World Inventia Publishers

Journal of Pharma Research

http://www.jprinfo.com/

Vol. 7, Issue 2, 2018 ISSN: 2319-5622 USA CODEN: JPROK3

Research Article

A VALIDATED STABILITY INDICATED RP-HPLC METHOD FOR DUTASTERIDE

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Received on: 07-01-2018; Revised and Accepted on: 28-01-2018

ABSTRACT

A Simple, Stability indicating, Isocratic, reverse phase High Performance Liquid Chromatographic (RPLC) related substance method was developed for Dutasteride in API. This method separates the impurities which are co-eluting in the pharmacopeia method. Successful separation of degradation impurities and synthetic impurities was achieved by YMC Triat phenyl column. Chromatographic was carried out on YMC Triat phenyl (150 X 4.6 mm, 3.0µm) column using 0.01M Potassium Dihydrogen Phosphate, pH to 2.5 with 1mL of trimethylamine as additive and acetonitrile. The developed HPLC method was validated with respect to linearity, accuracy, precision, ruggedness, robustness and specificity.

KEYWORDS: *Dutasteride, Impurities, HPLC, UV Detector and Validation.*

INTRODUCTION

Dutasteride,5α,17β-N-{2,5bis(trifluoromethyl)phenyl}-3-oxo -4-azaandrost-1ene-17-carboxamide [1], is a selective inhibitor of 5α-reductase, an intercellular enzyme that convert testosterone to DHT and is used for the treatment of patients with symptomatic benign prostatic hyperplasia [2-4]. Several bio-analytical and analytical methods have been reported for the analysis of Dutasteride. Recently stability indicated related substance by HPLC method was published in European Pharmacopoeia and United States Pharmacopeia, with six potential impurities. Three of the in-house process impurities were not separated by pharmacopeia method. So, comprehensive study was taken to develop a single method for all these impurities and it should be stability indicating method. In this newly developed HPLC method included three in-house impurities other than the listed pharmacopeia impurities. This method is accurate, linear, specific, reproducible and stability indicating HPLC method with good Limit of Quantification and Limit of Detection of Dutasteride in the presence of its degradation products of API. Impurity-E was not included in validation of this method due to the non-availability of required quantities, but shown specificity for these two impurities along with all impurities.

Method Development:

As a part of development, experiments were performed by using waters HPLC system with UV-Visible detector to achieve higher sensitivity. The main objective of the chromatographic method is to separate Dutasteride and from content of Impurity-A , Impurity-B and Impurity-C, Impurity-E, Impurity-F, Impurity-G, Desmethyl, Isomer and Dihydro impurity were co eluted using different stationary phases such as C18,C8, PFP, Phenyl, Cyno as well as different mobile phases. The chromatographic separation was achieved on YMC Triat phenyl 150 x 4.6mm, 3.0µm column. The peak shape of Dutasteride was found to be symmetrical; Impurity-A, Impurity-B, Impurity-C, Impurity-E, Impurity-F, Impurity-G, Desmethyl, Isomer and Dihydro impurity were separated with resolution greater than 1.5. The details of resolution for each parameter are tabulated in table-1.Dutasteride and its impurities retention times and the specificity for Dutasteride and its nine impurities are shown in the table-2.

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DOI: https://doi.org/10.5281/zenodo.1182694

MATERIALS AND METHODS

Samples and reagents:

The development samples of Dutasteride and all nine impurities (Six Pharmacopeial impurities, which are Impurity-A, Impurity-B, Impurity-C, Impurity-E, Impurity-F, and Impurity-G as well as three in-house impurities, which are Desmethyl, Isomer, and Dihydro impurity) were obtained from synthetic R&D laboratory of Dr. Reddy's Laboratories Ltd., CTO-III, and Hyderabad, India. Reagents used for analysis, i.e., Potassium dihydrogen orthophosphate, Tri ethylamine and Ortho phosphoric Acid (AR grade) and acetonitrile (HPLC grade) were obtained from Merck (India) Limited. Milli-Q grade water was used.

Instruments:

A Waters Model Alliance 2695-separation module (Waters corporation, Milford, MA, USA) equipped with a waters 2998-photo diode array UV detector was used. Data was processed through Waters empower software.

Chromatographic Conditions:

The analysis was carried out on YMC Triat phenyl column, 150 x 4.6mm x3.0µ particle size (Advanced Chromatography Technologies., Scotland) with a mobile phase consisting of Isocratic mixture of buffer and acetonitrile in the ratio of 580: 420 (v/v) 0.01 M potassium dihydrogen orthophosphate and 1mL tri ethyl amine pH adjusted to 2.5 with ortho phosphoric acid as buffer. The column temperature was maintained at 35°C. Flow rate was kept at 1.2 mL min^{-1} and the column eluent was monitored at 210 nm for 60 minutes. Water and Acetonitrile in the ratio of 20: 80 (v/v) is used as diluent.

Standard and Sample Preparation:

Assay performed with 0.5 mg/mL test concentration and related substance was performed with 1.0 mg/mL test concentration.

Resolutions in related substance, all the nine impurities are spiked 0.15% with respect to 1.0mg/mL test concentration.

RESULTS AND DISCUSSION

Analytical method validation:

Analytical method validation for the estimation related substance by HPLC and assay by HPLC of Dutasteride API was performed according to ICH Tripartite guide on Validation of Procedures: Text and Methodology and USP/NF current monograph recommendations [5-7].

System Suitability Evaluation:

Relative standard deviation for the area of six replicate injection of standard solution was not more than 2.0% for assay. Resolution between Impurity-A and Impurity-B should not be less than 1.2.

Forced Degradation Studies:

According to ICH stress testing of the drug substance can help the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used. The standard conditions for photo stability testing are described in ICH Q1B [8]. Degradation study of Dutasteride under different stress conditions suggested these degradation behaviors. In base degradation, 0.2N NaOH and heat at 70°C temperature for 12 hours, major degradation peak was observed at RRT: 0.89. It is degraded in 3N HCl at 70°C temperature for 48 hours, some degradation products were observed (1.11%) at RRT :0.44 and 0.60% of Impurity-A and 0.61% of Desmethyl impurity (figure 1) in acid conditions. In neutral (water), no major degradation products were observed after 48 hours at room temperature. The drug was also stable in water on heating at 70°C for 48 hours. No major degradation products were observed when the drug was exposed to 10% hydrogen peroxide at room temperature for 48 hours in oxidative degradation conditions. In thermal degradation, the drug was stable even when drug was exposed to dry heat at 105°C for 7 days. No degradation was observed. In UV light also drug was stable, when sample exposed to 254 nm and 365 nm under UV light for 7 days.

The mass balance of stressed samples was close to unaffected in the presence of Impurity-A, Impurity-B, Impurity-C, Impurity-E, Impurity-F, Impurity-G, Desmethyl, Isomer and Dihydro impurity and its degradation products conforms the stability indicating power of the developed method. In all above conditions Dutasteride is pure and peak purity is passing.

Precision:

The Precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Precision for Assay:

This is done to establish the ruggedness of the method was environmental factors like instruments, analyst, column and day are considered. Six sample preparations were prepared by two different analysts on two different days using two different lots of analytical columns. The assay and the % RSD for each six preparations and overall twelve results were calculated and found to be well within the predefined acceptance criteria. The results are tabulated in Table-2.

Precision for Related Substance:

The repeatability of the related substance method was checked by six-fold analysis by spiking all the seven impurities at 0.10% to 1.0 mg/mL of Dutasteride test sample as well as the same study was performed on different day with different analyst for the evaluation of inter day and Intra-day variation and analyst. The % RSD of percent area of all the impurities seven impurities in each six preparations and the overall twelve preparations were found well within the set acceptance limit, which conforms that the method is precise. The results are tabulated in Table-2.

Limit of Detection and Limit of Quantification:

The limit of detection (LOD) and limit of detection (LOQ) of Dutasteride and all its seven impurities were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of diluted solution of known concentration. The LOQ values were conformed by performing precision and accuracy verification.

The LOD of all the seven impurities along with Dutasteride in the range of 0.117µg/mL to 0.215µg/mL mg/mL and LOQ of all the nine impurities along with Dutasteride in the range of 0.355µg/mL to 0.779µg/mL with 10µL injection volume**.** The % RSD of percent area of all the seven impurities in six preparations at LOQ concentration were found less than 10, which conforms that the method is precise at LOQ Concentration. The results are tabulated in Table-1.

The percentage recovery of each impurity ranged from 88 to 105, those values are listed in the Table-1. Recovery all the impurities are well within the acceptance limit.

Linearity:

Linearity of the response was carried out at in five test concentration levels from 50% to 150% *i.e., 0.25-0.75 mg/mL*$^{-1}$ concentration calibration plot for the assay method was obtained over the calibration ranges tested, *i.e. 0.25-0.75 mg/mL*$^{-1}$, record the Dutasteride peak response over the range of concentration and plotted the linearity curve. The correlation coefficient obtained was greater than 0.999 for Dutasteride.

Linearity of the response for all the seven impurities was carried out from limit of quantification (LOQ) to 150% concentration of the specification limit, where specification limit is 0.1% of each impurity with respect to test concentration. Peak responses for all the seven impurities were recorded and plotted the calibration curve for each impurity concentration verses response, the correlation coefficient obtained for each impurity was greater than 0.999 and results are listed in Table-1.

Accuracy:

Known amount of each impurity addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of all the seven impurities in the Dutasteride sample. The study was carried out in triplicate at LOQ, 0.075%, 0.015% and 0.1875% of the analyte concentration (1.0 mg/mL) and calculated the percentage recovery of all the seven impurities. The percentage recovery of each impurity ranged from 88 to 105, those values are listed in the Table-1. Recovery all the impurities are well within the acceptance limit.

Robustness:

To determine the robustness of the method, chromatographic parameters are deliberate varied and the evaluated the change of resolution between Impurity-A and Impurity-B. Experiments are conducted by varying the flow by ± 10%, pH by ± 0.2 units and column temperature by ±5ºC. Resolution between impurity-A and impurity-B is illustrating and established the robustness of the method. Data is evaluated in the Table-1.

Solution Stability:

The solution Stability of assay of Dutasteride and its impurities in related substance method was carried out by spiking all the seven impurities at 0.1% level. All the prepared impurity solutions in volumetric flask were tightly capped and kept at room temperature for 48 hours. Assay and content of all the seven impurities were determined initially, after 24hours and after 48hours. Results were indicated the sample solution is stable up to 48hours.

Mobile Phase solution stability was also established for both Dutasteride assay and related impurities with fresh sample solutions and holding the mobile phased for 48 hours. Freshly prepared sample solutions were analyzed initially, after 24 hours and after 48 hours with initially prepared mobile phase. Results were indicated the sample solution is stable up to 48hours.

Table No. 1: System Suitability Results

Parameter	% RSD for Dutasteride	Resolution Between Impurity- A & Impurity-B
System Suitability	0.24%	1.74
Robustness		
Flow Variation (1.0 mL/min)	0.61	1.66
Flow Variation (1.4 mL/min)	0.75	1.71
Column Temperature Variation (30º C)	0.98	1.71
Column Temperature Variation (40º C)	1.02	2.07
pH Variation (pH: 2.3)	0.87	1.65
pH Variation (pH: 2.7)	1.32	1.70

Table No. 2: Validation Data

Parameter	Impurity-A	Impurity-B	Impurity-C	Desmethy Impurity	Di Hydro impurity	Isomer Impurity	Impurity-G	Impurity-F
RT about	2.8	3.1	9.5	15.8	26.3	31.6	33.8	35.4
LOD (µg/mL)	0.130	0.144	0.120	0.117	0.352	0.160	0.130	0.215
LOQ(µg/mL)	0.397	0.438	0.395	0.355	1.175	0.510	0.393	0.779
LOQ (n=6)	0.86	1.91	0.93	1.38	6.23	6.99	1.13	5.65
100% (n=6)	0.57	1.01	0.20	1.05	1.52	1.89	1.25	0.60
100% (n=12)	1.04	1.15	0.49	0.87	1.95	2.23	0.98	1.03
r	0.9996	0.9990	0.9996	0.9999	0.9996	0.9995	0.9999	0.9999
Slope	36287	52881	30967	46728	24985	29818	52799	28000
%Y-Intercept	3.01	-4.62	-1.54	1.18	-4.23	4.92	-1.87	-4.82
LOQ (n=3)	98.9	102.0	98.9	96.1	103.2	92.9	97.9	105.4
50% (n=3)	106.1	109.3	91.7	94.7	101.2	94.2	95.1	101.2
100% (n=3)	102.8	96.6	93.8	89.6	100.2	88.2	97.6	100.4
150% (n=3)	102.1	100.0	96.8	95.0	99.8	94.2	96.0	99.4

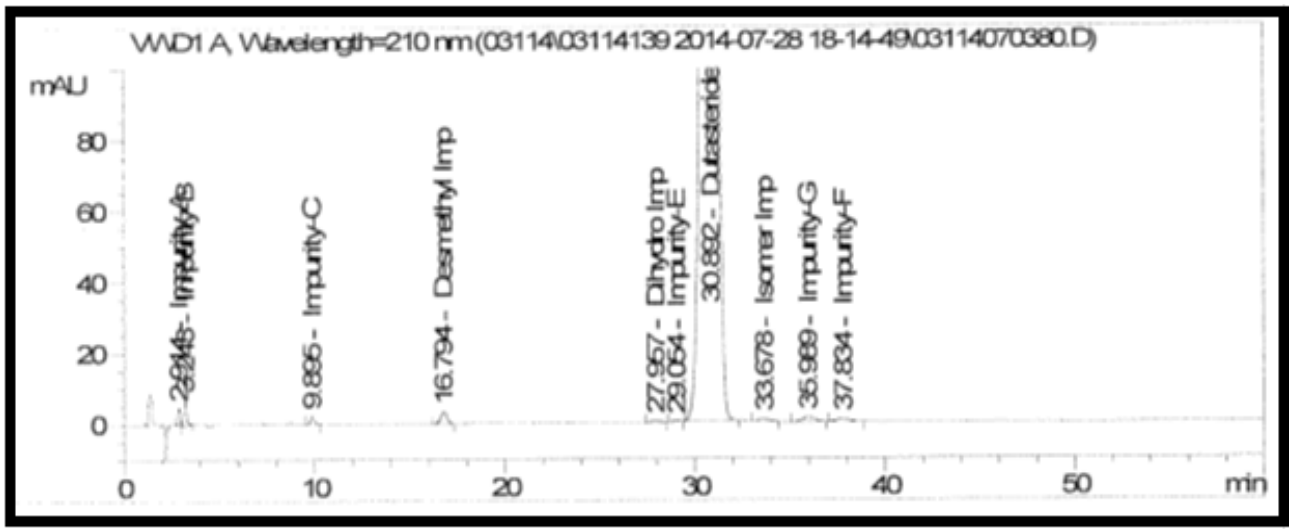

Fig 1: Typical HPLC chromatogram for specificity of Dutasteride

CONCLUSION

The LC method developed for quantitative and related substance determination of Dutasteride is precise, accurate, linear and specific. The method was fully validated, and the data found to be satisfactory for all the method validated parameters tested. The developed method is stability indicating and can be conveniently used by a quality control department to determine the related substance and the assay of regular Dutasteride commercial samples and also stability samples.

ACKNOWLEDGEMENTS

The authors wish to thank the management of Dr. Reddy's Laboratories Ltd., for permitting this work to be published. Cooperation extended by all the colleagues of Analytical R&D, CTO-II QC, and Process R&D division, is gratefully acknowledged.

REFERENCES:

1. *The Merck Index*, Merck Research Laboratories, Division of Merck & Co., Whitehouse Station, NJ, USA, 14th edition, **2006**.
2. Yamana K, Labrie F, Luu-The V. Human type 3 5α-reductase is expressed in peripheral tissues at higher levels than types 1 and 2 and its activity is potently inhibited by finasteride and dutasteride. Hormone Molecular Biology and Clinical Investigation **2010**;2(3).
3. 5-alpha reductase inhibitors (5-ARIs): Label Change - Increased Risk of Prostate Cancer U.S. Department of Health & Human Services.

4. S. C. Sweetman, Martindale. The complete Drug Reference, Pharmaceutical Press., The royal Pharmaceutical Society of Great Britain, 34th edition, **2005**.
5. ICH. Validation of Analytical Procedures: Text and methodology Q2 (R1): International Conference on Harmonization, IFPMA, Geneva, **2005**.
6. United States Pharmacopoeia (USP), XXVI. Validation of compendial methods. United States Pharmacopoeial Convention Inc.; Rockville, MD, USA, **2003**.
7. ICH harmonized tripartite guideline. Impurities in New Drug products Q2B (R2) current step 4 versions dated 2 June **2006**.
8. ICH. Q1B Stability Testing: Photo stability Testing of New Drug Substances and Products Geneva, November, **1996**.

How to cite this article:

D. Pavan Kumar et al. A VALIDATED STABILITY INDICATED RP-HPLC METHOD FOR DUTASTERIDE. J Pharm Res 2018;7(2):19-22.
DOI: https://doi.org/10.5281/zenodo.1182694

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil