UV SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF ANASTRAZOLE BULK AND TABLET DOSAGE FORM BY DERIVATIVE SPECTROSCOPY

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Two simple and sensitive spectrophotometric methods (Method A and Method B) were developed for the estimation of Anastrazole in pharmaceutical formulations. The method allows rapid analysis of binary pharmaceutical formulation with accuracy. Analysis was validated by statistically and recovery studies which was found satisfactory. The method describes simultaneous determination of Anastrazole dosage form. UV spectrophotometric method involves first derivative and Absorption Maxima spectroscopy using 393 nm & 358 nm as Method A and Method B respectively. For spectrophotometric method, Ferric Chloride was used as a solvent. Linearity was observed in concentration range of 5-40 µg/ml of Anastrazole .

1. Introduction
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Anastrazole 2 ‐ [3(1 ‐ cyano ‐ 1 ‐ methyl ‐ ethyl) ‐ 5 ‐ (1H ‐ 1, 2, 4 ‐ triazol ‐ 1 ‐ yl

methyl) phenyl] ‐ 2 ‐ methyl ‐ propinenitrile is a potent and selective non‑steroidal aromatase inhibitor [1]. Anastrazole is available as 1 mg tablet and it is usually taken once a day with or without food [2 ‐ 6]. As per the literature survey it is revealed that very few analytical methods for the separation and estimation anastrazole have been reported such as UV ‐ Spectrophotometer method [7]. An analytical method for quantification of drug in human plasma by HPLC, stress stability behaviour, LC assay method and LC ‐ MS/MS for low dose anastrazole tablets were developed and reported [8].

2. Material and methods

UV Visible spectrophotometer (Shimadzu Model 1601) was employed with spectral bandwidth of 1 nm and wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells). All chemicals used in this study were analytical grade and used without further purification. $FeCl_3$ 6H2O (0.8%) and 1×10^{-3} M solution in ethanol standardized against standard KMnO4 after reduction [9].

2.1 Preparation standard solution

Accurately transfer volumes of standard drug solution in ethanol (1mg/ml) equivalent to 0.4-1mg Anastrazole into a series of 10ml volumetric flask add 0.5ml of 0.8% $FeCl_3$ and heated in

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a water bath at 55±3ºC for 30 minutes, cool and complete volume with ethanol. Measure the absorbance of an orange chelate of Anastrazole with Fe (II) at 372 nm against reagent blank. Stock solutions were prepared separately in 0.5 mg Ferric Chloride to obtain 100 μg/ml of all drugs. The five working mixed standard were prepared by dilution of stock solution in same solvent system in concentration range 5-40 μg/ml of Anastrazole.

2.2 Selection of Wavelength

From the standard stock solution further diluted with Ferric Chloride to obtain the concentration of 20μg/ mL each solution were scanned in UV range (200-400 nm) in 1.0 cm cell against solvent blank. The overlain spectrum of drugs so recorded. The study of spectrum reveals that Anastrazole shows a well defined λmax at 387.0 nm.

2.3 Method A: First Order Derivative Spectroscopic method

The term derivative spectroscopy refers to a technique in which the rate of change of spectral intensity with wavelength is the slope of the spectrum is measured. It represents an elegant way of resolving overlapping spectra and has been successfully used for the determination of drugs alone or in mixture. Stock solution of Anastrazole (100 μg/ml) were prepared with distilled water. The solution was scanned in UV-region of 200-500 nm, the spectra was derivatized to first order and measured at 393 nm. The different concentrations from stock solutions were prepared and absorbance were measured was linear with concentration in the range of 5-40μg/ml.The calibration curves for Anastrazole was plotted in the concentration range of 5-40 μg/mL at wavelength 393 nm The concentration of the drug present in the mixture was determined against the calibration curve in quantitation mode.

2.4 Method B: Absorption Maxima Method

This method is based on the measurements of absorbance of Anastrazole at its λ max was found to be 358 nm. Stock solution of Anastrazole (0.5 – 5 ml of 100 μg/ml) were transferred into 50ml volumetric flask and made up to mark with distilled water. The absorbances of resulting solutions were measured at 365 nm using Fe (II) as blank. Calibration curve was plotted by using concentration versus absorbance.

2.5 Analysis of Formulation:

2.5.1 Sample preparation

Twenty tablets was taken for analysis, and average net weight was found out, powdered and equivalent to 50mg of Anastrazole was weighed accurately and dissolved in Fe(II) for method A and B by sonication for 3 minutes.

2.5.2 Assay procedure

The volumetric flask were made up to volume to 50 ml with respective solvents and prepared concentration was 100μg/ml for method A and B, the solutions were made up to the volume with appropriate solvents, then the solutions were centrifuged at 3500rpm for 15 minutes . The volume was made up to mark to get final concentration of 1mg/ml. Frequent shaking given and volume was made up to 100ml mark with FeCl₃. The solution was then filtered through Whattman filter paper #41.This filtrate was diluted suitably with FeCl₃ to get the solution of 100μg/ml concentration. the clear supernatant liquids were taken and The nominal concentration were prepared from calibration graph and the absorbance of the formulation were measured as per the above methods. To ensure the precision of the method, the repeated analysis of formulation were carried out for six times and the amount were calculated. The working solution of drug (100μg/ml) was prepared from standard stock solution in FeCl₃. The absorbance of this solution

was measured and amount of Anastrazole was calculated from the calibration curve. The readings were taken in triplicate. In Method-A, the concentration of Anastrazole was determined by measuring the absorbance of the sample at 338 nm in zero order spectrum modes. By using the calibration curve, the concentration of the sample solution can be determined. Method-B, the concentration of Anastrazole was determined by measuring the absorbance of the sample at 379nm, in first order derivative mode. The results of the tablet analysis were calculated against the calibration curve in quantitation mode. Results of tablet analysis are shown in Table- 1.

Table -1 Results of Analysis of Tablet Formulation.

Method	Label claim	Amount of drug estimated	%Label claim ±SD	Recovery % ±SD
A	1.0	0.997	99.97 ± 0.015	99.99
B	1.0	0.968	99.96 ± 0.032	99.95

3. Validation of the developed methods

The developed methods for simultaneous estimation of Anastrazole validated as per ICH guidelines.

3.1 Accuracy

Accuracy To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels 80%, 100% & 120%.The mean percent recovery for Anastrazole by all the two methods was found in the range of 99.91% to 100.15 %. The results were reported in Table 2.

Table 2- Result of tablet dosage form containing Anastrazole.

Parameters	Method-A	Method-B
Label claim (mg/Tab)	1.0	1.0
Found (mg/Tab)	1.03	0.98
Drug content[a]	99.97	99.94
±S.D	0.032	0.043
%COV	0.12	0.28
SE	0.18	0.26

3.2 Precision

To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Six samples of the tablet formulations were analyzed for the repeatability study. The standard deviation, coefficient of variance and standard error was calculated. The results were reported in Table 3.

3.3 Intermediate Precision (inter-day and intra-day precision)

The intra and inter-day precision was calculated by assay of the sample solution on the same day and on different days at different time intervals respectively. The results are presented in Table 3.

3.4 Linearity

The linearity of measurement was evaluated by analyzing different concentration of the standard solution of Anastrazole. For method A and method B the curve method, the Beer-Lambert's concentration range was found to be 5-40 µg/mL for both Anastrazole.

Table 3.Intraday, Interdays, formulation.

Method	Drug	Intra day precision %COV (n =6)	Interday precision %COV		
			Day 1[a]	**Day 2[a]**	**Day 3[a]**
Method A	1.0	0.864	0.711	0.653	0.488
Method B	1.0	0.795	0.683	0.541	0.437

[a] Mean of Six determinations, COV: Coefficient of variance,

4. Results and discussion

Standard deviation and coefficient of variance for six determinations of tablet sample, by all the methods, was found to be less than ± 2.0 indicating the precision of both the methods. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as % recovery. Percent recovery for DIA and ACE, by all the methods, was found in the range of 99.91 % - 100.15 %, values of standard deviation and coefficient of variation was satisfactorily low indicating the accuracy of both the methods. Based on the results obtained, it is found that the proposed methods are accurate, precise, reproducible & economical and can be employed for routine quality control of Anastrazole in dose tablet formulation.

Analytical validation parameters:

Parameter	Result	
	Method A	**Method B**
Absorption maxima(nm)	393 nm	358 nm
Linearity Range (µg/ml)	5-40	5-40
Standard Regression Equation	$Y = 0.004x + 0.0407$	$Y = 0.008x + 0.0754$
Correlation Coefficient (r2)	0.9998	0.9996
Accuracy (% recovery ±SD)	98.85±0.32	98.77±0.13
LOD (µg/ml)	1.48	6.10
LOQ (µg/ml)	6.32	9.65
%Drug found in tablet formulation	99.93	99.98

5. Conclusion

The proposed methods are economic, simple, sensitive, reproducible and accurate and can be used for the routine analysis of Anastrazole in bulk as well as in its pharmaceutical preparations.

Acknowledgement

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