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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

R. Suresh Kumar[a,c,*], M. Narasimha Naidu[a], Kasa Srinivasulu[a], K. Raja Sekhar[a], M. Veerender[a], M.K. Srinivasu[b]

[a] Integrated Product Development Organization, Dr. Reddy's Laboratories, Bachupally, Hyderabad 500072, India
[b] Discovery Research, Dr. Reddy's Laboratories, Miyapur, Hyderabad 500049, India
[c] Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University, Hyderabad – 500 072, India

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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

* Corresponding author at: Integrated Product Development, Dr. Reddy's Laboratories Ltd, Survey No.: 42, 45 & 46, Bachupalli, Qutubullapur, RR District, A.P., India. Tel.: +91 04044642473.
*E-mail addresses:* sureshk@drreddys.com, rsureshkumar11@yahoo.in (R. Suresh Kumar).

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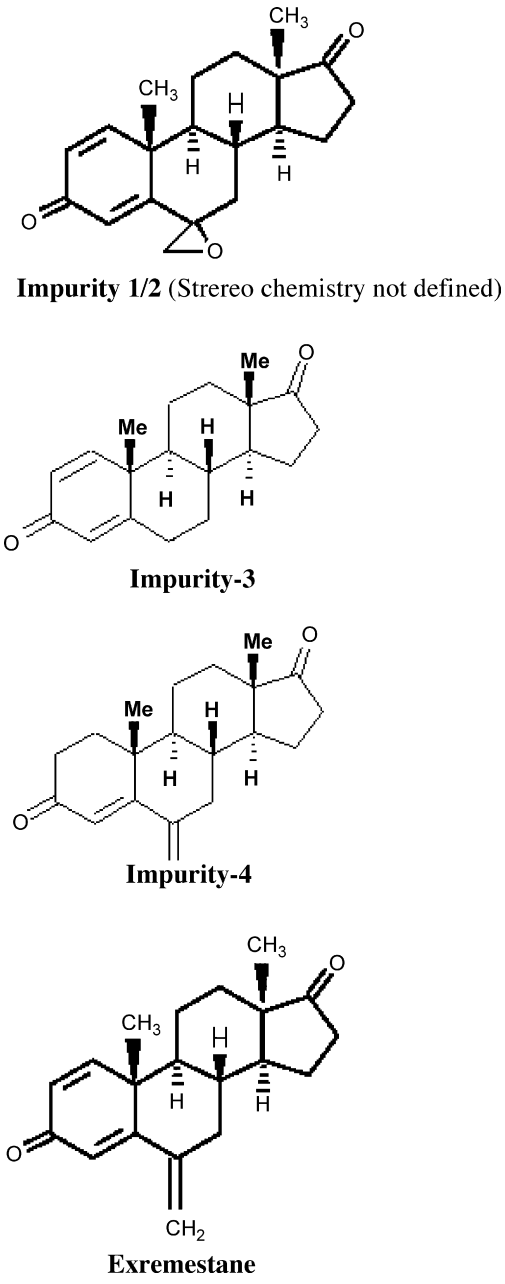



**Fig. 1.** Structure of impurities and Exemestane.

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.

**Table 1**
Factors and level studied for robustness testing.

| Factors | Level | | |
|---|---|---|---|
| | −1 | 0 | +1 |
| A. Temperature (°C) | 40 | 45 | 50 |
| B. Flow rate (ml/min) | 0.8 | 1.0 | 1.2 |
| C. Initial ratio of mobile phase A:mobile phase B | 68:32 | 70:30 | 72:28 |

*2.6.3. Linearity*

Linearity test solutions for assay were prepared at six concentration levels from 25% to 150% of assay analyte concentration (25, 50, 75, 100, 125 and 150 μg/ml). The peak area versus concentration data was performed by least-squares linear regression analysis.

Linearity test solutions for related substance method were prepared by diluting the impurity stock solution and Exemestane standard solution to the required concentrations. The solutions were prepared at six concentration levels from LOQ to 0.30% (w/w) of the analyte concentration. The calibration curve was drawn by plotting the peak areas of impurities and Exe versus its corresponding concentration.

Linearity test was performed for three consecutive days in the same concentration range for both assay and related substance method. The % R.S.D. value of the slope and *Y*-intercept of the calibration curve was calculated.

*2.6.4. Accuracy*

The accuracy of the assay was evaluated in triplicate at three-concentration levels, i.e. 50, 100 and 150 μg/ml in bulk drug sample. The % recovery was calculated from 100 μg/ml of reference standard preparation.

Bulk samples received from process research department of Dr. Reddy's Laboratories show the presence of Imp-1, Imp-2, Imp-3 and Imp-4 in between 0.02% and 0.04% levels. Standard addition and recovery experiments were conducted to determine accuracy of the related substance method for the quantification of all four impurities in bulk drug samples. The study was carried out in triplicate at 0.075%, 0.15% and 0.25% (w/w) of the related substances test concentration.

*2.6.5. Robustness*

A Box–Behnken design was generated for the study of robustness. The factors and level considered for the study are shown in Table 1.

A precision solution was prepared by spiking the impurities Imp-1, Imp-2, Imp-3 and Imp-4 at 0.15% (w/w) with respect to Exe related substances analysis concentration.

A standard and sample of Exe were prepared in assay concentration.

Recovery of four impurities and assay of Exe were studied as response surface.

## 3. Results and discussion

### 3.1. Method development

The main target of the chromatographic method is to get the separation of impurities and degradants generated, from Exe. It was also aimed that, method should be capable of resolving all impurities from each other. Early stage of development indicated that pH of the mobile phase has no influence on the retention or resolution between individual components or peak symmetry and BDS C-18 column shown quick elution of Exe and impurities than ODS column. Usage of BDS C-18 column for similar steroidal drug was found in literature [14]. Hence, BDS C-18 column with 3 μ particle size was used for the development.

**Table 2**
Mobile phase B composition for different trials.

| Trial number | Mobile phase B | Composition ratio of mobile phase B |
|---|---|---|
| 1 | Acetonitrile:methanol | 75:25 |
| 2 | Acetonitrile:methanol | 50:50 |
| 3 | Acetonitrile:methanol | 25:75 |
| 4 | Methanol | 100 |
| 5 | Acetonitrile | 100 |

A preliminary forced degradation was done with acid, alkali, peroxide, and thermal. These forced degradation samples were injected in the initial trial method (trial-1). In this method, the degradants generated in the forced degradation samples were designated as Imp-5 (base hydrolysis), Imp-6 (oxidation), Imp-7 (acid hydrolysis) and Imp-8 (second major in oxidation). The forced degraded samples were subjected to LC–MS analysis and the molecular weight of the impurities, Imp-5, Imp-6, Imp-7 and Imp-8 were found as 298.4, 312.4, 296.2 and 312.4, respectively. Impurities 5–8 are not formed during the synthetic process. Moreover these impurities were not observed during the accelerated stability studies (40 °C, 75% RH, 3 months) condition. Hence these impurities were not potential impurities; further attempts were not given for either isolation or identification of impurities.

In trial-1, mobile phase A is water and mobile phase B is mixture of acetonitrile and methanol (75:25, v/v), column temperature was maintained at 25 °C and all other chromatographic conditions adopted were, as described in Section 2.3. In this trial, it is observed that separation between Imp-1 and Imp-2 was not adequate (resolution < 1.5) and separation between Exemestane and Imp-8 was poor. Attempts were given to modify the gradient program to increase the resolution. Altering the gradient program simply increased the retention time and no improvement in resolution was observed.

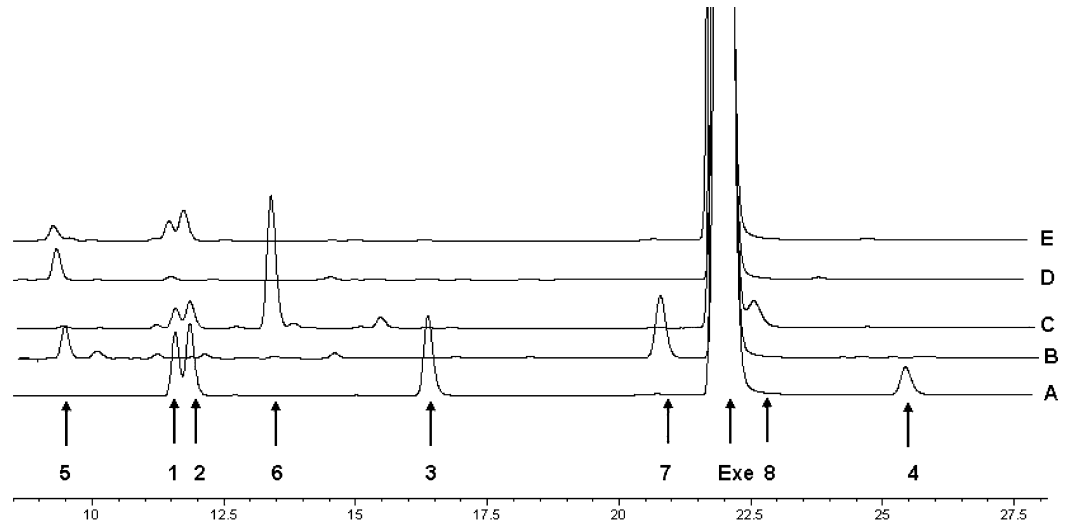
It was decided to adopt mobile phase A as water and a fixed gradient program mentioned in Section 2.3 for further trials. Flow rate was 1 ml/min in all trials. Column temperature was kept at 45 °C for trial-4 to reduce the back pressure and to improve the peak symmetry and in all other trials it was maintained at 25 °C. Organic modifier (mobile phase B) was changed in each trial. The mobile phase B used in different trials was tabulated in Table 2.

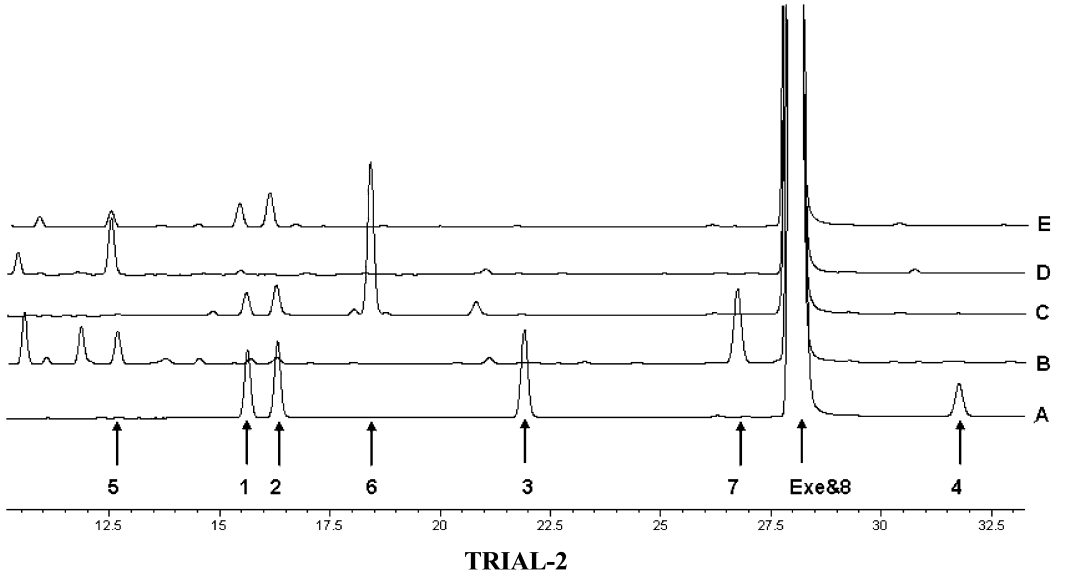
In each trial the forced degradation samples were injected. Attention was given for the separation of all eight impurities and Exemestane. Chromatograms of peak identification solution, thermal, acid, base and oxidation degradation obtained with each trial is presented in Fig. 2. Retention factor of each impurities and Exe obtained with each trial is presented graphically in Fig. 3.

The outcome of each trial is discussed below.

Trial-2: It was observed that the Imp-8 co-eluted with Exe.
Trial-3: Imp-8, was separated from Exe peak. However it was eluted at the same RT of Imp-7.
Trial-4: All impurities were separated from each other and with Exemestane.
Trial-5: Result from trial-4 encouraged to use a single organic modifier, instead of two. Hence this trial was conducted with only acetonitrile as an organic modifier. Imp-1 and Imp-2 were co-eluted. These two are major impurities in thermal degradation, and their separation is necessary for the method to become selective. Few attempts were made to modify the gradient program in trial-5, but there was no improvement in the resolution between Imp-1 and Imp-2.

Based on above, it was concluded that trial-4 was highly selective for the quantification of impurities, degradants as well as Exe.



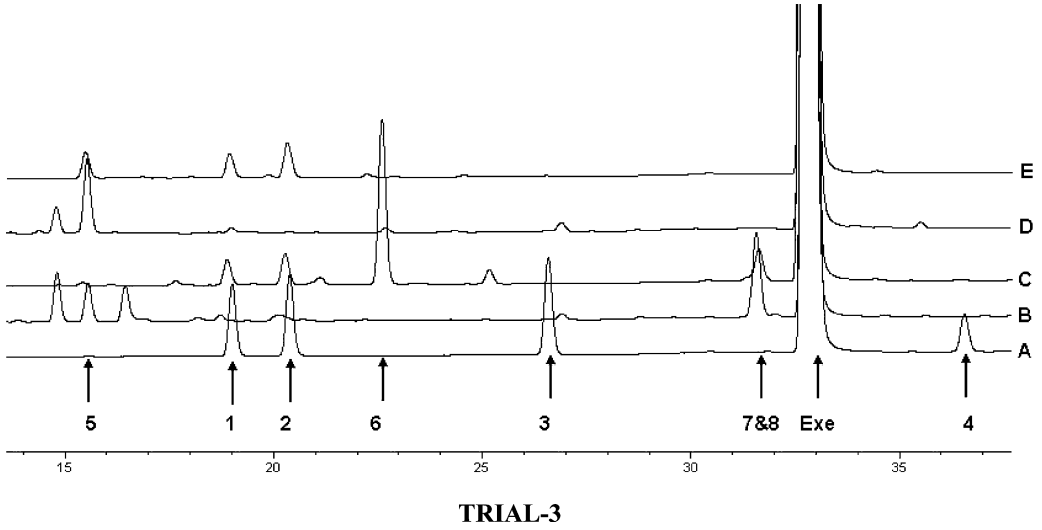
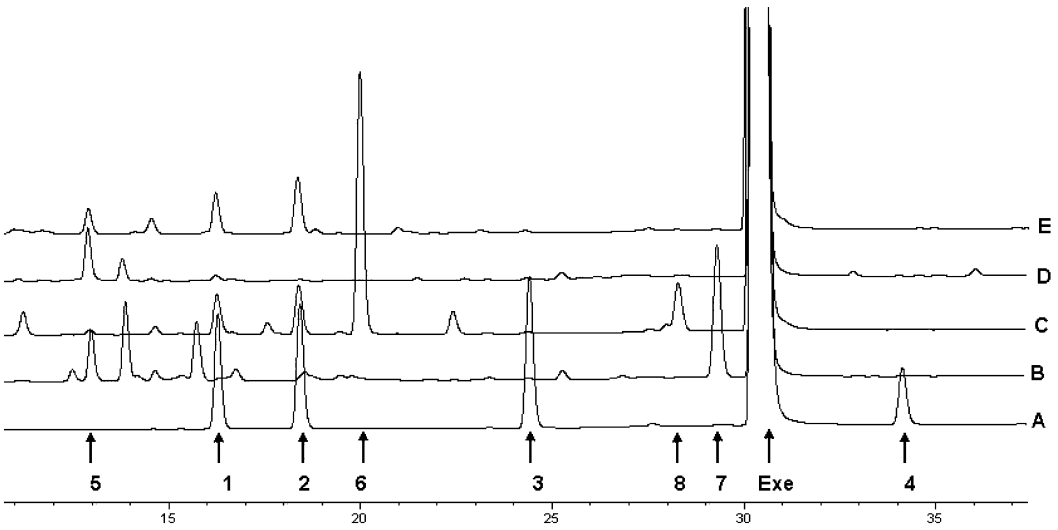
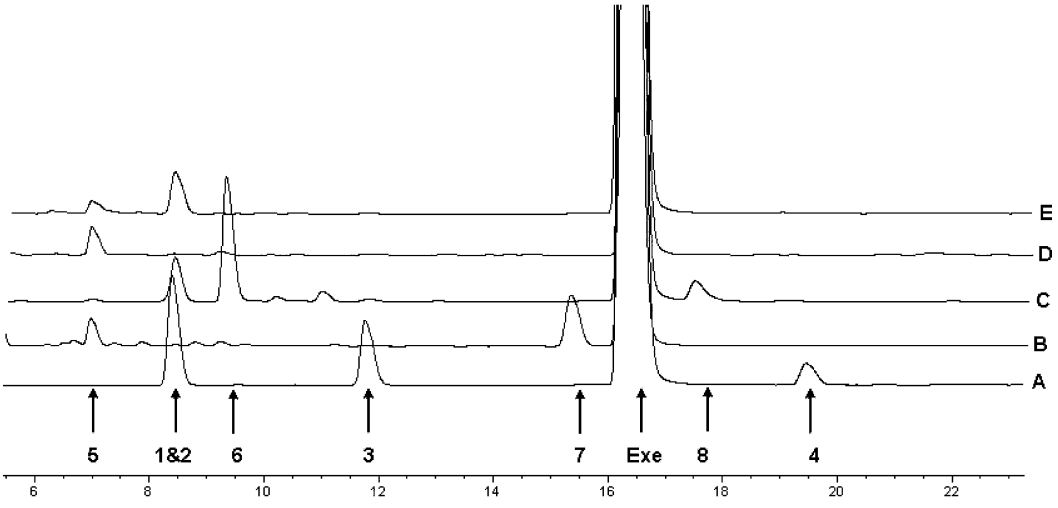




**Fig. 2.** Chromatograms of different trials. (A) Peak identification solution (mixture of Imp-1, Imp-2, Imp-3 and Imp-4 with Exe). (B) Acid degradation. (C) Oxidative degradation. (D) Base degradation. (E) Thermal degradation. 1. Impurity-1; 2. Impurity-2; 3. Impurity-3; 4. Impurity-4; 5. Impurity-5 (major in base degradation); 6. Impurity-6 (major in oxidative degradation); 7. Impurity-7 (major in acid degradation); 8. Impurity-8 (second major in oxidative degradation).




TRIAL-4




TRIAL-5

**Fig. 2.** (*Continued*).

It is also understood that, mixture of methanol and acetonitrile as organic mobile phase, is not suitable for Exe related substances analysis, especially in a gradient elution technique. In trial-4 all the impurities and degradants were eluted with in 35 min. After 35 min of run time, the organic phase was raised from 60% to 90% with in 5 min and maintained for 10 min. This gradient program ensured the elution of all other impurities found in crude API. Method used for trial-4 was finalized for mass balance study and further validation.

### 3.2. Specificity and mass balance study

No considerable degradation was observed in Exemestane bulk samples, under stress conditions such as photolytic stress and water

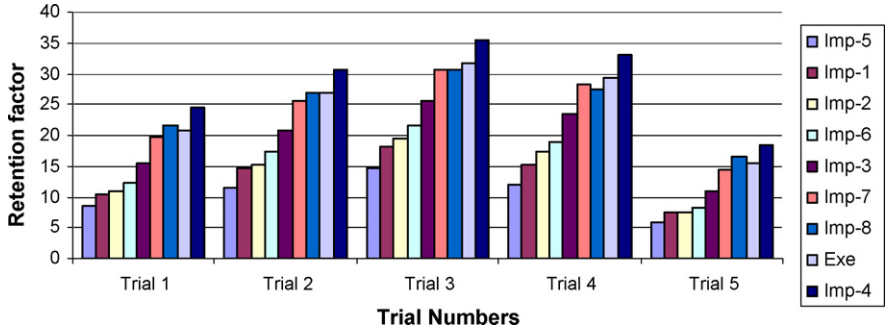



**Fig. 3.** Graphical representation of retention factor of components in different trials.

**Table 3**
Mass balance study.

| Stress condition | Time | Assay of active substance (%, w/w) | Total impurities (%, w/w) | Mass balance (assay + total impurities) (%, w/w) | Remarks |
|---|---|---|---|---|---|
| Thermal treatment (105 °C) | 10 days | 98.2 | 1.6 | 99.8 | Imp-1 and Imp-2 were major degradation products |
| Acid hydrolysis (2 M HCl, 70 °C) | 3 days | 95.5 | 4.0 | 99.5 | Unknown degradation products formed. Major degradation product designated as Imp-7 |
| Base hydrolysis (2 M HCl, 70 °C) | 3 days | 97.1 | 2.6 | 99.7 | Unknown degradation products formed. Major degradation designated as Imp-5 |
| Oxidation (3% $H_2O_2$, 60 °C) | 3 days | 95.2 | 4.4 | 99.6 | Unknown degradation products formed. Major degradation designated as Imp-6 and second major degradation product is Imp-8 |

hydrolysis. Stress study conditions and mass balance data are given in Table 3.

Peak purity test results obtained from PDA confirm that the Exemestane peak was homogeneous and pure in all the analyzed stress samples. The mass balance of stressed samples was close to 99.5%, which confirms the stability indicating power of the method.

### 3.3. Relative response factor

Relative response factor (RRF) was established for impurities 1, 2, 3 and 4 as the ratio of slope of impurities and slope of Exe. Slope value obtained with linearity calibration plot (Section 3.4.3) was used for RRF determination. Established RRF value for Imp-1, Imp-2, Imp-3 and Imp-4 are 1.04, 1.07, 1.08 and 0.65, respectively.

### 3.4. Results of method validation experiments

#### 3.4.1. Precision

The % R.S.D. of assay of Exemestane during assay method precision study was well within 0.6%. The % R.S.D. of area of Imp-1, Imp-2, Imp-3 and Imp-4 in related substance method precision study was within 5%. The % R.S.D. of assay results obtained in intermediate precision study was within 1.0%, confirming good precision of the method.

#### 3.4.2. Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) of Imp-1, Imp-2, Imp-3 and Exe was 0.003%, and for Imp-4 it was 0.007% (of analyte concentration, i.e. 1000 μg/ml) for 10 μl injection volume. Limit of quantification for Imp-1, Imp-2, Imp-3 and Exe were 0.01% and for Imp-4 it was 0.02% (of analyte concentration, i.e. 1000 μg/ml) for 10 μl injection volume. The method precision for Imp-1, Imp-2, Imp-3, Imp-4 and Exe at LOQ level was below 10% R.S.D. LOD and LOQ established with Exe is applicable to unknown impurities. Recovery at LOQ level for the impurities was in the range of 91.2–107.6%.

#### 3.4.3. Linearity

Linear calibration plot for assay was obtained over the calibration ranges tested, i.e. 50–150 μg/ml and the correlation coefficient obtained was greater than 0.999. Linearity was checked for assay method over the same concentration range for three consecutive days. The % R.S.D. values of the slope and *Y*-intercept of the calibration curves were 2.9 and 5.2, respectively. The results show that an excellent correlation existed between the peak area and concentration of the analyte.

Linear calibration plot for related substance method was obtained over the calibration ranges tested, i.e. LOQ to 0.3% for Imp-1, Imp-2, Imp-3, Imp-4 and Exe. The correlation coefficient obtained was greater than 0.999. Linearity was checked for related substance method over the same concentration range for three consecutive days. The % R.S.D. values of the slope and *Y*-intercept of the calibration curves were 5.2 and 8.6, respectively. The results show that an excellent correlation existed between the peak area and concentration of Imp-1, Imp-2, Imp-3, Imp-4 and Exe. The linearity established with Exe is applicable to unknown impurities.

#### 3.4.4. Accuracy

The percentage recovery of Exe in bulk drug samples was ranged from 99.5 to 99.8 (Table 4). The percentage recovery of Imp-1, Imp-2, Imp-3 and Imp-4 in bulk drugs samples was ranged from 94.2 to 99.5 (Table 4). The percentage recovery and percentage R.S.D. for three preparations shows that this method is accurate for the determination of assay and related substances of Exe.

#### 3.4.5. Robustness

For an analytical method to be robust, it must be able to demonstrate that it can produce quantitative results despite small changes in the experimental parameters, which may occur in a typical testing laboratory. Water and methanol are the two mobile phases in this method, it is expected that only instrument related experimental parameters can affect the results. Common such instrumental parameters which may occur during routine analysis are column temperature, flow rate and gradient composition. The factors and level considered for robustness study is described in Section 2.6.5.

Standard run and run order generated by Design Expert software are given in Table 5. Percentage recovery obtained for each impurities and assay analysis are also indicated in Table 5.

A recovery of 95.4–98.4% (w/w) was obtained for impurities and the percentage assay of Exemestane was ranged from 99.1 to 100.3% (w/w).

By using a fitted full quadratic model Eq. (1), a response surface regression analysis for each response factor was performed using coded units. Table 6 shows the values calculated for the coefficients and *P*-values (*P*-value is the probability of the null hypothesis). Using a 5% significance level, a factor is considered to affect the

**Table 4**
Accuracy study.

| Added % ($n = 3$) | Assay | | | Related substances determination | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Added (in ppm) | Recovery % | R.S.D. % | Added (in ppm) | Imp-1 | | Imp-2 | | Imp-3 | | Imp-4 | |
| | | | | | Recovery % | R.S.D. % | Recovery % | R.S.D. % | Recovery % | R.S.D. % | Recovery % | R.S.D. % |
| 50 | 50 | 99.5 | 0.73 | 0.75 | 95.8 | 2.1 | 95.1 | 3.3 | 96.4 | 5.4 | 95.5 | 2.8 |
| 100 | 100 | 99.8 | 0.70 | 1.50 | 94.2 | 1.4 | 96.1 | 4.0 | 99.5 | 3.5 | 98.2 | 5.2 |
| 150 | 150 | 99.7 | 0.42 | 2.25 | 97.0 | 3.3 | 97.0 | 4.5 | 94.9 | 3.3 | 95.7 | 2.8 |

**Table 5**
Standard run, run order for Box–Behnken design and response obtained for robustness testing.

| Standard | Run | Level | | | % w/w recovery of impurities | | | | % w/w |
|---|---|---|---|---|---|---|---|---|---|
| | | Factor A | Factor B | Factor C | Imp-1 | Imp-2 | Imp-3 | Imp-4 | Assay |
| 15 | 1 | 0 | 0 | 0 | 95.8 | 97.2 | 96.9 | 96.2 | 99.2 |
| 13 | 2 | 0 | 0 | 0 | 95.9 | 98.4 | 95.8 | 97.1 | 99.4 |
| 12 | 3 | 0 | 1 | 1 | 95.6 | 96.5 | 96.2 | 96.9 | 99.5 |
| 14 | 4 | 0 | 0 | 0 | 96.3 | 97.4 | 97.3 | 97.4 | 99.8 |
| 10 | 5 | 0 | 1 | −1 | 95.7 | 97.6 | 95.6 | 95.4 | 100.2 |
| 17 | 6 | 0 | 0 | 0 | 98.1 | 96.5 | 98.1 | 97.3 | 99.8 |
| 1 | 7 | −1 | −1 | 0 | 98.2 | 97.2 | 95.4 | 96.5 | 99.2 |
| 3 | 8 | −1 | 1 | 0 | 95.6 | 97.3 | 96.3 | 96.8 | 100.1 |
| 5 | 9 | −1 | 0 | −1 | 97.1 | 98.1 | 95.7 | 97.9 | 99.5 |
| 2 | 10 | 1 | −1 | 0 | 98.4 | 96.9 | 95.4 | 96.9 | 100 |
| 11 | 11 | 0 | −1 | 1 | 97.1 | 97.9 | 98.1 | 97.9 | 99.9 |
| 4 | 12 | 1 | 1 | 0 | 96.8 | 97.2 | 97.8 | 97.2 | 100.3 |
| 9 | 13 | 0 | −1 | −1 | 95.6 | 95.8 | 97.7 | 95.8 | 99.4 |
| 16 | 14 | 0 | 0 | 0 | 96.2 | 96.7 | 96.3 | 96.8 | 99.2 |
| 6 | 15 | 1 | 0 | −1 | 96.4 | 96.8 | 96.4 | 96.7 | 100.1 |
| 8 | 16 | 1 | 0 | 1 | 96.9 | 96.2 | 95.6 | 96.3 | 99.1 |
| 7 | 17 | −1 | 0 | 1 | 97.6 | 96.3 | 96.9 | 96.7 | 99.3 |

**Table 6**
Regression coefficients and the associated probability values (*P*-value) for each response (recovery).

| Term | Imp-1 | | Imp-2 | | Imp-3 | | Imp-4 | | Exe | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Coeff. | *P*-value | Coeff. | *P*-value | Coeff. | *P*-value | Coeff. | *P*-value | Coeff. | *P*-value |
| Constant | 96.46 | 0 | 97.24 | 0 | 96.88 | 0 | 96.96 | 0 | 99.48 | 0 |
| Factor A | 0.000 | 1.0000 | −0.220 | 0.415 | 0.110 | 0.8018 | −0.100 | 0.7596 | 0.180 | 0.1397 |
| Factor B | −0.700 | 0.0609 | 0.100 | 0.712 | −0.088 | 0.8451 | −0.100 | 0.7596 | 0.200 | 0.0986 |
| Factor C | 0.300 | 0.3708 | −0.170 | 0.522 | 0.170 | 0.6972 | 0.250 | 0.4525 | −0.180 | 0.1397 |
| A × B | 0.250 | 0.5907 | 0.050 | 0.896 | 0.380 | 0.5583 | 0.000 | 1.0000 | −0.150 | 0.3463 |
| A × C | 0.000 | 1.0000 | 0.300 | 0.441 | −0.500 | 0.4396 | 0.200 | 0.6663 | −0.200 | 0.2202 |
| B × C | −0.400 | 0.3973 | −0.800 | 0.066 | 0.050 | 0.9370 | −0.150 | 0.7457 | −0.300 | 0.0832 |
| $A^2$ | 0.890 | 0.0773 | −0.095 | 0.799 | −0.700 | 0.2761 | 0.140 | 0.7477 | 0.085 | 0.5756 |
| $B^2$ | −0.110 | 0.8151 | 0.005 | 0.989 | 0.048 | 0.9386 | −0.250 | 0.5746 | 0.340 | 0.0539 |
| $C^2$ | −0.350 | 0.4388 | −0.290 | 0.437 | −0.028 | 0.9644 | −0.200 | 0.6505 | −0.065 | 0.6671 |

response if the coefficients differ from zero significantly and the *P*-value <0.050.

$$Y = X_0 + X_A F_A + X_B F_B + X_C F_C + X_{AB} F_A F_B + X_{AC} F_A F_C + X_{BC} F_B F_C + X_{AA} F_{AA} + X_{BB} F_{BB} + X_{CC} F_{CC} \quad (1)$$

where $Y$ is the experimental response, $X_0$ is constant, $X_x$ the coefficients of the factors and interactions and $F_x$ stands for each factor.

From Table 6 it can be seen that *P* value for any of the studied factors are above 0.05. It shows that for the quantitative determination of impurities and Exe, this method is highly robust for 5 °C variation in column temperature, 0.2 ml/min variation in flow rate and a small variation in the initial ratio of mobile phase A and B.

## 4. Conclusion

A simple and accurate stability indicating HPLC method for the determination of Exemestane in the presence of degradation products was described for the first time. This method is highly specific for the quantification of degradation products and process impurities of Exemestane. The behavior of Exemestane under various stress conditions were studied and presented. This method can be used for quality control and stability studies.

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