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A Validated stability indicating LC method of assay and related substances for Finasteride

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ABSTRACT

The present paper describes the development of a stability indicating reverse phase liquid Chromatography (RPLC) method for Finasteride in the presence of its impurities and degradation products generated from forced decomposing studies. The drug substance was subjected to stress conditions of hydrolysis, oxidation, UV and thermal degradation. The degradation of Finasteride was observed under oxidative hydrolysis. The drug was found to be stable to other stress conditions attempted. Successful separation of the drug from the synthetic impurities and degradation product formed under stress conditions was achieved on a Symmetry C18 column using a mixture of water and Acetonitrile (64:34, v/v) as mobile phase. The developed HPLC method was validated with respect to linearity, accuracy, precision, specificity and robustness. The developed HPLC method to determine the related substances and assay determination 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.

Key words : Reverse Phase Liquid Chromatography, Finasteride , Degradation products.

INTRODUCTION

Finasteride (FNS), chemically known as N-(1, 1-dimethylethyl)-3-oxo-(5 α , 17 β)-4-azaandrost-1-ene-17-carboxamide (Fig 1)[1] is an antiandrogen which acts by inhibiting 5 α - reductase, the enzyme that converts testosterone to dihydrotestosterone [2]. It is being used in prostatic

hyperplasia (BPH) in low doses and in prostate cancer in higher doses. Additionally, it is registered in many countries for male pattern-baldness. The International Conference on harmonization (ICH) guideline entitled "Stability testing of new drug substances and products" requires that stress testing be carried out to elucidate the inherent stability characteristics of active substances [3]. In literature spectroscopic method reported for determination of Finasteride in tablets [4] and HPLC method for determination of Finasteride in human plasma is reported [5]. LC/MS/MS method for determination of Finasteride in biological fluids and human plasma [6,7] is reported. The reported methods were not evaluated with respect to stability indicating. To our present knowledge no stability indicating methods were reported in the literature for the determination of Finasteride and its impurities. An ideal stability indicating method one that quantifies the standard drug alone and also resolves its degradation products. Consequently, the implementation of an analytical methodology to determine Finasteride in bulk samples. The proposed method is simple, accurate, Linear specific, repeatable, stability indicating, reduces the duration of analysis and suitable for routine determination of Finasteride in Pharmaceutical samples. The proposed method was validated in compliance with ICH guidelines [8, 9] and its updated international convention [10] Superior resolution between Finasteride and its impurities was observed on Symmetry column with in short run time using a mobile phase Water and Acetonitrile.

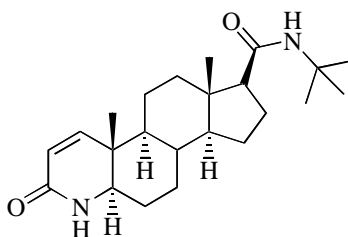


Fig.1

EXPERIMENTAL SECTION

Samples of Finasteride and its four impurities namely imp-A, imp-B, imp-C and imp-D (Fig.2) was received from Dr.Reddy's Laboratory, Hyderabad, India. HPLC grade Acetonitrile was purchased for Merck, Darmstadt, Germany. High purity water was prepared by using a Millipore Milli Q plus purification system.

Instrumentation and Chromatographic Conditions:

Waters Alliance 2695 separation module (Waters corporation, Milford, USA) equipped with 2695 PDA detector (for specificity and forced degradation studies) with Empower 2 software was used for the analysis. The column used was Symmetry C-18 (75mm X 4.6mm, 3.5 μ Waters Corporation, Milford, USA). Different mobile phases were tested in order to find the best conditions for the separation of Finasteride in presence of its potential impurities and degradation products. The optimum composition of mobile phase was determined to be Water: Acetonitrile (64:36, v/v).The flow rate was set to 1 mL min⁻¹,UV detection was carried out at 210 nm and 10 μ l injection volume were maintained .The mobile phase and samples were filtered using 0.45 μ m membrane filters. Mobile phase was degassed by ultrasonic vibrations prior to use. All determinations were performed at ambient temperature (25° C).

Method Validation:**Specificity:**

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. The specificity of the developed HPLC method for Finasteride was carried out in the presence of its impurities namely imp -A, imp-B, imp-C, and imp-D. Stress studies were performed for Finasteride bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions of UV light (254nm), acid (0.5N HCl)[11], base (0.5N NaOH), Oxidation (3.0 % H₂O₂) and heat (60°C) to evaluate the ability of the proposed method to separate Finasteride from its degradation products. Peak purity test was carried out of Finasteride peak by using PDA detector in stress samples. Assay studies were carried out of stress samples against qualified Finasteride reference standard. Assay was also calculated for Finasteride samples by spiking all four impurities at the specification level (i.e., 0.5%).

Precision:

The precision of the assay method was evaluated by carrying out six independent assays of Finasteride test samples against a qualified reference standard and calculate the % R.S.D of assay. The precision of the related substances method was checked by injecting six individual preparations of Finasteride (0.5 mg mL⁻¹) spiked with 0.5 % of imp -A, imp-B, imp-C and imp-D with respect to Finasteride analyte concentration[12,13]. % R.S.D of area for each imp -A, imp-B, imp-C and imp-D was calculated. The intermediate precision of the method was also evaluated using different analyst and different instrument in the same laboratory.

Limit of detection (LOD) and Limit of Quantification (LOQ):

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of Impurities and/or degradation products. The limit of detection (LOD) and limit of quantitation (LOQ) were separately determined at a signal to noise ratio (S/N) of 3 and 10.

Linearity:

Linearity test solutions for the assay method were prepared from Finasteride stock solutions at six concentration levels from 50% to 200% of assay analyte concentration (50%, 75%, 100%, 125%, 150%, and 200%). The peak area verses concentration data was treated by least squares linear regression analysis.

Linearity test solutions for the related substance method were prepared by dilution of stock solution to the required concentrations [14,15]. The solutions were prepared at six concentration levels from LOQ to 200% of specification level (25, 50%, 75%, 100%, 150% and 200%). Above test were carried out of 3 consecutive days in the same concentration range for both assay and related substances method. The % RSD value for the Slope and Y-intercept of the calibration curve was calculated.

Accuracy:

The accuracy of the assay method was evaluated in triplicate at three concentration levels 50%, 100% and 150 % of test concentration (0.5 mg mL^{-1}). The percentage of recoveries was calculated from the Slope and Y- intercept of the calibration curve obtained in the linearity study. The accuracy study of impurities was carried out in triplicate at 50%, 100% and 150 % of specification level (0.5%) to the Finasteride analyte concentration ($500 \text{ } \mu\text{g mL}^{-1}$). The percentages of recoveries for impurities were calculated from the slope and Y- Intercept of the calibration curve.

Robustness:

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution between Finasteride, imp -A, was recorded. The flow rate of the mobile phase was 1.0 mL min^{-1} . To study the effect of flow rate on the resolution, flow was changed by 0.1 units from 0.9 to 1.1 mL min^{-1} . The effect of the column temperature on resolution was studied at 20 and 30°C instead of 25°C . The effect of the percentage organic strength on resolution was studied by varying Acetonitrile by -3 to $+3$ % while other mobile phase components were held constant as stated in Chromatographic conditions.

Solution stability and Mobile phase stability:

The solution stability of Finasteride in the assay method was carried out by leaving both the solutions of sample and reference standard in tightly capped volumetric flasks at room temperature for 48 hours. The same sample solutions were assayed for 6 hours interval up to the study period. The mobile phase stability was also carried out by assaying the freshly prepared sample solution against freshly prepared reference standard solution for 6 hours interval up to 48 hours. Mobile phase prepared was kept constant during the study period. The % R.S.D for the assay of Finasteride was calculated during mobile phase and solution stability experiment.

The solution stability of Finasteride and its impurities in the related substance method was carried out by leaving spiked sample solution in tightly capped volumetric flasks at room temperature for 48 hours. Content of imp -A, imp-B, imp-C, and imp-D were determined for 6 hours interval up to the study period. The mobile phase stability was also carried out for 48 hours by injecting the freshly prepared sample solutions for every 6 hours interval. Content of imp -A, imp-B, imp-C and imp-D were checked in the test solutions.

RESULTS AND DISCUSSION

Optimization of Chromatographic conditions

The main objective of chromatographic method is to separate Finasteride from Imp -A, Imp-B, Imp-C and Imp-D. Impurities were co-eluted using different stationary phases such as C8, Cyno and Phenyl as well as different mobile phases. The chromatographic separation was achieved on an Symmetry C18, 75mm X 4.6 mm I.D with 3.5μ particles column using mixture of water and Acetonitrile (64:36v/v) as a mobile phase. The flow rate of the mobile phase was 1.0 mL min^{-1} , at 25°C column temperature, the peak shape of the Finasteride was found to be symmetrical. In optimized chromatographic conditions of Finasteride, Imp -A, Imp-B, Imp-C and Imp-D were separated with resolution greater than 2, typical retention times were about 5.9, 7.3, 7.9, and 8.9,

respectively (Fig 3). Developed HPLC method was found to be specific for Finasteride and its four impurities namely Imp -A, Imp-B, Imp-C and Imp-D (Fig 3).

Results of forced degradation studies:

Degradation was not observed in Finasteride sample when subjected to stress conditions like light, heat, acid and base hydrolysis (Fig.4). Degradation was observed only in oxidative conditions (Fig.4). Peak purity test results confirmed that the Finasteride peak is homogeneous in all the stress samples.

Precision

The %RSD of assay of Finasteride during the assay method precision study was within 0.12% and the %RSD for the area of Imp -A, Imp-B, Imp-C, and Imp-D in related substances method precision study was within 2.6 %. The %RSD of the assay results obtained in the intermediate precision study was within 1.5 % ,%RSD for the area of Imp -A, Imp-B, Imp-C, and Imp-D were well within 2.5% conforming to good precision of the method.

Limit of detection (LOD) and Limit of Quantification (LOQ):

The limit of detection for all impurities namely Imp -A, Imp-B, Imp-C and Imp-D were achieved 0.007, 0.010, 0.0019 and 0.014 $\mu\text{g mL}^{-1}$ respectively for 10 μL injection volume. The limit of quantification for all impurities namely -A, Imp-B, Imp-C and Imp-D are 0.021, 0.030, 0.059 and 0.041 $\mu\text{g mL}^{-1}$ respectively for 10 μL injection volume. The precision at the LOQ concentrations for Imp-A, Imp-B, Imp-C, Imp-D were performed and %RSD obtained were below 7.5%.

Linearity:

The linearity calibration plot for the assay method was obtained over the calibration ranges tested, i.e. 250-1000 $\mu\text{g mL}^{-1}$ and correlation coefficient obtained was greater than 0.99. Linearity was checked for assay method over same concentration range for 3 consecutive days. The % RSD value of the Slope and Y-Intercept of calibration curve were 1.4 and 2.5 respectively. The result shows that an excellent correlation existed between the peak area and concentration of the analysis.

Linear calibration plot for the related substances method was obtained over the calibration ranges tested i.e. LOQ (0.01%) to 1.0 % for impurity Imp -A, Imp-B, Imp-C and Imp-D. The correlation coefficient obtained was greater than 0.998. Linearity was checked for the related substances method over the same concentration ranges for 3 consecutive days. The %RSD values of the Slope and Y-intercept of calibration curve were 3.2 and 2.8 respectively. The above results show that an excellent correlation existed between the peak areas and the concentrations of Imp -A, Imp-B, Imp-C and Imp-D.

Accuracy

The percentage recovery of Finasteride in bulk drug samples was ranged from 99.8 to 100.3 %. The percentage recoveries of all four impurities in Finasteride samples varied from 99.3% to 102.7%. The HPLC chromatograms of unspiked and spiked sample at 0.5 % level of all impurities in Finasteride bulk drug samples are shown in Fig.3.

Robustness

In all the deliberate varied chromatographic conditions (flow rate, composition of organic solvent & column temperature) the resolution between critical pair, i.e. Finasteride and imp-A was greater than 1.5, illustrating the robustness of the method.

Solution stability and Mobile phase stability

The %RSD of assay of Finasteride during solution stability and mobile phase stability experiments were within 0.3%. No significant changes were observed in the content of impurities namely imp -A, imp-B, imp-C and imp-D during the solution stability and mobile phase stability experiments when performed using the related substance method. The solution stability and mobile phase stability experiment data confirms that the sample solution and mobile phases used during the assay and the related substance determination were stable for 48 hours.

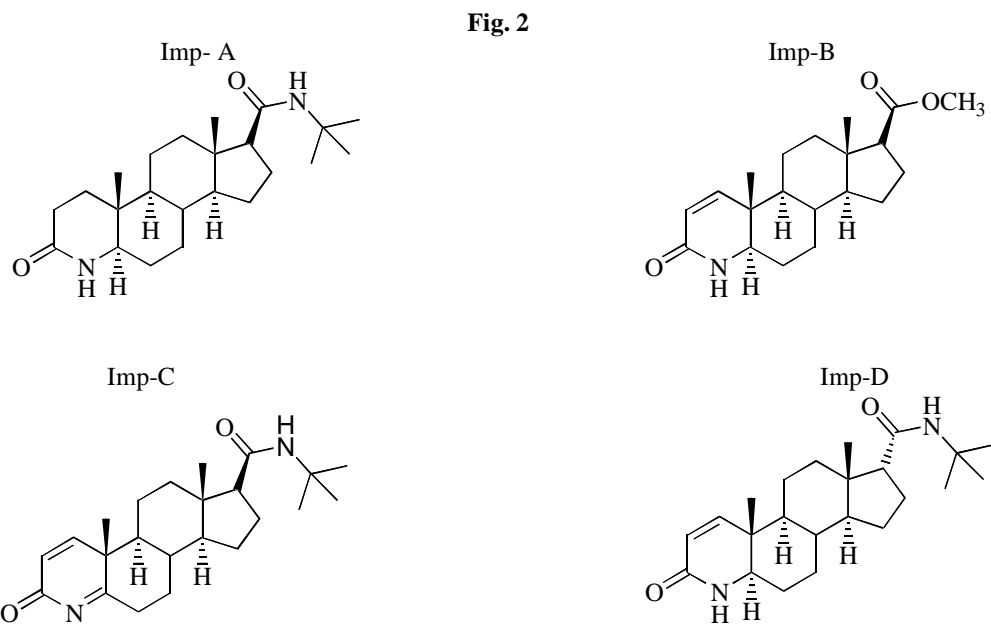
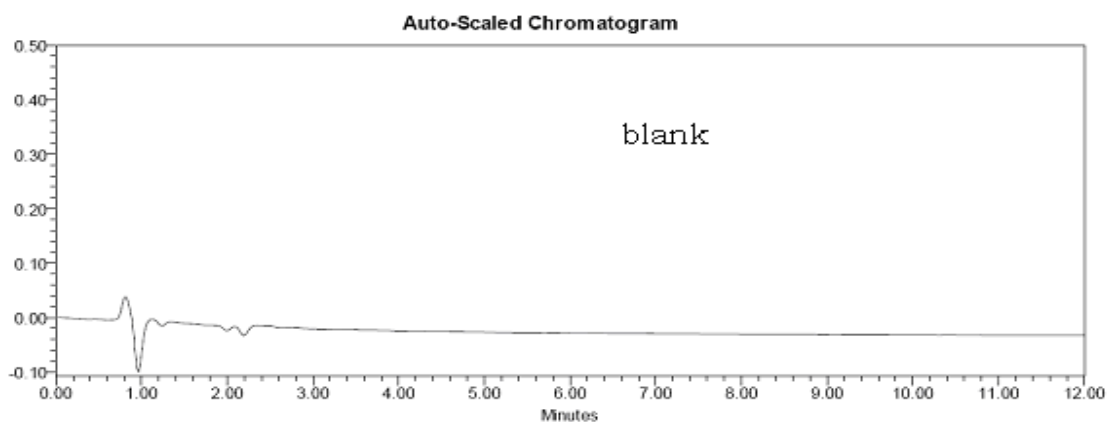


Fig.3



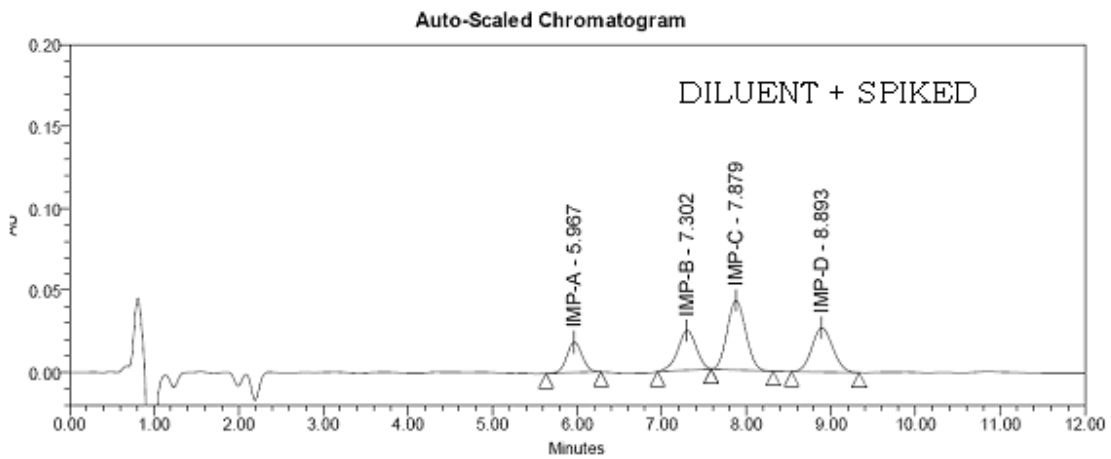
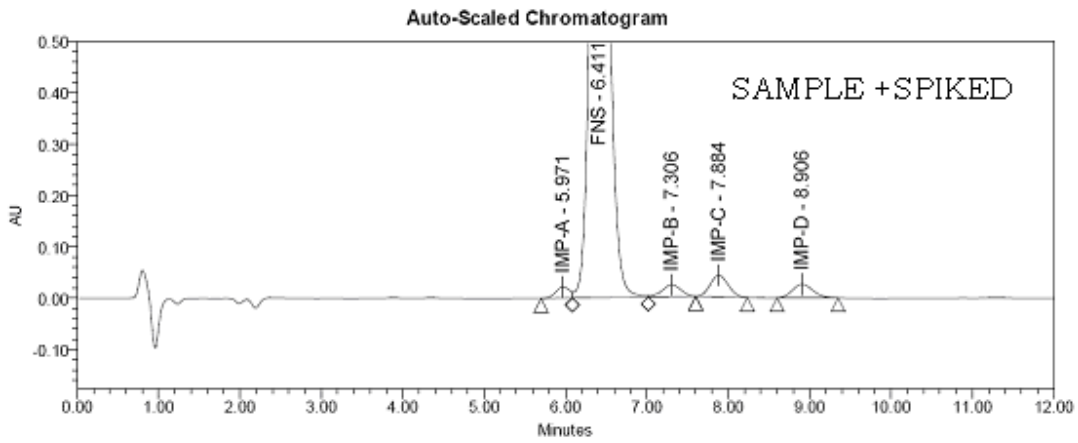
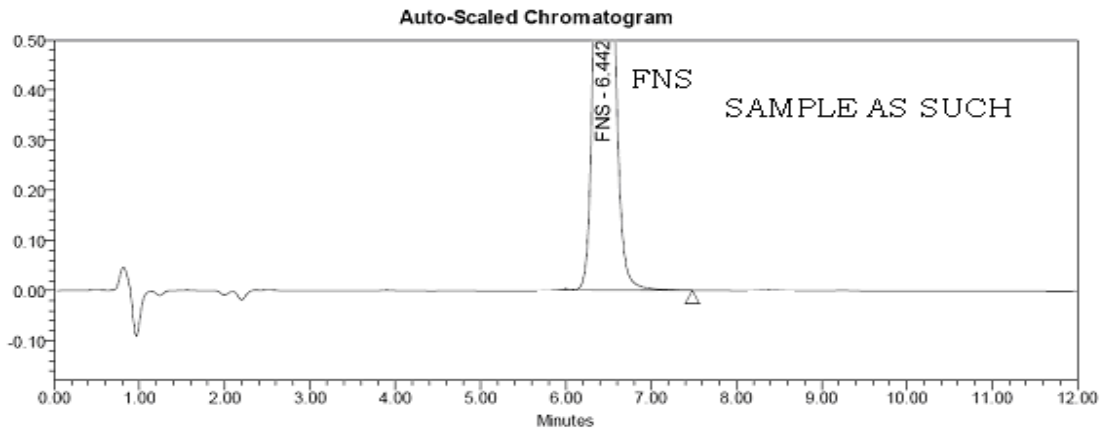
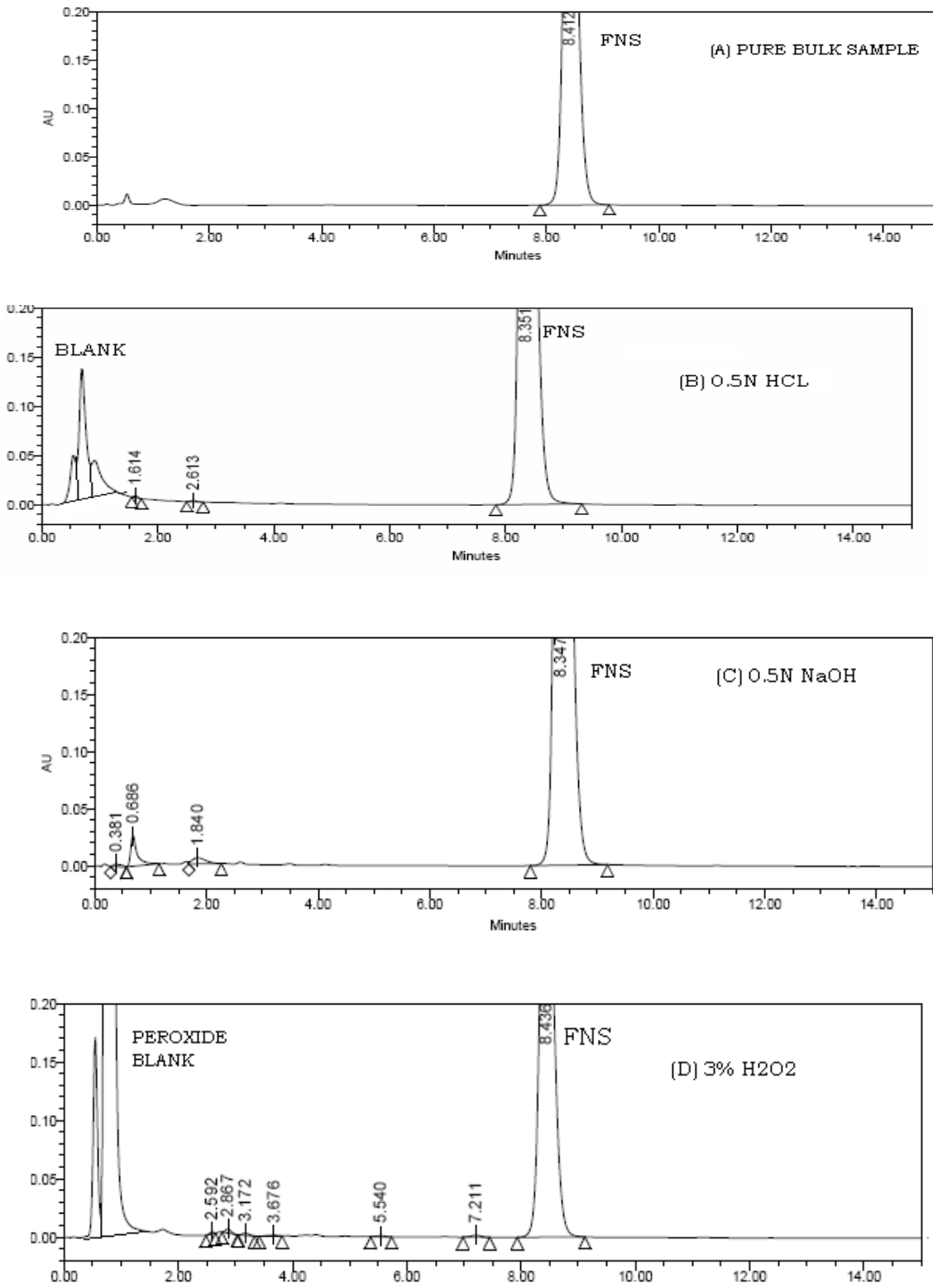
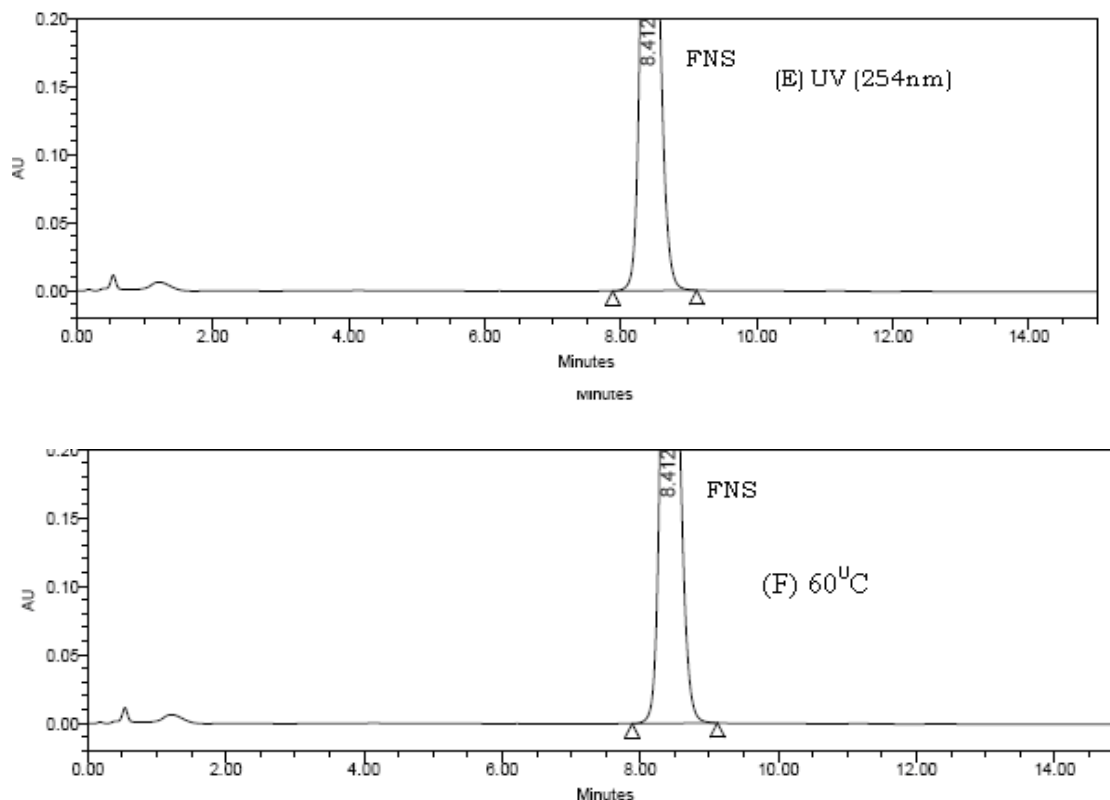


Fig. 4





CONCLUSION

The HPLC method developed for quantitative and related substance determination of Finasteride is linear, accurate, precise, rapid and specific. The method was fully validated showing satisfactory data for all method validation parameters tested. The developed method is stability indicating and can be conveniently used by quality control department to determine the related substance and assay in regular Finasteride production samples and also stability samples.

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