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RESEARCH ARTICLE

A Validated Stability-Indicating Liquid Chromatographic Method for the Determination of Exemestane

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Abstract: A stability indicating liquid chromatographic method was developed for the determination of Exemestane in presence of degradation products using Zorbax SB C18 (150 mm × 4.6 mm *i. d*., 3.5 µm particle size) column with a flow rate 1.2 mL/min (UV detection 247 nm). Linearity was observed over a concentration range 0.1-200 µg/mL with regression equation y = 56288 x - 3004 (R^2 = 0.999). Forced degradation studies were performed and Exemestane is reported to be highly sensitive towards oxidation. The method was validated as per ICH guidelines.

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

Introduction

Exemestane (6-methylen-androsta-1, 4-diene-3, 17-dione) is a lipophilic steroid drug. It is an orally active irreversible steroidal aromatase inhibitor used for the therapy of metastatic postmenopausal breast cancer, with estrogen-dependent pathological conditions[1-3]. It was shown to be a potent and selective-inhibitor of aromatase vitro and *in vivo*. The introduction of a 1, 2-double bond in the ring of steroid molecule increase the aromatase inactivator affinity for the aromatase enzyme, which leads to increased therapeutic potency[4].

Literature survey reports that Exemestane can be determined by different analytical techniques such as HPTLC[5], UV-spectrophotometry[6], GC-MS[7], LC-MS[8-12], LC-radio immuno assay[13], HPLC[14-17] and UPLC[18] in pharmaceutical dosage forms and in biological fluids. So far only two stability indicating HPLC[19-20] methods were reported for the determination of Exemestane and at present the authors have proposed a robust, precise and accurate stability indicating liquid chromatographic method for the determination of Exemestane in pharmaceutical dosage forms.

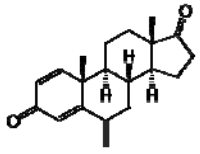

Figure 1. Chemical structure of Exemestane

Experimental

Exemestane standard (purity 99%) was obtained from Natco Pharma Ltd. (India) and was used as it is without further purification. All other chemicals were of analytical grade (Merck).

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

Preparation of phosphate buffer (pH 4.0*) solution*

5.04 g of Di sodium hydrogen phosphate and 3.01 g of potassium dihydrogen phosphate was accurately weighed and dissolved in HPLC grade water in a 1000 mL volumetric flask. pH was adjusted to 4.0 using glacial acetic acid.

Instrumentation and chromatographic conditions

Chromatographic separation was achieved by using Zorbax SB-C18 column (150 mm × 4.6 mm i.d., 3.5 µm particle size) for HPLC system of Shimadzu Model CBM-20A/20 Alite, equipped with SPD M20A prominence photodiode array detector, maintained at 25 ºC.

Isocratic elution was performed using phosphate buffer: acetonitrile (40:60, v/v) as mobile phase. The overall run time was 10 min. with flow rate 1.2 mL/min with UV detection at 247 nm. 20 µL of sample was injected into the HPLC system.

Preparation of stock solution

Stock solution was prepared by accurately transferring about 10 mg of Exemestane in to a 10 mL volumetric flask with mobile phase.

Further dilutions were made from the stock solution with mobile phase (phosphate buffer: acetonitrile (40:60, v/v). Prior to injection all 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[21].

Linearity

Linearity test solutions for the assay method were prepared from a stock solution at different concentration levels of the assay analyte concentration (0.1-200 µg/mL). 20 µL of each solution was injected in to the HPLC system and the peak area of the chromatogram obtained 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 based on the standard deviation of the response and the slope of the constructed calibration curve as described in International Conference on Harmonization guidelines Q2 (R1)[21].

Precision

The intra-day precision of the assay method was evaluated by carrying out 9 independent assays of a test sample of Exemestane at three concentration levels (10, 20 and 50 µg/mL) against a qualified reference standard. The % RSD of three obtained assay values at three different concentration levels was calculated.

The inter-day precision study was performed on three different days *i.e*. day 1, day 2 and day 3 at three different concentration levels (10, 20 and 50 µg/mL) and each value is the average of three determinations. The % RSD of three obtained assay values on three different days was calculated.

Accuracy

The accuracy of the assay method was evaluated in triplicate at three concentration levels (80, 100 and 120%) and the percentage recoveries were calculated. Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of Exemestane in the drug product. The study was carried out in triplicate at a total concentration 18, 20 and 22 µg/mL. The percentage recovery in each case was calculated.

Robustness

The robustness of the assay method was established by introducing small changes in the HPLC conditions which included wavelength (245 and 249 nm), percentage of acetonitrile in the mobile phase (58 and 62%), flow rate (1.1 and 1.3 mL/min) and pH (3.9 and 4.1). Robustness of the method was studied with 50 µg/mL of Exemestane.

Analysis of commercial formulations (Tablets)

Twenty tablets were procured from the local pharmacy store, weighed and crushed in to fine powder. Powder equivalent to about 10 mg Exemestane was accurately transferred into a 10 mL volumetric flask and made up to volume with acetonitrile. The contents were sonicated for 30 min to enable complete dissolution of Exemestane and then the solution was filtered. The filtrate was further diluted with mobile phase to yield 50 µg/mL.

Forced degradation studies/Specificity

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method[22]. All solutions for use in stress studies were prepared at an initial concentration of 1 mg/mL of Exemestane and refluxed for 30 min at 80 ºC in thermostat and then diluted with mobile phase to give a final concentration of 50 µg/mL All the solutions were analysed after 24 hours.

The acidic and alkaline degradations were performed using hydrochloric acid (0.1 M) and in sodium hydroxide (0.1 M) at 80 ºC in a thermostat and the stressed samples were instantly cooled with a mixture of ice and water, neutralized and diluted with mobile phase as per the requirement. Oxidation was performed using H_2O_2 solution where as thermal degradation was performed at 80 ºC in a thermostat for 6 hours.

Results and Discussion

The authors have developed a validated stability indicating RP-HPLC method for Exemestane in presence of degradation products. The performance characteristics of the present stability indicating liquid chromatographic method was compared and discussed with the previously published methods in Table 1.

HPLC method development and optimization

Initially the drug samples were analyzed using a mobile phase consisting of phosphate buffer solution: acetonitrile (50:50, v/v) with a flow rate of 1.0 mL/min where a sharp peak was eluted at 8.49 min. Therefore the flow rate was modified to 1.2 mL/min for which the retention time of the drug was less than 2 min (UV detection at 247 nm) and therefore the

mobile phase was slightly changed as (40:60, v/v) with the same flow rate and selected as the suitable chromatographic conditions for the entire study.

Table 1. Comparison of the performance characteristics of Exemestane of the present method with the published HPLC methods

Method/Reagent	Linearity µg/mL	Remarks	Ref.
Chloroform: methanol (9.2:0.8) (v/v)	100–500	HPTLC	[5]
Ammonium acetate: acetonitrile (pH 3.5)	10-100	LC/MS (Human urine)	[8]
Acetonitrile (100%)	0.05-25	LC/MS/MS (Human plasma)	[9]
Ammonium acetate: acetonitrile (pH 4.5) (60:40, v/v)	1 to 50	LC/MS	[10]
Formic acid: acetonitrile	0.1-40	LC/MS- (Human plasma)	[11]
Formic acid: acetonitrile	-	LC/MS (Human Urine)	[12]
Acetonitrile-water (34:66)	10-500 20-1000	LC and Radio Immunoassay (Human plasma)	[13]
Acetonitrile: KH_2PO_4 (pH 4.5) (35:65, v/v)	10-1000	Not Stability indicating HPLC	[14]
Acetonitrile: water (44:56, v/v)	2.5-50	Low linearity range	[15]
Methanol: phosphate buffer	20-100	Low linearity range (Gradient mode)	[16]
Water and methanol (50:50, v/v)	25-150	Low linearity range	[17]
Acetonitrile-water	-	UPLC	[18]
Water: methanol	25-150	Low linearity range	[19]
Acetonitrile-water (60:40, v/v)	6 to 14	Low linearity range	[20]
Phosphate buffer: acetonitrile (pH 4.0) (40:60, v/v)	0.1-200	Stability indicating HPLC	Present work

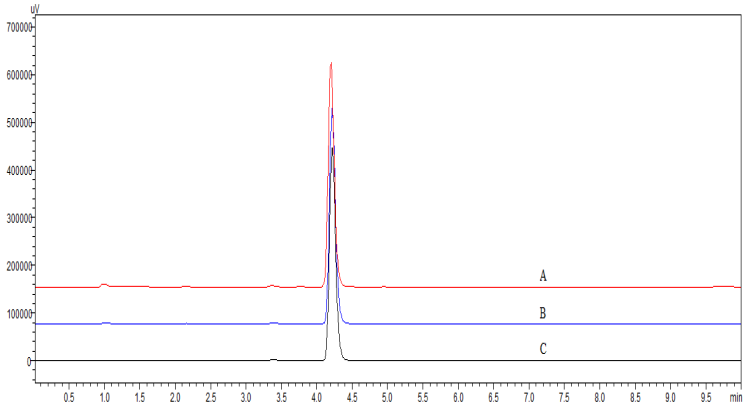

Figure 2. Typical Chromatograms of Exemestane (50 µg/mL) (A), X'CEL® (B) XTANE® (C) (Label claim: 25 mg)

The typical chromatogram obtained for Exemestane was shown in Figure 2A. Exemestane obeys Beer-Lambert's law over the concentration range 0.1-200 µg/mL (Table 2) with regression equation y = 56288 x + 3004 (r^2 = 0.999) (Figure 3).

Table 2. Linearity of Exemestane

Conc. µg/mL	*Mean peak area ± SD	RSD, %
0.1	5621±19.16	0.341
0.5	28231±121.95	0.432
1	56602±347.53	0.614
5	282600±740.41	0.262
10	567214±4327.84	0.763
20	1124228±3338.95	0.297
50	2841120±13694.19	0.482
100	5672150±20079.41	0.354
150	8523261±70913.53	0.832
200	11313321±25228.70	0.223

**Mean of three replicates*

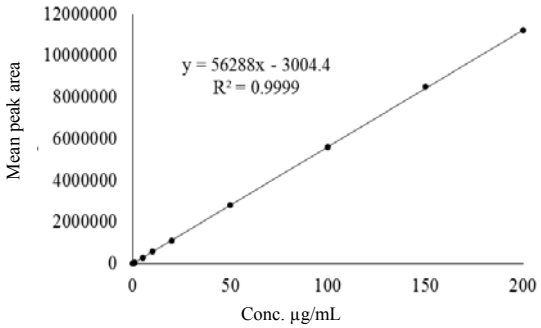

Figure 3. Calibration curve of Exemestane

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 0.0823 µg/mL and the LOD is found to be 0.0272 µg/mL.

Table 3. Precision and accuracy studies of Exemestane

Conc. µg/mL	Intra-day precision	Inter-day precision
	*Mean peak area ± SD (%RSD)	*Mean peak area ± SD (%RSD)
10	561256±2245.02 (0.4)	560089±3752.59(0.67)
20	1115512±3569.63(0.32)	1106782±5976.62 (0.54)
50	2814289±15197.16 (0.54)	2803299±23267.38 (0.83)

Accuracy			
Conc. µg/mL	*Mean peak area ± SD (% RSD)	Drug found µg/mL	*Recovery %
18	992898.4 ± 4567.33 (0.46)	17.49	97.16
20	1104332 ± 3644.29 (0.33)	19.76	98.80
22	1204776.2 ± 7710.56 (0.64)	21.53	97.86

**Mean of three replicates*

The % RSD was found to be 0.32-0.54 (intra-day) and 0.54-0.83 (inter-day) in precision studies where as in accuracy studies the percentage RSD was found to be 0.33-0.64 with a percentage recovery of 97.16-98.80% indicating that the method is precise and accurate (Table 3). The percentage RSD in robustness study was found to be 0.15-1.17 which is less than 2.0% indicating that the proposed method is robust (Table 4).

Table 4. Robustness study of Exemestane

Parameter	Condition	*Mean peak area	*Mean peak area ± SD (% RSD)
Flow rate (± 0.1 mL/min)	1.1	2819545	2835697.67±14238.16 (0.50)
	1.2	2841120	
	1.3	2846428	
Detection wavelength (±2 nm)	245	2814014	2834343.67±17929.12 (0.63)
	247	2841120	
	249	2847897	
Mobile phase composition phosphate buffer: acetonitrile (±2 %, v/v)	38:62	2843125	2840480.33±3015.82 (0.11)
	40:60	2841120	
	42:58	2837196	
pH (±0.1 unit)	3.9	2812945	2837951.33±23582.21 (0.83)
	4.0	2841120	
	4.1	2859789	

**Mean of three replicates*

Analysis of commercial formulations (Tablets)

The proposed method was applied to the determination of Exemestane tablets and the assay was calculated as 95.89-96.65% (Table 5) and no interference was observed with the excipients (Figure 2B-2C).

Table 5. Analysis of Exemestane commercial formulation (Tablets)

Formulation	Labeled claim, mg	*Amount found, mg	*Recovery %
XTANE	25	24.16	96.65
X'CEL	25	23.97	95.89

**Mean of three replicates*

Forced degradation studies/Specificity

The representative chromatograms obtained during the forced degradation studies of Exemestane were shown in Figure 4B-4E. Exemestane was eluted at 4.195 min and the overall runtime was 10 min (Figure 4A). 58.26% Exemestane was more destroyed in oxidative environment (Figure 4D) with a degradant peak at 1.149 min. 3.89% Exemestane has undergone alkaline degradation. Exemestane has undergone 3.51, 5.20 and 4.97% degradation during acidic, thermal and photolytic degradations respectively which is less than 10% (Table 6). In all the studies Exemestane has reported theoretical plates more than 2000 and the tailing factor less than 1.5 indicating that the proposed method is selective. When exposed to stress conditions Exemestane retains its symmetry and did not interfere with the degradant peaks indicating that the method is specific.

Table 6. Forced degradation studies of Exemestane

Stress conditions	*Mean peak area	*Drug recovered %	*Drug decomposed %	Theoretical plates	Tailing factor
Standard Drug	2841120	100	-	9206.195	1.297
Acidic degradation	2689320	94.66	5.34	9250.263	1.311
Alkaline degradation	2730488	96.11	3.89	9134.839	1.308
Oxidative degradation	1185987	41.74	58.26	6978.593	1.440
Thermal degradation	2526241	88.92	11.08	8802.654	1.301

**Mean of three replicates*

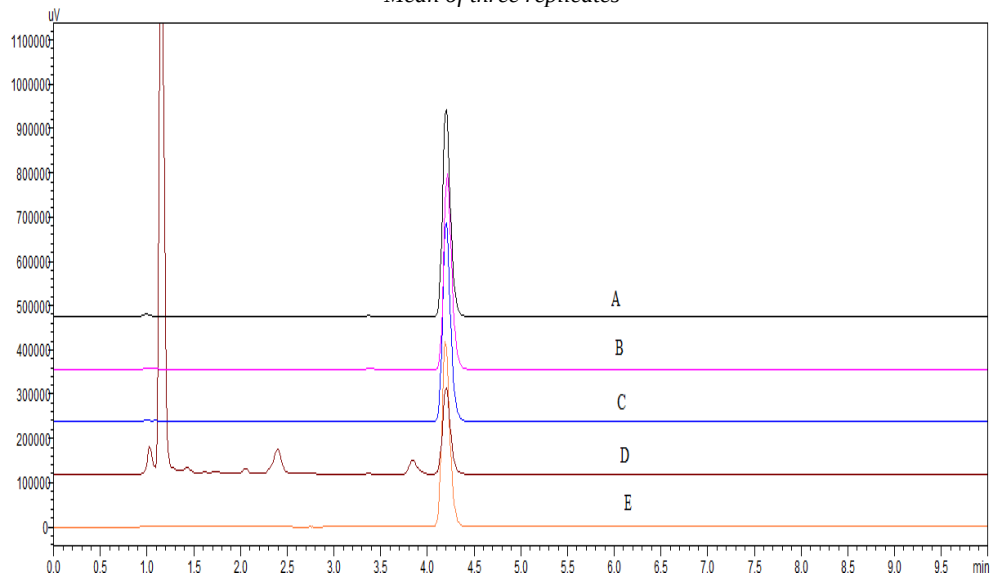

Figure 4. Typical Chromatograms of Exemestane [A], acidic [B], alkaline [C], and oxidative [D], thermal [E] degradations

Conclusion

This stability-indicating and validated HPLC method is selective, precise and accurate and can be applied for the determination of Exemestane.

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