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Development of validated RP-HPLC method for determination of letrozole in bulk and its pharmaceutical dosage forms

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ABSTRACT

A simple, sensitive and rapid reverse phase high performance liquid chromatographic method was developed for the estimation of Letrozole (Let) in bulk and pharmaceutical dosage forms. A Gemini C18 phenomenex column (250x4.6mm, 5µ) was used with a mobile phase containing a mixture of Acetonitrile and water in the ratio of 50:50. The flow rate was 1.1ml/min and effluents were monitored at 265nm and eluted at 4.53 min. The assay was validated for the parameters like accuracy, precision, robustness and system suitability parameters. The proposed method can be useful in the routine analysis for the determination of letrozole in bulk and pharmaceutical dosage forms.

Key Words: Letrozole, RP-HPLC, Validation, Pharmaceutical dosage form.

INTRODUCTION

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. Letrozole [4, 4'-(1H-1, 2, 4-triazol-1-ylmethylene) dibenzonitrile] is used as anticancer drug. Letrozole is a nonsteroidal competitive inhibitor of the aromatase enzyme system [1]. The suppression of estrogen biosynthesis in peripheral tissue and in the cancer tissue itself can therefore be achieved by specifically inhibiting the aromatase enzyme [2, 3]. Literature reveals that an HPLC method was developed for the determination of Letrozole in human plasma and urine. Also an HPLC method was reported for the determination of Letrozole in plasma and its metabolites in urine. Various analytical methods for the estimation of drug have been found in the literature such as UV spectrophotometric method [4], GC-MS for the identification of

letrozole in urine [5], capillary gas chromatography method for the analysis of tamoxifen, anastrozole and letrozole in pharmaceutical preparations [6], TLC and HPLC methods for the estimation of drug and related components in tablets [7] and HPLC method of the drug and its metabolites in biological fluids with automated liquid-solid extraction and fluorescence detection [8].This paper describes a simple, sensitive, validated and economic method for the determination of Letrozole in bulk and pharmaceutical dosage forms.

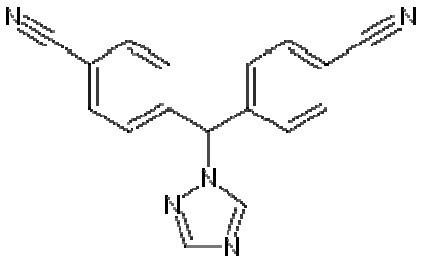


Fig. 1. Chemical structure of letrozole

MATERIALS AND METHODS

Chemicals and reagents

Letrozole was obtained from Micro labs, Bangalore, India. HPLC grade acetonitrile were purchased from MERCK, India. Water for RP-HPLC was prepared by triple distillation in a glass still and filtered through a nylon 0.45µm membrane filter (Gelman Laboratory, India). Other reagents were of AR grade.

Instruments

The HPLC system consisted of a Shimadzu (Japan) LC-10AT vp and LC-20AD pumps connected with SPD-10A vp UV-Visible detector. The data acquisition was performed by Spincotech 1.7 software. Analysis was carried out at 255nm using a phenomenex C18reverse phase column of 150x 4.6 mm i.d., 5 µm dimensions at ambient temperature.

Method development

The sensitivity of the HPLC Method depends upon the proper selection of the detection wavelength. An ideal wavelength is one that's give good response to the detector. The maximum peak area with letrozole solution (200µg/ml) was observed at 265nm with the acetonitrile:water (50:50). A number of trials were made to find out the ideal solvent system (mobile phase) for eluting the drug. The mobile phase containing acetonitrile: water different ratios (70:30, 60:40, 50:50) were tried. Better peak resolution with less tailing was obtained with the ratio of Acetonitrile (HPLC grade): water (50:50). Initially different C_8 and C_{18} columns were tried for selected composition of mobile phase and the quality of peaks were observed for letrozole, finally the C18 column was fixed based upon the satisfactory results of various system suitability parameters such as column efficiency, retention time, tailing/ asymmetry of the peaks. Keeping the mobile phase ratio 50:50 (acetonitrile and water), the chromatograms were recorded at a flow rate of 1.1 ml/min. At this flow rate, the peaks were sharp with good resolution. So 1.1 ml/min was kept constant for the analysis (flow rate 0.2ml/min, 0.4ml/min, 0.6 ml/min were also tried, but did not give any satisfactory results). In the present study acetonitrile:water (50:50) HPLC grade solution is used as diluents for dissolving the sample and standard drug.

Standard Preparation

About 50 mg of Letrozole was accurately weighed and transferred to a 50ml standard flask and add small quantity of mobile phase to dissolve the drug and make up the volume with the help of mobile phase to dissolve the drug and make up the volume with the help of mobile phase to dissolve the drug and make up the volume with the help of mobile phase which gives 1 mg/ml stock solution of Letrozole, from this stock solution take 4ml, 4.5ml,5 ml, 5.5ml, 6ml of solution was pipetted out and transferred to a series of 25ml of standard flask and make up the volume with mobile phase to get concentration of 160 µg/ml, 180 µg/ml, 200 µg/ml, 220 µg/ml & 240 µg/ml. From this each 20 µl of standard solution were injected to the chromatographic system for linearity study. The chromatograms of standard preparation were recorded and peak responses were noted and tabulated. A plot of concentration in percentage Vs peak area was drawn and it was found to be linear in the concentration range of 160 µg/ml to 240µg/ml.

Sample Preparation

20 tablets were weighed, powdered and powder equivalent to 50mg was transferred into a 50ml standard flask. Small amount of mobile phase was added and kept on rotary shaker to dissolve, makeup the volume and mixed well. Centrifuged a portion of the sample at 3500 rpm for 20 minutes. From this 5ml was taken and diluted to 25ml with mobile phase to get 200µg/ml.

Validation of the method

The optimized method is evaluated in accordance with the ICH guidelines. System precision and system suitability was determined by analyzing, in six replicate, standard drug solution and injected six times into the HPLC system. Linearity of detector response was determined by plotting a graph of "Concentration in percentage" versus "area" in linearity section. A study of Placebo interference from excipients was conducted by taking placebo in 100ml of volumetric flask in duplicate, equivalent to about the weight of placebo as per the test method. The precision of test procedure was evaluated for Letrozole in 2.5 mg by performing the assay as per the test method for five times. Accuracy of the system is established by carrying out the drug assay in triplicate by spiking with equivalent amount of Letrozole raw material into each volumetric flask, for each spike level to get the concentration of Letrozole equivalent to 80%, 100%, and 120% of the standard concentration of Letrozole as per the test method.

RESULTS AND DISCUSSION

Method development

A reversed-phase column procedure was proposed as a suitable method for the determination of Letrozole in bulk and pharmaceutical dosage forms. The chromatographic conditions were optimized by changing the mobile phase composition, pH, and buffers used in the mobile phase. Different ratios were experimented to optimize the mobile phase. Finally a mixture of Acetonitrile and water in the ratio of 50:50 was used. A typical chromatogram obtained by using the aforementioned mobile phase from 20 µL of the assay preparation. The retention times of Letrozole was found to be 4.53 min.

Validation of the method

System precision and system suitability.- The system suitability parameters were evaluated and found to be within the limits. The % RSD for peak areas from six replicate injections of

Letrozole was found to be 0.9708 %. The tailing factor for Letrozole is found to be 1.321, (Chromatogram No.1). The results are summarized in Table I.

Linearity of detector response.- The correlation co-efficient was found to be 0.9997. The results are summarized in Table II. From the above study it was established that the detector linearity is from 160 µg/ml to 240 µg/ml of Letrozole (Chromatogram No. 2).

Specificity.- There in no interference at RT of Letrozole peak, (Chromatogram No. 3). The results are summarized in Table III.

Repeatability.-The precision of test procedure was evaluated for Letrozole in 2.5 mg by performing the assay as per the test method for five times. The % Relative standard deviation, recovery of Letrozole was found to be within the limits, (Chromatogram No. 4). The results are summarized in Table IV.

*Accuracy***.-**The average % recovery of Letrozole was found to be within the limits, (Chromatogram No. 5). The results were summarized in Table V.

Table I. System Precision data of the proposed RP-HPLC method

Injection Number	Letrozole	Acceptance Criteria
1	1047.204	
2	1048.418	
		The % Relative Standard Deviation of peak areas of Letrozole
3	1031.258	should not be more than 2.0.
4	1029.882	
5	1050.92	
6	1049.305	
Mean	1042.831	
%RSD	0.9708	

Mean±SD, n=6

Table II. Data of linearity of detector response

Spike Level or Concentration (µg/ml)	Peak areas
160	833.124
180	924.928
200	1005.452
220	1157.555
240	1222.036
Coefficient of Correlation (r)	0.9997
Slope (m)	10.2588
Intercept (c)	0.6249

Acceptance Criteria
Coefficient of Correlation shall be not less than 0.999

Table III. Specificity data for the proposed RP-HPLC method

Sample No.	% Interference found in 2.5 mg tablets
1	No
2	No

Acceptance Criteria
No interference at the RT of Letrozole peak.

Table IV. Repeatability data for the proposed RP-HPLC method

Sample No.	% Drug recovered for 2.5 mg
1	99.68
2	101.48
3	99.2
4	100.64
5	100.72
Average	100.34
% RSD	0.9008

Mean±SD, n=5

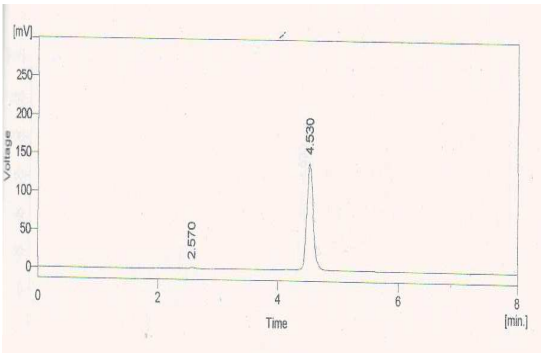
Acceptance Criteria
The % relative standard deviation of individual % Letrozole from the six units should be not more than 2.0%. The assay of Letrozole should be not less than 98.0%.

Table V. Accuracy data for the proposed RP-HPLC method

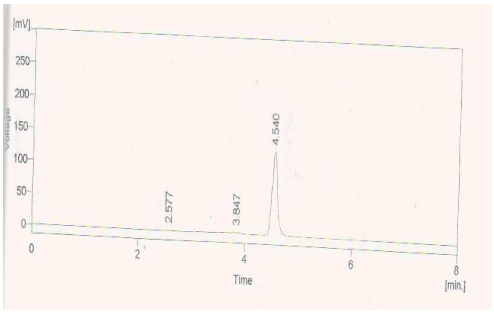
Sample No.	Spike Level	Mean % Recovery	S.D *	% RSD *
1	80%			
2	80%			
3	80%	100.24	0.7249	0.7232
4	100%			
5	100%			
6	100%	98.29	0.3843	0.3909
7	120%			
8	120%			
9	120%	99.02	0.4406	0.445

Mean±SD, n=3

Acceptance Criteria
The mean % recovery of the Letrozole at each level should be not less than 95.0% and not more than 105.0%.



(1)



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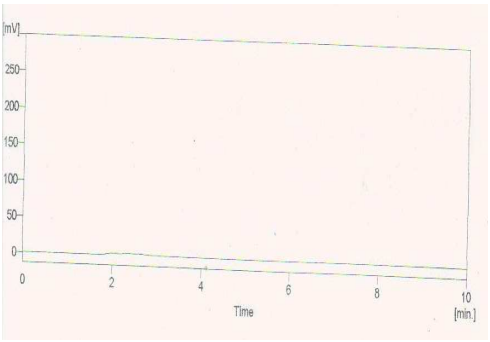

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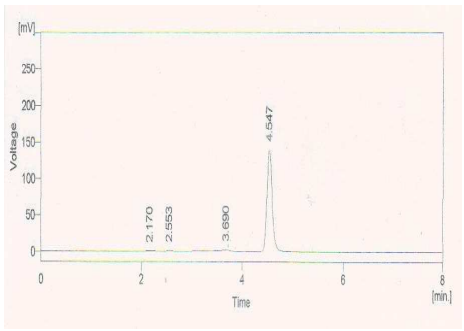

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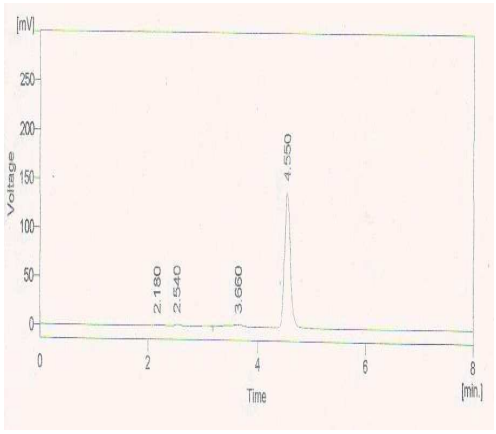

(5)

Fig. 2. HPLC chromatograms showing resolution of letrozole

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Letrozole from pure and in pharmaceutical dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations 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 and conveniently adopted for routine analysis of Letrozole in pharmaceutical dosage forms.

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