Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India

Development and validation of a spectrophotometric method for estimation of letrozole in bulk and pharmaceutical formulation

N. Mondal, T. K. Pal, S. K. Ghosal

Received December 28, 2006, accepted January 26, 2007

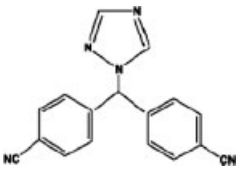
Prof. (Dr.) S. K. Ghosal, Division of Pharmaceutics, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700 032, India
profskghosal_ju@yahoo.co.in

Pharmazie 62: 597–598 (2007) *doi: 10.1691/ph.2007.8.6305*

A simple, sensitive and accurate UV spectrophotometric method has been developed for the determination of letrozole, a new aromatase inhibitor, in raw material and tablets. The drug shows maximum absorption at 238 nm. Beer's law was obeyed in the concentration range 2–20 μg/mL for the drug. Results were validated statistically according to ICH guidelines. Validation of the method yielded good results concerning range, linearity, precision and accuracy. It was found that the excipients present in the commercial formulation did not interfere with the method.

1. Introduction

Letrozole is a highly selective, non-steroidal, third generation aromatase inhibitor approved for first-line and extended adjuvant therapy in postmenopausal women with hormone-responsive, early stage breast cancer. In postmenopousal women, estrogens are mainly derived from the action of the aromatase enzyme, which converts adrenal androgens to estrone and eatradiol. The suppression of estrogen biosynthesis in peripheral tissues and in the cancer tissue itself can therefore be achieved by specifically inhibiting the aromatase enzyme. Letrozole inhibits the aromatase enzyme by competitively binding to the haeme of the cytochrome P450 subunit of the enzyme, resulting in reduction of estrogen biosynthesis in all tissues (Scott and Keam 2006).



Letrozole (CAS number 112809–51-5)

Chemically letrozole is 4,4′-(1H-1, 2,4-triazol-1-ylmethylene) bisbenzonitrile (Budavari, 2001) with a molecular weight of 285.31. The drug is official in the US pharmacopoeia wherein the estimation of letrozole in bulk and formulation is done by HPLC using UV detector taking a mixture of degassed water and acetonitrile at a ratio of 70 : 30 as mobile phase (USP-27 2004). A literature survey has not revealed availability of any UV spectrophotometric method for the determination of the drug in pharmaceutical formulations whereas reports are available for the estimation of the drug and related components by TLC and HPLC in tablets (Gu Ping et al. 2001), by HPLC in biological fluids with automated liquid-solid extraction and fluorescence detection (Marfil et al. 1996), and by GC/MS for identification of letrozole in urine (Mareck et al. 2005).

In the present study, a simple, economic precise and accurate analytical method for the estimation of letrozole in pure form and in solid dosage form was developed. The results of the analysis were validated by statistical methods and recovery studies.

2. Investigations, results and discussion

Letrozole was analyzed by UV spectrophotometric method both as a raw material and as a pharmaceutical tablet formulation. The linear regression equation was calculated to

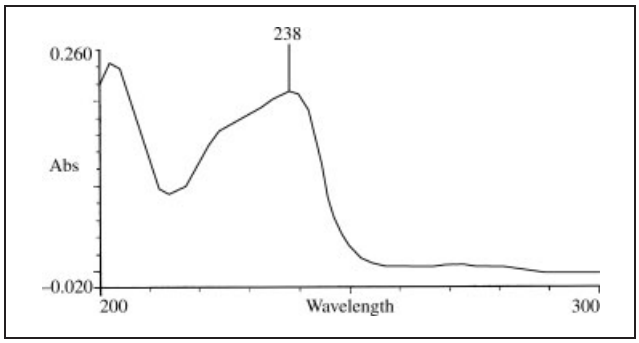


Fig.: UV spectrum of Letrozole measured in methanol

Table 1: Analysis of letrozole tablets (2.5 mg)

Sl. No	A*	%Analysis ± S.D.	SEM	% C.V.
1	101.07	100.61 ± 0.68	0.279	0.679
2	100.88			
3	99.64			
4	99.83			
5	101.07			
6	101.16			

*Absorbance – Average of three determinations, S.D.: Standard deviation, SEM: Standard error of mean, C.V.: Coefficient of variance

Table 2: Recovery studies of letrozole tablets

Sl. No.	Drug amount in tablet solution (μg)	Reference Added (μg)	Total amount (μg)	Recovery amount (μg)	Recovery (%)	Recovery (%) ± S.D.
1	12	0	12	12.06	100.5	99.9 ± 0.71
2	12	2	14	13.87	99.0	
3	12	4	16	15.89	99.3	
4	12	6	18	18.12	100.7	
5	12	8	20	20.03	100.1	

Table 3: ANOVA of intra- and inter-day assay of letrozole tablets

Source of Variation	SS	df	MS	F_{stat}*	F at level 1%	F at level 5%
Rows	0.14095	5	0.02819	0.04021	5.64	3.33
Columns	0.00702	2	0.00351	0.00501	7.56	4.10
Error	7.01150	10	0.70115			
Total	7.15947	17	0.02819			

* $F_{stat} < F$ at level 1% and 5% in both cases

be $Y = 0.0877X + 0.0223$ where X and Y are concentration in μg/mL and absorbance, respectively.

A standard calibration curve of the drug was constructed by plotting absorbance versus concentration. The UV absorption spectrum (Fig.) was monitored at 238 nm. Agreement with Beer's law was evident from the concentration range of the final dilution of 2–20 μg/mL. The correlation coefficient obtained for the line was 0.9997 indicating very good linearity. The experimental results obtained for the determination of letrozole tablets are shown in Table 1. The method has excellent reproducibility for a standard solution of 100 μg/mL. The average purity obtained was 100.7%.

The detailed accuracy is shown in Table 2. In this test the observed concentrations of letrozole reference substance in the tablets were not significantly different from the stated concentrations by Student's t test, $P = 0.05$ (100.614%, $n = 6$).

No interfering intensity was found in the UV spectra due to the tablet excipients. Letrozole was shown to be stable during all the procedure.

3. Experimental

3.1. Chemicals

Letrozole reference substance was obtained from Sun Pharma Advanced Research Centre (Vadodara, India). Tablets of brand Letoval (Batch No PHK40222B, Sun Pharma Industries Ltd., Andheri, Mumbai, India) containing 2.5 mg of letrozole were procured from a local pharmacy. The solvent used for the experiment was methanol (AR grade, Merck, India).

3.2. Equipment

A double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) connected to a computer loaded with spectra manager software UV Probe was employed with spectral bandwidth of 1 nm and wavelength accuracy of ±0.3 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (Precisa 310M, Switzerland).

3.3. Standard solution

The standard solution of letrozole was prepared by dissolving 10 mg of the drug in methanol and diluted to 100 mL. Aliquots of the standard solution were further diluted to give a range of final concentrations of 2–20 μg/mL. The absorbance of each solution was determined at 238 nm.

3.4. Sample preparation

For the analysis of the dosage form, twenty tablets of letrozole (2.5 mg) were ground to fine powder and mixed thoroughly. Powder equivalent to 10 mg of drug was transferred to a 100 ml volumetric flask and dissolved in about 40 ml methanol by shaking on a rotary flask shaker for 2 h. The solution was filtered through Whatman filter paper (No. 41). The filter paper was washed with the blank. The washings were added to the filtrate and the final volume was made up to 100 ml with the blank. After suitable dilution, the absorbance of final sample corresponding to 12 μg/ml was recorded against the blank at 238 nm. All determinations were conducted in triplicate.

The data were analyzed by linear simple regression by the least-squares method. The recoveries were determined by adding known amounts of letrozole reference substance (0, 20, 40, 60 and 80 μg) to the samples at beginning of the process. A recovery exercise was then performed.

The precision and accuracy of the assay as well as linearity of the calibration curve were determined for intra- and inter-day on three different days. The precision was expressed as the percent coefficient of variation of each curve. The statistical data were calculated by ANOVA (Table 3).

Acknowledgements: One of the authors, N. Mondal is grateful to UGC, Govt. of India [F.10-17/2004(SA-I)] for financial assistance to carry out the research work. Authors are also grateful to Sun Pharma Advanced Research Centre (Vadodara, India) for sending letrozole as gift sample.

References

Budavari S (ed.) (2001) The Merck Index, An Encyclopedia of Chemicals, drugs and biologicals, Merck and Co., 13th Edn, White house Station, NJ, 974.

Gu P, Li Y, Wu Y, Jiang S (2001) Determination of letrozole and related substances in tablets by TLC and HPLC. Zhongguo Yiyao Gongye Zazhi 32: 317–318.

International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use: Harmonized Tripartite Guideline on Validation of Analytical Procedures: Methodology Recommended for Adoption at step 4 of the ICH process on November 1996 by the ICH Steering Committee, Published by IFPMA, Switzerland.

Mareck U, Sigmund G, Opfermann G, Geyer H, Thevis M, Schaenzer W (2005) Identification of the aromatase inhibitor letrozole in urine by gas chromatography/mass spectrometry. Rapid Commun Mass Spectrom 19: 3689–3693.

Marfil F, Pineau V, Sioufi A, Godbillon J (1996) High-performance liquid chromatography of the aromatase inhibitor, letrozole, and its metabolite in biological fluids with automated liquid-solid extraction and fluorescence detection. J Chromatogr B 683: 251–258.

Scott LJ, Keam SJ (2006) Letrozole: in postmenopausal hormone-responsive early-stage breast cancer. Drugs 66: 353–362.

United States Pharmacopoeia (2004), 27th United State Pharmacopoeial Convention Inc., Rockville, MD, 1071.