Analytical Method Development and Validation of Exemestane Tablet by UV Spectrophotometry

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Abstract: The present research work discusses the development and validation of a UV spectrophotometric method for Exemestane. Simple, accurate and cost efficient spectrophotometric method has been developed for the estimation of Exemestane in Tablet dosage form. The optimum conditions for the analysis of the drug were established. The maximum wavelength (λ_{max}) was found to be 246 nm. The percentage recovery of Exemestane was in the 98.7 ± 0.4 . Beers law was obeyed in the concentration range of 2-14 $\mu\text{g/mL}$. Calibration curves shows a linear relationship between the absorbance and concentration. The line equation $y=0.05954x+0.0000$ with r^2 of 0.9938 was obtained. Validation was performed according to ICH guidelines for Linearity, accuracy, precision, LOD and LOQ. The sample solution was stable up to 36 hours. The proposed method may be suitable for the analysis of Exemestane in tablet formulation for quality control purposes.

Key word: Exemestane, validation, precision, accuracy, LOQ, LOD, ICH guidelines.

Introduction

Aromatase is one of the first molecular targets for rational drug development in the treatment of cancer¹. Aromatase is present in breast tissue and intratumoral aromatase is the source of local estrogen production in breast cancer tissues. Inhibition of aromatase is an important approach for reducing growth-stimulatory effects of estrogens in estrogen dependent breast cancer. Aromatase inhibitors are more effective than tamoxifen in post menopausal women with metastatic estrogen dependent breast cancer². Exemestane is a third generation steroidal inhibitor of aromatase inhibitor³.

Exemestane is not official in any of the pharmacopoeia and the USP pending monograph was available. Very few methods are available for the estimation of exemestane

and its related products. Burcin Yavuz *et al.*, (2007) were developed HPLC method for the determination of exemestane in oral anticancer drugs⁴. S Persiani *et al.*, (1995) was performed the determination of exemestane in biological fluids by HPLC followed by radio-immune assay⁵. Most of these methods are uneconomic and involving complex sample preparation⁶⁻⁸. So, there is a need for the development of simple sensitive effective and economic methods and hence the present work was planned to validate the UV spectroscopic method for exemestane in tablet formulations by using following parameter like accuracy, precision, linearity and range, limit of detection, limit of quantification, specificity, robustness, ruggedness and system suitability as per ICH guidelines⁹⁻¹². The aim of present work is to find out a simple, sensitive, specific, spectrophotometric method for the detection of Exemestane in pharmaceutical tablet formulation.

Experiment

Exemestane (Gift sample by Tocris bioscience (Missouri, USA), Methanol A.R grade were purchased from Qualigens Fine Chemicals, New Delhi. UV-Visible double beam spectrophotometer (UV-1800, SHIMADZU Limited, Japan) with 1cm matched quartz cells, Micropipette of Variable volume 10-1000 μL (Gene Pete Co.) and Digital balance (Axis LC)

Method Development

Standard Exemestane (10 mg) was accurately weighed and transferred to 10 mL volumetric flask. It was dissolved properly and diluted up to the mark with methanol to obtain concentration of 1 mg/mL. This solution was used as working standard solution. From this solution, by suitably dilution, 5 $\mu\text{g/mL}$ concentrations was obtained and used as standard solution. For calibration curve, dilution was made from 100 $\mu\text{g/mL}$. The absorbance of the solutions containing Exemestane at 5 $\mu\text{g/mL}$ was determined in the UV range 200 - 400 nm using an appropriate blank. The λ_{max} was found to be 246nm (Table 1). At these wavelength maxima, calibration curve was drawn by plotting graph between absorbance and concentrations (Figure 1).

Figure 1. Absorption Maxima of Standard Exemestane.

Table 1. Calibration Curve Parameter.

Concentration $\mu\text{g/mL}$	Absorbance
2	0.119
4	0.239
6	0.347
8	0.437
10	0.622
12	0.704
14	0.850

Validation of Method Parameters

Linearity

The aliquots of concentration ranging 2-14 $\mu\text{g/mL}$ were prepared in triplicate, but linearity was found to be between 2-10 $\mu\text{g/mL}$ concentrations. The linearity was calculated by the least square regression method (Table 2).

Table 2. Validation Parameters.

S. No	Parameter	Result
1.	Absorption Maxima(nm)	246
2.	Linearity Range ($\mu\text{g/mL}$)	2-14
3.	Standard Regression Equation	$y=0.05954x+0.0000$
4.	Correlation Coefficient (R^2)	0.9938
5.	Accuracy(% Recovery $\pm$ SD)	98.7 $\pm$ 0.4
6.	LOD $\mu\text{g/mL}$	1.14
7.	LOQ $\mu\text{g/mL}$	3.47

Precision

The precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day) and reported as RSD %. For this, 3.5 $\mu\text{g/mL}$, 5.5 $\mu\text{g/mL}$ and 7.5 $\mu\text{g/mL}$ concentration solution was measured three times in day and RSD% was calculated. (Table 3)

Table 3. Results of Recovery and Precision.

Ingredient	Amount of drug from formulation $\mu\text{g/mL}$	Amount Added $\mu\text{g/mL}$	Amount Recovered $\mu\text{g/mL}$	% Recovery*	Precision (Intra Day)*	Precision (Inter Day)*
Exemestane	4	3.5	3.504	99.88	0.732	0.480
Exemestane	4	5.5	5.447	99.03	0.470	0.777
Exemestane	4	7.5	7.525	100.03	0.223	0.932
* Percentage RSD of Three Samples.						

Accuracy (Recovery)

The accuracy of the method was evaluated through standard addition method. In this, known amount of standard Exemestane was added in pre-analyzed sample. This was done for 3.5 $\mu\text{g/mL}$, 5.5 $\mu\text{g/mL}$ and 7.5 $\mu\text{g/mL}$ and in triplicate. (Table 3)

Stability

The standard stock solutions of Exemestane concentration 5.5 $\mu\text{g/mL}$ was subjected to heat treatment on 40 $^{\circ}\text{C}$, 50 $^{\circ}\text{C}$ and absorbance were measured. The absorbance for 40 $^{\circ}\text{C}$ for 1hr was same while for 50 $^{\circ}\text{C}$, the absorbance was decreasing which was indicative that Exemestane is stable at 40 $^{\circ}\text{C}$ and but at 50 $^{\circ}\text{C}$ Exemestane solutions unstable.

Sample Preparation

The proposed method was applied to analyze commercially available Exemestane tablets. The tablet was having content of Exemestane equivalent to 80 mg. Twenty tablets were weighed and weight equivalent to 80 mg was dissolved in methanol. By frequent shaking volume was made up to mark methanol. The solution was then filtered through Whatman filter paper #41. The filtrate was diluted suitably with solvent to get the solution of 4 $\mu\text{g/mL}$ concentration. The absorbance was measured against solution blank. Amount of Exemestane was calculated from the calibration curve. The readings were taken in triplicate by performing the same experimentation in three times.

Result and Discussion

In the start of the method development for this drug, different solvents were tested such as ethanol, chloroform, acetone, 0.1N HCl, 0.1N NaOH and methanol. Due to greater solubility and reproducible readings of maximum absorbance, methanol was taken under consideration for further work. By serial dilution of 100 $\mu\text{g/mL}$, the different dilutions of standard drug having concentration 2, 4, 6, 8, 10, 12 and 14 $\mu\text{g/mL}$ were prepared and calibration curve was prepared by plotting graph between absorbance and concentration ($\mu\text{g/mL}$) (Figure 2). The results of linearity are presented in table 2. The data was statistically validated by means of least square regression method. The detection and quantization limits as LOD ($k=3.3$) and LOQ ($k=10$) were calculated and these were found to be 1.14 $\mu\text{g/mL}$ and 3.47 $\mu\text{g/mL}$ respectively. The precision (measurements of intraday and interday) results showed good

reproducibility with percentage relative standard deviation (% RSD) is below 2.0. This indicated that method is highly precise.

Table 4. Determinations of Active Ingredients in Tablets.

Sample	Label Claim	Amount Found mg /Tab*.	% Labelled Claim*
Exemestane	25	24.7	98.87
* Average of Three Determinations.

Standard Table							
	Sample ID	Type	Date	Time	Conc	WL246.0	WgtFactor
1	STD1	Standard	17-09-2011	16:18:16	2.000	0.119	1.000
2	STD2	Standard	17-09-2011	16:18:27	4.000	0.239	1.000
3	STD3	Standard	17-09-2011	16:23:00	6.000	0.347	1.000
4	STD4	Standard	17-09-2011	16:24:58	8.000	0.437	1.000
5	STD5	Standard	17-09-2011	16:26:18	10.000	0.622	1.000
6	STD6	Standard	17-09-2011	16:27:07	12.000	0.704	1.000
7	STD7	Standard	17-09-2011	16:28:10	14.000	0.850	1.000
8							

Sample Table						
	Sample ID	Type	Date	Time	Conc	WL246.0
1	SAMPLE	Unknown	17-09-2011	16:31:31	4.149	0.247
2						

Figure 2: Calibration Curve of Standard Exemestane.

The evaluation of accuracy of the method was performed by standard addition method. The test solution was prepared as 4µg/mL with the addition of 3.5µg/mL, 5.5µg/mL and 7.5µg/mL concentrations standard solution was added and recovery was found to be 98.7%. This indicated accuracy of proposed method.

The proposed method was also applied for the assay of Exemestane in tablet formulation (in triplicate) and the results as tabulated in Table 4. The results obtained were good agreement with the label claims.

Conclusion

From the results and discussion the method described in this paper for the determination of Exemestane from tablet formulation is simple, accurate, sensitive and reproducible. The proposed methods can be successfully applied for Exemestane assay in tablet dosage forms without any interference in quality control. Analysis of the tablets by this method were reproducible reliable and in good agreement with label claim of the drug.

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