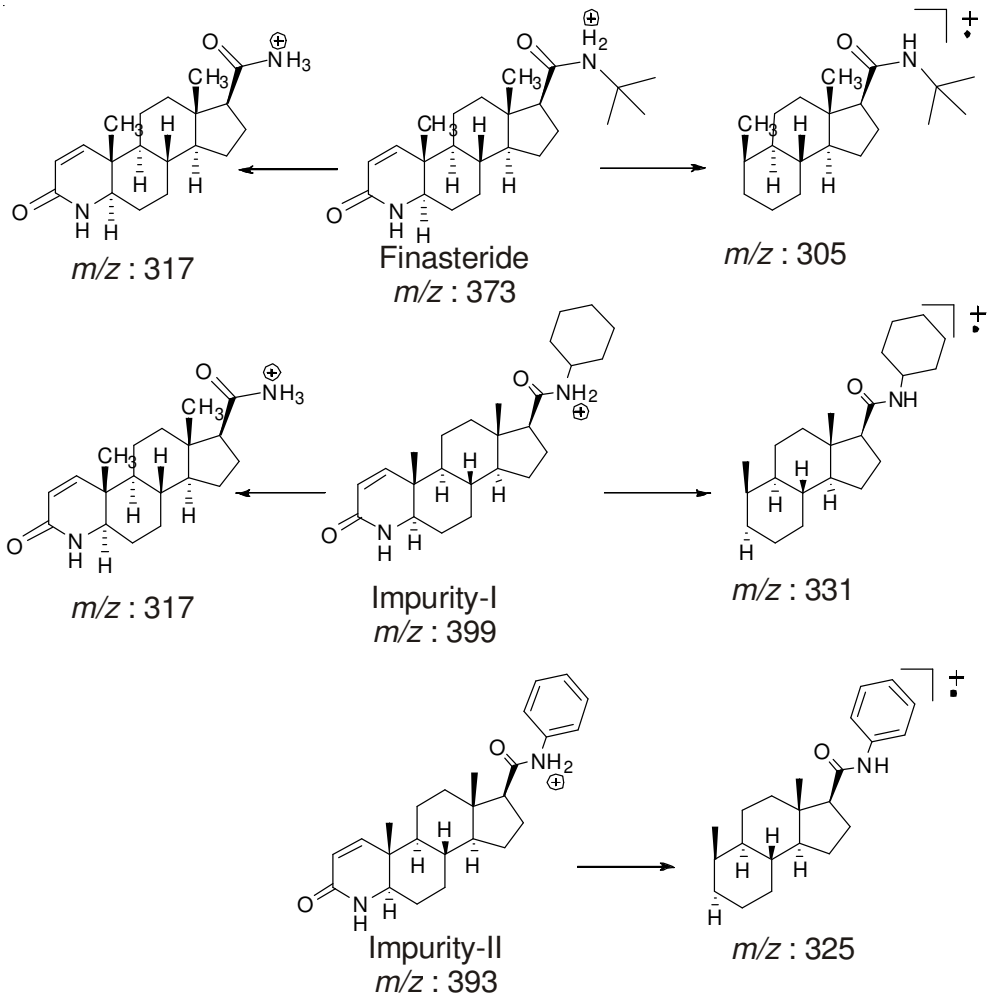


Fig. 9. Mass fragmentation of impurity-I and impurity II

Formation of impurities: Since the compound (**4**) was a key intermediate for finasteride (**1**) hence its quality is critical for the subsequent stages in the manufacturing process. In this regard a comprehensive study was carried out for identification of the two impurities. impurity in reaction mass was isolated with preparative HPLC and structures were substantiated by ^{1}H NMR, MS and MS-MS. Impurities were synthesized (**Scheme-II**), RRT's were established by HPLC co-injection. Based on the spectral data structure of impurity I and impurity II were characterized as cyclohexyl and phenyl analog of finasteride. Noteworthy, to mention that aniline[9] and cyclohexyl amine[10] are the basic building block for synthesis of N,N'-Dicyclohexylcarbodiimide (DCC). Hence during our investigation we focused on the quality of the commercial lots used in the manufacturing process of finasteride. We envisaged that if our assumption is true commercial lots of DCC would show residual impurities 5 and 6. Samples (DCC) were drawn from plant and subjected to Gas Phase Chromatography. Interestingly we observed varied percentage of 5 and 6 in the commercial lots and this was further confirmed by co-injection of working standard (5 and 6) with DCC.

DCC was purified[11] by reduced pressure to give a slightly coloured oil which solidifies at room temperature. Reaction with purified afford compound (**1**) with impurities I and II lest than 0.02 %.

Conclusion

In conclusion, we have isolated and characterized two process related impurities of finasteride at crude stage. Structures of these compounds were confirmed based on their MS and NMR spectral data followed by their independent synthesis. The identified impurities were synthesized to obtain sufficient quantities for final confirmation by co-injection in HPLC.

ACKNOWLEDGEMENTS

The authors thank the management of R&D-CTO and IPDO, Dr.Reddy's Laboratories Ltd., for supporting this work. Cooperation from the colleagues from analytical development is highly appreciated.

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