Analytical Method Development And Validation Of Finasteride By Using RP- HPLC

Chaitanya Pathak[1], Amit Kasabe[1], Sujit Kakade[1], Vaibhav Shilimkar[1], Ashok Bhosale[1], Dhairysheel Ghadge[2]

[1]PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune, Maharashtra, India, [2]Gourishankar Institute of Pharmaceutical Education & Research, Limb, Satara, Maharashtra, India
Corresponding author:
Mr. Chaitanya Dashrath Pathak
Department of Pharmaceutical Quality Assurance,
PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre,
Kharadi, Pune, Maharashtra, India
Email- chaitanyapathak2001@gmail.com

Keyword
Finasteride, reverse phase HPLC, validation

ABSTRACT
In case of Benign prostatic hyperplasia (BPH) for men with an enlarged prostate, finasteride is used to reduce the size of the prostate potentially prevent surgery. Finasteride tablets are used for the treatment of androgenetic alopecia, or male-pattern hair loss, with the aim to gradually promote hair growth and volume. High-performance liquid chromatography (RP-HPLC) was utilized to develop a rapid and specific stability indicating technique for finasteride detection. Separation was carried out by using a Acetonitrile mobile phase consisting of 0.01M Potassium phosphate monohydrate buffer (KH2PO4) pH 3.50 buffer: Acetonitrile in the ratio of 90:10. C18, (150×4.6) mm, 5µm, 1.0 ml/min flow rate, and 25°C temperature were utilized in the column. Finasteride was eluted at approximately 6.5 minutes after the detection, it was performed at 222 nm. Specificity, precision, linearity, accuracy, repeatability, and robustness were all analyzed according with ICH recommendations. It was found that the calibration curves for the concentration ranges of 10–60 ppm were linear. It was found that the drug's typically working curve equation was y = 25126x + 65930, with a correlation value of R^2 = 0.9864. The method's accuracy and precision are within an acceptable range. The results of the study indicated the validated method's simplicity and accuracy, confirming its efficacy for finasteride measurement.

INTRODUCTION

Finasteride is a medication used to treat pattern hair loss and Benign Prostatic Hyperlasia (BPH) in men. In case female it also used to promote excessive hair growth. Finasteride is a 5 ∝ reductase inhibitor. Finasteride is mostly taken oral route but there are topical formulation is also available for patients with hair losss, designed to minimize systemic exposure by acting specifically on hair follicles. It is also used to prostate cancer as well as enlarged of prostate. IUPAC name of Finasteride is (9aR,11aS)-N-tert-butyl-9a,11a-dimethyl-7-oxo-1,2,3,3a,3b,4,5,5a,6,9b,10,11-dodecahydroindeno[5,4-f]quinoline-1-carboxamide. The molecular formula of Finasteride is C23H36N202 and its molecular weight is 372.549 g/mol.

.
.

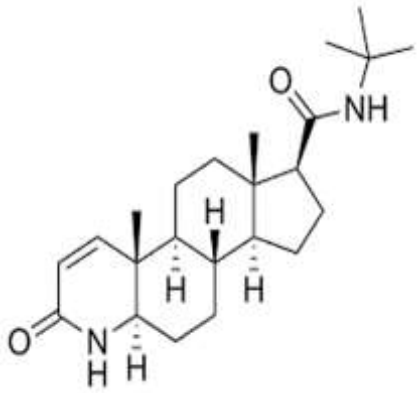

Fig. No. 1 Chemical Structure of Finasteride

Finasteride is white crystalline powder and it is soluble in chloroform, ethanol, methanol and n-propanol. It is 5 ∝ Reductase Inhibitor and also inhibits 5-alpha reductase enzymes which converts testosterone into dihydrotestosterone (DHT). Finasteride undergoes extensive metabolism in the liver (hepatic metabolism) via the cytochrome P450 enzyme system. Finasteride is store in room temperature and away from excessive heat and moisture. Half life of Finasteride is 6 to 8 hours. The drug is excretion through the renal. Food decreases slightly absorption. The side effects of the drugs are Anaphylaxis (Serious allergic reaction), Breast Cancer, Dizziness, Depression, Back Pain etc.

MECHANISM OF ACTION

The nuclear-bound steroid intracellular enzyme Type II 5α-reductase, which typically occurs in the prostatic stromal cell, is effectively and specifically reduced by finasteride. The enzyme transfers the androgen testosterone into the more active metabolite 5α-dihydrotestosterone (DHT). The main androgen considered to be involved in the growth and development of the prostate gland is DHT. As it builds up inside the prostate gland, it acts as the hormonal mediator for the hyperplasia. DHT exhibits a greater affinity for the prostate gland's androgen receptors. By influencing these receptors, DHT affects genes that regulate cell division.. The type II 5α-reductase isozyme, which is mostly located in the liver, prostate, seminal vesicles, epididymides, and hair follicles, is responsible for producing DHT when combined with type I 5α-reductase. Despite finasteride's 100-fold greater selectivity for type II 5α-reductase compared to the type I isoenzyme, long-term use of the medication may have some impact on type I 5α-reductase, which is primarily expressed in the liver and sebaceous glands in the majority of skin regions, including the scalp. Finasteride's selective inhibition of Type II 5α-reductase through the formation of a stable complex with the enzyme in both vitro and in vivo its basis for its mode of action. Finasteride exhibits a 100-fold selectivity for the human Type II 5α-reductase enzyme compared to the type I enzyme. The peripheral conversion of testosterone to DHT is inhibited by Type II 5α-reductase, which leads to a substantial increase in prostatic testosterone concentrations, a minimal to moderate increase in serum testosterone concentrations, and a significant decrease in tissue and serum DHT concentrations.

As DHT tends to be the primary androgen that induces prostatic growth, a drop in levels of DHT will lead to the prostatic volume to decrease (by about 20–30% after 6–24 months of continuous treatment). It has been suggested that raised DHT levels may result in potentiated prostaglandin D2 transcription, which promotes the growth of prostate cancer cells. Although the exact mechanism of action in males with androgenic alopecia is unknown, finasteride has been demonstrated to lower serum DHT, promote hair regrowth, halt hair loss, and lower scalp DHT concentration to levels seen in the hairy scalp.

According to another study, by inhibiting the prostate's vascular endothelial growth factor (VEGF) form working properly, finasteride can decrease bleeding of prostatic origin by causing shrinkage and programmed cell death. Patients who experience idiopathic prostatic bleeding, bleeding after anticoagulation, or bleeding during instrumentation may benefit from the drug's therapeutic effects.

MATERIAL AND METHOD

The standard medication finasteride was purchased from the Aster Analytics laboratory. To ensure accuracy and precision, the analytical study utilized calibrated instruments and high-purity reagents. Acetonitrile and Water filtering tools including PVDF syringe filters and 0.45 µm nylon membrane disc filters were between the reagents.

INSTRUMENTS AND SOFTWARE

The JASCO BS 4600S (ChromNAVCFR Chromatography software Version 2.0) HPLC system with a UV detector was used for chromatographic analysis. Using a JASCO UV- 1800 double-beam spectrophotometer with 10 mm matched quartz cells and Spectra Manager software, UV spectroscopic studies were conducted. A computerized analytical balance (Shimadzu, Japan Model AY 120) was used for the weighing. FT-IR spectrophotometer related study was conducted Shimadzu (FTIR 8400 S).

EXPERIMENTAL WORK

1. Drug Selection :

The finasteride sample was analyzed for its organoleptic characteristics, such as colour, appearance and odour and its melting point was determined using melting point apparatus. A little amount of material was placed into a capillary that was connected to a calibrated thermometer in order to determine the melting point. The assembly was then continuously heated while it was suspended in the paraffin bath. The temperature was recorded as 254°C.

2. UV Spectroscopy and Calibration curve of Finasteride :

The stock solution of Finasteride was prepared methanol the UV spectrum of 10µg/ml solution of Finasteride was taken to determine its absorption maxima.
Accurately weighed 10 mg of the drug was dissolved in 100 ml of methanol (100µg/ml). From the stock solution 1,2,3,4 and 5 ml of solution is pipette out into 10 ml volumetric flasks and volume is made up to 10 ml to form concentration of 10, 20, 30, 40 and 50 µg/ml with methanol. The absorbance values were recorded using UV visible spectrophotometer at 220 nm by taking methanol as a reference solution.

3. FT-IR Spectroscopy :

By using an FT-IR spectrophotometer, the FT-IR spectra of finasteride were taken at a resolution of 4 cm at potassium bromide powder for the purposes of authentication and to examine the primary peaks. The sample was validated by comparing the measured peak to the indicated principal peaks of the infrared spectrum.

PROCEDURE

1. Instrumentation and Chromatographic Conditions

HPLC system used was JASCO system equipped with model BS 4600S RHPLC pump, Rheodyne sample injection port (20 µl), JASCO UV visible detector and ChromNAV CFR chromatography software. Separation was carried out on Phenomenex Luna (250 mm x 4.6 mm, 5 µm) column using mixture of Methanol and water in the ratio of 50:50, v/v as mobile phase at flow rate of 1.0 mL/min. Samples were injected using Rheodyne injector with 20 µL loop. Detection was carried out at 222nm. All weighing was done on Shimadzu balance (Model AY-120).

2. Diluent Preparation

Mobile phase (Acetonitrile and water) used as Diluent.

3. . Preparation of standard Stock Solution

10 mg of Finasteride standard was accurately weighed and transferred into 25ml volumetric flask. 15ml of diluent was added and then sonicated in ultrasonic water bath for 30 minutes. The solution was cooled and volume was made up to the mark with mobile phase. Filtered through 0.45µ syringe filter.

4. Preparation of test solution

10mg of Sample was accurately weighed and transferred into 100 ml volumetric flask. 70 ml of diluent was added and then sonicated in ultrasonic water bath for 30 minutes. The solution was cooled and volume was made up to the mark with diluent. Filtered through 0.45 µ syringe filter.

5. Selection of analytical wavelength

It is the characteristics of a compound which helps to provide the electronic structure of the compound or analyte. The structural analysis of Finasteride was carried out under UV ranging from 200-400 nm using the standard solution.

RESULT AND DISCUSSION

UV SPECTROSCOPIC METHOD

Selection of wavelength

Blank Spectra

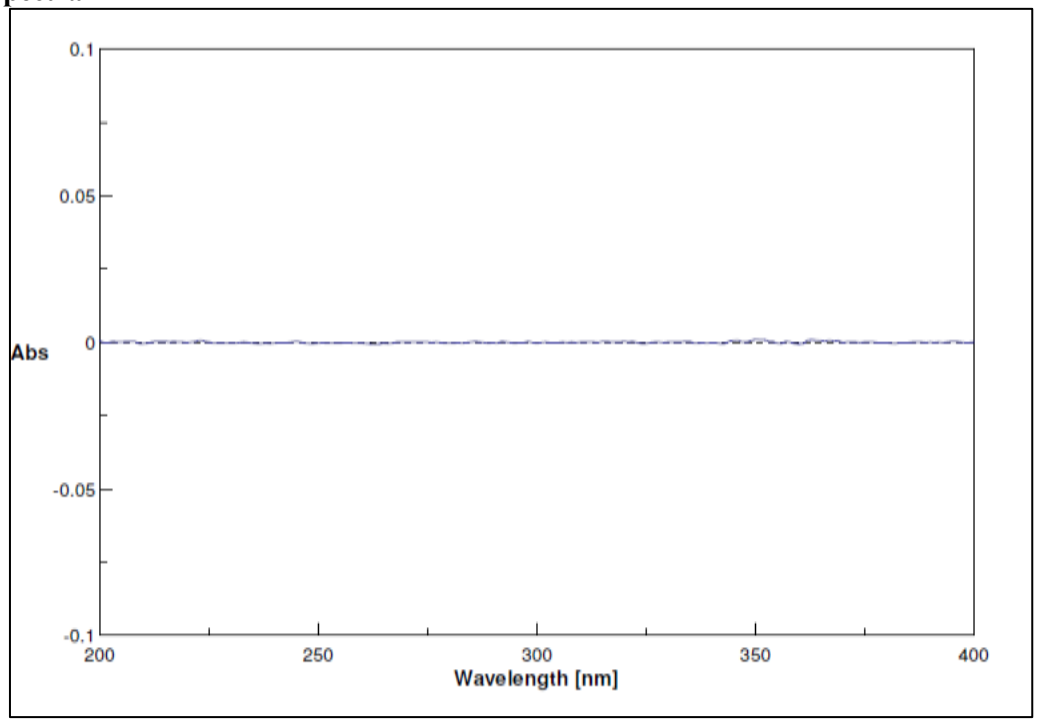

Fig. 2 UV spectrum of Blank

Standard Spectra

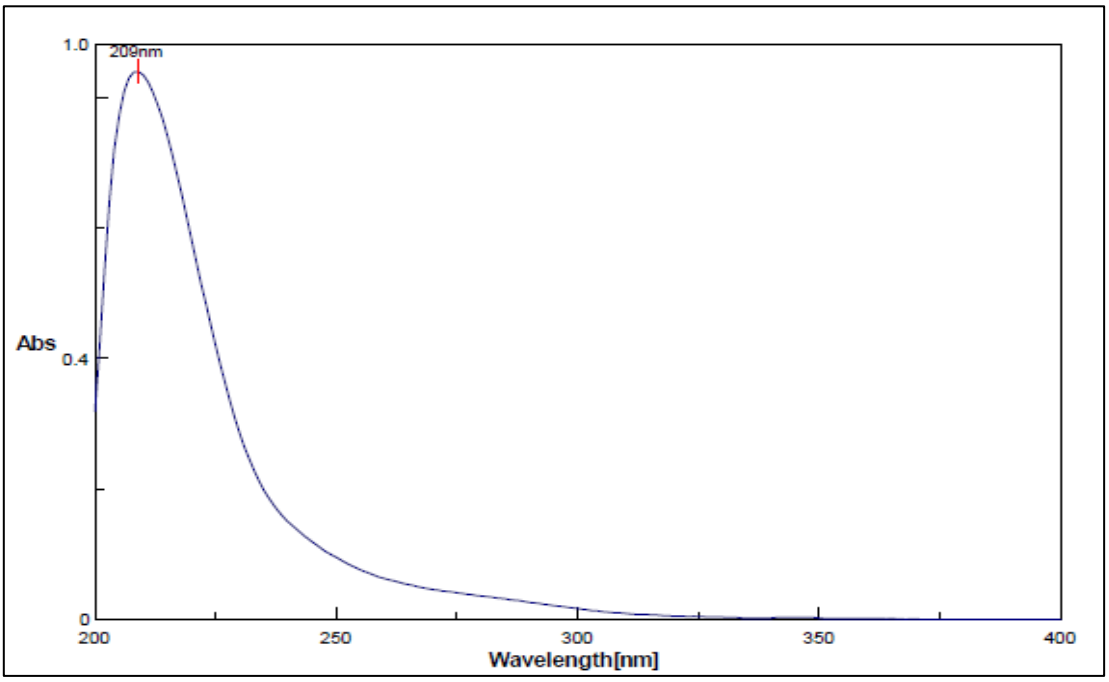

Fig. 3 UV spectrum of Standard Solution

Observation :- The standard solution was scanned from 400 nm to 200 nm. Wavelength of maximum absorption was determined for drug.It showed absorbance at 209 nm. 209 nm considered as an analytical wavelength for further determination.

Sr. No.	Wavelength (nm)	Absorbance
1.	209	0.8334 A^0

Table No: 1 UV Spectroscopic method

Optimized chromatographic condition :-

Sr.No	Parameters	Conditions used for analysis
1	Column	Phenomenex Luna
2	Mobile Phase	90:10 (ACN: Water)
3	Flow rate	1.0 ml/min
4	Detection Wavelength	209 nm
5	Sample injector	(20 µl)
6	Column temperature	25℃
7	Retention time	6.5 to 7 min.

Table No: 2 Optimized chromatographic condition

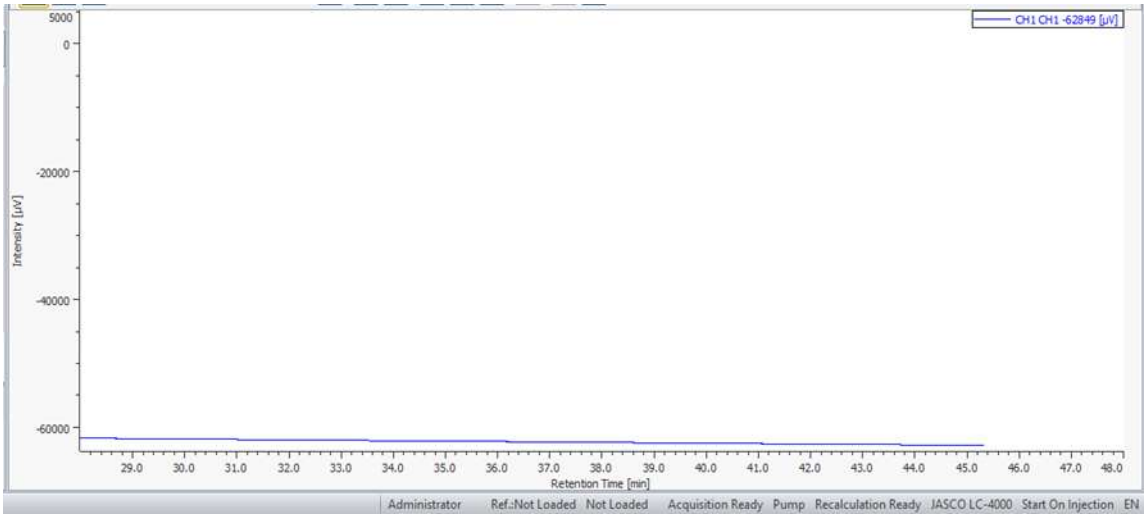

Figure No: 4 HPLC Chromatogram of Blank

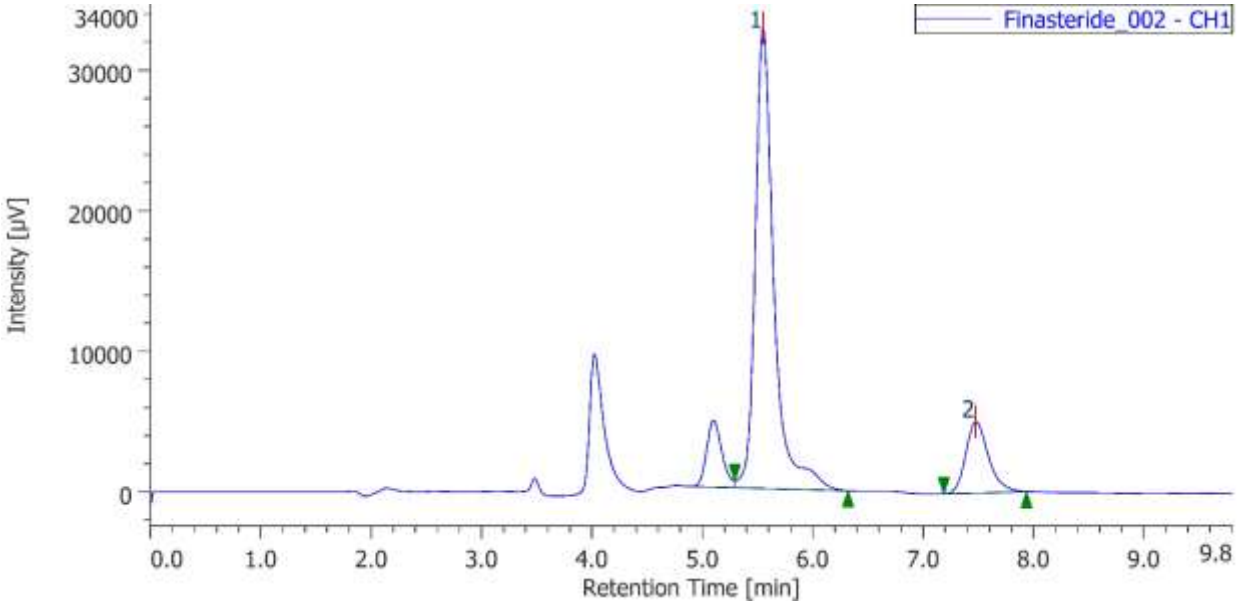

Figure No: 5 HPLC Chromatographic of Standard Finasteride

METHOD VALIDATION

The following parameters were considered for the analytical method validation of title ingredients.

- ➢ System Suitability.
- ➢ Specificity.
- ➢ Linearity.
- ➢ Accuracy.
- ➢ Precision.
- ➢ Method Precision.
- ➢ Intermediate Precision.
- ➢ Robustness.

Characterization of pure drug sample :

Organoleptic Properties :-

Sr. No	Identification	Observation	Inference
1	Appearance	White	Complies with IP
2	Colour	White, colourless	Complies with IP
3	Odour	Odourless	Complies with IP

Table No: 4 Organoleptic Properties of Finasteride

Organoleptic characters reveals that sample of Finasteride obtained complied with standards.

Sr. No	Solvent	Solubility
1	Acetonitrile	Highly soluble
2	Water	Highly soluble
3	Chloroform	Slightly soluble

Table No: 5 Solubility of Finasteride

INFRARED SPECTROSCOPIC METHOD

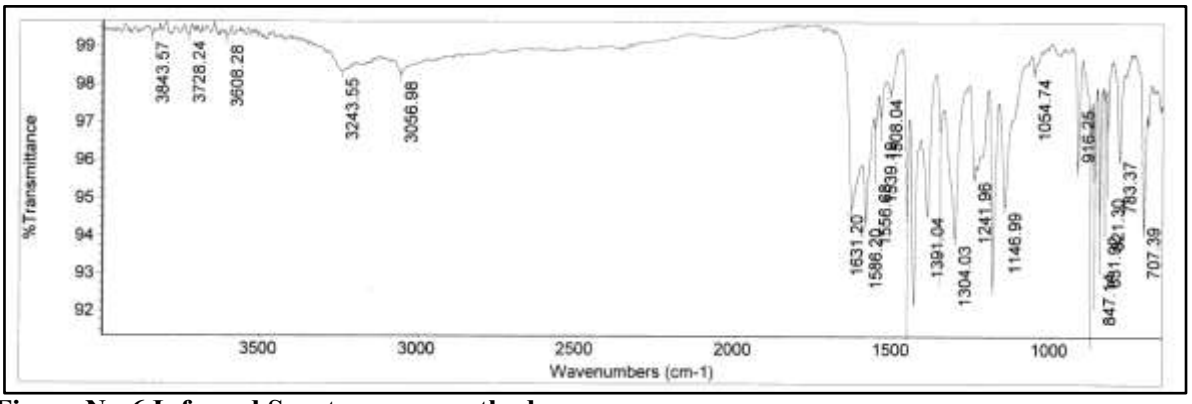

Figure No:6 Infrared Spectroscopy method

Identification of Compound :-

Sr. No	Standard IR Ranges (cm^{-1})	IR Ranges (cm^{-1})	Functional Group
1	3700- 3584	3608.28	O-H Stretching
2	3300- 2700	3243.55	N-H Stretching
3	1650- 1600	1631.20	C=C Stretching
4	1450- 1375	1391.04	C-H Bending

Table No.6 Functional group present in Finasteride
Differential Scanning Calorimetry (DSC) :-

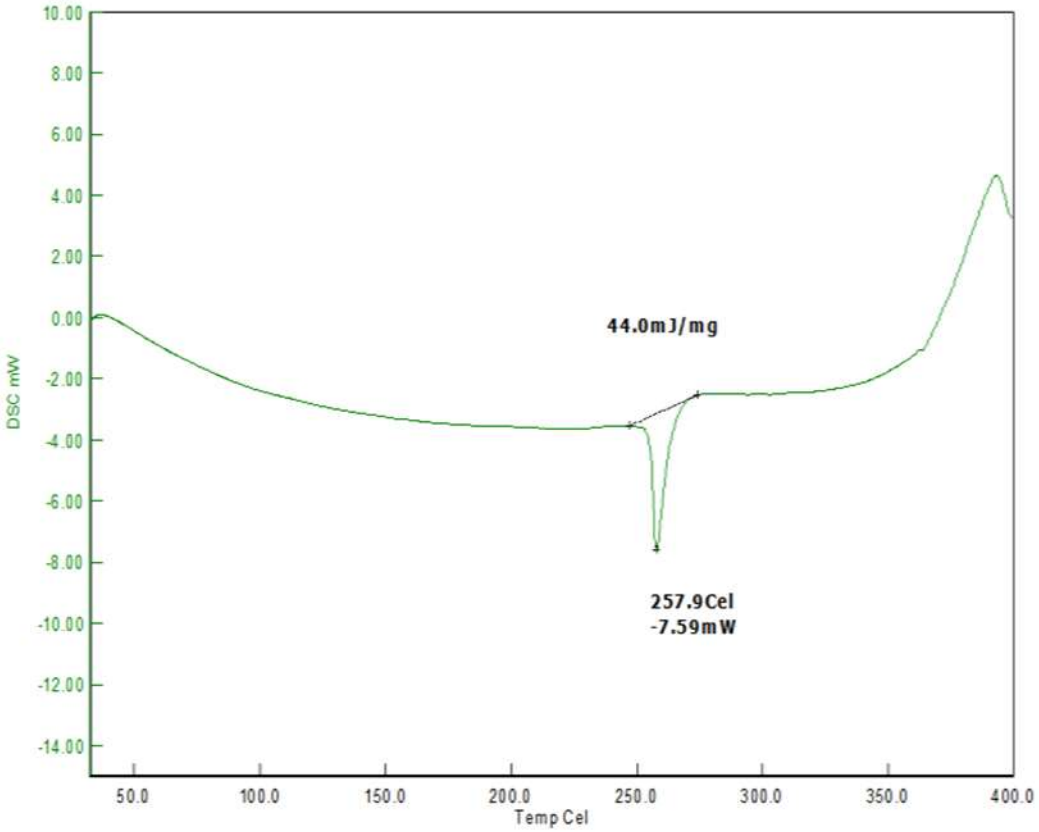

Fig.No.7 Differential Scanning Calorimetry

Melting Point :-

Sr. No	Reported Value	Observed Value
1	252-258℃	257℃

Table No. 6 Melting point of Finasteride

Melting point reveals that the sample of Finasteride obtained was complied with standards.

Area under curve (AUC) of Finasteride :-

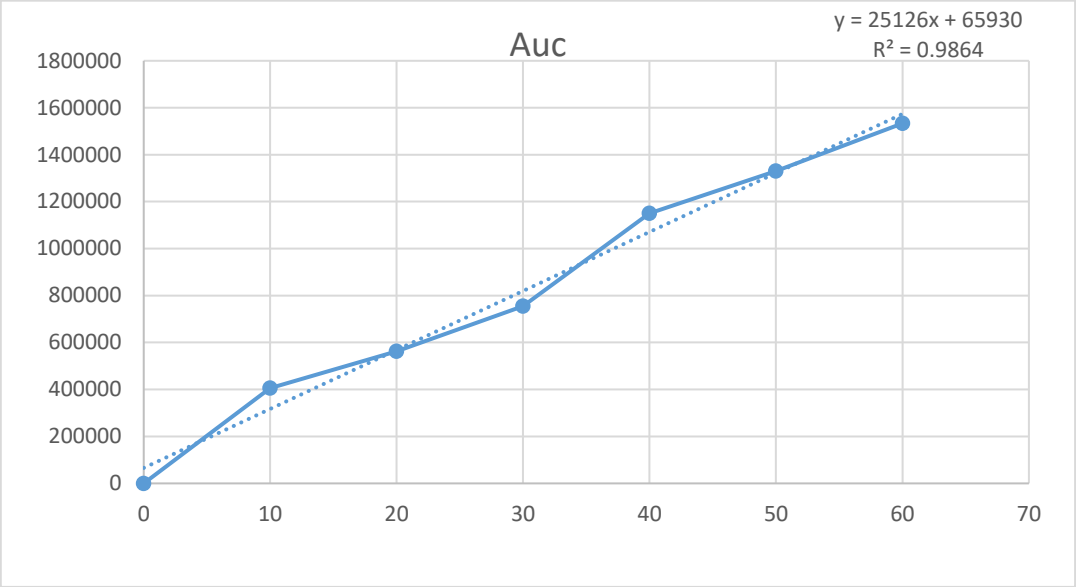

Figure No: 8 Area under curve of Finasteride

Sr. No	Concentration (ppm)	Area under curve (AUC)
1	0	0
2	10	406124
3	20	562884
4	30	755384
5	40	1149958
6	50	1330332
7	60	1533242

Table No. 7 Area under curve (AUC) of Finasteride

Recovery Studies : Accuracy was determined from recovery studies.

The mean % recovery at 80, 100, 120 % of the test concentration along with its statistical validation of drug Finasteride given in table. The % recovery at 80,100, 120 % is given below. It was confirmed that the developed method was accurate as the percent recovery was in the range of 100 %.

Level (%)	% Recovery	% Average Recovery
80	101.331	99.590
80	99.343	
80	98.097	
100	101.470	99.837
100	100.89	
100	97.153	
120	102.530	102.153

120	101.766	
120	102.165	

Table No.8 Recovery data of Finasteride

- **System Precision :-**

Inject No.	**Peak Area of Finasteride**
1	4143086
2	4131447
3	4165464
4	4162547
5	4170263
Average Area	**4154561.4**
% RSD	**0.39 %**

Table No. 9 System Precision of Finasteride

Method precision :-

Inject No.	**% Assay of Sample**
1	96.252
2	96.107
3	96.938
4	96.544
5	97.205
6	97.107
Average Assay	**96.692**
% RSD	**0.47 %**

Table No. 10 Method Precision of Finasteride

Intermediate precision

Inject No.	**Assay (% W/W, analysis-1)**	**Assay (% W/W, analysis-2)**
1	96.252	96.111
2	96.107	96.996
3	96.938	96.501
4	96.544	93.819
5	97.205	96.272
6	97.107	95.483
Average Assay	**96.692**	**95.8636**
SD	**0.4590**	**1.1173**
% RSD	**0.47**	**1.17**
Overall % RSD	**0.83**	

Table No.11 Intermediate Precision of Finasteride

- **Robustness**

Robustness of method was measured by multiple injections of a homogenous sample containing Finasteride by changing flow rate 0.9 ml/min, 1.0 ml/min and 1.1 ml/min, wavelength i.e 217 nm, 222 nm, and 227 nm. The method was found to be Robust in the range of deliberate changes method.

Change in Wavelength (nm)			
	218	**220**	**222**
1	1175247	79023	1095874
2	1154784	80115	1125487
3	1165245	78766	1126548
Mean	**1165092**	**79301.3**	**111596**
SD	**10232.3**	**716.3**	**17411.4**
% RSD	**0.87**	**0.90**	**1.56**

Table No. 12 Robustness study with change in wavelength of Finasteride

Change in Flow (min)			
	0.9	**1.0**	**1.1**
1	59029	1145781	1102584
2	58660	1145784	1121457
3	59261	1142154	1113652
Mean	**58983.3**	**1144573**	**1112564**
SD	**303.1**	**2094.9**	**9483.3**
% RSD	**0.51**	**0.18**	**0.85**

Table No. 13 Robustness study with change in flow rate of Finasteride

Conclusion :-

- A RP-HPLC method for estimation of Finasteride was developed employing Acetonitrile (ACN): Water as Mobile phase and Flow rate was set at 1.0 ml/min with UV detection at 220 nm.
- The development was found to be Precise, Accurate and linear over concentration range tested with correlation coefficients of 0.9864.
- Thus proposed method is rapid selective require simple, preparation procedure and represent good procedure for analysis of Finasteride.
- The method proved to be convenient and effective for quality control of Finasteride in bulk form.

References :-

1. Amer SM. 2003, Polarographic behavior and determination of Finasteride. Farmaco feb; 58(2):159-63
2. ICH Q2(R1). Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonisation, 2005.
3. Foye William O 1989, Principles of medicinal chemistry, 3rd Edn., Varghese Bombay 802.
4. Bakshi M, Singh S. Development of validated stability-indicating methods. J Pharm Biomed Anal. 2002;28(6):1011–1040.Rudra A, Chatterjee S, Sengupta S, Wankhede R, Nandi B, Maitra G, Mitra J. Management of obstetric hemorrhage. Middle East J Anaesthesiol 2010;20:499-507.
5. Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. 6th ed.
6. Barrish A Olah TV, Gilbert JD, Gerber TF, Meloughlin DA. 1994, A. sensitive and specific assay of Finastride based on combined LC-MS/MS, J.Pharm Biomed anal May 12(5):705—12.