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Development and Validation of stability indicating RP-HPLC method for estimation of Anastrozole in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

An accurate, highly sensitive, precise, simple, efficient and reproducible, isocratic Reversed Phase High Performance Liquid Chromatography method was developed and validated for the quantitative determination of Anastrozole in bulk and tablet dosage forms. RP-HPLC method was developed using Inertsil ODS (250x4.6mm) C_{18} column using a mixture of Water and Acetonitrile (55:45) as the mobile phase at a flow rate 1.1 ml/min. The detection was made at 215nm. The Retention time of the drug was 4.399±0.5min. Validation parameters such as specificity, linearity, precision, accuracy and robustness were evaluated for the method according to the International Conference on Harmonization (ICH) Q2 R1 guidelines. Linearity was established for anastrozole with in the range of 2.5-15.0µg/ml with correlation coefficient r^2=0.999. The percentage recovery was found to be 98.45% to 100.7%. LOD and LOQ were found be 0.615µg/ml and 1.863µg/ml respectively. Forced degradation studies were carried out at different stress conditions such as Acid degradation, Alkaline degradation, Oxidative degradation and Thermal degradation. Anastrozole was found to be unstable at alkaline pH. The proposed method was found to be accurate, precise and rapid for the analysis of anastrozole in bulk and pharmaceutical dosage forms.

Keywords: Anastrozole; RP-HPLC; Isocratic method; Validation; Forced degradation

INTRODUCTION

Anastrozole is an anticancer drug, which is indicated for adjuvant treatment of both pre and post menopausal women with hormone receptor-positive early breast cancer. The Reverse phase High performance liquid chromatography (RP-HPLC) is very useful for the determination of anastrozole drug substance in pharmaceutical dosage form. This paper describes a simple RP-HPLC method for estimation of anastrozole in bulk and tablet dosage form.

The chemical name for anastrozole [1] is 2-[3-(1-cyano-1-methyl-ethyl) -5-(1H-1,2,4-triazol-1-ylmethyl) phenyl] -2-methyl-propanenitrile (fig 1). The molecular formula of anastrozole is $C_{17}H_{19}N_5$ and molecular weight is 293.366 g/mol. It is a new generation non-steroidal aromatase - inhibitor. Anastrozole binds [2-3] reversibly to the aromatase enzyme through competitive inhibition and inhibits the conversion of androgens to estrogens in peripheral tissues (outside the CNS), and a few CNS sites in various regions within the brain. Anastrozole works [4] by lowering oestrogen hormone levels to shrink tumours and slows their growth.

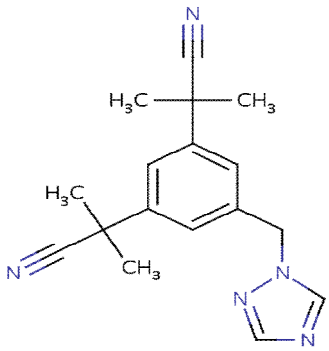

Figure 1. Structure of anastrozole

Literature survey revealed that very few analytical methods for the separation and estimation anastrozole

have been reported such as UV-Spectrophotometric method [5-6], LC MS/MS [7-11], UPLC-tandem mass spectrometry [12], Electro spray ionization tandem mass spectrometric analysis [13-14], Capillary gas chromatography [13-14], HPTLC [15]. Very few analytical HPLC [16-18] 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. So attempt was taken to develop and validate an economic, rapid reversed-phase high performance liquid chromatographic method [19-22] for the estimation of Anastrozole in pharmaceutical preparations with short analytical run time that will allow the analysis of a large number of samples in a short period of time. The method was validated [23-24] and found to be accurate, precise and reproducible. Forced degradation studies [25] of the drug revealed that the drug shows alkaline instability.

MATERIALS AND METHODS

Samples of anastrozole standards were obtained as gift samples from Celon laboratories, Hyderabad. Tablet formulation was purchased from local market. HPLC-grade methanol and acetonitrile were obtained from Merck.

Instrument

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

Chromatographic condition

Mobile phase consists of water and acetonitrile in the ratio 55:45%v/v. The mobile phase was pumped from the solvent reservoir in the ratio 55:45 to the column at a flow rate of 1.1ml/min whereas run time set was 8min. The separation was performed on Inertsil ODS column. The column was maintained at 30°C and the volume of each injection was 50µL. 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.

Preparation of standard stock solution

50mg of anastrozole was taken in a 50ml volumetric flask and the volume was made up to 50ml with diluents (water and acetonitrile in 55:45 ratio) to obtain 1mg/ml of anastrozole. The resultant solution was filtered through 0.45µ membrane filter. From this, 5ml of solution was transferred into 50ml volumetric flask and volume was made up to 50ml with diluent to obtain 100 µg/ml of anastrozole.

Preparation of sample solution

The Anastrozole tablets were crushed to give finely powdered material. Powder equivalent to 50mg of anastrozole was taken in a 50ml of volumetric flask containing 30ml diluent and sonicated for 15min. Then the volume was made up to the mark with diluents. The resultant solution was filtered through 0.45µ membrane filter. From this, 5ml of solution was transferred into 50ml volumetric flask and the volume was made up to the mark with diluent to obtain 100µg/ml of anastrozole.

Method validation procedure

The method is validated for linearity, precision, accuracy, robustness, system suitability etc. Standard plots were constructed with concentrations 2.5-15µg/ml prepared 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 on the same equipment on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on other day to determine the intermediate precision. Peak area of the anastrozole was determined and precision was reported as %RSD (Relative Standard Deviation).

VALIDATION OF THE PROPOSED METHOD

The developed method of analysis was validated as per the ICH guidelines for the parameters like system suitability, specificity, linearity, accuracy, limit of detection, limit of quantitation, precision and robustness.

LINEARITY

Linearity for the developed method was checked by injecting six solutions of different concentrations ranging from 2.5-15µg/ml and chromatograms were recorded. A linear relationship between peak area vs. concentration was observed in the range of study. The slope, y-intercept and correlation coefficient were found to be 70888, 13209 and 0.9993 respectively. The chromatograms were developed and the results were given in Table-2 and figure 2.

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

Parameter	Chromatographic conditions
Instrument	WATERS 2695- High performance liquid chromatography
Column	INERTSIL OctadecylSilane C_{18} column (150 x 4.6 mm, 5µ).
Detector	WATERS 2996 PDA detector
Diluent	Water:Acetonitrile (55:45 % v/v)
Mobile phase	Water:Acetonitrile (55:45 % v/v)
Flow rate	1.1ml/min
Detection wavelength	215nm
Run time	8min
Retention time	4.4±0.5min
Injection volume	50µl
Mode of separation	Isocratic mode
Column temperature	30^0C
Theoretical plates	2886
Tailing factor	1.34

Table 2. Linearity data of Anastrozole

Linearity level	Concentration (µg/ml)	Peak area
1	2.5	190051
2	5.0	378130
3	7.5	551875
4	10.0	730269
5	12.5	901158
6	15.0	1062607
Slope		70888
y-intercept		13209
R^2-value		0.9993

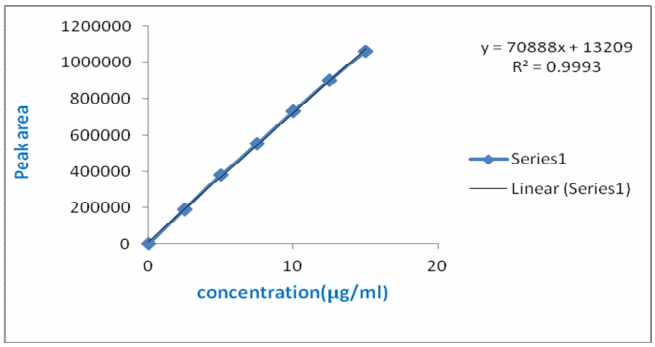

Figure 2. Linearity plot of anastrozole

PRECISION

Intraday and interday precision study of anastrozole was carried out by estimating corresponding responses for 6 times on the same day and on consecutive days for the concentration of 100µg/ml. The percent relative standard deviation (%RSD) was calculated which was within the acceptable criteria of not more than 2. The results were shown in table no.3 and 4.

ACCURACY (Recovery studies)

To determine the accuracy in sample preparation method of standard additions was made for measuring the recovery of the drugs. A fixed amount of sample was taken and standard drug was added at 50%, 100% and 150% levels. The results were analyzed and the results were found to be within the limits. The accuracy was expressed as the percentage of the analytes recovery. The mean percentage recovery of anastrozole at each level was not less than 98.45% and not more than 100.7%. The results were shown in table no.5.

Table 3. Results of precision study (Intraday)

Sample	Conc (µg/ml)	Inj. no.	Peak area	%RSD (Acceptance criteria<2.0)
Anastrozole	100	1	731267	0.3
		2	724008	
		3	735724	
		4	738401	
		5	732770	
		6	730846	

Table 4. Results of precision study (Interday)

Sample	Conc (µg/ml)	Inj. no.	Peak area	%RSD (Acceptance criteria<2.0)
Anastrozole	100	1	737329	0.5
		2	733379	
		3	738198	
		4	729111	
		5	737134	
		6	736088	

LIMIT OF DETECTION AND LIMIT OF QUANTIATION

LOD and LOQ were calculated using the 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. Limit of Quantification and Limit of Detection were found to be 1.863µg/ml and 0.615µg/ml respectively as per ICH guidelines. The results were shown in table no.6.

ROBUSTNESS

Robustness was carried by varying three parameters from the optimized chromatographic conditions such as making small changes in flow rate (±0.1ml/min), mobile phase composition (±5%) and column temperature (±5^{0}C). The %RSD was not more than 2. No significant changes were observed. The results were shown in table no.7

SYSTEM SUITABILITY TESTS

System suitability test parameters were checked by repetitively injecting the drug solution at the concentration level 10µg/ml for anastrozole to check the reproducibility of the system. System suitability parameters like number of theoretical plates (N), Tailing factor, resolution and relative standard deviation of peak height or peak area or repetitive injections were studied. The %RSD values are below 2%, theoretical plate count is above 2000 and tailing factor is less than 2, indicating that the method is suitable.

FORCED DEGRADATION STUDUES

For forced degradation analysis, aliquots of stock (0.1mg/ml) were separately treated with 10ml of 2N HCl (Acid stability), 10ml of 2N NaOH (Alkaline stability), 10ml of 30% H_2O_2 (Oxidative degradation) and thermal degradation at 80^{0}C for 2 hours. Stability of these samples was compared with fresh sample on the day of analysis. The HPLC chromatograms of degraded products show no interference at the analyte peaks, hence the method was specific and anastrozole shows alkaline instability. The chromatograms were shown in figures 5 to 8 and the results were shown in table no.8

RESULTS AND DISCUSSION

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 and acetonitrile 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 water and acetonitrile (55:45) and C18 analyzed column were selected which gave a sharp and symmetrical peak with 1.36 tailing factor. Forced degradation studies concluded that anastrozole shows alkaline instability. Calibration

graph was found to be linear in the range 2.5-15ppm. Six 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 70888 and 13209 respectively. A plot is 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 0.9993. The LOQ and LOD of anastrozole were found to be 1.863ppm and 0.615ppm respectively indicating the sensitivity of the method. The low values of standard deviation and coefficient of variation at each level of the recovery experiment indicated high precision of the method.

Table 5. Accuracy data of Anastrozole

Concentration level	Amount added	Amount found	Percent Recovery	Average %recovery
50%	5	4.918	98.36	
	5	4.926	98.52	98.456
	5	4.926	98.5	
100%	10	10.06	100.6	
	10	10.07	100.7	100.71
	10	10.084	100.84	
150%	15	14.866	99.106	
	15	14.864	99.09	99.088
	15	14.861	99.07	

Table 6. Limit of detection (LOD) and Limit of quantification (LOQ)

Limit of detection (LOD)	0.615µg/ml
Limit of quantification (LOQ)	1.863µg/ml

Table 7. Robustness study of anastrozole

S No	Parameter	Optimized	Used	Peak area	Retention time (min)	Plate count	Tailing factor
1	Flow rate (±0.1ml/min)	1.1ml/min	1.0	673155	4.06	2545	1.40
			1.1	737329	4.462	2886	1.34
			1.2	801957	4.88	2628	1.38
2	Column temperature(±5^{0}C)	30^{0}C	25^{0}C	725510	4.3	2495	1.42
			30^{0}C	737329	4.462	2886	1.34
			35^{0}C	728019	4.27	2571	1.39
3	Mobile phase composition (±5%)	55:45v/v	60:40	788873	4.59	2448	1.49
			55:45	737329	4.462	2886	1.34
			50:50	788873	4.59	2448	1.49

Table 8. Forced degradation study of anastrozole

Condition	Percent degradation	Percent of drug present after degradation
Control sample	-	100
Acid degradation	21	79
Alkaline degradation	90	10
Peroxide degradation	13	87
Thermal degradation	3	97

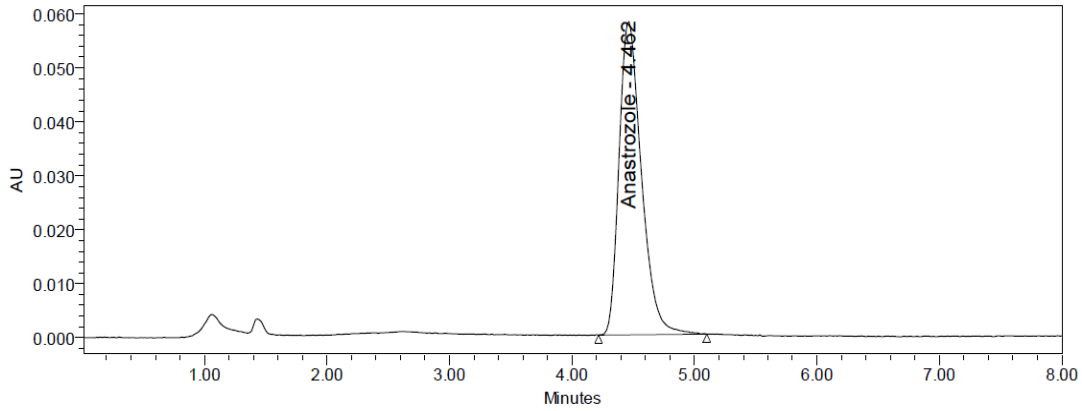

Figure 3. HPLC chromatogram of standard

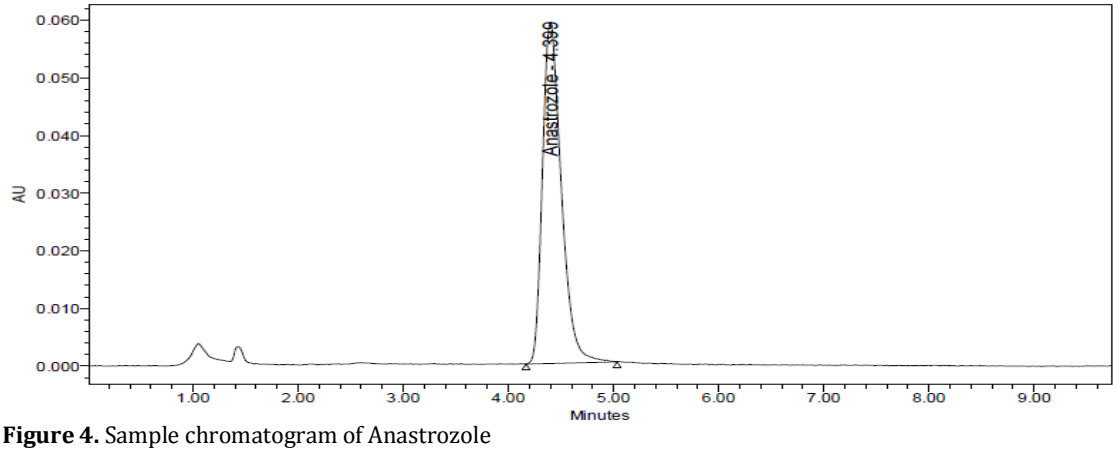

Figure 4. Sample chromatogram of Anastrozole

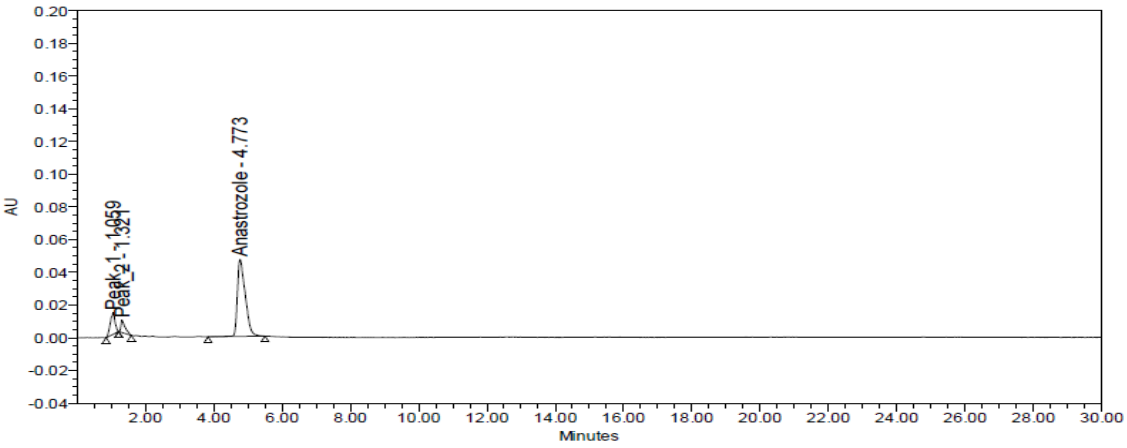

Figure 5. Acid degradation chromatogram of anastrozole

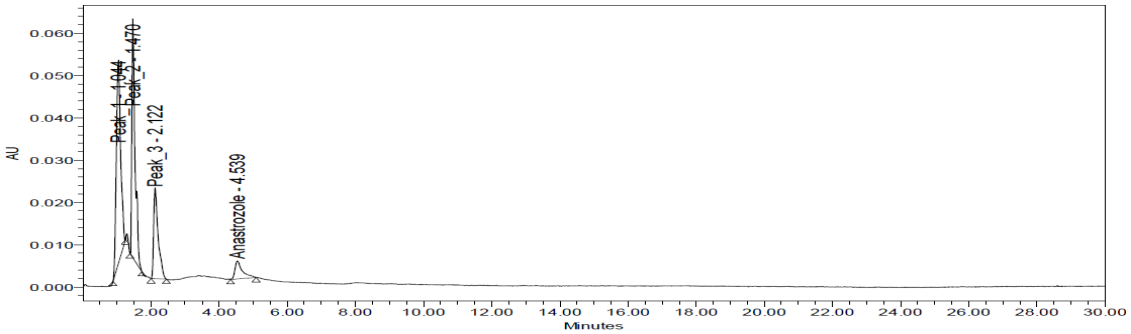

Figure 6. Alkaline degradation chromatogram of anastrozole

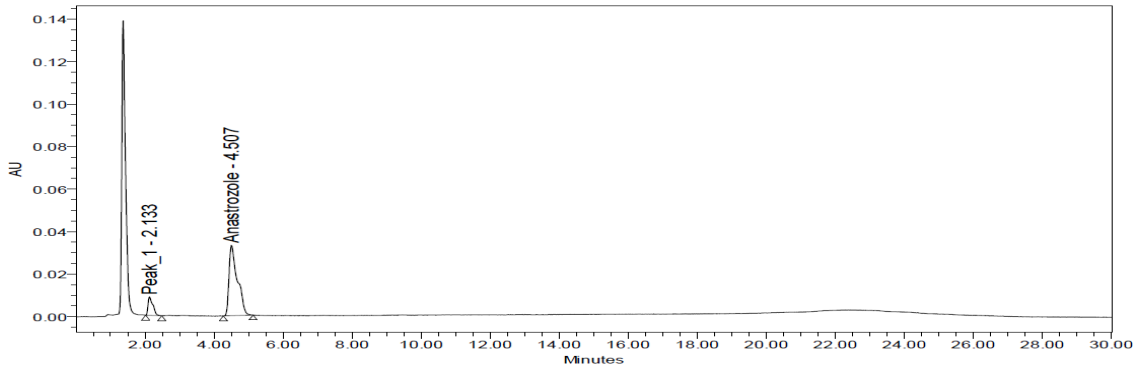

Figure 7. Peroxide degradation chromatogram of anastrozole

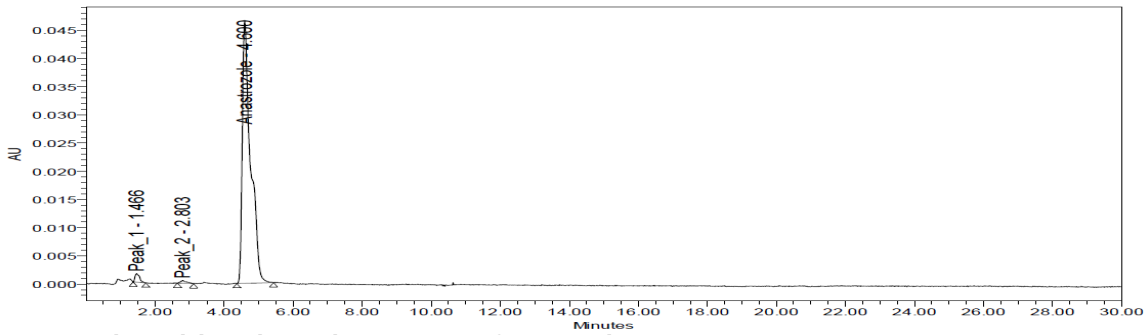

Figure 8. Thermal degradation chromatogram of anastrozole

CONCLUSION

A new RP-HPLC method has been developed for the quantitative determination of anastrozole in bulk and tablet dosage forms. The method was found to be precise, accurate, linear and robust during validation with satisfactory results. Hence it can be concluded that this method can be employed for the routine quality control analysis of anastrozole in bulk and pharmaceutical preparations.

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