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Research Article

FORCED DEGRADATION STUDY OF LETROZOLE-A VALIDATED STABILITY INDICATING HPLC ASSAY FOR BULK AND TABLET DOSAGE FORM

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ABSTRACT

The aim of the present work was to develop and validate a stability indicating liquid chromatographic method for the assay of Letrozole in bulk and tablet dosage form in presence of its degradation products and to establish the possible stable conditions using related compound A standard. The determination was carried out using an isocratic mobile phase system consisting of Water, Acetonitrile and Phosphate buffer (pH 5.8; 0.1M) in the ratio of 70:20:10 (v/v/v). The column used was Inertsil ODS 3 column, with a flow rate of 1.0 ml / min under UV detection at 230 nm. The retention time of Letrozole was found to be 9.454 mins. The described method was found to be linear over a concentration range of 600-1400 µg/ml (r^2=0.9997), with intraday and inter day precision values of 0.36% and 0.41%. Limit of detection and Limit of quantification was found to be 8 and 25 ng /ml, respectively. The results of the study revealed that, Letrozole was found to degrade under base hydrolysis (1M NaOH for 2hours) evident from the concentration of related compounds and mean difference in the recovery study of letrozole and it was found to stable for One hour in a solution of 0.01M NaOH. The developed method could effectively separate letrozole in presence of degradation products and formulation excipients, and the degraded compound was found to be related compound A, hence stability indicating. The developed method has the advantage in terms of stability indicating, less proportion of buffer and organic phase in the mobile phase.

Keywords: Letrozole, Stability-indicating method, RP-HPLC, Related compound A.

INTRODUCTION

The Regulatory aspect of International Conference on Harmonisation (ICH) guidelines insists the development of stability indicating assay method a mandatory one. An ideal stability indicating assay method should resolve all the degradation products from the drug. The establishment of the forced degradation conditions such as stress agent concentration, time of stress was determined in a trial and error method so that they effect a degradation of 10-20% of the parent compound [1-6]. Letrozole, chemically known as 4, 4 - (1 H-1, 2, 4-Triazol-1- yl methylene) dibenzo nitrile, [M.F: $C_{17}H_{11}N_5$, M.W:285.31, Mp: 184-185 °C] is a white, crystalline powder, freely soluble in chloroform, dichloromethane, slightly soluble in methanol, practically insoluble in water. Letrozole is a potent and selective non-steroidal aromatase inhibitor, approved for use in post-menopausal women who have breast cancer that has progressed after antiestrogen therapy [7–10].

The literature survey revealed, only few methods of analysis of letrozole have been reported using different techniques such as HPLC, HPTLC, capillary gas chromatography in bulk form, tablet dosage form and in biological fluids [10-16]. Even though the methods are simple, they are not stability indicating and cannot be used for stability studies. The aim of the present study was to develop a simple, precise, rapid repeatable and accurate, stability indicating reverse phase HPLC method for the estimation of letrozole in bulk form and tablet dosage form in presence of its degradation products and formulation excipients.

MATERIALS AND METHODS

Chemicals

A bulk drug sample of Letrozole (Batch no: FX6134) were provided by Cipla Laboratories Ltd., Bangalore and used for study. Water (HPLC Grade), Acetonitrile Runa (HPLC grade), and all the other chemicals were purchased from Merck, India and the solutions were prepared from Millipore purification system.

Instruments

Sartorius balance [Model: CP225], Metrohm pH meter, Sonica Ultrasonic cleaner, Millipore purification system, Photo stability chamber: Make: Caron, Model: 6540E, Shimadzu HPLC [LC 2010 A_{HT}, Injector: Auto sampler, Detector: Prominence Diode Array, Software: Chromeleon]

Chromatography

The chromatography was done in a reverse phase mode using a Stationary phase of stainless steel Inertsil ODS 3 column, L 1 of dimension, 250 mm x 4.6 mm i.d, 5µm. The Mobile phase consisted of Water, Phosphate buffer (pH 5.8; 0.1M), Acetonitrile in the ratio of 70:10:20 (v/v/v), filtered through a 0.45µm membrane filter and degassed by sonication. The Flow rate was maintained at 1.0 ml/min, with an Injection volume of 20µL in Room temperature of 20 ±2° C, and detected by UV detector at 232nm.

Preparation of Standard and bulk drug

10 mg of Letrozole [Working Standard, bulk drug sample] was taken in 100ml volumetric flask and it is made upto volume with the diluent. Then 10ml of this solution is taken in a 100ml volumetric flask and it is made upto volume with the diluent. This gave a final concentration of 10µg/ml of working standard Letrozole and sample Letrozole. That was filtered through 0.45-micron membrane filter. Each solution was injected and a chromatogram was recorded.

Preparation of formulation solution

Twenty tablets of Fempro (Each tablet contains 2.5mg of Letrozole) from cipla were crushed and powdered in a mortar. A quantity of powder equivalent to 10mg was taken in 100ml volumetric flask and the drug was extracted with the diluent for 30 minutes in a sonicator and the solution was filtered through a 0.45µm membrane filter and the volume was made upto the mark with the same. From this 10ml of the solution was taken in a 100ml flask and made upto the volume with the diluent and the solution was again filtered through a 0.45µm membrane filter. The above solution was injected and the chromatogram was recorded.

Forced degradation of letrozole

Forced degradation of the sample was done to determine the intrinsic stability of the drug and to assess the stability of the developed method as per ICH guidelines. Forced degradation was attempted using light, heat, acid, base, oxidation, reduction and hydrolysis. Liquid state degradation study was carried out by five different methods [Acid hydrolysis, Base hydrolysis, Oxidation, Reduction and Hydrolysis][17]. 5 different samples of 25mg of the sample were transferred to 5 different round bottomed flasks for the two hour exposures. Then 5 to 10ml of different agents (1.0M Hcl, 1.0M NaOH, 10% hydrogen peroxide, 10% aqueous sodium bi –

sulphate and purified water) were added to the above, and refluxed for two hours in a boiling water bath. At the end of the exposure, the solution was cooled and transferred into 250 ml round bottomed flask and the volume was made up to the mark with diluent. Then the solution was diluted to a concentration of 10µg/ml, and the solution was injected and the amount of degradation was checked by recovery studies and avoiding a degradation of more than20%.The degradation is limited to not more than 20% by reducing the concentration of the stress agent. Thermal degradation of the sample was attempted by keeping the normal sample protected from light and the forced degradation sample in an oven at 80˚C and analyzed at 24 and 48 hours. Photolytic degradation was done by exposing the sample to visible and UV light providing an overall illumination of1.2 million Lux hours and integrated ultraviolet energy of not less than 200 watt hours\sq meter.

Blank of all the above conditions were first injected under the chromatographic condition mentioned under assay, followed by 6 replicates of the sample solution of forced degradation study to rule out the possible degradation of refluxing.

Validation of the assay method

The method was validated as per ICH guidelines in terms of linearity and range, precision, accuracy, LOD and LOQ, solution stability, specificity and robustness.

Linearity and Range

20mg of Letrozole standard was taken in a 100ml volumetric flask and it is diluted upto the mark with the diluent. From this solution 3, 4, 5, 6, and7ml of the solution was taken and diluted to 100ml with the diluent to give concentration level of60, 80,100, 120,140% respectively.The standard drug solutions in varying concentrations ranging from 600µg to 1400µg were injected and calibration curves were plotted using peak area Vs concentration of the standard solutions.

Precision

The system precision of the method was determined by studying repeatability by injecting the 10µg/ml standard solution six times and reproducibility (Method precision) was done by injecting the formulation sample solution. The area of drug peaks and % RSD of intraday precision were calculated. The inter day studies (Ruggedness) was performed with different column (same make but with different lot no) BLHV030 to BLHS028, with different analyst on a different day. The peak area for the standard solution and the assay for the sample solution were determined and the %RSD was calculated.

Accuracy

The accuracy of the method was determined by recovery experiments. A known quantity of the pure drug was added to the pre-analyzed sample at 50%, 100% and 150% levels. The recovery studies were carried out 6 times and the % recovery and % RSD of the recovery studies were determined for each level separately.

Limit of detection and limit of quantification

The LOD and LOQ values were determined based on the signal to noise ratio of 3:1 and 10:1 respectively.

Robustness

Condition 1: Change the gradient mobile phase A by + 2.0 %(72:28)

Condition 2: Change the gradient mobile phase A by -2.0 %(68:32)

Condition 3: Change the flow rate +0.1ml\min (1.1ml\min)

Condition 4: Change the flow rate -0.1ml\min (0.9ml\min).

Under these conditions the standard (6) and the sample solution were injected (3) and the peak area ratio of the standard and sample were compared. The %RSD of the assay value for the standard was calculated.

Solution Stability

The standard solution at definite concentration was injected at predetermined time intervals and % RSD of the peak area of the standard solution was noted. The sample solution was injected and the % of assay was calculated. The solution used in precision study was used.

Specificity

The resolution, capacity factor, theoretical plates/meter, tailing factor, for the formulation sample solution was determined.

RESULTS AND DISCUSSION

Assay validation of Letrozole in bulk and tablet dosage form

The optimized chromatographic conditions gave well resolved chromatograms, evident from the representative chromatogram, from Fig.1, 2, and 3 of 10µg/ml standard solution, bulk sample solution and formulation sample solution respectively. There was no interference from the other substances and peaks were well resolved with adequate peak symmetry. The retention time of Letrozole in all the chromatograms was found to be 9.54 minutes and the analysis time would be less than 15 minutes.

With the established optimized assay method the validation of the developed method was done and the calibration curve of Letrozole was found to be linear over a concentration range of 600µg to 1400µg.From the regression analysis (n=3), the slope was found to be 1221.227±0.0040, y-intercept of 32699.8±0.0043and correlation coefficient of Letrozole was found to be 0.9997. The calibration curves were plotted using peak area in Y-axis Vs concentration of the standard solutions in X-axis (Fig-4)

Three different concentrations (600µg, 1000 µg and 1400µg) of the pure drug standard and the sample solutions were analysed in 5 replicates to determine the values of intraday and interday precision A summary of the intraday (System and Method) precision and interday (Ruggedness) is listed in Table 1. The % RSD values of precision included 0.18, 0.34 and 0.41 for system, method and ruggedness respectively. The average accuracy values for the recovery study includes 96.41% .Based on the signal to noise ratio, the limit of detection of the analyte was found to be 9ng and limit of quantification was found to be 30ng.The bench top stability of the sample solution was given in Table 1 and the solution was stable upto 25 hours.

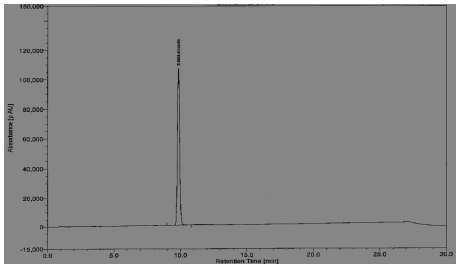

Fig. 1: Chromatogram of Standard Letrozole 10µg/ml

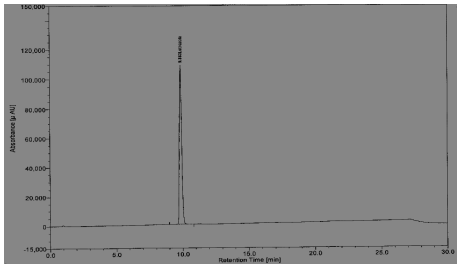

Fig. 2: Chromatogram of Sample Letrozole 10µg/ml

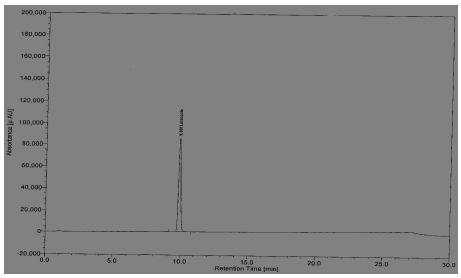

Fig. 3: Chromatogram of Letrozole formulation sample

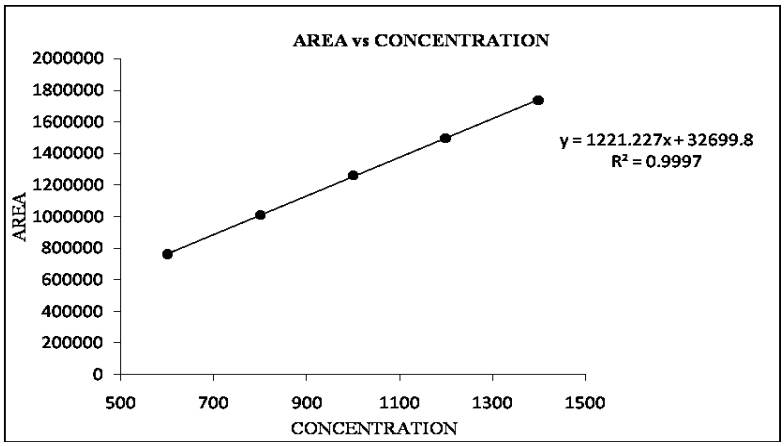

Fig. 4: Linearity curve of Letrozole

Forced degradation of letrozole and its estimation in presence of degradation products

The specificity of the developed method to identify the degradation products of Letrozole under stress conditions was analysed by forced degradation study. The results of the forced degradation of the sample drug for 2 hours under the above mentioned conditions from the Fig-(5-11) indicated that, the recovery of the drug was more than 80% in all the conditions except in the case of base hydrolysis condition. The retention time of Letrozole was found to be 9.7mins and a major degradation peak at a retention time of 6.2 mins was found in all the chromatograms. There was no letrozole peak in the base hydrolysis chromatogram indicating a major degradation.

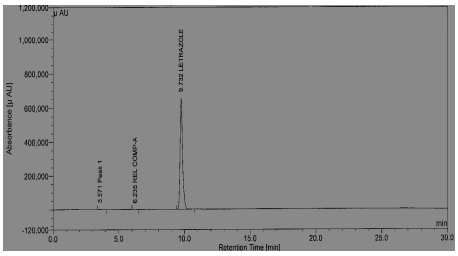

Fig. 5: 1 Chromatogram of Acid hydrolysissample at 2nd hour

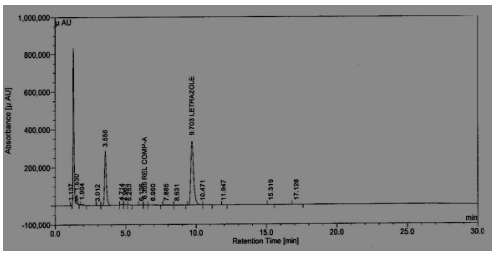

Fig. 6: Chromatogram of Oxidationsample at 2nd hour

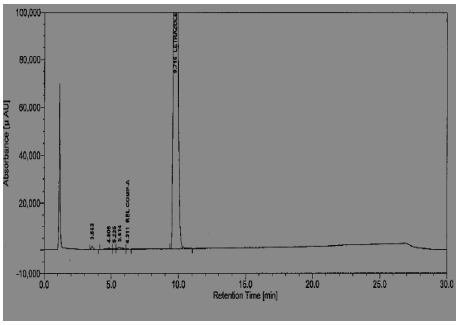

Fig. 7: Chromatogram of Reductionsample at 2nd hour

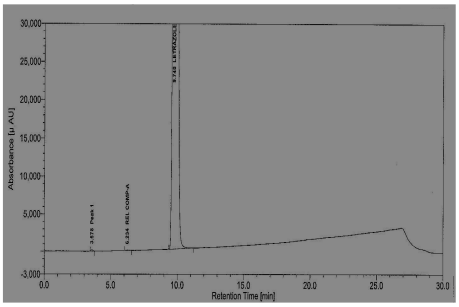

Fig. 8: Chromatogram of HydrolysisSample at 2nd hour

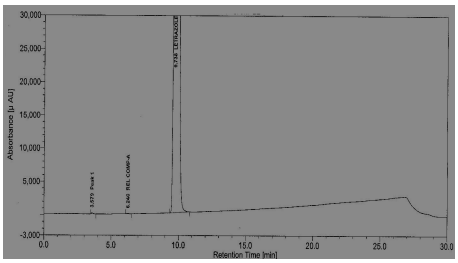

Fig. 9: Chromatogram of Elevated sample

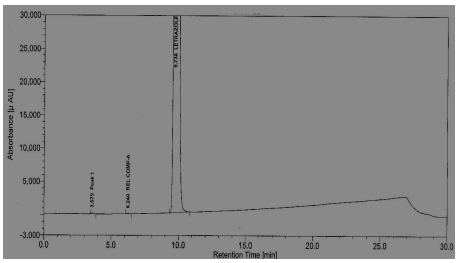

Fig. 10: Chromatogram of Photolysis UV sample

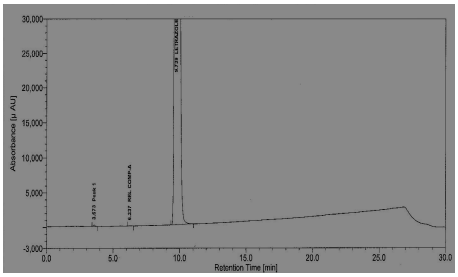

Fig. 11: Chromatogram of Photolysis Visible sample

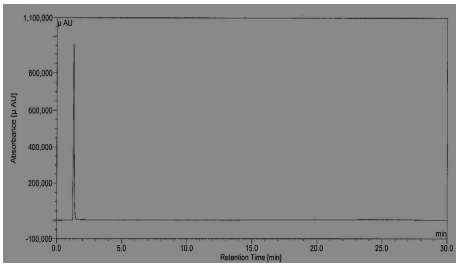

Fig. 12: Chromatogram of Base hydrolysis sample at 2nd hour (1.0MNaOH)

The base hydrolysis of the drug for 2hours with a solution of 1M NaOH resulted in complete degradation of letrozole (Fig-12). No letrozole peak was detected. The degradation was limited by decreasing the concentration of the stress agent and the time of exposure using a solution of 0.1M NaOH for 1 Hour (Fig-13). This limited exposure showed a degradation of about 43%. The chromatogram shows a lot of additional peak and less intense analyte peak. Since the degradation is more than 20%, a further reduction of the sample condition, using a solution of 0.01M NaOH for 1hours was tried (Fig-14). The degradation in this condition was less than 20% and thus a stable basic condition of Letrozole was established.

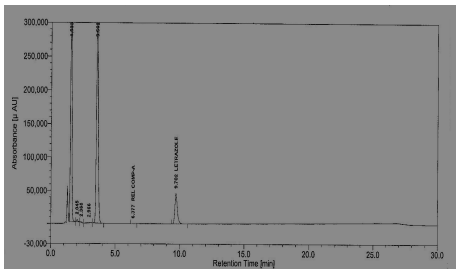

Fig. 13: Chromatogram of Letrozole treated with 0.1M NaOH for 1 Hour

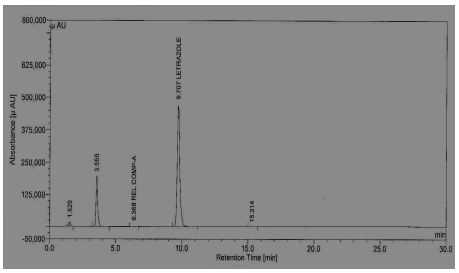

Fig. 14: Chromatogram of Letrozole treated with 0.01 M NaOH for 1 Hour

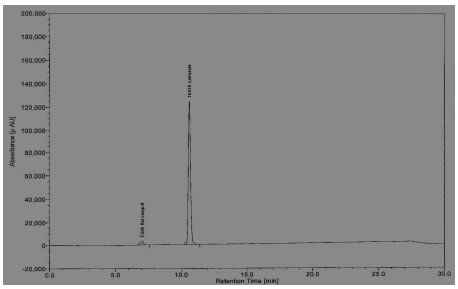

Fig. 15: Chromatogram of Letrozole and related compound A.

The degradation peak at the retention time of 6.2Mins was found to be related compound A, using the related compound A standard (Fig-15)

The recovery after stress and the stable conditions of Letrozole were given in the Table 2

The retention time of Letrozole was found to be 9.8mins with no interfering peaks and the developed method has the advantage over the already reported method in having a less proportion of organic phase of acetonitrile (20%).

The stability of the developed method was tested by subjecting the sample solution to the forced degradation studies such as liquid and solid state degradation using the above conditions. The chromatograms and the recovery studies suggest that the sample was stable in all the other conditions except base hydrolysis condition. The recovery of the drug was more than 80% in all the cases. The stable basic condition, estabilised with a reduction in the concentration of the stress agent and its time, was found to be 1hour in a solution of 0.01M NaOH. Hence the long time exposure of the

sample Letrozole to basic conditions is not recommended. The developed method has the advantage of detecting the degradation peaks and the major degradation peak at the retention time of 6.2mins was found to be related compound A using the related compound A standard. Hence the method is highly reliable to establish a stability profile for the drug Letrozole.

Table 1: Results of Validation

Validation of Letrozole	Validation Parameter	Results
System	Theoretical Plates	16116
Suitability	Tailing Factor	1.1
	Resolution	12.0
	Capacity factor	7
	LOD	8.0
	LOQ	25.0
Accuracy	% Recovery	96.41
Linearity	Co-eff. of variation	0.9997
Precision	Intra day: % RSD	System : 0.18 Method : 0.34
	Inter-day:%RSD	0.41
Robustness	Mobile Phase.Ratio: % RSD	0.36
	FlowRate: % RSD	0.43

Table 2: Summary of Percentage Recovery after stress

Stress Conditions	%Recovery
Protected Sample(as Such)	99.89
Photolysis (UV sample)	93.22
Photolysis (Visible sample)	96.00
Elevated sample**(**80˚ C)	92.55
Acid hydrolysis (1.0 M Hcl)	86.65
Base hydrolysis(0.01MNaoH)	82.32
For one hour	
Oxidation(10%H_2O_2) 1 hour	88.27
Reduction($NaHSO_3$)	93.54
Hydrolysis(purified water)	94.32

CONCLUSION

A simple stability indicating HPLC method for determination of Letrozole in its bulk and formulation was developed and validated as per ICH guidelines. The accuracy, precision, repeatability, reproducibility and specificity of the method were good as per the ICH guidelines. The developed method has the advantage over the already reported method of having a less proportion of organic component in the mobile phase, Well resolved degradation and analyte peaks, established stable basic condition and the identified degradation peak of related compound A, using pure standards. The method is reliable and can be used for establishing the stability profile of Letrozole.

Abbrevations

ICH, International Conference on Harmonisation; MW, Molecular weight; MF, Molecular formula; MP, Melting point; RP-HPLC, Reverse phase High performance Liquid chromatography; HPTLC, High performance thin layer chromatography; ODS, Octa decyl silane; LOD, Limit of detection; LOQ, Limit of quantification; RSD, relative standard deviation.

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