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Impurity profile study of dutasteride*

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Received February 2, 2007, accepted February 27, 2007

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Pharmazie 62: 743–746 (2007) *doi: 10.1691/ph.2007.10.7040*

During 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). These impurities were synthesized individually and characterized based on the spectroscopic data (HPLC, IR, NMR and MS). The structures of these impurities were 17β-*N*-[2,5-bis(trifluoromethyl) phenyl]carbamoyl-3-hydroxyl-4-azaestra-1,3,5,7,9-pentaene (desmethyl of dutasteride **2**), 17β-*N*-[2,5-bis(trifluoromethyl)phenyl]carbamoyl-4-aza-5α-androstane-3-one (dihydro impurity of dutasteride **3**), and 17β-*N*-[2,5-bis(trifluoromethyl) phenyl] carbamoyl-4-aza-5β-androst-1-ene-3-one (β-isomer of dutasteride **4**), respectively. The formation and synthesis of dutasteride impurities are discussed.

1. Introduction

Dutasteride is a 5α-reductase inhibitor (Rasmusson et al. 1984, 1986), which inhibits the conversion of testosterone to dihydrotestosterone, there by alleviating growth of prostate gland. It is useful for the treatment of benign prostatic hyperplasia (BPH) (Gormley et al. 1992). Dutasteride is being developed for the treatment of male pattern baldness (MPB) (Imperato-McGinley et al. 1979).

During the analysis of different laboratory batches of dutasteride (**1**), three impurities peaks along with the dutasteride peak were observed whose area percentage ranged from 0.05 to 0.1% in HPLC. A thorough study has been undertaken to synthesize and characterize these impurities by spectroscopic techniques. As per the stringent regulatory requirements, the impurities $\geq$0.1% must be identified and characterized. The identification, formation and synthesis of dutasteride impurities are discussed in this paper.

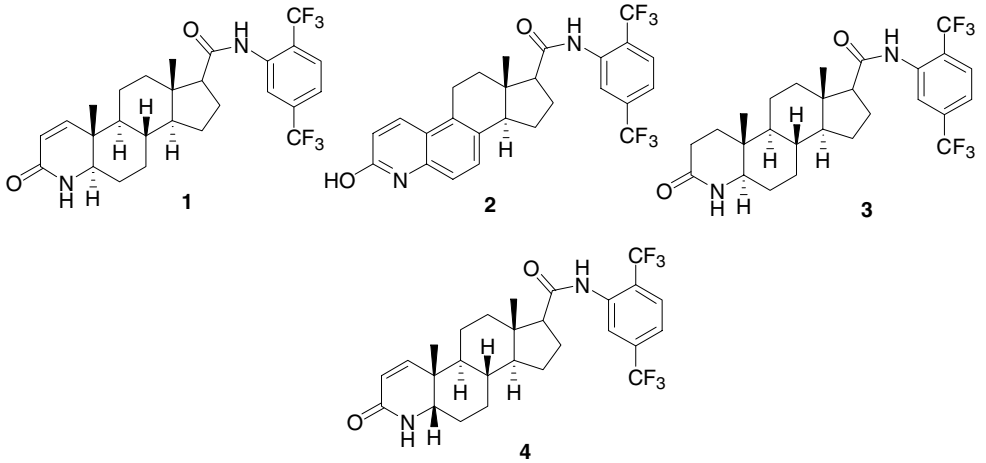
2. Investigations, results and discussion

2.1. Detection of impurities 2, 3 and 4

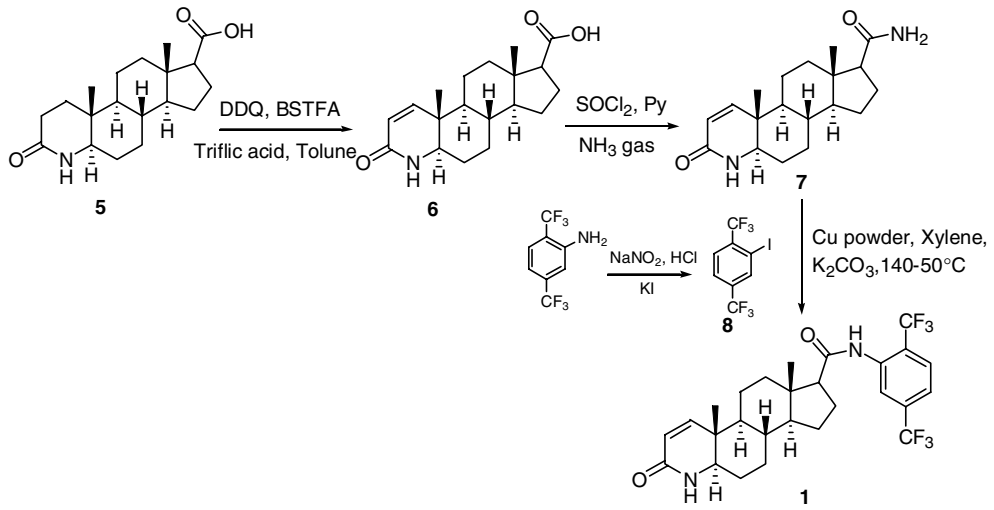
A typical analytical LC chromatogram of laboratory batch of dutasteride bulk drug recorded using the LC method is shown in the Fig. The impurities are marked as desmethyl dutasteride (**2**), dihydro dutasteride (**3**), and β-isomer dutasteride (**4**). These impurities were synthesized in the laboratory for structure elucidation.

2.2. Structure elucidation of desmethyl dutasteride (2)

The mass spectrum showed the molecular ion peak at m/z 508 atomic mass units (amu). The IR spectrum showed a peak at 3467 cm^{-1}. A deuterium exchangeable peak at δ 9.8 in the ^1H NMR spectrum, suggested that the proton could be due to a phenolic hydroxyl group. Based on this preliminary spectral information, the molecular formula of



Scheme 1



the compound **2** was found to be $C_{26}H_{22}F_6N_2O_2$ with 5 double bond equivalents (DBE). This formula matched well with the observed molecular ion peak 508 in the CI mass spectra. In the ^{1}H NMR signal disappearance at δ 0.99 ppm indicates the absence of methyl group. Based on these data, the structure of the compound has been characterized as 17β-*N*-[2,5-bis(trifluoromethyl)phenyl]carbamoyl-3-hydroxyl-4-azaestra-1,3,5,7,9-pentaene (desmethyl dutasteride **2**).

2.3. Structure elucidation of dihydro dutasteride (3)

In the mass spectrum, the molecular ion peak exhibited at m/z 530 atomic mass units (amu). The ^{1}H NMR spectrum showed disappearance of −HC=CH− signals in ring A at 1,2-position. Based on these preliminary spectral information, the molecular formula of the compound could be $C_{27}H_{32}F_6N_2O_2$. This formula matched well with the observed molecular ion of 530.5 in the CI spectra. The methyl groups yielded a singlet in the ^{1}H NMR spectrum at δ 0.8 ppm and δ 0.99 ppm. Based on these data, the structure of the compund has been characterized as 17β-*N*-[2,5-bis(trifluoromethyl)phenyl]carbamoyl-4-aza-5α-androstane-3-one (dihydro dutasteride **3**).

2.4. Structure elucidation of β-isomer of dutasteride (4)

The mass spectrum of impurity **4** exhibited a molecular ion at m/z 528.5 atomic mass units (amu). A signal at δ 6.1 in the ^{1}H NMR spectrum was found to be exchangeable nature. IR peaks at 1683 & 1607 cm^{-1} clearly indicate the presence of two carbonyl groups. The NOESY experiment shows that the proton position at 5 and methyl protons at position 19 are on the same side, indicating that proton at 5th position is β. Based on this preliminary spectral information, the molecular formula of compound **4** could be $C_{27}H_{30}F_6N_2O_2$. This formula matched well with the observed molecular ion of 528.5 in the CI spectra. The position of methyl groups which yielded singlets in the ^{1}H NMR spectrum at δ 0.8 ppm and δ 0.99 ppm. The SOR of compound showing $[\alpha]_D^{20}$ D = 280° (C = 1.0% in methanol), whereas dutasteride (**1**) SOR is +41.58°. Based on these data, the structure of the impurity **4** has been characterized as 17β-*N*-[2,5-bis(trifluoromethyl)phenyl] carbamoyl-4-aza-5β-androst-1-ene-3-one (β-isomer of dutasteride **4**).

2.5. Formation of impurities

The condensation of **7** and **8** in the presence of copper-powder, K_2CO_3 and xylene yields dutasteride **1**. During oxidation of **5** in the presence of DDQ, BSTFA and triflic acid yields **6** (Bhattacharya et al. 1988). The over oxidation (Williams et al.1995) of **5** will carry over along with **6** and further it will react with thionylchloride and ammonia gas in the presence of pyridine and will be condensed with **8** leading to the formation of dutasteride **1** and desmethyl dutasteride **2**. During the oxidation of **5** with DDQ, BSTFA and triflic acid give **6** along with trace amount (1.5−2% by HPLC) of **5**, which is carried over to **7**. Further it condenses with **8** in the presence of copper-powder, K_2CO_3 and xylene leads to formation of dihydro dutasteride **3**. Compound **5** having β-isomer impurity in the range of 1-2%, will carry over up to **7** leading to formation of β-isomer of dutasteride **4** in the presence of copper-powder, K_2CO_3 and xylene.

3. Experimental

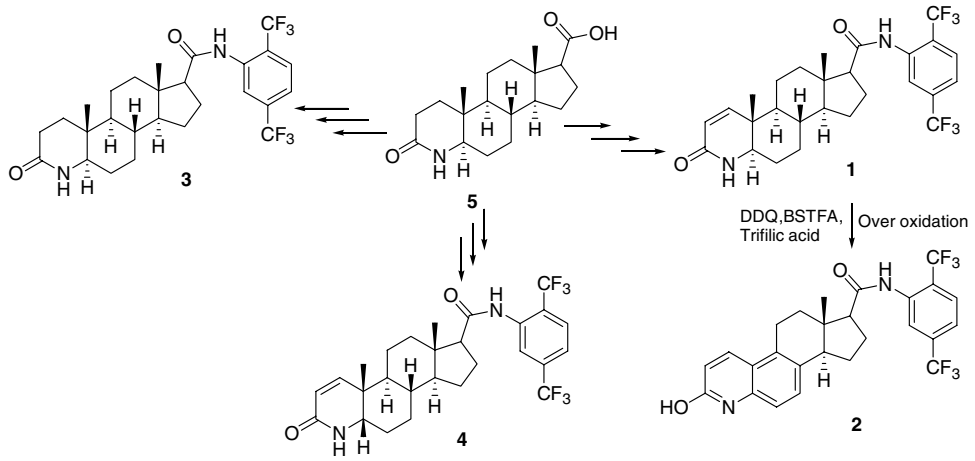
3.1. Synthesis of dutasteride and impurities

Dutasteride and its impurities were synthesized as shown in Scheme 1 (Reddy et al. 2005).

3.1.1. Synthesis of 17β-N-[2,5-bis(trifluoromethyl)phenyl]carbamoyl-3-hydroxyl-4-azaestra-1,3,5,7,9-pentaene (desmethyl dutasteride **2***)*

A mixture of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 10.5 g) and toluene (180 mL) was taken into a clean and dry round bottom flask equipped with a Dean-Stark apparatus followed by heating to 110 °C. The suspension was stirred for about 1 h, and cooled to 55 °C, followed by addition of dutasteride (12 g) and bis (trimethylsilyl) trifluoroacetamide (BSTFA, 23.3 g). The resultant suspension was heated to 105 °C and was stirred for 24 h. The suspension was then cooled to 80 °C and washed with 10% sodium sulphite solution (2 × 60 mL), followed by water washing (2 × 50 mL). The organic layer was dried on anhydrous sodium sulphate. The solvent was distilled completely at 60 °C under vacuum. The obtain residue was dissolved in a mixture of petroleum ether (105 mL) and ethyl acetate (45 mL) was stirred for about 5 h. The separated solid was filtered and was dried at 70 °C for 6 h to afford 1.3 g of the title compound as a solid. The solid obtained was purified by flash chromatography on a silica gel column by eluting with a mixture of petroleum ether and ethyl acetate (9 : 1). Fractions were collected and the solvent was distilled completely under vacuum to afford 0.9 g of the title compound.

Scheme 2



IR (KBr, cm^{-1}): 3467 (NH stretching); 2875 (C-H stretching); 1662 (C=O stretching); ^1H NMR ($CDCl_3$): 9.80 (br s, 1H, NH); 8.80 (s, 1H, Ar-H); 8.00 (d, 1H, Ar-H); 7.78 (d, 1H, Ar-H); 7.48 (s, 1H, OH); 7.45(d, 1H, Ar-H); 7.21 (d, 1H, Ar-H); 7.10 (d, 1H, Ar-H), 6.70 (d, 1H, Ar-H); 3.15 (m, 2H, CH_2); 2.91 (t, 1H, CH); 2.61 (t, 1H, CH); 2.30 (m, 2H, CH_2); 2.20 (m, 2H, CH_2); 1.80 (m, 2H, CH_2); 0.72 (s, 3H, CH_3).

3.1.2. Synthesis of 17β-N-[2,5-bis(trifluoromethyl)phenyl]carbamoyl-4-aza-5α- androstane-3-one (dihydro dutasteride ***3****, Batchelor et al. 1996)*

A mixture of 3-oxo-4-aza-5α-androstane-17β-carboxylic acid (**5**, Rasmusson et al. 1988, 1986, 50 g) and toluene (750 mL) was taken into a clean and dry round bottom flask equipped with a Dean-Stark apparatus followed by heating to reflux for 30 to 60 min. The resulting solution was cooled to 25 to 35 °C, pyridine (6.3 mL) was added under the nitrogen atmosphere followed by thionylchloride (14 mL) was added slowly. The reaction mass was stirred at 25 to 35 °C for 2–3 h. Ammonia gas was passed into reaction mass for 8–10 h. The separated solid was filtered and washed with toluene (100 mL). The resultant compound was slurred in water (500 mL) for 2 h. The reaction mass was filtered and washed with water (50 mL). The obtained solid was dried at 70–75 °C, to get 3-oxo-4-aza-5α-androstane-17β-carboxamide (**7**).

A mixture of potassium carbonate (11 g) and xylene (125 mL) was heated to azeotropic reflux for 2 h. The mixture was cooled to about 30–40 °C, to which 3-oxo-4-aza-5α-androstaneo-17β-carboxamide (**7**, 25 g), copper powder (15 g) and 2-iodo-1,4-bis(trifuoromethyl)benzene (**8**, Ross et al. 1953, 81 g) were added. The resulting mixture was heated to 140–50 °C and stirred for 50–60 h. The reaction mixture was cooled to 55–65 °C, and, after addition of ethyl acetate (750 mL) was stirred for 10–15 min. The reaction mass was filtered and washed with hot ethyl acetate (50 mL) to remove copper powder. The filtrate was washed with aqueous hydrochloric acid (12.5 mL) solution followed by 5% aqueous sodium bicarbonate solution and finally washed with water (2 × 125 mL). The organic layer was distilled under vacuum, and ethyl acetate (100 mL) was added and distilled to 60% of its volume. The reaction mass cooled the reaction mass to 25–35 °C, filtered and the solid was washed with chilled ethyl acetate (25 mL) to afford 17β-*N*-[2,5-bis(trifluoromethyl)phenyl]carbamoyl-4-aza-5α-androstane-3-one (dihydro dutasteride **3**). The product was dried at 60–70 °C, yield 9.6 g, m.p. 245–247 °C.

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

A solution of 3-oxo-4-aza-5α-androstane-17β-carboxylic acid (**5**, 100 g, which is having 28% of its β-isomer (Young et al. 2006; Solomons et al. 1974) by HPLC) and ethyl acetate (1000 mL, β-isomer is soluble in ethyl acetate where as α-isomer is not soluble) was heated to 65–70 °C, and stirred for 10–15 min. The solid obtained was filtered and washed with hot ethyl acetate (50 mL); the filtrates were distilled off completely (the residue having β-isomer of compound).

A mixture of DDQ (42.5 g) and toluene (750 mL) was taken into a three neck round bottom flask and heated to azeotropic reflux for 2 h. The reaction mass was cooled to 25–35 °C, the above β-isomer of **5** (30 g), bis (trimethylsilyl) trifluoroacetamide (BSTFA, 161 g) and trifilic (Scheme 3) acid (0.8 mL) were added. The reaction mixture was heated to 105–108 °C for 20–24 h and cooled to 50–60 °C, and water (250 mL) was added. The obtained solid was filtered; wet material was slurred with water (500 mL) at 50–60 °C for 1–2 h. The solid was filtered, and washed with water (100 mL). The solid was further purified in 8 : 2 dichloro methane and methanol, to afford the 3-oxo-4-aza-5β-androst-1-ene-17β-carboxylic acid (18 g).

A mixture of 3-oxo-4-aza-5β-androst-1-ene-17β-carboxylic acid (18 g) and toluene (275 mL) was heated at azeotropic reflux for 60 min. To the resulting solution was cooled to 25 to 35 °C, pyridine (2.3 mL) was added under nitrogen atmosphere, followed by thionylchloride (5.0 mL) for 20 min. The reaction mass was stirred at 25 to 35 °C for 3 h. Ammonia gas was passed into the reaction mass for 8–10 h. The obtained solid was filtered, washed with toluene (100 mL). The resultant solid was slurred in water (500 mL), and stirred for 2 h. The solid filtered was washed with water (50 mL) to get the pH neutral, and was dried at 70–75 °C to get the 3-oxo-4-aza-5β-androstane-17β-carboxamide. The obtained compound was further purified in a mixture of dichloromethane and methanol (8 : 2, 900 mL), dried at 60–70 °C, yield 16 g.

Potassium carbonate (4 g) was taken in xylene (45 mL) and heated to azeotropic reflux for 2 h, and then cooled to 30–40 °C. 3-Oxo-4-aza-5β-androstane-17β-carboxamide (16 g), copper powder (5 g) and compound **8** (28 g) were added and **t**he resulting mixture was heated to 140–50 °C and stirred for 50–60 h. After cooling the reaction mixture to 55–65 °C, ethyl acetate (250 mL) was added and stirred for 10–15 min. The reaction mass was filtered and the copper powder was washed with hot ethylacetate (25 mL). The filtrate was washed with aqueous hydrochloric acid (5.0 mL of HCl in 50 mL of water) at 60–65 °C, and followed by 5% aqueous sodium bicarbonate solution and finally washed with water (2 × 50 mL) at the same temperature. The organic layer was distilled off under vacuum. To the residue added ethyl acetate (25 mL) was added and distilled to 60% of its volume, the reaction mass cooled to 25–35 °C and the solid was filtered and washed with chilled ethyl acetate (15 mL) to afford the 17β-*N*-[2,5-bis (trifluoromethyl) phenyl] carbamoyl-4-aza-5β-androst-1ene-3-one (β-isomer of dutasteride **4**), m.p. 243–244 °C.

3.2. Samples

The analyzed samples of dutasteride material (B.No. DUS – Pharma) were prepared in Dr. Reddy's Laboratories Ltd., Bulk Actives – III, Hyderabad, India.

3.3. High-performance liquid chromatography (HPLC)

A water model alliance 2695 separation module equipped with a waters 2996 photo diode array UV detector was used. An in-house LC method was developed for the analysis of dutasteride, consisting of an zorbax SBC18 250 × 4.6 mm column (5 microns equivalent) with a mobile phase mixture of 0.01 M KH_2PO_4 buffer and acetonitrile in a gradient run set at a flow of 1.0 ml/min for the resolution of all impurities detection was done at $\lambda = 210$ nm.

3.4. Mass spectroscopy

Mass spectra were obtained using a Shimadzu QP-8000α mass spectrometer with an electron energy set to 1.5 kV. The sample was introduced via the mass inlet using a LC pump (LC 10 ADVP series) and an auto l injector (SIL 10ADVP). The heat block crossed dissolution line temperature at 230 and 250 °C respectively.

3.5. FT-IR Spectroscopy

The IR spectra recorded in the solid state as KBr dispersion using Perkin Elmer 60428 FT IR spectrophotometer.

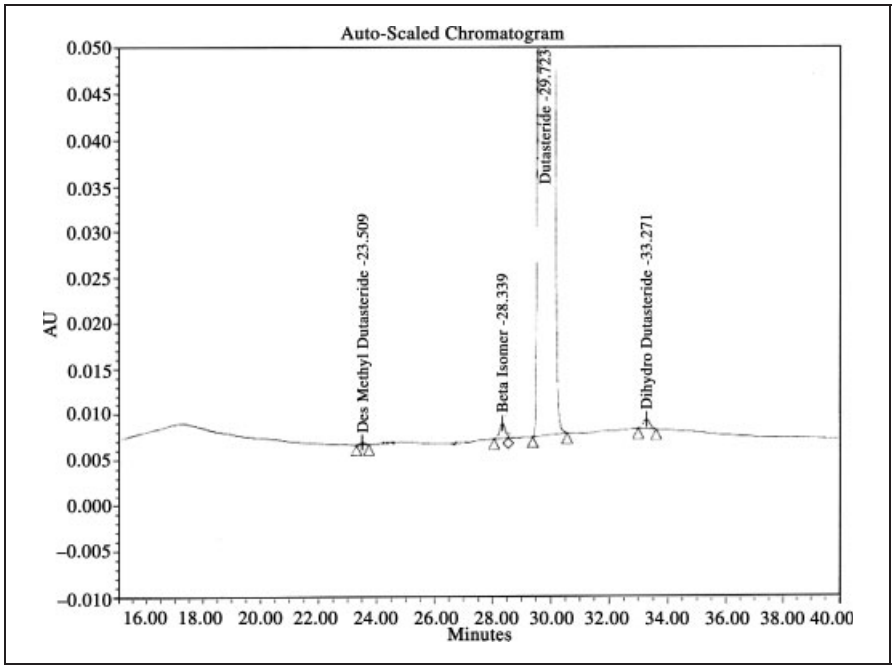


Fig.:
HPLC blend chromatogram of dusteride drug spiked with impurities

All the synthesized compounds were co-injected/spiked with dutasteride, the RRT are exactly matching with impurities.

Acknowledgements: The authors thank the management of Integrated Product Development division, analytical research and development, Unit-III of Dr. Reddy's Laboratories Ltd., for supporting this work.

* Dr. Reddy's communication No. IPDO-IPM-00053

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