Satyannarayana.P.V.V et al

I S S N 2249-1236

Research Article

VOL 1, ISSUE (2)

INTERNATIONAL JOURNAL OF RESEARCH AND REVIEWS IN PHARMACY AND APPLIED SCIENCES

DEVELOPMENT AND VALIDATION METHOD FOR QUANTIFICATION OF ANASTROZOLE IN FORMULATION ANALYSIS BY USING RP-HPLC

Krishnaveni.G[1],Satyannarayana.P.V.V.[2] , [1]Department of Chemistry,K.B.N.College,Vijayawada. [2]Professor of Chemistry(Rtd), Acharya Nagarjuna University, Nagarjunanagar.

Article Received on: 10.05.2011 *Article Accepted on: 26-06-2011*

Name: P.V.V. Satyannarayana

Address: Vijayawada

Email: chemperuri@yahoo.co.in

Phone: 919491130111

Corresponding Author

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 C_{18} 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 anastozole 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 anastozolel in formulation.

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

INTRODUCTION

Anasrozole 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 anastozole is $C_{17}H_{19}N_5$ and molecular weight is 293.37. IUPAC name is 2-[3-(1-cyano-1-methyl-ethyl)- 5-(1*H*-1,2,4-triazol-1-ylmethyl)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 at50%, 100% and150%, 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 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.

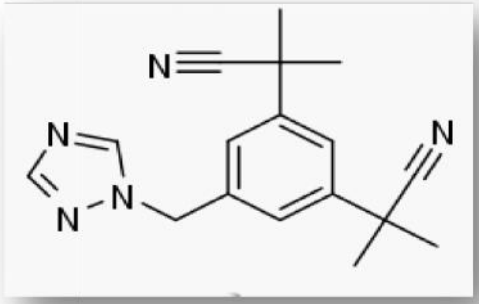

Figure.1 Structure of Anastrozole

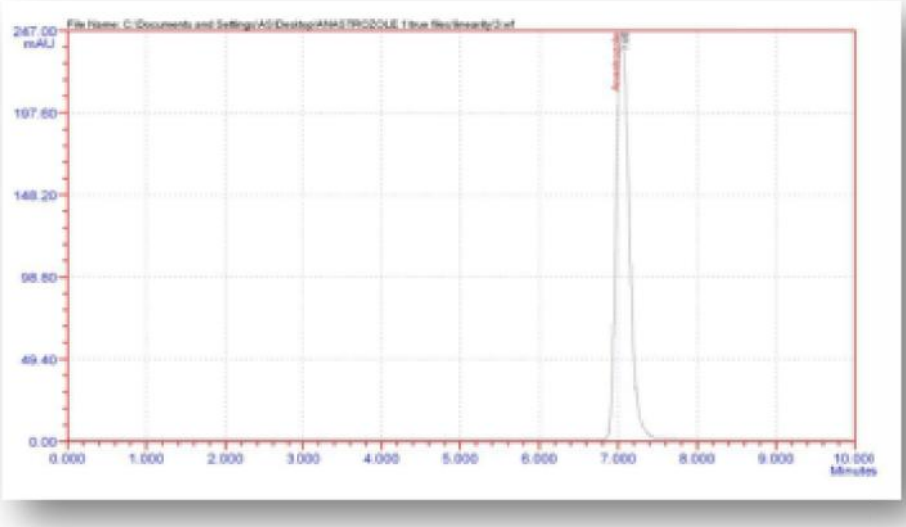

Figure.2 A Typical RP-HPLC Chromatogram for Anastrozole

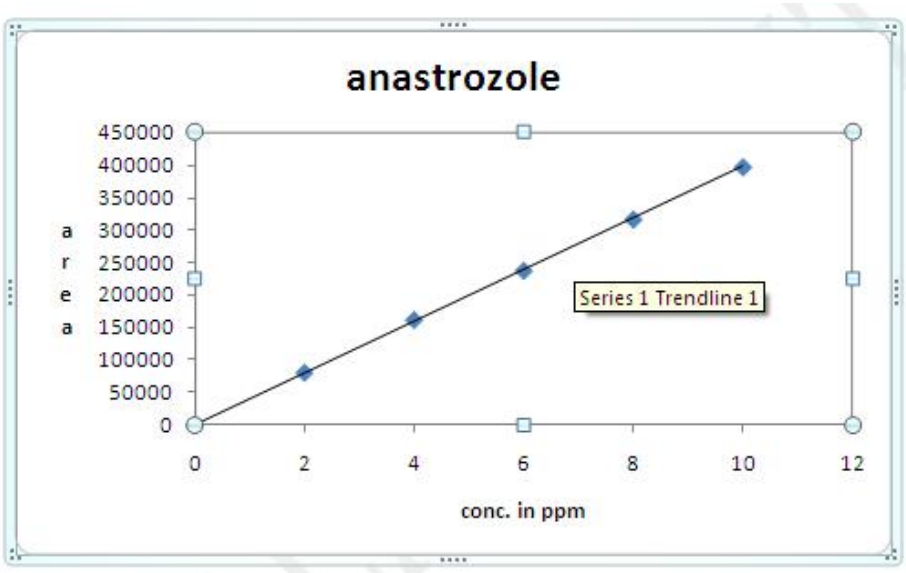

Figure 3 Linearity Graph

Linearity level	Concentration	Area
1	2.0	80548.6
2	4.0	161967.5
3	6.0	237749.8
4	8.0	316754.1
5	10.0	397347.0

Table.1

Linearity level	Intra day	Area
1	162742.2	159234.1
2	160814.7	155217.2
3	161458.2	158056.7
4	160535.0	158956.3
5	162189.0	157360.7
6	160395.3	157360.7

Table.2

REFERENCES

1. Saravanan G, Suryanarayana M V, Jadhav M J, Ravikumar M, Someswararao N and Acharyulu P V R, Chromatographia, 2007, 66, 435-438.
2. D.Sathis Kumar, A.Harani, D.Sridhar, David Banji, KNV Rao, Guruviah and Yogeswaran, E-Journal of chemistry, 2011, 8(2), 794-797.
3. Sethi P D, HPLC quantitative analysis of pharmaceutical formulation, CBS publication, New Delhi, 1996, 11-160.
4. Gustova D Mendes, Hamamoto D, Ilha J, Pereira A D S and Nucci G D, *J* Chromatogr *B*, 2007, 850, 553-559.Plourde P V, Breast Cancer Res Treat., 1994, 30,103-111
5. Matheson AJ and CM Perry, Glucosamine: a review of its use in the management of osteoarthritis, Drugs Aging, 2003, 20, 1041–1060.
6. Persiani S, Rotini R, Trisolino G, Rovati LC, Locatelli M, Paganini D, Aantonioli D and A Roda, Synovial and plasma glucosamine concentrations in osteoarthritis patients following oral crystalline glucosamine sulphate at therapeutic dose, Osteoarthritis Cartilage, 2007, 15, 764–772.
7. Largo R *et al.,* Glucosamine inhibits IL-1beta-induced NF kappa B activation in human osteoarthritis chondrocytes, Osteoarthritis Cartilage, 2003, 11, 290–298.
8. Joseph ZZ, Ted W and M Felicia, Determination of Glucosamine in raw materials and dietary supplements containing Glucosamine sulfate and/or Glucosamine hydrochloride by High-Performance Liquid Chromatography with FMOC-Su Derivatization: collaborative Study, Journal of Association of Analyt Chem Int, 2005, 88, 1048-1058.
9. Pashkova E, Pirogov A, Bendryshev A, Ivanaynen E and O Shpigun, Determination of underivatized Glucosamine in human plasma by high-performance liquid chromatography with electrochemical detection: Application to pharmacokinetic study, Journal of Pharmaceutical and Biomedical Analysis, 2009, 50, 671-4.
10. Wayne KW, Kathleen G and GB Andrew, Determination of Glucosamine in nutritional supplements by Reversed-Phase ion-pairing HPLC, Journal of Liquid Chromatography and Related Technologies, 2000, 23, 2861-2871.
11. PastoriniE *et al.,* Development and validation of a HPLC-ES-MS/MS method for the determination of Glucosamine in human synovial fluid, Journal of Pharmaceutical and Biomedical Analysis, 2009, 50, 1009-14.
12. Useni Reddy Mallu, K Hussain Reddy, Varaprasad Bobbarala and Somasekhar Penumajji, HPLC Method Development for Glucosamine Sulphate and Diacerein Formulation, Journal of Pharmacy Research, 2010, 3(2), 361-363.
13. International Conference on Harmonization, Q2A: Text on Validation of Analytical Procedures, Federal Register, 1995, 60 (40), 11260–11262.
14. International Conference on Harmonization, Q2B: Validation of Analytical Procedures: Methodology and Availability, Federal Register, 1997, 62 (96), 27463–27467.
15. FDA, Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls Documentation, Availability, Federal Register (Notices), 2000, 65(169), 52776–52777.
16. www.fda.gov/cder/guidance/cmc