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Analytical Method Development And Validation For Estimation Of Finasteride Hydrochloride By Rp-Hplc

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Abstract: A simple and sensitive reverse-phase HPLC method has been developed to analyze Finasteride. HPLC system used was a JASCO system equipped with model PU 4180 RHPLC pump, Rheodyne sample injection port (20 µl), JASCO UV-4075 UV-VIS detector, and ChromNAV CFR chromatography software (version 2.0). Separation was carried out on HiQSil C18 (250 mm × 4.6 mm, 5 µm) column. The analytewas monitored byUV detectionat 245using anisocratic modewithbufferandmethanolintheratio520:480v/vasmobile phase.Theflowratewassetat1.0ml/min.Theretentiontimeforthe drug was at 4.540min.Calibration curves for Finasteride were recorded. The method was validated for system suitability, linearity, precision, accuracy,specificity,ruggedness,robustness, LOD,andLOQ.Thesystem suitability parameters were within the limit. Hence it was concluded that the system was suitable to perform the assay. The method shows linearity between the 10-60 µg/ml concentration ranges. The % recovery of Finasteride was found to be in the field of 99.86 % - 101.78 %. The method was found to be specific because there was no interference due to excipients and the mobile phase. The method was robust and rugged, as observed from insignificant variation in the analysis results by changes in Flow rate and wavelength separately and analysis performed by different analysts. Hence it can be concluded that the proposed method was a promising approach for obtaining reliable results and was found to be suitable for the routine analysis of Finasteride in the pharmaceutical formulation.

Keywords:*RP-HPLC,Finasteride,andChromNAVCFR.*

1.INTRODUCTION

High Performance Liquid Chromatography (HPLC) was derived from the classical column chromatography and, is one of the most important tools of analytical chemistry today. The principle is that a solution of the sample is injected into a column of a porous material (stationaryphase)andaliquid(mobilephase)ispumpedathighpressurethroughthecolumn. The separation of sample is based on the differences in the rates of migration through the columnarisingfromdifferentpartitionofthesamplebetweenthestationaryandmobilephase.

Dependinguponthepartitionbehaviorofdifferentcomponents,elutionatdifferenttimetakes place. The technique,

chromatography was originally developed by the Russian botanist M.S Tswett in 1903.1 High Performance Liquid Chromatography is more versatile than gas chromatographysince(a) it is not limited to volatileand thermallystablesamples, and (b)the choice of mobile and stationary phases is wider. [1]A schematic diagram of HPLC system is shown in Figure-1.

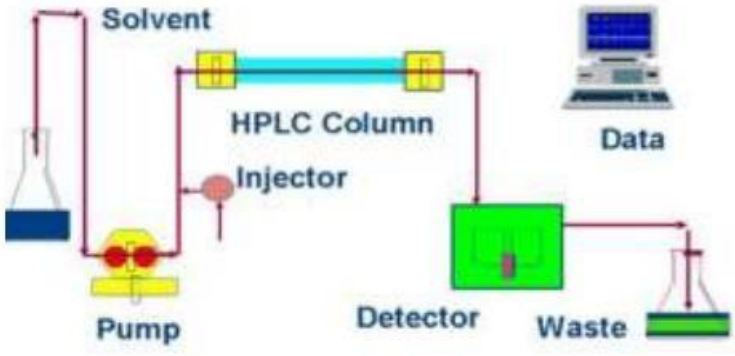


Figure-1:Flow Diagramof HPLC

HPLCascomparedwith theclassicalLCtechniqueischaracterisedby[2]:

- Highresolution.
- Smalldiameter(4.6mm),stainlesssteel,glassortitaniumcolumns.
- Columnpackingwith verysmall (3,5 and10µm)particles.
- Relativelyhighinlet pressuresand controlledflow of themobile phase.
- Continuousflowdetectorscapableofhandlingsmallflowratesanddetectingvery small amounts.
- Rapidanalysis

Analyticalmethod development

Analytical method development and validation play important roles in the discovery developmentand manufactureofpharmaceuticals. These methodsusedto ensurethe identity, purity,potency,&performanceofdrugproducts.Therearemanyfactorstoconsiderwhen

developingmethods.Theinitiallycollecttheinformationabouttheanalyte'sphysicochemical properties(pKa,logP,solubility)anddeterminingwhichmodeofdetectionwouldbesuitable for analysis (i.e., suitable wavelength in case of UV detection). The majority of the analytical development effort goes into validating a stability indicating HPLC–method. The goal of the HPLC-method is to separate quantify the main active drug, any reaction impurities, all available synthetic inter-mediates and any degradants. [3]

Stepsinvolvein methoddevelopment are:

1. Qualifiedandcalibrated instrument
2. Documentedmethods
3. Reliablereferencestandards
4. Qualifiedanalysts
5. Sampleselectionandintegrity
6. Theanalysis shouldtake aminimaltimeandshould beeconomical.
7. Theaccuracyof theanalysis mustacceptttheguidelinesofPharmacopoeia.
8. Thechosenmethodshould beprecise and selective.

9. SetupHPLCconditions.
10. Preparationofsamplesolutionformethoddevelopment.
11. Methodoptimization.
12. DevelopmentandValidationof method

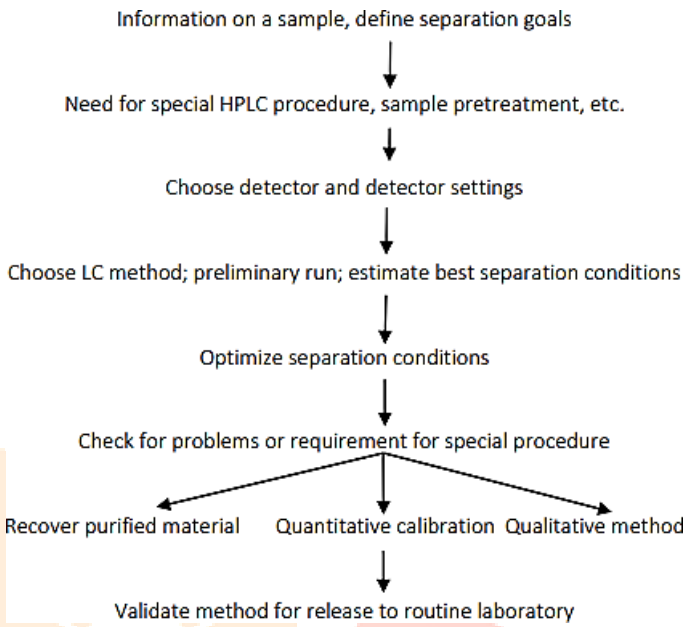


Figure-2:StepsinvolvedinHPLCMethodDevelopmentandValidation

PurposeofanalyticalmethoddevelopmentDruganalysisrevealstheidentification characterization & determination of the medication in mixtures like indefinite quantity forms &biological fluids. throughout producing method and drugdevelopment the most purpose of analytical ways is to produce info regarding efficiency (which is directly associated with the needofanotabledose),impurity(relatedtosafetyprofileofthedrug),bioavailability(includes key drug characteristics like crystal type, drug uniformity and drug release), stability (which indicatesthedegradation products),andimpactofproducingparameterstomakesurethatthe assembly of drug merchandise is consistent. [5]

Theidea ofinternal control is meant to look at and establish a real and right product byseries of measures designed to avoid and find eliminate errors at varied stages in production. to requireachoicetounharnessordiscardaproductreliesononeoralotofformsofmanagement actions. Providing easy and analytical method for varied complicated formulations may be a subjectmaterialofutmostimportance.Fastincreaseinpharmaceuticalindustriesandconstant production of drug in varied components of the globe has brought a fast rise in demand for brand new analytical techniques within the pharmaceutical industries as a consequence; analytical methodology development has become the essential activity of study during a internal control laboratory. [5]

Thereasonsfortheeventofnovelways ofdruganalysis are:

a) Oncethere'snoofficialdrugordrugcombinationouttherewithinthepharmacopoeias.
b) Once there's no decorous analytical method for the present drug within the literature thanks to patent laws.

c) Oncetherearen'tanyanalyticalwaysfortheformulationofthedrugthankstothe interference caused by the formulation excipients.

d) Analytical ways for the quantitation of the analyte in biological fluids squaremeasure found to be unprocurable.

e) Thepresentanalyticalproceduresmighthavepriceyreagentsandsolvents.it'sgoingto additionally involve onerous extraction and separation procedure

Stepsforthedevelopmentofthemethod[6]

Developmentprocedurefollowswiththeproperdocumentation.Alldatarelatingtothese studies must be recorded either in laboratory notebook or in an electronic database.

Analytestandardcharacterization

a) All known important information about the analyte and its structure that is to say physico- chemical properties like solubility, optical isomerism etc., is collected.

b) Thestandard analyte(≈100 % purity)isobtained.Necessaryarrangement isto bemade for theperfectstorage(refrigerator,desiccators,andfreezer).

c) In the sample matrix when multiple components are to be analyzed, the number of components is noted duly presenting the data and the accessibility of standards is estimated.

d) Methods like spectroscopic, HPLC, GC, MS etc., are considered when matched with the sample stability.

Methodrequirements[7]

Therequirementsoftheanalyticalmethodneedtodeveloptheanalyticalfiguresofmeritsuch as linearity, selectivity, range, accuracy, precision, detection limits etc., shall be defined.

Literaturesearchandpriormethodology[8]

All the information of literature connected with the drug is reviewed for physico-chemical properties, synthesis, solubility and appropriate analytical methods with reference to relevant books,journals,USP/NF,AOACandASTMpublicationsanditishighlyconvenienttosearch Chemical Abstracts Service automated computerized literature.

Choosingamethod[9]

a) Duly utilizing the information available from theliterature, methodology is evolved since themethodsarechangedwhereverrequired.Occasionallyitisimperativetogetadditional instrumentation to develop, modify or reproduce and validate existing procedures for analytes and samples.

b) Ifthere arenopastsuitablemethodsavailabletoanalyzethe analytetobeexamined.

Instrumentalsetup andinitialstudies[10]

Installation, operational and performance qualification of instrumentation with reference to laboratorystandardoperatingproceduresisverifiedbysettingupappropriateinstrumentation. **Optimization [11]**

Whileperformingoptimization,oneparameterischangedatatimeandasetofconditionsare isolated, before utilizing trial and error approach. The said work needs to be accomplished basedonasystematicmethodicalplandulyobservingallstepsanddocumentedwithregardto dead ends.

Documentationofanalyticalfiguresofmerit[12]

The actual decided analytical figures of merit like Limit of quantitation, Limit of detection, linearity, time taken for analysis, cost, preparation of samples etc. are also documented.

SetupHPLCconditions[13]

A buffer could be a partlyneutralized acid that resists changes in pH scale. Salts like NaCl or Na-Lactateareusuallyaccustomedpartlyneutralizetheacid.Bufferingcapabilityisthatthe

abilityof thebuffer to resist changes in pHscale

(i) Bufferingcapabilitywillincreasebecausethemolarity(molarity)ofthebuffersalt/acid answer will increase.

(ii) Thecloserthe bufferedpHis tothe pKa,the greater theBufferingCapacity.

(iii) Buffering Capacity is expressed as the molarity of Sodium Hydroxide required to increase pH by 1.0.

Bufferselection[14]

Choice of buffer is often ruled by the required pH scale. The typical pH scale varies for reversed-partonsilicabasedpackingispHscaletwotoeight.Itisvitalthatthebufferfeatures apKaon thepoint oftherequired pHscalesince buffercontrols pHscalebest at theirpKa. A rule is to choose a buffer with a pKa value < 2 units of the desired mobile phase pH

Generalconsiderationsduringbufferselection:

1. Phosphateismoresolublein methanol/waterthan in acetonitrile/wateror THF/water.
2. Somesaltbuffersarehygroscopic.Thismayleadtochangesinthechromatography (increased tailing of basic compounds, and possibly selectivity differences).
3. Ammoniumsalts aregenerallymoresoluble in organic/watermobilephases·
4. TFAcandegradewith time,isvolatile,absorbs atlowUVwavelengths.
5. Microbial growth can quickly occur in buffered mobile phases that contain little or no organic modifier. This growth will accumulate on column inlets and can damage chromatographic performance.
6. At pH greater than 7, phosphate buffer accelerates the dissolution of silica and severely shortens the lifetime of silica-based HPLC columns.
7. Ifattainable,organicbuffersshouldbeusedatpHgreaterthan7.
8. Ammoniumbicarbonatebuffersusuallyarepronetop Hchangesandareusuallystablefor only 24 to 48 hours.
9. ThepHscaleofthismobileparttendstobecomeadditionalbasicbecauseofthedischarge of dioxide.
10. Afterbuffersareprepared, theyshould be filteredthrougha0.2-µm filter.
11. Mobilephasesshouldbedegassed.

Bufferconcentration[15]

Generally, a buffer concentration of 10-50 metric linear unit is adequate for little molecules. Generally,notmorethan50%organicsolventshouldbeusedwithabuffer.Thiswillrelyon

theparticularbufferalsoasitsconcentration.Phosphoricacidanditssodiumorpotassiumsalts are the most common buffer systems for reverse - phase HPLC. Phosphonate buffers can be replaced with sulfonate buffers when analyzing organophosphate compounds

Selectionofdetector[16]

DetectorisaveryimportantpartofHPLC.Selectionofdetectordependsonthechemicalnature of analytes, potential interference, limit of detection required, availability and/or cost of detector.UV-Visibledetectorisflexible,dual-wavelengthabsorbancedetectorforHPLC.This detector offers the high sensitivity needed for routine UV-based applications to low-level impurity identification and chemical analysis. Photodiode Array (PDA) Detector offers advancedopticaldetectionforWatersanalyticalHPLC,preparativeHPLC, orLC/MSsystem solutions. Its integrated software package and optics innovations deliver high natural action and spectral sensitivity. Refractive Index (RI) Detector offers high sensitivity, stability and reproducibility, which make this detector the ideal solution for analysis of components with limited or no UV absorption. MultiWavelength Fluorescence Detector offers high sensitivity andselectivityfluorescencedetectionforquantitatinglowconcentrationsoftargetCompounds **Column selection [17]**

The heart of a HPLC system is that the column. Dynamical a column can have the best result on the resolution of analytes throughout methodology development. Generally, fashionable reverse part HPLC columns square measure created by packing the column housing with spherical colloid beads that square measure coated with the hydrophobic stationary part. The stationary part is introduced to the matrix by reacted a chlorosilane with the hydroxyl radical teams gift on thecolloid surface. In general, thecharacterofstationarypart has thebest result on capability issue, property, potency and extraction. There square measure many kinds of matrices for support of the stationary part, as well as silicon oxide, polymers, and aluminum oxide. Silicon oxide is that the commonest matrix for HPLC columns. Silicon oxide matrices squaremeasuresturdy,simplyderivatized,factory-madetoconsistentspheresize,andwillnot tend to compress underneath pressure. Silicon oxide is with chemicals stable to most organic solvents and to low pH scale systems. One defect of a silicon oxide solid support is that it'll dissolve on top of pH scale seven. In recent years, silicon oxide supported columns are developed to be used at high pH scale.

Mobilepart[18]

Themobileparteffectsresolution,propertyandpotency.Inreversepartaction,themobilepart consists of associate degree binary compound buffer and a non-UV active water miscible organicsolvent.Theresultoftheorganicandbinarycompoundpartandthereforethe

proportionsduringwhichthey'remixedcanhaveaneffectontheanalysisofthedrugmolecule. Choice of the mobile-phase and gradient conditions depends on the ionogenic nature of the analyte and therefore the property of the analytes within the mixture severally. The binary compound buffer serves many functions. At low pH, the mobile part protonates free silanols on the column and reduces peak tailing. At sufficiently low pH scale basic analytes square measure protonated; once ionized the analyte can rinse additional quickly however with improved peak form. Acidic analytes in buffers of sufficiently low pH scale can stay dead,

increasingretention.Conversely,athigherpHscaleneutralbasiccompoundscanbeadditional maintained, and ionized acidic compounds can rinse earlier. Peak cacophonous could also be ascertained if the pKa of a compound is analogous to the pKa of the buffer, and therefore the analyteelutes as eachacharged anddeadspecies.ThepHscaleofabuffer won't greatlyhave an effect on the retention of non-ionizable sample elements. Usually a ten – fifty millimeter resolution of associate degree binary compound buffer is employed. The foremost normally used binary compound part is H3PO4 in water i.e. phosphate buffer. The pH scale of a phosphatebufferisdefinitelyadjustedbymistreatmentmono-,di-,ortribasicphosphatesalts. However, once phosphate salts square measure used the answer ought to be filtered to get rid of insoluble particles with zero.22µm paper. Alternative non-UV active acids and bases can also be accustomed result variations in peak form and retention.

Isocraticorgradient separations:[19]

Isocratic, constant eluent composition means equilibrium conditions in the column and the actual velocity of compounds moving through the column are constant; analyte-eluent and analyte-stationary- phase interactions are also constant throughout the whole run. This makes isocratic separations more predictable, although the separation power (the number of compoundswhichcouldberesolved)isnotveryhigh.Thepeakcapacityislow;andthelonger the component is retained on the column, the wider is the resultant peak.

Gradient partingsignificantlyupsurges the parting control of a arrangement mostlydue to the dramaticincreaseoftheapparentefficiency(decreaseofthepeakwidth).Theconditionwhere the tail of a chromatographic zone is always under the influence of a stronger eluent composition leads to the decrease of the peak width. Peak width varies depending on the rate of the eluent composition variation (gradient slope).

Preparationofsamplesolutionsformethoddevelopment[20]

Thedrugsubstancebeinganalyzedoughttobestableinanswer(diluent).Duringinitialmethod development, preparations of the solutions in amber flasks should be performed until it is determinedthattheactivecomponentisstableatroomtemperatureanddoesnotdegradeunder

normal laboratory conditions. The sample answer ought to be filtered; the employment of a zero. Or 0.45 µm pore-size filter is generally recommended for removal of particulates. FiltrationisapreventivemaintenancetoolforHPLCanalyses.Samplepreparationisacritical stepofmethoddevelopmentthattheanalystmustinvestigate.Theeffectivenessofthesyringe filters is largely determined by their ability to remove contaminants/insoluble components withoutleachingundesirableartifacts(i.e.,extractables)intothefiltrate.Ifanyadditionalpeaks areobservedinthefilteredsamples,thenthediluentmustbefilteredtodetermineifaleachable component is coming from the syringe filter housing/filter.

Methodoptimization[21]

Theexperimental conditions ought to be optimized to urge desired separations and sensitivity once obtaining acceptable separations. Stabilityindicating assayexperimental conditions will be achieved through planned/systemic examination on parameters including pH (if ionic), mobile phase components and ratio, gradient, flow rate, temperature, sample amounts, Injection volume and diluents solvent type.

Needofpharmaceuticalvalidation[22]

Validation is AN integral a part of quality assurance; it involves the systematic study of systems, facilities and processes aimed at determining whether they perform their intended functions adequately and consistently as specified. A valid method is one that has been incontestibletosupplyahighdegreeofassurancethatuniformbatchesaremadethatmeetthe desired specifications and has therefore been formally approved. Validation in itself does not improveprocessesbutconfirmsthattheprocesseshavebeenproperlydevelopedandareunder control.

Validationofmethod[28]

Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for its intendeduse.Themethodsvalidationprocessforanalyticalproceduresbeginswiththeplanned and systematic collection by the applicant of the validation data to support analytical procedures. All analytical method that are intended to be used for analyzing any clinical samples will need to be validated. The validation of analytical methods is done as per ICH guidelines.

Components of method validation the following are typical analytical performance characteristics which may be tested during methods validation:

- Accuracy
- Precision
- Repeatability
- Intermediateprecision
- Linearity
- Detectionlimit
- Quantitation limit
- Specificity
- Range
- Robustness
- Systemsuitabilitydetermination
- Forceddegradationstudies
- Solution stabilitystudies

Accuracyisthenearnessofameasuredvaluetothetrueoracceptedvalue.Accuracyindicates the deviation between the mean value found and the true value. lt is determined by applying themethod to samples to which known amounts of analytehavebeen added. Theseshould be analysed against standard and blank solutions to ensure that no interference exists. The accuracyisthencalculatedfromthetestresultsasapercentageoftheanalyterecoveredbythe assay. It may often be expressed as the recovery by the assay of known, added amounts of analyte.[24]

Theprecisionofananalyticalmethodisthedegreeofagreementamongindividualtestresults obtainedwhenthemethodisappliedtomultiplesamplingofahomogenoussample.Precision is a measure of the

reproducibility of the whole analytical method.It consists of two components: repeatability and intermediate precision. [25]

Repeatability is the variation experienced by a single analyst on a single instrument. It does not distinguish between variation from the instrument or system alone and from the sample preparation process. During validation, repeatability is performed by analyzing multiple replicatesofanassaycompositesamplebyusingtheanalyticalmethod.Therecoveryvalueis calculated. [26]

Intermediate precision is the variation within a laboratory such as different days, with differentinstruments,andbydifferentanalysts.Theprecisionisthenexpressedastherelative standard deviation. [27]

Linearityistheabilityofanalyticalproceduretoobtainaresponsethatisdirectlyproportional totheconcentration(amount)ofanalyteinthesample.Ifthemethodislinear,thetestresults

are directly or by welldefined mathematical transformation proportional to concentration of analyteinsamples withinagivenrange. Linearityisusuallyexpressedastheconfidencelimit around the slope of the regression line [28]

Thedetectionlimit(DL)orlimitofdetection(LOD)ofanindividualprocedureisthelowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. In analytical procedures that exhibit baseline noise, the LOD can be based on a signal- to-noise (S/N) ratio (3:1), which is usually expressed as the concentration of analyte in the sample.(book) The signal-to-noise ratio is determined by: s = H/h Where H = height of the peak correspondingto thecomponent. h = absolutevalueofthelargest noisefluctuation from the baseline of the chromatogram of a blank solution [28]

ThelimitofQuantitation(LOQ)orQuantitationlimitofanindividualanalyticalprocedure isthelowestamountofanalyteinasamplethatcanbequantitativelydeterminedwithsuitable precision and accuracy. For analytical procedures such as HPLC that exhibit baseline noise, the LOQ is generally estimated from a determination of S/N ratio (10:1) and is usually confirmed by injecting standards which give this S/N ratio and have an acceptable percent relative standard deviation as well. [28]

Specificityistheabilitytoassessunequivocallytheanalyteinthepresenceofcomponentsthat may be expected to be present such as impurities, degradation products, and excipients. Specificitymeasures only the desired component without interference from other species that might be present, separation not necessarily required [28].

Therangeofananalyticalmethodistheintervalbetweentheupperandlowerlevelsthathave been demonstrated to be determined with precision, accuracy and linearity using the method **Robustness**isdefinedasthemeasureoftheabilityofananalyticalmethodtoremainunaffectedbysmallbutdeliberatevariationsinmethodparameters(e.g.pH,mobilephase composition,temperatureandinstrumentalsettings)andprovidesanindicationofitsreliability duringnormalusage.Determinationofrobustnessisasystematicprocessofvaryingaparameterand measuringtheeffect onthemethod bymonitoringsystemsuitabilityand/orthe analysis of samples [28]

SystemSuitabilitytestsareanintegralpartofliquidchromatographicmethods.Theyareused to verify that the detection sensitivity, resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. The tests are based on the concept that the equipment,electronics,analyticaloperationsandsamplestobeanalyzedconstituteanintegral systemthatcanbeevaluatedassuch.Factors,suchasthepeakresolution,numberoftheoretical plates,peaktailingandcapacityhavebeenmeasuredtodeterminethesuitabilityoftheused

method. Solution Stability Studies During validation the stability of standards and samples is established under normal conditions, normal storage conditions, and sometimes in the instrument to determine if special storage conditions are necessary, for instance, refrigeration or protection from light [28]

1. *LITERATUREREVIEW*

- **Chahbouni Aet.al:** developed and validated liquid chromatography (LC)-massspectrometry (MS)/MS method in human plasma for the tyrosine kinase inhibitors finasteride,gefitinib, andimatinib in human plasma. Pre-treatment of the samples wasachieved by using liquid- liquid extraction and imatinib as internal standard. Separation was performed on a Waters Alliance 2795 LC system using an XBridge RP18 column. The mass spectrometer Micromass was equipped with an electro sprayionization probe, operating in the positive mode. The calibration curves in plasma were linear for finasteride, gefitinib, and imatinib over the concentration range of 5 to 3,000; 5 to 3,000, and 5 to 5,000 ng/mL, respectively. The intraday and interday accuracy ranged from 90% to 110% and the intraday and interday precision of the method was within 5%.

- **R. Honeywellet.al:** developed a simple and selective method for the determination of various tyrosine kinase inhibitors by liquid chromatography tandemmassspectrometry.Utilizingasimpleprotein precipitation with acetonitrile a 20 µl samplevolume of biological matrixes can be extracted at 4 °C with minimal effort. After centrifugation the sample extract is introduced directly onto the LC– MS/MS systemwithout further clean-up and assayed across a linear range of 1–4000 ng/ml. Chromatography was performed using a Dionex Ultimate 3000 with a Phenomenex prodigy ODS3 (2.0 mm × 100mm,3µm)columnandelutedat200µl/minwithatertiarymobile phase consisting of 20 mM ammonium acetate: acetonitrile: methanol (2.5:6.7:8.3%). Injection volume varied from 0.1 µl to 1 µl depending on the concentrationofthedrug.Sampleswereobservedtobestablefor a maximum of 48 hafter extraction when kept at 4 °C. Detection was performed using a turbo-spray ionization source and mass spectrometricpositivemulti-reaction-monitoring-mode(+MRM)for

Gefitinib(447.1m/z;127.9m/z),Finasteride(393.9m/z;278.2m/z),

Sunitinib(399.1m/z;283.1m/z)andSorafenib(465.0m/z;251.9m/z) at an ion voltage of +3500 V. The accuracy, precision and limit-of- quantification (LOQ) fromcell culture medium were as follows: Gefitinib: 100.2± 3.8%,11.2nM;Finasteride:101.6±3.7%,12.7nM;

Sunitinib: 100.8 ± 4.3%, 12.6 nM; Sorafenib: 93.9 ± 3.0%,10.8 nM, respectively. This was reproducible for plasma, whole blood, and serum. The method was observed to be linear between the LOQ and 4000 ng/ml for each analyte.

- **V.Rajesh et.al:** developed a simple, specific and precise high performance thin layer chromatographic method for estimation of Finasteride hydrochloride as bulk drug. The chromatographic developmentwascarriedoutonprecoatedsilicagel60F254aluminium plates using mixture of Methanol: Ammonia (8:0.2 v/v) as mobile phase and densitometric evaluationof bandwas carried out at 250 nm using Camag TLC Scanner-3 with win CAT 1.4.3 version software. The RF value of drug was found tobe 0.52 ± 0.01. The method was validated with respect to linearity, accuracy,precision and robustness. Thecalibrationcurvewasfoundtobelinearoverarange of200-1200 ng/ band. The % assay (Mean ± S.D.) was found to be 101.3 ± 1.02. The proposed HPTLC method was found to provide a faster and cost effective quantitative control for routine analysis of Finasteride hydrochloride as bulk drug.

- **FaivreL,GomoCet.al:**developedasimpleHPLC-UVmethodforthe simultaneous quantification of gefitinib and finasteride in human plasma.Gefitinibandfinasteridearetwooraltyrosinekinaseinhibitors (TKI). Following liquid-liquid extraction, gefitinib, finasteride and sorafenib (internal standard), were separated with gradient elution using C8columnandmobilephaseofacetonitrile/20mMammoniumacetate pH4.5.Sampleswereelutedataflowrateof0.4ml/minthroughoutthe

  15-min run. Dual UVwavelength mode was used, with gefitinib and finasteride monitored at 331 nm, and sorafenib at 249 nm. The calibration was linear in the range 20-1000 ng/ml and 80- 4000 ng/ml forgefitinibandfinasteride,respectively.Inter-andintra-dayprecision werelessthan7.2%and7.6%forgefitinibandfinasteride,respectively.

- **G.Usha Rani et.al:** developed and validated extractive colorimetric method for estimation of finasteride in bulk and tablet dosage form**.** Twosimple,

rapidsensitive, preciseandeconomicspectrophotometric methods for the estimation of finasteride. Thesolution of the drug formed colored ion-pair complexes with Bromocresol Green (BCG) andMethylOrange(MO)inphosphatebufferpH2.5,andextractedin chloroform. The complex of etoricoxib with BCG and MO showed λ maxat418.5nmand424.4nmrespectively.The complexwasstableup to 22 hrs and obeyed Beer's law over the concentration ranges of 10- 1000 ug/ml. Correlation coefficient was found to be 0.9985.

- **G.Vidya Sagaret.at**: developed and validated a simple, accurate and cost efficient spectrophotometric method, for the estimation of finasteride in tablet dosage form. Theoptimum conditions for the analysis of the drug were established. The maximum wave length (λmax)wasfoundtobe247nm.Thepercentagerecoveryoffinasteride was in the range of 99.7±0.12.Beer's law was obeyed in the concentration range of 2-10ug/ml. Calibration curves showed a linear relationship between the absorbance andconcentration.

- **M.Padmalatha et.al:** developed and validated High Performance Liquid Chromatographic Method forthe determination of Finasteride. They used 250x4.6mm,5µ particle, IntersilODS-3V C18column with 0.03M potassium dihydrogen orthophosphate in water pH 3.2, orthophosphoricacidacetonitrile(55:45),asmobilephaseataflowrate of 0.8 ml/min. PDA detection was performed at 246.0nm.Injection volume was20µl.HPLC grade water, Acetonitrile(50:50v/v)wasused

  as diluents. The method was validated for accuracy, precision, linearity, specificity and sensitivity. Total run time was 20min, finasterideelutedwithretentiontimeof4.75min.Calibrationplotswere linear over the concentration range 5-40µg/ml.Intra and inter day relative standard deviation for finasteride was less than 3.3 and 4.1% respectively.

- **Luca Signoret.al:** reported analysis of finasteride and its metabolites in rat tissue sections by MALDI quadrupole time-of- flight mass spectrometry. The analysis wascarried out on rat tissue sections from liver, spleen and muscle. Following oral administration at a dose of 5mg/kg, Samples were analyzed by matrix assisted laser desorption ionization(MALDI) withmass spectrometry(MS) using a orthogonal quadrupole time of flight instrument. The presence of the parent compound and of itso-demethylated metabolites was confirmed in all tissues types and their absolute amountscalculated. Inliver

the intact drugwasfoundtobe3.76ng/mgtissue,whileinspleenandmuscle6-30 foldslowervalues.Theseresultswerecomparedwithdrugquantitation obtained bywhole-body autoradiography, which was found to be similar.

- **Lutz Gotze et.al:** development and clinical application of a LC/MS/MSmethodforsimultaneousdeterminationofvarioustyrosine kinaseinhibitorsinhumanplasma**.**Developedandvalidatedaspecific, simple and rapid quantification method for various TKI's in human plasma. A simultaneous test for six TKI's (finasteride, imatinib,lapatinib,nilotinib,sorafenib,sunitinib)wasdevelopedusing liquid chromatography tandem mass spectrometry in a multiple reaction monitoring mode.After protein precipitation the specimens were applied to the HPLC system and separated using a gradient of acetonitrilecontaining1%formicacidwith10mMammoniumformate on an analytic RP C18 column. The calibration range was 10- 1000ng/ml for sunitinib and 50-5000ng/ml for the other TKI's with coefficient of determination ≤15% and the chromatographic run time was12min.Plasmaspecimenswerestableformeasurementforatleast 1 week at 4°c.

- **S.S Pujeri et.al:** developed and validated stability – indicating chromatographic method for the assay of finasteride active pharmaceutical ingredient in the presence ofits degradation products on a C18 column using a mobile phase of 0.01M ammoniumformate- acetonitrile-containing formic acid with a flow rate of 1.0ml/min. Selectivitywasvalidatedbysubjectingthestocksolutionoffinasteride to acidic, basic,photolysis, oxidative and thermal degradation. The linearity range and values for limit of detection (LOD) and quantification (LOQ) were found to be 1-198, 0.33, and 1.1ug/ml, respectively.Theanalysisofthetabletcontainingfinasteridewasquite precise (relative standard deviation<1%).

- **ErrinR.Lepper et.al:** developed and validated a high- performance Liquid Chromatographic (HPLC) assay with U.V detection for the quantitative determinationoffinasterideinhumanplasma.Quantitative extraction was achieved bya single- solvent extraction involving a mixtureofacetonitrileandn-butylchloride(1:4v/v).Finasterideandthe internal standard hydrochloride salt (OSI-597) were seperated on a column packed with NOVA-PAK C18 material and a mobile phase composed of acetonitrile and water, pH 2.0

(60:40,v/v).The column effluent was monitored with dual UV detection at wavelengths of 348nm finasteride and 383nm finasteride hydrochloride. The calibrationgraphwaslinearintherangeof100-4500ng/ml,withvalues for accuracy and precision ranging from 87.9 to 96.2% and 2.13 to 5.10%respectively,forthreedifferentsetsofqualitycontrol samples.

- **Rasoulzadeh F[38]et.al:** studied the mutual interaction of anticancer drugfinasteridehydrochloridewithbovineserumalbumin (BSA)using fluorescence and UV /VIS spectroscopy. The BSA solution(0.1Mm) wasprepareddailyintrisbuffer(0.05mol-1,ph=7.4)andtreatedatfinal

  concentration of $1.67x10^{-5}$M with different amount of finasteride hydrochloridetoobtainfinalconcentrationof0,0.2,0.4,0.8,1,2,4,6,8,20 and42µmrespectively.Themixturewasallowedtostandfor5minand the fluorescencequenching spectra were recorded at 298,303, 308 and 313k. It was found that finasteride hydrochloride caused the fluorescence quenching of BSA by the formation of a BSA–FINASTERIDEHYDROCHLORIDEcomplex.Themechanismofthe complexformationwas thenanalysedbydeterminationofthe number of binding sites the apparent binding constant Ka, and calculation of the corresponding thermodynamic parameters.Suchasthefreeenergy (ΔG),enthalpy(ΔH)andentropychanges(ΔS)atdifferenttemperatures. Results showed that binding of finasteride hydrochloride to BSAwas spontaneous and the hydrophobic forces played a major role in the complex formation. The distance r between donar (BSA) and the acceptor (FINASTERIDE HYDROCHLORIDE) was found to be less than 8nm. Non-radioactive energytransferring and static quenching betweenthesetwomolecules.Thepresenceofsingle bindingsiteonBSA and pKa values for the association of BSA with FINASTERIDE HYDROCHLORIDE increased by the increase in temperature.

- **Jiongwei Pan et.al:** developed a novel bioanalytical method and validated for the quantitative determination of finasteride in human plasmabyusingthesupportedliquidextraction (SLE), sample cleanup coupled with hydrophilic interaction liquid chromatography and tandemmassspectrometricdetection(HILIC-MS/MS).TheSLEextract could be directly injected into the HILIC-MS/MS system for analysis without the solvent evaporation and reconstitution steps. Finasteride was used as the internal standard. The SLE extraction recovery was 101.3%.The validated

linear curve rangewas 2 to 2,000 ng/mL based on a sample volume of 0.100-mL, with a linearcorrelationcoefficient of>0.999.Thevalidationresultsdemonstratedthatthepresentmethod gaveasatisfactoryprecisionandaccuracy:intra-dayCV<5.9%

(<8.4% forthe lower limit of quantitation, LLOQ) with n = 6 and the accuracy of 98.0–106.0%;inter-day CV < 3.2% (<1.5% for LLOQ) withn=18andtheaccuracyof100.0–103.2%.Adilutionfactorof10 with blank plasma was validated for partial volume analysis. The stability tests indicated that the finasteride in human plasma is stable forthreefreeze-thawcycles(100.0–104.5%ofthenominalvalues),or 24-h ambient storage (100.0–\104.8% of the nominal values), or 227- day frozen storage at both -20ºC (91.5–94.5% of the nominal values) and -70 ºC (93.3–93.8% of the nominal values). The results also showed no significant matrix effect (<6.3%) even with directinjection of organic extract into the LC-MS/MS system.

- **Fouad Chiadmi et.al:** developed and validated an isocratic high- performance liquidchromatographic method for the determination of finasteride in human plasma with detection at 348 nm. Quinine was usedasinternalstandard.Areversed-phasesymmetryC18column(250 mmx4.6mm,5µm),wasequilibratedwithamobile phasecomposed ofpotassiumdihydrogenphosphate0.05Mandacetonitrile(60:40,v/v) with a final pH of 4.8 and having a flow rate of 1 mL/minute. The elutiontimeforfinasterideandinternalstandardwasapproximately7.4 and 2.6 minutes, respectively. Calibration curves of finasteride in human plasma were linear in the concentration range of 50-1,000 ng/mL. Limits of detection and quantification in plasma were 6.3 and 21ng/mL,respectively.Intra-andinter-dayrelativestandarddeviation for finasteride in plasma was less than 3.3 and 4.1%, respectively.

- **Hanqing Li et.al:** developed a new synthetic and differential antiproliferative activity of two active isomeric metabolites of Finasteride were investigated. This synthetic process had demonstrated to avoid the unstable 4- chloro-quinazoline intermediates and long procedures. New intermediates and final compoundswereidentified by ^{1}H NMR, ^{13}C NMR and their purities

were determined by HPLC. Invitroproliferative assay indicates that thesetwometabolitespossessedantiproliferativeactivityagainstsome conventional tumor cell lines and EGFR tyrosine kinase over-

expressiontumorcelllinesascomparedtoFinasteridecontrolandtheir antitumor activity in cellular level was reported.

- **Han-QingLiet.al**:developedandvalidatedanewHPLC-UVmethod forthequantitativedeterminationofepidermalgrowthfactorrreceptor inhibitorfinasterideintheplasmaoftumorbearingBALB/cnudemice. Finasteride and its internal standard 1-(3-((6,7-bis (2-methoxyethoxy) quinazolin-4-yl) amino) phenyl) ethanone were extracted from mice plasma samples using liquid-liquid extraction with a mixed solvent of methylt-butyletherandethylacetate(9:1,v/v).LunaC18column(4.6 mm×250 mm, 5 µm) with acetonitrile: 5 mM potassium phosphate buffer pH = 5.2 (41:59, v/v) as the mobile phase. UV detector was set at the wavelength of 345 nm, and the flow rate was 1.0 mL/min. The calibration curve was linear over the range of20–10 000 ng/mL with acceptable intra- and inter-day precision and accuracy. The intra-day and inter-day precisions were within the range of 1.69%–5.66%, and the accuracies of intra- and inter-day assays were within the range of 105%–113%.The mean recoveries were 85.2% and 96.1% for finasteride and internal standard, respectively.

- **RAJESH et.al:** developed a simple and sensitive spectrofluorimetric method forthe estimation of finasteride hydrochloride in pure and pharmaceutical dosage forms. Finasteride hydrochloride exhibits maximumfluorescence intensity in methanol and theBeer's law was obeyedintherangeof1-5µg/mLatanexcitationwavelength(λex)of 295nmandanemissionwavelength(λem)of339nm.Stabilitystudies withrespecttotimeandtemperaturewerealsocarriedout.Theresults obtained were in good agreement with the labelled amounts of the marketedformulations.Thismethodhasbeenstatisticallyevaluated andfoundtobeaccurateandprecise**.**

- **Patel, N., et al. (2015):** A simple, precise, rapid, accurate RP-HPLC method has beendevelopedandvalidatedforthesimultaneousdeterminationofMinoxidiland Finasteride in pharmaceutical dosage form. The chromatographic separation was achieved on ODS C18column (25 cm × 4.6 mm, 5 µ particle size) using a mobile phasecomprisingmethanol:wateralongwith0.5%triethylamine(TEA),pH6.38 adjusted with ortho phosphoric acid (OPA) in a ratio of 70:30 v/v. The flow rate was1ml/minandeluentsweredetectedbyUVdetectorat210nm.Retentiontimes were found to be 4.661 min and 10.005 min of Finasteride and Minoxidil respectively. The calibration curve was linear over the range of 12-24 µg/ml of Minoxidil and 0.4-0.8 µg/ml of

Finasteride. The results of all the validation parameters were well within their acceptance values. The developed method was successfully applied for determination of the two drugs from its pharmaceutical formulation. The excipients in the formulation do not pose any hindrance in determinationofthetwodrugs.Theproposedmethodissuitableforroutinequality control analysis.[29]

- **Thimmaraju, M., et al. (2011):** A simple, sensitive, precise and specific reverse phase high performance liquid chromatographic method was developed and validated for the determination of finasteride in bulk and tablet dosage forms. It was found that the excipient in the tablet dosage forms does not interfere in the quantification of active drug by proposed method. The HPLC separation was carried out by reverse phase chromatography on Shimadzu HPLC, 10-At detector with hypersil ODS C 18 Column 250 X 4.6 mm (particle size of 5µ) and constant flow pump. Rheodyne injector with 20 µl loop with a mobile phase composed in theratioacetonitrile:(0.05M)KH2PO4buffer(50:50)atflowrate1.8ml/min.The detectionwasmonitoredat208nm.Thecalibrationcurveforfinasteridewaslinear from 10-50□g/ml and internal standard (Bromhexine) 10□g/ml were prepared by suitabledilutionsofthestocksolutionwithappropriatemobilephase.Theinterday and intraday precision was found to be within limits. The proposed method has adequate sensitivity, reproducibility and specificity for the determination of finasterideinbulkanditstabletdosageforms. LODand LOQforfinasteridewere

  found to be 0.172 and 0.461.Accuracy (recoveries: 99.8-103.2%) and reproducibility were found to satisfactory.[30]

- **Shah, D.S.,etal. (2021):** Atopical solution comprisingofMinoxidil (MXL)and Finasteride(FNS)foralopeciaisformulatedinthepresentwork,whichessentially contains a lipid- Lauroglycol FCC as a penetration enhancer. The objective of the proposed work was to develop a rapid, simple, and robust reverse phase high performance liquid chromatographic (RP-HPLC) method to determine MXL and FNS in the said formulation. Herein, the chromatographic conditions were optimized based on the theoretical principles of separation and physicochemical properties such as pKa and log P of both the Active Pharmaceutical Ingredients (APIs).TheseparationwasaccomplishedonanInertsil®ODS-3C18column(150 mm*4.6mm;5µmofparticlesize)at25℃byusingamobilephasecomposedof 70:30 v/v ratio of Methanol and Milli-Q water along with 0.5% Triethylamine at pH6.4adjustedwithOrthoPhosphoricAcid.Drugpeaksshowedagoodresolution at 210 nm. The retention times for MXL and FNS were found to be 2.40 min and

  6.39min,respectively.Thedevelopedmethodwasfoundtobelinear(R2≥0.998) in a concentration range of 5-100 µg/mL for both the drugs. The method was validated according to the ICH guidelines Q2 (R1). The ability of the method to differentiate between

the types formulations was demonstrated by the in-vitro diffusion data performed using a highly sophisticated Strat-M® membrane. The cumulative amount of drug released (MXL and FNS) at the end of 24 hours was maximum for the topical formulation containing lipids prepared using isopropyl alcohol and propylene glycol as the base.[31]

- **Thimmaraju,M.K.,etal(2011):**Asimple,sensitive,preciseandspecificreverse phase high performance liquid chromatographic method was developed and validated for the determination of Finasteride and Tamsulosin in bulk and tablet dosage forms. It was found that the excipient in the tablet dosage forms does not interfere in the quantification of active drug by proposed method. The HPLC separation was carried out byreverse phase chromatographyon Shimadzu HPLC, 10-AtdetectorwithhypersilODSC18Column250X4.6mm(particlesizeof5µ) and constant flow pump. Rheodyne injector with 20 µl loop with a mobile phase composedintheratioacetonitrile:(0.05M)KH2PO4buffer(45:55)atflowrate1.8

  ml /min. The detection was monitored at 240nm. The linearity range was found between 125-625µg/ml for Finasteride 10-50 µg/ml for Tamsulosin and internal standard (Bromhexine) 40µg/ml were prepared by suitable dilutions of the stock solution with appropriate mobile phase. The interday and intraday precision was found to be within limits. The proposed method has adequate sensitivity, reproducibilityandspecificityforthedeterminationofFinasterideandTamsulosin in bulk and tablet dosage forms. LOD and LOQ for Finasteride and Tamsulosin were found to be 1.25, 4.166 and 0.495 and1.635.Accuracy (recoveries: finasteride100.76% & Tamsulosin 99.06%) and reproducibility was found to be satisfactory.[32]

- **Basavaiah, K., et al. (2007):** A rapid, highly sensitive high performance liquid chromatographic method has been developed for the determination of finasteride(FNS) in bulk drug and in tablets. FNS was eluted from a ODS C18 reversed phase column at laboratory temperature (30 ± 2º C) with a mobile phase consistingofmethanol and water(80+20)at aflowrateof1 mLmin -1 with UV detection at 225 nm. The retention time was ~ 6.1 min and each analysis took not morethan10min.Quantitationwasachievedbymeasurementofpeakareawithout using anyinternal standard. Calibration graph was linear from 2.0 to 30 µg mL -1 withlimitsofdetection(LOD)andquantification(LOQ)being0.2and0.6µgmL

  -1 , respectively. The method was validated according to the current ICH guidelines. Within-day coefficients of variation (CV) ranged from 0.31 to 0.69% and between-day CV were in the range 1.2-3.2%. Recovery of FNS from the pharmaceutical dosage forms ranged from 97.89 – 102.9 with CV of 1.41-4.13%. The developed method was compared with the official method for FNS determination in its tablet forms.[33]

- **Venkata, K. B., et al (2013)** in this work a rapid, specific, and accurate isocratic HPLC method was developed and validated for the assay of Finasteride in pharmaceutical dosage

forms. The assay involved an isocratic-elution of Finasteride in Grace C18 column using mobile-phase composition of 0.1% ortho phosphoric acid with triethyl amine as modifier buffer and acetonitrile in the ratio of 50:50 (v/v).The wavelength of detection is 294nm. The method showed good linearityintherangeof2.0–50.2×10^{-3}g/Lt.Theruntimeofthemethodis5mins.

The developed method was applied to directly and easily analyse of the pharmaceuticaltabletpreparations.Thepercentagerecoverieswerenear100%for given methods. The method was completely validated and proven to be rugged. Theexcipientsdidnotinterfereintheanalysis.Theresultsshowedthatthismethod can be used for rapid determination of Finasteride in pharmaceutical tablet with precision, accuracy, and specificity.[34]

- **Pallikonda, S.K.,et al.(2011)** in this work simple, sensitive, rapid, robust and reproducible method for the determination of Finasteride in bulk and pharmaceutical formulation (Tablets) was developed using reverse phase high performanceliquid chromatographicmethod (RP-HPLC).TheRP-HPLC analysis was performed isocratically on XTERRA C18 (4.6X150mm), analytical column using a mobile phase consisting of ortho phosphorus buffer and acetonitirle in the Ratio of 60:40v/v, with aflow rate of 0.6ml/min.The analyte was monitored with UV detector at 290nm. The developed method Finasteride elutes at a run time of 10 min. The proposed method is having linearity in the concentration range from 40 to 80 µg/mL of Finasteride. The present method was validated with respect to system suitability, linearity, and precision, limit of detection (LOD) and limit of quantification (LOQ), accuracy (recovery), ruggedness, and robustness. The proposed method can be readily utilized for bulk drug and pharmaceutical formulations.[35]
- **Chandra,Raju,etal.(2016)**inthisworkreliableandreproduciblereversed-phase high performance liquid chromatography (RP-HPLC) was developed for the quantitative determination of Finasteride from marketed bulk tablets. The active ingredientofFinasterideseparationachievedwithC18columnusingthemethanol water mobile phase in the ratio of 30:70 (v/v). The active ingredient of the drug content quantify with UV detector at 359 nm. The retention time of Finasteride is

  5.27 min. A good linearity relation (R2=0.999) was obtained between drug concentration and average peak areas. The limit of detection and limit of quantificationoftheinstrumentwerecalculated0.02and0.06µg/mL,respectively.

  Theaccuracyofthemethodvalidationwasdeterminedwiththeinter-day(100.28

  %)andintra-day(100.48%) recoveriesofthedrug.Thequantificationcorrelation

  range was 5-50 ppm. The new method was validated according to international conference harmonization guidelines.[36]
- **Nagaraju, P. et al (2015)** in this work a simple, rapid, accurate and precise RP-

HPLCmethodwasdevelopedforthedeterminationofQuetiapinefumarateinpure and tablet dosage forms. Separation of the drug was achieved on aisocratic ShimadzuprominenceHPLCinstrumentonaWatersXterraC18column(250x4.6 mm, 5µ). The method showed a linear response for concentration in the range of 50–150 µg/mL using buffer (9.2 ± 0.05) and acetonitrile in the ratio of 51:49 v/v withdetectionat254nmwithaflowrateof1.0mL/minandretentiontimewas

6.588min.Themethodwasstatisticallyvalidatedforlinearity,accuracy,precision and selectivity. Quantitative and recovery studies of the dosage form were also carried out and analyzed, the %RSD from recovery studies was found to be less than 1[37]

- **Rosa, P. C. P.,et al (2013)** in this work a simple and sensitive high performance liquidchromatographicmethodhasbeendevelopedforthedeterminationofassay quantitativeofrelatedcompoundsandquetiapinehemifumarateinrawmaterialand tablets. Quetiapine hemifumarate is used for the treatment of schizophrenia and therearesome genericmedicinesavailableinbrazilianmarketingpharmaceutical, it´s necessary evaluate the quality control of raw material used in the production. EfficientchromatographicseparationwascarryoutonaC18stationaryphasewith mobile phase consisting in a mixture of phosphate buffer pH 6.6:Acetonitrile:Methanol(45:40:15),flowrateof1.0mLmin-1,injectionvolume of 20 µL, temperature of 25 °C and ultraviolet detection at 220 nm. All of the chromatographic parameters were attended, with resolution greater than 2.9 betweenquetiapinehemifumarateandimpurities.TheHPLCmethodwasvalidated according ICH guidelines, evaluating selectivity, limits of detection and quantification,linearity,accuracy,precisionandrobustness.Therelativeretentions timeswereabout0.58,0.69and0.88torelatedcompounds,piperazine,andlactam andethanolcompound,respectively.Impuritieswerefound<0.1%insamplesand the assay of quetiapine hemifumarate was > 98.15%. The method can be used for the routine analysis of the impurities in Quetiapine hemihumarate (QH) without any interference.[38]
- **Nakamura,M.,etal.(2004)**InthisworkanewHPLCmethodhasbeendeveloped for measuring clonazepam (CZP) in plasma, using a reversed-phase non-porous silica column packed with 2 µm particles. CZP in plasma was first purified with a column extraction technique and injected onto a non-porous silica column. The calibration curve was linear from 5—200 ng/ml. The recoveries of CZP added to plasmaweremorethan94.0%,withacoefficientofvariationintherangeof5.1—13.8%.Wedevelopedarapidroutinemethodusinganon-poroussilicacolumnthat was accurate and improved solvent consumption in the measurement of CZP.[39]
- **Sallustio, B. C., (1994)** The present method was developed employing a rapid solid-phase extraction, thus minimising sample workup and providing analytical sensitivitydownto2µg/Lusing1mlofplasma.Plasmasampleswereloadedonto C18solid-phase

extraction columns, and clonazepam and its internal standard (methyl-clonazepam)wereelutedwithmethanol,dried,andreconstitutedin130µl of mobile phase. Chromatographic separation was achieved using a 3-µm RP18 columnat40°Candamobilephaseof32%acetonitrileand0.5%glacialaceticacid in distilled water at 0.5 ml/min. Detection was carried out using ultraviolet absorbanceat306nm.Retentiontimesforclonazepamandmethyl- clonazepam were~7and 12min,respectively.Standardcurveswerelinearovera range of 5–200 µg/L with intraassay coefficients of variation of 1.2 and 4.8% at 200 and 5 µg/L, respectively. Plasma concentrations measured in patient samples were not statistically different from those obtained using an established gas chromatographic method, and quality control specimens from the Heathcontrol EQASchemewereconsistentlywithin±1.2SDofthegroupmeans. Therewasno chromatographic interference from other benzodiazepines or other drugs used for the treatment of epilepsy.[40]

- **Foudah, Ahmed I., et al. (2022)** the study aimed to develop a new reverse-phase high-performance liquid chromatography (RP-HPLC) method with diode array detection(DAD)detectionforsimultaneousestimationofescitalopram(EST)and clonazepam(CZP)intabletdosageformswithaqualitybydesign(QbD)approach. The chromatographic conditions were optimized by Box-Behnken design (BBD) anddevelopedmethodwasvalidatedforthelinearity,systemsuitability,accuracy, precision, robustness, sensitivity, and solution stability according to International Council for Harmonization (ICH) guidelines. EST and CZP standard drugs peaks were separated at retention times of 2.668 and 5.046 min by C-18 column with dimension of 4.6 × 100 mm length and particle size packing 2.5 µm. The mobile phase was methanol: 0.1% orthophosphoric acid (OPA) (25:75, *v*/*v*), with a flow rate of 0.7 mL/min at temperature of 26 °C. The sample volume injected was 20 µLandpeaksweredetectedat239nm.Usingthestandardcalibrationcurve,the% assay of marketed tablet was founded 98.89 and 98.76 for EST and CZP, respectively. The proposed RP-HPLC method was able to detect EST and CZP in the presence of their degradation products, indicating the stability-indicating propertyofthedevelopedRP-HPLCmethod.Thevalidationparameter'sresultsin terms of linearity, system suitability, accuracy, precision, robustness, sensitivity, and solution stability were in an acceptable range as per the ICH guidelines. The newly developed RP-HPLC method with QbD application is simple, accurate, time-saving, and economic.[41]
- **Singh, Sonu Sundd, et al. (2004)** in this work novel liquid chromatographic– electrospray ionisation mass spectrometric (LC–ESI-MS) method has been developed for the determination of escitalopram, an antidepressant in human plasma using paroxetine as internal standard. The method involved liquid–liquid extraction of the analyte from human plasma with a mixture of diethyl ether and dichloromethane (70:30, v/v). The

chromatographic separation was achieved within 7.0 min byusing 2.0 mM ammonium acetate (pH 5.0)–acetonitrile (54:46, v/v)asmobilephaseandaODSYMC™AQ150mm×4.6mmanalyticalcolumn; theflow-ratewas1.0ml/min.Ionsignals*m*/z325.0and330.0forescitalopramand internalstandard,weremeasuredinthepositivemode.Adetailedvalidationofthe method was performed as per USFDA guidelines and the standard curves were foundtobelinearintherangeof1.0–200ng/mlwithameancorrelationcoefficient more than 0.99. The absolute recovery was more than 75% for both escitalopram andinternalstandard.Themethodwassimple,sensitive,precise,accurateandwas successfully applied to the bioequivalence study of escitalopram in healthy, male, human subjects.[42]

- **Spell, J. C., et al (1998)** in ths work a stability indicating, reversed phase high-performance liquid chromatographic method utilizing a smallbore HPLC column

has been developed for the determination of clonazepam in a commercial tablet dosage form. The use of a small bore column results in a substantial solvent savings, as well as a greater mass sensitivity, especially in the identification of degradationpeaksinachromatogram.Themethodinvolvesultravioletdetectionat 254nmandutilizeda150×3.0mmi.d.columnpackedwith3µmoctyldecylsilane particleswithamobilephaseofwater–methanol–acetonitrile(40:30:30,v/v/v)ata flow rate of 400 µl min^{-1} at ambient temperature, with and withoutthe useof 1,2-dichlorobenzeneastheinternalstandard.ThecurrentUSPmethodfortheanalysis ofclonazepamusinga300×3.9mmi.d.conventionaloctyldecylsilanecolumnwas utilized as a comparison to the smallbore method. The retention times for clonazepamandtheinternalstandardonthe3.0mmi.d.columnwere4.0and12.5 min,respectively.Theintra-andinterdayRSDsonthe3.0mmi.d.columnwere

<0.55% (*n*=4) using the internal standard, and <0.19% (*n*=4) without the internal standard at the lower limit of the standard curve, 50 µg ml^{-1} and had a limit of detection of 24 ng ml^{-1}. The assay using the 3.0 mm i.d. column was shown to be suitable for measuring clonazepam in a tablet dosage form.[43]

2. *AIMAND OBJECTIVE*

Theliteraturesurveyrevealedthatspectrophotometricmethod,HPTLC methodand Reverse phase HPLC method were used forthe determination of drug inthe tablet dosage form and also for its determination in biological specimens.

In the present study the aim was to develop a new RP HPLC method for the determination of the drug in its API form and its validation. The plan of the work can be represented as follows.

PlanofWork

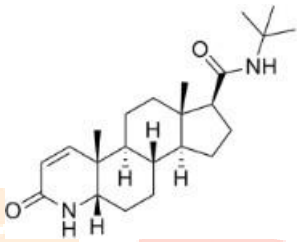
- ToobtainthroughknowledgeinpracticalHPLCmethoddevelopment.
- Toimplementastep-by-stepprocedureformethoddevelopment and to setinitial chromatographic conditions for the assay of Finasteride API.
- To conduct trails for the initial chromatographic conditions and to findoptimum conditions.
- TovalidatethedevelopedRP-HPLCmethod.

3. *DRUG PROFILE*

Name-Finasteride

ChemicalName-*N*-(2-methyl-2-propyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide,

Structure-



Figno.:structureofFinasteride **Molecular Formula**- C23H36N2O2

MolecularMass-372.549g/mol

Solubility-Freelysolinchloroform,DMSO,ethanol,methanol,n-propanol;sparinglysol in propylene glycol, polyethylene glycol 400; very slightly sol in 0.1N hydrogen chloride, 0.1N sodium hydroxide,water.

MeltingPoint-252-254°C

Pka value: (Strongest Acidic) **4.86** **Category**-5-alphareductase inhibitors.

Mechanism of action- Finasteride acts as a competitive and specific inhibitor of Type II 5α- reductase, a nuclear-bound steroid intracellular enzyme primarily located in the prostatic stromal cell that converts the androgen testosterone into the more active metabolite, 5α- dihydrotestosterone (DHT).[1] DHT is considered to be the primary androgen playing a role in thedevelopmentandenlargementoftheprostategland.Itservesasthehormonalmediatorfor the hyperplasia upon accumulation within the prostate gland.[7] DHT displays a higher affinity towards androgen receptors in the prostate gland compared to testosterone[10] and byacting on theandrogenreceptors,DHTmodulatesgenesthatareresponsibleforcell proliferation.[9] Responsible for the production of DHT together with type I 5α-reductase, the typeII5α-reductaseisozymeisprimarilyfoundintheprostate,seminalvesicles,epididymides, and hair follicles as well as liver.[11] Although finasteride is 100-fold more selective for type II 5α-reductase than for the type I isoenzyme,[3] chronic treatment with this drug may have some effect on type I 5α-reductase, which is predominantly expressed in sebaceous glands of most

regionsofskin,includingthescalp,andliver.ItisproposedthatthetypeI5α-reductaseand typeII5α-reductaseisresponsiblefortheproductionofone-thirdandtwo-thirdsofcirculating DHT, respectively.

Protien binding: Approximately 90% of circulating finasteride is bound to plasma proteins **Half-life:** In healthy young subjects receiving finasteride, the mean elimination half-life inplasma was 6 hours ranging from 3 to 16 hours. In elderly patients over the age of 70 years, the half-life is prolonged to 8 hours.

Use: Finasterideisused totreatmenwithan enlarged prostate (benign prostateenlargement). It can help ease your symptoms if: it's difficult to start peeing. You need to pee urgently or frequently more often.

4. *MATERIALSANDMETHODS*

A simple and sensitive reverse-phase HPLC method has been developed for the analysis of Finasteride.HPLCsystemusedwasJASCOsystemequippedwithmodelPU4180RHPLCpump, Rheodynesampleinjectionport(20µl),JASCOUV-4075UV-VISdetectorandChromNAVCFR chromatographysoftware (version 2.0). Separation was carried out on HiQSil C18 (250 mm × 4.6 mm, 5 µm) column. The analyte was monitored by UV detection at 245nm using an isocratic mode withbufferandmethanolinthe ratio520:480v/vas mobile phase.The flow rate wasset at 1.0ml/min. The retention time for the drug was at 4.540min.Calibration curves for Finasteride were recorded.

EquipmentandApparatusused:

- Analyticalbalance(MetllerToledoAG-245)
- Vacuumfilterpump
- Ultrasonicator(sonarex)
- Membranefilter(0.45and0.2microns)
- pH-Meter(LabIndia)

ChemicalsandReagentsused:

a) Acetonitrile(HPLCgrade)

b) Ortophosphoricacid(HPLCgrade).

c) Triethylamine(HPLCgrade)

d) Water(HPLCgrade)

e) Methanol(HPLCgrade)

Referencestandards: Finasteride Hydrochloride	--	Supplier

%purity	--	99.5%

The reference standard was obtained as gift sample and the authenticity andpurityof the sample was certified.

Finasteride-25mg

METHODDEVELOPMENT

The objective of this experiment was to optimize the assay method for the estimation of Finasteride. The trials were done to optimize the chromatographic conditions.

Preparationofmobilephaseandstandardsolutionofthedrugfortrails:-

The mobile phase was prepared with a composition according as indicated inthe table no 6 for the purpose of different trails. The mobile phase was filtered through 0.4µ filter and sonicated. The mobile phase was used as a diluent to preparethe standard solution.

Standardsolutionwaspreparedforeachtrailintherespectivemobilephase.

WorkingstandardsolutionofFinasterideHydrochloride:

About 50 mg of working standard of Finasteride Hydrochloride was weighedandtransferred into a clean and dry 50 ml standard flask. The sample wasdissolvedinasmallvolumeofmobilephasebysonicationforabout10min, and the volume was madeupwiththemobilephase.(1000µg/ml).0.5mlofthe stocksolutionwaspipettedinto a10 ml standard flask and diluted to mark with mobile phase (concentration 50 mcg/ml).

The standard solution was injected into the column in each trail. The Retentiontime at each trail was determined. The column, mobile phase and results obtained inthe trails have been indicated in the table 6. The table also revealstheresultofthe chromatogramsintermofretentiontime.Thetrailno5 employed was found to be satisfactory in which column, mobile phase orthophosphoric acid, and triethylamine buffer: methanol (520:480) were used to obtain adequate results.

Table:1

Differentcombinationsofbufferedsolventsystemtriedwithdifferentcolumnintrails

Sr.no/ Trail. no	Stationary phase	Mobile phase	Flow rate	Temp.	Wave length	Retention Time	Remarks

1.	OEM Column, C18(250X4.6X5µ)	Water:Methanol 65 : 35	1 ml/min	40ºC	245nm	16.043	Symmetric peak,More retention time
2.	Inertsil ODS C18 (250X4.6X 5µ)	Water:Acetonitrile 60: 40	1 ml/min	40ºC	245nm	11.870	Small tailing
3.	Inertsil ODS C18 (150X4.6X 5µ)	0.1MAmmonium Phosphatebuffer: Acetonitrile 40 : 60	1 ml/min	40ºC	245nm	5.587	Less Retention time
4.	Kromasil C18 (150X4.6X 5µ)	Phosphatebuffer: Acetonitrile : Methanol 650 : 210 : 140	1.5 ml/min	Ambient	250nm	8.302	Peak broadening
5.	Develosil ODSHG-5 (250X4.6X 5µ)	Ortho phosphoric acid, Triethylamine buffer:Methanol 520 : 480	1 ml/min	40ºC	245nm	4.543	Well resolved

PeakofFinasteridewaswellresolvedwiththecolumnwiththesolventsystemoforthphosphoricacid, triethylamine buffer: methanol in the ratio of 520: 480 as shown in following Fig 4.

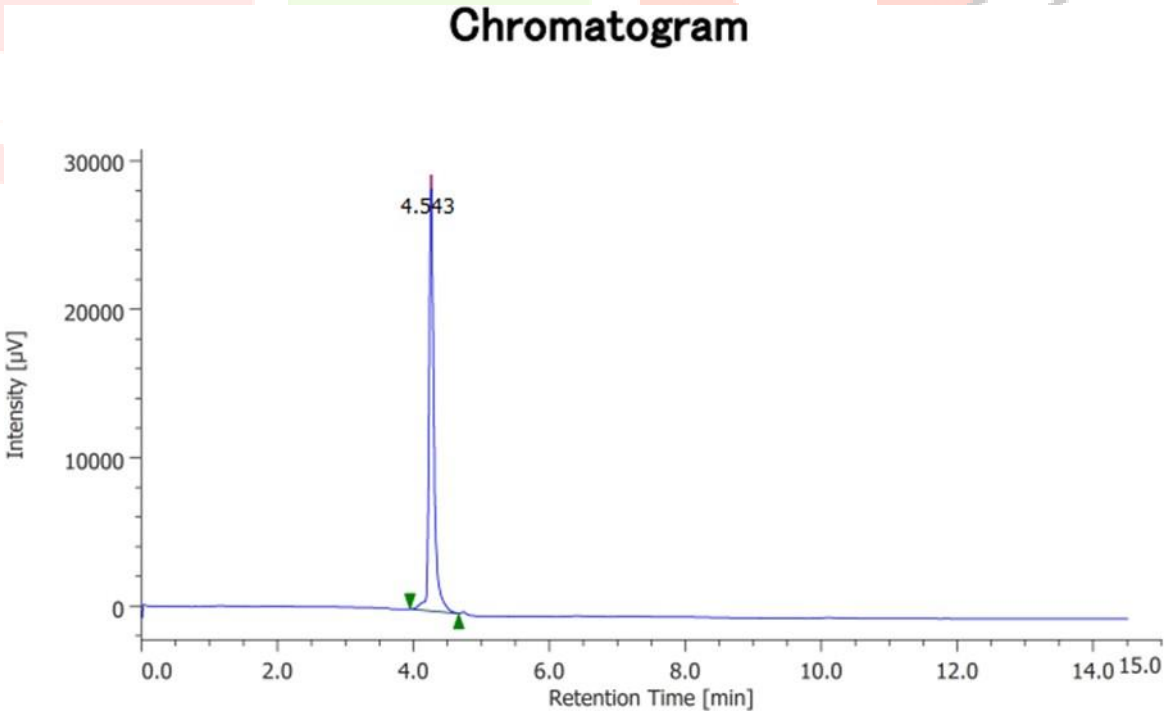


Figure4:-Chromatogramof drug(Optimised mobile phase)

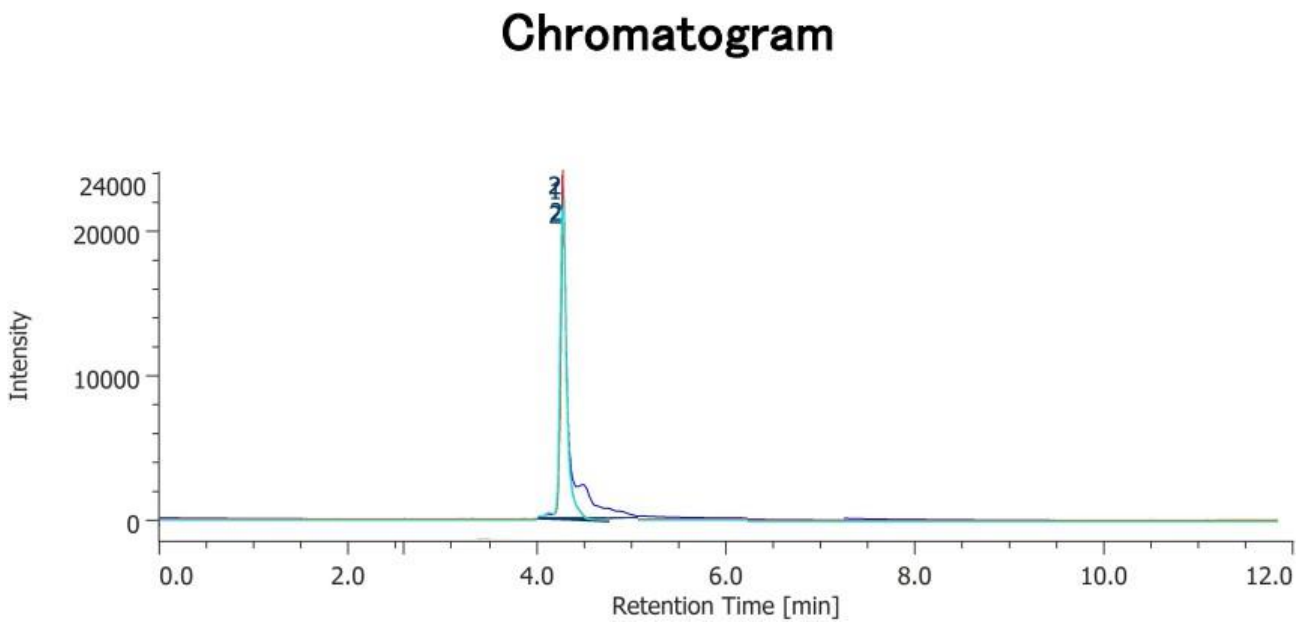


Figure5:-Overlayof HPLCgraph

OptimizedmethodPreparationmobilephase:

Preparationofbuffer:

Thebuffersolutionwaspreparedbymixing2mloftriethylamineand2 ml oforthophosphoric acid in water and the volume was madeup to 1000 ml.

Preparationofmobilephase:

The mobile phase was prepared by mixing buffer and methanol in the ratio 520:480v/v respectively and filtered through 0.45µ filter. The mobile phasewasthensonicatedusingUltra-sonicatortoremove the dissolvedgases.

PreparationofDiluent:

Mobilephasewasusedasthediluent.

DeterminationofRetentiontime:

PreparationofFinasterideHydrochloridestandardstocksolution:

WorkingstandardsolutionofFinasterideHydrochloride:

About 50 mg of working standard of Finasteride Hydrochloride was weighedandtransferred into a clean and dry 50 ml standard flask, the sample was dissolved in a small volume of mobile phase by sonication for about 10 minandthevolumewasmadeupwiththemobilephasefilteredthrough0.45µ filter.

(1000µg/ml).0.5 ml of the stock solution was pipetted into a10 ml standard flask and diluted to mark with mobile phase (concentration-50 mcg/ml).

TheretentiontimeofFinasterideHydrochloridewasfoundtobe4.540minwheninjectedand chromatograms are shown in the Fig.11 and Fig.12.

Applicationofstandardmethodforthesample:

Sample : Finasteridetablet.

Labelclaim : Finasteride25mg.

Mfg.by : NatcoPharmaPvtLimited.

Preparationofworkingsamplesolution:

Average weight of the tablet was computed from the weight of 20 tablets.Thetabletswerepowdered.Thetabletpowderequivalentto100mgof Finasteride was accurately weighed and transferred into a clean and dry 100 ml standard flask. The sample was dissolved in a small volume of mobile phase by sonication for about 10 min and the volume was made up with the mobile phase. The solution was filtered byusing Whatmann filter paper. (Concentration 1000µg/ml).0.5 ml of the stock solutionwas pipetted into a10 mlstandardflaskanddilutedtomarkwithmobilephase.Itwasfilteredthrough 0.45µ filter (Concentration-50 mcg/ml).

100mgFinasteride ⟹ 100ml(1000µg/ml)

⇩

0.5ml ⟹ 10ml(50µg/ml)

Thesamplewasinjectedandchromatogramswererecordedandshowninthe

Fig.13.

TheamountofFinasteridepresent ineach tabletformulationwascalculated bycomparingthepeakareaofthetestwiththatofthestandard.

Assay:

Assay of formulation available in the market was carried by preparing the samplesolutionasindicatedaboveprocedureinjectedintoHPLCsystem.The

percentagepuritywasfoundoutbyusingfollowingformula.Recoverystudies werealso carried out. The results were discussed.

ThecontentofFinasteridepresentinthetabletofaverageweight:

$$\frac{AT}{AS} \times \frac{WS}{25} \times \frac{0.5}{10} \times \frac{10}{0.5} \times \frac{393.3}{429.9} \times \frac{P}{100} \times \frac{Avg.Wt.}{LC} \times 100$$

Where,

AT = average area counts of sample preparation.

AS =averageareacountsofstandardpreparation. WS =weight of working standard taken in mg.

P =Percentagepurityofworkingstandard LC =Label Claim

TW= weight of sample takenAvgwt=averageweightoftabletinmg

393.9=MolecularweightofFinasteride

429.9=MolecularweightofFinasteridehydrochloride

Tabletaverageweight	45.8	mg
Weightofstandard	25.4	mg
Weightofsample	42.6	mg
Labelclaim	25	mg
Std. purity	99.5	%

Factor for calculation: 393.9/429.9mg

Avg.standardArea	2015.879
Avg.samplearea	2014.965

$$\frac{2014.965}{2015.879} \times \frac{25.4}{25} \times \frac{0.5}{10} \times 42.6 \times \frac{393.3}{429.9} \times \frac{99.5}{100} \times \frac{45.8}{25} \times 100$$

= 99.5%.

TheamountofFinasteridepresentinthetabletofaverageweight

=99.5/100X 25

=24.875mg.

VALIDATION

ValidationofanalyticalmethodfortheassayofFinasteride:

Validation of analytical method is a process to establish that the performancecharacteristics of the developed method meet the requirement of the intended analytical application.

Design oftheexperiment:

Typicalanalyticalparametersusedinassayvalidationare:

METHODVALIDATION

1. SYSTEMSUITABILITYSTUDIES

2. SPECIFICITY

3. LINEARITYANDRANGE

4. PRECISION

a) SYSTEMPRECISION

b) METHODPRECISION

5. RUGGEDNESS

6. ACCURACY

7. ROBUSTNESS

8. LOD

9. LOQ

1. SYSTEMSUITABILITY

Preparationofstandardstocksolution:

About 50 mg of working standard of Finasteride Hydrochloride was weighed andtransferredintoacleananddry50mlstandardflask, thesample was dissolved ina small volume of mobile phase by sonication for about 10 min and the volume wasmade up with the mobile phase. (1000µg/ml)

0.5mlofthestocksolutionwaspipettedintoa10mlstandardflaskand dilutedto mark with mobile phase (concentration-50 mcg/ml) and filtered through 0.45µ filter.

Procedure:

Thestandardsolutionwasinjectedforfivetimesandmeasuredthearea for allfive injections in HPLC. The %RSD for the area of five replicate injections was foundto be within the specified limits. The Chromatograms were shown in Fig.14. The results were discussed in the following table 7.

TableNo.2

DataforSystemSuitability

Injection	**tR**	**PeakArea**	**USP Plate count**	**USP Tailing**
1	4.547	2036.567	6470	1.147
2	4.553	2032.964	6865	1.65
3	4.53	2036.427	6911	1.69
4	4.55	2028.420	6932	1.69
5	4.547	2037.567	6890	1.65
Mean	4.5454	2034.389	6906.6	1.67
SD	0.00896	3.7635222	46.418	0.02
%RSD	0.20	0.18	0.672	1.43

2. Specificity

The specificity of the method was evaluated by analyzing the sample solutionspiked with the excipients at appropriate levels. The assay result was unaffected by the presence of extraneous materials.

Preparationofplacebo:

Placeboispreparedbymixingallexcipientswithoutactiveingredients.

Determination:

About 100mg of placebo was weighed accurately and transferred in to 100mlofvolumetricflask,mixedthoroughlywithsufficientmobilephaseand thevolumewasmadeupto100mlwithdiluent.Thesolutionwasfiltered.0.5 ml of this solutionwas diluted to 10 ml with mobile phase. The solution was again filtered through millipore filter and 10 µl of this solution was injected and chromatogram was recorded shown in the Fig.15.

About 50 mg of Finasteride working standard was weighed accurately and transferred into 100 ml standard flask, dissolved in small volume of the mobilephase.100mgofplacebowasmixedwithabovesolutionandmadeup the volume with mobile phase, filtered through millipore filter, 10µl of this solutionwasinjectedandchromatogramwasrecordedshowninFig.16,17and reports were shown in table 8.

TableNo. 3Specificityfor Finasteride

Sr. No.	Sample	Area obtained	%Contentof drugw/v
1	Standard	2037.567	99.81%w/v
2	Standard+ placebo	2028.420	99.43%w/v
3	Placebo	0	0

3. *LINEARITYANDRANGE*

Linearity was assessed by performing measurement at several analyte concentrations. A minimum five concentrations were recommended for linearity studies.

The linearity of an analytical method is its ability to show test results thatisdirectlyproportionaltotheconcentrationofanalyteinsamplewithina given range. The linearity of an analytical method was determined by mathematical treatment of test result obtained by analysis of samples with analyte concentration across claimedrange of peak area Vs concentration is plotted and percentage curve fitting iscalculated.

Acceptancecriteria	:	Percentagecurvefittingshouldnotbeless than99.7%

Preparationofworkingstandardsolution

Finasteride was weighed accurately and stock solution was prepared. Different volumesofstocksolutionweredilutedtogetaconcentrationrangeof 10 to 60 µg/ml.

TableNo.4Preparationofworkingstandardsolution

S.No	Volume of stock solutiontaken(ml)	Volumetric flasktaken (ml)	Concentration of Solution(mcg/ml)
1	0.1	10	10
2	0.2	10	20
3	0.3	10	30
4	0.4	10	40
5	0.5	10	50
6	0.6	10	60

Procedure:

10µl of working standard solution were injected in duplicate and thechromatograms were recorded and shown in Fig.18 to 23.

Thecorrelationco-efficientandpercentagecurvefittingwere calculated fromthe following formula.

$$R=\frac{3(x-\bar{x})^2(y-\bar{y})^2}{(n-1)S_xS_y}$$

Where

x=concentration

y = instrumental

responseSx=standard

deviationofxSY=

standarddeviationofy

Percentagecurvefitting=100xcorrelationcoefficient

Acceptancecriteria	:	Correlation coefficient should not be less than 0.99%
	:	Curvefittingshouldnotbelessthan99.7%

The linearity data and analytical performance parameters of Finasteride wasshownintableandcalibrationcurveofFinasteridewasshowninFig.24.

Fig.24CalibrationcurveofFinasteride.

TableNo.5 DataforLinearity

Conc. (µg/ml)	Avg. Area
10	464.112
20	860.935
30	1222.921
40	1612.944
50	2011.541
60	2395.321

TableNo.6LinearityresultsforFinasteride

Conc.(µg/ml)	10	20	30	40	50	60
Peakarea	464.112	861.433	1222.921	1612.944	2011.541	2395.321
Correlation	0.999					

TableNo.7CalibrationparametersforFinasteride

Parameter	Results
Slope	38.56
Intercept	78.40
Correlationco-efficient	0.999
Percentagecurve fitting	99.9%

4. *PRECISION*

Precision of an analytical method is the degree of agreement among individualtest result when the procedure is applied repeatedly to multiple samplingsofahomogenoussample.Precisionofanalyticalmethodisusually expressed as the standard deviation or relative standard deviation.

Determination:

The precision of an analytical method was determined by assaying sufficient number of sample and relative standard deviation is calculated.

The precision of the instrument is determined byassaying the samples consecutively number of times and relative standard deviation is calculated.

Acceptancecriteria	:	Therelativestandarddeviationshouldbe within 2%

A. *SYSTEMPRECISION*

Preparationofstandardsolution:

About 50 mg of working standard of Finasteride Hydrochloride was weighed andtransferredintoacleananddry50mlstandardflask, thesample was dissolved ina small volume of mobile phase by sonication for about 10 minandthevolumewasmadeupwiththemobilephase.(1000µg/ml).0.5mlof thestocksolution waspipettedinto a10 ml standard flask and diluted to mark with diluent and filtered through 0.45µfilter (concentration-50 mcg/ml).

Procedure:

Thestandardsolutionwasinjectedforfivetimesandmeasuredthearea for allfive injections in HPLC. The %RSD for the area of five replicate injections was foundto be within the specified limits. The Chromatograms were shown in Fig 25. The results were discussed in the Table 13, 14.

B. *METHODPRECISION*

Preparationofworkingsamplesolution

Average weight of the tablet was computed from the weight of 20 tablets.Thetabletswerepowdered.Thetabletpowderequivalentto100mgof Finasteridewasaccuratelyweighedandtransferredintoacleananddry100ml standard flask. The sample was dissolved in a small volume of mobile phase by sonication for about 10 minand the volume wasmadeupwiththe mobile phase. The solution was filtered byusing Whatmann filter paper (Concentration 1000µg/ml).0.5mlofthestocksolutionwaspipettedintoa10mlstandardflask and diluted to mark with mobile phase and filtered through 0.45 µ filter (concentration-50 mcg/ml).

Procedure:

The sample solution was injected for five times andmeasuredthe area for allfive injections in HPLC. The %RSD for the area of five replicate injections was foundto be within the specified limits. The Chromatograms were shown in the Fig.26. Theresults were discussed in the Table 15, 16.

The standard deviation and relative standard deviation were calculated from statistical formula.

Standarddeviation(σ)=

$$\sigma = \sqrt{\frac{\sum(x_i - \mu)^2}{N}}$$

Where,

x=sample,

xi=meanvalueofsamples,

n = number of samples

RelativeStandardDeviation(%)=σ/xi×100

TableNo.8Precisiondataofthesystem

Injection No	**PeakArea**	**% Recovery**
1	2036.567	100.7
2	2032.964	100.5
3	2036.427	100.7
4	2028.420	100.3
5	2037.567	100.7
Mean	2034.389	100.58
SD	3.7635222	0.1789
%RSD	0.18	0.18

TableNo.9SystemprecisionreportforFinasteride

Relativestandard deviation	**Finasteride**	**Acceptancecriteria**
	0.18	**<2.0%**

TableNo.10MethodprecisionofFinasteride

Injectionno	Peak Area	% Recovery
1	2018.593	100.1
2	2014.965	99.96
3	2013.985	99.92
4	2015.879	100.0
5	2011.118	100.3
Mean	2017.031	100.056
SD	3.140105	0.151912
%RSD	0.16	0.15

TableNo.11MethodprecisionreportforFinasteride

	Finasteride	Acceptance criteria
Relativestandard deviation	**0.16**	**<2.0%**

5. *RUGGEDNESS*

The ruggedness of an analytical methodis degree of reproducibility of test result obtained by the analyst under a variety of normal test condition. Suchasdifferentlaboratories,differentanalysts,differentinstruments,lotsof reagents, different elapsed assay times, different temperature, different days etc.

Theruggednessofananalyticalmethodisdeterminedbyaliquotsfrom homogenous lots by different analyst using operational and environmental conditionsthat may differ but are also with in the specified parameters of the assay. The degreeof reproducibility of test results is then determined as functionoftheassayvariables.Thisreproducibilitymaybecomparedwiththe precision of the assay under normal condition to obtain a measure of the ruggednessoftheanalyticalmethod.Theassay ofFinasteridewasperformed in different days.

Procedure:

Workingstandardsolutionandworkingsample solutionof Finasteride werepreparedbydifferentanalystandondifferentdaysand10µlof working sample solution was injected and chromatograms were recorded shown in Fig.27,28and ruggednessofthemethodandreportofFinasteridewasshown in Table 17,18.

TableNo.12RuggednessresultsforFinasteride:(Day-1,Analyst-1)

Parameter	Peak Area	% Assay
Avg	2054.018	99.68
%RSD	0.04	0.43

TableNo.13RuggednessresultsforFinasteride:(Day-2,Analyst-2)

Parameter	Peak Area	%Assay
Avg	2056.393	100.865
%RSD	0.03	0.26

6. *ACCURACY*

TheAccuracyofananalyticalmethodistheclosenessofthetestresult obtained by that method to the true value

Accuracy is measured as the percentage of the analytes recovered by theassay.Spikedsampleswerepreparedintriplicateatthreeintervalsarange of 100-150%ofthetargetconcentration,andinjectedintotheHPLCsystem.

Acceptancecriteria	:	Percentage recovery should be within 90-110%w/w

PreparationofSampleStockSolution:

Average weight of the tablet was computed from the weight of 20 tablets.Thetabletswerepowdered.Thetabletpowderequivalentto100mgof Finasteridewasaccuratelyweighedandtransferredintoacleananddry100ml standard flask. The sample was dissolved in a small volume of mobile phase by sonication for about 10 minandthevolumewasmadeupwiththemobile phase. The solution was filtered byusing whatmann filter paper. (Concentration 1000µg/ml).0.5mlofthestocksolutionwaspipettedintoa10mlstandardflask and diluted to mark with mobile phase and filtered through 0.45 µ filter. (Concentration-50 mcg/ml)

The stock solution was diluted with mobile phase. Further to obtain a concentration ranging from 45mcg to 65 mcg/ml. The dilution made was shown in theTable 15.

TableNo.14

Sr.No.	From stock sample solution volume taken (ml)	50 mcg/ml solution taken(ml)	Volumetric flasktaken (ml)	Concentration ofsolution (mcg/ml)
1	0.4	1	10	45
2	0.5	1	10	55
3	0.6	1	10	65

Procedure:

Thestandardsolution,45mcg/ml,55mcg/mland65mcg/mlsolutions were separately injected into the HPLC. The individual recovery and mean recoveryvalueswerecalculated.Thechromatogramswereshowninfig.29 to 32.TheresultswerediscussedintheTable20.

TableNo.15PercentageRecoverydataforFinasteride

Sr.No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	%Recovery	Mean% Recovery
1	100 %	45	45.85	101.88	101.78
	100 %	45	45.77	101.77	
	100 %	45	45.78	101.75	
2	125 %	55	54.8	99.68	99.77
	125 %	55	54.84	99.72	
	125 %	55	54.87	99.77	
3	150 %	65	64.05	98.55	99.86
	150 %	65	64.23	98.81	
	150 %	65	64.12	98.70	

7. *ROBUSTNESS*

Robustnessofananalyticalmethodismeasureofitscapacitytoremain unaffected by small but deliberate variation in method parameters and provides on indication of its reliability during normal usage.

Determination:

The robustness of an analytical method is determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay. For example change in physical parameters like flow rate and wavelength.

a) *EffectofvariationofFlowrate:*

A study was conducted to determine the effect of variation in flow rate by injecting 0.9 ml/min and 1.1ml/min. Sample solution was prepared and injectedintotheHPLCsystem.Theretentiontime valuesweremeasured.The chromatograms wereshownintheFig.33to35.Theresultswerediscussedin the Table 22 to 24.

b) *Effectofvariationofwavelength:*

A study was conducted to determine the effect of variation in wavelength. Standard solution was prepared and injected into the HPLC system at 248nm and 244nm. The effects of variation in wavelength were measured. The chromatograms were shown in the Fig.36 & 37.The results were discussed in the Table 25 to 28.

TableNo.16ChromatographicconditionforRobustnessChangein flow rate0.9 ml/min

Changein flow	**0.9ml/min**
Instrument	HPLC Shimadzu Separation Module LC-20ATProminenceLiquid
Column	DevelosilODSHG-5 250X4.6,5µm
Wavelength	245nm
Injection volume	10µl
Columnoven	40°c
Runtime	10min

TableNo.17ReportofRobustness

Drug	AverageRt in 0.9ml/min	AverageRt in 1.0ml/min	Average Asymmetry in0.9ml/min	%RSD
Finasteride	5.180	4.540	1.105	0.019

TableNo.18ChromatographicconditionforRobustnessChangein flow rate1.1 ml/min

Changein flow	1.1 ml/min
Instrument	HPLCShimadzuSeparationModuleLC-20AT Prominence Liquid
Column	DevelosilODSHG-5 250X4.6,5µm
Wavelength	245nm
Injection volume	10µl
Columnoven	40°c
Runtime	10min

TableNo.19ReportofRobustness

Drug	AverageRt in 1.1ml/min	AverageRt in 1.0ml/min	Average Asymmetry in1.1ml/min	%RSD
Finasteride	4.113	4.540	1.161	0.21

TableNo.20ChromatographicconditionforRobustnessChangeinwavelength244nm

Change in Wavelength	244nm
Instrument	HPLCShimadzuSeparationModuleLC-20 ATProminence Liquid
Colum n	DevelosilODSHG-5 250X4.6,5µm
Flowrate	1.0ml/min
Injectionvolume	10µl
Columnoven	40°c
Runtime	10min

TableNo.21ReportofRobustness

Drug	Average Rtin244nm	Average Rtin 245nm	Average Asymmetry in244 nm	%RSD
Finasteride	4.543	4.547	1.167	0.043

TableNo.22ChromatographicconditionforRobustnessChange inwavelength248nm

Change in Wavelength	248nm
Instrument	HPLCShimadzuSeparationModuleLC-20 ATProminence Liquid
Column	DevelosilODSHG-5 250X4.6,5µm
Flowrate	1.0ml/min
Injectionvolume	10µl
Columnoven	40°c
Runtime	10min

TableNo.23ReportofRobustness

Drug	Average Rt in 248 nm	Average Rtin 245nm	Average Asymmetry in248 nm	%RSD
Finasteride	4.543	4.547	1.114	0.015

8. LimitofDetection(LOD)

It is the lowest amount of analyte in a sample that can be detected, but not necessarily quantities as an exact value, under the stated experimental conditions. Thedetection limit is usually expressed as the concentration of analyte (percentage partsper million) in the sample.

Itisdeterminedbybasedonthestandarddeviationofresponseandtheslope. Thedetectionlimitmaybeexpressed as

The LOD was determined by the formula:

$$LOD=3.3\sigma/S$$

Where

σ = standarddeviationofthe response

S = slopeofcalibrationcurve

LOD = 3.3(0.00896/38)

= 0.000766µg/ml

Fromtheformulalimitofdetectionwasfoundtobe=0.000766µg/ml

9. *LimitofQuantification(LOQ)*

It is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. Quantification limit is expressed as the concentration of analyte (e.g: % ppm) in the sample.Whichcanbequantitatedwithsuitable precision and accuracy.

Based on the deviation of the response and the

slope.Quantitationlimit(QL)maybeexpressed as:

LOQ=10σ/S

Where

σ = Standarddeviation of the response

S = slopeofcalibrationcurve

LOQ = 10(0.00896/38.56)

= 0.002323µg/ml.

Fromtheformulalimitofquantitationwasfoundtobe=0.002323µg/ml.

6. *RESULTSANDDISCUSSION*

Validation of analytical method for determination of assay of Finasteride 25 mg tablets was performed for the parameters including – Specificity, Linearity and Range, Precision (System precision, Method precision), Intermediate precision (Ruggedness), Accuracy andRobustness. The summary of results obtainedis appended below.

Parameter	AcceptanceCriteria	Results
Specificity	Thereshouldnotbeany interference from placebo, blank and mainpeak. (Active)	Thereisnointerference fromblank,placebo and sample peak.
Linearity andRange	Correlationcoefficientshould be not less than 0.995 over workingrange.	Correlationcoefficient =0.9999.
Precision Repeatability System precision	%RSDshouldnotbe more than2.0%	SD=0.1789 %RSD=0.18
Repeatability Method precision	%RSDshouldnotbe morethan2.0%	SD=0.1519 %RSD=0.15
Intermediate precision Ruggedness	%RSDshouldnotbemore than2.0% Thedifferencebetweenassay ofmethod precision and intermediateprecisionshould notbemorethan2.0%	Day1andAnalyst1 %Assay=99.68 %RSD=0.43 Day2andAnalyst2 %Assay=100.865 %RSD=0.26
Accuracy	Recoveryateachleveland %meanrecoveryshouldbe between100%to150% with %RSDshouldnotbemore than2.0%	Recovery ateach level98.55to101.88. Mean Recovery99.86 to101.78. %RSD= 0.18
Systemsuitability	%RSDshouldnotbemore than2.0%	SD=3.76352 %RSD=0.18

Robustness:	**Bychangeinflowrate**

a) 0.9ml/min	%RSDshouldnotbe morethan2.0% Asymmetryfactorshould notbe more than2.0%.	%RSD =0.019 Asymmetryfactor=1.105
b) 1.1ml/min	%RSDshouldnotbe morethan 2.0%. Asymmetry factor should notbemorethan2.0%	%RSD=0.21 Asymmetryfactor=1.161

Bychangein wavelength		
a)248nm	%RSDshouldnotbemore than2.0% Asymmetryfactorshouldnot bemorethan2.0%	%RSD=0.015 Asymmetryfactor=1.114.
b)244nm	%RSDshouldnotbemore than2.0% Asymmetryfactorshouldnot be morethan2.0%	%RSD=0.043 Asymmetryfactor=1.167.

DISCUSSION:

The observations and results obtained for each parameter including Specificity, Linearity and Range, Precision (System precision, Method precision),Intermediateprecision(Ruggedness),AccuracyandRobustnesslie wellwithin theacceptancecriteria.

7. *CONCLUSION*

Forroutineanalyticalpurposeitisdesirabletoestablishmethods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation step. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool.

Finasteride was slightly soluble in methanol and very slightly soluble in water. Methanol and Mixture of Buffer was chosen as the mobile phase. The run time of theHPLC procedure was 10 minutes.

The method was validated for system suitability, linearity, precision,accuracy,specificity,ruggednessrobustness,LODandLOQ. The system suitability parameters were within limit, hence it was concludedthatthesystemwassuitabletoperformtheassay.Themethod showslinearitybetweentheconcentrationrangeof10-60µg/ml.The% recoveryofFinasteridewasfoundtobeintherangeof99.86%-101.78 %.Astherewasnointerferenceduetoexcipientsandmobilephase,the methodwasfoundtobe specific.Themethodwasrobust andruggedas observed from insignificant variation in the results of analysis by changes in Flow rate and wave length separatelyand analysis being performed by different analysts.

Goodagreementwasseenintheassayresultsofpharmaceutical formulation by developed method. Hence it can be concluded that the proposedmethodwasagoodapproachforobtainingreliableresultsand found to be suitable for the routine analysis of Finasteride in the pharmaceutical formulation.

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