Development of an Analytical Method and Validation of Exemestane Tablet by UV Spectrophotometry

Chapter · January 2023

DOI: 10.9734/bpi/ctcb/v8/9034F

5 authors, including:

Angala Parameswari
ratnam institute of pharmacy
23 PUBLICATIONS **286** CITATIONS

SEE PROFILE

Alagusundaram Muthumanickam
ITM University
55 PUBLICATIONS **869** CITATIONS

SEE PROFILE

Chapter 1
Print ISBN: 978-81-19039-31-9, eBook ISBN: 978-81-19039-30-2

Development of an Analytical Method and Validation of Exemestane Tablet by UV Spectrophotometry

S. Angala Parameswari [a*], M. Alagusundaram [b], Priyanka Keshri [b], Goli Venkateswarlu [b] and M. Venkata Ratnam [c]

DOI: 10.9734/bpi/ctcb/v8/9034F

ABSTRACT

This chapter discusses the development and validation of a UV spectrophotometric method for the estimation of Exemestane tablets. The determination of exemestane in tablet dosage form has been created using a straightforward, accurate, and economical spectrophotometric approach. The ideal circumstances for the drug's analysis were developed. The maximum wavelength (λ max) was found to be 246 nm. The percentage recovery of Exemestane was noticed to be 98.7±0.4. Beers law was obeyed in the concentration range of 2-14 µg/mL. The absorbance and concentration have a linear relationship, according to calibration curves. The line equation y=0.05954x+0.0000 with r^2 of 0.9938 was obtained. Validation was carried out in accordance with ICH guidelines for linearity, accuracy, precision, LOD, and LOQ. The sample solution was stable for 36 hours. The suggested technique may be appropriate for the study of Exemestane in tablet formulation for quality control purposes.

Keywords: Exemestane; UV method; validation; ICH guidelines.

1. INTRODUCTION

Aromatase is one of the first molecular targets identified for rational drug development in cancer treatment [1]. Aromatase is found 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 estrogents in estrogen dependent breast cancer. In

[a] *Department of Pharmaceutical Analysis, Ratnam Institute of Pharmacy, Nellore-524346, Andhra Pradesh, India.*
[b] *School of Pharmacy, ITM university, Gwalior-474001, Madhya Pradesh, India.*
[c] *Department Chemical Engineering, School of Engineering Technology, Mettu University, Oromia Region, Ethiopia.*
**Corresponding author: E-mail: eswarialagusundaram@gmail.com;*

postmenopausal women with metastatic estrogen-dependent breast cancer, aromatase inhibitors are more effective than tamoxifen [2]. Exemestane is a third-generation steroidal inhibitor of aromatase inhibitor [3].

Exemestane is official in USP pharmacopoeia. Exemestane is an irreversible (Type 1), steroidal aromatase inhibitor. Aromatase catalyses the conversion of androgens to estrogens in peripheral tissues. This occurs mainly in adipose tissue, but also in normal and malignant breast tissues and provides the main source of estrogen in postmenopausal women. The goal of hormone therapy in breast cancer is to deprive tumour cells of estrogens, which are implicated in the development or progression of tumours. Maximal estrogen suppression is produced by a 25 mg dose. It occurs after 2-3 days and returns to baseline in 10-14 days [4].

A very few methods are available for the estimation of exemestane and its related products. Burcin Yavuz *et al.,* (2007) were developed analytical method to determine the amount of soluble EXE in phase solubility studies and in vitro dissolution studies of EXE. An HPLC method with UV detection was validated in methanol medium, using a Hichrom Nucleosil 100 C18 column (150mmx4.6mm). EXE peak area was linear (r2 > 0.9996) over the concentration range 2.5 - 50 µg/mL. This validation included specificity, range, linearity, precision, accuracy, LOD and LOQ; all results were acceptable and confirmed that the method is suitable for its intended use [5].

S Persiani et al. was performed the determination of exemestane in biological fluids by HPLC followed by radio-immune assay [6]. Exemestane is extracted from human plasma and urine by solid-phase and liquid-liquid extraction, respectively. The test compound is then isolated from endogenous steroids, its metabolites and/or degradation products by HPLC. The exemestane containing fraction is collected and its exemestane content measured by radioimmunoassay (RIA) [6].

Konda B et al. [7]. Development and validation of stability indicating method for the determination of exemestane by reverse phase high performance liquid chromatography. Chromatographic separation of exemestane and its degraded products were achieved by using C18 reverse phase column (Phenomenex, size: 250 × 4.60 mm, particle size 5 µm) at ambient temperature with isocratic elution at a flow rate of 1.0 mL/min. The mobile phase used for the analysis was acetonitrile-water (60:40, %v/v) with UV visible detection at 242 nm. The RP-HPLC method was used to study the degradation behaviour of drug under various stress conditions as per ICH recommended guidelines. Mukthinuthalapati et al. [8] developed a novel stability indicating liquid chromatographic method for the determination of Exemestane using HPLC system of Shimadzu Model CBM-20A/20 Alite, equipped with PDA and Zorbax SB C18 (150 mm × 4.6 mm i.d., 3.5 µm particle size) column. A mixture of sodium acetate buffer and acetonitrile (30:70, v/v) was used as a mobile phase with 1.0 ml/min flow rate and the method was validated as per ICH guidelines. Forced degradation studies were

performed in different stress conditions such as acidic, basic, oxidation and thermal degradations.

Most of these methods are uneconomic and involving complex sample preparation [9-11]. 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 guidelines [12-15]. The purpose of present work is to find out a simple, sensitive, specific, spectrophotometric method for the detection of Exemestane in pharmaceutical tablet formulation.

2. MATERIALS AND METHODS

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) with1cm matched quartz cells, Micropipette of Variable volume 10-1000 µL (Gene Pete Co.) and Digital balance (Axis LC)

2.1 Method Development

Standard Exemestane (10 mg) was accurately weighed and transferred to 10 mL volumetric flask. It was soluble 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, 5µg/mL concentrations were obtained and used as standard solution. For calibration curve, dilution was made from 100 µg/mL and to obtained range between 2-14 µg/mL. The absorbance of the solutions containing Exemestane at 5 µg/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 (Fig. 1).

3. METHOD VALIDATION

3.1 Linearity

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

3.2 Precision

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

3.3 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 3.5µg/mL, 5.5 µg/mL and 7.5µg/mL and in triplicate (Table 3).

3.4 Stability

The standard stock solutions of Exemestane concentration 5.5µg/mL was subjected to heat treatment on 40^0C, 50^0C and absorbance were measured. The absorbance for 40^0C for 1hr was same while for 50^0C, the absorbance was decreasing which was indicative that Exemestane is stable at 40^0C and but at 50^0C Exemestane solutions unstable.

3.5 Sample Preparation

The proposed method was applied to analyse commercially available Exemestane tablets. The tablet was having content of Exemestane equivalent to 80 mg. Twenty tablets were weighed, powdered 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 4µg/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.

Table 1. Calibration Curve Parameter

Concentration	Absorbance
2	0.119
4	0.239
6	0.347
8	0.437
10	0.622
12	0.704
14	0.850

Table 2. Validation Parameters

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

Table 3. Results of Recovery and Precision

Ingredient	Amount of drug from formulation µg/mL	Amount Added µg/mL	Amount Recovered µg/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*

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*

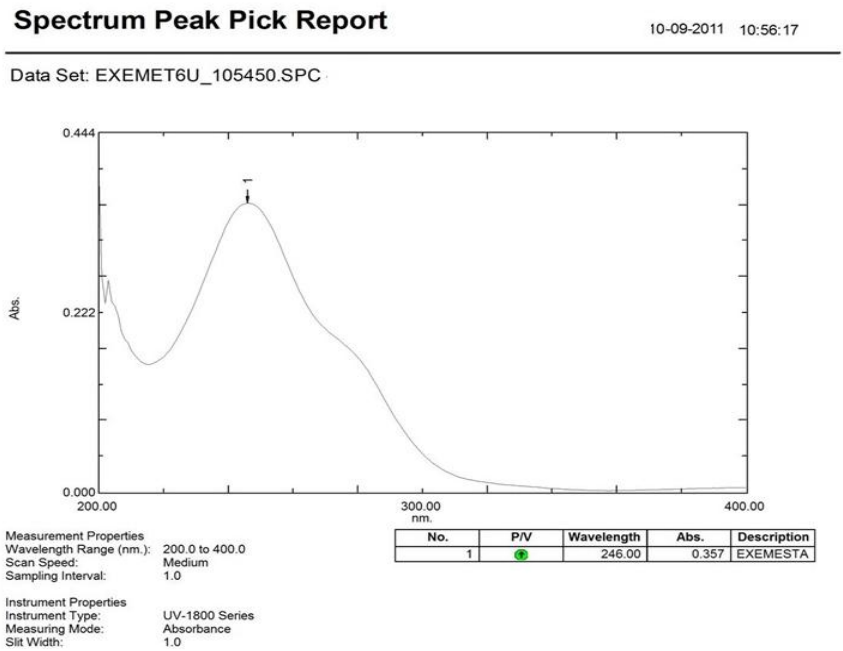
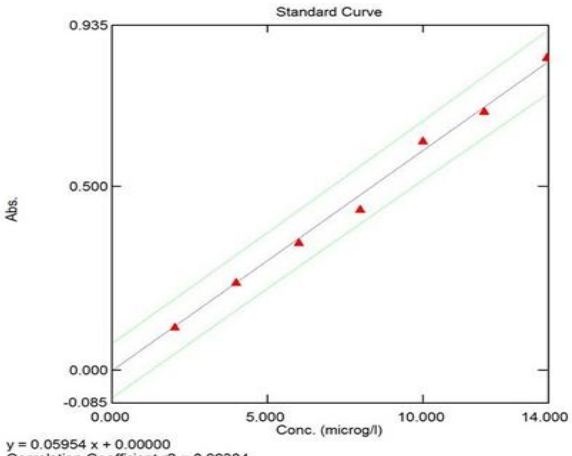


Fig. 1. Absorption Maxima of Standard Exemestane



Standard Table

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

Fig. 2. Calibration Curve of Standard Exemestane

4. RESULTS 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 100µg/mL, the different dilutions of standard drug having concentration 2, 4, 6, 8, 10, 12 and 14µg/mL were prepared and calibration curve was prepared by plotting graph between absorbance and concentration (µg/mL) (Fig. 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 1.14µg/mL and 3.47µg/mL respectively. The precision (measurements of intraday and inter-day) results showed good reproducibility with percentage relative standard deviation (% RSD) is below 2.0. This indicated that method is highly precise.

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.

5. CONCLUSION

The developed and validated method for the estimation of Exemestane from tablet formulation is simple, accurate, sensitive and reproducible by the evident of the results. This present method was successfully applied for Exemestane assay in tablet dosage forms without any interference in quality control. Analysis of the tablets by this method were provides reproducible results with more reliable and in good agreement with label claim of the drug.

ACKNOWLEDGEMENT

I would like to thank BP international for giving me an opportunity to writing a book Chapter. I'd also like to thank Annamacharya college of pharmacy to provide facility to complete this research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Smith IE, Dowsett M. Aromatase inhibitors in breast cancer. N Engl J Med. 2003;348(24):2431-42.
   DOI: 10.1056/NEJMra023246. PMID: 12802030
2. Brueggemeier RW. Aromatase, aromatase inhibitors, and breast cancer. Am J Ther. 2001;8(5):333-44.
   DOI: 10.1097/00045391-200109000-00007. PMID: 11550075
3. Susana M. Campos, Aromatase Inhibitors for Breast Cancer in Postmenopausal Women, The Oncologist.2004;9(2):126-36.
   Available: https://doi.org/10.1634/theoncologist.9-2-126.
4. Njar VC, Brodie AM. Comprehensive pharmacology and clinical efficacy of aromatase inhibitors. Drugs 1999;58(2):233-55.

5. Yavuz, B; Bilensoy, E; Şumnu, M, Analytical method validation for HPLC Assay of Oral Anticancer drug Exemestane. Fabad Journal of Pharmaceutical Sciences. 2007;32:15-22.
6. Persini S, Broutin F,Cicioni P,Stefanini P,Benedetti MS. Determination of the new aromatase inhibitor exemestane in biological fluid by automated high-performance liquid chromatography followed by radioimmunoassay. European Journal of Pharmaceutical Sciences. 1996;4(6):331-340.
7. Konda B, Tiwari RN, Fegade H. Development and validation of stability indicating method for the determination of exemestane by reverse phase high performance liquid chromatography. J Chromatogr Sci. 2011;49(8):634-9.
DOI: 10.1093/chrsci/49.8.634. PMID: 21859539
8. Mukthinuthalapati MA, Bukkapatnam V. A Novel Validated Stability-Indicating RP-HPLC Method for the Determination of Exemestane (Steroidal Aromatase Inhibitor). J Bioequiv Availab. 2015;7:288-292. doi:10.4172/jbb.1000256.
9. Per E. Lonning, Jurgen Geisler, Lars E. Krag, Bjørn Erikstein, Yngve Bremnes, Anne I. Hagen Ellen Schlichting, Ernst A. Lien, Erik S. Ofjord, Jolanda Paolini, Anna Polli, and Giorgio Massimini. Effects of Exemestane Administered for 2 Years Versus Placebo on Bone Mineral Density, Bone Biomarkers, and Plasma Lipids in Patients with Surgically Resected Early Breast Cancer. Journal of Clinical Oncology. 2005;23(22):5126-5137.
DOI:10.1200/JCO.2005.07.097.
10. Cenacchi V, Barattè S, Cicioni P, Frigerio E, Long J, James C. LC-MS-MS determination of exemestane in human plasma with heated nebulizer interface following solid-phase extraction in the 96 well plate format. J Pharm Biomed Anal. 2000;22(3):451-60.
DOI: 10.1016/s0731-7085(00)00235-1. PMID: 10766362
11. Giuseppe corona, Caterina Elia, Bruno Castta, Crivellari Diana, Sara Rosalen, Mario Bari and Giuseppe Toffoli. A liquid chromatography-tandem mass spectrometry method for the simultaneous determination of exemestane and its metabolite 17-dihydroexemestane in human plasma.J Mass Spectrom. 2009; 44(6):920-928.
Available: https://doi.org/10.1002/jms.1566.
12. Vessman J. Selectivity or specificity? Validation of analytical methods from the perspective of an analytical chemist in the pharmaceutical industry. J. Pharm and Biomed. Anal. 1996; 14:867 – 869.
13. ICH, QIA Stability Testing of New Drug Substances and Products, Int. Conf on Harmonization, Geresa, November; 1996.
14. International conference on Harmonisation, Guidance for Industry in Q2B validation on analytical procedures: methodology. Switzerland IFPMA. 1996;1-8.
15. Wegscheider, Validation of Analytical Methods, in: Accreditation and Quality Assurance in Analytical Chemistry, H. Guenzler, Springer (Ed.) Verlag, Berlin; 1996.

Biography of author(s)

Prof. S. Angala Parameswari
Department of Pharmaceutical Analysis, Ratnam Institute of Pharmacy, Nellore-524346, Andhra Pradesh. India.

Research and Academic Experience: She is a Professor, Department of pharmaceutical analysis, Ratnam institute of pharmacy, Nellore. Andhra Pradesh, India. She has 15 years of Experience.

Research Area: Her Research Area includes Analytical method development, validation and interpretation of analytical data.

Number of Published Papers: She has 30 Published papers.

Special Award: She received awards of Best teacher in 2015 by AKSHARA KEERTHI PATTABHADRULA SAMKSHEMA SEVA SAMITHI, Nellore, Andhra Pradesh.

Any other Remarkable Point(s): She Guided 4 Ph.D students, 44 PG students and 25 UG students. She is a Life member in APTI.

Dr. M. Alagusundaram
School of Pharmacy, ITM university, Gwalior-474001, Madhya Pradesh, India.

Research and Academic Experience: He has 18 years of research experiences.

Research Area: Drug delivery systems.

Number of Published Papers: He has published 58 papers in national journal and international journal of repute.

Special Award: He received Vidyaratna and Best teacher in 2015 by AKSHARA KEERTHI PATTABHADRULA SAMKSHEMA SEVA SAMITHI, Nellore, Andhra Pradesh.

Any other Remarkable Point(s): He Guided 10 Ph.D students, 52 PG students and 32 UG students. Life member of APTI.

Mrs. Priyanka Keshri
School of Pharmacy, ITM University, Gwalior-474001, Madhya Pradesh, India.

Research and Academic Experience: She has 4 Years of Experience.

Research Area: Drug delivery system.

Number of Published Papers: She has 8 Published papers.

Special Award: She received Gold Medalist award in M. Pharm.

Dr. Goli Venkateswarlu
School of Pharmacy, ITM university, Gwalior-474001, Madhya Pradesh, India.

Research and Academic: He has 16 years of research Experience.

Research Area: His research area includes in Biotechnolgy, Phytochemistry and Pharmacology.

Number of Published Papers: He has published 65 papers in national and international journal of repute.

Special Award: He received Best Researcher in 2015 by Grabs educational society, Chennai, Tamil Nadu.

Any other Remarkable Point(s): He Guided 2 Ph.D students, 23 PG students and 60 UG students. Life member of APTI.

Dr. M. Venkata Ratnam
Department Chemical Engineering, School of Engineering Technology, Mettu University, Oromia Region, Ethiopia.

Research and Academic Experience: He is an Associate Professor,
Department of Chemical Engineering,
Mettu University, Ethiopia
He has 15 years of Experience.

Research Area: His Research Area includes Wastewater treatment, Process modeling, and simulation using simulation tools.

Number of Published Papers: He has 35 Published papers.

DISCLAIMER
This chapter is an extended version of the article published by the same author(s) in the following journal.
Analytical Method Development and Validation of Exemestane Tablet, 9(4): 2068-2073, 2012.

Peer-Review History: During review of this manuscript, double blind peer-review policy has been followed. Author(s) of this manuscript received review comments from a minimum of two peer-reviewers. Author(s) submitted revised manuscript as per the comments of the peer-reviewers. As per the comments of the peer-reviewers and depending on the quality of the revised manuscript, the Book editor approved the revised manuscript for final publication.