ABSTRACT

A simple, rapid, precise and accurate RP-HPLC method was developed and validated for rapid assay of anastrozole in tablet dosage form. Chromatographic separation of Anastrozole was performed by using a C18 column as stationary phase with a mobile phase comprising of acetonitrile, 0.1% orthophosphoric acid and methyl alcohol (30:50:20) at flow rate of 1.0 ml/min and U.V detection at 256 nm. The linearity of anastrozole is in the range of 2.0-10 ppm/ml. The limits of detection (LOD) and quantification (LOQ) were found to be 0.05 and 0.165ppm respectively. The recovery was calculated by standard addition method. The proposed method was found to be accurate, precise and rapid for the analysis of anastrozole in formulation.

Keywords: Anastrozole, RP-HPLC, Linearity, Precision, Accuracy.
INTRODUCTION

Anastrozole is used to treat breast cancer after surgery and for metastases in both pre and post menopausal women. Anastrozole is an aromatase inhibitor which means that it interrupts a critical step in the body’s synthesis of estrogen. The molecular formula of anastrozole is C_{17}H_{19}N_{5} and molecular weight is 293.37. IUPAC name is 2-[3-(1-cyano-1-methyl-ethyl)- 5-(1H-1,2,4-triazol-1-ymethyl)phenyl]-2-methyl propanenitrile. The half life is 72 hrs. This drug is frequently used in the treatment of children with growth disorder to stop or slow the onset of puberty and in the treatment of moderate-to-severe pubertal gynecomastia. Anastrozole is marketed under the trade name Arimidex.

MATERIALS AND METHODS

Acetonitrile, orthophosphoric acid and methanol used were of analytical grade. Chromatographic separation was performed with PEAK High performance Liquid Chromatography having isocratic pump equipped with PEAK LC-UV7000 variable wavelength detector. Chromatograms and data were recorded by means of PEAK Chromatographic software version 1.06.

PREPARATION OF STANDARD SOLUTION

1mg of anastrozole was taken in a 10ml volumetric flask and 10ml of mobile phase was added to obtain 100ppm of anastrozole. Two ml of the stock solution was pipetted out into a 100ml volumetric flask and made up to the mark. The resulting solution was filtered through nylon filter paper. The calibration curve was plotted with the five concentrations of 2.0-10ppm of working standard solutions. Calibration solutions were prepared daily and analyzed immediately after preparation.

CHROMATOGRAPHIC CONDITIONS

Mobile phase : Acetonitrile, 0.1% Ortho phosphoricacid and methanol (30:50:20)
P_{H} : 3.0
Analytical Column : C18
U.V. detection : 225nm
Flow rate : 1.0ml/min
Injection volume : 20μl
Temperature : ambient
Runtime : 10min
Retention time :7.045min

METHOD VALIDATION PROCEDURE

The method is validated for linearity, precision and accuracy. Standard plots were constructed with concentrations 2.0-10ppm prepared in triplicate to test linearity. The peak area of anastrozole was plotted against the concentration and a linear graph was obtained. The precision of assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from 6 replicate injections of freshly prepared anastrozole test solution in the same equipment on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine the intermediate precision. Peak area of the anastrozole was determined and precision was reported as %RSD(Relative Standard Deviation)
LINEARITY

Linearity for the developed method was checked by preparing five solutions of different concentrations ranging from 2.0-10ppm. The chromatograms were developed and the peak areas are given in Table-1. A linear relationship between area vs. concentration was observed in the range of study.

PRECISION

The Intra-day and Inter-day precisions were determined by taking sample solution of 4.0ppm concentration. The RSD values for Intra-day and inter-day were found to be 0.588 and 0.918 respectively. The values were given in the Table-2.

ACCURACY AND RECOVERY

To study the accuracy of the proposed method recovery studies were carried out at different spiked levels. A fixed amount of pre analysed samples were taken and standard drug was added at 50%, 100% and 150%, levels. Each level is repeated times. The lower value of RSD of assay indicated that the method is accurate. The results are given in Table-3.

LOQ AND LOD

Limit of Quantification and Limit of Detection were calculated at 0.165ppm and 0.05ppm respectively as per ICH guidelines

ROBUSTNESS

Robustness was carried by varying two Parameters from the optimized chromatographic conditions. No significant change was observed.

SPECIFICITY

The specificity was determined by comparing test results obtained from analysis of sample solution containing exclusive ingredients with that of test results obtained from standard drugs.

FORMULATION

The commercially available Redest tablet was powdered and a solution of 10ppm was prepared. The solution was filtered and injected into the chromatographic system and chromatogram was recorded. The percentage of anastrozole in tablet was found to be about 0.99.

RESULTS AND DISCUSSIONS:

RP-HPLC method developed for determination of drugs has great importance in the quality control analysis. The chromatograms for pure drug were obtained by using different mobile phases like methanol, acetonitrile, THF and different buffers like ortho phosphoric acid in different volume ratios. Different columns like C8, C18, phenyl, cyano with different dimensions were used. The retention time and tailing factor were calculated. Finally acetonitrile, 1% ortho phosphoric acid and methanol (30:50:20) and C18 analyzed column were selected which gave a sharp and symmetrical peak with 1.16 tailing factor. Calibration graph was found to be linear in the range 2.0-10ppm. Five different concentrations of anastrozole in the given range were prepared and injected into HPLC. The slope(m) and intercept(c) obtained were found to be 39419.17 and 2358.38 respectively. A plot drawn between peak area and concentration of drug solution in the range studied was found to have excellent linear correlation with a correlation coefficient of 0.999.
The LOQ and LOD of anastrozole were found to be 0.165ppm and 0.05ppm respectively indicating the sensitivity of the method. The percentage assay or average amount of anastrozole in formulation was found to be 0.99. The low values of standard deviation and coefficient of variation at each level of the recovery experiment indicated high precision of the method.

CONCLUSION:

RP-HPLC method for the determination of anastrozole from their formulation was found to be accurate and precise. Thus the proposed HPLC method can be successfully applied for the routine quality control analysis of anastrozole formulations.

Figure.1 Structure of Anastrozole

Figure.2 A Typical RP-HPLC Chromatogram for Anastrozole
Figure 3 Linearity Graph

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<th>Linearity level</th>
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<tr>
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<tr>
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<td>10.0</td>
<td>397347.0</td>
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Table 1

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<td>157360.7</td>
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Table 2
REFERENCES

16. www.fda.gov/cder/guidance/cmc