



New RP-HPLC Method for the Quantification of Letrozole (An Anti-Cancer Agent)

Mukthinuthalapati Mathrusri Annapurna*, Sistla Mounica Pratyusha and Raghu Raj Naik

Department of Pharmaceutical Analysis and Quality Assurance GITAM Institute of Pharmacy, GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh, India.

***Corresponding Author:** Mukthinuthalapati Mathrusri Annapurna, Department of Pharmaceutical Analysis and Quality Assurance GITAM Institute of Pharmacy, GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh, India.

Received: June 26, 2019; **Published:** July 22, 2019

DOI: 10.31080/ASPS.2019.03.0348

Abstract

Letrozole is an oral non-steroidal aromatase inhibitor used for the treatment of cancer. A simple and new liquid chromatographic method has been developed for the assay of Letrozole tablets and the method was validated as per ICH guidelines. Shimadzu Model CBM-20A/20 Alite HPLC system equipped with PDA detector with C8 column (250 mm × 4.60 mm i.d. 5µm particle size) was used with a flow rate of 1.0 mL/min (Detection wavelength 240 nm). Letrozole has shown linearity 1–100 µg/mL with linear regression equation $y = 93299x - 2999.3$ ($r^2 = 0.9999$). The LOD and LOQ were found to be 0.2799 µg/ml and 0.8691 µg/ml respectively.

Keywords: RP-HPLC; Letrozole; Assay; Validation; ICH Guidelines

Introduction

Letrozole is an anti-cancer agent especially used for the treatment of estrogen-dependent breast cancers [1]. Letrozole is chemically 4-[(4-cyanophenyl) - (1, 2, 4-triazol-1-yl) methyl] benzotrile (Figure 1). It is an oral non-steroidal aromatase inhibitor that has been introduced for the adjuvant treatment of hormonally-responsive breast cancer. It is readily and completely absorbed from the gastrointestinal tract. It is slowly metabolized in the liver to an inactive carbinol metabolite, which is then excreted as glucuronide in the urine [2]. Letrozole was determined by different analytical techniques such as Spectrophotometry [3,4], HPLC [5-12] in formulations and biological fluids and also with fluorescence detection [13], LC-MS [14] in human plasma and GC-MS [15] in urine and in the present study the authors have proposed a reverse phase isocratic liquid chromatographic method for the determination of Letrozole in tablets and the method was validated as per ICH guidelines [16].

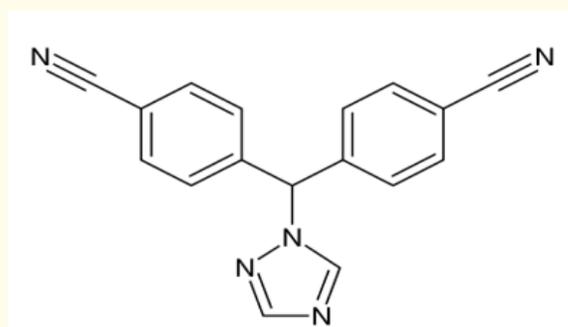


Figure 1: Chemical structure of Letrozole.

Materials and Methods

Instrumentation

Chromatographic separation was achieved by Shimadzu Model CBM-20A/20 Alite UFLC system (Shimadzu Co., Kyoto, Japan) equipped with SPD M20A prominence photodiode array detector

on C18 column (250 mm × 4.60 mm i.d. 5µm particle size) maintained at room temperature.

Preparation of Letrozole drug solution

25 mg of Letrozole was accurately weighed and dissolved in a 25 mL volumetric flask and volume was made up to the mark with HPLC grade acetonitrile (1000 µg/mL) and dilutions were made with mobile phase and filtered.

Method validation

Linearity, precision and accuracy

A series of solutions (1–100 µg/mL) of Letrozole were prepared from its stock solution, diluted with mobile phase and 20 µL of each of these solutions were injected in to the HPLC system. The peak area of Letrozole was noted (n=3) and the mean peak area was calculated from the chromatograms obtained and a calibration curve was drawn by taking the concentration of the Letrozole solutions on the x-axis and the corresponding mean peak area values on the y-axis.

Intraday and interday precision was studied on the same day and on three consecutive days respectively at three different concentration levels (10, 20 and 50 µg/mL) and the % RSD was calculated. The accuracy of the assay method was evaluated in triplicate at three concentration levels (50, 100 and 150 %), and the percentage recoveries were calculated.

Assay of letrozole tablets

Letrozole is available in India as tablets with brand names FEMARA (Novartis), FERTOLET (Cipla Ltd.) and HERHOPE (Torrent Pharmaceuticals) with labelled claim 2.5 mg. The API of Letrozole was obtained as gift sample from Cipla Limited (India). Twenty tablets were procured, crushed and powdered. 25 mg Letrozole tablet powder was extracted with acetonitrile and sonicated for half an hour and filtered through 0.45 mm membrane. Later suitable solutions were prepared on dilution with the mobile phase and 20 µL of these solutions were injected in to the HPLC system and the peak area was noted along with the retention time from the resultant chromatogram and the percentage purity was determined from the linear regression equation.

Results and Discussion

A new reverse phase liquid chromatographic method was developed for the quantification of Letrozole tablets. Mobile phase composition acetic acid: acetonitrile: water (0.1: 50: 50 v/v) with flow rate 1.0 mL/min (UV detection at 240 nm) for the determination of Letrozole. A sharp peak was observed at 3.403 min (Run time 10 min) with all the system suitability parameters acceptance.

The theoretical plates were more than 2000 and the tailing factor was less than 1.5. The LOD and LOQ were found to be 0.2799 µg/ml and 0.8691 µg/ml respectively. The chromatographic conditions (optimized) were shown in Table 1.

| Parameter | Optimized chromatographic conditions |
|----------------------|--|
| Mobile Phase | Glacial acetic acid: Acetonitrile: Water (0.1: 50: 50) |
| Flow Rate | 1.0 mL/min |
| Detection wavelength | 240 nm |
| Column temp. | (25°±2°C) |
| Injection Volume | 20 µL |
| Detector | SPD M20A prominence photodiode array detector |
| Elution | Isocratic mode |
| Total Run Time | 10 mins |
| Retention time | 3.403 mins |

Table 1: Optimized chromatographic conditions.

Method validation

Letrozole has shown linearity over the concentration range 1–100 µg/mL (Table 2) with linear regression equation $y = 93299x - 2999.3$ ($R^2 = 0.9999$) (Figure 2). The % RSD was found to be 0.158-0.862 (Intraday) and 0.108-0.753 (Inter day) (Table 3) which is less than 2.0 stating that the method is precise. The % recovery in accuracy study was found to be 99.52-99.67 % (Table 4) and the % RSD obtained was 0.253-0.491 which is less than 2.0 stating that the method is accurate. The chromatograms obtained for Letrozole (API) was shown in Figure 3.

| Conc. (µg/ml) | Mean peak area |
|---------------|----------------|
| 0 | 0 |
| 1 | 93469 |
| 5 | 467345 |
| 10 | 934690 |
| 20 | 1869382 |
| 30 | 2804172 |
| 40 | 3738530 |
| 50 | 4583490 |
| 60 | 5618444 |
| 80 | 7457063 |
| 100 | 9346903 |

Table 2: Linearity of Letrozole.

*Mean of three replicates

| Conc. µg/ml | Interday precision |
|--------------------|-------------------------------------|
| | * Mean ± standard deviation (% RSD) |
| 10 | 935746.3 ± 7046.1 (0.753) |
| 20 | 1871375.3 ± 7204.7 (0.385) |
| 50 | 4674604.6 ± 5048.5 (0.108) |
| Intraday precision | |
| 10 (Day 1) | 935746.3 ± 1993.14 (0.213) |
| 10 (Day 2) | 937862.3 ± 4858.12 (0.518) |
| 10 (Day3) | 926951.3 ± 7990.32 (0.862) |
| 20 (Day 1) | 1871375.3 ± 6007.11 (0.321) |
| 20 (Day 2) | 1864683.3 ± 9360.71 (0.502) |
| 20 (Day 3) | 1879823.3 ± 9192.33 (0.489) |
| 50 (Day 1) | 4674604.6 ± 7385.87 (0.158) |
| 50 (Day 2) | 4575802.6 ± 9975.24 (0.218) |
| 50 (Day 3) | 4551583.6 ± 9012.13 (0.198) |

Table 3: Intraday precision study of Letrozole.

*Mean of three replicates

| Spiked Conc. (µg/ml) | Formulation (µg/ml) | Total Conc. (µg/ml) | *Conc. Obtained (µg/ml) [%Recovery] (RSD) |
|----------------------|---------------------|---------------------|---|
| 10 (50%) | 20 | 30 | 29.857 [99.52] (0.326) |
| | 20 | 30 | |
| | 20 | 30 | |
| 20 (100%) | 20 | 40 | 39.868 [99.67] (0.491) |
| | 20 | 40 | |
| | 20 | 40 | |
| 30 (150%) | 20 | 50 | 49.829 [99.66] (0.253) |
| | 20 | 50 | |
| | 20 | 50 | |

Table 4: Accuracy study of Letrozole.

*Mean of three replicates

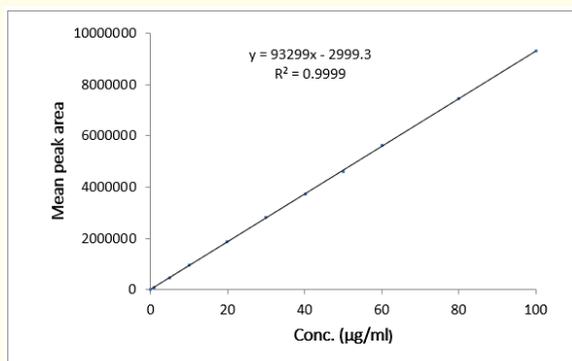


Figure 2: Calibration curve of Letrozole.

Assay of Letrozole tablets

Assay was performed by using two different brands of Letrozole tablets consisting of 2.5 mg API and found that the amount of Letrozole was 99.28-99.88 (Table 5) and there is no interference of excipients (Figure 3).

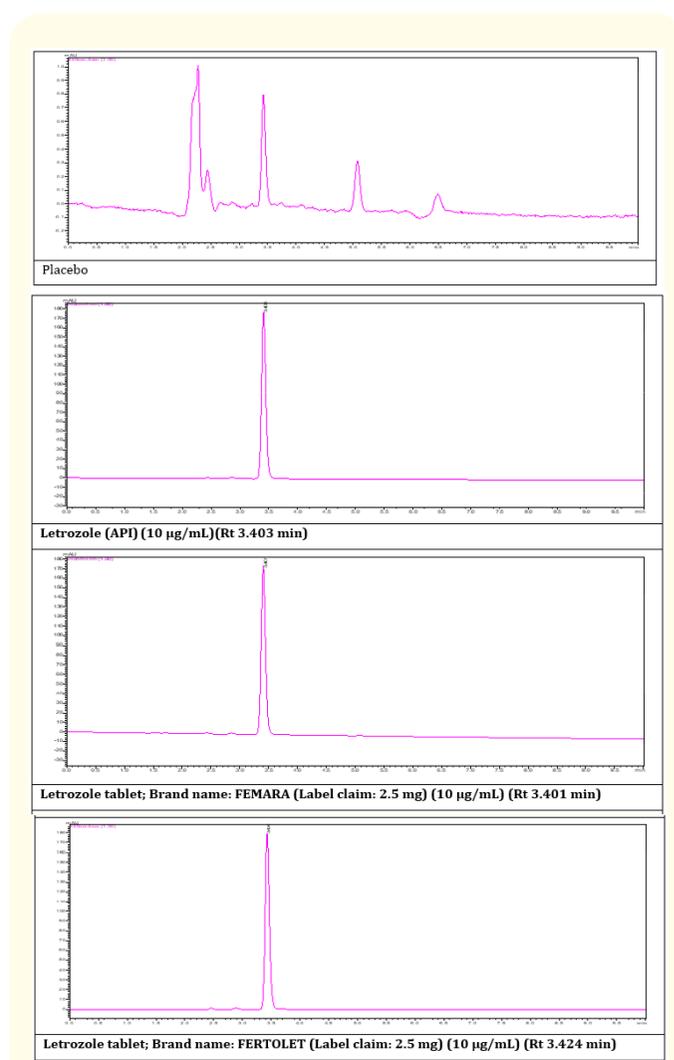


Figure 3: Representative chromatograms of Letrozole.

| Brand name | Label claim (mg) | Observed amount (mg) | *Recovery % |
|------------|------------------|----------------------|-------------|
| I | 2.5 | 2.493 | 99.72 |
| II | 2.5 | 2.482 | 99.28 |
| III | 2.5 | 2.497 | 99.88 |

Table 5: Assay of Letrozole tablets.

*Mean of three replicates

Conclusion

The proposed liquid chromatographic method for the assay of Letrozole tablets was validated and the method is precise and accurate. There is no interference of the excipients.

Acknowledgement

The authors are grateful to M/s GITAM (Deemed to be University), Visakhapatnam for providing the research facilities and Cipla Limited (India) for supplying gift samples of Letrozole. The authors declare no conflict of interest.

Bibliography

- Iveson TJ, *et al.* "Phase 1 study of the oral non-steroidal aromatase inhibitor CGS 20267 in Healthy post-menopausal women". *Journal of Clinical Endocrinology and Metabolism* 77.2 (1993): 324-331.
- Lamb HM, *et al.* "Letrozole a review of its use in post-menopausal women with advanced breast cancer". *Drugs* 56.6 (1998): 1125-1140.
- Acharjya SK, *et al.* "Spectrophotometric methods for the determination of Letrozole in bulk and pharmaceutical dosage forms". *Journal of Advanced Pharmacy and Technology and Research* 1.3 (2010) 348-353.
- Ganesh M, *et al.* "Validated UV spectrophotometric method for the determination of Letrozole in bulk and solid dosage form". *Rasāyan Journal of Chemistry* 1.1 (2008): 55-58.
- Mathrusri Annapurna M, *et al.* "Stability-indicating liquid chromatographic method for the determination of Letrozole in pharmaceutical formulations". *Journal of Pharmaceutical Analysis* 2.4 (2012): 298-305.
- Ganesh M, *et al.* "Determination of Letrozole in tablet formulations by reversed phase high performance liquid chromatography". *Tropical Journal of Pharmaceutical Research* 9.5 (2010): 505-510.
- Anand S, *et al.* "Development of validated RP-HPLC method for determination of Letrozole in bulk and its pharmaceutical dosage forms". *Der Pharmacia Sinica* 2.2 (2011): 263-269.
- Elkady EF, *et al.* "Preparation and characterization of two new forced degradation products of Letrozole and development of a stability-indicating RP-LC method for its determination". *Pakistan journal of pharmaceutical sciences* 28.6 (2015): 2041-2051.
- Mondal N, *et al.* "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 Poloniae Pharmaceutica ñ Drug Research* 66.1 (2009): 11-17.
- Acharjya SK, *et al.* "Development of high- performance liquid chromatography for determination of Letrozole in wistar rat serum and its applications in Pharmacokinetic studies". *Scientia Pharmaceutica* 80.4 (2012): 941-953.
- Sekar V, *et al.* "Bioanalytical method development and validation of Letrozole by RP-HPLC method". *International Journal of Pharmaceutical Research and Development* 1 (2009): 1-8.
- Shervin PH, *et al.* "Synthesis and characterization of PEGylated dendrimers based on magnetic nanoparticles for Letrozole extraction and determination in body fluids and pharmaceutical samples". *Microchemical Journal* 143 (2018): 190-197.
- Afshin Z, *et al.* "HPLC determination of Letrozole in plasma using fluorescence detection application to pharmacokinetic studies". *Chromatographia* 66.9-10 (2007): 747-750.
- Chidambara J, *et al.* "Validation and application of a high performance liquid chromatography- tandem mass spectrometry assay for Letrozole in human plasma". *Asian Journal of Pharmaceutical and Clinical Research* 4.2 (2011): 107-112.
- Mareck U, *et al.* "Identification of the aromatase inhibitor Letrozole in urine by gas chromatography / mass spectrometry". *Rapid Communications and Mass Spectrometry* 19.24 (2005): 3689-3693.
- ICH Validation of analytical procedures: Text and methodology Q2 (R1), International Conference on Harmonization (2005).

Volume 3 Issue 8 August 2019

© All rights are reserved by Mukthinuthalapati Mathrusri Annapurna, et al.