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Bioanalytical Method Development and Validation for Letrozole Tablet by LC-MS

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Abstract

The main objective of this work is to develop rapid, selective and sensitive LC-MS/MS method that have short and simple extraction procedures, consume small amounts of solvent and biological fluid for extraction and a short turn-around time. Need for the development of method and validation by HPLC Method for letrozole for better accuracy, precision and higher sensitivity and recovery rate from human plasma sample. The drug candidate letrozole API purchased from Biodeal Pharmaceutical (Himachal Pradesh). HPLC Model Shimadzu 2030, Column: 4.6mm×25cm Fine-pack C_8 Steel Column, Software: Cromalion 7.2, Analytical balance: Shimadzu, pH meter: Toshcon Industries and LC-MS: Sciex QTRAP 4500 used for the entire research work. Optimization of chromatography in HPLC Method for letrozole using Mobile Phase as Water: Acetonitrile: Methanol (50:30:20 v/v/v) any many trials has been taken for variation in mobile phase, flow rate 1 mL/min and other parameters till the optimized chromatogram was observed, Extraction Technique LLE completed and observed mean value 4502.00, SD value 7.19 and % RSD were found 0.16 for method precision. The Mean value 4499, and SD 3.49 and %RSD 0.08 for System Suitability found. Recovery at LOQ level 2.35 SD, 0.26%RSD, Recovery at 100% level 3.33 SD, 0.07%RSD and Recovery at 150% level 3.31 SD, 0.05%RSD was observed. For method development of tablet formulation 2.5 mg the mean value observed 4489, 9.19 SD and % RSD 0.20 observed. The Regression Statistics data LLOQ and LOD for regression analysis were found 1.369 ng/ml and 0.637 ng/ml respectively, so the sensitivity of developed methods was very high rather than other available methods.

Keywords: Letrozole, Bio-analytical Method Development, LLOQ, Method Validation.

Introduction

Pharmaceutical analysis determines the quality of drug products via analytical chemistry. This will introduce areas such as method validation, handling of raw materials and finished products, documentations, inspections that impact the development of pharmaceutical products. Throughout this, critical cGMPs regulations, FDA guidance and ICH quality guidelines will be discussed specially emphasizing procedures to help individuals to maintain a high level of compliance that rounds the laboratory environment. Importance of pharmaceutical analysis- Identify of the drug in formulated product, Determination of active ingredient or additional impurities, Stability of the drug, Rate of drug from its formulation Identify and purity of pure drug that meet specification, Concentration of specified impurities Concentration of drug in plasma or biological fluids, Determine pka values partition coefficients, solubility and stability of drug under development [1]**.** According to ICH

method validation is defined by establishing documented evidence which proves a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specification and quality characteristics [2-4].

The Validation parameters like Response function, Sensitivity, Precision, Accuracy, Limit of detection (LOD) Limit of quantification (LOQ), Robustness, Stability, System suitability all the parameter must be comply with the methods.

The drug candidate belongs to Polycystic ovary syndrome is a condition where you have few, unusual or very long periods. It often results in having too much of a male hormone called androgen. Many small sacs of fluid develop on the ovaries. They may fail to regularly release eggs. A condition marked by infertility, enlarged ovaries, menstrual problems, high levels of male hormones, excess hair on the face and body, acne, and obesity. Women with polycystic ovary syndrome have an increased risk of diabetes, high blood pressure, heart disease, and endometrial cancer. Basic need for the work was to extract the drug molecule from the human plasma by different extracted methods like SPE and LLE and obtained the accurate higher sensitivity method [5-7].

Materials and Methods:

Materials: The drug candidate letrozole API purchased from Biodeal Pharmaceutical (Himachal Pradesh), tablet mfg. by Cipla purchased from medical store. Methanol, Mili Q water in-house, triethylamine, acetonitrile and ammonium acetate from chemdyes corporations Gujarat. Instruments used during the research work as HPLC Model Shimadzu 2030, Column: 4.6mm×25cm Fine-pack C_8 Steel Column, Software: Cromalion 7.2, Analytical balance: Shimadzu, pH meter: Toshcon Industries and LC-MS: Sciex QTRAP 4500 used for the entire research work.

Methods: Optimization of chromatography in HPLC Method for Letrozole:

Mobile Phase A: 2 mM Ammonium Formate in 0.1% Formic Acid in water, Sonicate it for 5 Min. filter it through 0.45µm Millipore filter [8-9]. Mobile Phase B: Use 100% Methanol. Diluent-1: Methanol, Diluent-2: Methanol: Water (60:40).

Standard Stock Preparation: Diluted 50mg of STD API in to 200mL volumetric flask with Diluent-1 and mixed it well. Now, diluted above 2.0 mL of solution to make it up to 200mL with diluent-2 and mixed it well [10].

Standard Final Preparation: Diluted 0.2 mL (200µL) of above standard stock solution in to 100.0mL of volumetric flask with Blood Plasma and mixed well, centrifuge it for 5000 RPM for 10 Minutes. After that Filter the above supernatant with 0.45µ PTFE MDI filter collect the filtrate by discarding not less than 5mL of filtrate through it [11].

Sample Preparation: Weigh accurately 10 tablets and calculate the average weight. Crush it in to fine powder and take sample powder equivalent to about 50 mg of Letrozole in to 200 mL of volumetric flask. Add about 150mL of diluent-1 and Sonicate it for 30 minutes with shaking of every 5 minutes for a period of 30 seconds. Make it up to mark with diluent-1 and mixed well [12, 13].

Now diluted 2 mL above solution to 200 mL in to Diluent-2 and mixed it well. Then in dilueted above 0.2mL of solution in to 100 mL Blood Plasma and mixed it well [14-16]. Centrifuge it for 5000 RPM for 10 minutes. Filter the above supernatant with 0.45µ PTFE MDI filter collect the filtrate by discarding not less than 5mL of filtrate through it [17-19].

Table 1: Optimization of chromatography condition

Sr. No	Chromatography Parameter	Condition
1	Column	Biphenyl Kinetic(150mm×4.6mm,5.0µm)
2	Mode	MRM mode
3	Flow rate	1 mL/min

4	Injection Volume	20µL
5	Column Temperature	40°C
6	Auto Sampler Rinsing Volume	1000µL
7	Auto Sampler sampling speed	15µL/sec
8	Autosampler Temperature	10°C
9	Auto Sampler Rinsing Volume	35µL/sec
10	Auto Sampler needle Stock	54mm
11	Run Time	3.4 min

Result and Analysis:

Table 2: Gradient Program: Isocratic Method (Binary Pump)

Time (Min)	Module	%A Mobile Phase	%B Mobile Phase
0.01	Pumps	30	70
3.5	Pumps	30	70

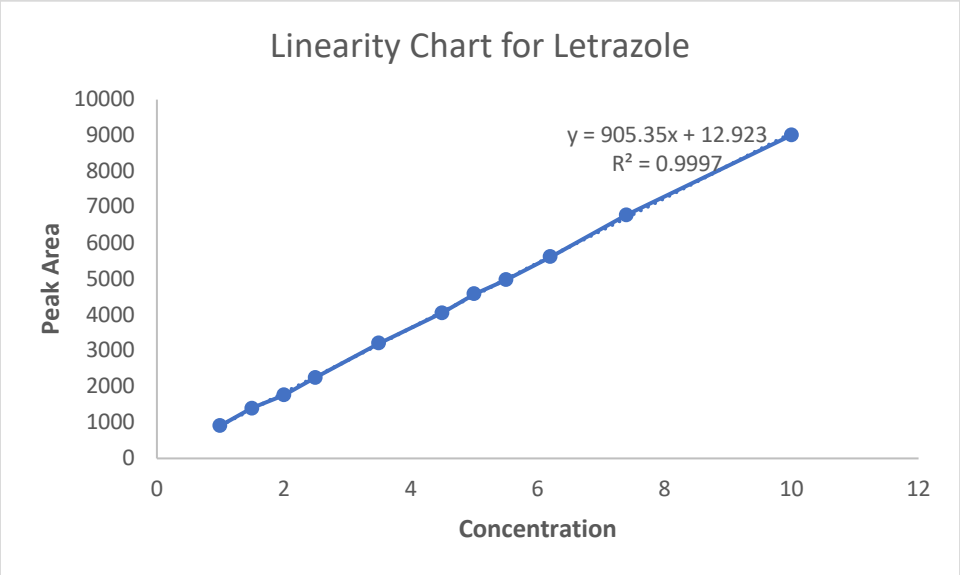

Fig. 1: Linearity profile for Letrazole

Table 3: Calculation for linearity

Sr. no	Concentration	peak Area
1	1	912
2	1.5	1402
3	2	1765
4	2.5	2258
5	3.5	3212
6	4.5	4059
7	5	4587
8	5.5	4977
9	6.2	5622
10	7.4	6791
11	10	9010

Trials for Optimization Chromatography:

Trial 1:

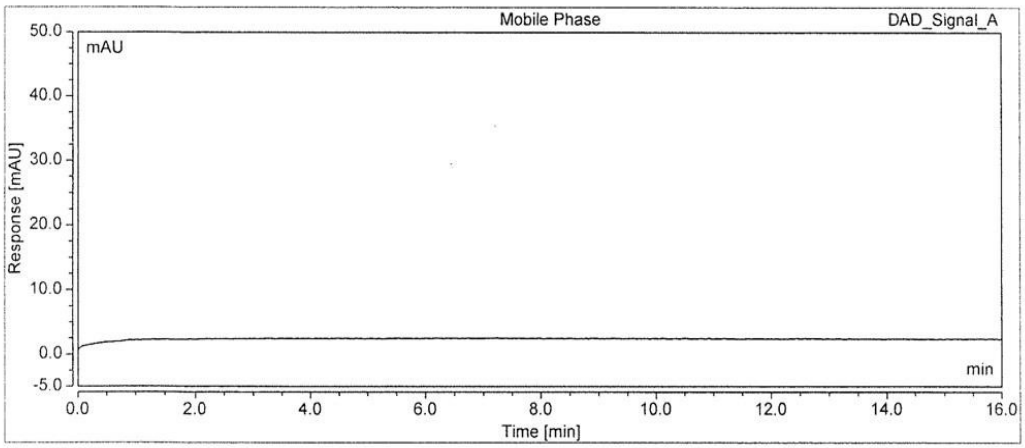

Fig. 2: Typical HPLC chromatogram of Mobile Phase Formic acid: Acetonitrile (90:10 v/v)

Trial 2:

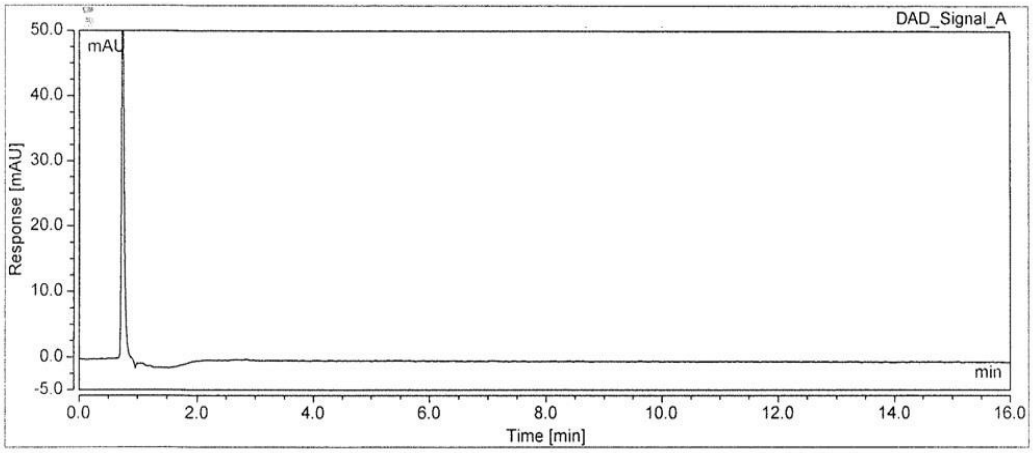

Fig. 3: Typical HPLC chromatogram of Mobile Phase Water: Acetonitrile (30:70 v/v)

Trial 3:

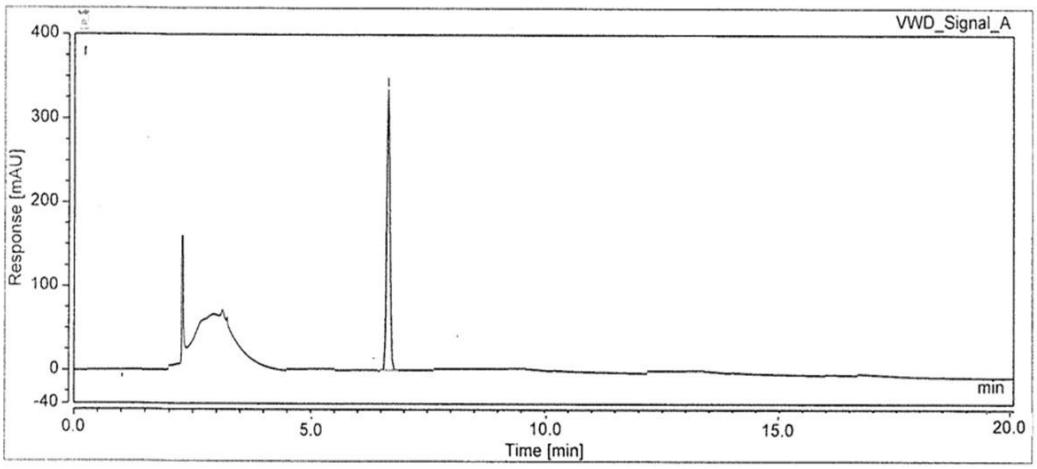

Fig. 4: Typical HPLC chromatogram of Mobile Phase Water: Methanol (30:70 v/v)

Trial 4:

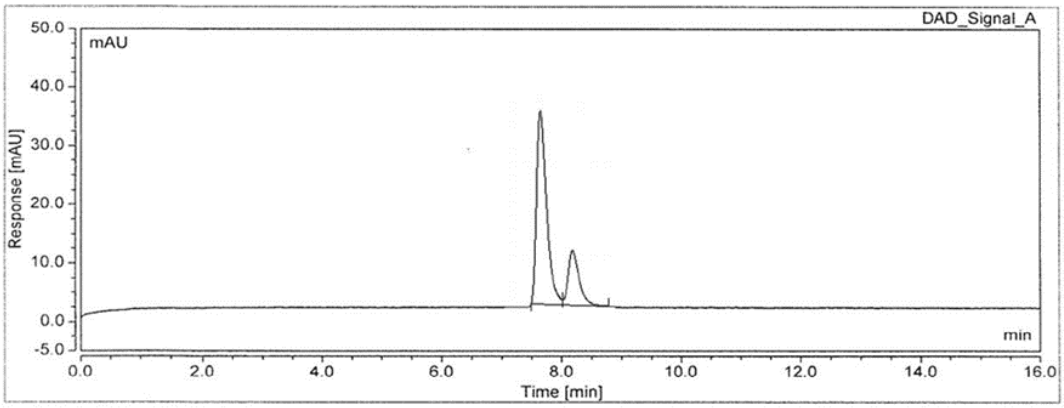

Fig. 5: Typical HPLC chromatogram of Mobile Phase Water: Acetonitrile: Methanol (20:40:40 v/v/v)

Trial 5:

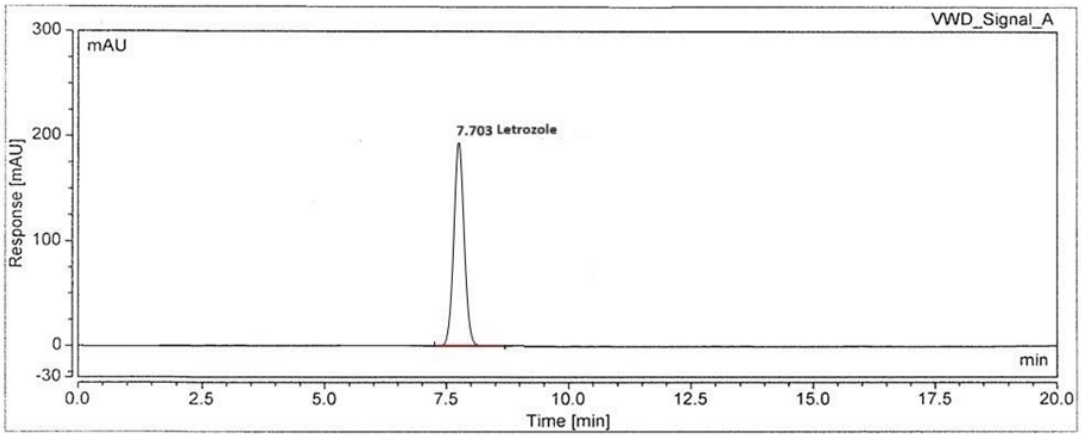

Fig. 6: Typical HPLC chromatogram of Mobile Phase Water: Acetonitrile: Methanol (50:30:20 v/v/v)

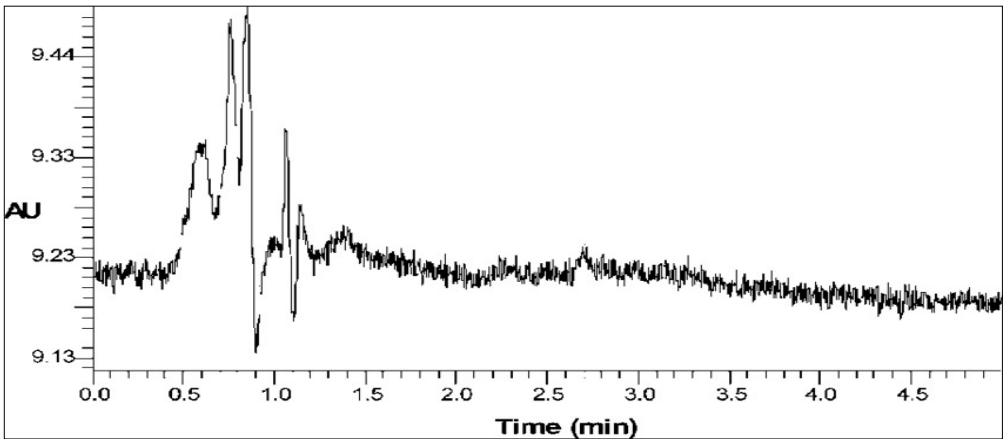

Fig. 7: Typical LC-MS Chromatogram of Blank blood plasma Sample

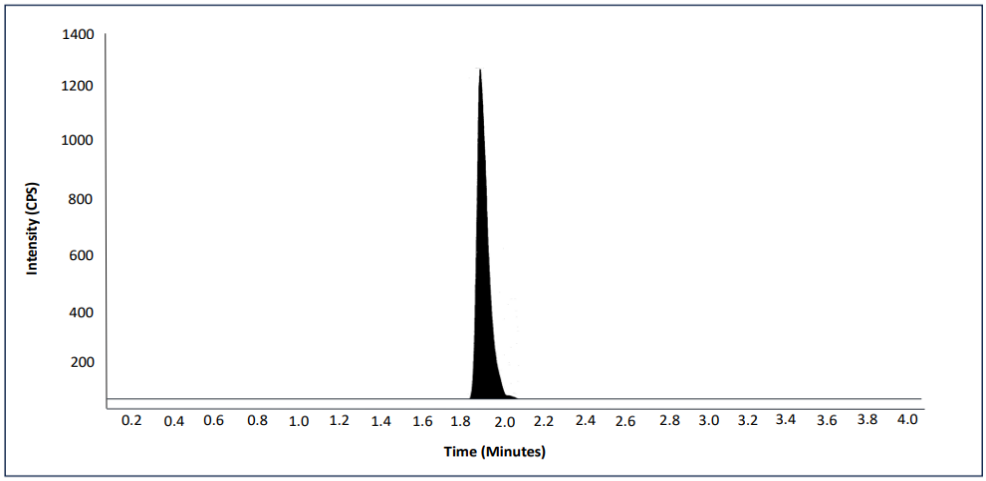

Fig. 7: Typical LC-MS Chromatogram of Standard Solution in Blood Plasma

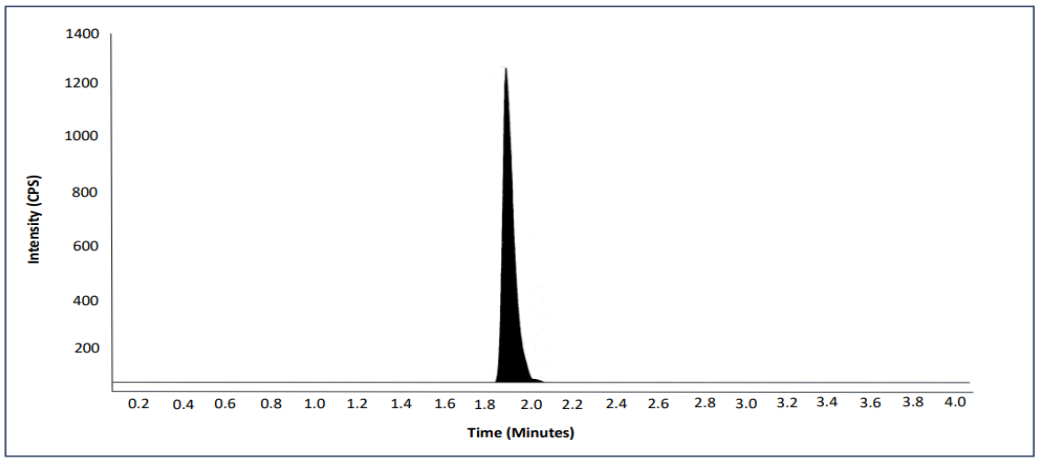

Fig. 8: Typical LC-MS Chromatogram of Sample solution in Blood Plasma

Table 4: Calculation for Recovery at Different Levels

	Recovery Data		
Sr. No.	**Recovery at LOQ**	**Recovery at 100%**	**Recovery at 150%**
1	902	4500	6751
2	904	4495	6750
3	901	4499	6745
4	898	4502	6748
5	900	4505	6755
6	904	4501	6750
Mean	**902**	**4500**	**6750**
SD	2.35	3.33	3.31
%RSD	**0.26**	**0.07**	**0.05**

Table 5: Table of Method Precision

Sr. No.	**Precision**	**Intermediate Precision**
1	4501	4508
2	4507	4501
3	4498	4497
4	4490	4499
5	4510	4505

6	4505	4501
Mean	**4502**	**4502**
SD	7.19	4.02
%RSD	**0.16**	**0.09**

Table 6: system Suitability

Sr. No.	**Standard SST**	**Standard CAL**
1	4496	4501
2	4500	4499
3	4498	
4	4495	
5	4502	
6	4504	
Mean	**4499**	**4500**
SD	3.49	1.41
%RSD	**0.08**	**0.03**

Table 7: Letrozole Tablet USP (2.5mg)

Multiple R	0.99915
R Square	0.99999
Adjusted R Square	0.99973
Standard Error	0.98575
Observation	5
LLOQ	1.369 ng/ml
LOD	0.637 ng/ml

Table 8: Summary Output for Letrozole Regression Statistics

Sr. No.	**Tablet Area**
1	4482
2	4495
Mean	**4489**
SD	9.19
%RSD	**0.20**

Conclusion:

In the present study a LC-MS/MS-ESI method was developed for detection and quantification of letrozole in human plasma. This developed method was also validated as per the EMA and USFDA guidelines. Letrozole is a weak acid. The retention of this drug observed only in acidic pH. Therefore, if the elution was carried out in isocratic method, the response found was less. Hence in the present study, a gradient method was applied with the use as Water: Acetonitrile: Methanol (50:30:20 v/v/v) for total 3.4 min run time. Matrix effect and the recovery considered as the critical phase in the analysis of plasma samples. From the data of the present method, it was evident that the plasma matrix effect was minimized more than 90%. Therefore, it can be concluded from the present study that the developed method by using LC-MS/MS and liquid-liquid extraction technique for letrozole in human plasma was found to be simple, specific, sensitive, reproducible and fully validated per EMA and USFDA guidelines. The present method was hence found superior enough to be applied to comparative pharmacokinetic studies in future.

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