





A validated method without derivatization for the determination of Letrozole by high performance liquid chromatography-fluorimetric method for pharmaceutical preparation

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ABSTRACT

A high performance liquid chromatography method has been developed for determination of letrozole in tablets. The method has developed in reverse phase column with acetonitrile-50.0 mM phosphoric acid solution, pH:7 (50:50, v/v) and 0.7 mL/min flow rate by using a fluorimetric detector. Letrozole has natural fluorescence properties, and there is no need for derivatization for this molecule. The detector was set at 256, 585 nm for excitation and emission wavelength respectively. The method was validated in accuracy, precision, specificity, linearity and system suitability parameters are studied. The linear range was found 50-700 ng/mL. The limit of detection and quantification for letrozole was found to be 14-50 ng/mL respectively. The recovery of drug is found to be 98.8%. The developed method has been validated and successfully applied to tablet analysis of drug substance. This method is simple, reproducible and can be used safely for routine analysis of letrozole in tablets.

Keywords: Letrozole, HPLC-FLD, pharmaceutical preparation

INTRODUCTION

Letrozole, 4,4'-((1H-1,2,4-triazol-1-yl)methylene) dibenzonitrile is a nonsteroidal competitive inhibitor of the aromatase enzyme system; it inhibits the conversion of androgens to estrogens (Figure 1). Letrozole (LTZ) is used for the treatment of breast tumor which is estrogen-dependent. Breast cancer is also responsible for 18% of deaths in cancer types (Lamb and Adkins, 1998; Annapurna et. al., 2012). In adult nontumor and tumor bearing female animals, letrozole is as good as ovariectomy in decreasing uterine weight, raising serum luteinizing hormone (LH), and resulting in the regression of estrogen dependent tumors. Unlike ovariectomy, treatment with letrozole does not cause an increase in serum follicle stimulating hormone (FSH). Letrozole inhibits gonadal steroidogenesis but has no important effect on adrenal mineralocorticoid or glucocorticoid synthesis.

Letrozole inhibits the aromatase enzyme by binding to the heme of the subunit of the cytochrome P450 enzyme competitively, concluding in a decrease of estrogen biosynthesis in all tissues. women suffering from breast cancer with treatment of letrozole effect by reducing serum estrone, estradiol and estrone sulfate and hasn't been shown to importantly affect adrenal corticosteroid synthesis, aldosterone synthesis, or synthesis of thyroid hormones (Annapurna et. al., 2012; Scott and Kearn, 2006).

Numerous drug substances of interest cannot be detected because the lack of the structural properties essential for the production of signals compatible with general detectors, such as ultraviolet (UV) absorbance and fluorescence. Derivatization can be used as an effective modification method that can improve the overall specificity and sensitivity of trace analyses. Besides these advantages, derivatization includes disadvantages such as lower yields and time-consuming experimental procedures. Letrozole has natural fluorescence properties to detect lower concentrations from various matrices.

In the literature, limited method can be found on letrozole analysis in pharmaceutical preparations. Mondal et al. developed a spectrophotometric technique for the determination of letrozole in pharmaceutical formulation (Mondal and Pal, 2009). The RP-HPLC method (Mondal and Pal, 2009; Pallavi et al., 2012) and spectrophotometric derivative method (Acharjya et al., 2010)

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have been reported for the analysis of letrozole from pharmaceuticals. Letrozole determination from biological materials were detected high performance liquid chromatography with fluorescence detector (HPLC-FLD) (Moussa et al., 2013; Rodríguez et al., 2013; Marfil et al., 1996; Zarghi et al., 2007) gas chromatography (Mareck et al., 2005), high performance liquid chromatography mass spectrometer (HPLC-MS) (Gomes and Bhosale 2013) and also stability studies of letrozole preparations were investigated (Mareck et al., 2005; Gomes and Bhosale 2013, Prasad and Govindrajulu, 2012). There is no publication of letrozole from pharmaceutical preparation by chromatographic method. The method developed can be applied to stability studies and also pharmacokinetic studies.

The aim of this study is to develop a simple and safe method based on fluorescence detection with a high performance liquid chromatography technique and an application of this method on pharmaceutical preparations.

MATERIALS AND METHODS

Apparatus

The HPLC system Shimadzu LC (liquid chromatography) 20 series (Shimadzu Analytical and Measuring Instruments, Kyoto, Japan) with fluorescence detector were used for the analysis of letrozole. The HPLC systems parts included a pump (Shimadzu

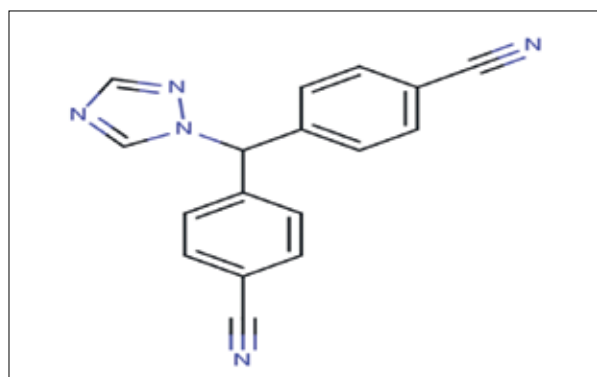


Figure 1. Structure of letrozole

LC-20AT) and an auto sampler (SIL-20HT). An instrument software (LC Solution) was also used.

Chemicals

All the chemicals were analytical grade. Letrozole was obtained from Natco Pharma Limited (India). Methanol which is HPLC grade was purchased from Sigma-Aldrich (Taufkirchen, Germany) and Acetonitrile which is HPLC grade was purchased from VWR (Vienna, Austria). HPLC grade solvents were also used. Femera[®] was used as a tablet which contains 2,5 mg LTZ.

Chromatographic conditions

Chromatographic separation was carried out at 25 °C temperature using a reversed phase Intersil ODS (octadecyl silane) 5 µm, 250x4.6 mm column (Tokyo, Japan). The mobile phase consists of acetonitrile: phosphate buffer 50 mM pH:7 (50:50) (v/v). The flow rate of the mobile phase was 0.7 mL/min. Letrozole has natural fluorescence properties and the detector was set at 256, 585 nm for excitation and emission wavelengths respectively. The injection volume chosen was 20 µL.

Preparation of standard solutions and quality control samples

A stock solution of LTZ (letrozole) (0.1 mg/mL) was prepared and diluted to LTZ2 (1 µg/mL) with methanol. All solutions were stored at 4°C until the end of the study. The five calibration standards were prepared with methanol between the concentration ranges of 50 and 700 ng/mL. The quality control (QC) samples at a concentration of 50; 200; 400 and 700 ng/mL. All calibration standards and QC (quality control) samples were stored at 4°C until the end of the study.

Analytical method validation

In order to prove the acceptable nature of analytical method, the following protocol was performed during the method evaluation. There was no need for ethics committee approval for this study.

RESULTS

The chromatographic separation of analytes and other ingredients of drugs was optimized to provide an acceptable reso-

Table 1a. System suitability parameters of letrozole

Title	Retention Time	Area	Height	Concentration	k'	Tailing Factor	Theoretical Plate
0,05ltz_kal1_4.lcd	9.991	189065	11477	0.050	2.223	1.120	7830.783
0,1ltz_kal1_4.lcd	9.989	428129	25201	0.100	2.222	1.134	7700.118
0,3ltz_kal1_4.lcd	9.980	1085090	63898	0.298	2.219	1.134	7760.786
0,5ltz_kal1_4.lcd	9.977	1783137	104200	0.499	2.218	1.133	7660.238
0,7ltz_kal1_4.lcd	9.970	2527196	148730	0.704	2.216	1.140	7752.800
Average	9.981	1202524	70701	0.330	2.220	1.132	7740.945
%RSD	0.086	80.329	80.119	83.067	0.125	0.651	0.837
Maximum	9.991	2527196	148730	0.704	2.223	1.140	7830.783
Minimum	9.970	189065	11477	0.050	2.216	1.120	7660.238
Standart deviation	0.009	965977	56645	0.274	0.003	0.007	64.780
ltz: Letrozole							
kal: Calibration standards							
%RSD: Relative standard deviation							
k': Capacity factor							

lution, a good peak shape and an intensity of the response. The mobile phase combination was changed consistently to establish chromatographic conditions giving an agreeable resolution. The retention time of letrozole is 9,9 minutes.

System suitability test

System suitability parameters summarized in Table 1a. were within acceptable limits. In Table 1b. system suitability acceptance limits are given according to FDA guidelines.

Selectivity

The selectivity of method was investigated by analyzing inactive ingredients of pharmaceutical preparations. Chromato-

grams were compared to the matrix for any interference or any of the method reagents (Figure 2).

Sensitivity

The lowest standard of concentration 50 ng/mL for LTZ on the calibration curve was identified as the lower limit of quantification (LOQ) with a precision of less than or equal to 20 %. LOD (limit of detection) was found 14 ng/mL. The method developed is sensitive enough for the determination of LTZ from biological materials.

Linearity

The calibration curve contains five calibration samples within the range of 50-700 ng/mL including lower limit of quantification (LOQ) for LTZ. The acceptance criteria of back calculated concentration of standard batch was 15% deviation from the nominal value excluding the LOQ (for LOQ, 20 % deviation was applied). The standard calibration curves was linear over the concentration range from 700 ng/mL with a mean of $r^2=0.9998$ for LTZ. Accuracy and precision results of calibration standard can be shown in Table 2. The LOQ was 50 ng/mL. The calibration curve had a regression equation of $y=223827 X + 56.3597$.

Table 1b. System suitability parameters in HPLC (According to FDA guideline)

Parameter	Limit
Capacity factor	$k' > 2$
Injection precision	$RSD < \%1, n \geq 5$
Resolution	R_s
Tailing factor	$T \leq 2$
Theoretical plate number	$N > 2000$

Table 2. Accuracy and precision results of calibration standards

Sample	Concentration ng/mL	Mean	Recovered amount	Deviation(SD)	%CV	n
ST1	0,05	0,0496	99,2	0,002191	4,417117	6
ST2	0,1	0,099167	99,2	0,003251	3,277957	6
ST3	0,3	0,295167	98,4	0,006765	2,291962	6
ST4	0,5	0,497	99,4	0,011367	2,287046	6
ST5	0,7	0,689333	98,5	0,020363	2,954067	6

Accuracy is evaluated by mean and the recovered amount and precision is evaluated by standard deviation (SD) and coefficient of variation (%CV).
 %CV: Coefficient of variation
 SD: Standard deviation
 n: Number of samples

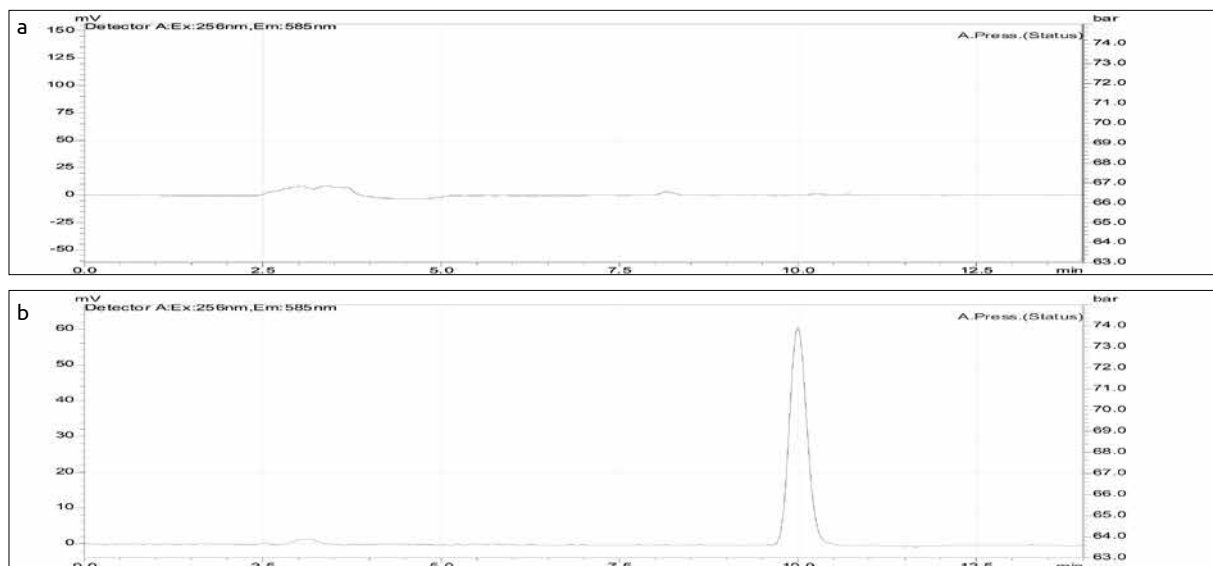


Figure 2. a, b. Placebo and letrozole (300 ng/mL) Chromatogram

Table 3. Results of accuracy and precision

Sample	Concentration ng/mL	Mean	Recovered amount	Deviation(SD)	%CV	n
QC1	50	0,048188	96.36	0,004665	9,680995	18
QC2	200	0,195722	97.85	0,006676	3,410818	18
QC3	400	0,402313	100.57	0,017843	4,435039	18
QC4	700	0,689056	98.43	0,013584	1,9714	18

Accuracy is evaluated by mean and the recovered amount and precision is evaluated by standard deviation (SD) and coefficient of variation (%CV).
 %CV: Coefficient of variation
 SD: Standart deviation
 n: Number of samples

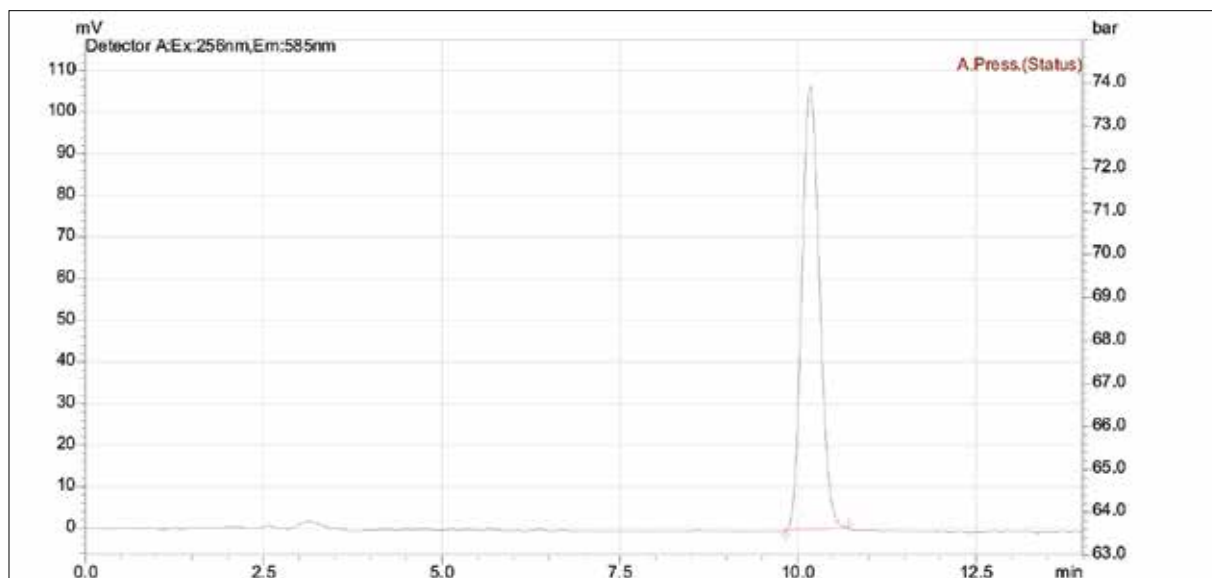


Figure 3. Chromatogram of 250 ng/mL tablet solution

Accuracy and precision

Precision and accuracy of intra-day were settled by analysis of six replicates of 5 concentrations including low, medium and high concentrations of quality control samples. Inter-day precision and accuracy were examined by the analysis of these quality control samples on three separate batches. The precision of the method was showed as the percentage of coefficient of variation and the accuracy of the method was showed in terms of relative errors. The precision was showed as the percentage of coefficient of variation. Table 3 presents a summary of the accuracy and precision.

Sample preparation

Tablet formulation containing 2.5 mg LTZ, ten tablets were powdered and one tablet weight was diluted to 100 mL flask to volume with methanol, 6 replicates were prepared in this way. Further dilution was carried out by transferring an appropriate amount for 250 ng/mL final concentration (Figure 3). Recovery from pharmaceutical preparation was found to be 98.8 %.

RESULTS AND DISCUSSION

Letrozole has natural fluorescence properties which allows it to be analyzed without derivatization. System suitability parameters were within acceptable limits. The method developed

has been validated. The method was found to be selective as seen in Figure 2. Chromatographic separation can differentiate the peak of analytes. The method was sensitive enough for the determination of letrozole from pharmaceutical preparations. Because of minority of papers on letrozole for pharmaceutical preparations, it is aimed to develop more sensitive and lower detection limits. Based on experiments and statistical analyses of letrozole, our data show that the proposed method demonstrates greater sensitivity to relevant studies including Mondal's (Mondal and Pal, 2009) and Narataj's (Nataraj et al. 2012). Considering the calibration curve and LOD and LOQ values, the proposed method is more sensitive than reported studies (Mondal and Pal, 2009; Pallavi et al., 2012). As a result, the correlation coefficient and the linear range of our method are more convenient and effective than the other studies (Prasad et al. 2012), (Acharjya et al., 2010). This method can be applied to pharmaceutical analysis of letrozole for quality control during the process as well as cleaning swap analysis due to lower detection limit of the method. For further studies this method can be used for biological fluids such as plasma.

The developed method for the determination of LTZ from pharmaceutical preparation has been found accurate, precise, selective, and suitable for the quality control analysis.

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Conflict of Interest: The authors have no conflict of interest to declare.

REFERENCES

- Acharjya S, Mallick P, Panda P, Kumar K, Annapurna M (2010). Spectrophotometric methods for the determination of letrozole in bulk and pharmaceutical dosage forms. *J Adv Pharm Technol Res* **1(3)**: 348–353. [CrossRef]
- Annapurna M, Mohapatra C, Narendrab A (2012). Stability-indicating liquid chromatographic method for the determination of letrozole in pharmaceutical formulations. *J Pharm Anal* **2**:298–305. [CrossRef]
- Gomes S, Bhosale S (2013). Application of sensitive rapid and validated liquid chromatography tandem mass spectrometry method for simultaneous determination of letrozole and metformin in human plasma. *Int J Adv Res Pharm Bio Sci* **3(2)**: 84-94.
- Lamb HM, Adkins JC (1998). Letrozole a review of its use in postmenopausal women with advanced breast cancer. *Drugs* **56**: 1125-1140. [CrossRef]
- Mareck U, Sigmund G, Opfermann G, Geyer H, Thevis M, Schänzer W. (2005). Identification of the aromatase inhibitor letrozole in urine by gas chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* **19(24)**: 3689-3693. [CrossRef]
- Marfil F, Pineau V, Sioufi A, Godbillon SJ (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 Biomed Sci Appl* **683**: 251-258. [CrossRef]
- Mondal N, Pal TK (2009). 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 Pol Pharm* **66(1)**: 11-17.
- Moussa B.A, El-Bagary R & Osman E (2013). Determination of letrozole in pharmaceutical preparation and human plasma based on fluorometric detection. *Anal Chem Lett* **3**: 139-146. [CrossRef]
- Nataraj K, Kumar D, Reddy S, Swamy B (2012). Stability indicating method development and validation for related substances for letrozole tablets by RP-HPLC. *Res J Pharm, Biol Chem Sci* **4**, 658-676
- Pallavi S, Vasanth PM, Ramesh T, Malothu R (2012). Development and validation of HPTLC method for the determination of letrozole in bulk and tablet dosage form. *Int Res J Pharm* **3**: 202-204.
- Prasad H, Govindrajulu G (2012). Forced degradation study of letrozole a validated stability indicating HPLC assay for bulk and tablet dosage form. *Int J Pharm Pharm Sci* **4(2)**: 582-586.
- Prasad H, Govindrajulu G, Palaniyappan V (2012). Forced degradation study of letrozole-A validated stability indicating HPLC assay for bulk and tablet dosage form. *Int J Pharm Pharm Sci* **4**, 582-586.
- Rodríguez J, Castañeda G, Muñoz L (2013). Rapid determination of letrozole, citalopram and their metabolites by high performance liquid chromatography-fluorescence detection in urine: Method validation and application to real samples. *J Chromatogr B Biomed Sci Appl* **913**-914: 12-18. [CrossRef]
- Scott LJ, Keam SJ (2006). Letrozole: in postmenopausal hormone-responsive early-stage breast cancer. *Drugs* **66**: 353-62. [CrossRef]
- Zarghi A, Foroutan S, Shafaati A, Khodam A (2007). HPLC Determination of letrozole in plasma using fluorescence detection: Application to pharmacokinetic studies. *Chromatographia* **66(9)**: 747–750. [CrossRef]