Analytical method development with validation of bulk drug – dutasteride employing relative impurity profile.

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ABSTRACT: Benign prostatic hyperplasia is currently treated with the drug dutasteride. Similar to the finasteride, nearly 50 % of serum prostate specific antigen is reduced at the period of 6 months and having a 25 % of prostate volume in 2 years. The aim of this study is to develop a new analytical method with RP-HPLC (Reverse Phase High Performance Liquid Chromatography) which is highly accurate and new precise method for analyzing the purity of dutasteride as both in pharmaceutical formulation and in bulk quantities. As stated, in this study a new method was developed and validated for analyzing dutasteride in oral dosage form, by using RP-HPLC. 30 % of 0.02M phosphate buffer with 70 % acetonitrile (30:70 v/v) ratio is used as mobile phase in Zorbax SB C18 (5 µm) having a diameter of 250 x 4.6 mm with a flow rate of 1.2 ml/min, and optimized wavelength of 225 nm. ICH guidelines were used to validate the method. Results of assay for impurity in bulk drug were in allowable limit as per ICH. The proposed data conclude that the studies carried out, shows the development of analytical method and validation of dutasteride bulk drug employing relative impurity profile.

Keywords: Dutasteride, RP-HPLC. impurity profile, ICH, Method development

INTRODUCTION

Dutasteride (DUT) is 5 ARI-androgen derivative which has a potential to inhibit 5α-reductase and used as 5α-reductase inhibitor(Patel et al., 2010; Ramakrishna et al., 2004). It is also chemically known as (1S,3aS,3bS,5aR,9aR,9bS,11aS)-N-[2,5-bis(trifluoromethyl)phenyl]-9a,11a-dimethyl-7-oxo-1,2,3,3a,3b,4,5,5a,6,9b,10,11-dodecahydroindeno[5,4-f] quinoline-1-carboxamide. Due to its potential to inhibit the 5α-reductase, it is used in the combination to treat the benign prostate hyperplasia (Lowe et al., 2003); with a soothing effect to urinate without any difficulties by diminishing the size of the prostate gland. Testosterone is a precursor of the dihydrotestosterone (DHT), which is converted to DHT by the enzyme 5α-reductase (Brooks, 1986; Harris et al., 1992; Serda et al., 1993). 5α-reductase exists as two isoforms – type 1 isoform which is active in the production of DHT in skin, and liver while type 2 primarily actives in the reproductive tissue. Fortunately, Dutasteride inhibits both the isoforms by stabilizing the complex form of the enzyme. With the extensive literature review, a research gap was found,

and this study fulfils it by developing a highly precisive analytical technique to estimate the dutasteride, and its impurities by RP-HPLC (Cha, 2007; GRIMM, 1995; Lyon et al., 1998; Rasmusson et al., 1985, 1986).

MATERIALS AND METHODS:

Chemical and reagents:

From Merck HPLC grade acetonitrile, and Phosphate buffer, i.e., Potassium dihydrogen orthophosphate from Qualigens fine chemicals were procured. HPLC grade water was acquired from Mill-Q RO system. Hydrochloric acid (HCl) AR grade obtained from Loba chemicals; Sodium hydroxide (NaOH) AR grade from Fisher scientific Ltd, and hydrogen peroxide (H2O2) AR from Loba chemicals were also procured. DUT (standard) from Inga Pharmaceuticals, Mumbai; impurities like Desmethyl dutasteride, Dihydro dutasteride, carboxylic acid impurity, with isomer were obtained from Dr Reddy's laboratories.

Instruments:

Digital balance R200D & 1702, Systronics Digital pH meter 335, HPLC 2695, HPLC 2695 - PDA detector, Shimadzu gradient HPLC system Shimadzu HPLC LC-10AT-VP, Shimadzu spectrophotometer Pharma spec UV Visible spectroscopy-1700, Mark ultra sonicator, Zorbax SB C18, Zorbax SB CN, Hibar ® C18 were used. The columns have a common diameter of 250 x 4.6 mm, i.e., 5 µm.

METHODOLOGY:

In this part, development of a new method to analyse DUT is carried out with RP-HPLC under optimized chromatographic conditions. The methodology includes the preparation of buffer solution, diluent, sample, and standard solutions(Divya & Thakkar, n.d.; Walfish, 2006).

Preparation of 0.02M potassium dihydrogen orthophosphate buffer:

The standard buffer solution is used to maintain standard pH of the standard, and test solution. The essential of the standard buffer solution is used reference solution which can be used for measuring the sample with the specified pH. Dissolve 2.72 grams of potassium dihydrogen orthophosphate in 1000 microlitre of millipore water, and pH is adjusted to 3.0 ± 0.05 using orthophosphoric acid(Willard et al., 1988).

Diluent preparation:

The diluent is used to dilute the standard, reference, or sample, to procure the data from the RP-HPLC. The diluent should have a property of complete solubilization of both standard, reference and sample. A suitable solvent should be identified and tested. After several combination, a noble diluent was identified. The diluent is prepared by combining acetonitrile, and water in the ratio of 8:2. In this ratio both the standard, and sample are completely soluble(Malissa, 1969; Sengupta et al., 2018).

Preparation of mobile phase:

In RP-HPLC, the mobile phase should be in polar or a mixture of polar with the organic solvent in C_{18} column attached to the silica surface or similar materials. If the organic solvent content is increased in the polar mixture, it will reduce the retention time of the column; if the polarity of the mixture is increased, then the retention time of the column also increases. In this study, the acetonitrile was used to prepare the mobile phase with buffer in the ratio of 30:70 v/v. It is then filtered with 0.45-micron membrane filter to increase its purity, also degassed with the ultrasonic bath (Handbook of Pharmaceutical Analysis by HPLC; Sethi, 2008).

Preparation of Standard Solution:

Standard solution is used to compare the results of the sample to identify its significance. The standard compound is dutasteride which is accurately weighed about 10 mg. The volumetric flask of about 10 ml was to dissolve the 10mg of the drug with the diluent of about 10 ml. This solution will be used as standard, to compare the sample.

Preparation of Sample Solution:

The sample, i.e., 10 mg of dutasteride was accurately weighed, transferred to the 10ml volumetric flask – where it is dissolved, and diluted with the diluent (*Fundamentals of Analytical Chemistry - Douglas A. Skoog, Donald M. West, F. James Holler, Stanley R. Crouch - Google Books*, n.d.). The volume is made up to the 10ml with the diluent. This will be used as the sample solution, and their impurities present in the dutasteride will be used in the reference solution.

Preparation of reference Solution:

Each of the impurities present in the dutasteride is procured, and 10mg was weighed accurately. They were dissolved, transferred into 10ml volumetric flask, and volume was made up to 10ml with the diluent. This is the stock solution of the impurities. 20 microlitre of the stock solution was diluted with the 10 ml of standard solution in the 10 ml volumetric solution to obtain the reference solution (Bari et al., 2007).

RESULTS AND DISCUSSION:

This study is designed to develop a sensitive method to identify and quantify the impurities in dutasteride. By following this procedure, the results were obtained, and the impurity profile were reviewed.

The standard impurities of the dutasteride are analysed for the profiles like melting point, functional group with the FTIR, purity, and structural analysis. The results of the spectra obtained from the FTIR of the standard dutasteride, sample dutasteride, and their impurities are shown in the Figure 1, 2, 3, and 4. The interpretation of the results were carried are shown in the Table 1, 2, and 3 respectively.

Wavelength selection for the detection of the solutions (λmax):

100 µg/ml of the dutasteride was scanned in UV spectroscopy. They are scanned in the range of 200 – 400 nm. The UV spectrum of the all the above-mentioned solutions were superimposed with the software UV-Probe 3.2. The isosbestic point were the spectra overlaid was found to be 225 nm. Only in this selected wavelength identified, the absorbance level was extremely good without any interference.

Validation of the HPLC methods:

The major challenge to this methodology is the validation. In this the validation of the protocol, and identification of the limits with allowable variability is the key to this study. It is the crucial place where the protocol is determined for its correctness. The proof of validation, and its component like methods, procedure is presented earlier, and optimized data are shown.

Stationary Phase	Zorbax SB ® C_{18} (250 x 4.6 mm i.d., 5µ)
Solvent A	Potassium dihydrogen orthophosphate buffer pH adjusted to 3.0 ± 0.05 using orthophosphoric acid.
Solvent B	Acetonitrile
Mobile Phase ratio	30:70% v/v
Detection	225nm
Flow rate	1.2ml/min
Injection Volume	20µl
Needle wash	Water HPLC grade
Column temperature	35 ºC
Auto sampler temperature	35 ºC
Diluent	Mobile phase
Syringe rinse diluent	Mixture of water and ACN in ratio of 30:70% v/v
Run time	10 min

Accuracy:

Assay values determine the accuracy of the method. About 6 times, the assays were carried out to identify the recovery percentage of the impurities, and its accuracy. The recovery percentage of the impurities present in the standard drug were identified within 99.75 % - 100 %. The results are presented in the Table 4 & 5.

Precision:

The inter-day, and intra-day studies were used to demonstrate the precision of the method. In intra-day studies, nearly 6 times the injection of the standard, sample, and reference solutions were prepared, and injected. The response factor of impurities for the above solutions was identified, and the percentage of the RSD were found between 0.27 to 0.38. The inter-day studies

were also repeated nearly 6 times for the consecutive period of three days. Like the intra-day, the inter-day response peak for the impurities, and percentage of the RSD were also calculated. The solutions are stored in 4 degrees Celsius, and it was stable for 72 hours. The developed method of RP-HPLC was found to be more precise. The results are represented in Table 6.

Linearity range:

Various concentration ranging from 0.2 to 1.1 µg/ml with µg/ml NIC of the standard solution were chromatogramed, and results are shown in the Table 7. The response factor (RF) was used to plot the calibration curve against concentration of the sample solution containing impurities. The linear response for the calibration graph was procured from the results over all ranges of concentration. The results were positive showing a good linearity from the concentration level 0.3 to 1.1 µg/ml with a level of r2 showing 0.997. These are shown in Figure 5, 6, 7 & 8. The slope equation was obtained. From the data obtained, the method was highly sensitive to identify the concentrations of impurities.

Estimation of impurities and dutasteride in bulk drug:

Impurities in dutasteride, and dutasteride in bulk drug estimation were carried out in RP-HPLC method. The sample solutions were prepared, chromatogramed, and estimated the impurities with a quantity presenting from 202.63 µg/30 mg of dutasteride. The chromatograms of the mixture were shown in the Figure 9, 10, 11, 12, 13 & 14. The relative retention time was obtained 10.00 min for drug is reported. There was no interference with impurity peak od the dutasteride, and in the standard chromatogram which show the high specificity of the designed method.

LOD and LOQ:

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the method which is developed were determined. The smallest concentration of the analyte can be measured in LOD which gives measurable response. LOD can be calculated using this formula.

Limit of Detection = (3.3 x S.D / Slope of calibration curve)

Using this formula, the data was obtained and presented in Table 7.

Ruggedness & robustness:

The method of ruggedness, and robustness were studied by changing column of chromatography, reagents used, instruments, wavelength with the data systems. From the results observed, there were no remarkable changes in the chromatogram showing that the RP-HPLC method had developed ruggedness and robustness.

Stability studies of the solvents, buffers, drug solution and the mobile phase additives were carried out for 3 days. This study is to identify any changes in chromatographic conditions, in accordance with the stability of the above solutions. From the obtained chromatographic patterns, stability of the above solutions was constant up to three days when it is stored at 4ºC. The efficiency, resolution, symmetry peak of the column was determined. The procured value of the system shows suitability for both standard, and sample solutions. Parameter showing the

system suitability may fall within ±3% of standard deviation during the performance of the routine method. The system suitability parameters showed in Table 8.

Table-1. IR Interpretation for Dutasteride

S. No	Functional groups	Standard Wave number (cm^{-1})	Wave number (cm^{-1})
1	N-H	3300-3500	3349
2	C=O	1700-1750	1705
3	C=C	1620-1670	1642
4	C-H	3000-3100	3047
5	C-N	1030-1260	1252
7	C-F	500-600	530
8	C-C	750-1100	751

Table-2. IR interpretation for desmethyl dutasteride

S. No	Functional groups	Standard wave number (cm^{-1})	Wave number (cm^{-1})
1	C=N	1590-1660	1579
2	C-C	750-1100	1095
3	C=C	1620-1670	1642
4	C=O	1680	1679
5	C-O	1000-1050	1016
6	O-H	2500-3500	3035
7	C-F	500-600	530

TABLE-3. FTIR spectra of isomer of dutasteride

S. No	Functional groups	Standard-Wavenumber (cm^{-1})	Wavenumber (cm^{-1})
1	N-H	3300-3500	3349
2	C=O	1700-1750	1705
3	C=C	1620-1670	1683,1607
4	C-H	3000-3100	3047
5	C-N	1030-1260	1252
7	C-F	500-600	530
8	C-C	750-1100	751

Table-4. Results of assay for impurity in bulk drug

S. No	Drug and osage form	IMP-1 present in 100 µg of drug (µg/ml)	IMP 2present in 100µg of drug(µg/ml)	IMP-3 present in 100µg drug (µg/ml)	Imp-4 present in 100µg drug (µg/ml)	Allowable limit for mpurities as per ICH (µg/ml)
1	Bulk drug	0.011	0,022	0.026	0.016	0.15

Table-5. Report for precision study(repeatability)

Amt of bulk drug conc	Peak areas of DUT	Peak areas of Imp-1	Peak areas of Imp-2	Peak areas of imp-3	Peak areas of imp-4
100	23356712	32872	67505	44421	36802
	23359051	32772	67443	44463	36776
	23234512	32653	67574	44254	36754
	23245322	32571	67453	44314	36654
	23354326	32324	67487	44376	36842
	23355432	32654	67856	44387	36845
Mean	23317559	32854	67553	44369	36779
%RSD	0.258	0.572	0.23	0.17	0.20

Table-6. Interday precision (0hrs,24hrs,48hrs)

AMOUNT OF DRUG CONC	PEAK AREA OF DUT	PEAK AREA OF IMP-1	PEAK AREA OF IMP-2	PEAK AREA OF IMP-3	PEAK AREA OF IMP-4
100µg/ml	23359056	32872	67505	44465	36802
	23359064	32762	67494	44543	35803
	23259045	32652	67123	44345	36567
	23258097	32173	67565	44234	36987
	23139106	32563	67484	44567	35897
	23359045	32653	67678	44578	36807
Mean	23288902	32612	67474	44455	36810
RSD	0.38	0.36	0.27	0.31	0.38

Table-7. linearity and range for impurity

S. No	Concentration of IMPURITIES(µg/ml)	Response factor for imp-1	Response factor for imp-2	Response factor for imp-3	Response factor for imp-4
1	0	0	0	0	0
2	0.3	0.2358	0.245	0.248	0.2523
3	0.4	0.333	0.343	0.348	0.3542
4	0.5	0.4043	0.4322	0.438	0.4432
5	0.6	0.4971	0.503	0.5052	0.5235
6	0.7	0.5718	0.582	0.590	0.600
7	0.8	0.6855	0.6962	0.7011	0.698
8	0.9	0.7508	0.7694	0.7702	0.77
9	1.0	0.8648	0.8783	0.8845	0.8932
10	1.1	0.9993	0.9995	0.9998	1.0024

Table-8: LOD and LOQ IMPURITIES and DUT in bulk drug

Name of the compound	LOD(µg/ml)	LOQ(µg/ml)
Dutasteride	0.125µg/ml	1µg/ml
Impurity 1	1.1ng/ml	4.0ng/ml
Impurity 2	2.2ng/ml	7ng/ml
Impurity 3	2.3ng/ml	7.5ng/ml
Impurity 4	1.5ng/ml	6ng/ml

Table-9: Robustness Study-comparison of RT and RRT:

PARAMETERS	DUT		IMP-1		IMP-2		IMP-3		IMP-4	
	RT	RRT	RT	RRT	RT	RRT	RT	RRT	RT	RRT
FLOW RATE(1.2ml/min)	10.00	1.00	2.76	1.02	6.31	1.01	9.22	1.02	13.3	1.01
FLOW RATE(1.1ml/min)	10.46	1.46	3.12	1.13	7.14	1.13	10.1	1.09	14.4	1.08
Column temp(35)	10.00	1.00	2.76	1.02	6.31	1.01	9.22	1.02	13.3	1.01
Column temp(45)	10.00	1.00	2.74	1.01	6.12	0.96	9.43	1.04	13.4	1.01
Mobile phase(phosphate buffer:ACN)30;70	10.00	1.00	2.76	1.02	6.31	1.01	9.22	1.02	13.3	1.01
Mobile phase(phosphate buffer:ACN)35;65	9.56	0.96	2.42	0.88	6.11	0.97	9.03	0.98	13.0	0.98

Table-10. Report form system suitability and method validation parameters

Validation parameters	Observation
Linearity and Range	0.993(imp-1),0.995(imp-2),0.998imp-3) 1.0024(imp-4)
Correlation co-efficient (r^2)	0.9967(IMP-1),0.9971(IMP-2),0.9975(IMP-3),0.9947(IMP-4)
Slope equation	0.8939(IMP-1),0.8949(IMP-2),0.8954(IMP-3) ,0.8905(IMP-4)
Limit of detection (LOD)	0.125µg/ml(dut),1.1ng/ml(imp1),2.2ng/ml(imp-2), 2.3ng/ml(imp-3),1.5ng/ml(imp-4)
Limit of quantification (LOQ)	1µg/ml(dut)4.0ng/ml(imp-1),7ng/ml(imp 2),7.5ng/ml(imp-3),6ng/ml(imp-4)
Number of theoretical plates	6370/meter
Asymmetric factor	0.92
Tailing factor	0.916

FIG NO:1 FTIR SPECTRA OF DUTASTERIDE

FIG NO:2 FT IR SPECTRA OF DESMETHYL DUTASTERIDE

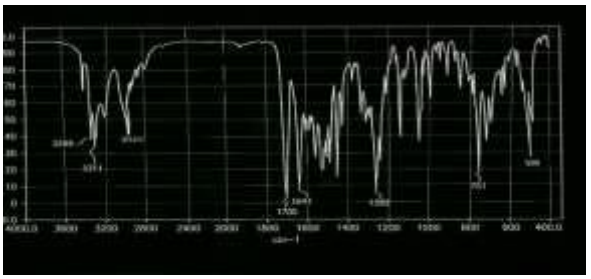


FIG NO:3 FT IR SPECTRA OF DIHYDRO DUTASTERIDE

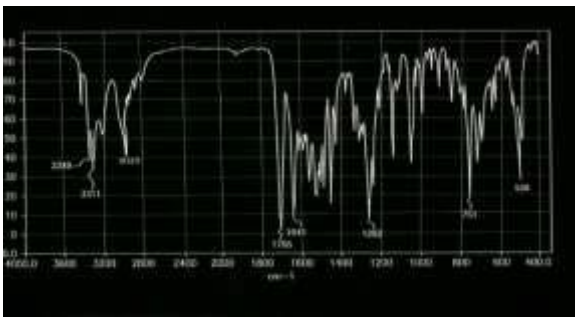


FIG NO:4 FTIR SPECTRA OF ISOMER OF DUTASTERIDE

FIG NO:5 LINEARITY RESPONSE FACTOR FOR IMPURITY 1

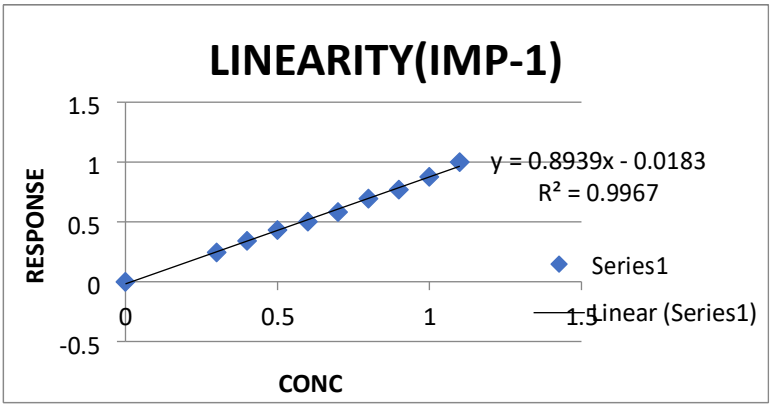


FIG NO:6 LINEARITY RESPONSE FACTOR FOR IMPURITY 2

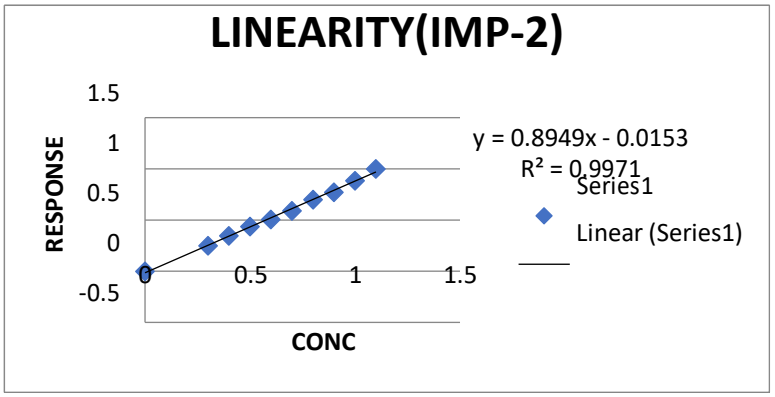


FIG NO:7 LINEARITY RESPONSE FACTOR FOR IMPURITY 3

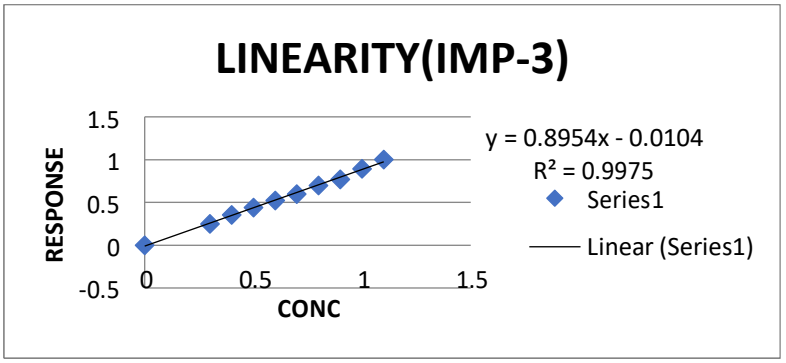


FIG NO:8 LINEARITY RESPONSE FACTOR FOR IMPURITY 4

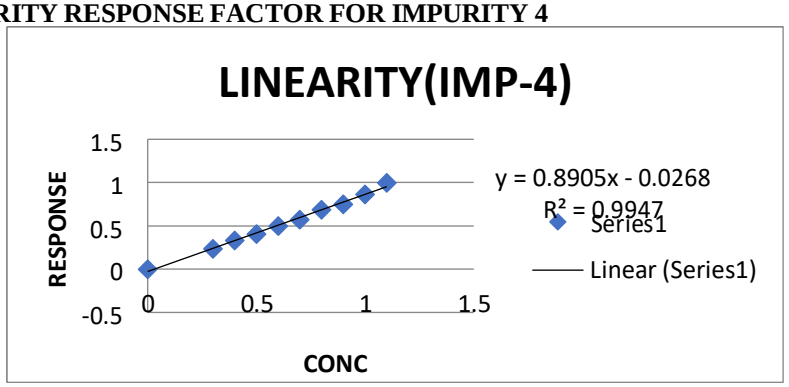


FIG NO:9 TYPICAL CHROMATOGRAM FOR DUTASTERIDE EMPLOYING RELATIVE IMPURITY PROFILE (100 µg/ml)

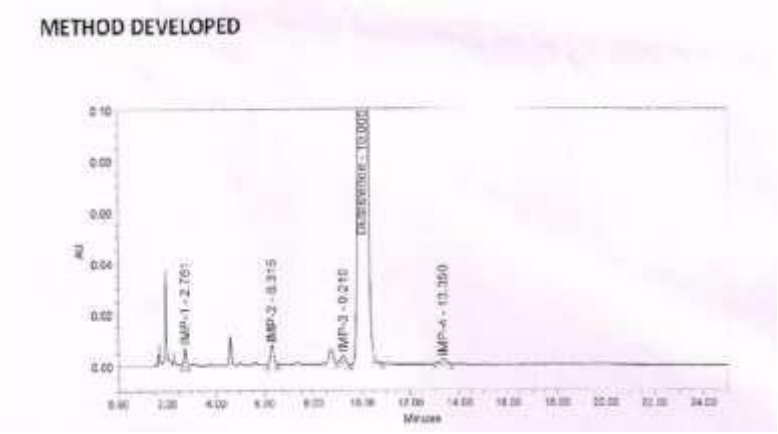


FIG NO: 10 TYPICAL CHROMATOGRAM STD DUTASTERIDE (100µg/ml)

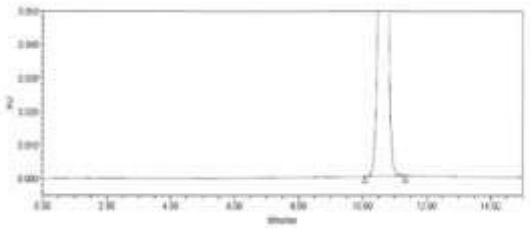


FIG NO:11 TYPICAL CHROMATOGRAM FOR DESMETHYL DUTASTERIDE

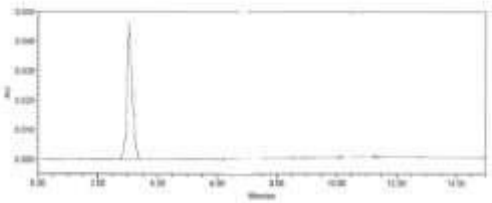


FIG 12: TYPICAL CHROMATOGRAM FOR DI HYDRO DUTASTERIDE

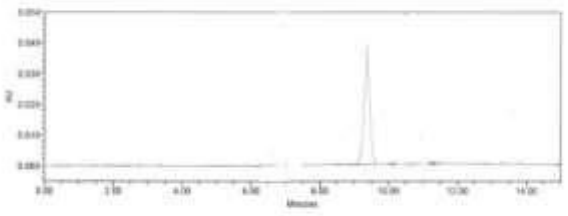


FIG 13: TYPICAL CHROMATOGRAM FOR ∞ ISOMER

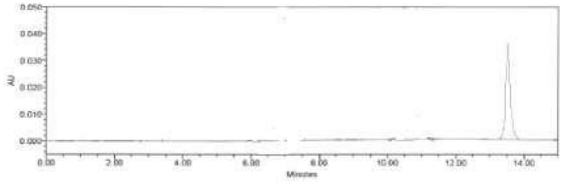
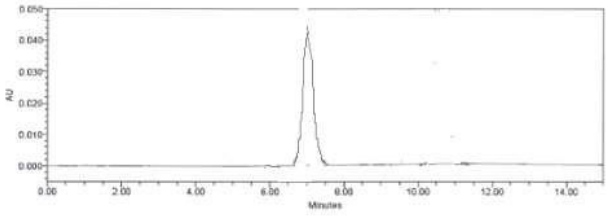


FIG 14: TYPICAL CHROMATOGRAM FOR CARBOXYLIC ACID IMPURITY



CONCLUSION:

Desmethyldutasteride, di hydro dutasteride, carboxylic acid impurity (DUT 1), ∞isomer are the process related impurities may present in the API dutasteride. ICH guideline Q3B(R2) helps in monitoring RI in the API (Dutasteride). This can be a simple method, but the developed method has high specificity, accuracy, and precision with stability. This method can be used for the quantification of RI present in the API. The API – dutasteride contains excess RI which is not within the acceptable range as per ICH guidelines. The impurity structural analysis may cause hyperplasia effect. It was proved that qualification limit does not shows the hyperplasia effect. This method can be used for the accurate quantification of impurities present in the drug dutasteride.

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