INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com ISSN 2230 – 8407

Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE DETERMINATION OF LETROZOLE IN BULK AND DOSAGE FORM

Pallavi S[1], Vasanth PM*[1], Ramesh T[2], Ramesh Malothu[2]

[1]Department of Pharmaceutical Analysis, UCEV-JNTUK, Vizianagaram, A.P, India

[2]Department of Biotechnology, UCEV-JNTUK, Vizianagaram, A.P, India

Article Received on: 07/09/12 Revised on: 09/10/12 Approved for publication: 02/11/12

*Email: vasanthpharma@gmail.com

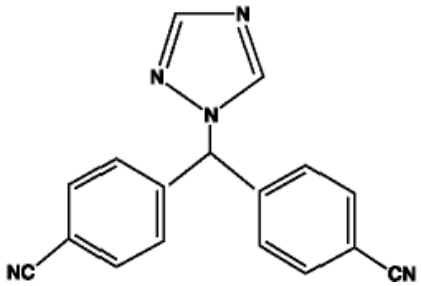
ABSTRACT

A Reverse phase high performance liquid chromatographic method was developed for the determination of Letrozole in bulk and dosage form. The separation was effected on a kromasil ODS C18 column (250mmX4.6mm, 5µ) using a mobile phase mixture of buffer and methanol in a ratio of 85:15 v/v at a flow rate of 1.0ml/min. The detection was made at 230nm. The retention time of letrozole was found to be 3.443±0.08 min. Calibration curve was linear over the concentration range of 20-120µg/ml of Letrozole. The propose method was validated as per the ICH guidelines. The method was accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug and dosage form.

Keywords: Method development and validation, letrozole, Tablets, Kromasil C18 column, RP-HPLC.

INTRODUCTION

Letrozole (benzonitrile, 4,4'-(1H-1,2, 4-triazol-1-ylmethlene) bis-, Femara) 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. A few methods of analysis of letrozole have been reported using different techniques such as microarray approach, capillary gas chromatographic method with flame ionization detector. However, there is no high performance liquid chromatographic (HPLC) method reported for letrozole in pharmaceutical dosage forms. The HPLC methods using the most commonly available columns and detector like UV are preferred. The present study describes the determination of letrozole in pharmaceutical dosage forms by using RP-C $_{18}$ column with UV detectors. Owing to the widespread use of HPLC in routine analysis, it is important that well validated HPLC methods are to be developed for estimating letrozole. The aim of this study is development of a simple, precise, rapid and accurate reverse phase HPLC method for the estimation of letrozole in different pharmaceutical dosage forms[1-4].



Chemical Structure of Letrozole

MATERIALS AND METHODS

Instrumentation

Shimadzu Class VP version 6.12 SPS data system employed with spinchrome software was used in the experiment. A 20uL Hamilton injection syringe was used in the sample injection. LC 20 AD Pump and Prominence SPD 20A UV-deuterium detector was employed in the study[5-8].

Materials required

HPLC grade solvents like methanol, water (Merck ltd) Analytical grade chemicals like potassium dihydrogen phosphate and ortho phosphoric acid (SD fine chem ltd).the letrozole API, and formulation sample was supplied by Taj pharmaceuticals, India.

Optimized conditions

The Mobile phase consisted of 85:15 %(v/v) of potassium dihydrogen phosphate adjusted to pH-3.5+0.2 with orthophosphoric acid and methanol. Operated on Isocratic mode. The flow rate is 1.0 ml/min. chromatographic separation of letrozole was performed on kromasil ODS C18 column (250mmX4.6mm, 5µ). The wavelength of detection is 230 nm. The column temperature was maintained at 25°C. The injection volume is 20µL.

Preparation of mobile phase

1.32gm of potassium dihydrogen phosphate dissolved in 600ml of water, then add 0.3g of ortho phosphoric acid to maintain pH of the mobile phase. The solution was filtered through 0.45um membrane filter and degassed. Mixture of potassium dihydrogen phosphate and methanol were prepared in the ratio of (85:15) V/V and filtered through 0.05u membrane filter and sonicated.

Stock and working standard solution

About 25mg of letrozole was weighed accurately and transferred into 25ml volumetric flask. The solution was sonicated and filtered through whattman filter paper and resulting solution was diluted with the mobile phase to get a working standard solution of 25µg/ml of letrozole.

Assay

Twenty tablets of letrozole were accurately weighed and powdered. Transfer powder equivalent to 100mg in a 100ml volumetric flask, sonicated to about 15min and final volume made upto 100ml with mobile phase and filtered through 0.45um membrane filter. From the above solution, 1ml of aliquot was diluted to 10ml with mobile phase and mixed well. This solution (20 µ L) was injected six times into the column and its peak area was compared with standard calibration plot for assay study. Results was shown in table 1.

Table 1: Analysis of marketed formulation data of Letrozole

Drug Name	Label claim(mg)	Amount Found(mg)	% Assay
letrozole	2.5	2.47	98.8

Table 2: System Suitability Parameters

Parameters	Value
Theoretical Plates(h)	7815
Tailing factor(T)	0.9
Resolution	0
Retention time	3.42

Table 3: Calibration Data of the proposed method

Concentration of letrazole (ug/ml)	Mean Peak area
20	1244.002
40	2371.692
60	3647.656
80	4977.042
100	6136.990
120	7299.147

Table 4: Accuracy data of Letrozole (triplicate values at 80, 100, 120 percent levels)

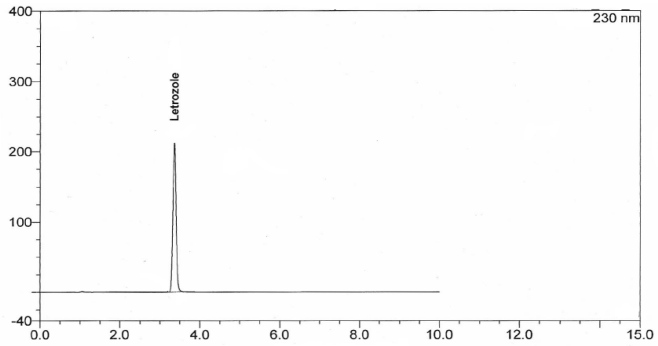
Amount taken (µg)	Amount found (µg)	Percent recovery	Mean percentage recovery
90	89.14	99.04	99.04
110	109.16	99.23	99.23
130	128.80	99.08	99.08

Table 5: Robustness data of Letrozole

Drug name	Variations	Chromatographic parameters				
	Change in wavelength at ± 2nm	Retention time	Area	Height	Theoretical Plates	Asymmetry
	1. wave length at 228nm	3.430	6247.732	728.039	3666	1.714
	2.wavelength at 230nm	3.437	6283.478	724.787	3503	1.655
	3.wavelength at 232nm	3.430	6363.381	735.321	3666	1.714
	Change in flow rate at ± 0.1ml/min					
	1.flow rate at 0.9ml/min	4.110	7498.414	739.806	3656	1.727
	2.flow rate at 1.0ml/min	3.437	6283.478	724.787	3503	1.655
Letrozole	3.folw rate at 1.1ml/min	2.940	5411.49	718.573	3518	1.680

Table 6: Forced degradation data of Letrozole

Drug name	Condition	Retention time (min)	Area	% Degradation	% of Active drug Present after Degradation
	Control Sample	3.437	6283.478	-	100
	Acid Degradation	3.437	6237.208	0.73	99.27
	Alkaline Degradation	3.460	5906.601	6.00	94.00
Letrozole	Thermal Degradation	3.427	5924.547	5.72	94.28



LETROZOLE - R_T 3.44

Figure 1: Standard Chromatogram of Letrozole

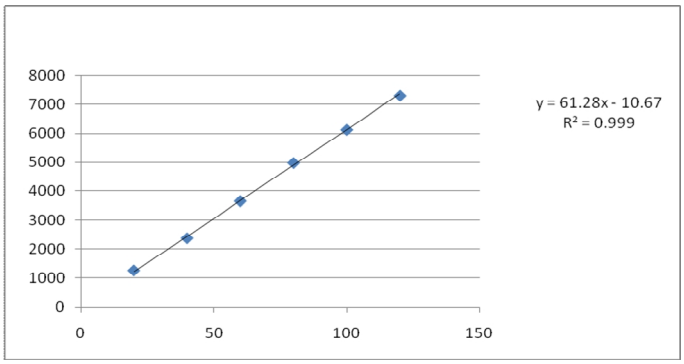


Figure 2: Linear Calibration Plot of Letrozole

RESULTS

System Suitability (SST)

SST is commonly used to verify resolution, column efficiency, and repeatability of the chromatographic system to ensure its adequacy for a particular analysis. It is performed by performing 6 injections on a sample mixture containing letrozole std. parameters such as tailing factor, retention time, theoretical plates and resolution etc. were determined. Results shown in table 2. [9-12]

Linearity & Range

Linearity is the method to obtained tests that are directy proportional to the analyte concentration within a given range. Range of analytical procedure is the interval between the upper and lower concentrations of the analyte in the analytical procedure has a suitable level of precision, accuracy, linearity.

Calibration curve standards were prepared in the range of 20-120µg/ml of letrozole. The calibration curves of peak areas against concentration were found to be linear and correlation coefficient (r) found to be 0.999. The results and the calibration graphs are shown below in the table 3 and figure 2.

Accuracy

The Accuracy is determined as the closeness of the calculated concentration to the nominal concentration.. The mean recoveries were calculated at three different levels i.e at 80%, 100%,120% solutions made from the standard solution. The % mean recoveries found to be 99.05. Results shown in table 4.

Robustness

Robustness is observed by evaluating the influence of Flowrate, and detection wavelength. Change in wavelength observed by + 2nm that of detection wavelength. And variation in flowrate was performed at + 0.1ml/min that of actual flowrate used in the experiment. Results were shown in table 5.

a) At Flow rate of 0.9ml/min – late elution of drug, Rt increases

b) At flow rate of 1.1ml/min -fast elution of drug, Rt decreases
c) Change in detection wavelength- variation in peak area was observed.

Forced Degradation Studies

For force degradation analysis, aliquots of stock are treated separately with 100uL of 0.1N HCl (Acid Stability), 0.1N NaOH (Alkaline Stability), and thermal degradation to 105°c for 24 Hrs. Stability of these samples are compared with fresh sample on the day of analysis. Letrozole shows alkaline instability. Results shown in table 6.

DISCUSSION

A new, Reversed-phase HPLC method for the determination of letrozole in formulation was said to be accurate, linear, and selective, proving the reliability of the method. The run time is relatively short , i.e. 10 min, which enables rapid quantitation of many samples in routine and Quality Control analysis of Tablet formulations. The method employed is isocratic and the results show the method could find practical application as a quality-control tool. Validation was performed according to ICH guidelines.. Validation parameters results include: 99.05% recovery, range of 20-120ug/ml, a good linear relationship (r2 = 0.99970) was observed . The regression curve was constructed by linear regression fitting and its mathematical expression was y = 61.287x-10.1785 (Where y gives peak area and *x* is the concentration of the drug). Robustness performed with variation in detection wavelength and flowrate. Letrozole shows significant alkaline instability. The above parameters like accuracy, linearity, robustness, forced degradation study results obtained are within the acceptance criteria.

ACKNOWLEDGEMENT

The author thanks to Taj pharmaceuticals.ltd, for providing gift sample.

REFERENCES

1. J. Rodriguez Flores, A.M. Contento Salved, M.J. Villaseñor Llerena, L. Muñoz Fernández, Micellar electrokinetic chromatographic method for the determination of letrozole, citalopram and their metabolites in human urine,Journal of chromatography A,2008,1185(2), 281-290.
2. Marfil, V. Pinea ,A.. Sioufi and J. Godbillon, High-performance liquid chromatography of the aromatase inhibitor, letrozole, and its metabolite in biological fluids with automated liquid-solid extraction and fluorescence detection, Journal of Chromatography B: Biomedical Sciences and Applications,1996,683(2),251-258.
3. Barrows LR. Antineoplastic and immunoactive drugs. In: Gennaro AR, editor. Remington: The Science and Practice of Pharmacy. 20th ed. Vol II. Philadelphia, PA: Lippincott Williams and Wilkins Company; 2000. pp. 1477–506
4. Lamb HM, Adkins JC. Letrozole: A review of its use in postmenopausal women with advanced breast cancer. Drugs. 1998;56(6):1125–40. PubMed.
5. Levien T, Baker DE. Letrozole and Budesonide Inhalation powder. Hosp Pharma. 1997;33(4):437–54
6. Mouridsen H, Gershanovich M, Sun Y. Superior efficacy of Letrozole versus Tamoxifen as first line therapy for postmenopausal women with advanced breast cancer. J Clin Oncol. 2001;19(10):2596–606. PubMed
7. Worthington I. Current drug topics: Letrozole. Newsletter. 1996;15:125
8. Itoh T, Karlsberg K, Kijima I, Yuan YC, Smith D. Letrozole: A microarray Approach. Mol Cancer Res. 2005;3(4):203–18. PubMed.
9. Berzas JJ, Rodriguez J, Contento AM, Cabello MP. Determination of drugs used in advanced breast cancer by capillary gas chromatography of pharmaceutical formulations. J Separation Sci. 2003;26(9):908–14
10. Bhatnagar AS, Hausler A, Schieweck K, Lang M, Bowman R. Highly selective inhibition of estrogen biosynthesis by CGS 20267, a new non-steroidal aromatase inhibitor J.Steroid. Biochem. Mol Biol. 1990; 37: 1021-1027
11. Bhatnagar AS, Batzl C, Hausler A, Schieweck K, Lang M, Trunet PF. Pharmacology of nonsteroidal aromatase inhibitors: In: Pasqualini JR, Katzenellenbogen BS (eds) Hormonedependent cancer. Marcel Dekker, New York, 1996; p 155
12. Miller WR. Biology of aromatase inhibitors: pharmacology/endocrinology within the breast Endocr Rela Cancer. 1999; 6: 187-195
13. Lu Q, Liu Y, Long BJ, Grigoryev D, Gimbel M, Brodie A. The effect of combining aromatase inhibitors with antiestrogens on tumor growth in a nude mouse model for breast cancer Breast Cancer Rel Treat. 1999; 57: 183-192.
14. Geisler J, Haynes B, Anker G, Dowsett M, Lønning PE. Influence of Letrozole and Anastrozole on Total Body Aromatization and Plasma Estrogen Levels in Postmenopausal Breast Cancer Patients Evaluated in a Randomized, Cross-Over Study. J Clin Oncol. 2002; 20: 751-757
15. Dixon JM, Renshaw L, Young O, Murray J,Macaskill EJ, McHugh M, Folkerd E, Cameron D, Dowsett M. Letrozole suppress plasma oestradiol(E2) levels more completely in postmenopausal women with breast cancer [abstract] .J Clin Oncol. 2006; 24(Suppl 18): 552
16. Rodr´ıguez FJ, Contento SAM, Villase˜nor LMJ, Mu˜noz FL. Micellar electrokinetic chromatographic method for the determination of letrozole, citalopram and their metabolites in human urine. J Chromatogra A. 2008; 1185: 281-290.
17. Ganesh M, Kamalakannan K., Rahul P, Satish U, Anand S, Sivakumar T, Swastika. A validated UV Spectrophotometric method for the determination of Letrozole in bulk and solid dosage form. Rasayan J Chem. 2008; 1: 55- 58
18. Nita M, Pal TK, Ghosal SK, Development and validation of RP-HPLC method to determine letrozole in different pharmaceutical formulations and its application to studies of drug release from nanoparticles. Acta Polo Pharmaceutican Drug Resear 2009; 661: 11- 17
19. Methodology. ICH Harmonised Tripartite Guideline "Validation of analytical procedures" Having reached Step 4 of the ICH Process. ICH Steering Committee Meeting, 6 November 2005
20. USP 30. The United States Pharmacopoeia, 29th Edn; United States, Pharmacopeial Convention.:Rockville. 2007; pp 680-683
21. Handbook of Pharmaceutical analysis by M.W. Dong by page 65
22. Beckett A.H. and Stanlake J.B. Practical Pharmceutical Chemistry, 4th Edn, Part 2, CBS publications and distributors, 2002,1
23. ICH, Validation of analytical procedures: methodology, (Q2B) Geneva 1996.

Source of support: Nil, Conflict of interest: None Declared