WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 2, 308-318. Review Article ISSN 2277– 7105

DEVELOPMENT OF NEW VALIDATED RP-HPLC METHOD FOR ESTIMATION OF ANASTRAOZOLE IN BULK AND TABLET DOSAGE FORMS

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Article Received on
26 Nov. 2019,

Revised on 16 Dec. 2019,
Accepted on 06 Jan. 2020,

DOI: 10.20959/wjpr20202-16590

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ABSTRACT

A simple, Précised, Accurate method was developed for the estimation of Anastrozole by RP-HPLC technique. Chromatographic conditions used are stationary phase Azilent C18 150mm x 4.6 mm, 5.µm, Mobile phase 0.01N Kh2po4: Acetonitrile in the ratio of 60:40 and flow rate was maintained at 1.0ml/min, detection wave length was 215nm, column temperature was set to 30^{o}C and diluent was mobile phase Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to 150% levels, R^2 value was found to be as 0.999. Precision was found to be 0.5 for repeatability and 0.6 for intermediate precision. LOD and LOQ are 0.086µg/ml and 0.261µg/ml respectively. By using above method assay of marketed formulation was carried out 99.57% was present. Degradation studies of Anastrozole were done, in all conditions purity threshold was more than purity angle and within the acceptable range. Full length method was not performed; if it is done this method can be used for routine analysis of Anastrozole.

KEYWORDS: HPLC Anastrozole, Method development. ICH Guidelines.

INTRODUCTION

Pharmaceutical Analysis is that core branch of pharmacy education and research, which is advancing very fast. It can be categorized as synthesis of new drugs molecules and pharmaceutical analysis. Analytical chemistry is the science of making quantitative and

qualitative evaluation. In practice, quantifying an analyte in a complex sample becomes an exercise in problem resolving. To be efficient and effective, analytical chemist must know the tools that are available to tackle a wide variety of problems. Analytical chemistry is divided into two branches qualitative and quantitative. In this qualitative method provides information about the identity of atomic or molecular species or functional groups in the sample. A quantitative method provides numerical information as to the relative amount of one or more of the components. Varieties of analytical methods are used for the analysis of drugs in bulk, formulations and bioanalytical samples. In pharma industry, spectrophotometric and chromatographic methods have gained the significance in recent studies. Spectrophotometric method is defined as a method of analysis that embraces the measurement of absorption by chemical species of radiant energy at definite and narrow wavelength approximating monochromatic radiation. There electromagnetic spectrum extends from 100-780 nm. Traditionally, analytical chemistry has been split into two main types.

Qualitative and Quantitative: Qualitative Inorganic Analysis seeks to establish the presence of an inorganic compound in a sample or given element. Quantitative analysis seeks to establish the amount of a compound in a sample or given element. Qualitative Organic Analysis seeks to establish the presence of a given functional group or organic compound in a sample. There are various techniques used for analysis of mixture of compounds. Spectroscopy used to measure the interaction of the molecules with electromagnetic radiation. Then chromatography is the collective term for a family of laboratory techniques for the separation of mixtures and comprise passing a mixture of samples dissolved in a "mobile phase" along a stationary phase. The analyte which is separated, to be measured from other molecules in the mixture and allows it is to be isolated. Analytical Chromatography is used to determine the existence and possibly also the concentration of analyte(s) in a compound. Analytical chemistry has played critical roles in the understanding of basic science to a variety of practical applications in industrial productions, biomedical engineering, environmental monitoring, forensic sciences and so on.

HPLC

The phenomenal growth in chromatography is largely due to the introduction of the versatile technique called high-pressure liquid chromatography, which is frequently called high-performance liquid chromatography. Both terms can be abbreviated as HPLC.

High-pressure liquid-solid chromatography (HPLC) is rapidly becoming the method of choice for separations and analysis in many areas. Most of the samples that are dissolved can be separated on some type of HPLC column.

Characteristics of HPLC method

- Efficient, highly selective, widely applicable
- Only small sample required.
- May be non-destructive of sample
- Easily flexible to quantitative analysis.
- High resolving power.

Modes of HPLC

1) Normal phase chromatography: The nature of stationary phase is polar and the mobile phase is non-polar in this mode. In this technique, non-polar compounds travel faster and are eluted first because of the lower affinity between the non-polar compounds and stationary phase. The time for polar compounds to elute takes longer time because of their higher affinity to the stationary phase, therefore generally this method is not used in the pharmaceutical applications because most of the drug molecules are polar in nature and hence take longer time to elute.

2) Reversed phase chromatography: Reversed phase mode is the most popular mode for analytical and preparative separations of compounds of concern in biological products, pharmaceutical formulations & API's, chemical substances, food and biomedical engineering. The stationary phase is non-polar hydrophobic packing with octal and octadecyl functional group bonded to silica gel and the mobile phase is a polar solvent, often a partially or fully aqueous mobile phase. Polar substances prefer the mobile phase and elute first. As the hydrophobic character of the solutes increases, retention increases. Generally, the lower the polarity of the mobile phase, higher is the eluent strength.

Method Development on HPLC

Method development and optimization in liquid chromatography is still an attractive field of research for theoreticians (researchers) and attracts also a lot of interest from practical analysts. Among all, the liquid chromatographic methods, the reversed phase systems based on modified silica offers the highest probability of successful results. However, a large number of (system) variables (parameters) affect the selectivity and the resolution. Alternate

analytical methods are developed for the drug product to reduce the cost and time. When alternative analytical methods are intended to replace the existing procedure, analyst should collect the literature for all types of information related to analyte and define the separation goal. Then estimate the best separation condition from trial runs. After optimizing the separation condition, validate the method for release to routine laboratory.

Getting Started on Method Development

"Best column, best mobile phase, best detection wavelength, efforts in separation can make a world of difference while developing HPLC method for regular analysis. Determining the ideal combination of these factors assures faster delivery of desired results – a validated method of separation."

a) The Mobile Phase: In reverse-phase chromatography mobile phase is more polar than the stationary phase. Mobile phase in these systems is usually mixtures of two or more individual solvents with or without additives or organic solvent modifiers. The usual approach is to choose what appears to be the most appropriate column, and then to design a mobile phase that will optimize the retention and selectivity of the system. Separations in these systems are considered to be due to different degrees of hydrophobicity of the solutes. The simple alteration of composition of the mobile phase or of the flow rate allows the rate of the elution of the solutes to be adjusted to an optimum value and permits the separation of a wide range of the chemical types. First isocratic run followed by gradient run is preferred.

b) The Detector: The next consideration should be the choice of detector. UV-visible detectors are the most popular as they can detect a broad range of compounds and have a fair degree of selectivity for some analytics.

c) The Column Length: Many chromatographers make the mistake of simply using what is available. Often this is a 150 mm×4.6 mm. These columns are able to resolve a wide variety of compounds. While many reverse phase separations can be carried out on such column. Method development can be streamlined by starting with shorter columns; 150, 100 or even 50 cm long.

d) The Stationary Phase: Selecting an appropriate stationary phase can also help to improve the efficiency of method development. For example, a reverse phased C_{18} column can provide a further time saving over a C_{18}, as it does not retain analytes as strongly as the C_{18}

phase. For normal phase applications, cyano (nitrile) phases are most versatile.

e) The Internal Diameter: By selecting a shorter column with an appropriate phase, run times can be minimized so that an elution order and an optimum mobile phase can be quickly determined.

f) Gradient Programming: The fastest and easiest way to develop a method is to use a mobile phase gradient. Always start with a weak solvent strength and move to a higher solvent strength. To begin, use a very fast gradient (e.g.10 minutes) and then modify the starting and finishing mobile phases to achieve a suitable separation.

g) Retention: Analytes may be too strongly retained (producing long run times). If this occurs, the solvent strength should be raised and in reverse phase mode analysis means a higher % of organic solvent in the mobile phase.

h) Poor Separation: Analytes often co-elute with each other or impurities. To overcome this, the analysis should be run at both higher and lower solvent strengths so the best separation conditions may be determined.

i) Peak Shape: This is a problem which occurs, especially for basic compounds analyzed by reversed phase HPLC. Use Wakosil II type of high purity silica phase to minimize any potential problems. These modern phases are very highly deactivated so secondary interactions with the support are minimal. To maximize the reproducibility of a method, it is best to use a column heater to control the temperature of the separation. A temperature of 35 – 40°C is recommended.

j) Buffer selection: In RP-HPLC method the retention time of analytes is related to their hydrophobic nature. More the hydrophobic nature of analyte, the longer it is retained. The analyte becomes less hydrophobic when it is ionized and, therefore its retention decreases. When separating mixtures containing acid and/or bases by reversed phase HPLC, it is necessary to control the pH of mobile phase using appropriate buffer in order to achieve reproducible results.

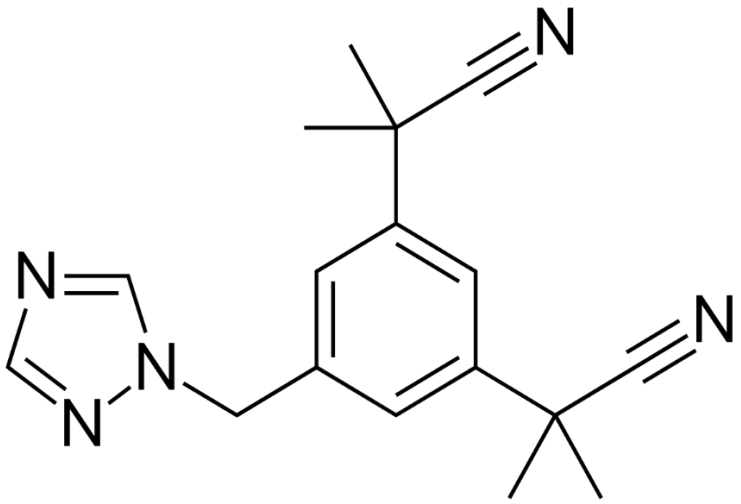
2. Buffers play an additional role in the reproducibility of a separation. The buffer salts reduce peak tailing for basic compounds by effectively masking the sialons and they also reduce the potential ion-exchange interactions with un protonated silanols.

DRUG PROFILE

NAME: Anastrozole

CHEMICAL NAME: 2,2-{5-(1H-1,2,4-triazol-1-ylmethyl)-1,3-Phenylene} Bis(2-methylpropanenitrile)

MOLECULAR STRUCTURE



MOLECULAR FORMULA: $C_{17}H_{19}N_5$

MOLECULAR WEIGHT: 293.4 g/mole

APPEARANCE: Crystalline solid.

SOLUBILITY: Freely soluble in methanol, acetone, ethanol, tetrahydrofuran; very soluble in acetonitrile.

CATEGORY: Anti-fungal.

MODE OF ACTION: Anastrozole reversibly binds to the aromatase enzyme. Through competitive inhibition blocks the conversation of androgens to estrogens in peripheral (extra-gonadal) tissues.

ROUTE: By mouth (tablets)

MELTING POINT: 81-82°

BOILING POINT: 469.7°C at 760mmHg

REFRACTIVE INDEX: 1.791P^{ka}:11.38

STORAGE TEMPERATURE: Store in original container in a cool dark place

ABSORPTION MAXINA: UV max (HCL buffer of PH-1.7: Acetonitrile) (1:1):264nm.

BRAND NAME: ARIMIDEX (Tablet), ALTROL (Tablet) REDSET (1mg-filmcoated tablets).

Pharmacokinetics

- Studies with Radiolabelled drug have demonstrated orally administrated Anastrozole is well absorbed into systemic circulation with 83% to 85% of Radiolabel recovered urine and feces.
- Anastrozole are linear over the dose range of 1 to 20mg and do not change with repeated dosing.

Pharmacodynamics

- It is a non-steroidal aromatase inhibitor that was reported to maximally suppress plasma Estradiol concentration at doses of 1 to 10 mg/day with both doses supressing Estradiol to the limits of detection used.
- The drug had a plasma B-phase elimination half-life of 38 to 68 hrs.

MATERIALS AND METHODS

Experimental

Materials and Methods

Pharmaceutical grade working standard Anastrozole was obtained from Syncorp Clincare Technologies Pvt.Ltd. Laboratories, Hyderabad, India. All chemicals and reagents were HPLC grade and were purchased from S D Fine-Chem Limited & Loba Chemie Pvt. Ltd, Mumbai, India.

Instrumentation

The analysis was performed using HPLC (Waters-717 series) with UV detector and data handling system Empower2 software, UV-Visible double beam spectrophotometer (Labindia), analytical balance 0.1mg Sensitivity (LabIndia), pH meter (LabIndia), Vaccum filtration, Ultra sonicator. The column used is Azilent C18 Column, 150 mm x 4.6 mm i.d. and 5µm particle sizewith the flow rate 1.0ml/min (isocratic).

Preparation of Potassium Dihydrogen Phosphate buffer

Accurately weigh 1.36084g of potassium dihydrogen phosphate (KH2PO4) and transferred into 1000 ml clean volumetric flask. Add 500ml of Hplc grade water and stir to dissolve the

buffer salt, and then complete the volume upto the mark with the HPLC water. Adjust the pH of the solution to 3.0 with orthophosphoric acid using pH meter. Then the solution is filtered and degassed for 30 minutes in order to eliminate air bubbles.

Preparation of mobile phase

Mix potassium dihydrogen Phosphate buffer 600 ml (60%) and 400 ml of Acetonitrile (40%) and degassed in the ultrasonicator water bath for 10minutes. Then filter through the 0.45µ filter below the vacuum filtration process.

Standard Preparation for the Analysis

25 mg of Anastrozole working standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase.

Further dilution was done by transferring 0.5ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

Sample Preparation for the Analysis

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Finally, the weighed tablets are powdered and triturated well by using mortar and pestle. A quantity of powder which is equivalent to the 100mg of drug was transferred to a clean and dry 100ml of volumetric flask and add 70 ml of diluent and the resulted solution was sonicated for 15 minutes by using ultra Sonicator. Then the final volume was make up to the mark with the same diluent. The final solution was filtered through a selected membrane filter (0.45 µm). From this above stock solution (1 ml) was transferred to five different 10 ml volumetric flask and volume was made up to 10ml with same solvent system. The prepared solution was injected in six replicates into the HPLC system and the observations were recorded.

Diluent

Mobile phase can be used as diluent.

Study of Spectra and selection of wavelength

Anastrozole working standard solution was scanned between the range 200-400 nm in 1cm cell against blank. Maximum absorbing wavelength of Anastrozole was selected from spectral data and wavelength selected from spectra of UV spectrophotometer. The λmax for

Anastrozole was found to be 241nm. UV spectrum and typical standard chromatogram of Anastrozole.

Optimization of HPLC Chromatographic Method

The chromatographic conditions were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength and different diluents for sample preparation etc. The selected and optimized mobile phase was Acetonitrile: Potassium dihydrogen orthophosphate buffer (pH-3.0) (60:40v/v) and conditions optimized were: flow rate (1.0 ml/minute), wavelength (215 nm, UV-detector), run time was 6.0 mins and injection volume was 20 µl.

Method Validation

Accuracy

Recovery study

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of ANASTROZOLE were taken and added to the pre-analysed formulation of concentration 10µg/ml. From that percentage recovery values were calculated.

Precision

Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Anastrozole (API). The percent relative standard deviation was calculated.

Linearity & Range

The calibration standard solution of Anastrozole was injected into the HPLC system and the chromatograms were recorded at 215nm and a calibration graph was obtained by plotting peak area versus concentration of Anastrozole.

Method Ruggedness

The Ruggedness of the proposed method was implemented for the 5 injections of Anastrozole drug on dissimilar days and by altered analysts.

Method Robustness

Influence of small changes in chromatographic conditions such as change in flow rate (□ 0.1ml/min), Temperature (□2^0C), Wavelength of detection (□2nm) & Acetonitrile content in mobile phase (□2%) studied to determine the robustness of the method are also in favour of (Table-5, % RSD < 2%) the developed RP-HPLC method for the analysis of Anastrozole (API).

LOD & LOQ

The minimum concentration of the analyte can be used to detect the sample by using all experimental conditions and the minimum concentration of the analyte can be used to quantify the sample by using all experimental conditions.

The LOD and LOQ were calculated by the use of the equations $LOD = 3.3 \times \sigma / S$

And $LOQ = 10 \times \sigma / S$

Where,

σ is the standard deviation of intercept of Calibration plot and S is the average of the slope of the corresponding Calibration plots.

System Suitability Parameter

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analysed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table-6**.**

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