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Research Article

Determination of dutasteride for analytical method development and validation in bulk as well as in pharmaceutical dosage form by using RP-HPLC

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ABSTRACT

Objective: The present work was undertaken with the aim to develop and validate a rapid and consistent RP-HPLC method in which the peaks will be appear with short period of time as per ICH Guidelines.
Methods: The proposed method was simple, fast, accurate and precise method for the Quantification of drug in the dosage form, bulk drug as well as for routine analysis in Quality control. Reversed-phase high-performance liquid chromatography (RP-HPLC) methods was developed and validated for simultaneous estimation of Dutasteride in bulk drug and in combined dosage forms. RP-HPLC separation was achieved on a Symmetry C18 (4.6 x 250 mm, 5µm, Make: XTerra) under an Isocratic Mode. The mobile phase was composed of Phosphate Buffer (40%) whose pH was adjusted to 3.0 by using Orthophosporic Acid & Acetonitrile (80%) [HPLC Grade]. The flow rate was monitored at 1.0 ml per min. The wavelength was selected for the detection was 280 nm. The run time was 8 min.
Results: The retention time found for the drugs Dutasteride were about 2.2 min. The linearity was established in the range of 20 to 150 µg/ml. The proposed method was adequate sensitive, reproducible, and specific for the determination of Dutasteride in bulk as well as in pharmaceutical dosage form. The validation of method was carried out utilizing ICH-guidelines. The described RP-HPLC method was successfully employed for the analysis of pharmaceutical formulations containing combined dosage form. In the research the proposed method was found to be suitable and accurate for the Quantitative determination of the drug in pharmaceutical dosage form.
Conclusion: The method was simple, precise, accurate and sensitive and applicable for the simultaneous determination of Dutasteride and Tamsulosin hydrochloride in bulk drug and in combined dosage forms by using the above said chromatographic conditions.

Keywords: Dutasteride, ICH Guidelines, HPLC

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INTRODUCTION

Dutasteride, a selective inhibitor of both, type 1 and type 2 isoforms of 5α- reductase (5-AR) enzyme that converts testosterone to 5α-dihydrotestosterone (DHT) which is responsible for enlargement of prostate, is used in treatment of benign prostatic hyperplasia, frequently occurring in men over the age of 50 years [1]. Chemically, DTS is (5α,17β)-N-{2,5 bis(trifluoromethyl) phenyl}-3-oxo-4-azaandrost-1-ene- 17-carboxamide with an empirical formula $C_{27}H_{30}F_6N_2O_2$, representing a molecular weight of 528.5 g/mol [2]. Literature survey revealed LC-MS and HPLC methods for estimation of DTS in human plasma and pharmaceutical dosage forms [3–5]. A LC-MS-MS method is reported for the simultaneous determination of Tamsulosin and Dutasteride in human plasma [6]. The intracellular enzyme that converts Testosterone to Dutasteride is used for the treatment of patients with symptomatic benign prostatic hyperplasia. Literature survey reveals that several analytical and Bioanalytical methods was reported for the analysis of Dutasteride. For Dutasteride, the methods reported were alone or in combination with other drugs. These include, HPLC [7-9] and HPTLC [10] methods in bulk and pharmaceutical dosage form, stability indicating LC methods [11- 12], LC-MS [13- 14] methods, spectrophotometric analysis of Dutasteride in tablets [15] were reported.

The present work deals with RP-HPLC method development and validation for the quantitative analysis of Tamsulosin and Dutasteride and its stress degradation products. The aim of the present work was to develop an economic, accurate, specific, reproducible RP-HPLC method using PDA detection for the determination of Tamsulosin and Dutasteride, either in bulk form or in pharmaceutical dosage form. The chemical structure of the drugs were represented in fig. no. 1.

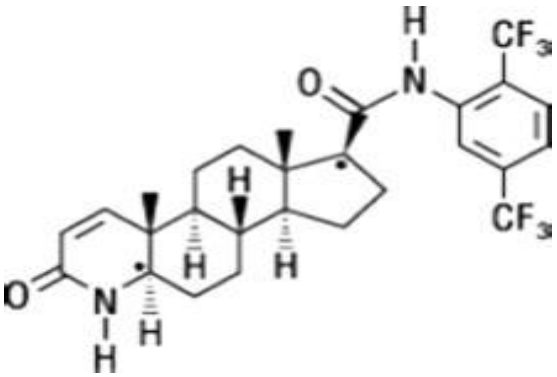

Fig. 1: It shows the chemical structure of Dutasteride

MATERIALS & METHOD

Chemicals and Reagents Used

The following chemicals were procured for the process: Water [HPLC Grade], Methanol [HPLC Grade], Acetonitrile [HPLC Grade], Dutasteride [Working standards] & KH_2PO_4 all the chemicals were procured from STANDARD SOLUTIONS and the tablets were collected from the Local market.

Apparatus and Chromatographic Conditions:

Equipment: High performance liquid chromatography equipped with Auto Sampler and DAD detector.

UV/VIS spectrophotometer: LAB INDIA UV 3000+

pH meter and Weighing machine: Schimadzu

Column: Symmetry C18 (4.6 x 150mm, 5µm, Make: XTerra) or equivalent

Phosphate Buffer: 6.8 grams of Potassium Dihydrogen Ortho Phosphate in 1000 ml Water [HPLC Grade] pH adjusted with Orthophosporic acid.

pH: 2.5

Mobile phase: Phosphate Buffer: Acetonitrile (20: 80 v/v)

Flow rate: 0.8 ml per min **Wavelength:** 280 nm **Injection volume:** 20 µl **Run time:** 7min.

Preparation of Phosphate buffer [21]: The buffer solution was prepared by dissolving accurately weighed 6.8 grams of potassium dihydrogen ortho phosphate and transferred into a clean and dry 1000 ml volumetric flask, dissolved and diluted with 1000ml water [HPLC Grade]. The final pH of the buffer was adjusted to 2.5 by using Orthophosporic acid.

Preparation of mobile phase: The Mobile Phase was prepared by mixing 200 ml (20%) of the above buffer and 800 ml of Acetonitrile [HPLC Grade] (80%) and degassed in an ultrasonic water bath for 10 minutes. Then the resultant solution was filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as Diluent.

Preparation of Dutasteride Standard & Sample Solution:

Preparation of Stock solution: The stock solution was prepared by weighing accurately 50 mg Dutasteride transferred into a clean and dry 100 ml volumetric flask. About 70 ml of diluent was added and sonicated. The volume was made upto the mark with diluent. From the above prepared Stock solution pipette out 1.5 ml of solution and transferred into a clean and dry 10ml volumetric flask, the diluent was made upto the mark to get concentration of 75µg/ml.

Preparation of Sample Solution: The sample solution was prepared by weighing equivalently 50 mg of Dutasteride and transferred into a 100 ml

clean and dry volumetric flask added about 70ml of diluent and sonicated to dissolve it completely and the volume made up to the mark with the same solvent. From above prepared stock solution pipette out 1.5ml.

of solution and transferred into a clean and dry 10 ml volumetric flask, the diluent was added upto the mark 10ml to get final concentration of 75µg/ml. The standard and sample solutions of 75 µg/ml were injected five times and the peak areas were recorded. The mean and percentage relative standard deviation were calculated from the peak areas.

System Suitability [22-24]: The Tailing factor for the peaks due to Dutasteride and Tamsulosin hydrochloride in Standard solution should not be more than 1.5. The Theoretical plates for the Dutasteride and Tamsulosin hydrochloride peaks in Standard solution should not be less than 2000. The system suitability of the method was checked by injecting five different preparations of the Dutasteride standard. The parameters of system suitability were checked.

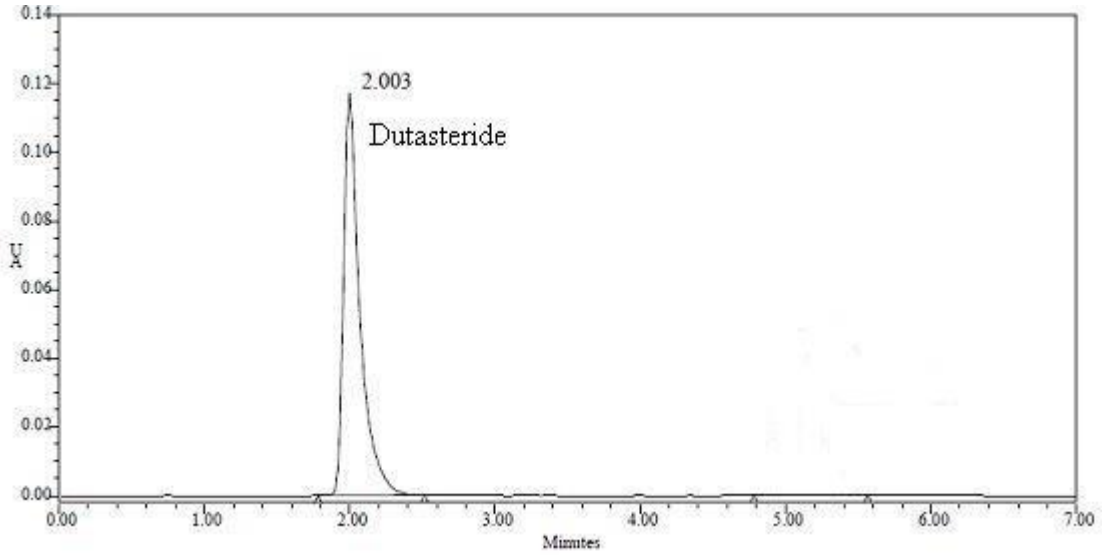

Fig. 3: It shows the Chromatogram for System Suitability

S. No.	Name	Retention time	Area (µV sec)	Height (µV)	USP tailing	USP plate count
1.	Dutasteride	2.00	987156	124568	1.6	3675

Acceptance criteria: The Resolution between two drugs should not be less than 2. The Theoretical plates should not be less than 2000. The Tailing factor should not be less than 0.9 and not more than 2. It was found from above data; that all the system suitability parameters for developed method were within the limit.

Precision: It is a measure of degree of repeatability of an analytical method under normal operation and it is normally expressed as % of relative standard deviation (% RSD). The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The chromatogram was represented in fig. no.6. (Table no. 2 & 3)

Intermediate precision/ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The chromatogram was represented in fig no. 7. (Table no. 4 & 5).

Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and value found. The standard solution with Accuracy - 50%, Accuracy -100% and Accuracy -150% were injected into chromatographic system and calculated the amount found and amount added for Dutasteride and further calculated the individual recovery and mean recovery values. The chromatograms were represented in fig. no. 8, 9 & 10. (Table no. 6). Linearity: It is the ability of the method to elicit test result that is directly proportional to analytic concentration within a given range. It is generally reported as variance of

slope or regression line. It is determined by series of three to six injections of five of more standards. Different levels of solution were prepared and injected to the chromatographic system and the peak area was measured. Plotted a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The calibration curve was represented in fig. no. 11 & 12. (Table no. 7). Limit of detection: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value. Limit of detection for the drugs dutasteride & tamsulosin: The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio. Limit of detection is the lowest concentration of the substance that can be detected, not necessarily quantified by the method. (Regression statistics) The minimum concentration at which the analyte can be detected is determined from the linearity curve by applying the following formula. Limit of detection (LOD) = $\sigma S \times 3.3$ Where S – slope of the calibration curve σ – Residual standard deviation

Limit of quantification: It is defined as lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy and reliability by a given method under stated experimental conditions. LOQ is expressed as a concentration at a specified signal to noise ratio.

Limit of quantification for the drugs dutasteride and tamsulosin: The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio. Limit of Quantification is the lowest concentration of the substance that can be estimated quantitatively. It can be determined from linearity curve by applying the following formula Limit of Quantification (LOQ) = $\sigma S \times 10$ Where S – slope of the calibration curve σ – Residual standard deviation The data were represented in Table No. 9, 10 & 11 and the chromatograms were represented in Fig. no. 13, 14, 15 & 16.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The standard and samples of Dutasteride were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The flow rate was varied at 0.7 ml/min to 0.9ml/min.: The Standard solution of Dutasteride was prepared and analysed using the varied flow rates along with method developed flow rate. On evaluation of the above results, it was concluded that the variation in flow rate does not affected the method significantly. Hence it was indicated that the method was robust even by change in the flow rate. The chromatograms were represented in fig. no. 17 & 18. (Table No. 11). The Organic composition in the Mobile phase was varied from 70% to 90%.: The Standard solution for the drug Dutasteride & Tamsulosin was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition. On evaluation of the above results, it was concluded that the variation in 10% Organic composition in the mobile phase does not affected the method significantly. Hence it was indicated that the method was robust even by change in the Mobile phase ±10. The chromatograms were represented in Fig. no. 19 & 20. (Table no. 12)

RESULTS AND DISCUSSION

The present work was undertaken with the aim to develop and validate a rapid and consistent stability indicating RP-HPLC in which the peaks will be appear with short period of time as per ICH Guidelines. The proposed method was simple, fast, accurate and precise method for the Quantification of drug in the pharmaceutical dosage form, bulk drug as well as for routine analysis in Quality control. Overall, the proposed method was found to be suitable and accurate for the Quantitative determination of the drug in pharmaceutical dosage form. The method was effectively separated the drug from its degradation product and it was employed as a stability- indicating one. The method was simple, precise, accurate and sensitive and applicable for the simultaneous determination of Dutasteride in bulk drug and in combined dosage forms. The Reversed-phase high-performance liquid chromatography (RP-HPLC) methods was developed and validated for simultaneous estimation of Tamsulosin hydrochloride and Dutasteride in bulk drug and in combined dosage forms. RP-HPLC separation was achieved on a Symmetry C18 (4.6 x 150mm, 5µm, Make: XTerra) in an Isocratic Mode. The mobile phase was composed of Phosphate Buffer (20%) whose pH was adjusted to 2.5 by using Orthophosporic Acid & Acetonitrile (80%) [HPLC Grade]. The flow rate was monitored at 0.8 ml per min. The wavelength was selected for the detection was 274 nm. The run time was 7min. The retention time found for the drugs Dutasteride is 2.003 min. It was represented in fig. no. 4 & 5.

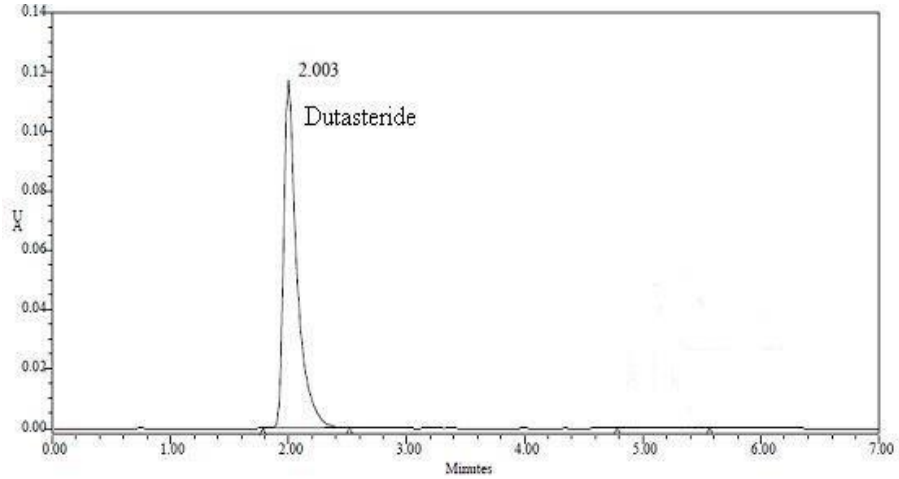

Fig. 4: Chromatogram for the optimized method development

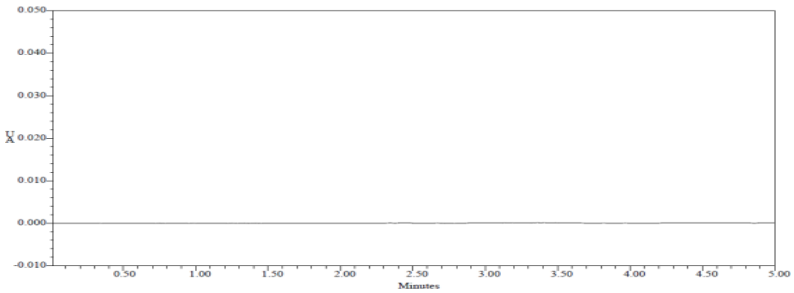

Fig. 5: It shows the Chromatogram for System Suitability

Table 2: Precision result for the drug Dutasteride

Sr. No.	Sample area	Standard area	Percentage purity
1.	**993375**	**973322**	**101.04**
2.	**995049**	**975043**	**101.03**
3.	**995219**	**976092**	**100.54**
4.	**992956**	**982956**	**100.44**
5.	**994145**	**993212**	**101.09**
Average			**100.84**
% RSD			**0.304**

Table 3: Ruggedness

Sr. No.	Sample area	Standard area	Percentage purity
1.	**981887**	**973322**	99.23
2.	**989049**	**975043**	99.11
3.	**991219**	**976092**	99.78
4.	**987956**	**982956**	99.02
5.	**989541**	**993212**	99.91
Average			**99.42**
% RSD			**0.31**

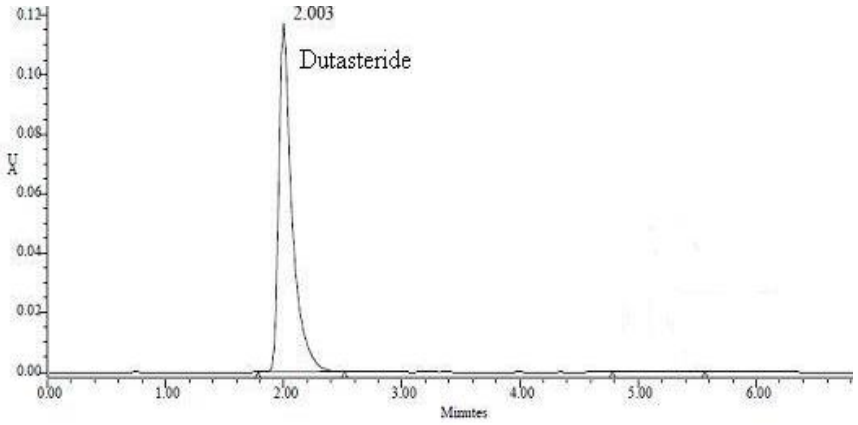

Fig. 7: Chromatogram for the Intermediate Precision

Acceptance criteria: The % RSD of five different sample solutions should not be more than 2. The %RSD obtained is within the limit, hence the method was rugged.

The Accuracy data were summarized in Table no. 6 and the chromatograms for Accuracy50%, 100% & 150%were represented in Fig. no. 8, 9 & 10.

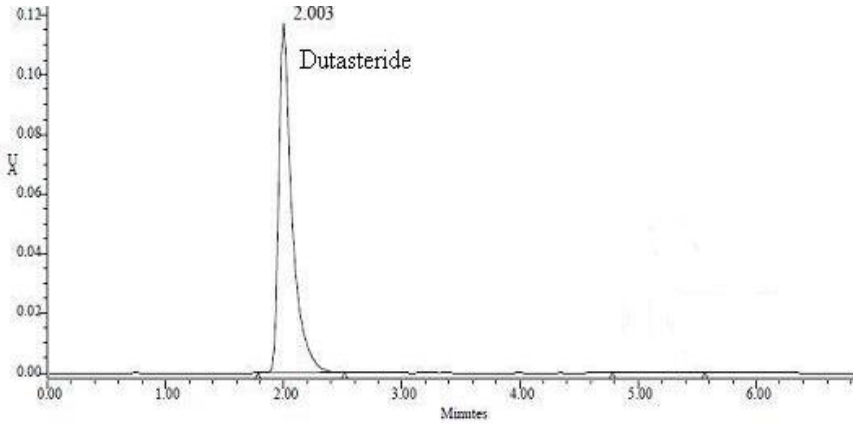

Fig. 8: Chromatogram for Accuracy (50%)

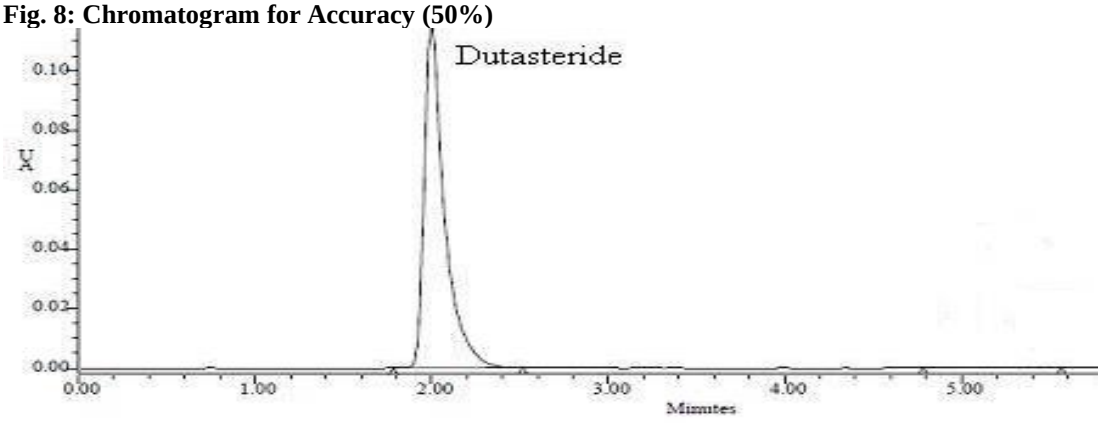

Fig. 9: Chromatogram for Accuracy (100%)

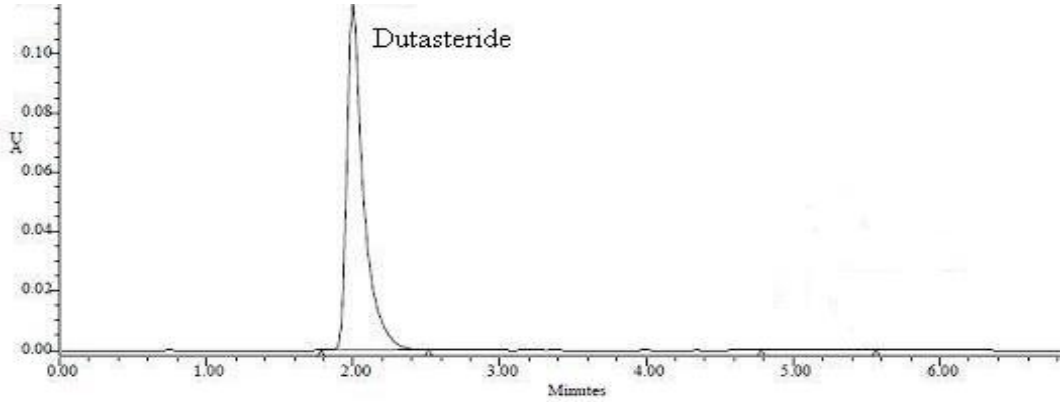

Fig. 10: Chromatogram represents for Accuracy (150%)

Table 7: It shows the recovery results for the drug Dutasteride

Sample concentration	Set no	Assay	% Recovery
50 %	1	24.8	99.8
	2	24.9	99.9
	3	24.8	99.8
Average recovery			99.9
100 %	1	49.9	99.9
	2	49.8	99.7
	3	49.7	99.7
Average recovery			99.8
150 %	1	74.8	99.8
	2	74.7	99.8
	3	74.9	99.9
Average recovery			99.9

Acceptance criteria: The percentage recovery at each level should be between (97-103%). The results obtained for recovery at 50%, 100% and 150% were within the limits. Hence the method was accurate. In order to test the linearity of the method, five dilutions of the working standard solutions for the drugs Dutasteride & Tamsulosin in the range of 25 to 125µg/ml were prepared. The data were represented in Table no. 7. Each of the dilution was injected into the column and the Linearity Curve was represented in Fig. no.11 & 12.

Sample concentration	Area
25	299800
50	663819
75	993775
100	1362536
125	1699876

Table 8: Analytical performance parameters for the drugs Dutasteride

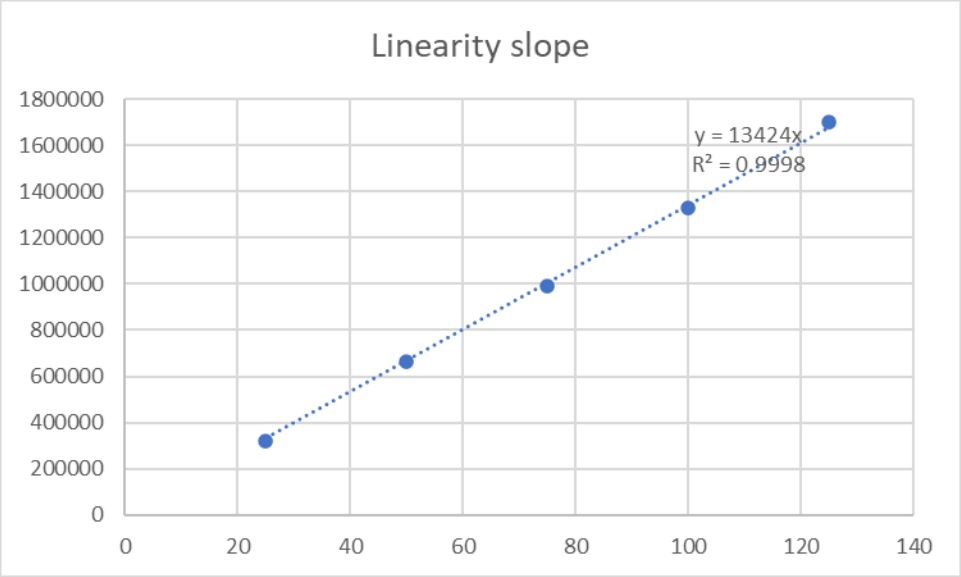

Fig. 11: Linearity curve for the drug Dutasteride

Acceptance criteria: The Correlation coefficient (R2) should not be less than 0.999. The correlation coefficient obtained was 0.999 which was in the acceptance limit. The linearity was established in the range of 25 to 150 µg/ml. The Limit of detection and limit of quantification of the method were calculated basing on standard deviation of the response and the slope (s) of the calibration curve at approximate levels of the limit of detection and limit of quantification.

The data were represented in Table No. 9 The Signal to noise ratio should be 3 for LOD. The results obtained were within the limit. The Signal to noise ratio should be 10 for LOQ solution. The results obtained were within the limit.

Table 9: LOD results for the drugs Dutasteride

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Dutasteride	56	176	3.14

Acceptance criteria: The Signal to noise ratio should be 3 for LOD. The results obtained were within the limit.

Table 10: LOQ results for the drugs Dutasteride

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Dutasteride	56	563	10.05

Acceptance criteria: The Signal to noise ratio should be 10 for LOQ solution. The results obtained were within the limit.

CONCLUSION

Development of new analytical methods for the determination of drugs in pharmaceutical dosage is important in pharmacokinetic, toxicological biological studies. Pharmaceutical analysis occupies a pivotal role in statuary certification of drugs and their formulations either by the industry or by the regulatory authorities. In industry, the quality assurance and quality control departments play major role in bringing out a safe and effective drug or dosage form. The current good manufacturing practices (CGMP) and the Food Drug Administration (FDA) guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility. Therefore, the complexity of problems encountered in pharmaceutical analysis with the importance of achieving the selectivity, speed, low cost, simplicity, sensitivity, specificity, precision and accuracy in estimation of drugs. It was concluded that the proposed new RP-HPLC method developed for the quantitative determination of Dutasteride & Tamsulosin in bulk as well as in its formulations was simple, selective, sensitive, accurate, precise and rapid. The method was proved to be superior to most of the reported methods. The mobile phases were simple to

prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence the method can be easily adopted as an alternative method to report routine determination of Dutasteride depending upon the availability of chemicals and nature of other ingredients present in the sample. The method also finds use in clinical, biological and pharmacokinetic studies for the drug Dutasteride. The method was validated as per ICH guidelines, and validation acceptance criteria were met in all cases. Application of this method for estimation of Dutasteride from tablet dosage form and stressed samples showed that neither the degradation products nor the excipients interfered in the estimation of drug. Hence, this method was specific, stability-indicating and can be successfully used for the estimation of drug in bulk and pharmaceutical dosage forms.

REFERENCES

1. Sweetman SC, editor. Martindale, The Complete Drug Reference. 34th ed. London: The Pharmaceutical Press; 2005. p. 1549.
2. Budavari S, editor. The Merck Index. 13th ed. Whitehouse station, NJ: Merck & Co, Inc; 2001. p. 3504.
3. Ramakrishna NV, Vishwottam KN, Puran S, Koteshwara M, Manoj S, Santosh M. Selective and rapid liquid chromatography-tandem mass spectrometry assay of dutasteride in human plasma. J Chromatogr B. 2004;809:117–24.[PubMed]
4. Kamat SS, Choudhari VB, Vele VT, Prabhune SS. Determination of dutasteride by LC: validation and application of the method. Chromatographia. 2008;67:911–6.
5. Subba Rao DV, Radhakrishnanand P. Stress degradation studies on dutasteride and development of a stability-indicating HPLC assay method for bulk drug and pharmaceutical dosage form. Chromatographia. 2008;67:9–10.
6. Agarwal S, Gowda KV, Sarkar AK, Ghosh D, Uttam B, Chattaraj T, et al. Simultaneous determination of tamsulosin and dutasteride in human plasma by LC-MS-MS. Chromatographia. 2008;67:11–2.
7. Ch. Amrutha Varshini, K Shantha Kumari, S Sushma, K Prakash. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Alfuzosin Hydrochloride and Dutasteride in Bulk and Pharmaceutical Dosage Form. Inventi Rapid: Pharm Analysis & Quality Assurance, 2012(4):1-4, 2012.
8. Dipti B Patel, N.J.Patel, A.M Prajapati and S.A.Patel. RP-HPLC method for the estimation of dutasteride in pharmaceutical dosage form. Journal of Planar Chromatography, 24(3):218-221, 2011.
9. D.B.Patel, N.J.Patel. Validated reverse phase high performance liquid chromatographic and high performance thin layer chromatographic method for simultaneous analysis of tamsulosin hydrochloride and dutasteride in pharmaceutical dosage form. Acta chromatographia, 22(3):419-431, 2010.
10. Shivprasad S.Deshmukh, Vaishali V.Musole, Vidhya K.Bhusari, Sunil R.Dhaneshwar. Validated HPTLC method for simultaneous analysis of Alfuzosin hydrochloride .
11. Dipti B.Patel, Natubhai J.Patel, Sejal k.Patel and Paresh patel. Validated stability indicating HPTLC method for the determination of dutasteride in pharmaceutical dosage form. Chromatography Research International, Article ID 278923, 2011.
12. Vishnu P.Choudhari and Anna Pratima Nikalije. Stability indicating TLC method for the determination of dutasteride in pharmaceutical dosage form.Chromatographia, vol 70(1-2), 309- 313, 2009.
13. N.V.S. Ramakrishna, K.N.Vishwottam, SPuran, M.koteshwara,S Manoj,M Santosh. Selective and Rapid liquid chromatographytandem mass spectrometry assay of Dutasteride in human plasma. Journal of Chromatography B, 809(1):117-114, 2004.
14. Noel A.Gomes, Ashutosh Pudage, Santosh S.Joshi, Vikas V.Vaidya, Sagar A.Parekh and Amod v,Tamhankar. Rapid and Sensitive LC-MS-MS method for the Simultaneous Estimation of Alfuzosin and Dutasteride in Human Plasma. Chromatographia, 69, (1-2), 9-18, 2009.
15. Md. Ruhul, Amin, Moynul, Hasan, Abdullah, AlMasu. Validated UV spectrophotometric estimation of dutasteride in tablet dosage form. International journal of comprehensive Pharmacy, 2(4):1-3, 2011.
16. Shivprasad S. Desmukh, Shweta S. Havele, Vaishali V. Musale, Sunil R. Dhaneshwar. Development and validation of RP-HPLC method for simultaneous estimation of alfuzosin hydrochloride and dutasteride in pharmaceutical dosage form. Der Pharmacia Lettre, 2(6):342-349, 2010.