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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 U.V. 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 205.9nm. The calibration curve was in concentration range 8-18µg/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. due its simplicity, rapidness, high precision and accuracy this method may be applied successfully for determining anastrozole in bulk and on tablet dosage formulation. The repeatability study was done for the precision of proposed method. All the results of analysis were validated according to the International Conference on Harmonization (ICH) guideline. This method has been successfully used to determine Anastrozole content in tablets of different origin and bulk mixtures.

KEYWORDS: Anastrozole, Spectrophotometric assay.

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] There is a need for developing newer methods in UV for developing a simple and economic method and so we proceeded with UV and validated as per the ICH guidelines. This thesis deals with the investigation carried out in laboratory on the development and validation of UV spectroscopical method for determination of anastrozole.

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.

EXPERIMENTAL

MATERIALS

Spectrophotometer analysis was carried out on a Shimadzu (pharmaspec-1700) U.V. visible spectrophotometer and systronic-2210 U.V. visible double beam spectrophotometer with spectral bandwidth of 2 nm and a pair of 1cm quartz cells. Pure drug samples of Anastrozole were procured as a gift sample from Dabur India Limited, Sahibabad, U.P. Tablets (Altraz, Alkhem Pvt. Ltd.) containing Anastrozole (1mg) were procured from local market.

METHODS

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.

Preparation of Standard Stock Solution

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 1ml was taken diluted to 10 ml with that solvent to get 50µg/ml.

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

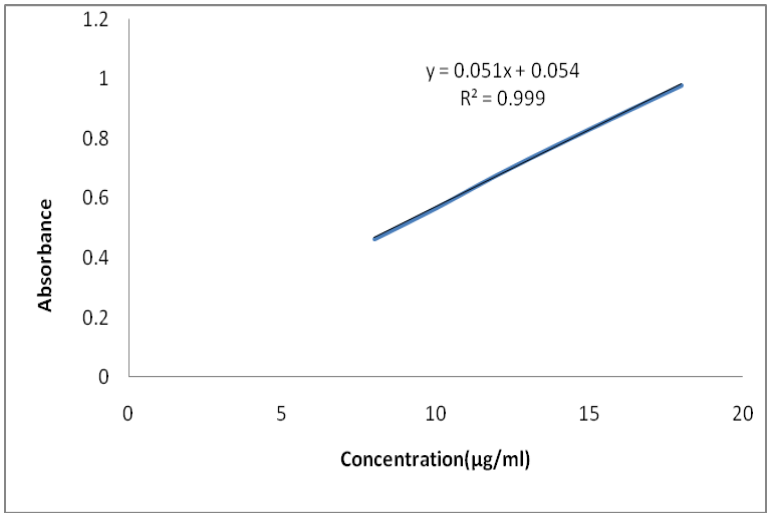
region 200-400 nm against 1N HCL as blank. The resulting spectra were shown in fig-1 and the absorption curve showed characteristic absorption maxima at 205.9 nm for Anastrozole.

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 2-20µg/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 8-18µg/ml. Results of analysis of tablets were shown in Table-1.

Table 1: linearity table of anastrozole.

Sl. 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



Preparation and Analysis of Tablet Sample

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 50ml solvent (1N HCL). Then the solution was sonicated for 30 minutes and filtered. From that solution 7ml 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.

Table 2: Results of analysis of tablets.

Formulation	Labeled amount of Anastrozole(µg/ml)	Amount obtained(µg)	% of drug present	% RSD
Altraz (Alkem)	**1000**	**998.93±0.051**	**99.97**	**0.0052**

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

Validation of Method

The method was validated in terms of linearity, accuracy, precision, specificity and reproducibility of the sample applications. The linearity of the method was investigated by serially diluting the stock solution of Anastrozole and measuring the absorbance values at 205.9 nm. Calibration curves were constructed by plotting absorbances against concentrations of drug in µg/ml.

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.

Table 3: Intraday and Interday 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
Interday 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**

For validity and reproducibility of the proposed method, recovery studies were carried out. A known amount of the standard drug was added to pre-analyzed tablet solution sample, at three levels (80%, 100%, 120%) and the resulting solutions were analyzed by the proposed method. Percentage recoveries were calculated and results are presented in Table 4.

Table 4: Recovery study of pure drug using tablet formulation.

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 S.D:0.1143 %RSD:0.1143
S2:80%	11.2	14	0.705	100.10	
S3:80%	11.2	14	0.703	99.82	
S4:100%	14	14	0.782	99.93	Mean:99.93 S.D:0.1061 %RSD:0.1062
S5:100%	14	14	0.783	100.06	
S6:100%	14	14	0.781	99.80	
S7:120%	16.8	14	0.860	99.91	Mean:99.98 S.D:0.0518 %RSD:0.0518

Repeatability is established by inter-day and intra-day precision. Intra-day precision was determined by analyzing, the three different concentration of drug for three times on the same day. Inter-day precision was determined by analyzing the three different concentration of the drug for three days in the same week. The results are presented in Table 4.

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	0.1411	10.00	0.2942
10	10.00		10.02	
10	10.00		9.95	
12	12.17	0.1386	12.12	0.1024
12	12.20		12.11	
12	12.21		12.09	
14	14.04	0.0882	14.06	0.1002
14	14.06		14.09	
14	14.07		14.06	

RESULTS AND DISCUSSION

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. Anastrozole is available as 1mg tablet and usually taken once a day with or without food. Spectral and absorbance measurements were made on Shimadzu(pharmaspec-1700) U.V. visible spectrophotometer and systronic-2210 U.V. visible double beam spectrophotometer. Contech digital balance was used for weighing of the sample. From the optical characteristics of the proposed method it was found that the drug obeys linearity within the concentration range 8-18µg/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. The results are presented in Table 6.

Table 6: Optical Characteristics and Statistical data.

Parameters	**Anastrozole**
Absorption Maximum(nm)	205.9
Beer's law limit(µg/ml)	8-18
Molar Absorptivity	0.0561×10^{4}
Sandell's sensitivity(µg/cm^2/0.001 absorbance unit)	17.819×10^{-3}
% Relative Standard deviation	0.2335
% Range of error 0.05 confidence limit 0.01 confidence limit	 0.174 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

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.

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