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# DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF ANTINEOPLASTIC AGENT ANASTROZOLE IN BULK AND TABLET DOSAGE FORM

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## ABSTRACT

*A Simple UV-Spectrophotometric method was developed for the estimation of Anastrozole in bulk and tablet dosage form by using 1N HCl. The maximum absorbance (λmax) was found to be 205.9 nm. The calibration curve was in concentration range 8-18 µg/ml with correlation co-efficient of 0.999. The procedure was validated as per ICH rules for accuracy, precision, detection limit, linearity, reproducibility and quantitation limit. The percentage recovery for Anastrozole was found to be 99.80% to 100.10 %. Due to its simplicity, rapidness, high precision and accuracy of the method it may be used for determining Anastrozole in bulk and tablet dosage form.*

***Keywords*** *– Anastrozole, UV- Spectrophotometry, ICH*

## 1. INTRODUCTION

Anastrozole chemically known as 2-[3(1-cyano-1-methyl-ethyl)-5-(1H-1,2,4-triazol-1-yl methyl) phenyl]-2-methyl-propinenitrile is a potent, nonsteroidal and reversible Aromatase inhibitor. It is useful as adjuvant therapy in early Estrogen receptor positive breast cancer. It is indicated for the treatment of advanced breast cancer in postmenopausal women with disease progression following Tamoxifen therapy and even in patients with Estrogen receptor negative disease [1-2]. Anastrozole is available as 1mg tablet and usually taken once a day with or without food [3-7].

In the present investigation an attempt has been made to develop accurate and precise UV spectrophotometric method for the estimation of Anastrozole in bulk and pharmaceutical formulations. The method is potentially suitable for drug monitoring and determination of pharmacokinetic profiles.

## 2. MATERIALS AND METHODS

### 2.1 Instrument

Spectral and absorbance measurements were made on Shimadzu (Pharmaspec-1700) UV-visible spectrophotometer and systronic-2210 U.V. visible double beam spectrophotometer. Contech digital balance was used for weighing of the sample.

**2.2 Reagents**

1N HCL, double distilled water, Anastrozole standard gifted by Dabur India Limited, Sahibabad, U.P., India were used.

**2.3 Selection of solvent**

Different solvents were tried for obtaining U.V. spectra for Anastrozole. Among the five solvents, 1N HCL shows greater absorbance 0.580 at λmax 205.9 nm. Due to greater absorbance shown by 1N HCL it was chosen as the solvent system for estimation of Anastrozole.

**2.4 Scanning and determination of maximum wavelength (λmax)**

In order to ascertain the wavelength of maximum absorption(λmax) of the drug, solution of drugs (10 µg/ml) in 1N HCL are scanned using spectrophotometer within the wavelength region 200-400 nm against 1N HCL as blank. The resulting spectra were shown in fig. 1 and the absorption curve showed characteristic absorption maxima at 205.9 nm for Anastrozole.

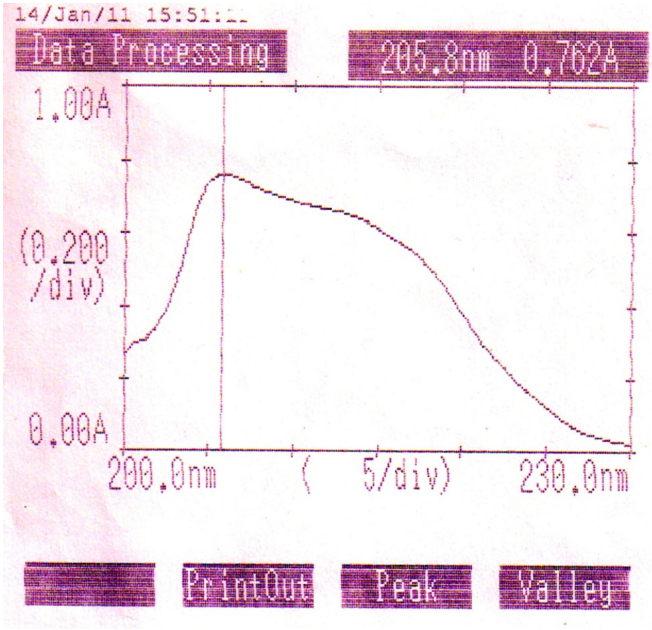


**Fig. 1: UV-Spectrum of Anastrozole**

**2.5 Preparation of working standard solutions**

Standard stock solutions of Anastrozole were prepared by dissolving 5 mg of drug in 10 ml of solvent (1N HCL) to get concentration of 500 µg/ml solutions. From this solution, 1 ml was taken diluted to 10 ml with that solvent to get 50 µg/ml.

**2.6 Construction of calibration curve**

Construction of Beer's Law plot for Anastrozole aliquots were taken separately in 10 ml volumetric flask and the volume was made up to the mark with 1N HCL to prepare a series of solution containing 2-20 µg/ml. The absorbance of all the above solutions were measured at 205.9 nm and the calibration curve was plotted by taking concentration of drug on X-axis and absorbance on Y-axis and was shown in the fig. 2. The drug has obeyed Beer's Law in the concentration range 8-18 µg/ml.

**Table-1: Results of Linearity study**

| Sr. No. | Concentration (µg/ml) | Absorbance |
|---|---|---|
| 1. | 8 | 0.4645 |
| 2. | 10 | 0.5680 |
| 3. | 12 | 0.6795 |
| 4. | 14 | 0.7825 |
| 5. | 16 | 0.8815 |
| 6. | 18 | 0.9790 |

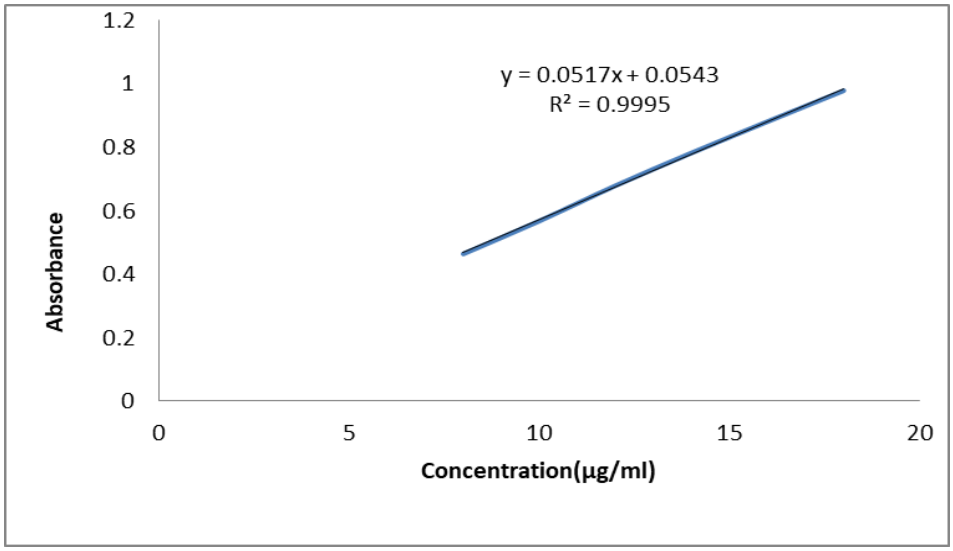


**Fig. 2: Calibration Curve**

**2.7 Estimation of Anastrozole in tablet formulation**

For analysis of commercial formulation content, 20 tablets of brand of Anastrozole were accurately weighed and average weight of powder per tablet were determined separately and mixed thoroughly. Drug equivalent to 1 mg of Anastrozole was accurately weighed and dissolved in 50 ml solvent (1N HCL). Then the solution was sonicated for 30 minutes and filtered. From that solution, 7 ml was taken and diluted to 10ml with that of solvent to get 14µg/ml. Further three dilutions (10-14µg/ml) were made and their absorbances were measured at 205.9 nm and concentration was determined from regression equation of calibration curve. Results of analysis of tablets were shown in Table-2.

**2.8 Validation**

**2.8.1 Precision**

The precision of the proposed method was ascertained by actual determination of eight replicates of fixed concentration of the drug within the Beer's range and finding out the absorbances by the proposed method. From these absorbances Mean, Standard deviation, % RSD and percentage range of errors (at 0.05 and 0.01 confidence limits) was calculated. The readings were shown in Table-3.

**2.8.2 Accuracy**

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%,100% and 120%) of bulk samples of Anastrozole within the linearity range was taken and added to the pre-analyzed tablet formulation of concentration 14 µg/ml for Anastrozole. From that, percentage recovery values were calculated and shown in Table-4.

**2.8.3 Repeatability**

Repeatability is given by inter-day and intra-day precision. Intra-day precision was determined by analyzing the three different concentrations of drug for three times in the same day. Inter-day precision was determined by analyzing the three different concentrations of drug for three days in a week. Results are presented in Table-5. From the data % RSD was determined.

## 3. RESULTS AND DISCUSSION

From the optical characteristics of the proposed method it was found that the drug obeys linearity within the concentration range 8-18 µg/ml. The slope and intercept was found to be 0.051 and 0.054 for 1N HCL. From the precession table for 1N HCL the % RSD value was found to be less than 1% which indicate that the proposed method has found reproducibility. It was found that the percentage recovery values of pure drug from the analyzed formulation was 99.80-100.10 for 1N HCL. The system suitability parameter also reveals that the values were within the specified limits for 1N HCL.

## 4. CONCLUSION

The proposed method was found to be simple, precise, accurate and sensitive. High percentage recovery showed that the method was free from interference of excipients used in the formulation. Values of LOD and LOQ showed that the proposed method was sensitive enough to analyze the drug in bulk as well as in its pharmaceutical formulation. Hence the proposed method renders suitable for routine analysis in quality control laboratories.

**Table-2: Results of analysis of tablets**

| Formulation | Labeled amount of Anastrozole (µg/ml) | Amount obtained (µg) | % of drug present | % RSD |
|---|---|---|---|---|
| Altraz (Alkem) | 1000 | 999.96±0.052 | 99.99 | 0.0052 |

**Each value is average of three determinations ± Standard deviation*

**Table-3: Intraday and Inter-day precision of determination of Anastrozole**

| **Amount taken (µg/ml)** | **8** | **10** | **12** | **14** | **16** | **18** |
|---|---|---|---|---|---|---|
| Intraday variation amount found (µg/ml) | 7.98 | 10 | 12.20 | 14.06 | 16.12 | 17.97 |
| % Found | 99.75 ± 0.0008 | 100.00 ± 0.001 | 101.69 ± 0.002 | 100.49 ± 0.0009 | 100.75 ± 0.0036 | 99.85 ± 0.001 |
| % Bias | -0.250 | 0 | 1.667 | 0.428 | 0.750 | -0.167 |
| % RSD | 0.173 | 0.301 | 0.296 | 0.117 | 0.411 | 0.103 |
| Inter-day variation amount found (µg/ml) | 7.98 | 9.98 | 12.14 | 14.05 | 16.04 | 17.97 |
| % Found | 99.75 ± 0.0008 | 99.80 ± 0.002 | 101.16 ± 0.0027 | 100.35 ± 0.0009 | 100.25 ± 0.0021 | 99.83 ± 0.0015 |
| % Bias | -0.250 | -0.200 | 1.167 | 0.357 | 0.250 | -0.167 |
| % RSD | 0.173 | 0.355 | 0.401 | 0.117 | 0.241 | 0.154 |

**Each value is average of three determinations ± standard deviation*

**Table 4: Recovery study of pure drug using tablet formulation (ALTRAZ, ALKEM LABORATORY LIMITED)**

| Sample ID | Concentration of Anastrozole | | Absorbance of Pure Drug and Formulation | % Recovery of Pure Drug | Statistical Analysis |
|---|---|---|---|---|---|
| | Pure Drug (µg/ml) | Tablet Formulation (µg/ml) | | | |
| S1:80% | 11.2 | 14 | 0.704 | 99.96 | Mean:99.96 |
| S2:80% | 11.2 | 14 | 0.705 | 100.10 | S.D: 0.1143 |
| S3:80% | 11.2 | 14 | 0.703 | 99.82 | % RSD: 0.1143 |
| S4:100% | 14 | 14 | 0.782 | 99.93 | Mean: 99.93 |
| S5:100% | 14 | 14 | 0.783 | 100.06 | S.D: 0.1061 |
| S6:100% | 14 | 14 | 0.781 | 99.80 | % RSD: 0.1062 |
| S7:120% | 16.8 | 14 | 0.860 | 99.91 | Mean:99.98 S.D:0.0518 |
| S8:120% | 16.8 | 14 | 0.861 | 100.02 | %RSD:0.0518 |
| S9:120% | 16.8 | 14 | 0.861 | 100.02 | |

**Table- 5: Results for Repeatability Studies**

| | Inter-day | | Intra-day | |
|---|---|---|---|---|
| Amount taken (µg/ml) | Amount found (µg/ml) | %RSD | Amount found (µg/ml) | %RSD |
| 10 | 9.97 | | 10.00 | |
| 10 | 10.00 | 0.1411 | 10.02 | 0.2942 |
| 10 | 10.00 | | 9.95 | |
| 12 | 12.17 | | 12.12 | |
| 12 | 12.20 | 0.1386 | 12.11 | 0.1024 |
| 12 | 12.21 | | 12.09 | |
| 14 | 14.04 | | 14.06 | |
| 14 | 14.06 | 0.0882 | 14.09 | 0.1002 |
| 14 | 14.07 | | 14.06 | |

**Table - 6: Optical Characteristics and Statistical data**

| Parameters | Anastrozole |
|---|---|
| Absorption Maximum(nm) | 205.9 |
| Beer's law limit(µg/ml) | 8-18 |
| Molar Absorptivity | $0.0561\times10^{4}$ |
| Sandell's sensitivity (µg/cm$^2$/0.001 absorbance unit) | $17.819\times10^{-3}$ |
| % Relative Standard deviation | 0.2335 |
| % Range of error<br>0.05 confidence limit<br>0.01 confidence limit | <br>0.174<br>0.133 |
| Limit of detection (LOD) | 0.0980 |
| Limit of quantitation (LOQ) | 0.3267 |
| Correlation coefficient($R^2$) | 0.999 |
| Slope(m) | 0.051 |
| Intercept(c) | 0.054 |

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