Journal of
Bioequivalence & Bioavailability
ISSN: 0975-0851

Mukthinuthalapati and Bukkapatnam, J Bioequiv Availab 2015, 7:6
DOI: 10.4172/jbb.1000256

Research Article Open Access

A Novel Validated Stability-Indicating RP-HPLC Method for the Determination of Exemestane (Steroidal Aromatase Inhibitor)

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Abstract

Background: Exemestane is an active irreversible lipophilic steroidal aromatase inhibitor used to treat breast cancer in addition to surgery and/or radiation in post-menopausal women. It is a white to slightly yellow crystalline powder with a molecular weight of 296.41. Exemestane is freely soluble in N, N-dimethyl formamide, soluble in methanol, and practically insoluble in water. The present robust RP-HPLC method supports the quantitative analysis of Exemestane in pharmaceutical formulations and for carrying out the forced degradation studies.

Methods: A novel stability indicating liquid chromatographic method was developed for the determination of Exemestane using HPLC system of Shimadzu Model CBM-20A/20 Alite, equipped with SPD M20A prominence 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.

Results: The proposed liquid chromatographic method has shown linearity over a concentration range 0.1–200 µg/ml with regression equation y = 59411x - 7316 with correlation coefficient 0.999. During the validation process i.e. the intra-day and inter-day precision studies, accuracy and robustness studies the method has shown an RSD of less than 2.0 %. Exemestane is found to be more stable during all the degradation studies because the percentage of degradation was reported to be less than 10.

Conclusions: The proposed method was found to be precise, accurate and robust and it can be applied for the determination of Exemestane in any formulations.

Keywords: Exemestane; Liquid chromatography; Validation; Stability-indicating; ICH

Introduction

Exemestane is an orally active irreversible lipophilic steroidal aromatase inhibitor used for the therapy of metastatic postmenopausal breast cancer [1-3]. Exemestane (Figure 1) is chemically 6-methylen-androsta-1, 4-diene-3, 17- dione ($C_{20}H_{24}O_2$; 296.403 g/mol). The presence of 1, 2-double bond in the ring of steroid molecule increased the aromatase in-activator affinity for the aromatase enzyme, which indirectly increased the therapeutic potency [4].

Exemestane has been determined by different analytical techniques such as LC-MS [5-9], GC-MS [10], LC-radio immunoassay [11], UV-spectrophotometry [12], HPTLC [13] and UPLC [14].

Breda et al., developed a HPLC method in plasma by high-performance liquid chromatography with ultraviolet detection [15] whereas Burcin Yavuz et al. developed cyclodextrin complexes of Exemestane to improve the solubility and here also the authors used HPLC method using acetonitrile and water mixture where the retention time was observed at more than 10 minutes [16] and [15-16] in pharmaceutical dosage forms and biological fluids. Uday et al. [17], Vijaya, Lakshmi et al. [18] proposed liquid chromatographic methods but they have not performed the stress degradation studies. Suresh Kumar et al. [19] have analysed Exemestane along with four impurities and the robustness was studied using Box-Behnken design.

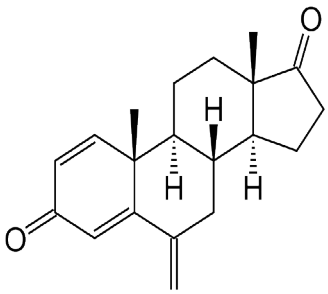

Figure 1: Chemical structure of Exemestane.

Till now only two stability indicating HPLC methods are available in the literature in which Bharath et al. [20] developed a stability indicating method in which UV detector was used and the retention time of Exemestane was at around 7.0 minutes. Mathrusri Annapurna et al. [21] observed the elution of Exemestane at about 4.5 minutes using phosphate buffer and acetonitrile mixture. In the present study the authors have proposed a new simple, economical and robust stability indicating liquid chromatographic method (Isocratic mode) for the determination of Exemestane in pharmaceutical dosage forms (Tablets).

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Received April 28, 2015; **Accepted** November 17, 2015; **Published** November 24, 2015

Citation: Mukthinuthalapati MA, Bukkapatnam V (2015) A Novel Validated Stability-Indicating RP-HPLC Method for the Determination of Exemestane (Steroidal Aromatase Inhibitor). J Bioequiv Availab 7: 288-292. doi:10.4172/jbb.1000256

Materials and Methods

Chemicals and reagents

Exemestane (purity 99%) was supplied as a gift sample from Natco Pharma Ltd. (Hyderabad, India) and was used without further purification. Methanol (HPLC grade), Sodium hydroxide (NaOH) and Hydrochloric acid (HCl), orthophosphoric acid and Hydrogen peroxide (H_2O_2) were purchased from Merck (India). All other chemicals were of analytical grade (Merck) and used as received.

Exemestane is available as tablets (Label claim 25 mg) with brand names X'CEL® (Celon Laboratories Ltd., India) and XTANE® (Natco Pharma Ltd, India) respectively.

Instrumentation

Chromatographic separation was performed on HPLC system of Shimadzu Model CBM-20A/20 Alite, equipped with SPD M20A prominence photodiode array detector by using Zorbax SB-C18 column (150 mm × 4.6 mm i.d., 3.5 µm particle size) as stationary phase for, maintained at 25°C and LC Solutions 1.25 software.

Chromatographic conditions

The mobile phase for the method was performed using Isocratic elution mode of sodium acetate buffer: acetonitrile (30:70, v/v). The flow rate was set as1.0 ml/min. The UV detection was done at 254 nm. 20 µL of sample was injected into the HPLC system. The overall run time was 10 min.

Preparation of sodium acetate buffer (pH 4.0) solution

The buffer solution was prepared by mixing 28.6 ml of glacial acetic acid with 10 mL of 50% w/v NaOH in to a 1000 ml volumetric flask, dissolving and diluting to volume with HPLC grade water.

Preparation of stock solution

Stock solution was prepared by dissolving about 10 mg of Exemestane in a 10 ml volumetric flask with the mobile phase and further dilutions were made with mobile phase (sodium acetate buffer: acetonitrile (30:70, v/v) and all the solutions were filtered through 0.45 µm membrane filter.

Method validation

The method was validated for linearity, limit of quantitation (LOQ), limit of detection (LOD), intra/inter-day precision, accuracy, robustness and specificity.

Linearity

A series of solutions (0.1-200 µg/ml) were made and 20 µL of each was injected in to the HPLC system and the peak area of the chromatogram was noted. A graph was drawn by taking the concentration of the drug on the x-axis and the corresponding peak area on the y-axis.

Limit of quantification (LOQ) and limit of detection (LOD)

The limit of quantification (LOQ) and limit of detection (LOD) were calculated as described in International Conference on Harmonization guidelines Q2 (R1) [22].

Precision study

The intra-day precision and inter-day precision study was performed at three concentration levels (20, 50 and 100 µg/ml) on three different days i.e. day 1, day 2 and day 3 respectively and the % RSD was calculated.

Accuracy study

The accuracy of the method was evaluated at three levels (80, 100 and 120%), and the percentage recoveries were calculated. Standard addition and recovery experiments were conducted for the quantification of Exemestane

Robustness study

The robustness of the assay method was performed to check the sensitivity of the method. In this small changes are made in the HPLC conditions which included wavelength detection range (252 and 256 nm), percentage of acetonitrile in the mobile phase (28 and 72 %), flow rate (0.9 and 1.1 ml/min) and pH (4.1 and 3.9). Robustness studies were done for the method with 100 µg/ml of Exemestane.

Forced degradation studies/specificity

Forced degradation studies were performed to evaluate specificity of the method [23,24] and the forced degradation studies were performed by refluxing the Exemestane drug solution (1 mg/ml) with 0.1 N HCl and 0.1 N NaOH respectively for 30 min at 80°C. The resulting solutions were then neutralized and diluted with mobile phase to give a final concentration of 50 µg/ml. Oxidation was performed using 6 % H_2O_2 solution whereas thermal degradation was performed by exposing the drug solution to 80°C in a thermostat for 30 minutes. Photolysis was performed by exposing the drug solution to UV rays for about 6 hours in the UV chamber (254 nm).

Assay of marketed formulations (Tablets)

Commercially available marketed formulations (Tablets) were procured and finely powdered and powder equivalent to 10 mg of Exemestane was transferred into a 10 ml volumetric flask and acetonitrile was added to make up to volume. The contents were sonicated for 30 minutes and the solution was filtered. The filtrate was further diluted with mobile phase as per the requirement.

Results and Discussion

In the literature only two stability indicating liquid chromatographic methods were available and therefore there is need for the analytical techniques. A more economical and simple stability indicating RP-HPLC method was proposed for the determination of Exemestane in presence of degradation products.

PDA detector is more advantageous rather than a UV detector in the chromatographic study. The peak area or peak height of the chromatogram can be measured at desired wavelength using PDA detector whereas UV detector can support only two different wavelengths. Also the PDA detector gives information about the purity index (peak purity and purity threshold) from which the impurity if any can be identified. The present proposed RP-HPLC method using PDA detector was compared with the previously published methods and summarized in Table 1.

HPLC method development and optimization

The drug samples were analyzed using different mobile phases with different pH and flow rates. Finally mobile consisting of sodium acetate: acetonitrile (30:70, v/v) with flow rate 1.0 ml/min has given a sharp peak with all acceptable system suitability parameters with short

Method/Reagent	Linearity (µg/ml)	Remarks	Ref.
Acetonitrile-water	-	UPLC	14
Acetonitrile: KH_2PO_4 (pH 4.5) (35:65, v/v)	10-1000	HPLC (Plasma) UV detector	15
Acetonitrile: water (44:56, v/v)	2.5-50	HPLC	16
Methanol: phosphate buffer	20-100	HPLC PDA detector (Gradient mode)	17
Water and methanol (50:50, v/v)	25-150	HPLC	18
Water: methanol	25-150	HPLC (Impurities) Box-Behnken design	19
Acetonitrile-water (60:40, v/v)	6 – 14	HPLC UV detector Very low linearity range	20
Phosphate buffer: acetonitrile (pH 4.0) (40:60, v/v)	0.1-200	Stability indicating HPLC PDA detector	21
Sodium acetate buffer: acetonitrile (pH 4.0) (30:70, v/v)	0.1-200	Stability indicating HPLC PDA detector	Present work

Table 1: Comparison of proposed method with the previously published liquid chromatographic methods

retention time (UV detection at 247 nm). The typical chromatogram so obtained for Exemestane was shown in Figure 2.

Beer-Lambert's law was obeyed over the concentration range:

0.1-200 µg/ml (Table 2) with regression equation y = 59411 x + 7315

(r2 = 0.999) (Figure 3)

The LOQ and LOD were determined based on the 10 and 3.3 times the standard deviation of the response, respectively, divided by the slope of the calibration curve. The LOQ is found to be 0.0906 µg/ml and the LOD is found to be 0.0299 µg/ml.

The % RSD in precision studies was found to be 0.29-0.67 and 0.67-0.81 for intra-day and inter-day respectively indicating that the method is precise. The percentage recovery in accuracy studies was found to be 98.52-99.01% with % RSD 0.12-0.22 (less than 2.0) indicating that the method is accurate (Table 3).

In robustness studies, parameters such as flow rate, detection wavelength, pH and mobile composition were slightly altered and the percentage RSD was found to be 0.94-1.80 indicating that the proposed method is robust (Table 4).

Forced degradation Studies/Specificity

Forced degradation studies were conducted for Exemestane with the optimized liquid chromatographic conditions and the typical chromatograms so obtained were shown in Figures 4A-4F. The PDA detector clearly represents the presence of degradants at a particular wavelength in the 3D chromatograms of Exemestane.

Exemestane has shown less than 10 % degradation in all stressed conditions such as 3.61% (acidic), 2.80% (basic), 1.71% (oxidative), 6.88% (thermal) and 1.03% (photolytic) degradation (Table 5). During the oxidation degradation a sharp degradation peak was observed at 1.402 minutes. The pure drug peak obtained with theoretical plates more than 2000 and tailing factor less than 1.5 indicating that the proposed method is selective and specific. So the proposed stability indicating liquid chromatographic method can be applied successfully for the determination of Exemestane in pharmaceutical formulations.

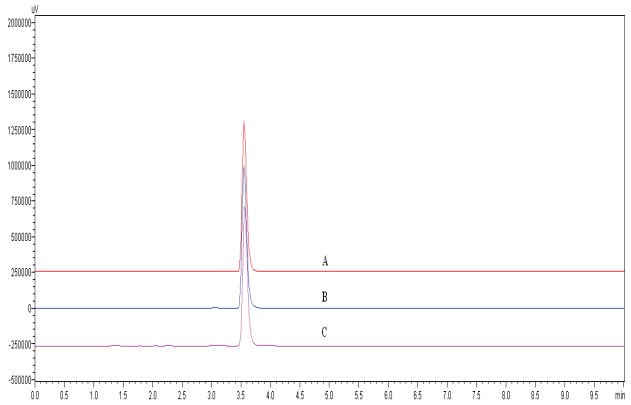

Figure 2: Representative Chromatograms of (A) Exemestane (50 µg/ml), (B) X'CEL® (Label claim: 25 mg) (C) XTANE® (Label claim: 25 mg)

Conc. (µg/ml)	*Mean Peak Area ± SD	RSD (%)
0.1	6097 ± 15.85	0.26
1	58790 ± 199.89	0.34
5	295544 ± 1182.18	0.40
10	611409 ± 3913.02	0.64
20	1240314 ± 4341.10	0.35
50	3025873 ± 3328.46	0.11
100	5935485 ± 17212.91	0.29
150	8725436 ± 32284.11	0.37
200	12024332 ± 73348.43	0.61

*Mean of three replicates

Table 2: Linearity of Exemestane.

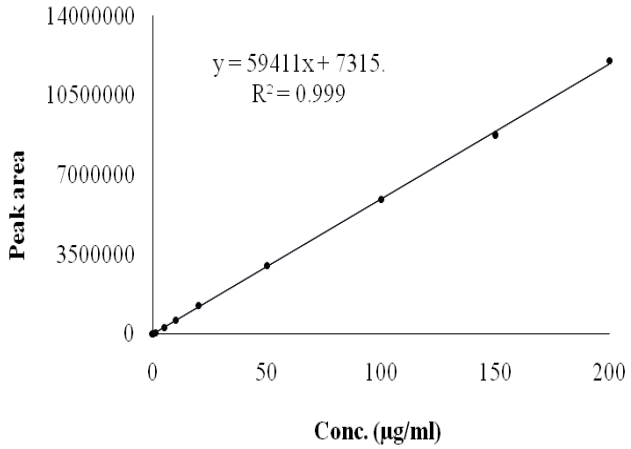

Figure 3: Calibration curve of Exemestane

Application of the proposed method for the analysis of available formulations in the pharmacy store (Tablets)

The proposed method was applied to the determination of Exemestane tablets and the assay was calculated as 94.11-96.13% (Table 6) and no interference was observed with the excipients (Figure 2B-2C).

Conclusion

The developed stability-indicating and RP-HPLC method is

Conc. (µg/ml)	Intra-day precision	Inter-day precision
	*Mean peak area ± SD (%RSD)	*Mean peak area ± SD (%RSD)
20	1244826.33 ± 8375.58 (0.67)	1250654.33 ± 9500.35 (0.76)
50	3043333.00 ± 8686.59 (0.29)	3000259.00 ± 24281.95 (0.81)
100	5878355.33 ± 19598.07 (0.33)	5838815.00 ± 39056.33 (0.67)
Accuracy		
Conc. (µg/ml)	***Mean peak area ± SD (% RSD)**	**Drug Found (µg/ml)**	***Recovery (%)**
18	1060838.67 ± 1314.82 (0.12)	17.73	98.52
20	1183261.67 ± 2533.63 (0.21)	19.79	98.97
22	1301425.67 ± 2866.37 (0.22)	21.78	99.01

*Mean of three replicates

Table 3: Precision and accuracy studies of Exemestane.

Parameter	Condition	*Mean peak area	*Mean peak area ± SD (% RSD)
Flow rate (± 0.1 ml/min)	0.9	5824121	5892413.33±59812.60 (1.02)
	1.0	5935485	
	1.1	5917634	
Detection wavelength (± 2 nm)	252	5942156	5971042.00±55908.86 (0.94)
	254	5935485	
	256	6035485	
Mobile phase composition phosphate buffer: acetonitrile (± 2 %, v/v)	28:72	6014897	5924915.33±95705.25 (1.62)
	30:70	5935485	
	32:68	5824364	
pH (± 0.1 unit)	3.9	5837459	5832885.67±104960.75 (1.80)
	4.0	5935485	
	4.1	5725713	

*Mean of three replicates

Table 4: Robustness study of Exemestane.

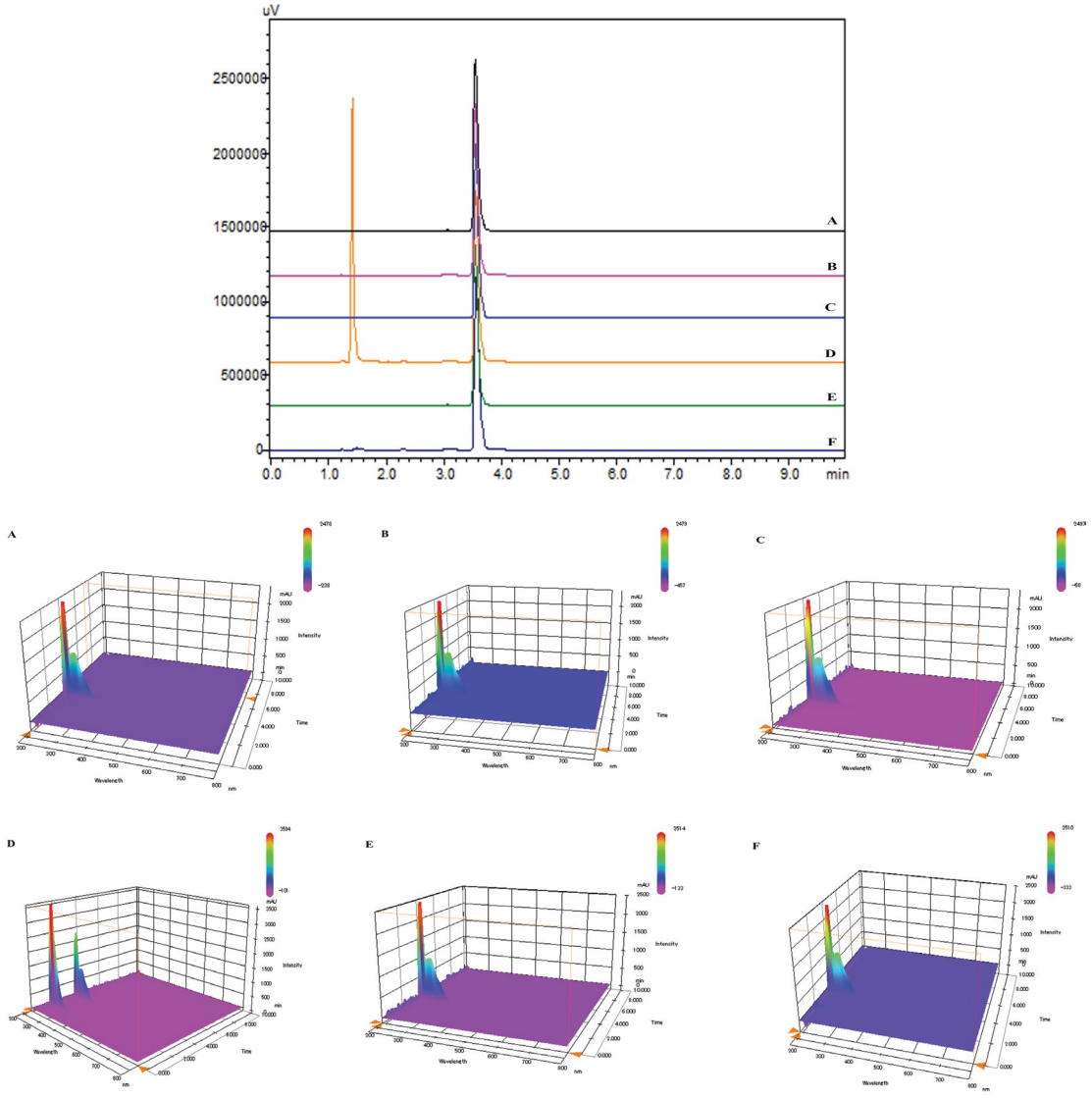

Figure 4: Representative Chromatograms of Exemestane [A] acidic [B], alkaline [C], oxidative [D], thermal [E] and photolytic [F] degradations

Stress Conditions	*Mean peak area	*Drug recovered (%)	*Drug decomposed (%)	Theoretical Plates	Tailing factor
Standard Drug	5935485	100	-	7222.409	1.421
Acidic degradation	5721046	96.39	3.61	7575.085	1.434
Alkaline degradation	5769237	97.20	2.80	7736.154	1.428
Oxidative degradation	5833858	98.29	1.71	7882.941	1.434
Thermal degradation	5526985	93.12	6.88	7643.248	1.417
Photolytic degradation	5874137	98.97	1.03	8022.939	1.441

*Mean of three replicates

Table 5: Forced degradation studies of Exemestane.

Formulation	Labeled claim (mg)	*Amount found (mg)	*Recovery (%)
XTANE	25	24.03	96.13
X'CEL	25	23.52	94.11

*Mean of three replicates

Table 6: Analysis of Exemestane commercial formulation (Tablets).

selective, precise and accurate. The stability studies of the drug show no degradation in any of the subjected conditions indicating that the drug molecule was very much stable in all the conditions. The developed method can be applied for the determination of Exemestane for marketed formulations.

Acknowledgments and Conflict of Interest

The authors are grateful to M/s GITAM University for providing necessary research facilities, NATCO Pharma Ltd. India) or providing the gift samples of Exemestane and University Grants Commission, New Delhi, India for their financial support. Authors have no conflict of interest.

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