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METHOD DEVELOPMENT AND VALIDATION OF ANASTROZOLE IN TABLET DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

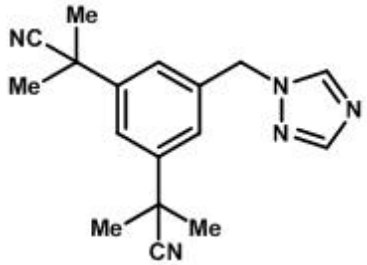
A new, simple, accurate and precise reversed phase high performance liquid chromatographic method was for method development and validation of Anastrazole. The method was based on reversed phase liquid chromatography and separation was achieved on a Water's Inertsil ODS C18 150×4.6 mm 5µ column. The Eluent was monitored by measuring the absorbance at wavelength 215 nm using a mixture of water and acetonitrile in the ratio of 60:40 (v/v) at pH 4 with a flow rate of 1.0 mL min-1. The Calibration curves were found to be linear in the concentration range of 16-60 µg mL-1 for Anastrazole. The proposed method was validated by testing for its linearity, recovery, precision and it was successfully employed for the rapid, specific and sensitive estimation of Anastrazole

INTRODUCTION:

Many breast cancers have estrogen receptors and growth of these tumors can be stimulated by estrogens. In post-menopausal women, the principal source of circulating estrogen (primarily estradiol) is conversion of adrenally-generated androstenedione to estrone by aromatase in peripheral tissues, such as adipose tissue, with further conversion of estrone to estradiol. Anastrozole is a potent and selective non-steroidal aromatase inhibitor [1-3]. It significantly lowers serum estradiol concentrations. As less estrogen reaches the cancer cells, they grow more slowly or stop growing altogether. It is used in the treatment of breast cancer after surgery, as well as for metastasis in both pre and post-menopausal women. It is also used as Adjuvant treatment (After surgery) of postmenopausal women with hormone receptor-positive early breast cancer (cancer that has not spread), to reduce the chance of the cancer coming back. It is used in first line treatment of postmenopausal women with hormone receptor-positive or hormone receptor unknown locally advanced or metastatic breast cancer. Anastrozole offers no clinical benefit to premenopausal women with breast cancer. Anastrazole is well absorbed into the systemic circulation with 83 to 85% of the radiolabel recovered in urine and feces [4]. Food does not affect the extent of absorption. Elimination of Anastrazole is primarily via hepatic metabolism (approximately 85%) and to a lesser extent, renal excretion (approximately

11%), and Anastrazole has a mean terminal elimination half-life of approximately 50 hours in postmenopausal women. A group of scientists employed different optical spectroscopic techniques viz., fluorescence, FTIR, circular dichroism (CD) and UV-vis absorption spectroscopy to investigate the mechanism of interaction of an anticancer drug, Anastrozole (AZ) with transport proteins viz., bovine serum albumin (BSA) and human serum albumin (HAS) [5,6]. The drug, AZ quenched the intrinsic fluorescence of protein and the analysis of results revealed the presence of dynamic quenching mechanism [7]. A simple and sensitive analytical method for simultaneous determination of anastrozole, bicalutamide, and tamoxifen as well as their synthetic impurities, anastrozole pentamethyl, bicalutamide 3-fluoro-isomer, and tamoxifen e-isomer, was developed and validated by using high performance liquid chromatography (HPLC) [8]. A rapid, simple and specific method for estimation of anastrazole in human plasma was validated using Letrozole as internal standard.

Fig 1: Structure of anastrozole



MATERIALS AND METHODS:

EXPERIMENTAL INSTRUMENTATION:

The HPLC system consisted of a LC Waters (Waters, Milford, MA, USA) using a Water's Inertsil ODS C18 150×4.6 mm 5µ column. The system was equipped with a photodiode array detector (Water, 2965 model) and auto sampler (Waters, model 717 plus). Data was processed using Empower Pro software (Waters, Milford, MA, USA). The mobile phase was pumped at a flow rate of 0.000 to 1.500ml/min. RT's were observed for Anisatrazole

CHEMICALS AND REAGENTS

Acetonitrile grade and Milli Q water supplied by Merck and Reference standards of Anastrozole were kindly supplied by Celon laboratories, Hyderabad, India with purity of 90-110%. Sample i.e., Armilon were kindly supplied by Celon laboratories, Hyderabad, India

PREPARATION OF MOBILE PHASE (60:40) V/V

Measure and transfer separately 600ml of Water and 400ml of Acetonitrile to a reagent bottle. Mix well, Sonicate for about 10mins and filter through a 0.45µ membrane filter.

PREPARATION OF NEEDLE WASHES SOLUTION

Measure and transfer separately 500ml of Acetonitrile and 500ml of Water to an appropriately sized reagent bottle. Mix well, Sonicate for about 10 mins.

METHOD DEVELOPMENT AND OPTIMISATION OF CHROMATOGRAPHIC CONDITIONS:

SELECTION OF WAVELENGTH

Selection of wavelength (λ_{max}) should be performed in order to determine the wavelength at which maximum absorbance is observed. The drug solution was scanned at 190 to 700nm [9].The maximum absorbance (λ_{max}) was found at 215nm, which is used for the present study.

MOBILE PHASE:

Mobile Phase was prepared by taking Water and Acetonitrile in the ratio 60:40. Then it was filtered and degassed.

METHOD DEVELOPMENT:

Mobile Phase: Water: Acetonitrile (60:40)

Flow rate: 1.5ml/min

Wavelength: 215nm

Column: Inertsil ODS C18 150×4.6 mm 5µ

Injection volume: 20µl

Mode : Isocratic

Run time: 10 min

PREPARATION OF STANDARD SOLUTION

Transfer accurately about 40mg of standard into a 100ml volumetric flask. Add 50ml of diluent and dissolve. Then make upto the mark with the diluent.Take 5ml of the resulting solution and make upto 50 ml with the diluent to obtain a concentration of 40µg/ml.

PREPARATION OF SAMPLE SOLUTION

Take 20 tablets and crush them. Transfer an amount equivalent to 4 tablets into a 100ml volumetric flask.To it add about 50ml of diluent and sonicate for about 15minutes. Then dilute it upto the mark with the diluent to obtain a concentration of 40µg/ml.

PREPARATION OF PLACEBO

Weigh and transfer accurately placebo equivalent to 4mg of Anastrozole into a 100ml volumetric flask. Add about 50ml of diluent and sonicate for about 15minutes with occasional stirring. Make the solution upto the mark with the diluent.

PREPARATION OF STOCK SOLUTION

Weigh and transfer accurately 40mg of Anastrozole standard into a 100ml volumetric flask. To it add about 70ml of diluent to dissolve and make upto the mark with the diluent and sonicate well.

QUANTITATIVE DETERMINATION OF THE DRUG BY USING THE DEVELOPED METHOD

SAMPLE: ANASTROZOLE

VALIDATION

Typical validation characteristics which should be considered are Linearity, Accuracy, Precision, and Robustness. After method development, the validation of the current method has been performed in accordance with USP requirements for assay determination which include accuracy, precision, selectivity, linearity, range, robustness and ruggedness.[10,11]

Accuracy Formula:

Percentage recovery = amount recovered /actual amount added X 100

Acceptance criteria

Percentage recovery in all the cases should be between 98.0 and 102.0 %.

Precision:

- System Precision
- Method Precision
- Intermediate Precision

% RSD Formula: $(\sigma / \mu) \times 100$

Specificity: Blank, placebo solution, standard and sample solutions were injected into the HPLC system. Anastrazole was homogenous and there was no interference at the retention time.

RESULTS

ACCURACY: The accuracy of a method is the closeness of the measured value to the true value for the sample. Accuracy is usually determined by recovery studies.

ROBUSTNESS

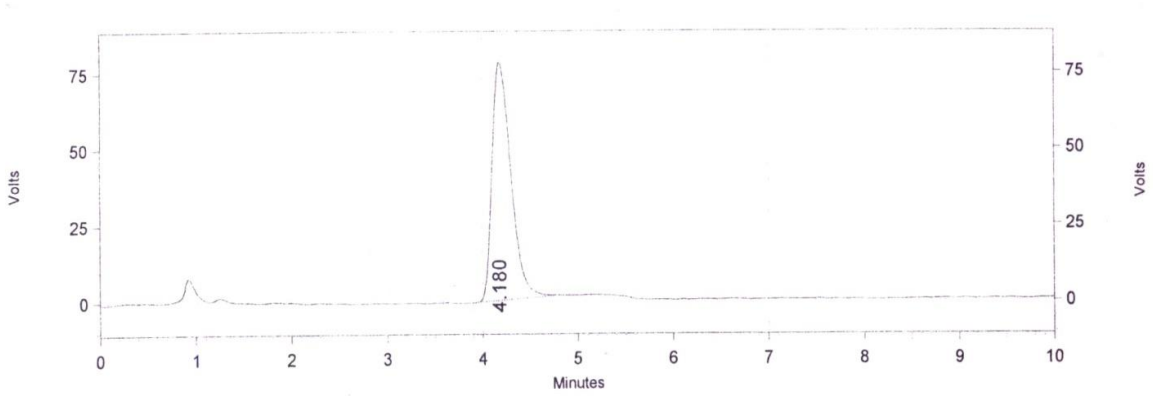
To establish the robustness of the HPLC method employed for analysis of Anastrozole tablets, the method was challenged for various parameters like effect of mobile phase flow, effect of wavelength change and effect of mobile phase composition change [12-14]. The observations in different conditions.

PRECISION

Repeatability: Six preparations of sample were prepared and injected into HPLC system.

SUMMARY AND DISCUSSION

Analytical method development and Validation has been developed for the determination of assay of Anastrazole dosage form was performed for the parameters including-specificity, linearity and range, precision (system precision, method precision), intermediate precision (ruggedness), accuracy and Robustness. The summary of results obtained is appended. The present work was developed and validated a HPLC method with PDA detector for the assay of Anastrazole to be employed in routine and stability tests [15,16].



Peak shape was good. The peak is sharply resolved with less tailing and hence the trial-4 method is optimized for analysis.

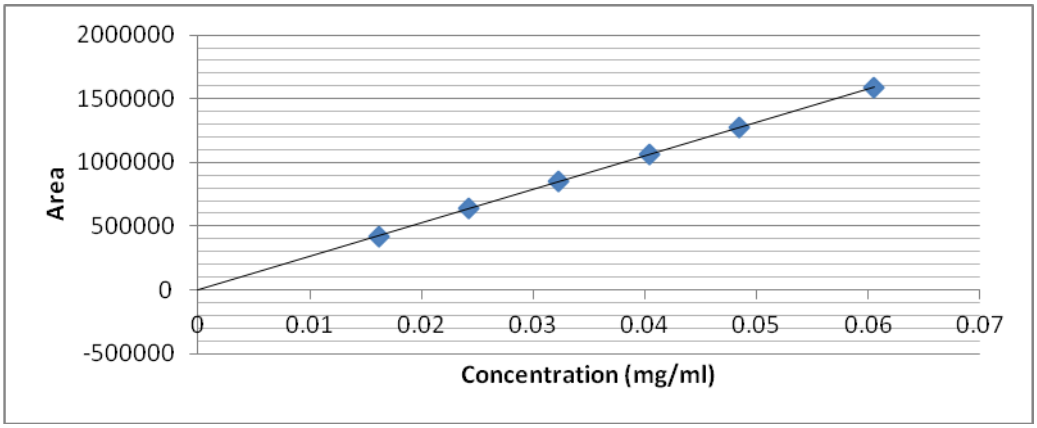


Fig 2: Linearity plot of Anastrozole

Table 1: Accuracy of Anastrozole

Spiked Level	Peak Area	Amount added (µg/ml)	Amount found (µg/ml)	% Mean recovery
80% Sample-1	854915	33.17	32.63	98.4
80% Sample-2	852592	33.17	32.54	98.1
80% Sample-3	851810	33.17	32.51	98.0
100%Sample-1	1065996	41.46	40.69	98.1
100% Sample-2	1099117	41.46	41.95	101.2
100%Sample-3	1091662	41.46	41.67	100.5
120%Sample-1	1323962	49.75	50.53	101.6
120%Sample-2	1274932	49.75	48.66	97.8
120%Sample-3	1278943	49.75	48.81	98.1
Mean	99.1			
Std deviation	0.89			
%RSD	0.89			

Table 2: Retention times of Anastrozole Standard and Sample

Name	Retention time	
	Standard	Sample
Anastrazole	4.180	4.207

Table 3: Results for System suitability of Anastrozole

S.No	Anastrazole			
	Area	Retention time	Tailing factor	Plate count
1	1061774	4.34	1.03	3245
2	1057836	4.30	1.04	3492
3	1054690	4.30	1.08	3884
4	1065825	4.36	1.04	3126
5	1045304	4.29	1.10	3379
Mean	1057086			
%RSD	0.74			

Table 4: Repeatability results of Anastrozole

Sample No.	% Assay
1	99.9
2	101.0
3	99.0
4	100.4
5	100.1
6	100.7
Mean	100.2
Standard deviation	0.75
% RSD	0.75

Table 5: Effect of change in flow rate (±0.1ml of Actual flow) & wavelength (±2nm)

PARAMETERS	ACCEPTANCE CRITERIA	RESULTS
Linearity and Range	Correlation coefficient should not be less than 0.999 over working range	Correlation coefficient = 0.9991
Accuracy	Recovery at each level and % mean recovery should be between 100% to 150% with % RSD should not be more than 2.0%	Mean% recovery =99.1% %RSD=0.89
Specificity	There shouldn't be interference from blank and main peak. (Active)	There is no interference from blank and sample peak
Precision (Repeatability) system & method precision	% RSD should not be more than 2.0%	Mean=100.2 %RSD=0.75
Intermediate precision	% RSD value is not more than 2.0	%RSD between two analysts = 1.02

In the method development of Anastrazole project work carried out by incorporating the Reverse phase High Performance Liquid Chromatography (HPLC). Finally the method was developed & validated according to ICH guidelines for its various parameters.

CONCLUSION

The method was validated for system suitability, linearity, precision, accuracy, specificity, ruggedness, robustness and stability in analytical solution. The system suitability parameters were within limit, hence it was concluded that the system was suitable to perform the assay. The method shows linearity between the concentration ranges of 16-60 µg/ml for Anastrazole. The % Recovery of Anastrazole was found to be 98.2%, 99.9% and 99.2% for accuracy 80%, 100% and 120% samples respectively. As there was no interference due to excipients and mobile phase and good resolution peaks of Anastrazole was found, the method was found to be specific. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in Flow rate, organic composition in mobile phase. Good agreement was seen in the assay results of pharmaceutical formulation by developed method & it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of Anastrazole in Bulk & Pharmaceutical formulations.

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