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A novel validated RP-HPLC method for the determination of anastrozole in bulk and pharmaceutical tablet dosage forms

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ABSTRACT

An accurate, highly sensitive, precise, simple, efficient and reproducible, isocratic Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the quantitative determination of anastrozole in pharmaceutical tablet dosage forms. RP-HPLC method was developed by using Welchrom C_{18} Column (4.6 X 250mm, 5µm), Shimadzu LC-20AT Prominence Liquid Chromatograph. The mobile phase composed of 10mM Phosphate buffer (pH-3.0, adjusted with triethylamine): acetonitrile (50:50 v/v). The flow rate was set to 1.0 mL/min with the responses measured at 215 nm using Shimadzu SPD-20A Prominence UV-Vis detector. The retention time of anastrozole was found to be 6.143 min. Linearity was established for anastrozole HCl in the range of 2-10 µg/mL with correlation coefficient 0.999. The percentage recovery was found to be 99.84 % to 100.2%. Validation parameters such as specificity, linearity, precision, accuracy, robustness, limit of detection (LOD) and limit of quantitation (LOQ) were evaluated for the method according to the International Conference on Harmonization (ICH) Q_2 R_1 guidelines. The developed method was successfully applied for the quantification of bulk and active pharmaceutical ingredient present in tablet dosage form.

Key words: Anastrozole, Isocratic RP-HPLC, UV-Vis detector, Method Validation.

INTRODUCTION

Anastrozole, 2-[3-(1-cyano-1-methyl-ethyl)-5-(1H-1,2,4-triazol-1-ylmethyl)phenyl]-2-methyl-propanenitrile is a new generation non-steroidal aromatase - inhibitor [1-3]. Anastrozole binds reversibly to the aromatase enzyme through competitive inhibition, inhibits the conversion of androgens to estrogens in peripheral tissues (outside the CNS), and a few CNS sites in various regions within the brain [4]. Anastrozole is used to treat advanced breast cancer [5-12] in women who have gone through "the change of life" (menopause) [13]. Anastrozole works by lowering estrogen hormone levels to help shrink tumors and slow their growth. Anastrozole has been tested for reducing estrogens, including estradiol, in men [14]. It can also contribute to decrease the risk of stroke, heart attack, chronic inflammation, prostate enlargement and prostate cancer [15]. As per the literature survey revealed that very few analytical methods for the separation and estimation anastrozole have been reported such as

UV-Spectrophotometric method [116]. HPLC [17,18], Electrospray ionization tandem mass spectrometric analysis [19], LC-MS/MS [20-26], Capillary gas chromatography [27], UPLC-tandem mass spectrometry [28], and Isolation and characterization of process related impurities in anastrozole active pharmaceutical ingredients have been determined [29]. Very few analytical HPLC methods were reported in literature for the determination of anastrozole in bulk and pharmaceutical dosage forms. The reported HPLC methods so far in the literature are considered to be uneconomical, time consuming and have poor symmetry. In fact there is a need for the development of a novel, simple, rapid, efficient RP-HPLC analytical method with reproducibility for determination of anastrozole in bulk and pharmaceutical dosage forms. The objective of this work was from the economic point of view and for the purpose of routine analysis it was decided to develop a novel, simple, rapid, economic, precise, efficient RP-HPLC method for quantitative analysis of anastrozole, and to validate the method according with ICH guidelines [30]. This method showed advantage of shorter retention time, runtime, simple mobile phase preparation.

MATERIALS AND METHODS

Chemicals and Reagents

The reference sample of anastrozole standard was kindly supplied as gift sample by Hetero Drugs Ltd., Hyderabad, Andhra Pradesh, India. All the chemicals were analytical grade. Potassium dihydrogen orthophosphate and phosphoric acid from Rankem Ltd., Mumbai, India, while acetonitrile (HPLC grade) and triethylamine (HPLC grade) from Merck Pharmaceuticals Private Ltd., Mumbai, India. Ortho phosphoric acid used was of HPLC grade and purchased from Merck Specialties Private Ltd., Mumbai, India. Commercial tablets of Anastrozole formulation was procured from local market. Armotraz 1mg tablets are manufactured by Cipla Ltd., Mumbai India and Anabrez are manufactured by Sun Pharma Pvt. Ltd., Bangalore, Karnataka.

Instruments

Quantitative HPLC was performed on a isocratic high performance liquid chromatograph (Shimadzu LC-20AT Prominence Liquid Chromatograph) with a LC-20AT VP pump, manual injector with loop volume of 20 µL (Rheodyne), programmable variable wavelength Shimadzu SPD-20A Prominence UV-Vis detector and Welchrom C_{18} Column (4.6 X 250mm, 5µm particle size). The HPLC system was equipped with "Spinchrome" software. In addition an electronic balance (Shimadzu TX223L), digital pH meter (Systronics model 802), a sonicator (spectra lab, model UCB 40), UV-Visible Spectrophotometer (Systronics model-2203) were used in this study.

Chromatographic conditions

Anastrozole was analyzed by various reversed phase columns like C_8 and C_{18} columns. Among C_8 and C_{18} columns, C_{18} (250mmX4.6mm, 5µm) column was selected. Various combinations of acetonitrile, phosphate buffer and methanol with triethylamine as column modifier were tested. The mixture of 10mM Phosphate buffer (pH adjusted to 3.0 using triethylamine) and Acetonitrile in ratio of 50:50 v/v was selected as mobile phase and UV detection wavelength was 215 nm with a flow rate of 1mL/min. Injection volume was 20µL, with ambient temperature, run time was 12 min. and retention time was 6.143 min. The resulting HPLC chromatogram was shown in Fig. 3.

Preparation of mobile phase

A 10mM Phosphate buffer was prepared by dissolving 6.056 g of potassium dihydrogen orthophosphate in 445 mL of HPLC grade water. To this 55mL of 0.1M phosphoric acid was added and pH was adjusted to 3.0 with triethylamine. The above prepared buffer and acetonitrile were mixed in the proportion of 50:50 v/v and was filtered through 0.45 µm nylon membrane filter and degassed by sonication.

Preparation of Standard solution

About 10 mg of pure anastrozole was accurately weighed and dissolved in 10 mL of mobile phase at to get 1 mg/mL stock solution. Working standard solution of anastrozole was prepared with mobile phase. The final volume was made with the mobile phase. The standard solution was filtered through 0.45 µm nylon membrane filter and degassed by sonicator.

Selection of detection wavelength

The UV spectra of various diluted solutions of anastrozole in mobile phase were recorded using UV spectrophotometer. The peak of maximum absorbance was observed at 215 nm. This wavelength was used for detection of anastrozole.

Calibration curve for Anastrozole Hydrochloride:
Aliquots of standard anastrozole stock solution were taken in a different 10 mL volumetric flasks and diluted up to the mark with the mobile phase such that the final concentration of Anastrozole are in the range of 2µg/mL, 4µg/mL, 6µg/mL, 8µg/mL,10µg/mL. Replicates of eachcalibration standard solutions of 2, 4, 6, 8, 10µg/mL were injected using a 20µL fixed loop system and the chromatograms were recorded at 215nm and a Calibration graph was obtained by plotting peak area versus concentration of Anastrozole. The calibration data is presented in Table 3.

Analysis of Marketed Formulations
The content of twenty tablets were accurately weighed and transferred into a mortar and ground to a fine powder. From this, tablet powder which is equivalent to 10 mg of anastrozole was taken and the drug was extracted in 10 mL of mobile phase. The resulting solution was filtered using Whatman Grade No. 1 filter paper and degassed by sonication. This solution was further suitably diluted for chromatography. The test solutions were injected into the system by filling a 20 µL fixed volume loop manual injector. The chromatographic run time of 12 min. was maintained for the elution of the drug from the column. The elutes were monitored with UV detector at 215 nm. A 20µL volume of standard and sample solutions were separately injected on HPLC system. From the peak area of anastrozole the amount of drug in the sample were computed. The content was calculated as an average of six determinations and experimental results were presented in Table 4. The representative standard and sample chromatograms of anastrozole were shown in Fig. 3 and 4.

VALIDATION OF THE PROPOSED METHOD
The developed method of analysis was validated as per the ICH for the parameters like system suitability, specificity, linearity, precision, accuracy, robustness and system suitability, limit of detection (LOD) and limit of quantitation (LOQ).

System suitability
System suitability tests are an integral part of chromatographic method which was used to verify reproducibility of the chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solution at the concentration level 10 µg/mL for anastrozole to check the reproducibility of the system.

At first the HPLC system was stabilized for 40 min. One blank followed by six replicates of a single calibration standard solution of anastrozole was injected to check the system suitability. To ascertain the systems suitability for the proposed method, the parameters such as theoretical plates, peak asymmetry, retention time and parameters were taken and results were presented in Table 1.

Specificity
The effect of wide range of excipients and other additives usually present in the formulations of anastrozole in the determinations under optimum conditions was investigated. The specificity of the RP-HPLC method was established by injecting the mo excipients such as lactose anhydrous, microcrystalline cellulose and magnesium stearate have been added to the placebo solution and injected and tested. The representative chromatogram of placebo was shown in Fig. 2. The specificity results were presented in Table 5.

Linearity
The linearity graphs for the proposed assay methods were obtained over the concentration range of 2-10 µg/mLof anastrozole. Method of least square analysis was carried out for getting the slope, intercept and correlation coefficient, regression data values and the results were presented in Table 2 and Table 3. The representative chromatograms indicating the Anastrozole were shown in Fig. 5 to 9. A calibration curve was plotted between concentration and area response and statistical analysis of the calibration curve is shown in Fig. 10.

Precision
Intraday and interday precision study of anastrozole was carried out by estimating corresponding responses 3 times on the same day and on 3 different days for the concentration of 10µg/mL. The percent relative standard deviation (% RSD) was calculated which is within the acceptable criteria of not more than 2.0. The results for intraday and interday precision were presented in Table 6 and Table 7 respectively.

Accuracy (Recovery studies)

The accuracy of the method was determined by calculating recovery of anastrozole by the method of addition. Known amount of anastrozole at 50%, 100% and 150% was added to a pre quantified sample solution. The recovery studies were carried out in the tablet in triplicate each in the presence of placebo. The mean percentage recovery of anastrozole at each level was not less than 99.84% and not more than 100.2%. The results were presented in Table 8.

Robustness

The Robustness was evaluated by the analysis of Itopride HCl under different experimental conditions such as making small changes in flow rate (± 0.2 mL/min), detection wavelength (±5nm) and Mobile phase composition (±5%). The results were presented in Table 9.

LOD and LOQ

Limit of Detection is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. The limit of quantitation is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. Limit of Detection and Limit of Quantitation were calculated using following formula LOD = 3.3(SD)/S and LOQ= 10 (SD)/S, where SD = standard deviation of response (peak area) and S = slope of the calibration curve. The LOD and LOQ values are presented in Table 10.

RESULTS AND DISCUSSION

The mobile phase consisting of 10mM phosphate buffer (pH-3.0): acetonitrile (50:50 v/v) at 1 ml/min flow rate was optimized which gave sharp peak, minimum tailing factor with short run time for anastrozole. The retention time for anastrozole was 6.143 min. UV spectra of anastrozole showed that the drug absorbed maximum at 215 nm, so this wavelength was selected as the detection wavelength. System suitability parameters and optimized chromatographic conditions are shown in Table 1. All the system suitability parameters were evaluated with the back ground of regulatory requirements which also suggests good chromatographic condition. The calibration curve for anastrozole was found to be linear over the range of 2-10 µg/mL. The data of regression analysis of the calibration curve is shown in Table 2 and Table 3. The developed method was applied to the assay of anastrozole tablets. The experimental results are given in Table 4. The results were very close to labeled value of commercial tablets. The representative standard and sample chromatograms of anastrozole are shown in Fig. 3 and 4 respectively. The regression equation was found to be Y=190.2x + 0.357 with correlation coefficient is r^2=0.999 which indicates this method has good linearity. The representative chromatograms indicating the anastrozole are shown in Fig. 5 to 9. The linearity of the graph is shown in Fig. 10. The specificity was studied for the examination of the presence of interfering components, while the comparison of chromatograms there was no interference from placebo (Fig 2) with sample peak. They do not disturb the elution or quantification of anastrozole; furthermore the well-shaped peaks also indicate the specificity of the method. Therefore, it was concluded that the method is specific. The specificity results are summarized in Table 5. Precision was studied to find out intra and inter day variations in the test methods of anastrozole for the three times on the same day and different day. The %RSD for intra-day and inter-day precision obtained was 0.2221 and 0.3583 respectively. The values of % RSD (< 2.0) indicate that the proposed method is quite precise and reproducible and results are shown in Tables 6 and 7. Recovery studies of the drug were carried out for the accuracy parameter at three different concentrations levels i.e., multiple level recovery studies. A known amount of anastrozole standard was added into pre-analyzed sample and subjected them to the proposed HPLC method. The % recovery was found to be within the limits as listed in Table 8. Generally the mean percentage recovery of Itopride HCl at each level was not less than 99% and not more than 101%. In this case percentage recovery of anastrozole was found to be in the range of 99.84 % to 100.2%. The method precision was done and the low % RSD (0.3583) values indicates that the proposed method which was in good agreement with precision. Robustness was done by small changes in the chromatographic conditions like mobile phase flow rate, temperature, mobile phase composition etc., It was observed that there were no marked changes in the chromatograms. Infact the parameters are within the limit which indicates that the method has robustness and suitable for routine use. The Robustness results are presented in Table 9. The limit of detection (LOD) and limit of quantitation (LOQ) was calculated based on the standard deviation (SD) of the response and the slope (S) of the calibration curve at levels approximating the LOD and LOQ. The limit of detection (LOD) was 0.157 µg/mL and the limit of quantitation (LOQ) was 0.476 µg/mL which shows that this method is very sensitive. The results are presented in Table 10.

Table 1: Optimized chromatographic conditions and system suitability parameters for proposed HPLC method for Anastrozole

Parameter	Chromatographic conditions
Instrument	SHIMADZU LC-20AT prominence liquid chromatograph
Column	WELCHROM C_{18} Column (4.6 X 250mm, 5µm)
Detector	SHIMADZU SPD-20A prominence UV-Vis detector
Diluents	10mM Phosphate Buffer(pH-3.0): Acetonitrile (50:50 v/v)
Mobile phase	10mM Phosphate Buffer(pH-3.0): Acetonitrile (50:50 v/v)
Flow rate	1mL/min.
Detection wave length	By UV at 215nm.
Run time	12 minutes
Column back pressure	121-130 kgf.
Temperature	Ambient temperature (25°C)
Volume of injection loop	20µL
Retention time (R_t)	6.143 min
Theoretical plates[th.pl] (Efficiency)	17783
Theoretical plates per meter[t.p/m]	355660
Tailing factor (asymmetry factor)	1.071

Table 2: Linear regression data of the proposed HPLC method of Anastrozole

Parameter	Method
Detection wavelength(λ max)	By UV at 215nm
Linearity range (µg/mL)	2-10µg/mL
Regression equation (Y=a+bx)	Y=0.357+190.2X
Slope(b)	190.2
Intercept(a)	0.357
Standard deviation of slope (S_b)	1.084263545
Standard deviation of intercept (S_a)	6.565541813
Standard error of estimation (Se)	0.146152858
Correlation coefficient (r^2)	0.999
% Relative standard deviation* i.e., Coefficient of variation(CV)	0.358302123
Percentage range of errors* (Confidence limits) 0.005significance level 0.001 significance level	 0.751395764 1.178618443

**Average of six determinations*

Table 3: Calibration data of the proposed HPLC method of Anastrozole

S. No	Concentration, µg/mL.	Retention time, (R_t) min.	Peak area, mV.s.
1.	0	-	0
2.	2	6.097	380.86
3.	4	6.070	763.09
4.	6	6.103	1146.94
5.	8	6.100	1506.78
6.	10	6.143	1910.48

Table 4: Assay results of Anastrozole formulation

S. No	Formulations	Labeled amount	Amount found	% Assay ±RSD*
1	Anabrez (Sun Pharmaceutical Industries Pvt. Ltd.,)	1 mg	1.016 mg	101.6±0.131

***** *Average of 6 determinations.*

Table 5: Specificity study

Name of the solution	Retention time (R_t) min.
Mobile phase	No peaks
Placebo	No peaks
Anastrozole 10 µg/mL	6.143 min.

Table 6: Results of Precision study (Intraday)

Sample	Concentration (µg/mL)	Injection no.	Peak area (mV.s)	%RSD (acceptance criteria < 2.0)
Anastrozole	10	1	1901.866	0.2221
		2	1904.458	
		3	1899.398	
		4	1903.544	
		5	1911.684	
		6	1906.264	

Table 7: Results of Precision study (Interday)

Sample	Concentration (µg/mL)	Injection no.	Peak area (mV.s)	%RSD(acceptance criteria < 2.0)
Anastrozole	10	1	1911.465	0.3583
		2	1908.862	
		3	1916.946	
		4	1904.788	
		5	1918.928	
		6	1901.386	

Table 8: Recovery data of the proposed Anastrozole by RP-HPLC method

S. No	Concentration level	Amount added (µg/mL)	Amount found (µg/mL)	Mean % Recovery ± SD*	% RSD #
	50%	5	4.96	99.87±0.0416	0.8337
1		5	5.04		
		5	4.98		
	100%	10	9.98	100.2±0.0871	0.8700
2		10	9.96		
		10	10.12		
	150%	15	15.06	99.84±0.0850	0.5678
3		15	14.89		
		15	14.98		

**SD is standard deviation*

 # % RSD is percentage of relative standard deviation.

Table 9: Robustness results of Anastrozole

S. no	Parameter	Optimized	Used	Peak area	Retention time (R_t), min	Plate count	Peak asymmetry
1.	Flow rate (±0.2mL/min)	1.0 mL/min	0.8 mL/min	1926.562	6.836	18076	1.086
			1.0 mL/min	1910.395	6.143	17783	1.073
			1.2 mL/min	1892.984	5.892	17568	1.078
2.	Detection wavelength (±5nm)	235 nm	230nm	1852.348	6.142	17492	1.146
			235nm	1910.395	6.143	17783	1.073
			240nm	1876.566	6.146	17306	1.168
3.	Mobile phase composition (±5%)	50:50v/v	55:45v/v	1894.552	6.438	18024	1.122
			50:50v/v	1910.395	6.143	17783	1.073
			45:55v/v	1868.686	5.896	17468	1.107

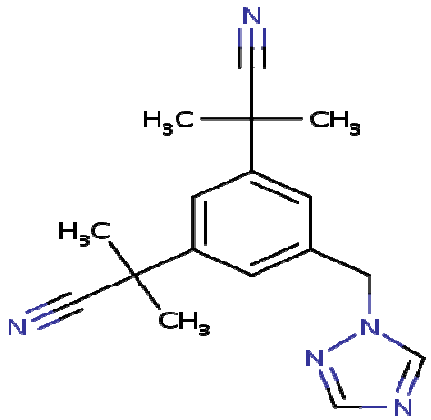

Fig. 1: Structure of Anastrozole

Table 10: Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of Detection(LOD)	0.157 µg/mL
Limit of Quantitation(LOQ)	0.476 µg/mL

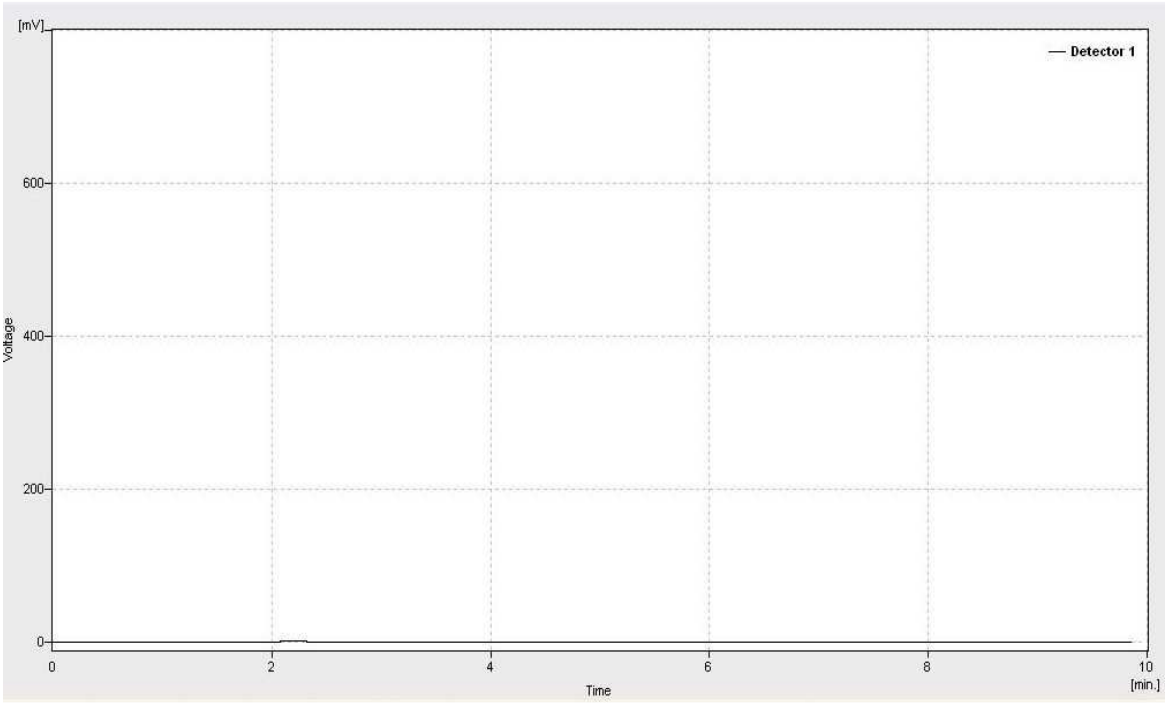

Fig 2: A Typical Chromatogram of Anastrozole placebo

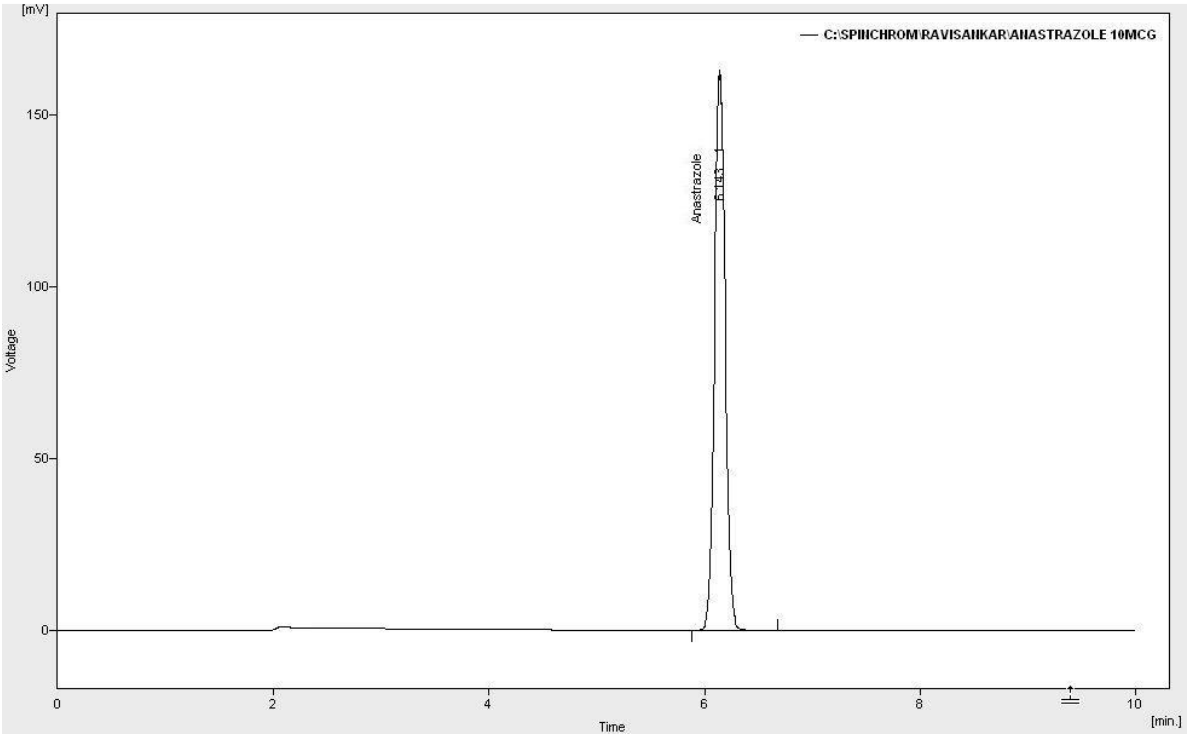

Fig. 3: A typical chromatogram of Anastrozole standard

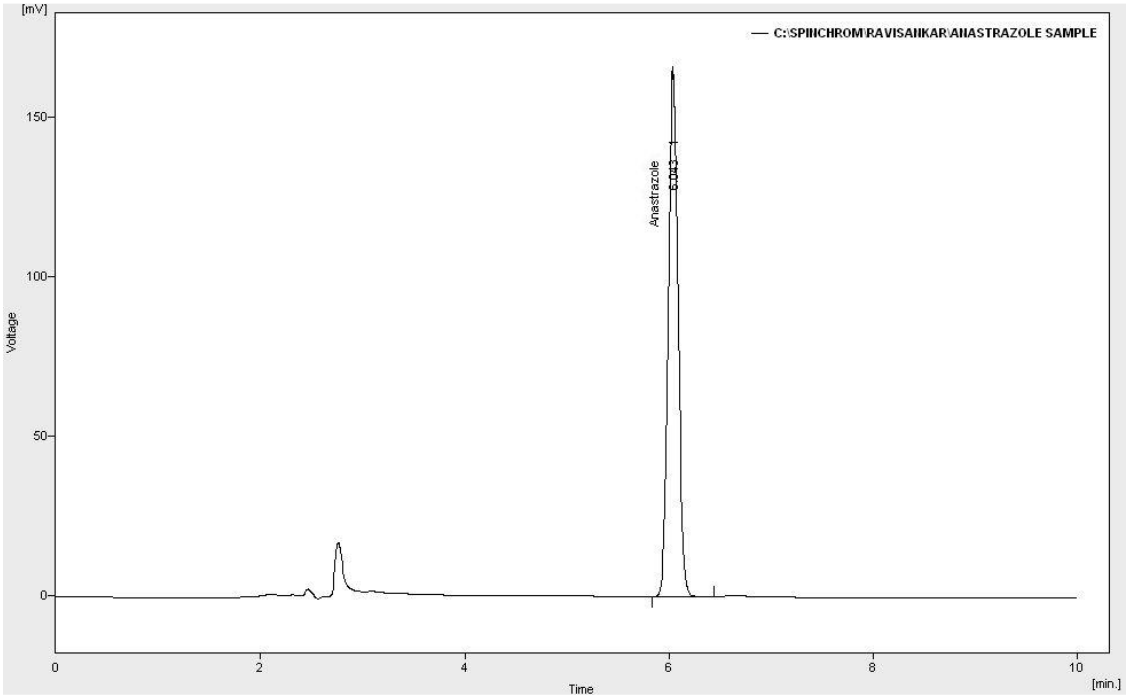

Fig. 4: Chromatogram of market formulation (ANABREZ Tablets) of Anastrozole

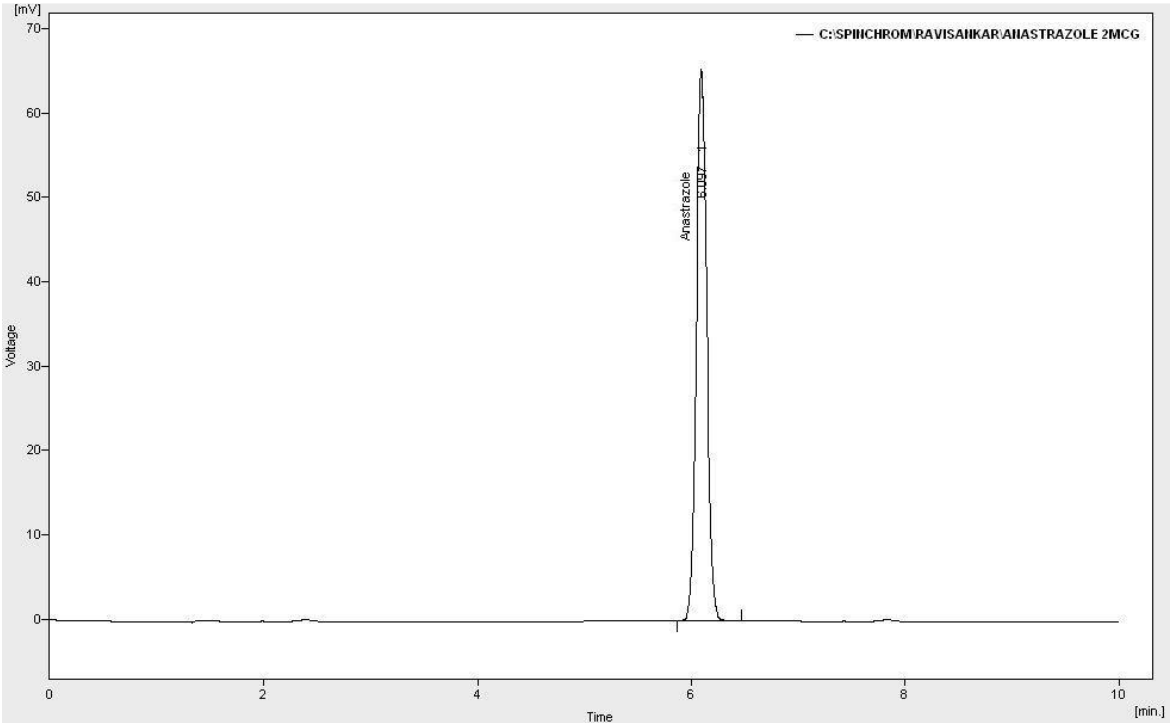

Fig. 5: Standard chromatogram of Anastrozole (2 µg/mL)

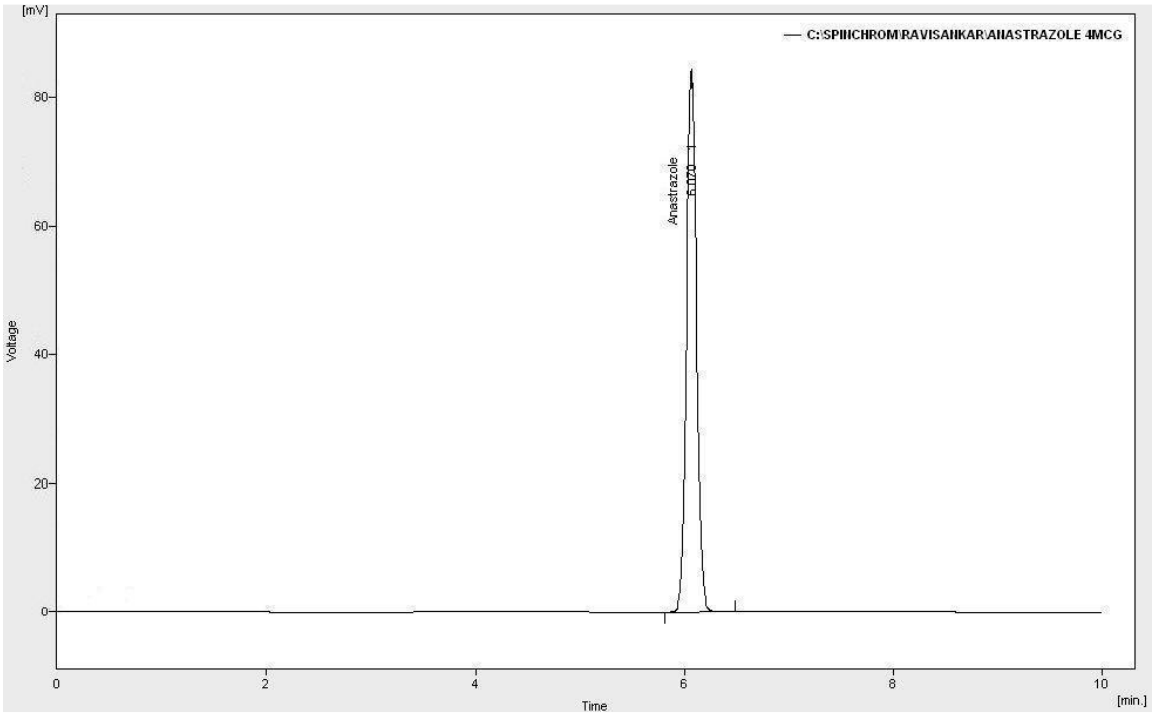

Fig. 6: Standard chromatogram of Anastrozole (4µg/mL)

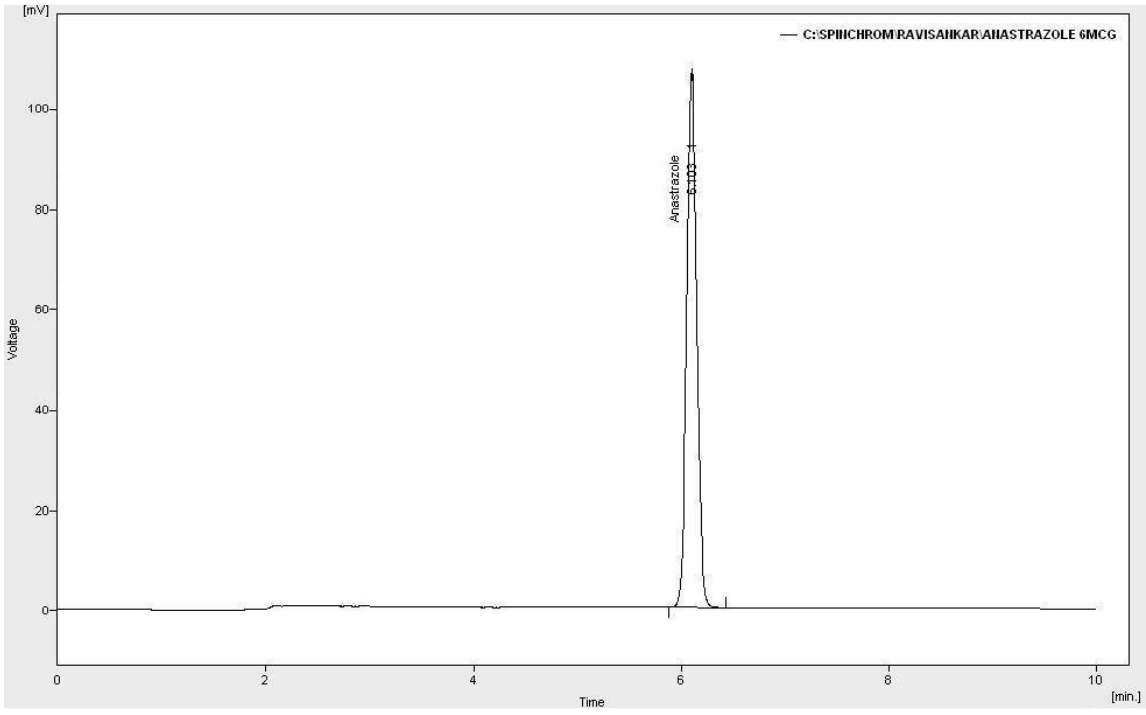

Fig. 7: Standard chromatogram of Anastrozole (6 µg/mL)

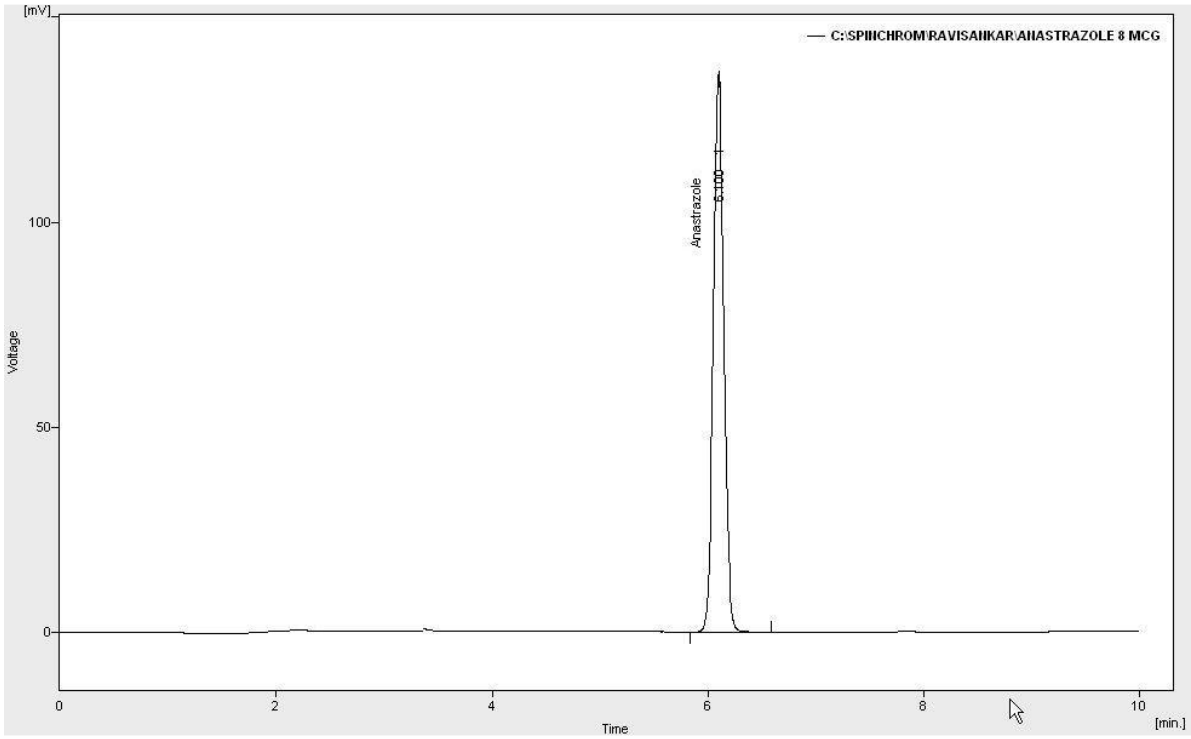

Fig. 8: Standard chromatogram of Anastrozole (8 µg/mL)

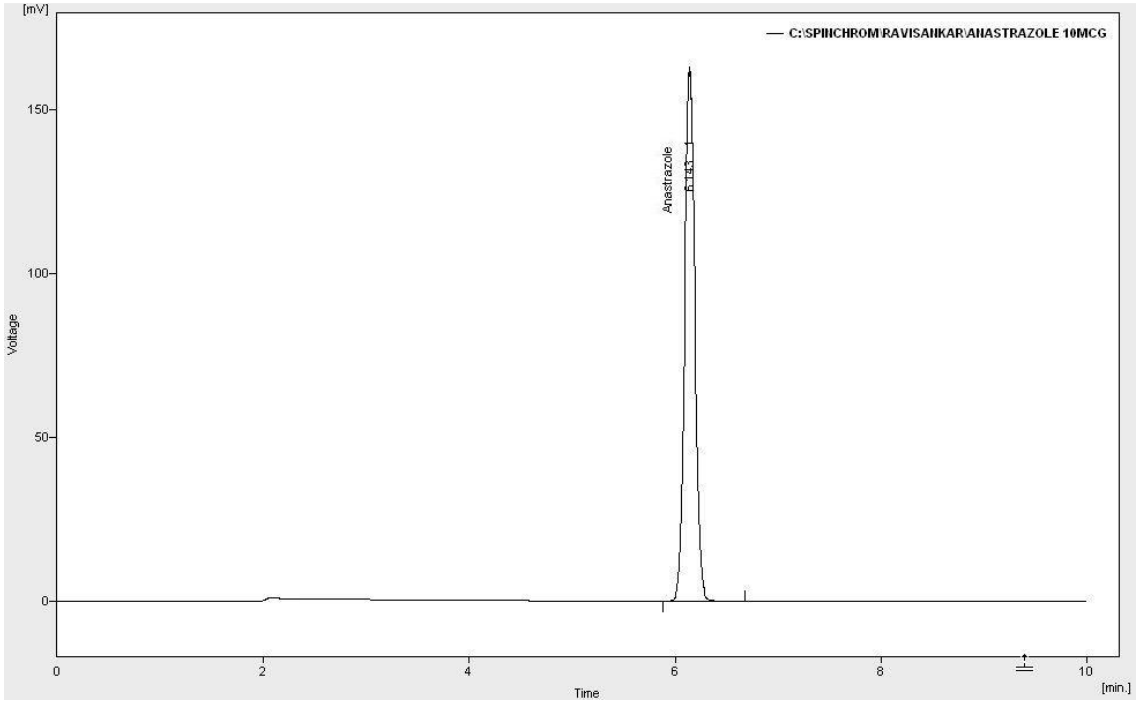

Fig. 9: Standard chromatogram of Anastrozole (10µg/mL)

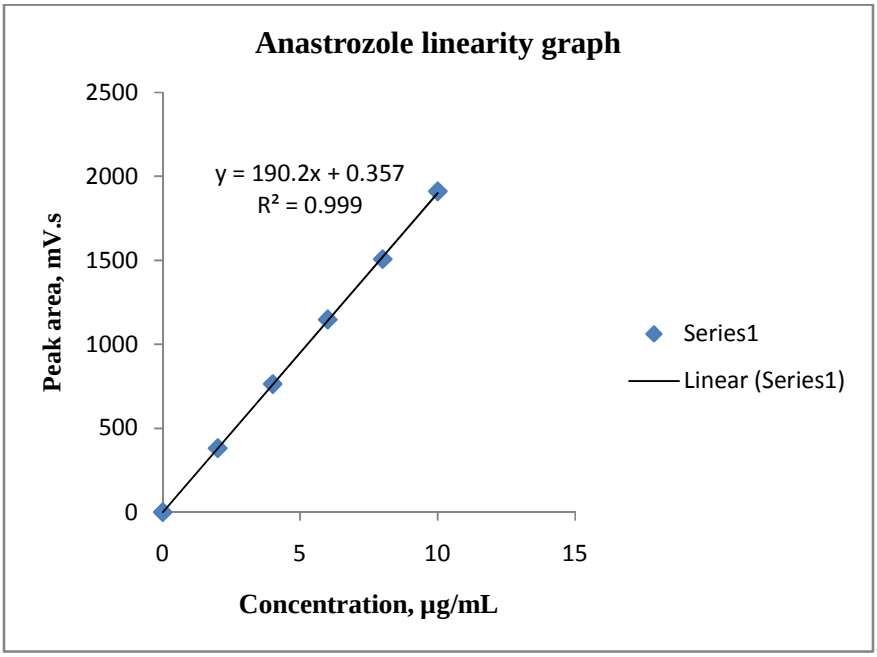

Fig. 10: Calibration plot of Anastrozole

CONCLUSION

A New validated RP-HPLC method has been developed for the quantitative determination of anastrozole in bulk and pharmaceutical tablet dosage forms. Statistical analysis of the results shows that the proposed procedure has good precision and accuracy. The method was completely validated shows satisfactory results for all the method validation parameters tested and method was free from interference of the other active ingredients and additives used in the formulation. Infact, results of the study indicates that the developed method was found to be simple, reliable, accurate, linear, sensitive, economical and reproducible and have short run time which makes the method rapid. Hence it can be concluded that this method may be employed for the routine quality control analysis of anastrozole in active pharmaceutical ingredient (API) and pharmaceutical preparations.

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