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 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].

Methods: A novel stability indicating liquid chromatographic method was developed for the determination of Exemestane using HPLC system of Shimadzu Model CBM-20A/20 Allie, 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 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-methyl-androsta-1, 4-diene-3, 17-dione (C20H24O2; 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.

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).
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 (H₂O₂) 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 XCEL® (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 25ºC 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 80ºC. 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 % H₂O₂ solution whereas thermal degradation was performed by exposing the drug solution to 80ºC 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
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 selective and specific. So the proposed stability indicating liquid chromatographic method can be applied successfully for the determination of Exemestane in pharmaceutical formulations.
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) |
| 20            | 1244826.33 ± 8375.58 (0.67) | 1250654.33 ± 9500.35 (0.76) |
| 50            | 3043333.00 ± 8666.59 (0.29) | 300259.00 ± 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 4: Robustness study of Exemestane.

| Parameter | Condition | *Mean peak area ± SD (% RSD) |
|-----------|-----------|-----------------------------|
| Flow rate | (± 0.1 ml/min) | 0.9 5824121 ± 59812.60 (1.02) |
| Detection wavelength | (± 2 nm) | 252 5942156 ± 5971042.00a ± 59908.86 (0.94) |
| Mobile phase composition | phosphate buffer: acetonitrile (± 2 %, v/v) | 28:72 6014897 ± 5924915.33 ± 95705.25 (1.62) |
| pH (± 0.1 unit) | 3.9 5837459 ± 5832885.67 ± 104960.75 (1.80) |

*Mean of three replicates

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

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Table 5: Forced degradation studies of Exemestane.

| 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        | 5526958         | 93.12               | 6.88                 | 7643.248           | 1.417         |
| Photolytic degradation     | 5874137         | 98.97               | 1.03                 | 8022.939           | 1.441         |

*Mean of three replicates

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

| 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