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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RELATED SUBSTANCES BY RP - HPLC METHOD FOR FINASTERIDE IN PURE AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The developed method was a simple, efficient, economical method for the Validation of Finasteride Impurities in Pure and Drug product by reverse phase high pressure liquid chromatography. For Finasteride Impurities Chromatography was performed on Waters Nova-Pak, C18, 250 × 4.6µ with mobile phase Water: Tetra hydro furan: ACN at a flow rate of 1.8 mL/min and eluents were monitored at 210 nm The retention times of Impurity - A, Impurity - B, Impurity - C and Impurity - D are 32.80, 40.86, 46.50 and 38.349 respectively and showed a good linearity in the concentration range of 15 - 300 µg/ml for all Impurities with a correlation coefficient of 0.999, 0.999, 0.999 and 0.999 respectively. The percent recoveries ranged between 92.1-108.2%. RSD for all Parameters are within the acceptance. The method could be successfully used for the analysis Impurities of Finasteride.

KEYWORDS

Finasteride, HPLC, Acetonitrile and Impurity profile.

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INTRODUCTION

Pharmaceutical analysis comprises those procedures necessary to determine identity, strength, quality and purity of the drug substances and drug products. Pharmaceutical analyst plays a major role in all quality controlling divisions of industry. Analytical chemistry involves separating. Identifying, and determining the relative amounts of components in a sample matrix.

Anti-infective agents treat infection by suppressing or destroying the causati -ve microorganisms like

bacteria, mycobacterium, fungi, protozoa, or viruses. Anti-infective agents derived from natural substances are called as antibiotics and those produced from synthetic substances are called antimicrobials. However, these two terms are now used interchangeably. An anti-infective agent should be chosen on the basis of its pharmacological properties and spectrum of activity as well as on various bost (patient) factors. A combination of drugs should be given only when clinical experience has shown such therapy to be more effective than single-agent therapy in a particular treatment. A multiple agent regimen can increase the risk of toxic drug effects and in a few cases result, a drug antagonism and subsequent therapeutic ineffectiveness.

The methods of estimation of drugs are divided into physical, chemical, physicochemical and biological ones. Of them, physical and physicochemical methods are used mostly. Physical methods of analysis involve the studying of the physical properties of a substance. They include determination of the solubility, transparency or degree of turbidity, color density or specific gravity (for liquids), moisture content, melting, freezing and boiling points. 3Physicochemical methods are used to study the physical phenomenon's that occur as a result of chemical reactions. Among the physicochemical. Methods are optical refractometry, Polarimetry, emission and fluorescent methods of analysis, photometry including photo colorimetry, spectrophotometry, nephelometry and turbidimetry, electrochemical (potentiometry, amperometry, coulornetry, voltametry, (column, paper, thin layer, gas-liquid, high performance liquid chromatography) methods are generally preferable. Methods involving nuclear reactions such as nuclear magnetic resonance (NMR) and paramagnetic resonance (PMR) are becoming more and more popular, the chemical methods include the gravimetric and volumetric procedures, which are based on complex formation. Acid-base, precipitation and redox reactions. Titrations in non-aqueous media and complexometry have been widely used in pharmaceutical analysis whenever the existing amounts are in milligram level and the interferences are negligible. The methods (HPLC, GLC, NMR and Mass Spectroscopy) of choice for assay involve sophisticated equipment that are very costly and pose problems of maintenance, Hence they are not in the reach of most laboratories and small-scale industries, which produce bulk drugs and pharmaceutical formulations. However this sophisticated equipment usage eliminate the difficulties encountered in the determination of minute amounts of degradation products or the analysis of the metabolites of drugs in body fluids[1].

METHOD DEVELOPMENT[2,3,4]

Instruments used

Instruments used in the method development are placed in Table No.1.

Reagents used

Tetrahydrofuran (HPLC grade), Water (Milli Q-Grade) and Acetonitrile (GR grade).

Preparation of solutions

Preparation of Diluent

Prepare required volume of water and Acetronitrile in the ratio of 50:50 mix well and Sonicated for 30 mints for degassing.

Standard Preparation

Weigh accurately 100mg of Finasteride working standard into a 10 ml volumetric flask, dissolve and dilute to volume with diluents and sonicate.

Test preparation (b)

Weigh accurately 100mg of Finasteride sample in to 10 ml volumetric flask, dissolve and dilute to volume with diluents, Prepare the test solution six times from the same homogenous sample.

Reference solution (c)

Dilute 2.0 ml of above test solution (b) to l00 ml with dilute. Further dilute 1.0 ml of this solution to 10 ml with the same diluents.

Method development[5,6]

Proper selection of the method depends upon the nature of the sample molecular weight, and solubility. The drug selected for the present study was polar. Polar compounds can be separated by Reverse base chromatography. Reverse phase chromatographic technique was selected for Initial

separations from the knowledge of properties of the compound. C_{18} column was chosen as stationary phase and different mobile phases were checked and the most suitable condition was optimized. The objective of this experiment was to optimize the Related Substances method for finasteride based on the literature survey. So here the trials mentioned describes how the optimization was done.

SELECTION OF WAVELENGTH FOR DETECTION BY SCANNING IN UV[7]

The working standard solution of Finasteride was scanned in the UV region and spectrum was recorded. Distilled water was used as the blank. Solutions were scanned on spectrophotometer in the UV range of 200-400nm. It was seen that at 210 nm maximum absorbance was found.

In HPLC, proper peak response was observed using 210nm. Hence, 210 nm was selected as the wavelength for estimation in HPLC.

Trial - I

Chromatographic system

A liquid chromatography is equipped with a UV-Visible detector.

Mobile phase

Water, Tetrahydrofuran and Methanol in the ratio of 50:25:25.

Chromatographic conditions

Flow rate : 1.2 ml/min
Column : Novpak C18, 250× 4.6mm, 4µ or equivalent
Detector wave length : 210 nm
Injection volume : 15µl
Temperature : 550C
Run time : 80 mints
Diluent : Mobile phase

Conclusion

In this trail the peak of finasteride was broad, tailing was observed, there is no sharpness of peak and impurities peaks are not eluted, hence futher studies are conducted.

Trial - II

Chromatographic system

A liquid chromatography is equipped with a UV-Visible detector.

Mobile phase

Water, Tetrahydrofuran and Methanol in the ratio of 60:20:20

Chromatographic conditions

Flow rate : 1.4 ml/min
Column : Novpak C18, 250 × 4.6mm, 4µ or equivalent
Detector wave length : 210 nm
Injection volume : 15µl
Temperature : 550C
Run time : 80 mints
Diluent : Prepare a solution of water and Acetronitrile in the ratio of 1:1(v/v)

Conclusion

In this trail, impurity A merged with unknown impurity and tailing was observed. Hence gone for further trail as it was not satisfactory.

Trial - III

Chromatographic system

A liquid chromatography is equipped with a UV-Visible detector.

Mobile phase

Water, Tetrahydrofuran and Acetonitrile in the ratio of 60:20:20

Chromatographic conditions

Flow rate : 1.8 ml/min
Column : Novpak C18, 250× 4.6mm, 4µ or equivalent
Detector wave length : 210 nm
Injection volume : 5µl
Temperature : 550C
Run time : 80 mints
Diluent : Prepare a solution of water and Acetronitrile in the ratio of 1:1(v/v)

Conclusion

In this trail Impurity C was not eluted, so next rail was conducted.

Optimized method

Chromatographic system

A liquid chromatography is equipped with a UV-Visible detector.

Mobile phase

Water, Tetrahydrofuran and Acetonitrile in the ratio of 80:10:10

Chromatographic conditions

Flow rate : 1.8 ml/min

Column : Novpak C18, 250× 4.6mm, 4μ or equivalent

Detector wave length : 210 nm

Injection volume : 15μl

Temperature : 550C

Run time : 80 mints

Diluent : Prepare a solution of water and Acetronitrile in the ratio of 1:1(v/v)

Conclusion

This method was finalized, because all peaks of Finasteride and its Impurities are well separated and have the better resolution.

METHOD VALIDATION[8,9,10]

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 the intended analytical applications.

Validation parameters

- System Suitability
- Specificity/selectivity
- Linearity
- Accuracy
- Precision
- Limit of Detection
- Limit of Quantificatiolution Stability
- Robustness
- Ruggedness

All the parameters are done and the results are placed in the Table No.4.

Table No.1: Instruments used

S.No	Name	Make/Model
		Waters 2695
1	HPLC instrument	Alliance
	• Series	Empower 2
	• Software	Isocratic
2	Pump	Waters
3	Column	Waters 2489 UV/Vis detector
4	Detector	Waters 2996 PDA detector
		Waters 2487 Dual wavelength detector

Table No.2: Chemical names for Impurities

S.No	Impurity Code	Chemical Name
1	Impurity A	N- (1, 1-dimethylethyl)-3-oxo-4-aza-5α- androstane - 17β-carboxamide.
2	Impurity B	Methyl 3-oxo-4-aza-5α-androst-1-ene-17β-carboxylate.
3	Impurity C	N-(1, 1-dimethylethyl)-3-oxo-4-azaandrosta-1, 5-diene-17β-carboxamade.
4	Impurity D	N-(1, 1-dimethylethyl)-3-oxo-4-aza-5α-androst-1-ene-17β- carboxamide.

Table No.3: Results of optimized method for Sample

S.No	Name of the Peak	Retention time (min)
1	Finasteride	34.511
2	Impurity - A	32.800
3	Impurity - B	40.860
4	Impurity - C	46.500
5	Impurity - C	38.349

Table No.4: Results of validation parameters

<table>
<tr><th>S.No</th><th>Validation Parameter</th><th>Acceptance Criteria</th><th colspan="4">Results</th></tr>
<tr><td rowspan="4">1</td><td rowspan="4">System Suitability</td><td>% RSD Standard solution should be not more than 1.0%.</td><td colspan="4">0.89</td></tr>
<tr><td>Theoretical plate count should not be less than 6000.</td><td colspan="4">15252.1</td></tr>
<tr><td>The tailing factor [Asymmetry] should be NMT 4</td><td colspan="4">1.27</td></tr>
<tr><td>Resolution should not be less than 1.5</td><td colspan="4">1.74</td></tr>
<tr><td>2</td><td>Specificity</td><td>The interference of the diluents/placebo is considered insignificant, if the chromatogram of the placebo shows no peak, at the retention time of analyte peak</td><td colspan="4">No peaks are eluted</td></tr>
<tr><td>3</td><td colspan="6">Precision</td></tr>
<tr><td></td><td>System precision</td><td>The % RSD calculated on 6 determinations should not be more than 1.0%</td><td colspan="4">0.32</td></tr>
<tr><td rowspan="2"></td><td rowspan="2">Method Precision</td><td rowspan="2">The % RSD calculated on 6 determinations should not be more than 10.0 %</td><td>Imp-A</td><td>Imp -B</td><td>Imp - C</td><td>Imp-D</td></tr>
<tr><td>0.39</td><td>1.37</td><td>1.35</td><td>4.32</td></tr>
<tr><td rowspan="2">4</td><td rowspan="2">Linearity</td><td rowspan="2">The correlation coefficient should be ≥0.999</td><td>Imp-A</td><td>Imp-B</td><td>Imp-C</td><td>Imp-D</td></tr>
<tr><td>0.999</td><td>0.999</td><td>0.999</td><td>0.999</td></tr>
<tr><td rowspan="2">5</td><td rowspan="2">Accuracy</td><td rowspan="2">Mean % recovery at each level should be between 85%-115%</td><td>Imp-A</td><td>Imp- B</td><td>Imp-C</td><td>Imp-D</td></tr>
<tr><td>103.4-107.4</td><td>92.1 -108.2</td><td>92.1-104.2</td><td>99.7-105.8</td></tr>
<tr><td>6</td><td>Robustness</td><td>The system suitability parameters should pass for all conditions</td><td colspan="4">The system suitability parameters passed for all the conditions</td></tr>
<tr><td rowspan="2">7</td><td rowspan="2">Limit of Detection</td><td rowspan="2">The Signal to noise ratio is in between 2 and 3</td><td>Imp-A</td><td>Imp-B</td><td>Imp - C</td><td>Imp-D</td></tr>
<tr><td>2.94</td><td>2.89</td><td>2.83</td><td>2.3</td></tr>
<tr><td>8</td><td>Limit of Quantification</td><td>The signal to noise ratio is in between 9.5 to 10.4</td><td>10.15</td><td>10.21</td><td>10.21</td><td>9.77</td></tr>
<tr><td rowspan="6">9</td><td rowspan="6">Ruggedness</td><td rowspan="6">%RSD should not be more than 2.0%</td><td colspan="4">Different Column - I</td></tr>
<tr><td>Imp-A</td><td>Imp- B</td><td>Imp- C</td><td>Imp-D</td></tr>
<tr><td>0.60</td><td>0.63</td><td>0.66</td><td>1.14</td></tr>
<tr><td colspan="4">Different Column- II</td></tr>
<tr><td>Imp-A</td><td>Imp-B</td><td>Imp- C</td><td>Imp- D</td></tr>
<tr><td>0.50</td><td>1.44</td><td>0.57</td><td>0.93</td></tr>
</table>

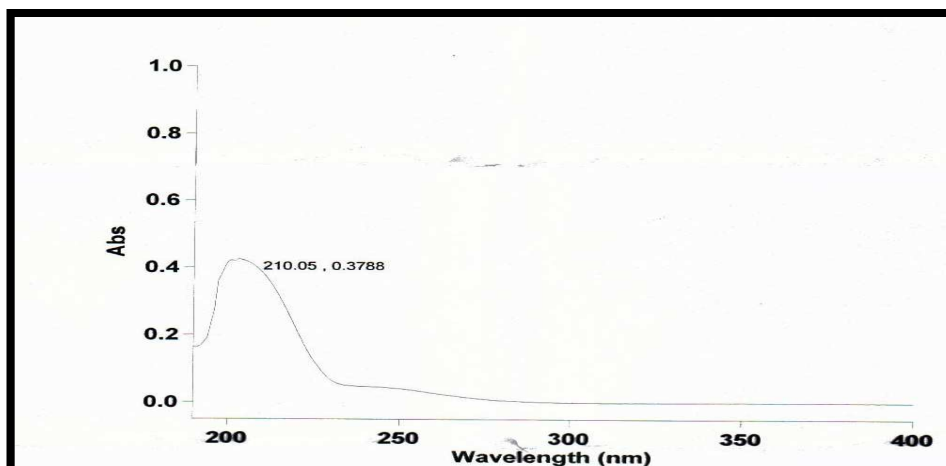

Figure No.1: Spectra of Finasteride showing λ max of 210nm

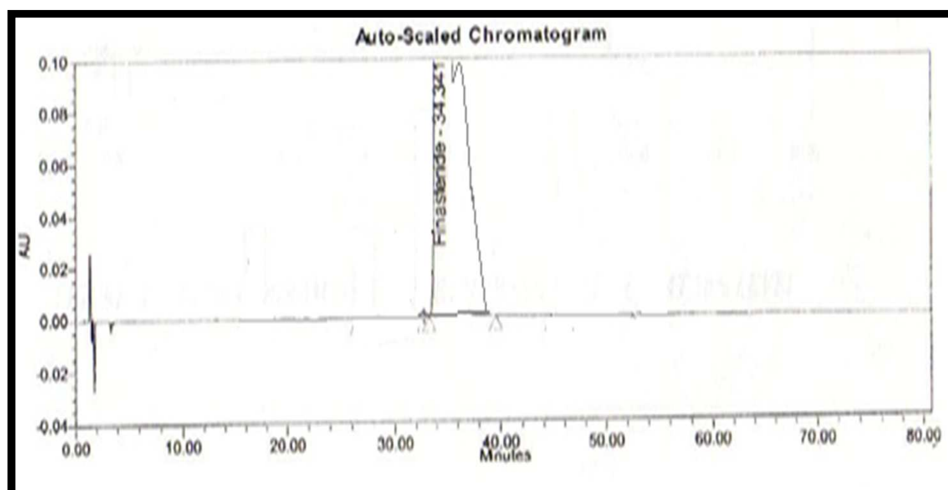

Figure No.2: Chromatogram for trial – 1

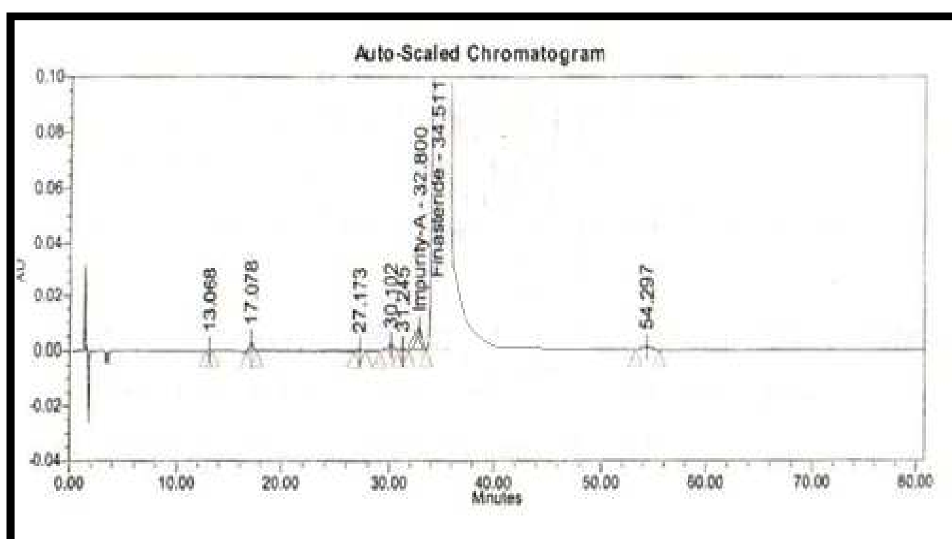

Figure No.3: Chromatogram for trial - II

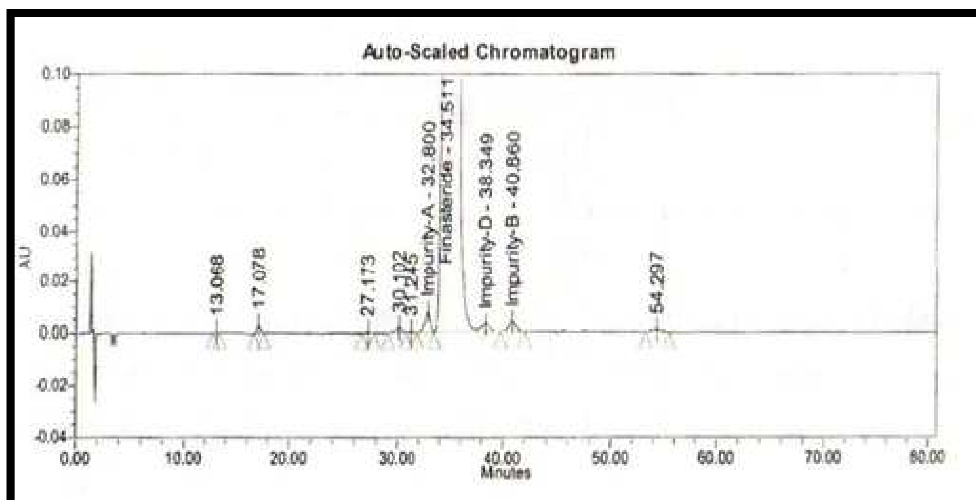

Figure No.4: Chromatogram for trial – III

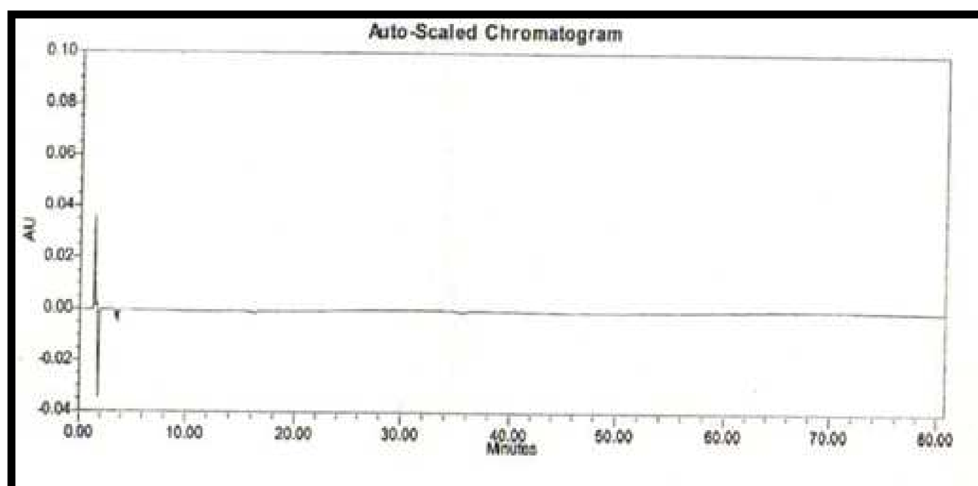

Figure No.5: Chromatogram of Finasteride by RP-HPLC Blank

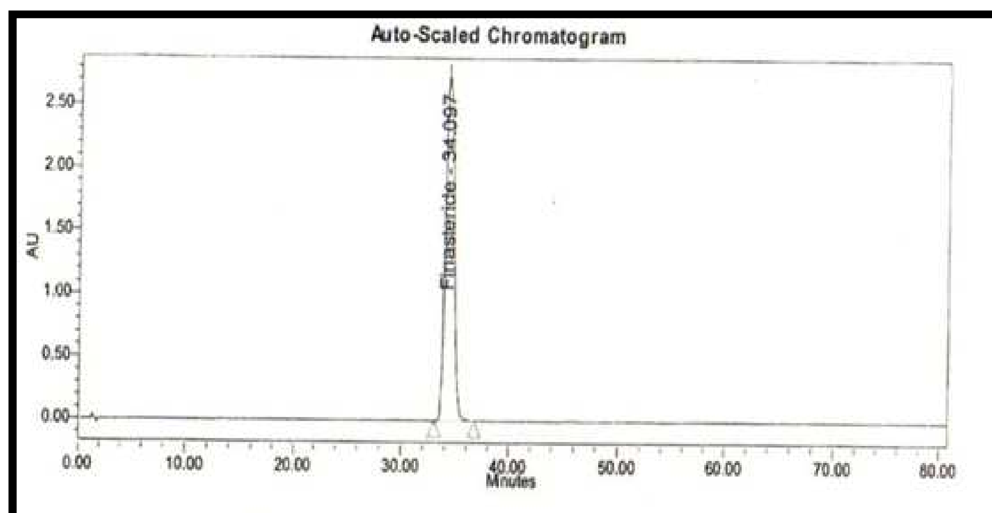

Figure No.6: Chromatogram of Finasteride by RP-HPLC Standard

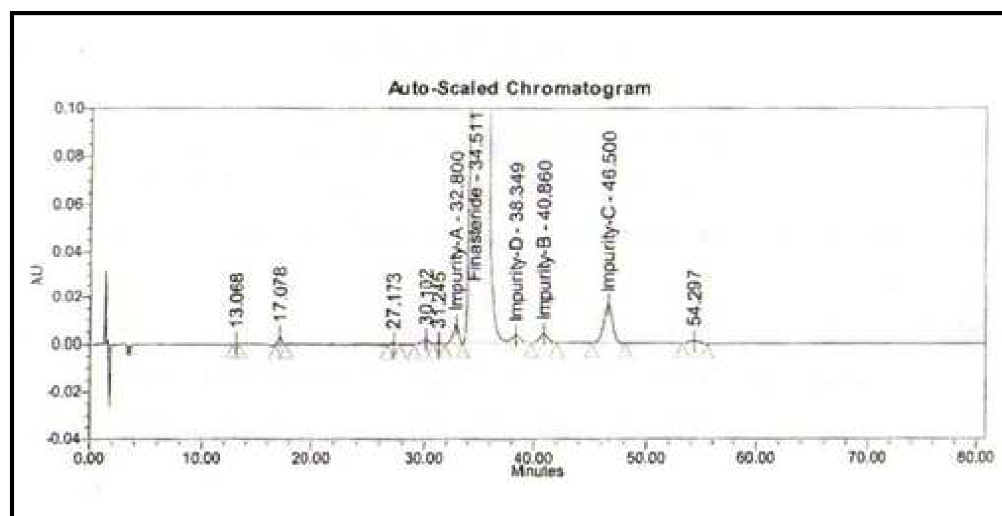

Figure No.7: Chromatogram of Finasteride by RP-HPLC Standard Sample

CONCLUSION

Several Related substance methods has been developed for the determination of Finasteride in formulation and biological fluids but literature survey shows few methods for the determination of impurity profile in Finasteride so this method is very useful for the determination of impurity profile in Finasteride bulk drug. This method was validated as per ICH guidelines and met the regulatory requirements for selectivity, accuracy and stability. To consider the obtained data, it was possible to affirm that the proposed method was fast, simple and suitable for the accurate determination of Finasteride and its impurities in bulk drug.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Basavaiah K. Determination of Finasteride in Tablets by High Performance Liquid Chromatography, *E-Journal of Chemistry,* 4(1), 2007, 109-116.
2. Akheel A Syed. LC determination of finasteride and its application to storage stability studies, *Journal of Pharmaceutical and Biomedical Analysis,* 15(5-6), 2001, 1015-101.
3. Segal A I. A stability-indicating HPLC method to determine finasteride in a tablet formulation, *Journal of Liquid Chromatography and Related technologie,* 25(20), 2002, 3167-3176.
4. Vishnu P. Simultaneous determination of Finasteride and Tamsulosin in pharmaceutical preparations by ratio derivative spectroscopy, *Choudhari Journal of Pharmacy Research,* 2(6), 2009, 1065-1067.
5. Ilango K, Valentine P, Lakshmi K S. Spectrophotometric Method for the Estimation of Finasteride in Tablets, *Indian Journal of Pharmaceutical Sciences,* 64(2), 2002, 174-175.
6. Sanjay B. Bari. HPTLC Method Validation for simultaneous determination of Tamsulosin Hydrochloride and Finasteride in Bulk and Pharmaceutical Dosage Form, *J Anal Bioanal Techniques,* 2(2), 2011, 1000119.

7. Noel A. Gomes, Ashutosh Pudage, Santosh S. Joshi, Vikas V. Vaidya, Sagar A. Parekh and Amod V. Rapid and Sensitive LC-MS-MS method for the simultaneous estimation of alfuzosin and Dutasteride in human plasma, *Chromatographia,* 69(1-2), 2009, 9-18.
8. Giuseppe Carlucci. Finasteride in biological fluids: extraction and separation by a graphitized carbon black cartridge and quantification by high-performance liquid chromatography, *Journal of Chromatography B: Biomedical Sciences and Applications,* 693(1), 1997, 245-248.
9. Carlin J R, Christofalo P, Arison B H, Ellsworth R E, Rosegay A, Miller R R, Chiu S H, and Vanden Heuvel W J. Disposition and metabolism of finasteride in dogs, *Drug Metab Dispos,* 25(1), 1997, 100-109.
10. Sevgi Tatar. A new spectrophotometric method for the determination of finasteride in tablets, *U/U Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* 67(3-4), 2007, 778-783.
11. Szabolcs Feketea. Validated UPLC method for the fast and sensitive determination of steroid residues in support cleaning validation in formulation area, *Journal of Pharmaceutical and Biomedical Analysis,* 49(3), 2009, 833-838.
12. Xiaohong Chen. Development and validation of an LC-MS assay for finasteride and its application to Prostate Cancer Prevention Trial sample analysis, *J Chromatogr Sci,* 46(4), 2008, 356-361.
13. Patel D B. Validated stability indicating HPTLC method for the determination of Tamsulosin hydrochloride in pharmaceutical dosage forms, *International Journal of Chemtech Research,* 2(1), 2010, 646-652.
14. Constanze M L. High Performance liquid chromatographic method for the dermination of finasteride in human plasma at therapeutic doses, *Journal of Chromatography B: Biomedical Sciences and Applications,* 566(1,3), 1991, 127-134.
15. Takano T. Determination of finasteride in human plasma using direct injection with column switching, *J Chromatogr B Biomed Appl,* 676(1), 1996, 141-146.

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