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Development of New Validated RP-HPLC Method for Estimation of Anastrazole in Bulk and Tablet Dosage Forms

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ABSTRACT

A simple, Precised, Accurate method was developed for the estimation of Anastrozole by RP-HPLC technique. Chromatographic conditions used are stationary phase Azilent C18 (150mm x 4.6mm, 5µm) Mobile phase 0.01N Kh2Po4:Acetonitrile in the ratio of 60:40 and flow rate was maintained at 1.0 ml/min, detection wave length was 215 nm, column temperature was set to 30^{o}C and diluent was mobile phase Conditions were finalized as optimized method. The retention time was found to be 2.248 min. 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% to150 % 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 100.11% 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.

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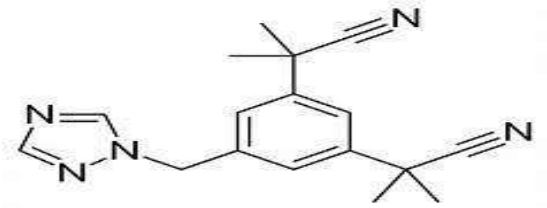
INTRODUCTION

Anastrozole is a non-steroidal aromatase inhibitor (AI), similar to Letrozole, used to decrease circulating estrogen levels in the treatment of postmenopausal women with estrogen-responsive breast cancer. Anastrozole was first approved for use in the United States in 1995. Anastrozole is also related to exemestane, a steroidal AI, but its non-steroidal nature provides stark advantages including a lack of steroid-associated adverse effects such as weight gain and acne. Aromatase inhibitor, including Anastrozole, have become endocrine drugs of choice in the treatment of postmenopausal breast cancer due to a more favourable efficacy and adverse effect profile as compared to earlier estrogen receprtor modulators such as tamoxifen.

Structure of Anastrozole:



MATERIALS AND METHOD

Materials:

Anastrozole pure drugs (API), Combination Anastrozole tablets (Arimidex), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium Dihydrogen Ortho Phosphate buffer, Ortho-Phosphoric acid. All the above chemicals and solvents are from Rankem.

Equipment and Apparatus used:

HPLC instrument used was of WATERS HPLC 2695 SYSTEM with Auto Injector and PDA Detector. Software used is Empower. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Anastrozole solutions.

- Sonicator (Ultrasonic sonicator)
- PH meter (Thermo scientific)
- Micro balance (Sartorius)
- Vaccum filter pump

Reagents used:

- Methanol HPLC Grade (RANKEM)
- Acetonitrile HPLC Grade (RANKEM)
- HPLC Grade Water (RANKEM)

- Glacial Acetic acid

Preparation of 0.01N Potassium Dihydrogen Phosphate Buffer:

Accurately weighed 1.36gms of Potassium Dihydrogen Ortho Phosphate in 1000ml of volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then add 1 ml of Triethylamine then P^H was adjusted to 3.0 with diluted Ortho Phosphoric acid solution.

Preparation of Mobile Phase

Into a 1000ml cleaned volumetric flask, HPLC grade, Acetonitrile 400ml and Potassium Dihydrogen Phosphate (0.01%w/v) which are filtered through 0.25mm membrane filters by vaccum filtration were slowly added, mixed well and Sonicated upto 20min. Cool the above solution. This solution is again Sonicated to 10 min. Cool the solution in room temperature and used for chromatography method

Preparation of Standard stock solutions:

Accurately weighed 5mg of Anastrozole transferred 50ml of volumetric flask, and $3/4^{th}$ of diluents was added and Sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (100µg/ml of Anastrozole)

Preparation of Standard working solutions (100% solution):

1ml of Anastrozole each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluents (10µg/ml of Anastrozole).

Preparation of Sample stock solution:

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 10ml of volumetric flask, 5ml of diluents was added and Sonicated for 25min, further the volume was made up with diluent and filtered by HPLC filters (100µg/ml of Anastrozole).

Preparation of Sample working solutions (100% solution):

1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent (10µg/ml of Anastrozole).

RP-HPLC METHOD DEVELOPMENT

Based on nature and solubility characteristics of Anastrozole, Reverse phase mode of HPLC was selected for chromatography. Among different RP-HPLC stationary phases tried, C18 column was found to be optimum. In order to get sharp peak with base line separation from interfering peaks carried out a number of experiments by varying the composition of solvents and mobile phase flow rate. To have an ideal separation of drug under isocratic conditions, mixtures of

solvents like Methanol, water and Acetonitrile with or without different buffers in different combinations were tested as mobile phase. A mixture of 0.01N KH2PO4 : Acetonitrile (60:40 v/v) was proved to be the most suitable of all the combinations, since the chromatographic peak obtained was better defined and resolved and almost free from tailing. This chromatographic conditions for the estimation of Anastrozole was discussed in below table.

Table 1: Optimized chromatographic conditions for estimation of Anastrozole

Parameter	Condition
Mobile phase	0.01N KH2PO4: Acetonitrile (60:40) (V/V)
Pump mode	Isocratic
Diluents	Mobile phase
Column	Azilent C18 Column (150 x 4.6 mm, 5µ)
Column Temp	30^0C
Wavelength	215nm
Injection Volume	10µL
Flow rate	1.0 ml/min
Run time	10min

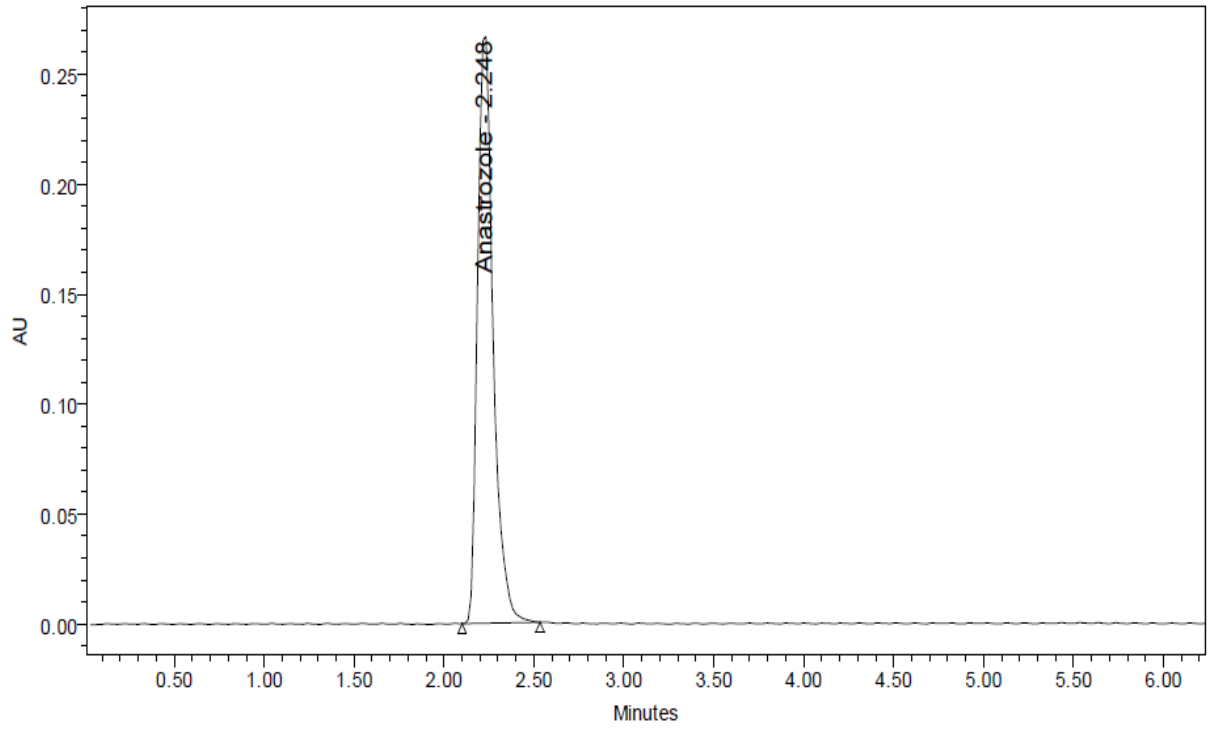
RESULTS AND DISCUSSION

Analysis of formulations:

The sample solution was injected and a chromatogram was recorded. The injections were repeated five times and the peak areas were recorded. The amount of drug present in the pharmaceutical formulation was calculated using standard calibration curve (concentration in µg/ml was taken on X –axis and average peak area on Y –axis). A representative chromatogram has been given in Fig. 1

VALIDATION OF PROPOSED METHOD

As an integral part of analytical method development is validation. The proposed method was validated as per ICH guidelines.



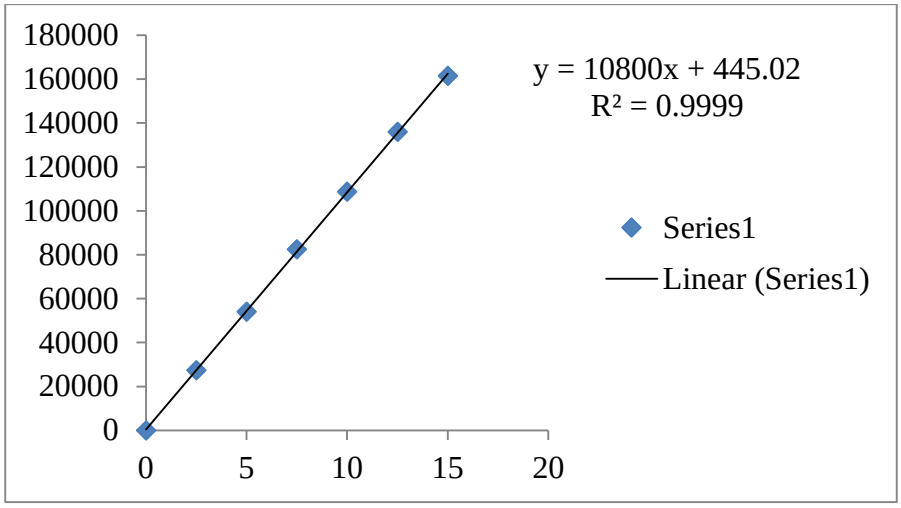
Chromatogram of Anastrozole

Linearity

To demonstrate the linearity of assay method, inject 6 standard solutions with concentrations of about 2.5ppm to 15ppm of Anastrozole. Plot a graph to concentration versus peak area. Slope obtained was 10820 Y-Intercept was 395.02 and Correlation Co-efficient was found to be 0.999 and Linearity plot was shown in table

Table 2: Linearity Results of Anastrozole

Linearity Level (%)	Concentration (ppm)	Area
0	0	0
25	2.5	27433
50	5	54057
75	7.5	82554
100	10	108660
125	12.5	135956
150	15	161446



Linearity Plot

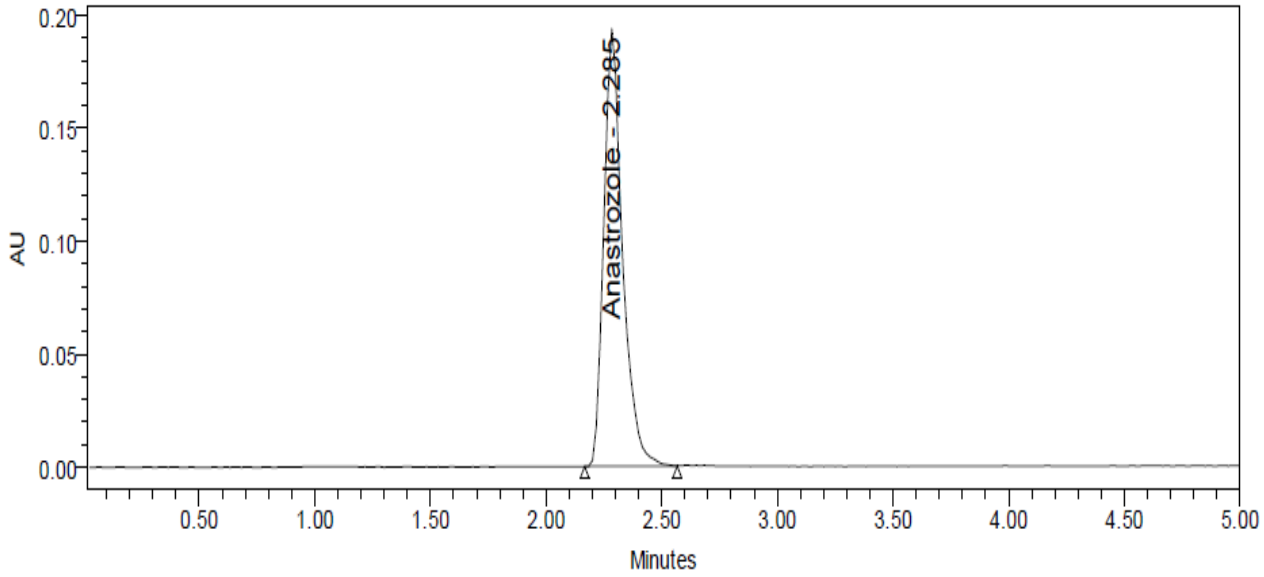
Precision:

Repeatability:

Six working sample solutions of 10ppm are injected and the % Amount found was calculated and %RSD was found to be 1.0

Table 3. Repeatability data

S.No	Peak Area
1	106637
2	104967
3	105889
4	105829
5	105479
6	105552
AVG	105726
STDEV	554.0
%RSD	0.5

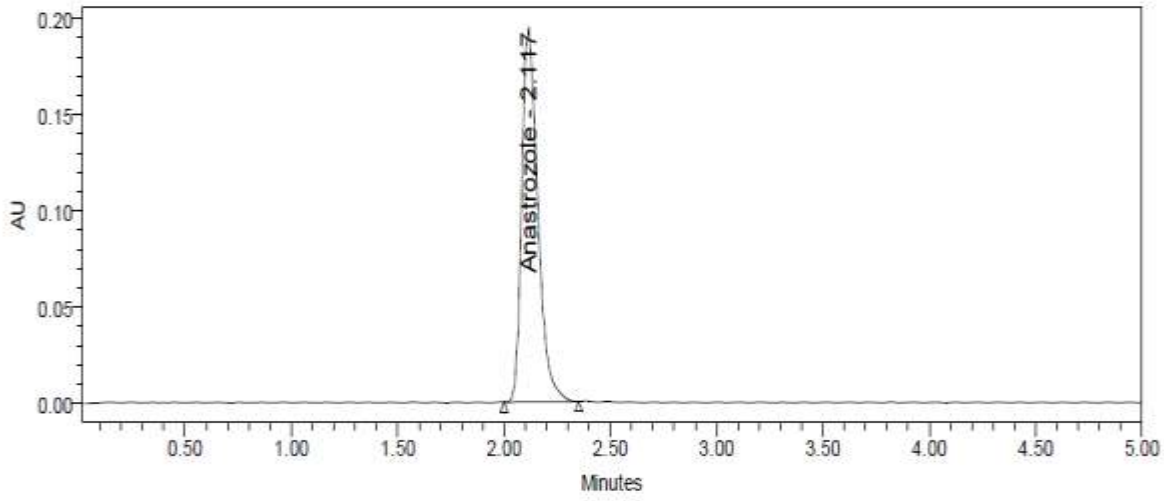


Intermediate precision:

Five working sample solutions of 10ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 1.0

Table 4. Intermediate precision data

S.No	Peak Area
1	102214
2	102481
3	103878
4	102095
5	103011
6	102693
AVG	102729
STDEV	652.6
%RSD	0.6



Limit of Detection and Limit of Quantification:

Calibration curve was prepared using concentrations in the range of 2.5-12.5µg/ml (expected detection limit range). The standard deviation of Y-intercepts of regression line was determined and kept in following equation for the determination of Detection limit and Quantitation limit. The results were reported in table 3.

$$\text{Limit of detection} = \frac{\sigma \times 3.3}{S}$$

$$\text{Limit of quantification} = \frac{\sigma \times 10}{S}$$

Where,

σ = the standard deviation of the response.

S = the slope of the calibration curve

Table 5. Limit of Detection and Limit of Quantification for Anastrozole

s	Values
Limit of Quantification	0.261µg/ml
Limit of Detection	0.086µg/ml

LOD: Detection limit of the Anastrozole in this method was found to be 0.086µg/ml.

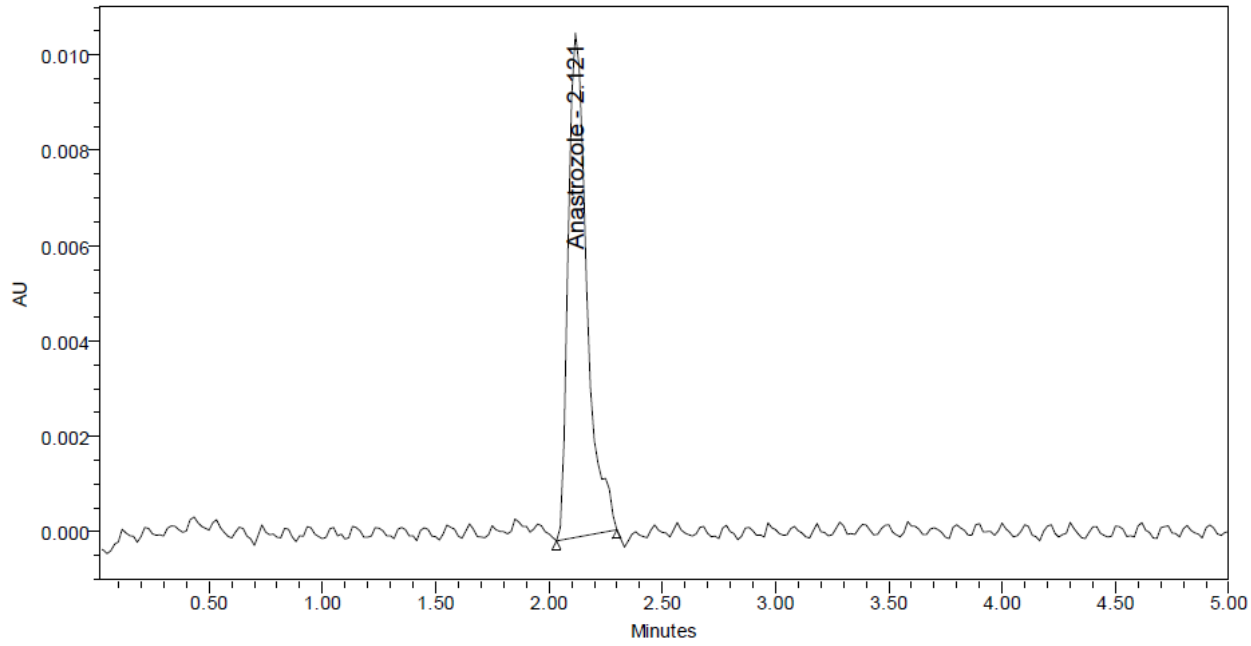


Figure LOD Chromatogram of Anastrozole

LOQ: Quantification limit of the Anastrozole in this method was found to be 0.261µg/ml.

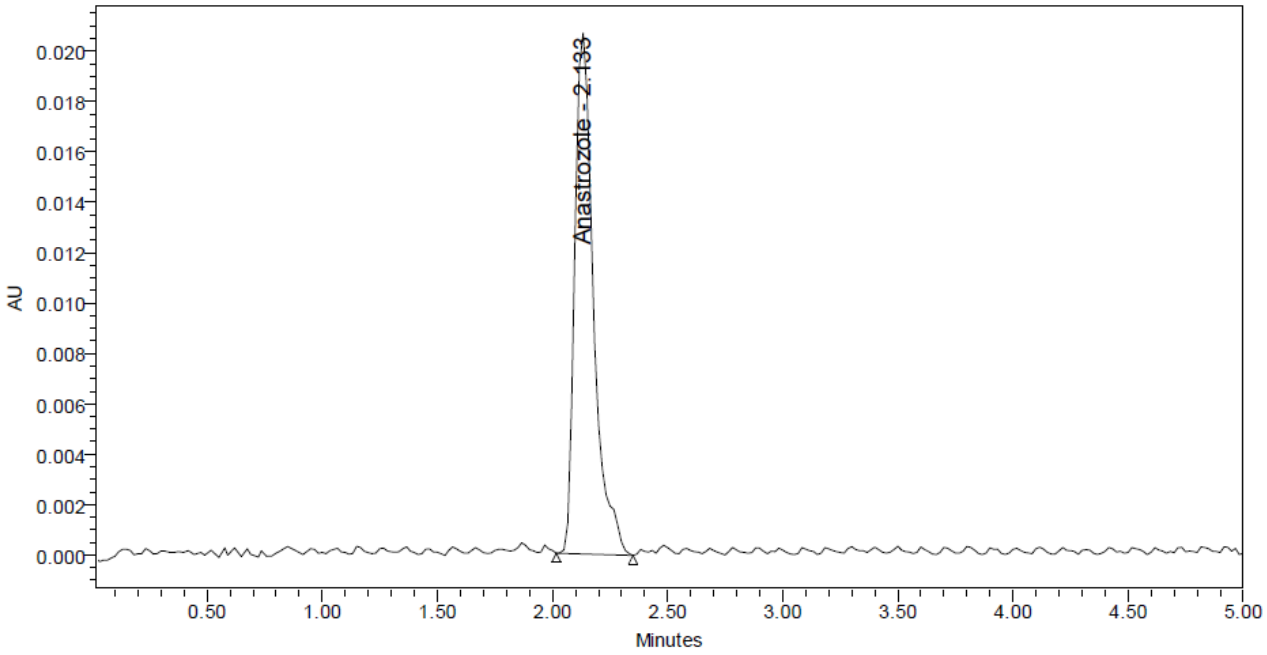


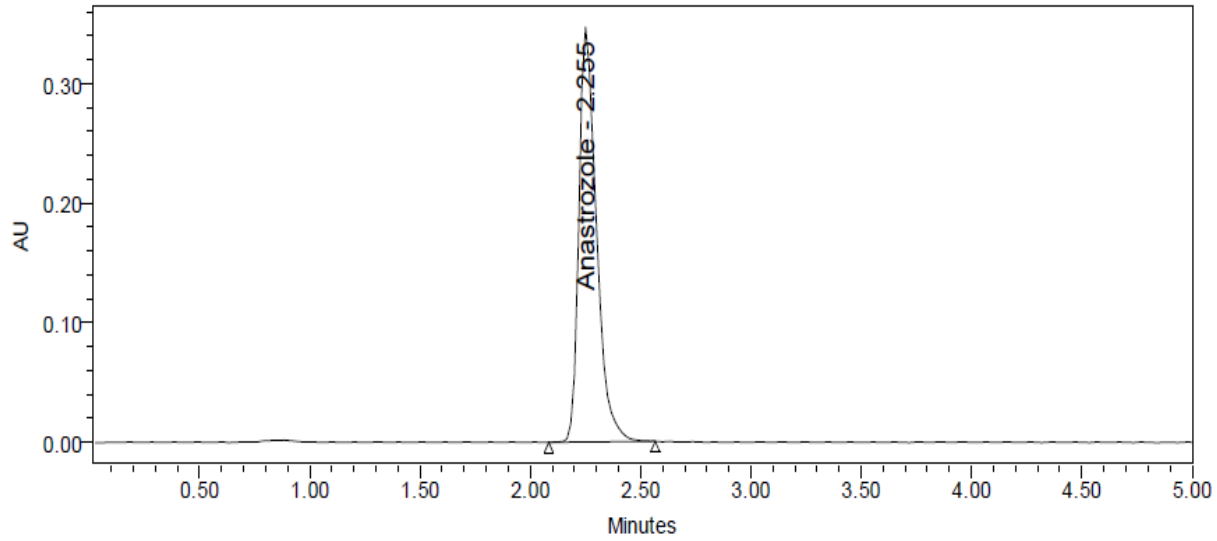
Figure LOQ Chromatogram of Anastrozole

Accuracy:

Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner and %Recovery was calculated as 100.26%.

Table 6: Accuracy data

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	5	5.04122	100.82	100.20%
	5	4.96414	99.28	
	5	5.047043	100.94	
100%	10	9.951109	99.51	
	10	10.08401	100.54	
	10	9.915434	99.15	
150%	15	14.9427	99.62	
	15	15.20342	101.36	
	15	15.04492	100.30	

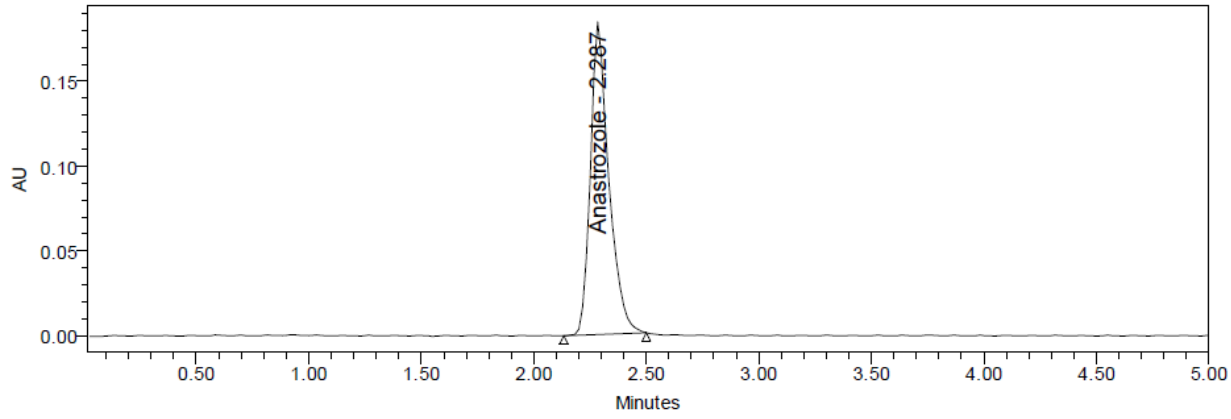


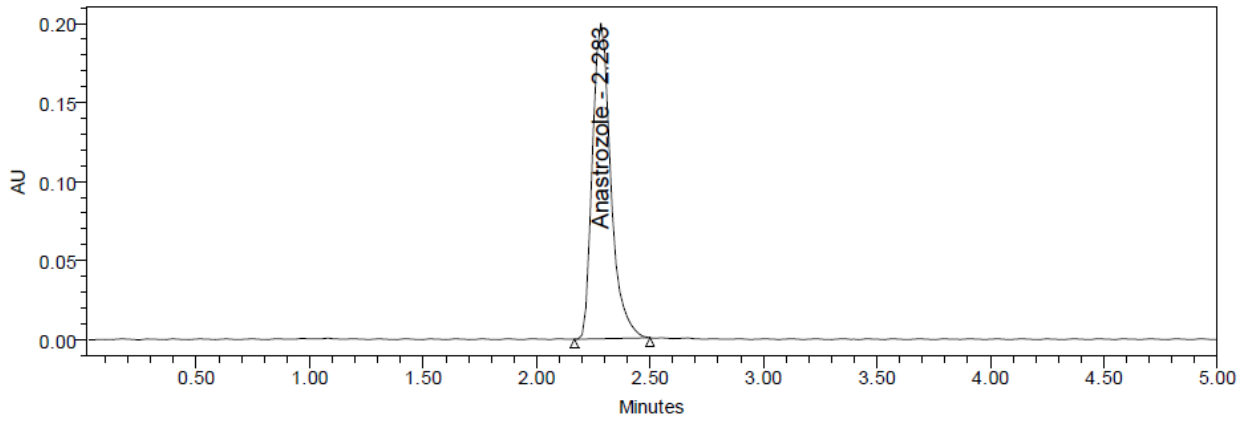
Robustness:

Small Deliberate change in the method is made like Flow minus, flow plus, Mobile phase minus, Mobile phase plus, Temperature minus, Temperature Plus. %RSD of the above conditions is calculated

Table 7: Robustness Data

Parameter	%RSD
Flow Minus (0.9misin)	1.0
Flow Plus(1.1ml/min)	1.4
Mobile phase Minus (50B:50A)	0.8
Mobile phase Plus (40B:60A)	1.4
Temperature minus (25°C)	1.3
Temperature plus (35°C)	1.1



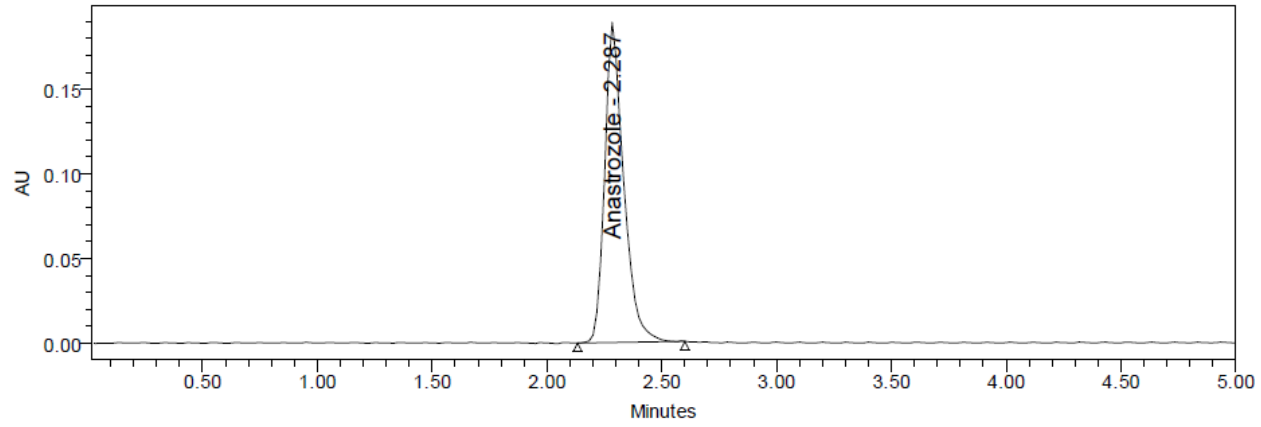


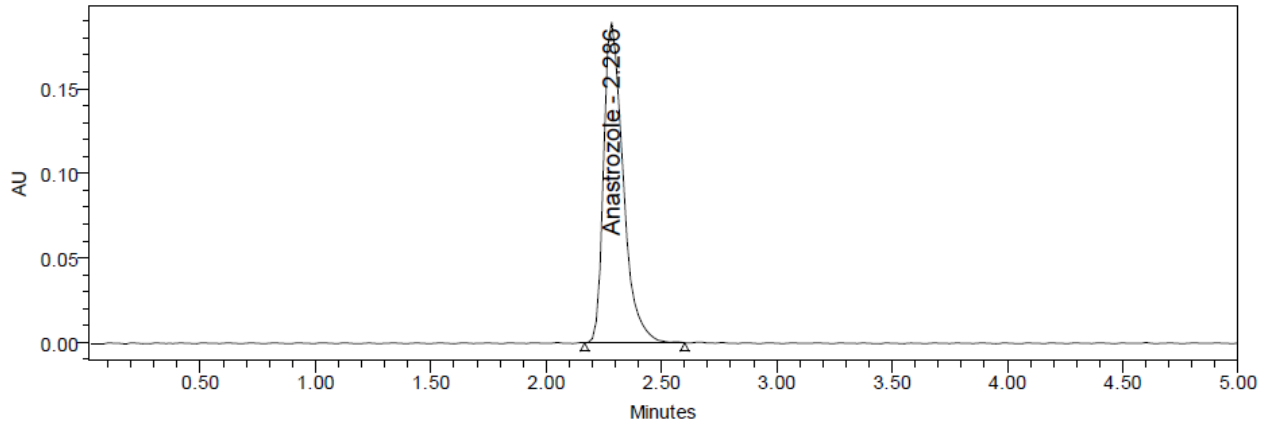
Assay of Marketed Formulation

Standard solution and sample solution were injected separately into the system and chromatograms were recorded and drug present in sample was calculated using before mentioned formula.

Table 8: Assay of Formulation

Sample No	Standard	Sample	%Assay
1	106624	106637	100.18
2	105536	104967	98.61
3.	103754	107889	101.36
4.	107752	105829	99.42
5.	107270	106479	100.04
6.	105797	107552	101.04
AVG	106122	106559	100.11
STDEV	1434.1	1080.3	1.015
%RSD	1.4	1.0	1.01



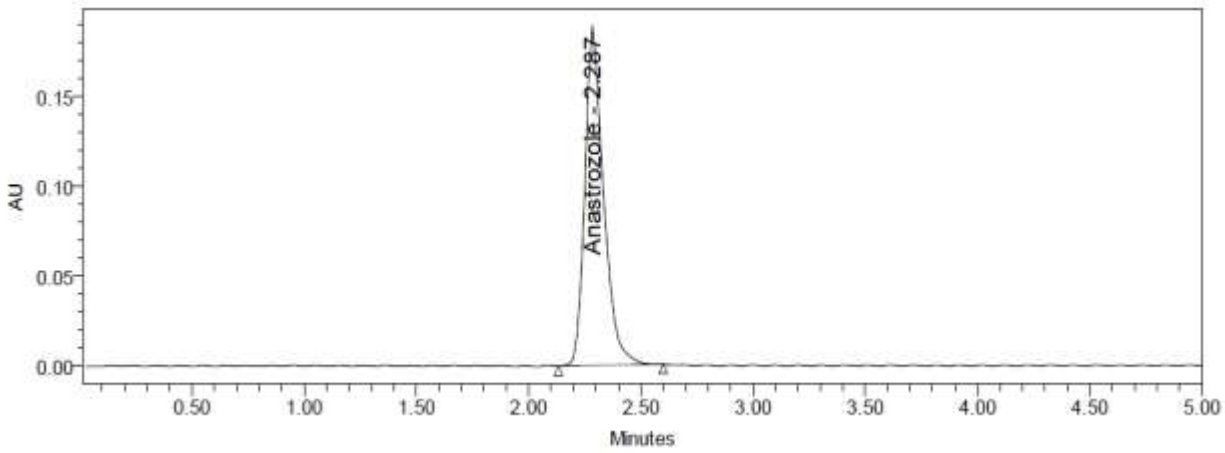


System Suitability

A Standard solution of Anastrozole working standard was prepared as per procedure and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention time, tailing factor, theoretical plates and peak areas from five replicate injections are within range and Results were shown in table

Table 9: System Suitability Parameters

S no Inj	Anastrozole RT(min)	SP Plate Count	Tailing
1	2.274	3725	1.45
2	2.276	4031U	1.37
3	2.282	3923	1.41
4	2.284	3411	1.35
5	2.286	3427	1.43
6	2.287	3495	1.3



Degradation Studies:

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation

Degradation procedure:

Oxidation:

To 1 ml of stock solution ofAnastrozole1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain (10ppm) solution and10µlwere injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1 ml of stock solution Anastrozole1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 1c. The resultant solution was diluted to obtain (10ppm) solution and 10µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 ml of stock solution Anastrozole1 ml of 2 N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain (10ppm) solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105^0c for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to (10ppm) solutionand10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the (100ppm) solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain (10ppm) solutions and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

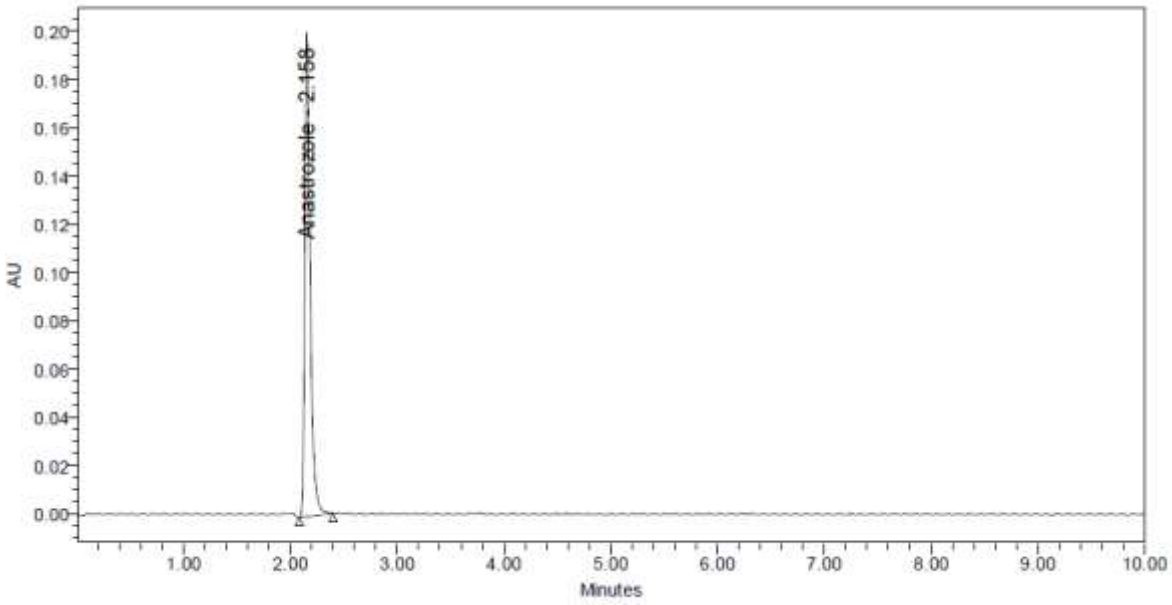
Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60ºc. For HPLC study, the resultant solution was diluted to (10ppm) solution and 10µl were injected into the system and the chromatograms were recorded to assess the

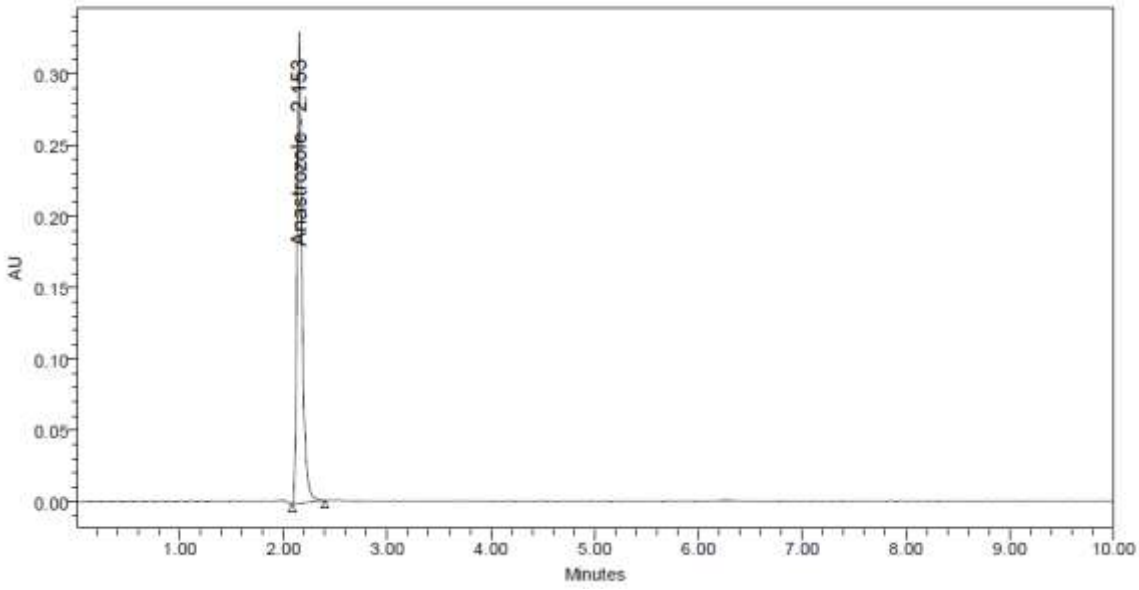
stability of the sample**.**

Table 10: Degradation Data of Anastrozole

S.NO	Degradation Condition	% Drug UN Degraded	% Drug Degraded
1	Acid	92.95	7.05
2	Alkali	93.15	6.85
3	Oxidation	95.55	4.45
4	Thermal	97.44	2.56
5	UV	98.40	1.60
6	Water	99.14	0.86



Acid degradation chromatogram



Base degradation chromatogram

Table 11: Summary of validated parameters for proposed method:

Parameters		Anastrozole	Limit
Linearity :Range (µg/ml)		2.5-15 µg/ml	R< 1
Regression coefficient		0.999	
Slope(m)		10800	
Intercept(c)		445.0	
Regression equation (Y=mx+c)		y = 10800x + 445.0	
Assay(% mean assay)		100.11%	90-110%
Specificity		Specific	No interference of any peak
System precision %RSD		1.4	NMT 2.0%
Method precision %RSD		0.5	NMT 2.0%
Accuracy %recovery		100.20%	98-102%
LOD		0.08	NMT 3
LOQ		0.26	NMT 10
Robustness	FM	1.0	
	FP	1.4	%RSD NMT 2.0
	MM	0.8	
	MP	1.4	
	TM	1.3	
	TP	1.1	

SUMMARY AND CONCLUSION

Chromatographic conditions used are stationary phase Azilent C18 (150mm*4.6mm 5µm),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% to150 % levels, R^2 value was found to be as 0.999. Precision was found to be 0.5for 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 100.11% 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.

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