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Development and validation of a stability indicating LC method for the assay and related substances determination of Exemestane, an aromatase inhibitor

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ABSTRACT

A selective stability indicating HPLC method was developed and validated for quantification of impurities (process related and degradants) and assay determination of Exemestane. Stability indicating power of the method was established by forced degradation experiments and mass balance study. The chromatographic separation was achieved with Hypersil BDS-C-18 using gradient elution. The developed method is validated for parameters like accuracy, linearity, LOD, LOQ, ruggedness. Box–Behnken experimental design was applied to check the robustness of the method.

1. Introduction

Estrogens are the most important hormones involved in human breast hormone-dependent cancer [1]. In post-menopausal women, estrogens are produced essentially by the conversion of androstenedione to estrone, via the aromatase enzyme in peripheral tissues [2]. Exemestane (Exe), 6-methylen-androsta-1,4-diene-3,17-dione, is a highly specific and irreversible steroidal aromatase inhibitor. Exe binds covalently to the active site cytochrome P450, making it inactive [3].

In the literature, several LC methods were reported for determination of Exemestane in biological samples [4–7]. As per our knowledge, no stability indicating method was found in literature search for quantification of Exe and related impurities.

Box–Behnken designs are response surface methods used to examine the relationship between one or more response variables and a set of quantitative experimental parameters [8]. Box–Behnken designs do not have axial points, thus all design points fall within the safe operating zone. These designs also ensure that all factors are never set at their high levels, simultaneously [8,9]. Box–Behnken designs were used in optimization and robustness testing of CE method [10], optimization of condition for anion exchange LC [11], were some of the works found in literature.

The main target of this work was to develop a stability indicating LC method, which is selective for the quantification of all possible degradants, process impurities and assay of Exe. The developed method is validated as per ICH guidelines for impurities and Exe [12]. Box–Behnken design was also applied to check the robustness and ensured that, the developed method is highly robust.

2. Experimental

2.1. Materials and reagents

HPLC grade methanol and acetonitrile were purchased from Rankem. Sodium hydroxide, hydrochloric acid, hydrogen peroxide were purchased from Merck. HPLC grade water was obtained from Milli-Q water purification system (Millipore, Milford, USA)

Exemestane drug substance, reference standard and impurities were obtained from Process Research department of Dr. Reddy's Laboratories, Hyderabad, India. Impurities were designated as Imp-1 (6 α/β-Spirooxiranandrosta-1,4-diene-3,17-dione), Imp-2 (6 α/β-Spirooxiranandrosta-1,4-diene-3,17-dione), Imp-3 (Androst-1,4-diene-3,17-dione) and Imp-4 (6-methylene-4-Androstene-3,17-dione). Imp-1 and Imp-2 are confirmed by the

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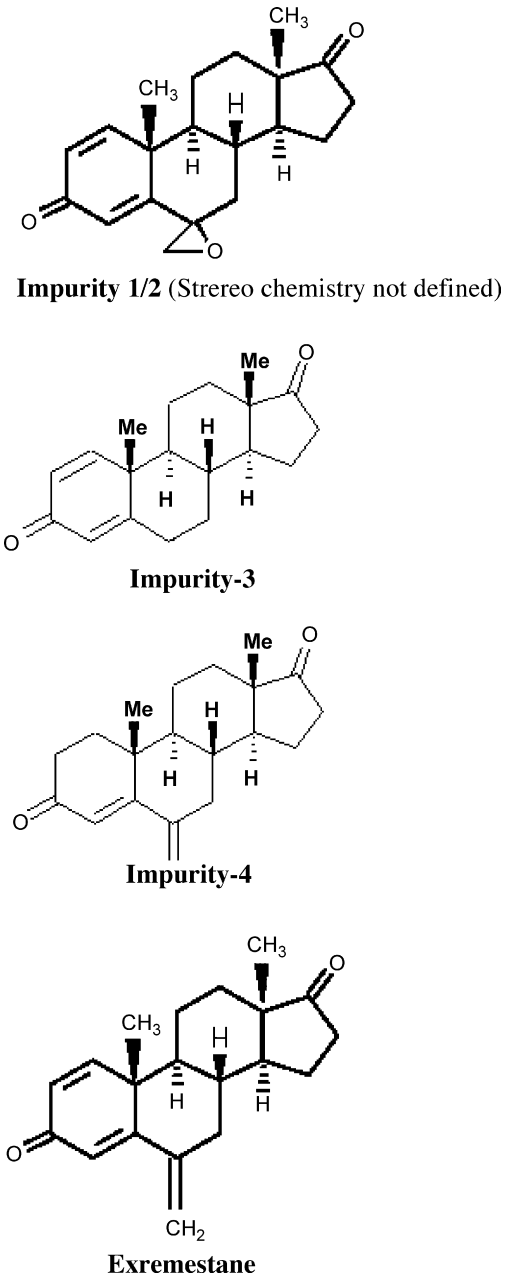

Fig. 1. Structure of impurities and Exemestane.

retention time in LC and the stereo chemistry was not assigned. These impurities are related substances of Exemestane with a specification limit of ≤0.15%.

The structure of Imp-1, Imp-2, Imp-3, Imp-4 and Exe were shown in Fig. 1.

2.2. Instrumentation and software

Two LC systems, LC1 (for development and specificity studies) and LC2 (for validation) were used.

LC1: Waters 2695 separation module with 996 PDA detector. The out put signal was monitored and processed using Empower software (Waters Corporation, Milford, MA, USA).
LC2: Agilent 1100 series LC with a variable wavelength detector. The out put signal was monitored and processed using Chemstation software (Agilent Technologies, Waldbronn, Germany).

Design Expert (Version 7.1.6) was used to generate Box–Behnken design for robustness study.

2.3. Chromatographic conditions

The chromatographic column used was Hypersil BDS, C-18 150 mm × 4.6 mm column with 3 μm particles of Thermo scientific make. The mobile phase consists of water (solvent A), and methanol (solvent B). The separation was achieved by gradient elution. The HPLC gradient was set as: T/%B: 0/30, 35/60, 40/90, 50/90, 52/30, and 60/30. The flow rate of the mobile phase was kept at 1.0 ml/min and the column temperature was maintained at 45 °C and the chromatogram was monitored at a wavelength of 247 nm. The injection volume was 10 μl. A mixture of acetonitrile and water (1:1, v/v) was used as diluent.

2.4. Preparation of standard solutions

Exe was prepared at 1000 μg/ml for analysis of related substances and 100 μg/ml for assay determination. Diluted standard solution of Exe at a level of 1 ppm was prepared from reference standard to quantify the impurities in related substances analysis. A stock solution of impurities (mixture of Imp-1, Imp-2, Imp-3 and Imp-4) at 100 μg/ml was also prepared in diluent.

2.5. Specificity and mass balance study

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products [13]. The specificity of the developed LC method for Exemestane was carried out in the presence of its impurities namely Imp-1, Imp-2, Imp-3 and Imp-4.

Sample was subjected to acid hydrolysis, alkaline hydrolysis and oxidation conditions. Sample was also subjected to thermal and photo degradation in dry state. Different stress conditions were followed to achieve about 1–10% degradation are shown in Section 3.2. The degraded sample was diluted to get 1000 μg/ml and 100 μg/ml solutions and determined the total impurities and assay, respectively.

2.6. Method validation

2.6.1. Precision

Assay method precision was evaluated by carrying out six independent assays of test sample of Exemestane against qualified reference standard and calculated the % R.S.D.

The precision of the related substance was checked by injecting six individual preparations of (1.0 mg/ml) Exemestane spiked with 0.15% of Imp-1, Imp-2, Imp-3 and Imp-4 with respect to Exe concentration. % R.S.D. of area for each Imp-1, Imp-2, Imp-3 and Imp-4 was calculated.

The intermediate precision of the method was also evaluated using different analyst, on a different day with different make instrument in the same laboratory.

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

The LOD and LOQ for Imp-1, Imp-2, Imp-3 and Imp-4 and Exemestane were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively by injecting a series of diluted solutions with known concentration. Precision study was also carried at the LOQ level by injecting six individual preparations of Imp-1, Imp-2, Imp-3, Imp-4 and Exemestane and calculating the % R.S.D. of the area. Accuracy at LOQ level was evaluated in triplicate for the four impurities by spiking the impurities at the estimated LOQ level to test solution.