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**Research Article**

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# Method Development and Validation for Estimation of Anastrozole in Pharmaceutical Dosage Form by RP-HPLC

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**Abstract** HPLC (LC-2010-HT, Shimadzu, Singapore) method for analysis of Anastrozole was carried out by the C-18 column (Inertsil, ODS 3V, 250mm X 4.6mm; 5µ) using a mobile phase consisting of buffer-acetonitrile (1:1) with the pH 2.5 adjusted by using buffer. 0.02 M $KH_2PO_4$. The mobile phase was pumped at the rate of 1.1 ml/ min and the detection was carried out at 233 nm. The linearity was found in the range of 10-30 µg/mL hence 20µg/mL was selected as optimum working concentration for the assay of Anastrozole with regression coefficient ($r^2$> 0.99). The peaks obtained were sharp having clear baseline separation with a retention time of 7.1 minute for Anastrozole. The optimized method is precise, accurate and robust and so it can be applied as stability indicating for the estimation of Anastrozole in tablet dosage form.

**Keywords** Anastrozole, method development, linearity, regression coefficient, HPLC, validation

## 1. Introduction

Analytical chemistry is a branch of chemistry that deals with the separation, identification and determination of components present in a sample. Conventionally, analytical chemistry has been split into two main types, qualitative and quantitative seeks to establish the presence and amount of a given element or compound in a sample respectively [1].

Anastrazole is chemically 2-[3-(2-cyanopropan-2-yl)-5-(1,2,4-triazol-1- ylmethyl) phenyl]-2-methylpropanenitrile, exerts its anti-estrogenic effects via selective and competitive inhibition of the aromatase enzyme found predominantly in the adrenal glands, liver, and fatty tissues [2]. As a fourth-generation aromatase inhibitor, anastrozole selectively binds to and reversibly inhibits aromatase, a cytochrome P450 enzyme complex found in many tissues including those of the premenopausal ovary, liver, and breast; aromatase catalyzes the aromatization of androstenedione and testosterone into estrone and estradiol, the final step in estrogen biosynthesis [3]. It is freely soluble in methanol, ethanol, acetone, tetrahydrofuran and very soluble in acetonitrile [4].

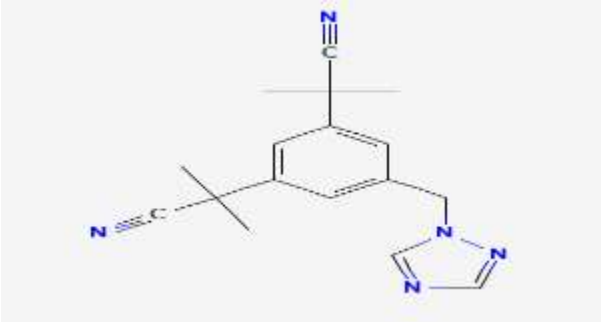


*Figure 1: Anastrazole*

## 2. Materials and Methods

A Shimadzu's HPLC (LC-2010-HT, Shimadzu, Singapore) equipped with UV-Visible detector & Diode array detector, with LC Solution software. Column used was Inertsil ODS C18, 250 mm x 4.6mm; 5µ, Column temperature was set 40 °C. The phosphate buffer is an universal buffer and trials were taken to adjust the molarity of the buffer. 0.02 M $KH_2PO_4$, at the pH adjust on 2.5-3.0. moboile phase were taken with account of forced degradation, buffer-acetonitrile (1:1) at c18 in different solvents like methanol and acetonitrile at $\lambda_{max}$ 233 nm.

### 2.1. Standard preparation:

Weigh and transfer accurately about 10 mg of Anastrozole reference / working standard into a 100 ml volumetric flask, add to it about 75 ml of diluent and sonicate to dissolve. Make up the volume with diluent and mix well. Further dilute 5 ml of solution to 50ml with diluent and mix.

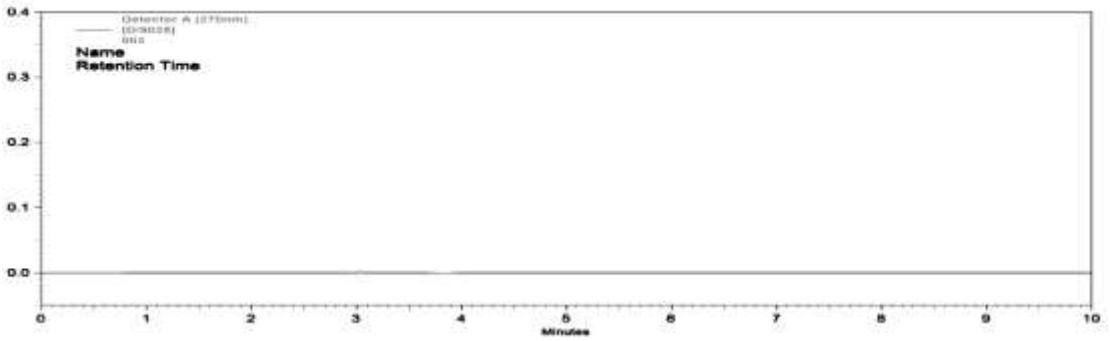
### 2.2. Sample preparation:

Weigh and transfer 10 tablets (each tablet contain 1mg of anastrozole) into 100 ml volumetric flask. Add about 70 ml of diluent and sonicate for 30 minutes with intermittent shaking, dilute up to the mark with diluent and mix. Filter through 0.45µ nylon filter. Further dilute 5 ml to 50ml with same diluent and mix.

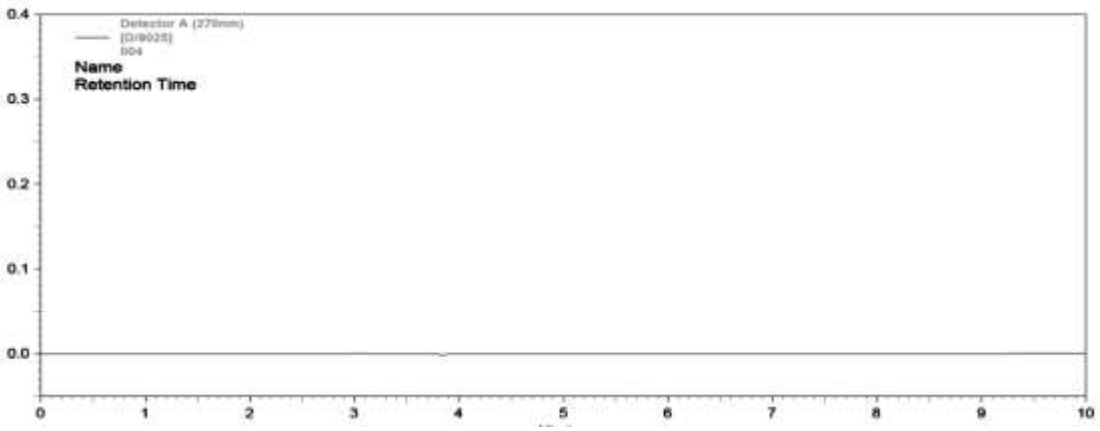
### 2.3. Analytical Method Validation

#### 2.3.1. Specificity

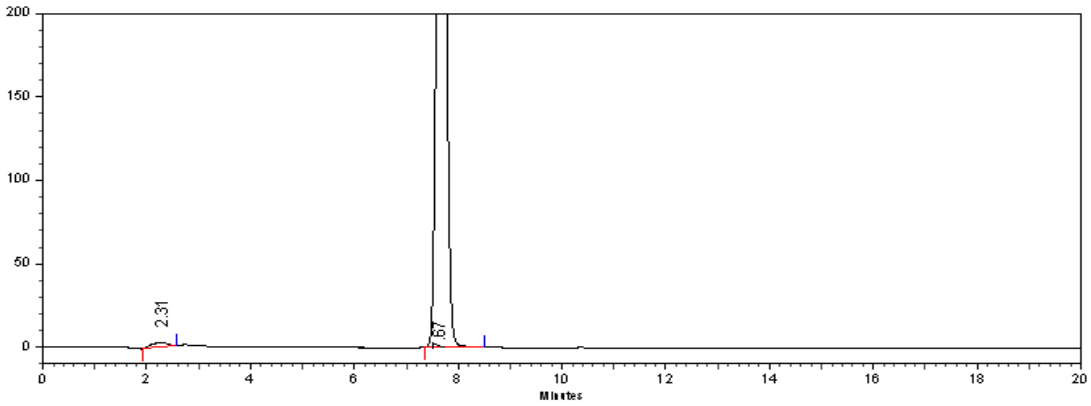
The peak purity index for the main peak in standard preparation and sample preparation was determined and recorded in Table.



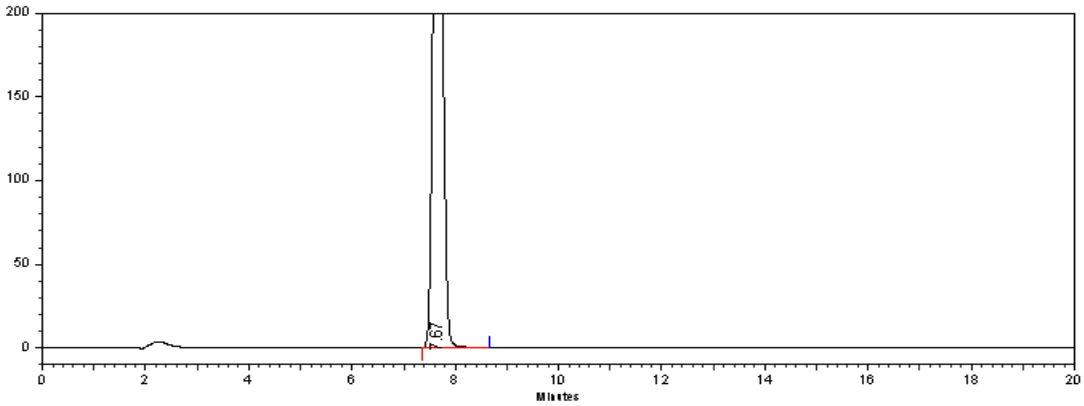

(A)




(B)




(C)




(D)

*Figure 2: Chromatograms of (a) diluent, (b) placebo, (c) standard and (d) Sample preparation*

### 2.3.2. Linearity and range

The mean area at each level was calculated and a graph of mean area versus concentration was plotted. The correlation co-efficient, Y intercept and slope of regression line were calculated and recorded.

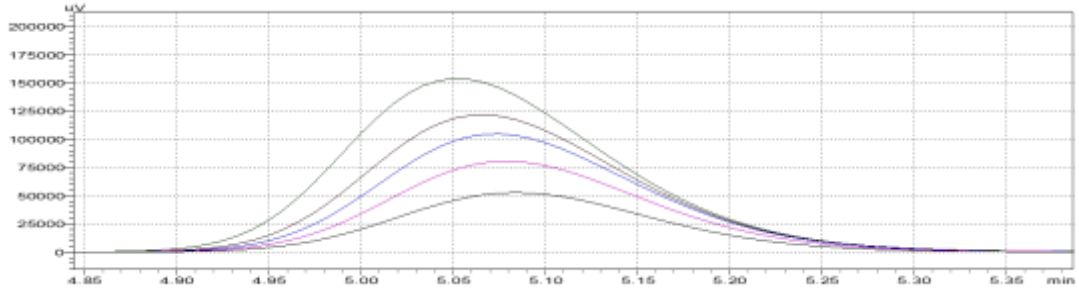



*Figure 3: Chromatograms of Linearity*

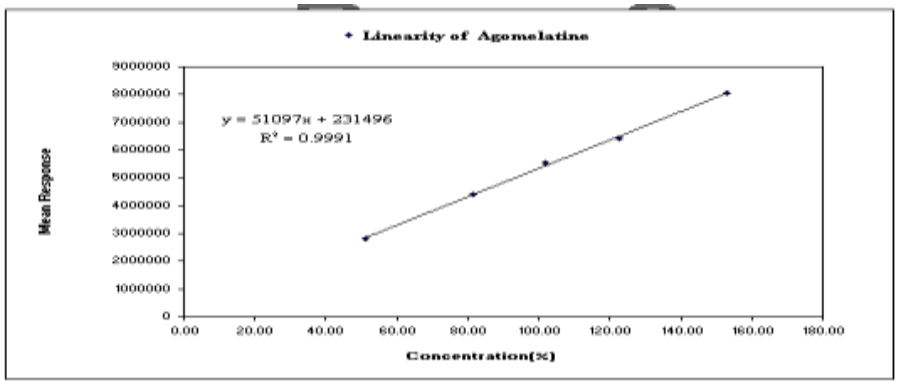



*Figure 4: Calibration curve of Anastrozole*

**Table 1:** Linearity of Anastrozole by RP-HPLC Method

| **Linearity level** | **Anastrozole** | |
|---|---|---|
| | **Conc. (µg/ml)** | **Mean area (n=2)** |
| 50 | 10 | 2812332 |
| 80 | 16 | 4417528 |
| 100 | 20 | 5529251 |
| 120 | 24 | 6406905 |
| 150 | 30 | 7982262.5 |
| Correlation coefficient | 0.999 | |

### 2.3.3. Accuracy (%recovery)

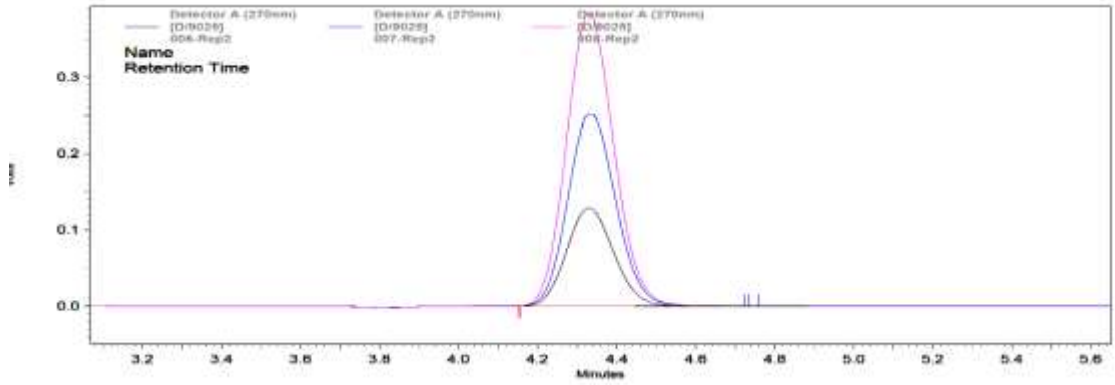



*Figure 5: Curve showing recovery studies*

% recovery, mean% recovery and %RSD were calculated at each level and recorded. The % recovery is within limit (98.0 – 102.0 %) so the method is accurate.

**Table 2:** Data indicating recovery study of Anastrozole

| S. No. | %Level | Amount Added (mg) | Area | | Mean Area | %Recovery | Mean | RSD |
|---|---|---|---|---|---|---|---|---|
| 1 | 50% | 12.9 | 2826936 | 2797728 | 2812332 | 100.6 | 100.7 | 0.6 |
| | | 13.1 | 2819226 | 2867699 | 2843463 | 101.2 | | |
| | | 12.7 | 2798313 | 2780644 | 2789479 | 101.3 | | |
| 2 | 100% | 25.36 | 5516743 | 5541759 | 5529251 | 100.6 | 101.0 | 0.4 |
| | | 24.12 | 5515466 | 5524365 | 5519916 | 101.4 | | |
| | | 25.20 | 5521073 | 5511036 | 5516055 | 101 | | |
| 3 | 150% | 37.5 | 7994414 | 7970111 | 7982263 | 98.2 | 99.8 | 1.8 |
| | | 37.45 | 8494293 | 8027361 | 8260827 | 101.8 | | |
| | | 38.5 | 8494293 | 8115462 | 8304878 | 99.5 | | |

### 2.3.4. Precision

Individual % assay, mean % assay and % RSD were calculated and recorded. The % RSD is 0.127 % for Anastrozole which indicate that the method is precise.

**Table 3:** Method precision data for analysis of Anastrozole in tablet dosage form

| Sample No. | Area | Mean Area | % Assay |
|---|---|---|---|
| 1 | 5456371 | 5476440 | 99.61% |
| | 5496510 | | |
| 2 | 5434761 | 5427440 | 98.73% |
| | 5420119 | | |
| 3 | 5452151 | 5460768 | 99.34% |
| | 5469386 | | |
| 4 | 5412788 | 5367850 | 98.46% |
| | 5322913 | | |
| 5 | 5521903 | 5496020 | 99.98% |
| | 5470138 | | |
| 6 | 5472039 | 5476505 | 99.08% |
| | 5420972 | | |
| Mean Assay | | | 99.2% |
| % RSD | | | 0.825% |

### 2.3.4.1 Intermediate precision (ruggedness)

The mean % assay value was calculated and compared with the mean % assay value obtained in method precision study. The difference of the mean assays obtained was calculated and recorded. The low % RSD indicates that method is precise.

**Table 4:** Intermediate precision data for analysis of Anastrozole in tablet dosage form

| Sample No. | Area | Mean Area | % Assay |
|---|---|---|---|
| 1 | 5425595 | 5430034 | 99.61% |
| | 5434474 | | |
| 2 | 5424565 | 5426840 | 98.7% |
| | 5429115 | | |
| 3 | 5523598 | 5477266 | 99.6% |
| | 5430934 | | |
| 4 | 5424563 | 5422371 | 98.68% |
| | 5420179 | | |
| 5 | 5433799 | 5429683 | 98.77% |
| | 5425567 | | |
| 6 | 5521965 | 5475774 | 99.6% |
| | 5429583 | | |
| Mean Assay | | | 99.17% |
| % RSD | | | 0.47% |
| Difference* | | | 0.355% |

*Difference in the mean % assay value obtained in intermediate precision study and method precision study.

**2.3.5. Robustness:**

The low % RSD value (< 2%) reveal that the proposed method is robust for this variation as shown in the Table 5.

**2.3.6. Limit of detection**

LOD is the lowest concentration of the drug which can be detected by instrument.

LOD= 0.262ppm

**2.3.7. Limit of quantification**

LOQ is the lowest concentration of the drug which can be quantified by instrument.

LOQ= 0.794ppm

**Table 5:** Summary of validation parameters for optimized HPLC method

| **Parameters** | | **Anastrozole** |
|---|---|---|
| Linearity Range (µg/ml) | | 10-30 |
| Regression equation y=mx+c | | 51097X+231496 |
| Slope | | 51097.3423 |
| Intercept | | 231469.483 |
| Correlation coefficient ($r^2$) | | 0.9995 |
| LOD(µg/ml) | | 0.262 ppm |
| LOQ(µg/ml) | | 0.794 ppm |
| %Recovery (accuracy %RSD, | Level 1 | 100.7% |
| n=3 for each level | Level 2 | 101% |
| | Level 3 | 99.8% |
| Repeatability (%RSD, n=6), | | 99.2% |
| Interday (%RSD) (n = 6) | | 99.17% |
| % Assay ± SD (n= 5) | | 99.05% |

## 3. Conclusion

Anastrozole is a nonsteroidal inhibitor of estrogen synthesis, used for the treatment of breast cancer. Till today, no official method has been reported for the estimation of Anastrozole in tablet dosage form and in bulk by RP- HPLC method. So, it was planned to develop HPLC method for the same.

The method was developed using several trials of different column and different mobile phases. It was found that Buffer: Acetonitrile (50:50) gave good resolution of peaks and satisfied retention time (7.1 minute) for estimation of Anastrozole.

After developing the HPLC method, it was validated for standard and marketed formulation of Anastrozole in terms of linearity, range, precision, accuracy, LOD, LOQ, specificity, and robustness as per ICH guidelines. The developed method was found to be accurate and precise. The % RSD values were within limits. So, this method can be used for the routine analysis of Anastrozole in bulk and tablet dosage form.

## References

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