



REVERSE PHASE HPLC METHOD FOR THE ANALYSIS OF ANASTRAZOLE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple and precise RP-HPLC method was developed and validated for the determination of anastrozole in pharmaceutical dosage forms. Chromatography was carried out on an Inertsil ODS (250x4.6mm) C18 column using a mixture of Buffer:Acetonitrile (60:40) as the mobile phase at a flow rate 1.0 ml/min. The analyte was monitored using UV detector at 215 nm. The Retention time of the drug is 6.431 min for anastrozole. The proposed method is found to be having linearity in the concentration range of 0.1-0.6 µg/ml with correlation coefficient of $r=0.9999$. The developed method has been statistically validated and found simple and accurate. The mean recoveries obtained for anastrozole are in the range 99.8-100.2%. Due to its simplicity, rapidness, high precision and accuracy of the proposed method it may be used for determining anastrozole in bulk and dosage forms.

Keywords: Anastrozole, RP-HPLC.

INTRODUCTION

Anastrozole 2-[3(1-cyano-1-methyl-ethyl)-5-(1H-1, 2, 4-triazol-1-yl methyl) phenyl]-2-methyl-propinenitrile is a potent and selective non-steroidal aromatase inhibitor used to treat breast cancer in post-menopausal women [1]. Anastrozole decreases the amount of estrogen in the body and can also stop the growth of many types of breast cancer cells. Anastrozole is available as 1 mg tablet and it is usually taken once a day with or without food [2-6]. As per the literature survey it is revealed that very few analytical methods for the separation and estimation anastrozole have been reported such as UV-Spectrophotometer method [7]. An analytical method for quantification of drug in human plasma by HPLC, stress stability behavior, LC assay method and LC-MS/MS for low dose anastrozole tablets were developed and reported [8,9,10]. A simple reliable and reproducible RP-HPLC method was developed, validated and recovery studies were conducted and studied by using various statistical parameters according to ICH guidelines [11].

EXPERIMENTAL

Instrumentation

Analysis was performed using isocratic high performance liquid chromatography system (HPLC) Waters 2695 model equipped with a UV-Visible detector. The output signal was monitored and processed using Empower software.

Chemicals and reagents

Anastrozole was obtained as a gift sample from Cipla limited, Mumbai. Ammonium acetate (AR grade) used for preparing buffer and acetonitrile HPLC grade was purchased from Merck.

Chromatographic conditions

Mobile phase consists of buffer and acetonitrile in the ratio 60:40. Buffer was prepared by dissolving 0.077g of ammonium acetate in 1000 ml water and filtered through 0.45µ membrane filter. The mobile phase was pumped from the solvent reservoir in the ratio 60: 40 to the column at a flow rate 1.0 ml/min where as run time set was 10min. The column was maintained at 25°C and the volume of each injection was 20µL. Prior to injection of the solutions, column was equilibrated for at least 30min with mobile phase flowing through the system. The eluents were monitored at 215 nm.

Procedure

50 mg of anastrozole was weighed, transferred to 50 ml volumetric flask containing 20ml of mobile phase. The solution was sonicated for 15 min to dissolve the drug completely and the volume made up with mobile phase to get the concentration of 1 mg/ml solution.

Subsequent dilutions of this solution ranging from 0.1-0.6 µg/ml were made with the mobile phase in 10 ml volumetric flasks. The solutions prepared were filtered through 0.45 µ membrane filter. 20 µL of the filtrate was injected 6 times into the column and the corresponding chromatograms were obtained. Drug was analyzed at 215 nm. Retention time and mean peak area ratios were recorded for all the concentrations obtained from the chromatograms. A calibration curve of peak area ratio to respective concentration was plotted. The regression of the drug concentrations over the peak area ratio was computed using least squares method of analysis. Regression equation was used to estimate the amount of anastrozole in tablet formulations.

Estimation of anastrozole in tablet dosage forms

20 tablets were taken and crushed to a fine powder. Dose equivalent to 10mg of anastrozole was transferred to a 50 ml volumetric flask containing 30 ml of mobile phase. This powder was dissolved with intermittent sonication for 15 min to ensure complete solubility. The solution was made up to the mark with mobile phase and filtered through 0.45 µ membrane filter. 5 ml of this solution was pipetted into 10ml volumetric flask and diluted with the mobile phase to get concentration of 100 µg/ml. Each of these solutions was injected twice into the system and the chromatograms were recorded. The mean peak area of the drug of five such determinations was calculated and the drug content in the tablets was quantified using the regression equation obtained for the pure sample.

RESULTS AND DISCUSSION

After several systematic trials the present study was aimed at developing a sensitive, precise and accurate RP-HPLC method for the analysis of anastrozole in pharmaceutical dosage forms. The present method contains mobile phase 10 Mm ammonium acetate buffer and acetonitrile in the ratio 60:40 v/v which was found to be the most suitable as the chromatographic peaks obtained with this system were better defined and resolved and all almost free from tailing. Under the above conditions the retention time obtained for anastrozole was 6.431 min. A model Chromatogram is shown in Fig. 1.

The calibration curve obtained showed linearity over a concentration range of 0.1-0.6 µg/ml, with a correlation coefficient $r=0.9999$ the representative linear regression equation being $Y=2062978X+68554$ as shown in Table.1. and Fig. 2.

The accuracy of the proposed method was determined by adding known quantities of the drug to the previously analyzed formulations and they were reanalyzed by the proposed method. The accuracy of the method was supported by high recovery values

obtained from the developed method. The % recovery results of the method are given in Table 2. The developed HPLC method in the present study has also been used to quantify anastrozole in the tablet dosage forms. Anastrozole tablets were quantified using the proposed analytical method and the results are given in Table 3.

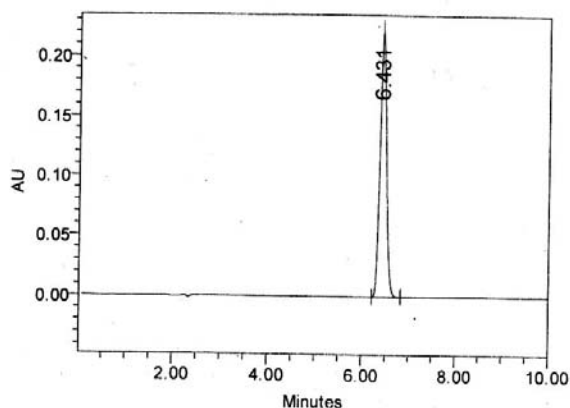


Fig. 1: Sample chromatogram of Anastrozole

Table 1: Calibration of the proposed HPLC method

Concentration ($\mu\text{g/ml}$)	Mean Peak Area(N=3)
0.1	2169504
0.2	4249127
0.3	6304521
0.4	8275026
0.5	10336215
0.6	12468021

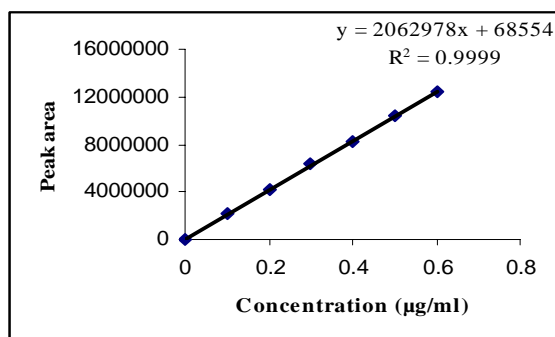


Fig.3: Linearity graph of Anastrozole

Table 2: Recovery data of standard solutions added to the samples analyzed by using the proposed HPLC method

Amount of drug added ($\mu\text{g/ml}$)	Amount found ($\mu\text{g/ml}$) (N=3)	%Recovery(N=3)
0.05	0.049	99.8
0.1	0.099	99.7
0.15	0.151	100.2

Table 3: Assay of anastrozole in tablet dosage forms by proposed HPLC method

Labeled amount (mg/tablet)	Observed amount (mg/tablet)	% Purity
Brand-1 1.0	0.998	99.8
Brand -II 1.0	1.013	101.2

From the obtained results it can be concluded that this method is quite precise and accurate. The absence of additional peaks in the Chromatogram indicated that there is no interference of the common excipients used in the tablets. The proposed HPLC method is sensitive and reproducible for the analysis of anastrozole in pharmaceutical dosage forms. The method was duly validated by using required statistical parameters.

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