International Journal of Pharmacy and Analytical Research (IJPAR)

IJPAR /Vol. 14 / Issue 4 / Oct - Dec -2025

www.ijpar.com

ISSN: 2320-2831

DOI : https://doi.org/10.61096/ijpar.v14.iss4.2025.1250-1257

Research

ANALYTICAL METHOD VALIDATION FOR THE ASSAY OF FINASTERIDE BY HPLC

Palle Arun Kumar[1],Anjali[2], Mohammad Omar[3]

[1,2,3]*Arya college of Pharmacy, Kandi, Sangareddy, affiliated to Osmania University, Hyderabad, Telangana, India.*

*Author for Correspondence: Palle Arun Kumar
Email: drparam18@gmail.com

Published on: 08.12.25

Published by:
Futuristic Publications

Abstract

A reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for the determination of purity of Finasteride using 3.0mm x 3.0cm, 3µm Packing L7 or quivalent column maintained at 25°C with a mobile phase of a water and tetrahydrofuran (4:1). The mobile phase flow rate was 3.0 mL/min, and the detection wavelength was 215 nm. The developed HPLC method was validated with respect to linearity, accuracy, precision, specificity, and robustness. The developed HPLC method to determine the assay of Finasteride can be used to evaluate the quality of regular production samples. It can be also used to test the stability samples of Finasteride.

Keywords:RP-HPLC, Validation, Finasteride, Assay method

INTRODUCTION

Analytes in the pharmaceutical analysis sector range from basic chemicals to complex Biomolecules in structure. A wide range of techniques to use to develop reliable analytical methods for these substances.

Qualitative analysis: It deals with the identification of substances It deals with the determination of elements or compounds present in the sample. [1]

Quantitative analysis: It provides numerical information concerning the quantity of sample species (the analyte) in a measured amount of matter the sample. [2]

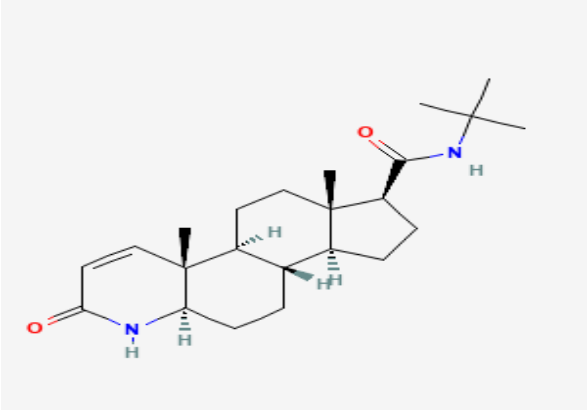
High-Performance Liquid Chromatography(HPLC): The HPLC is the method of choice in the field of analytical chemistry [4]since this method is specific, robust, linear, precise, and accurate and the limit of detection analyses calculations are performed by the integrator itself.

Principles of Separation [5] High-surface-area particles are used in adsorption chromatography to adsorb molecules of interest. If you're using non-polar mobile phases such as chloroform or heptane, you're going to need to use adsorption solid like silica gel or alumina, or even porous glass beads. The competition model and the solvent interaction model are used to describe the adsorption process in adsorption chromatography.
Instrumentation: The essential parts of the High-Performance Liquid Chromatography are: Solvent reservoir and treatment system Mobile phase Pump system Sample injection system Column Detector

Figure 1: An example of an HPLC flow chart.

MATERIALS AND METHOD

Details of Instruments, Column, Chemicals and Specification limits
Instruments: HPLC with UV-Visible or PDA detector, Analytical Balance, Glassware Class-A. Column: 3.0mm x 3.0cm, 3µm Packing L7 or equivalent
Chemicals: Tetrahydrofuran, Acetonitrile, HPLC grade Water Specification limits: Not less than 95.0 % and Not more than 105.0 %, Description of Analytical method (Methodology), Chromatographic Conditions: HPLC Column: 3.0mm x 3.0cm, 3µm Packing L7 or equivalent.
Wavelength: 215 nm, Flow rate: 3.0 mL/minute, Injection Volume : 10 µL
Validation Plan for Validation of Finasteride USP, following parameters shall be verified.
Validation Parameters: 1 System Suitability, 2 Specificity, 3. Precision i) System Precision, ii) Method Precision (repeatability), 4 Linearity, 5 Accuracy
DRUG PROFILE: Finasteride: Molecular Formula C23H36 N2O2, Molecular weight 372.5 g/mol, Solubility Insoluble in Water



Description of Analytical Method: Chromatographic Conditions:
HPLC Column : 4.6mm x 30.0cm, 4µm Packing L1 or equivalent.

Wavelength	: 210 nm
Flow rate	: 1.5 mL/minute
Column oven temperature	: 60°C
Injection Volume	: 15 µL
Run time	: 10 minutes
Diluent	: Acetonitrile and water (50:50%v/v)

Preparation of Mobile Phase: Prepared a filtered and degassed mixture of water, tetrahydrofuran and Acetonitrile (8:1:1). Make adjustments if necessary (As per USP <621>)

Preparation of Standard solution: Dissolve an accurately weighed quantity of USP Finasteride RS in diluting solution and dilute quantitatively and stepwise if necessary, with Diluting solution to obtain a solution having a known concentration of about 1.0 mg per mL.

Preparation of Sample stock solution: Transfer about 100 mg of Finasteride, accurately weighed to a 100 mL volumetric flask, dissolve in and dilute with diluting solution to volume and mix.

Procedure

1. Equilibrate the column using mobile phase to get a stable base line.
2. Inject diluent as a blank (one injection), standard preparation (five injections) and check the system suitability parameters.

System suitability:

a. The tailing factor for Finasteride peak is NMT 1.3.
b. The Theoretical plates for Finasteride peak is NLT 10,000.

Calculation:

Calculation:

Calculate the % of each impurity from the portion of Finasteride taken by the following formula. % = 100 (r_i/r_s)

r_i : peak area response of each impurity from the sample chromatogram

r_S : Sum of Peak area responses of all peaks in the chromatogram.

Validation Results: System Suitability

As per methodology, injected blank and six replicate injections of standard solution intoHPLC system. Calculated the % RSD for six replicate injections.

Results: Table 10: System suitability

Parameter	System suitability	
Result	1.1	12210
Acceptance Criteria	Tailing factor NMT 1.3	Theoretical plates NLT 10,000

Acceptance criteria

a. The tailing factor for Finasteride peak is NMT 1.3.
b. The Theoretical plates for Finasteride peak is NLT 10,000.

Conclusion

The above results reveal that the system meets the required system suitability criteria.

Specificity: Interference of Blank: As per methodology, injected Blank, Standard solution, individual impurities solutions, Sample solution and spiked sample solution checked the peak interference of blank at the retention time of Finasteride and its related impurities.

Results: Table 11: System suitability

Parameter	System suitability	
Result	1.2	10537
Acceptance Criteria	Tailing factor NMT 1.3	Theoretical plates NLT 10,000

Table 12: Blank Interference Data

S. No.	Name	Interference Due to blank (Yes/No)
1	Finasteride	No

Table 13: Impurity Interference Data

S. No.	Name	Retention time (mins)	
		Individual solution	Spiked sample
1	Impurity-A	19.47	20.04
2	Impurity-B	17.84	18.09
3	Impurity-C	16.95	17.30
4	Finasteride	14.600	14.92

Note: For Finasteride retention time for individual solution considered from standard retention time.

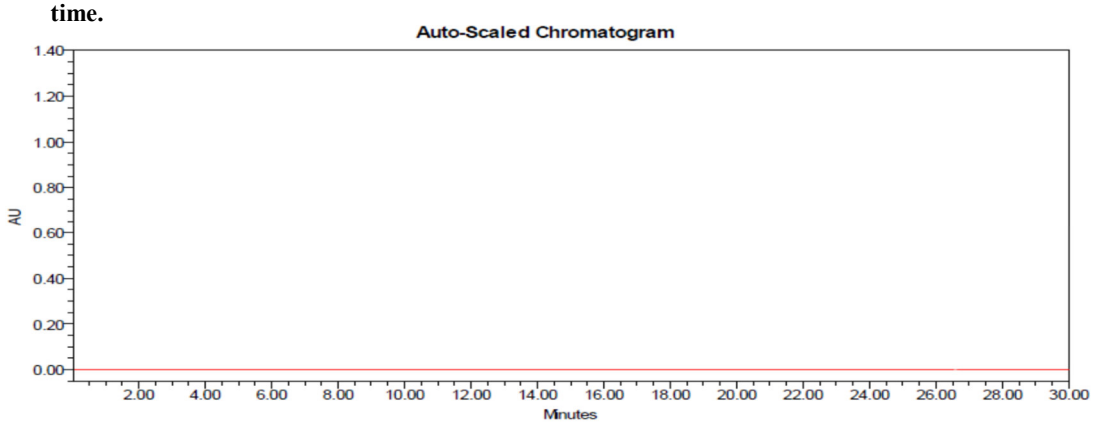


Figure 4: Typical chromatogram of Blank

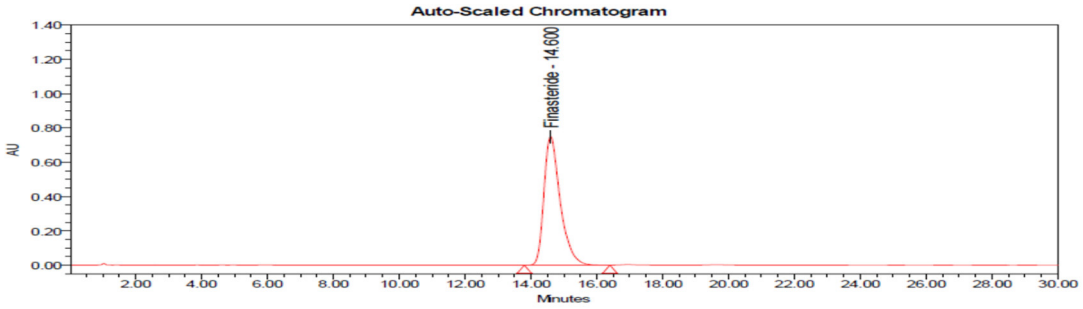


Figure 5: Typical chromatogram of Standard

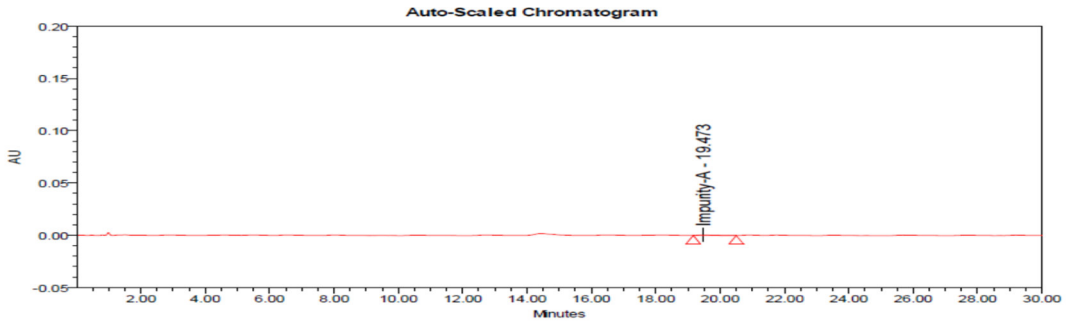


Figure 6: Typical chromatogram of Impurity-A

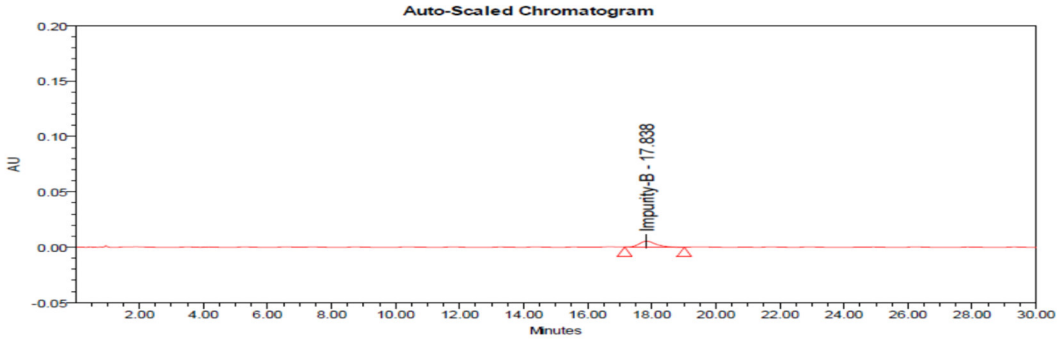


Figure 7: Typical chromatogram of Impurity-B

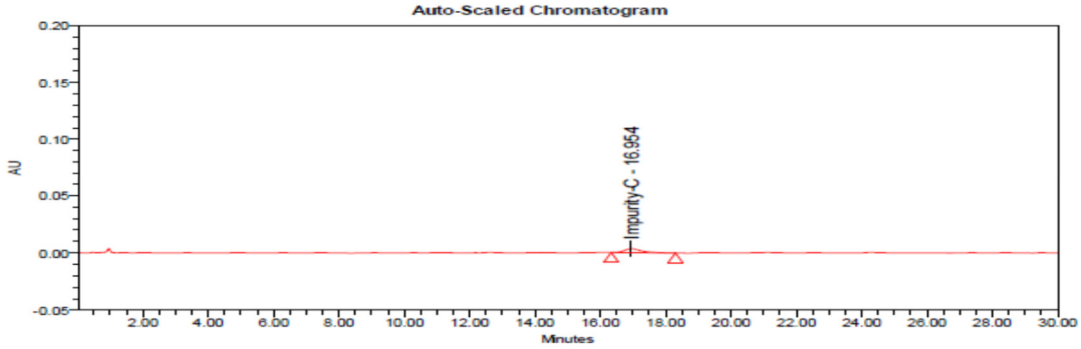


Figure 8: Typical chromatogram of Impurity-C

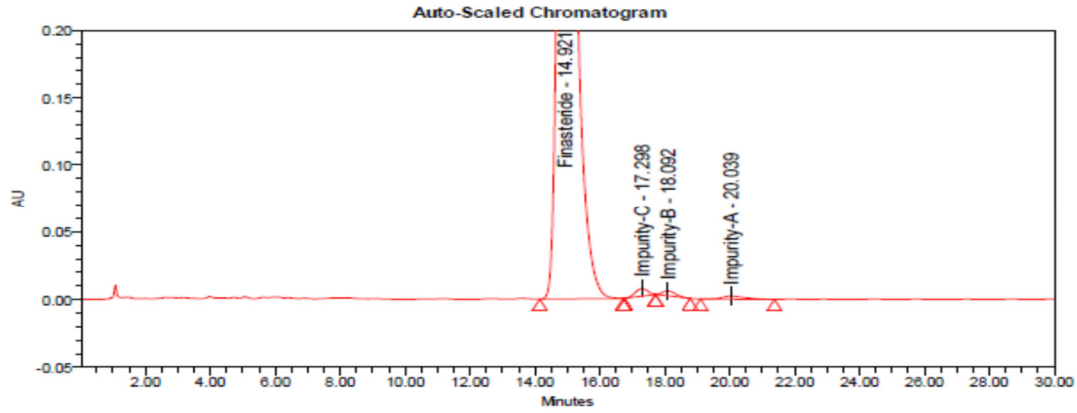


Figure 9: Typical chromatogram of Spiked sample

Acceptance criteria: The tailing factor for Finasteride peak is NMT 1.3. The Theoretical plates for Finasteride peak is NLT 10,000. The blank should not show any interference at the retention time of Finasteride peak in the standard and sample solution.

Conclusion: There is no interference observed from blank at the retention time of Finasteride peak and no interference was observed with each individual impurity as well as with analyte peak.

Precision: System Precision: As per methodology, injected blank, standard solution for six times into the HPLC system.

Results: Table 14: System suitability

Parameter	System suitability	
Result	1.1	12210
Acceptance Criteria	Tailing factor NMT 1.3	Theoretical plates NLT 10,000

- The % RSD of Retention time for six replicate injections of Standard preparation is 0.7.

Table 15: System Precision

Injection No.	Finasteride Area

01	20452883
02	20467070
03	20328973
04	20272468
05	20117580
06	20245900
Average	20314146
STDEV	132579.27
% RSD	0.7

Acceptance criteria: The tailing factor for Finasteride peak is NMT 1.3.The Theoretical plates for Finasteride peak is NLT 10,000.

Conclusion: The above results reveal that the system is precise.

Method Precision: Analysed six test preparations of Finasteride as per the methodology and determined the % RSD of six sample preparations for Chromatographic purity of Finasteride.

Results: Table 16: System suitability

Parameter	System suitability	
Result	1.1	12210
Acceptance Criteria	Tailing factor NMT 1.3	Theoretical plates NLT 10,000

Table 17: Method precision Results

Sample	Finasteride (%w/w)
01	100.7
02	100.3
03	100.0
04	99.5
05	99.3
06	100.9
Average	100.1
S.D	0.65
%RSD	0.7

Note: No Impurity was identified during the method precision samples (%w/w)

Acceptance criteria: The tailing factor for Finasteride peak is NMT 1.3.The Theoretical plates for Finasteride peak is NLT 10,000.The % RSD for the Chromatographic purity of Finasteride from the six preparations of the method precision solutions should be not more than 2.0.

Conclusion: The above results reveal that the method is precise.

Linearity: Linearity for Finasteride was determined in the concentration range from 25 to 150 % levels of test concentration levels.

Results: Table 18: System suitability

Parameter	System suitability	
Result	1.1	12210

Acceptance Criteria	Tailing factor NMT 1.3	Theoretical plates NLT 10,000

Table 19: Linearity Results of Finasteride

Level (%w/w)	Finasteride Concentration (ppm)	Finasteride Peak Area
25 (L1)	250.03	4979054
50 (L2)	500.05	9863384
75 (L3)	750.08	14714427
100 (L4)	1000.10	19674496
150 (L5)	1500.15	29555313
Correlation Coefficient	0.99999	
Slope	19663.33318	
Y-Intercept	25095.18919	
% Y-Intercept	0.2	
Residual Sum of Squares	0.9999	

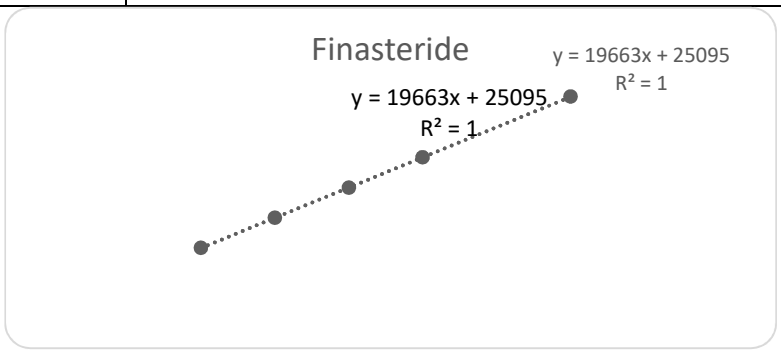


Figure 10: Linearity graph for Finasteride

Acceptance criteria: The tailing factor for Finasteride peak is NMT 1.3.The Theoretical plates for Finasteride peak is NLT 10,000.Report the slope &intercept, bias at 100 % level should be between ± 2.0 %. The Correlation coefficient should be not less than 0.99.

Conclusion: The above results reveal that the method is linear over the range of 25 % to 150% of working level concentration.

Accuracy: As per methodology, injected blank, 50%, 100% and 150% sample solutions and injected into the HPLC system and demonstrated the accuracy of the method. Calculated the system suitability parameters and % Individual recovery and % mean recovery.

Results: Table 20: System suitability

Parameter	System suitability	
Result	1.1	12210
Acceptance Criteria	Tailing factor NMT 1.3	Theoretical plates NLT 10,000

Table 21: Accuracy of Finasteride

Sample No	Spike level	Added ppm	Found ppm	'%' Recovery	'%' Mean recovery	%RSD
1	50%	500.1	498.9	99.8	100.0	0.4
2	50%	500.1	501.3	100.2		
3	50%	500.1	501.1	100.2		
4	50%	500.1	501.9	100.4		
5	50%	500.1	496.9	99.4		

6	50%	500.1	500.0	100.0		
1	100%	1000.2	1007.3	100.7	100.3	0.4
2	100%	1000.2	1002.7	100.2		
3	100%	1000.2	1000.3	100.0		
1	150%	1500.3	1490.1	99.3	99.6	0.3
2	150%	1500.3	1495.4	99.7		
3	150%	1500.3	1500.2	100.0		
4	150%	1500.3	1487.2	99.1		
5	150%	1500.3	1493.0	99.5		
6	150%	1500.3	1497.2	99.8		

Acceptance criteria

The tailing factor for Finasteride peak is NMT 1.3. The Theoretical plates for Finasteride peak is NLT 10,000. Individual % recovery and mean % recovery value for Finasteride at each level should be in between 97 to 103. % RSD for Individual % recovery at each level should not be more than 2.0

CONCLUSION

The above results reveal that the method is accurate.

CONCLUSION: The current analytical method was validated according to the protocol, and it passes the acceptance criteria. Thus, it was determined that the analytical approach is particular, precise, linear, accurate, rugged, and robust. As a result, the current, analytical approach is suitable for regular analysis and serves its intended function.

REFERENCES

1. Sharma B, Instrumental methods of chemical analysis, 19th edition, Goel PublishingHouse, 2003.
2. Willard H, Instrumental method of analysis, 7th edition, CBS Publishers andDistributors, New Delhi, 1986.
3. Remington, The Science and practice of pharmacy, 21st edition, (1), 2006.
4. Satoshkar R, Bhandarkar S and Ainapure S, Pharmacology and pharmacotherapeutics,15th edition, Popular Prakashan, Mumbai, 1996.
5. Wolff ME. Berger's, A Medicinal Chemistry, 4th edition, Wiley Interscience, NewYork, 1981.
6. Doserge RF. Wilson and Griswold's, Text book of organic medicinal andpharmaceutical chemistry, 8thedition, Lippincott Company, 1982.
7. AshutoshKar, Pharmaceutical analysis, 1st edition, Vol I, CBS Publishers andDistributers, New Delhi, 2007.
8. Beckett AH and Stenlake JB, Practical pharmaceutical chemistry, 4th edition, CBSPublishers and Distributers, New Delhi, 2007.
9. Indian Pharmacopoeia, Indian Pharmacopoeia commission, Ghaziabad, 2007.
10. British Pharmacopoeia, Medicines and health care product regulatory agency, London,2010.
11. Reynolds JEF, Martindale- The Extra Pharmacopoeia. 28thedition, ThePharmaceutical press, London, 1982.
12. Willard H, Merritt L, John A. Dean and Frank A. Settle, Instrumental methods ofanalysis, 7th edition, CBS Publishers and Distributers, New Delhi, 1986.
13. Fulton GK, Barbara S. Larsen and Charles N. McEwen, Gas chromatography andmass spectroscopy-A practical guide, Academic Press, London, 1996.
14. Basett J, Denney RC, Jerry GH and Mendham J, Vogel's text book of quantitativeinorganic analysis, 4th edition, London, 1986.
15. Howard GA and Martin AJP, the separation of the C12- C18 fatty acids by reversed-phase partition chromatography, Biochemical Journal, 1950; 46(5): 532-538.
16. Krusulovic AM and Brown RP, Reverse Phase High performance liquidchromatography, Theory practice and biomedical applications, 1982.
17. GurdeepChatwal and Sham Annand, Instrumental methods of chemical analysis, 1st Edition, Himalaya publishing House, Delhi, 1999.
18. Joachim Ermer and John H, Method validation in pharmaceutical analysis- A guide tobest practice, Wiley – VCH VerlagGmbh and Weinheim, 2005.
19. Thomas H, Stout, John G and Dorsey, High-Performance Liquid Chromatography,Eurand America, Inc. Vandali, Ohio, 2002.
20. Synder LR and Kirkland JJ, Introduction to modern liquidchromatography, Wileyinterscience, New York, 1979.