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Research Article

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

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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] benzonitrile (Figure 1). It is 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 glucoronide 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].

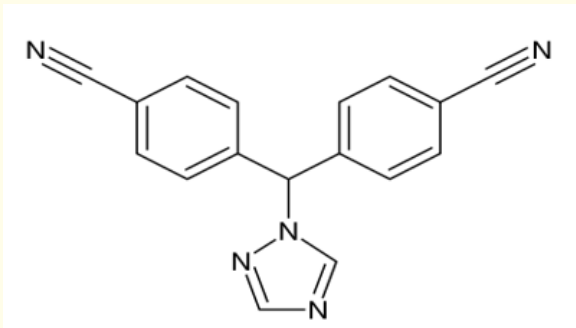

Figure 1: Chemical structure of Letrozole.

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 20 20	30 30 30	29.857 [99.52] (0.326)
20 (100%)	20 20 20	40 40 40	39.868 [99.67] (0.491)
30 (150%)	20 20 20	50 50 50	49.829 [99.66] (0.253)

Table 4: Accuracy study of Letrozole.

*Mean of three replicates

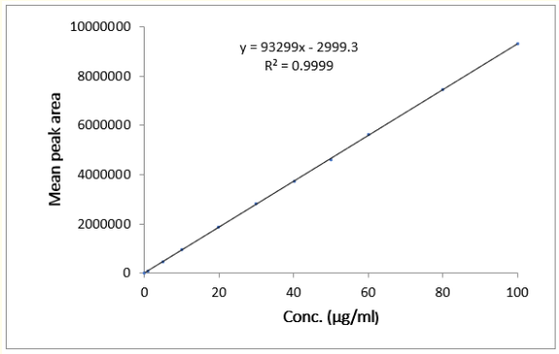

Figure 2: Calibration curve of Letrozole.

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.

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