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DEVELOPING AND VALIDATING AN HPLC METHOD TO QUANTIFY SIMULTANEOUSLY DUTASTERIDE AND THEIR RELATED MOLECULES (DUTASTERIDE ACID, 2,5 BIS- (TRI FLUORO METHYL)-ANILINE AND DUTASTERIDE 17 α-EPIMER) IN CAPSULES

Pola Srinivas[1*] and Kancharlapalli Venkataramana[2]

[1]Department of Pharmacy, Acharya Nagarjuna University, Andhra Pradesh, India.

[2]Principal, ASN College of Pharmacy, Tenali, Andhra Pradesh, India.

ABSTRACT

Stability indicating HPLC method was suggested to simultaneously estimate dutasteride and their related molecules (dutasteride acid, 2,5 bis-(tri fluoro methyl)-aniline and dutasteride 17 α-epimer) in capsules. Stationary phase used was Inertsil ODS-3, 250 mm × 4.6 mm column with 3 µ particle dimension for separation, monitor and quantification. Mobile phase A (MP a) is a mixture of 0.42% perchloric acid buffer, acetonitrile and methanol in proportion 40:10: 50 (*v/v/v*). Mobile phase B (MP b) is a mixture of acetonitrile and water in proportion 90:10 (v/v). Elution was gradient mode with flow rate of 1.0 mL/min. The validation was carried out as stated by USP and ICH guiding principle. Linearity was obtained with concentration range of 0.466 µg/mL (LOQ level) - 7.6414 µg/mL for dutasteride acid, 0.3493 µg/mL (LOQ level) - 7.5599 µg/mL for 2,5 bis-(tri fluoro methyl)-aniline, 0.3962 µg/mL (LOQ level) - 7.6551µg/mL for dutasteride17α-epimer and 0.4012 µg/mL (LOQ level) - 7.5294 µg/mL for dutasteride. The precision, accuracy, system suitability ruggedness and robustness results are within the criteria of acceptance. Hence, the developed and validated methodology stands for usage in routine analysis and stability sample analysis.

Keywords: Dutasteride, 2,5 Bis-(tri fluoro methyl)-aniline, Dutasteride 17 α-epimer.

INTRODUCTION

Dutasteride is an antiandrogenic 4-azasteroid molecule and belongs to medication category of 5 alpha- reductase inhibitors[1,2]. Dutasteride is chemically termed as (1*S*,3*aS*,3*bS*,5*aR*,9*aR*,9*bS*,11*aS*)-*N*-[2,5-bis(trifluoromethyl)phenyl]-9*a*,11*a*-dimethyl-7-oxo-1,2,3,3*a*,3*b*,4,5,5*a*,6,9*b*,10,11-dodecahydroindeno[5,4-f]quinoline-1-carboxamide (Figure 1). 5 alpha reductase is an intracellular enzyme which actually involved in transforming testosterone to 5 alphadihydrotestosterone[3,4]. Declining concentrations of dihydrotestosterone may alleviate or inhibit enlargement in prostate gland [5]. Dutasteride is used in males with an overactive prostate gland and to manage benign prostatic hyperplasia and baldness[6,7]. Few methods to quantitatively assess Dutasteride have already been published. Contractor et al.[8], Ranjani et al.[9] and Myung et al.[10] have developed LC-MS methods to quantify dutasteride in plasma of humans and rats. Dipti et al.[11] described densitometric TLC for determining dutasteride in presence of degradants of acid, oxidative, alkali, photolytic,

wet heat and dry heat. Kamila et al.[12] and Amin et al.[13] reported spectrophotometry methods for dutasteride estimation in pharmaceutical forms. Patel et al.[14] and Navaneeswari et al.[15] reported HPLC methods to separate and assay dutasteride in formulations. Reddy et al. proposed UPLC method to estimate dutasteride and impurities in bulk[16].

This investigation was aimed at developing and validating a stability indicating HPLC method to monitor and quantify dutasteride and its impurities (dutasteride acid, 2,5 bis-(tri fluoro methyl)-aniline and dutasteride 17 α-epimer) simultaneously. The structures of studied impurities are shown in Figure 1.

MATERIALS AND METHODS

Apparatus

The chromatographic system used to perform development and validation of this method was comprised of a Make: Agilent; Model: 1200 Series, quaternary pump (G1311A model), auto sampler (G1329A model), Diode array detector (G1315C model) thermostated column (G1316A). System was controlled by version 4.0 EZChrome Elite software. Stationary phase used was Inertsil ODS-3, 250 mm × 4.6 mm column with 3 µ particle dimension.

Drug, impurities and formulation

Dutasteride (99.3% purity) reference drug and Dutas capsule formulation (0.5 mg dutasteride per capsule) was gained as gift samples from Dr. Reddy's Laboratories (Hyderabad, India). Impurities like dutasteride acid (99.4% purity), 2,5 bis-(tri fluoro methyl)-aniline (99.8% purity) and dutasteride 17α-epimer (97.3% purity) were obtained from SynThink Research Chemicals (Pune ,India).

CHEMICALS

Analytical grade reagents hydrogen peroxide, perchloric acid, sodium hydroxide, phosphoric acid, hydrochloric acid are bought from Merck chemicals (Mumbai, India).

SOLVENTS

High performance chromatography grade solvents like methanol and acetonitrile are from Merck chemicals, Ramkem (Mumbai, India), respectively.

Mobile phase and diluent

Mobile phase A (MP a) is a mixture of 0.42% perchloric acid buffer, acetonitrile and methanol in proportion 40:10: 50 (*v/v/v*). Mobile phase B (MP b) is a mixture of acetonitrile and water in proportion 90:10(v/v). MP a and b was degassed using sonication for about 5 min and filtering via 0.45 µ Nylon membrane filter. Acetonitrile was used as diluent and also as blank sample.

Chromatographic conditions

Flow rate in column	:	1.0 mL/min
Wavelength for detection	:	210
Temperature at column	:	50^{o}C
Run time	:	75 min
Sample size for analysis	:	15 µL
Elution mode	:	Gradient

Gradient Program was set as follows

Time (min)	0	15	40	50	55	60	65	75
MP a (%)	95	85	65	35	0	0	95	95
MP b (%)	5	15	35	65	100	100	5	5

Standard stock solutions

Stock solutions of dutasteride (25 µg/mL), dutasteride acid (25 µg/mL), 2,5 bis-(tri fluoro methyl)-aniline (25 µg/mL) and dutasteride 17α-epimer (25 µg/mL) were prepared separately with diluent.

Solutions for linearity study

The stock solutions were mixed and diluted in appropriate proportions to get linearity solutions with concentration range of 0.466 µg/mL - 7.6414 µg/mL (dutasteride acid), 0.3493 µg/mL - 7.5599 µg/mL (2,5 bis-(tri fluoro methyl)-aniline), 0.3962 µg/mL - 7.6551 µg/mL (dutasteride17α-epimer) and 0.4012 µg/mL - 7.5294 µg/mL (dutasteride).

Solutions for precision

The stock solutions were mixed and diluted in appropriate proportions to get standard solution with concentration 2.5 µg/mL (dutasteride acid), 2.5 µg/mL (2,5 bis-(tri fluoro methyl)-aniline), 2.5 µg/mL (dutasteride17α-epimer) and 2.5 µg/mL (dutasteride).

Solutions for accuracy

The stock solutions were mixed and diluted in appropriate proportions to prepare solutions for accuracy. Solutions to study accuracy were prepared with diluent at 4 concentration levels (LOD level; 50% level, 100% level and 150% level). Twenty capsules were emptied, and contents were transferred to dry beaker. Accurately weighed 3000 mg of oily solution equal to dutasteride 5 mg were transferred to a 10 mL volumetric flask with dropper. Added 4 mL of diluent and sonicated for 10 min at room temperature, added appropriate

volume of each (for LOD level – 0.1 mL; for 50% level - 0.5 mL; for 100% level – 1.0 mL; for 150% level – 1.5 mL) impurity stock standard solutions, diluted the volume to mark with diluent and mix carefully. Filtered sample solution through 0.45 µ Nylon membrane filter.

Concentrations at different levels are as follows

LOD level

0.466 µg/mL (dutasteride acid), 0.3493 µg/mL (2,5 bis-(tri fluoro methyl)-aniline) and 0.3962 µg/mL (dutasteride17α-Epimer).

50% level

2.4875 µg/mL (dutasteride acid), 2.5449 µg/mL (2,5 bis-(tri fluoro methyl)-aniline), 2.5420 µg/mL (dutasteride17α-Epimer).

100% level

4.9750 µg/mL (dutasteride acid), 5.0898 µg/mL (2,5 bis-(tri fluoro methyl)-aniline) and 5.0839µg/mL (dutasteride17α-Epimer).

150% level

7.4625 µg/mL (dutasteride acid), 7.6347 µg/mL (2,5 bis-(tri fluoro methyl)-aniline) and 7.6259µg/mL (dutasteride17α-Epimer).

Solutions for degradation study

The placebo, dutasteride standard (2.5 µg/mL) and test sample (dutasteride - 2.5 µg/mL + placebo) were subjected to different stress degradation conditions based on ICH guidelines [17].

Acid stressed sample solutions

The placebo (3005.32 mg), dutasteride standard (5 mg) and test sample (dutasteride – 5.2 mg + placebo – 3005.25 mg) were added to 5 mL of methanol. 45 mL of 2N HCl was added to all flasks. The flasks were refluxed at 90^{o}C for 2 hr. 50 mL of 2N NaOH was added to all flasks to neutralize the samples. The sample mixture was centrifuged at 3500 rpm for 10 min. 5 mL of supernatant solution was diluted to 100 mL with diluent and filtered via 0.45µm Nylon membrane filter.

Alkali stressed sample solutions

The placebo (3000.05 mg), dutasteride standard (5 mg) and test sample (dutasteride – 5.0 mg + placebo – 3000 mg) were added to 5 mL of methanol. 45 mL of 1N NaOH was added to all flasks. The flasks were refluxed at 90^{o}C for 5 min. 50 mL of 1N HCl was added to all flasks to neutralize the samples. The sample mixture was centrifuged at 3500 rpm for 10 min. 5 mL of supernatant solution was diluted to 100 mL with diluent and filtered via 0.45 µm Nylon membrane filter.

Peroxide stressed sample solutions

The placebo (3000.59 mg), Dutasteride standard (5.05 mg) and test sample (Dutasteride – 5.05 mg + placebo – 3000.78 mg) were added to 2 mL of methanol. 48 mL of 0.01% peroxide was added to all flasks. The flasks were refluxed at 90^{o}C for 2 hr. 48 mL of 0.01% sodium meta bisulphate was added to all flasks. The sample mixture was centrifuged at 3500 rpm for 10 min. 5 mL of supernatant solution was diluted to 100 mL with diluent and filtered via 0.45 µm Nylon membrane filter.

Thermal stressed sample solutions

The placebo (3000.84 mg), dutasteride standard (5.01 mg) and test sample (dutasteride – 5.01 mg + placebo – 3005.0 mg) were placed in watch glass and subjected to 105^{o}C for 48 hr in a oven. After degradation time period, the samples were mixed with 70 mL of diluent, sonicated for 10 min and made to 100 mL with diluent. The sample mixture was centrifuged at 3500 rpm for 10 min. 5 mL of supernatant solution was diluted to 100 mL with diluent and filtered via 0.45 µm Nylon membrane filter.

UV stressed sample solutions

The placebo (3000.64 mg), dutasteride standard (5.20 mg) and test sample (dutasteride – 5.10 mg + placebo – 3005.84 mg) were placed in watch glass and subjected to UV light for 72 hr in a UV chamber. After degradation time period, the samples were mixed with 70 mL of diluent, sonicated for 10 min and made to 100 mL with diluent. The sample mixture was centrifuged at 3500 rpm for 10 min. 5 mL of supernatant solution was diluted to 100 mL with diluent and filtered via 0.45 µm Nylon membrane filter.

Humidity stressed sample solutions

The placebo (3000.81 mg), dutasteride standard (5.10 mg) and test sample (dutasteride – 5.14 mg + placebo – 3005.91 mg) were placed in watch glass and subjected to 27^{o}C or 90% humidity for 10 days. After degradation time period, the samples were mixed with 70 mL of diluent, sonicated for 10 min and made to 100 mL with diluent. The sample mixture was centrifuged at 3500 rpm for 10 min. 5 mL of supernatant solution was diluted to 100 mL with diluent and filtered via 0.45 µm Nylon membrane filter.

RESULTS AND DISCUSSION

Optimization of chromatography conditions

For the simultaneous detection and quantification of dutasteride acid, 2,5 bis-(tri

fluoro methyl)-aniline, dutasteride 17α-epimer and dutasteride in bulk and capsule formulations with good system suitability values, the conditions like column type, mobile phase composition, solvents ratio in mobile phase, mode of elution, flow rate of mobile phase, column temperature, wavelength for analysis injection volume of sample for analysis and total run time for one analysis were optimized through several trail operations. Finally, good system suitability values (resolution, peak tailing, peak shape and area response) were obtained in conditions given below:

- Inertsil ODS column - 3, 250 mm × 4.6 mm and 3 µ size of particle
- MP a: 0.42% perchloric acid buffer, methanol and acetonitrile in ratio 40:50:10 (*v/v/v*)
- MP b : Acetonitrile and water in ratio of 90:10 (*v/v*).

Gradient elution mode. The gradient programme is as follows

Time (min)	0	15	40	50	55	60	65	75
MP a (%)	95	85	65	35	0	0	95	95
MP b (%)	5	15	35	65	100	100	5	5

- 1.0 mL/min flow rate
- 50 ^{o}C temperature
- 210 nm as wavelength for analysis
- 15 µl as sample injection for analysis
- 75 min as run time

The chromatogram of sample using optimized conditions is shown in Figure 2. The retention times are dutasteride acid - 8.04 min, 2,5 bis-(tri fluoro methyl)-aniline - 16.92 min, dutasteride 17α-epimer - 36.75 min and dutasteride - 38.83 min. Clear resolution was seen between dutasteride and its impurities like dutasteride acid, 2,5 bis-(tri fluoro methyl)-aniline and dutasteride 17α-epimer with optimized conditions.

With optimized chromatographic conditions, the relative retention time and relative responsive response factor for dutasteride acid, 2,5 bis-(tri fluoro methyl)-aniline, dutasteride 17α-epimer and dutasteride were calculated as using formula given below:

$$\text{Relative Retention Time} = \frac{\text{Retention time of impurity peak}}{\text{Retention time of main peak}}$$

$$\text{Relative Response Factor} = \frac{\text{Slope of Impurity peak}}{\text{Slope of main peak}}$$

The relative retention time and relative responsive response factor for dutasteride acid, 2,5 bis-(tri fluoro methyl)-aniline, dutasteride 17α-epimer and dutasteride are given in Table 1: Relative retention time and relative responsive response factor values of Dutasteride and its impurities.

Validation

The method described was validated in compliance of ICH guidelines for parameters - accuracy, linearity, specificity, precision, selectivity, limit of detection, limit of quantification and robustness[18].

System suitability

Diluent (acetonitrile) and standard solution (dutasteride – 2.5 µg/mL + dutasteride 17α-epimer 2.5 µg/mL) was infused (n = 6 times) into the system and chromatographs were recorded. Criteria parameters for system suitability were computed. The criteria for system suitability shall always to be met. The acquired values (Table 2) illustrated the system's suitability to analyze dutasteride acid, 2,5 bis-(tri fluoro methyl)-aniline, dutasteride 17α-epimer and dutasteride simultaneously. (Table 2: Results for system suitability)

Specificity

Specificity was confirmed through placebo & diluent interference study and impurity interference study.

Placebo and diluent interference study

Placebo was prepared using mono- and di-glycerides, medium chain triglycerides, butylated hydroxy toluene, gelatin, tween 80 and FDC Red 40. Placebo and dilent solutions were infused into the chromatography system. The chromatograms obtained were checked for interference peaks at retention times of dutasteride, dutasteride acid, 2,5 bis-(tri fluoro methyl)-aniline, dutasteride 17α-epimer. No placebo (Figure 3a) and diluent (Figure 3b) related peaks were noted at retention times of dutasteride, dutasteride acid, 2,5 bis-(tri fluoro methyl)-aniline, dutasteride 17α-epimer. Therefore, there was no placebo and diluent interference.

Impurity Interference study

To check interference from impurities, all individual impurities are injected at

specification level (dutasteride acid – 2.5 µg/mL, 2,5 bis-(tri fluoro methyl)-aniline– 2.5 µg/mL, dutasteride 17α-epimer – 2.5 µg/mL). Impurities are added to test sample at specification level and Injected into HPLC system. Control samples were also injected. The impurities were separated from each other and from dutasteride peak. The peak purity of dutasteride peak in control sample and the peak purity of dutasteride, dutasteride acid, 2,5 bis-(tri fluoro methyl)-aniline, dutasteride 17α-epimer in added samples was more than 0.990. No additional peaks of blank and placebo were observed at retention times of dutasteride, dutasteride acid, 2,5 bis-(tri fluoro methyl)-aniline, dutasteride 17α-epimer. Peaks of dutasteride related substances are not interfered with dutasteride and also from each other. The chromatograms of this test were given in Figures 4a – 4f. Hence, the method was selective and specific.

Linearity

Linearity test was tested from the LOQ level concentration to 150% level concentration of dutasteride, dutasteride acid, 2,5 bis-(tri fluoro methyl)-aniline and dutasteride 17α-epimer. Linearity was seen in range of 0.466 - 7.6414 µg/mL (dutasteride acid), 0.3493 - 7.5599 µg/mL (2,5 bis-(tri fluoro methyl)-aniline), 0.3962 - 7.6551 µg/mL (dutasteride 17α-epimer) and 0.4012 - 7.5294 µg/mL (dutasteride). The regression statistic values for the linearity were given Table 3. It is obvious in the above information that perhaps the response was linear. Coefficients of correlation are >0.990. Furthermore, the residual assessment revealed that the values were spread randomly around zero, which fits well within linearity model. The quantitative method is therefore linear. (Table 3: regression statistic values for dutasteride and its impurities).

Limit of detection and quantification

Detection (LOD) and quantification (LOQ) limit was determined by the slope and linearity curve
residual standard deviation as framed by ICH set of laws. The computed values are given in Table 4.

The quantitative method was adequate enough for the intended use (Table 4: Sensitivity results for dutasteride and impurities).

Precision

Method precision

Six times evaluated the samples (dutasteride acid – 2.5 µg/mL, 2,5 bis-(tri fluoro methyl)-aniline – 2.5 µg/mL, dutasteride 17α-epimer – 2.5 µg/mL and dutasteride – 2.5 µg/mL) according to the procedure established. The quantity of dutasteride and total impurities as well as their relative standard deviation. Relative standard deviation calculated were less than 10% (Table 5) and proved method precision (Table 5: Method precision findings for individual impurities and dutasteride).

Intermediate precision

Six times evaluated the samples (dutasteride acid – 2.5 µg/mL, 2,5 bis-(tri fluoro methyl)-aniline – 2.5 µg/mL, dutasteride 17α-epimer – 2.5 µg/mL and dutasteride – 2.5 µg/mL) as per the procedure established by distinct analyst using separate column lots on distinct devices and distinct days. The quantity of dutasteride and total impurities as well as their relative standard deviation. Relative standard deviation calculated were less than 10% (Table 6) and proved intermediate precision and ruggedness (Table 6: Intermediate precision/ruggedness findings for individual impurities and dutasteride).

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Accuracy

Accuracy was tested by spiking the known amount of dutasteride, dutasteride acid, 2,5 bis-(tri fluoro methyl)-aniline and dutasteride 17α-epimer in capsule sample with different (4) levels varying from LOQ to 150% specification level. Recovery was conducted at each level in triplicate. The recovery of dutasteride, dutasteride acid, 2,5 bis-(tri fluoro methyl)-aniline and dutasteride 17α-epimer was calculated. Percent recoveries determined were within 70% - 130% (acceptance criteria) at LOQ level and within 85% - 115% (acceptance criteria) at other levels (Table 7). Percent relative standard deviation got for 12 determinations were not more than 15%. The method is therefore accurate Table 7: Accuracy for impurities at varying concentration levels.

Robustness

The method's robustness was researched through intentional method modifications such as modifications in flow rate, temperature, organic solvent ratio, and wavelength. Values for RRT are measured in all modified conditions. It has been noted that there have been no noticeable changes in RRT values. The values acquired are given in Table 8 and showed that the procedure is robust Table 8: Robustness findings – RRT and their differences).

Specificity (forced degradation)

Forced degradation of dutasteride capsules is performed to ensure that any degradation

product, if found, will not clash with dutasteride peak and with each other during the stability testing. The forced degradation research was done through exposing the sample to oxidative lysis, alkali lysis, acid lysis, photolytic, dry heat and humidity. The results of degradation are given in Table 9. The chromatograms are given in Figures 5a – 5f. The known and unknown degradants are separated from dutasteride peak and with each other. The peak purity of dutasteride peak in stressed sample was <0.990 and mass balance was <95%. The procedure is therefore considered specific and stability indicating (Table 9: Forced Degradation results of dutasteride capsules).

CONCLUSION

Stability indicating HPLC method was suggested to simultaneously estimate dutasteride and related molecules of dutasteride in capsules. The validation for the estimation of related molecules of dutasteride capsules is carried out as stated by USP and ICH guiding principle. The precise and robust protocol is found as specific for estimation of known impurities, unknown impurities and degradation products. The method proved stability indicating through degradation study. The procedure is found as linear and accurate in specified range determined. The limit of quantification values established was less than of the reporting threshold. Hence the suggested procedure stands for usage of routine and stability sample analysis.

Table 1: Relative retention time and relative responsive response factor values of Dutasteride and its impurities

Impurity or drug	Relative retention time	Relative responsive response factor
Dutasteride Acid	0.21	0.78
2,5 Bis-(tri fluoro methyl)-aniline	0.42	0.97
Dutasteride 17α-Epimer	0.92	0.95
Dutasteride	1.00	1.00

Table 2: Results for system suitability

System suitability	Observed value	Acceptance criteria
Resolution between Dutasteride and Dutasteride 17α-Epimer	3.2	≥ 1.5
Asymmetry for Dutasteride peak from standard	1.1	≤ 2.0
% RSD for area of six replicate injections of standard preparation	0.5	≤ 5.0

RSD – relative standard deviation

Table 3: regression statistic values for dutasteride and its impurities

Parameter	Dutasteride	Dutasteride 17α-epimer	2,5 Bis-(tri fluoro methyl)-aniline	Dutasteride
Slope	67142.0	81567.9	82986.0	85566.5
Intercept	-7237.6	3494.8	1156.1	5205.4
Correlation Coefficient	0.999	1.000	1.000	1.000
Percent Bias at 100% Level	-2.2	0.8	0.3	1.2
RRF	0.78	0.95	0.97	1.00

RRF – relative response factor

Table 4: Sensitivity results for dutasteride and impurities

Drug/impurity name	LOD (µg/mL)	LOQ (µg/mL)
Dutasteride acid	0.1538	0.4660
2,5 Bis-(tri fluoro methyl)-aniline	0.1153	0.3493
Dutasteride 17α-epimer	0.1307	0.3962
Dutasteride	0.1324	0.4012

Table 5: Method precision findings for individual impurities and dutasteride

Sample No.	Dutasteride Acid		2,5 Bis-(tri fluoro methyl)-aniline		Dutasteride 17α-Epimer		Percent total impurities	Percent dutasteride
	RRT	%	RRT	%	RRT	%		
1	0.20	1.093	0.42	1.093	0.92	1.033	3.219	0.934
2	0.20	1.084	0.42	1.061	0.92	1.031	3.176	0.934
3	0.20	1.067	0.42	1.037	0.92	1.011	3.115	0.944
4	0.20	1.043	0.42	1.060	0.92	1.011	3.114	0.960
5	0.20	1.016	0.42	1.050	0.92	1.019	3.085	0.977
6	0.20	1.073	0.42	1.029	0.92	1.001	3.103	0.995
Average	0.20	1.063	0.42	1.055	0.92	1.018	3.135	0.957
RSD	-	2.7	-	1.093	-	1.2	1.6	2.6

RRT – relative retention time; RSD – relative standard deviation

Table 6: Intermediate precision/ruggedness findings for individual impurities and dutasteride

Sample No.	Dutasteride Acid		2,5 Bis-(tri fluoro methyl)-aniline		Dutasteride 17α-Epimer		Percent total impurities	Percent dutasteride
	RRT	%	RRT	%	RRT	%		
1	0.20	1.095	0.42	0.993	0.92	1.051	3.139	0.887
2	0.20	1.074	0.43	1.007	0.92	1.044	3.125	0.928
3	0.20	1.067	0.43	1.081	0.92	1.034	3.182	0.931
4	0.20	1.050	0.43	1.019	0.92	1.078	3.147	0.912
5	0.20	1.089	0.43	1.042	0.92	1.062	3.193	0.920
6	0.20	1.039	0.43	1.031	0.92	1.053	3.123	0.911
Average	0.20	1.069	0.43	1.029	0.92	1.054	3.152	0.915
RSD	-	2.0	-	3.0	-	1.4	0.9	1.7

RRT – relative retention time; RSD – relative standard deviation

Table 7: Accuracy for impurities at varying concentration levels

Spike Level	µg/mL quantity added	Mean percent recovery	% RSD
Dutasteride acid			
LOQ	0.4660	90.1	1.0
50%	2.4875	97.4	1.3
100%	4.9750	102.2	2.4
150%	7.4625	100.5	1.3
Overall percent RSD			5.1
2, 5 Bis-(tri fluoro methyl)-aniline			
LOQ	0.3493	87.2	6.6
50%	2.5449	102.0	0.8
100%	5.0898	100.9	1.5
150%	7.6347	98.4	0.4
Overall percent RSD			6.8
Dutasteride17α-Epimer			
LOQ	0.3962	95.3	3.5
50%	2.5420	101.5	0.7
100%	5.0839	103.4	0.8
150%	7.6259	101.9	0.5
Overall percent RSD			3.6

Table 8: Robustness findings – RRT and their differences

Flow rate	Dutasteride acid		2,5 Bis-(tri fluoro methyl)-aniline		Dutasteride 17α-Epimer	
	RRT	Diff	RRT	Diff	RRT	Diff
Variation in pH						
1.0	0.20	NA	0.41	NA	0.92	NA
0.9	0.21	0.01	0.43	0.02	0.92	0.00
1.1	0.19	0.01	0.40	0.01	0.91	0.01
Variation in temperature						
50°C	0.20	NA	0.41	NA	0.92	NA
45°C	0.20	0.00	0.42	0.01	0.92	0.00
55°C	0.20	0.00	0.41	0.00	0.91	0.01
Variation in organic solvent ratio						
2% lower	0.20	NA	0.41	NA	0.92	NA
Actual	0.20	0.00	0.41	0.00	0.92	0.00
2% higher	0.20	0.00	0.42	0.01	0.91	0.01
Variation in wavelength						
205	0.20	NA	0.41	NA	0.92	NA
210	0.20	0.00	0.41	0.00	0.92	0.00
215	0.20	0.00	0.41	0.00	0.92	0.00

RRT – relative retention time; Diff - difference

Table 9: Forced Degradation results of dutasteride capsules

		Control	Degradation					
			Acid	Base	Peroxide	Light	Thermal	Humidity
Peak Purity		**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**
Compounds	**RRT**	**% impurity**						
Dutasteride	1.00	NA	NA	NA	NA	NA	NA	NA
Dutasteride acid		ND	ND	ND	ND	ND	ND	ND
2, 5 Bis-(tri fluoro methyl)aniline	0.43	0.021	ND	0.008	0.016	0.022	0.025	0.020
Dutasteride 17α-Epimer	0.92	0.039	ND	ND	0.263	0.035	0.033	0.054
Chloro Dutasteride	1.05	0.017	0.024	ND	0.025	0.040	0.021	0.026
Total impurities		0.060	0	0.008	0.279	0.057	0.058	0.074
% Net degradation			0	0	0	0	0	0
Mass Balance (%)			101	100	101	101	101	102

NA – not available; ND – not detected; RRT – relative retention time

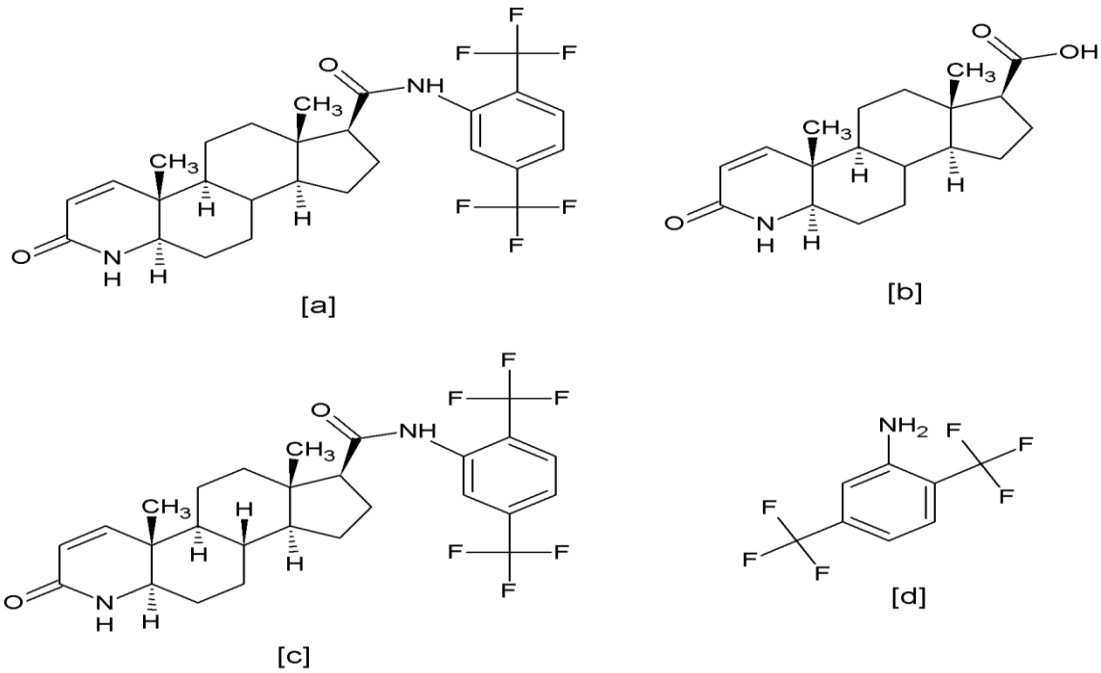

Fig. 1: [a] Dutasteride [b] Dutasteride acid [c] Dutasteride 17 α-Epimer [d] 2,5 Bis-(tri fluoro methyl)-aniline

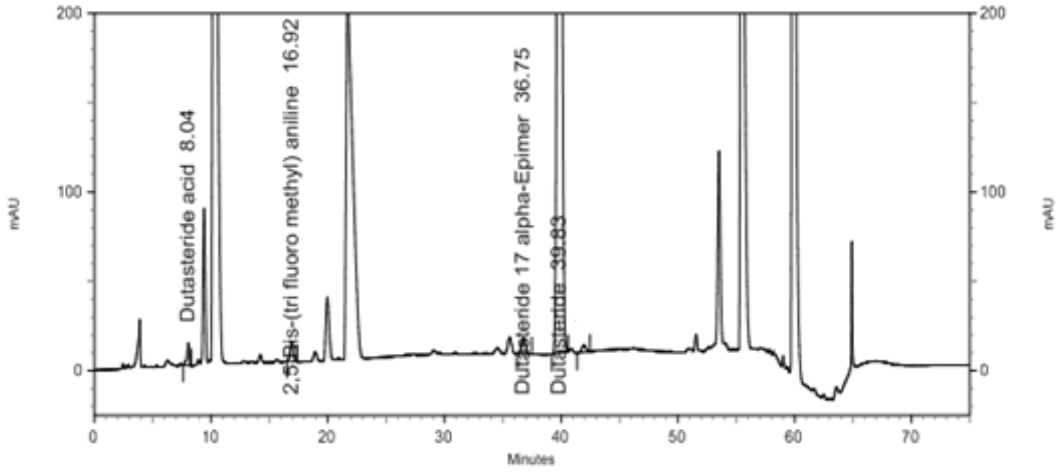

Fig. 2: Dutasteride acid (RT 8.04 min), 2,5 Bis-(tri fluoro methyl)-aniline (RT 16.92 min), Dutasteride 17α-epimer (RT 36.75 min) and Dutasteride (RT 38.83 min) chromatogram

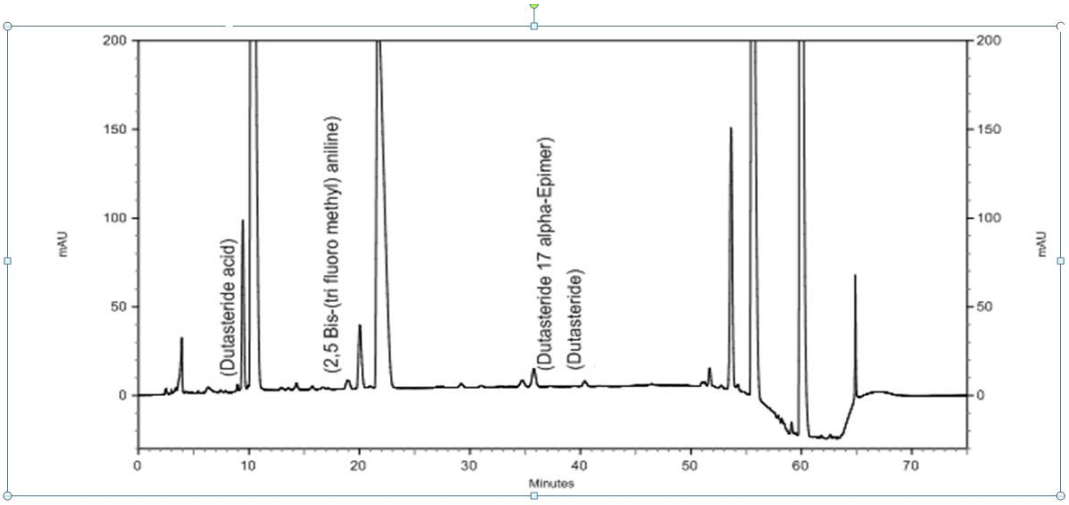

Fig. 3a: Placebo chromatogram

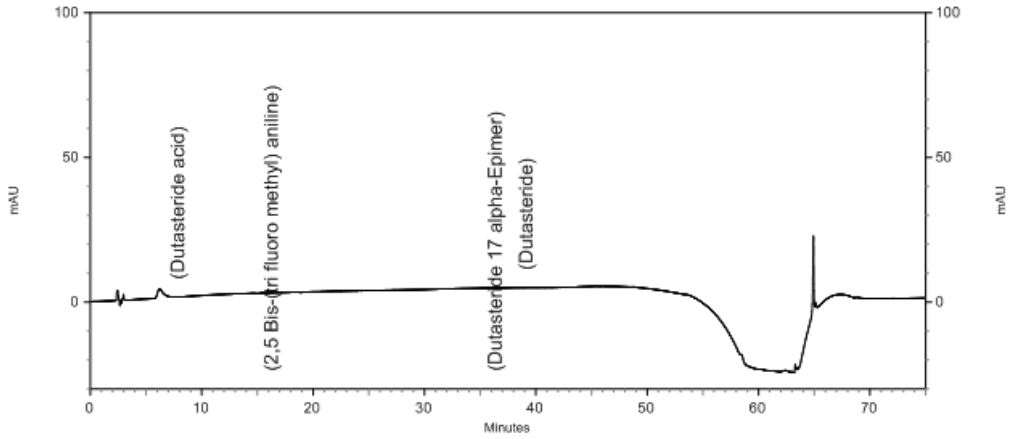

Fig. 3b: Diluent chromatogram

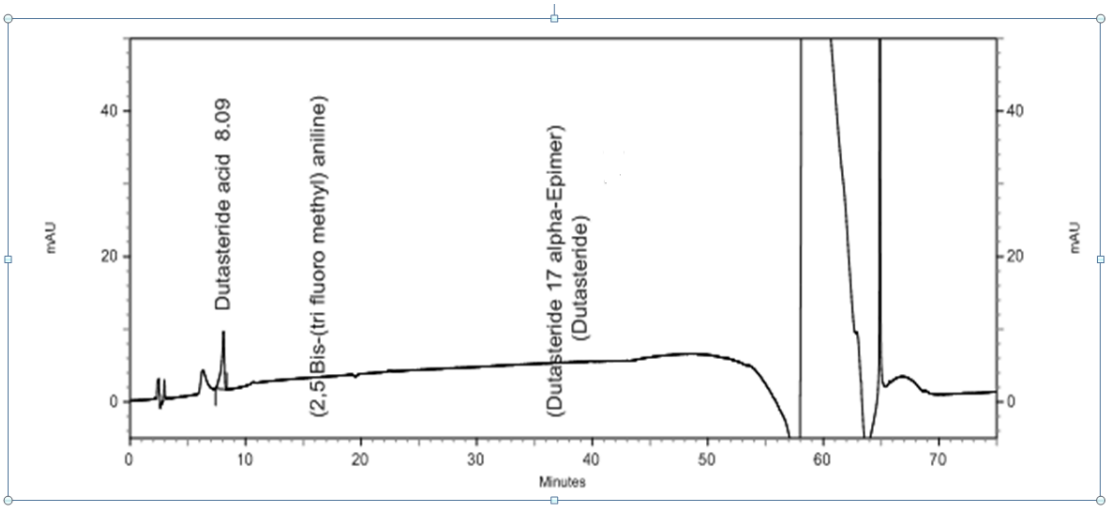

Fig. 4a: Dutasteride acid chromatogram

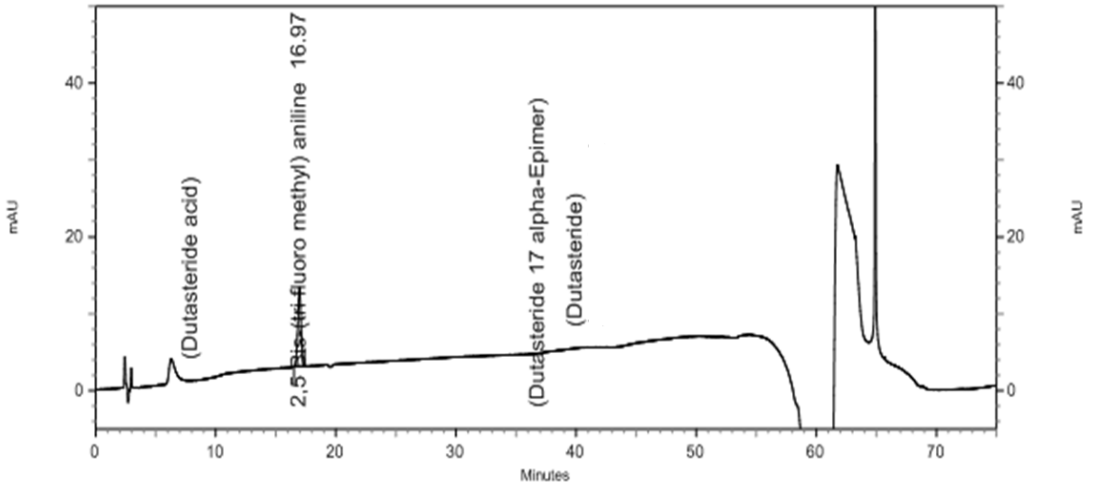

Fig. 4b: 2, 5 Bis-(tri fluoro methyl)-aniline chromatogram

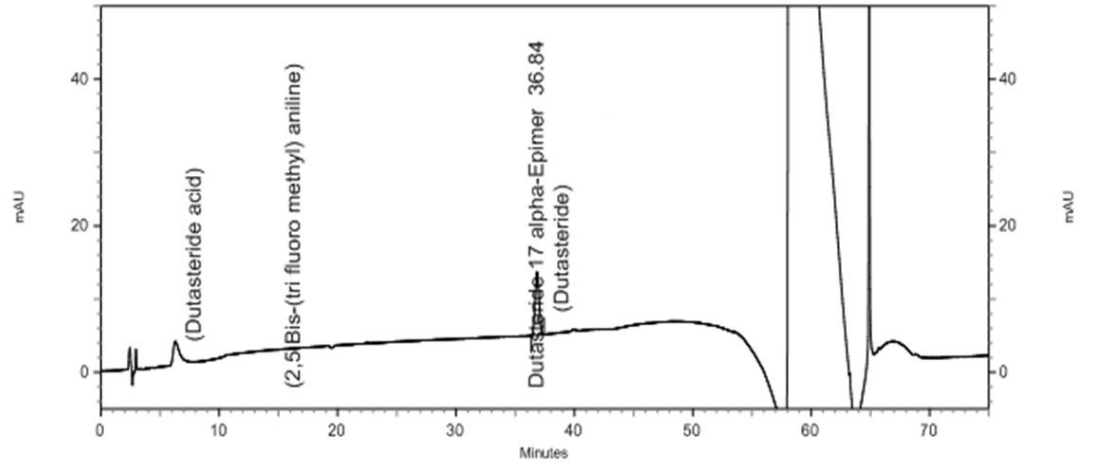

Fig. 4c: Dutasteride 17α-Epimer chromatogram

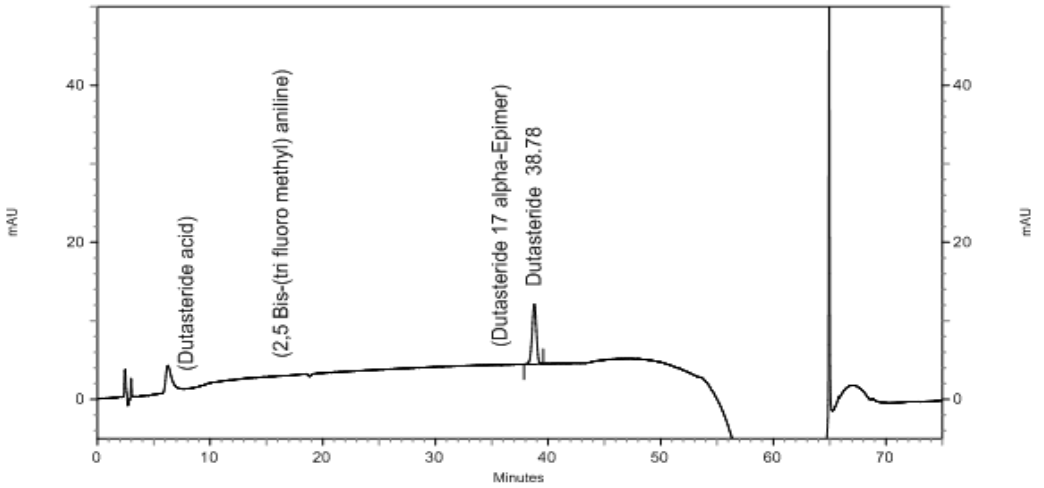

Fig. 4d: Dutasteride chromatogram

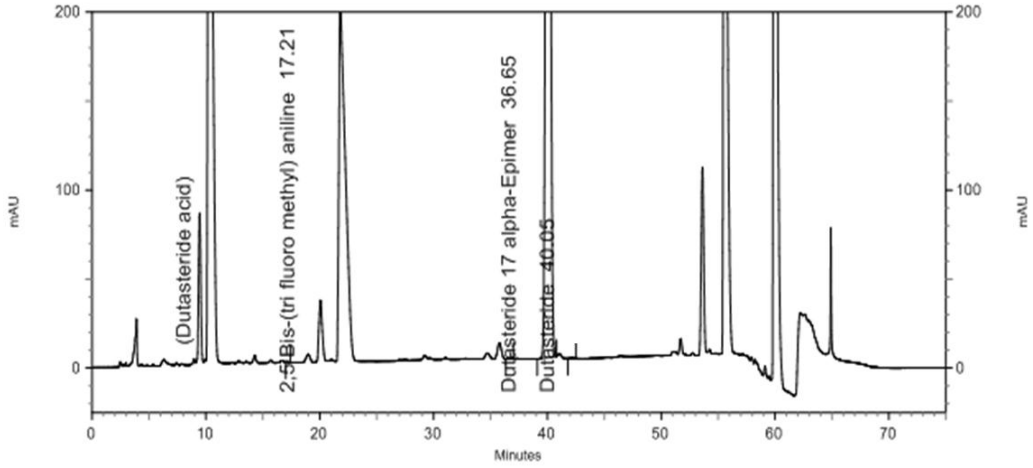

Fig. 4e: Control test chromatogram

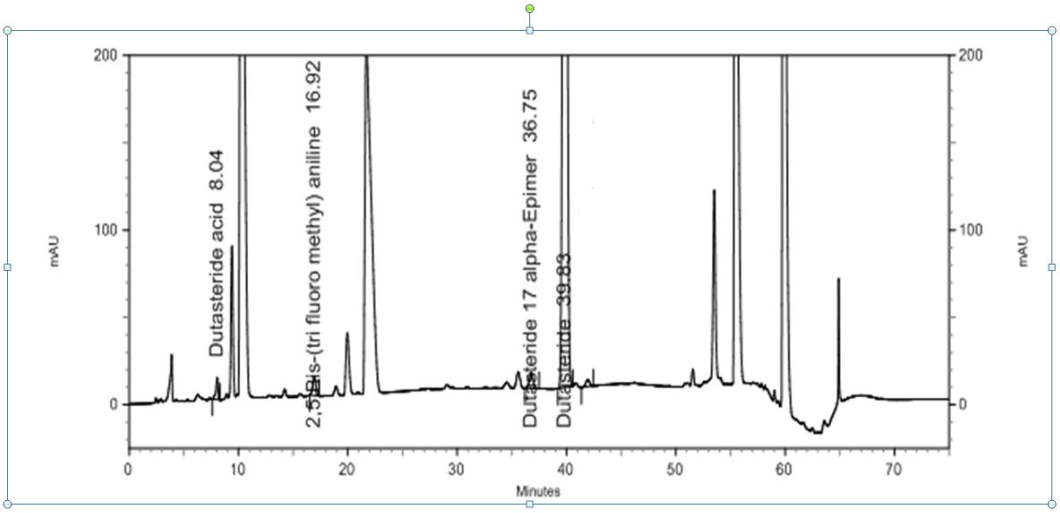

Fig. 4f: Spiked test chromatogram

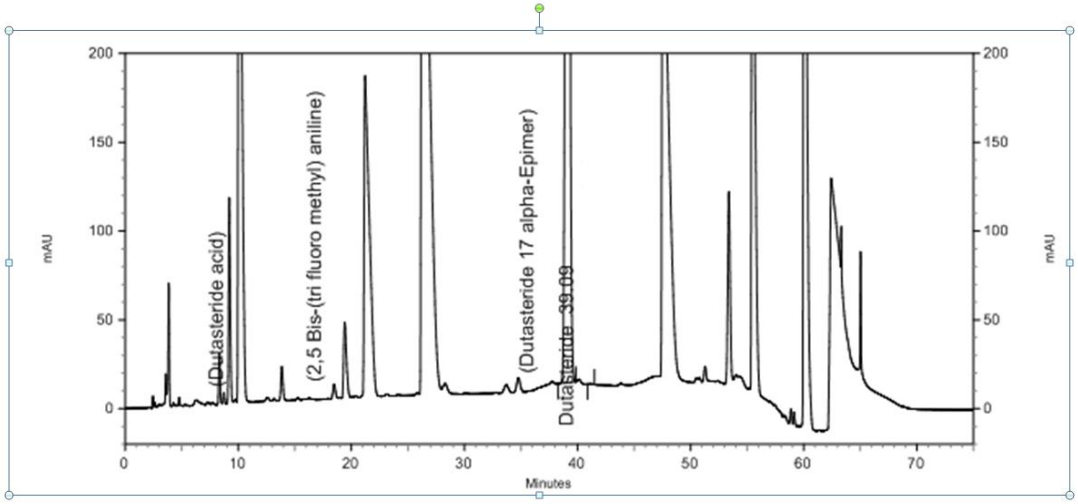

Fig. 5a: Chromatogram of acid stressed capsule

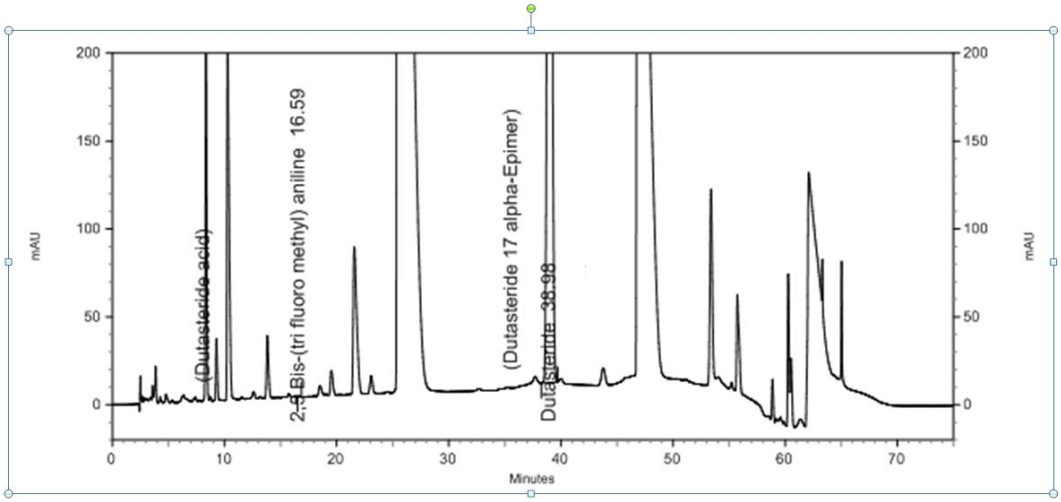

Fig. 5b: Chromatogram of alkali stressed capsule

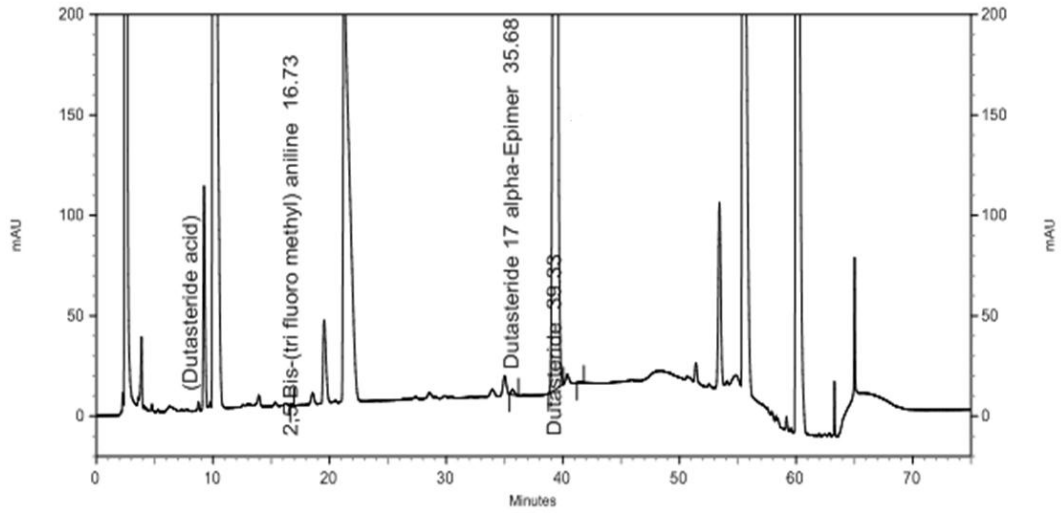

Fig. 5c: Chromatogram of peroxide stressed capsule

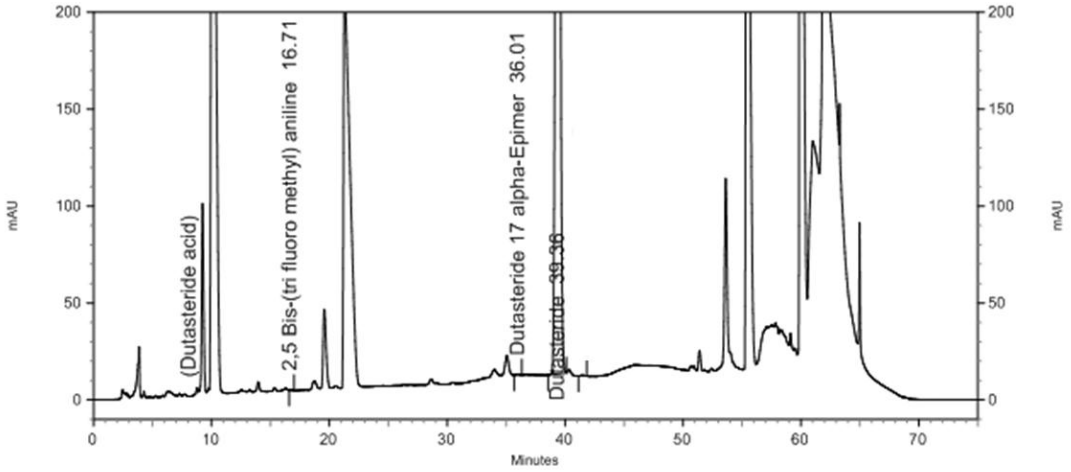

Fig. 5d: Chromatogram of photo stressed capsule

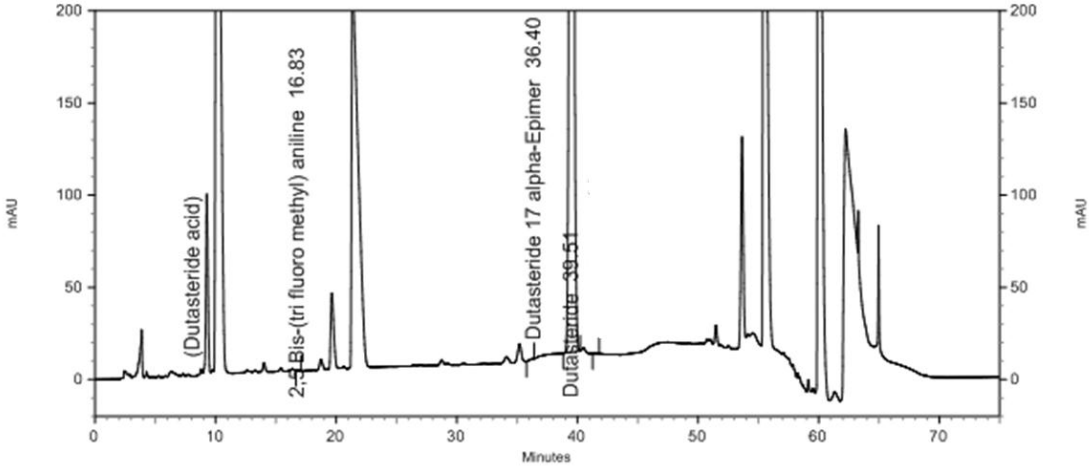

Fig. 5e: Chromatogram of thermal stressed capsule

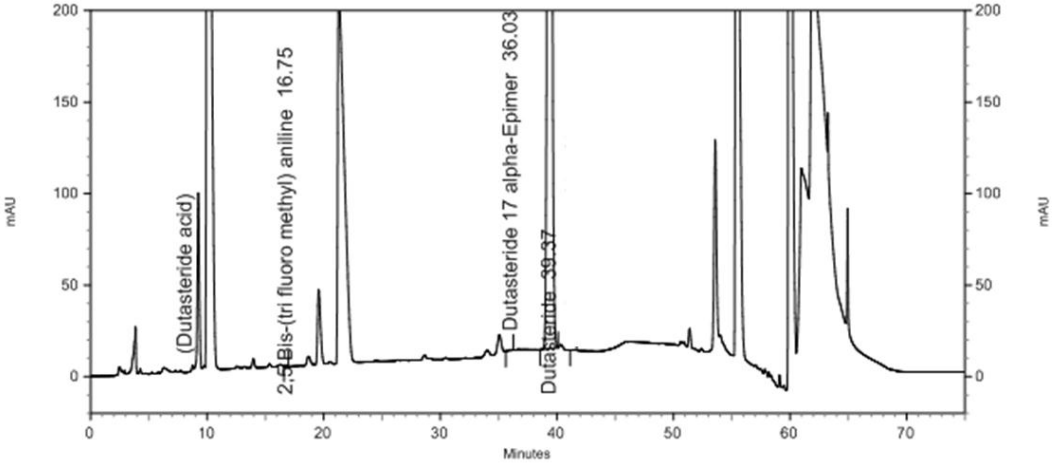

Fig. 5f: Chromatogram of humidity stressed capsule

REFERENCES

1. Dutasteride, Drug bank, Accessed on July 2019. Available at: https://www.drugbank.ca/drugs/DB01126
2. Dutasteride, Pubchem, Accessed on July 2019. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/Dutasteride
3. Kim EH, Brockman JA and Andriole GL. The use of 5-alpha reductase inhibitors in the treatment of benign prostatic hyperplasia. Asian J Urol. 2018;5(1):28–32.
4. Hirshburg JM, Kelsey PA, Therrien CA, Gavino AC and Reichenberg JS. Adverse effects and safety of 5-alpha reductase inhibitors (Finasteride, Dutasteride): A Systematic Review. J Clin Aesthet Dermatol. 2016;9(7):56–62.
5. Ehdaie B and Touijer KA. 5-alpha reductase inhibitiors in prostate cancer: from clinical trials to clinical practice. Eur Urol. 2013;63(5):788–791.
6. Traish A, Haider KS, Doros G and Haider A. Long-term dutasteride therapy in men with benign prostatic hyperplasia alters glucose and lipid profiles and increases severity of erectile dysfunction. Horm Mol Biol Clin Investig. 2017;30 (3): pii: /j/hmbci.2017.30.issue-3/hmbci-2017-0015/hmbci-2017-0015.xmL.
7. Shanshanwal SJ and Dhurat RS. Superiority of dutasteride over finasteride in hair regrowth and reversal of miniaturization in men with androgenetic alopecia: A randomized controlled open-label, evaluator-blinded study. Indian J Dermatol, Venereol Leprol. 2017;83(1):47-54.
8. Contractor P, Kurani H, Guttikar S and Shrivastav PS. Reliable and sensitive determination of dutasteride in human plasma by liquid chromatography-tandem mass spectrometry. Biomed Chromatogr. 2013;27(9):1168-1176.
9. Ranjani VA, Karthik K, Kumar JP, Kumar KSB and Prabhakar T. A validated method development of dutasteride in human plasma using LC-MS/MS. Int J Pharma Chem Sci. 2013; 2(1):266-272.
10. Myung JK, Ha RC, Dong HL, Dong WY, Young WC and Yong SC. A method to monitor dutasteride in rat plasma using liquid-liquid extraction and multiple reaction monitoring: comparisons and validation. Mass Spectr Lett. 2014;5(3);79–83.
11. Dipti BP, Natubhai JP, Sejal KP and Paresh UP. Validated stability indicating HPTLC method for the determination of dutasteride in pharmaceutical dosage forms. Chromatogr Res Int 2011; 2011: Article ID 278923, 5 pages.
12. Kamila MM, Mondal N and Ghosh LK. A validated spectrophotometric method for determination of dutasteride in bulk drug and pharmaceutical formulations. Int J PharmTech Res. 2010;2(1): 113-117.
13. Amin Md R, Moynul H, Al Masud A, Hanifuddin Md, Hasanuzzaman Md and Islam Md K, validated UV spectrophotometric method for estimation of dutasteride in tablet dosage form. Pharmacie Globale. Int J Compre Pharma. 2011;2(4):1-3.
14. Patel DB, Patel NJ, Patel SK, Prajapati AM and Patel SA. RP-HPLC method for the estimation of dutasteride in tablet dosage form. Indian J Pharma Sci. 2010;72(1):113-116.
15. Navaneeswari R and Reddy PR. Development and validation of a RP-HPLC method for dutasteride and its impurities in bulk drug. African J Sci Res. 2011;6(1):318-324.
16. Reddy YK, Reddy GVS, Veera KNJ and Hotha LL. A stability indicating UPLC method for dutasteride and its related impurities. American J PharmTech Res. 2012; 2(6): 702-715.
17. International Conference on Harmonization (ICH) of technical requirements for the registration of pharmaceutical for human use stability testing of new drugs substance and products Q1A (R2). 2003.
18. International Conference on Harmonization, ICH Guidelines, Validation of analytical procedures technical requirements for registration of pharmaceuticals for human use: Text and Methodology Q 2 (R1), International Conference on Harmonization, Geneva, Switzerland, 2005.