Determination of Letrozole in formulation by a Validated RP-HPLC method

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Abstract

A simple, rapid and validated reversed phase liquid chromatographic method has been developed for the determination of Letrozole (LZ) in pharmaceutical dosage. LZ was separated on ODS analytical column with a mixture of acetonitrile, water in the ratio 50:50 (v/v) as mobile phase at a flow rate of 1.0 mL /min. The effluent was monitored by UV detection at 265nm. Calibration plot was linear in the range of 160 to 240 μg/mL with the linear regression (r) = 0.999. The method was validated for recovery, precision, specificity.

1. Introduction

Letrozole(LZ) is denoted chemically as 4, 4′-[(1H-1,2,4-triazol-1-yl) methylene] bis-benzonitrile (Figure 1), is a highly potent and selective third generation aromatase inhibitor (AI) used for treatment of hormone-sensitive breast cancer in postmenopausal women,[1]which blocks effectively the synthesis of estrogen, a causative agent for cancer.[2,3] LZismorepo -tentthanotherAIanditinhibitthearomataseenzymeinperiphe raltissuecompletely.[4-7] Several analytical methods have been described for the estimation of letrozole in plasma and urine by LC -MS/MS,[8] HPLC with fluorescence detection using fully automatedliquid - solidextraction,[9] Micellarelectrokineticchro mato graphic,[10] estimation of LZinpharmaceuticalformulation by UV method,[11] Nita,et.al.,[12] recently explained a method based on HPLC with UV detection, but in this method even the analyte waselute data bout ninth min which leads to longer runtime for single sample, and triple solvent system. Herein we depict a simple, validated HPLC by UV detection, which required only 8 min for a single run and the LZ was eluted at 4.5 min.

2. Experimental

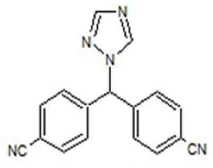
Chemicals and reagents.

LZ reference standard was obtained from Sigma laboratories (Bangalore,India), pharmaceutical product containing 2.5mg/tablet LZwaspurchasedfromlocalmarket. Acetonitrile(HPLC) purchased from J.T.Baker(NewJercy, USA). Milli-Qobtained from Millipore water system (Billerica, USA). All other chemical were purchased from Rankem India ltd (Bombay,India)and used a ssuch.

Chromatographic conditions. Analysis was performed with a Shimadzu LC-10 AT VP system (Japan) equipped SPD-1 0UV-visible detector, and Rheodyne-7125 inject or with 20μl sample loop. Letrozole was separated on a PhenomenexODSanalytical column(250×4.6mm,5μm) under reversed phase condition. The mobile phase was a mixture of acetonitrile, water in 50:50(v/v) with 1.0mL flow rate and the analyte was monitored at 265nm.

Calibration. Calibration plots were constructed by plotting peak area response of appropriate working standards of LZ in mobile phase Vs their concentrations. The curve was observed to be linear from 160, 180, 200, 220, 240 μg/mL with linear regression of 0.999(r).

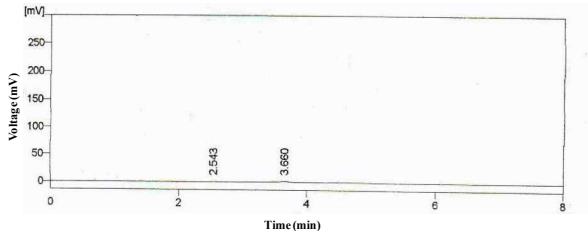
Assay sample preparation. A uniform mixture of tablet powder was obtained by weighing, powdering and mixing twenty tablets. From that, amount of tablet powder equivalent to 50 mg LZ was weighed, transferred to 50 mL standard flask. Small amount of mobile phase was added; sonicated to dissolve, volume made-up with mobile phase, filtered with 0.45 μ syringe filter, dilute 5mL of this to 25 mL with mobile phase to get 200 μg/mL. From this 20 μL, the solution was injected into HPLC.



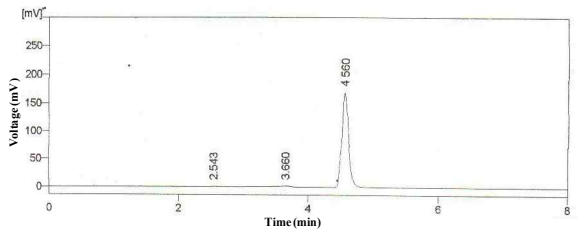
[Fig. 1] Structure of Letrozole.

3. Results and Discussion

Chromatography. Symmetrical peaks were observed for LZ at 4.5 min RT, a typical chromatogram of the blank and LZ standard was illustrated in Figure 2 and Figure 3 respectively. They also shows the less tailing when compare to the reported methods.

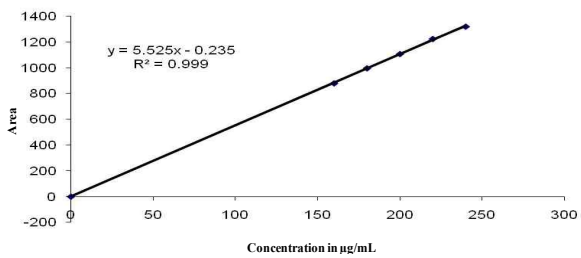


[Fig. 2] Blank Chromatogram.



[Fig. 3] Standard Chromatogram.

Linearity. The linearity of the peak area with respect to the concentration of standards was examined under optimal HPLC/UV conditions and is described as a regression equation y=5.5255c-0.235, where 'c' is the concentration with a linear correlation coefficient of 0.999. LZ was linear in the concentration range over 160 to 240 μg/mL (Figure 4).



[Fig. 4] Standard Curve.

Method validation. Method validation was performed by following the ICH and USP guideline for analytical method validation.[13,14]

Intraday and inter-day accuracy and precision. For intra-day precision, standard solution of fixed concentration was injected at various time interval and % RSD was found to be 0.83 % (limit % RSD < 2.0%). In addition, the day-to-day precision was studied by injecting the same concentration of standard solution at various days and the % RSD was 0.78 (limit %RSD <2.0 %) and results are provided in Table 1. The accuracy of the method was carried out by recovery of LZ in dosage formulation at three concentration levels (80, 100 and 120 % with reference to label claim of tablet), percentage recoveries was found to be 98.57 % to 99.2 % (Table 1).

[Table 1] Results of Linearity, Precision and Accuracy

Linearity		Precision of the method		Accuracy (Recovery studies)	
Concentration (μg/mL)		Inter day*	Intra day* (3 days)	Spike level	% Recovery**
160		0.83	0.78	80%	99.20
180				100%	98.57
200				120%	98.95
220					
240					
(r)	0.999				
Intercept	-0.235				
Slope	5.525				

*RSD of 10 determination, **RSDof6determinationateachlevel

Specificity. Placebo and blank run were carried out to prove the specificity of the chromatographic method towards the LZ (Figure 2 and Figure 6), which were free from interference/suppression peak at the RT of LZ due to solvent and/ commonly used tablet excipients.

Limits of detection and quantification. The LOD is the indication of sensitivity of the method which was found to be 136μg/mL(S/N>3). InadditiontheLOQ was 160μg/mL (S/N>10) for LZ.

Stability. Bench top stability was carried out to find the stability of LZ standard in mobile phase up to 24h. The results of the study showed that LZ was stable up to 24h without degradation (Table 3).

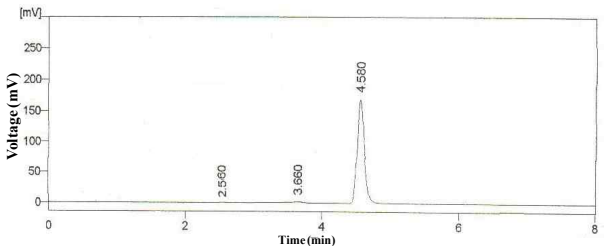
Ruggedness. The ruggedness was established by determining LZ in dosage formulation using the different chromatographic system and by two analysts. The percentage % RSD, for analyst variation and inter system variation were0.75 - 0.8 (limit <2.0 %), and 0.86 -1.01 (Limit <2.0 %). This indicates that the method was rugged (Table 3).

[Table 2] Results of Formulation Assay

Formulation	Label claim mg/tab	Amount found* mg/tab	% found*	% RSD*
F-1	2.5	2.48	99.2	0.781
F-2	2.5	2.51	100.2	0.568

*mean of 6 determinations

Application of the method to tablets: The method was used for determination of LZ in tablet formulation by as described above. The results obtained (Table 2) showed percentage found were high and RSD (%) values were low which confirms the method is suitable for routine determination of these components in their pharmaceutical preparation. Figure 5 shows a typical chromatogram obtained from analysis of a tablet formulation.

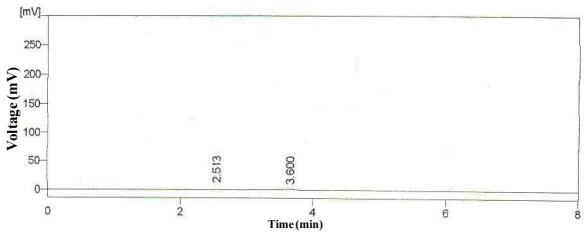


[Fig. 5] Sample Chromatogram.

[Table 3] Results of ruggedness and bench top stability

Bench top stability				Ruggedness			
Initial Assay	12h	24h	Deviation from the initial assay	Analyst*		System*	
				1	2	1	2
99.54%	99.60	100.89	0.6 to1.35	100.6	99.86	99.96	99.65
%RSD (limit NMT 2.0%)				0.75	0.8	0.86	1.01

*n=6.



[Fig. 6] Chromatogram of Placebo (absence of peak at the RT of LZ)

4. Conclusion

A simple and reliable HPLC method for measuring LZ in pharmaceutical formulation has been developed and validated. However many methods have reported already, have less sensitive ,longer run time, more tailing in the analyte peak and partially validated this method overcome all the insufficiency in the reported method .Our method is advantageous in terms of shorter runtime, less tailing, cost effective solvent with simple sample preparation and fully validated .Hence, we conclude that our method is suitable for the routine quality control of this drug.

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