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RP-HPLC Estimation of Exemestane in Tablet Dosage Form

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A rapid, sensitive and precise HPLC method was developed for the estimation of exemestane in pure and tablet dosage forms. Separation of the drug was achieved on a reverse phase C_8 column using a mobile phase consisting of water and methanol in the ratio of 50:50 v/v. The flow rate was 1 mL/min and the detection wavelength 249 nm. The linearity was found in the range of 25-150 µg/mL with a correlation coefficient of 0.9998. The proposed method was validated for its sensitivity, linearity, accuracy and precision. This method can be employed for routine quality control analysis of exemestane in tablet dosage forms.

Key Words: Exemestane, Estimation, Tablets, RP-HPLC.

INTRODUCTION

Exemestane, (6-methyleneandrosta-1,4-diene-3,17-dione)[1], is a steroidal aromatase inhibitor used in the adjuvant treatment of breast cancer in postmenopausal women. It acts as a false substrate for the aromatase enzyme and is processed to an intermediate that binds irreversibly to the active site of the enzyme causing its inactivation. As a result, the circulating estrogen levels are significantly lowered. A few HPLC[2-4] and LC-MS[5,6] methods were reported earlier for the determination of exemestane in biological samples. In the present study the authors report a rapid, sensitive, accurate and precise HPLC method for the estimation of exemestane in bulk samples and in tablet dosage forms.

EXPERIMENTAL

The reference sample of exemestane was supplied by Natco Pharma Ltd., Hyderabad. HPLC grade water and methanol of were purchased from E. Merck (India) Ltd., Mumbai.

Chromatographic conditions: The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase C_8 column (150 mm × 4.6 mm; 5 µm), a 2695 binary pump, a 10 µL injection loop and a 2487 dual absorbance detector and running on Waters Empower software.

A mixture of water and methanol in the ratio of 50:50 v/v was found to be the most suitable mobile phase for ideal separation of exemestane. The solvent mixture was filtered through a 0.45 µ membrane filter and sonicated before use. It was

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pumped through the column at a flow rate of 1.0 mL/min. The column was maintained at ambient temperature. The pump pressure was set at 1850 psi. The column was equilibrated by pumping the mobile phase through the column for at least 0.5 h prior to the injection of the drug solution. The detection of the drug was monitored at 249 nm. The run time was set at 6 min. Under these optimized chromatographic conditions the retention time obtained for the drug was 3.0 min. A typical chromatogram showing the separation of the drug is given in Fig. 1.

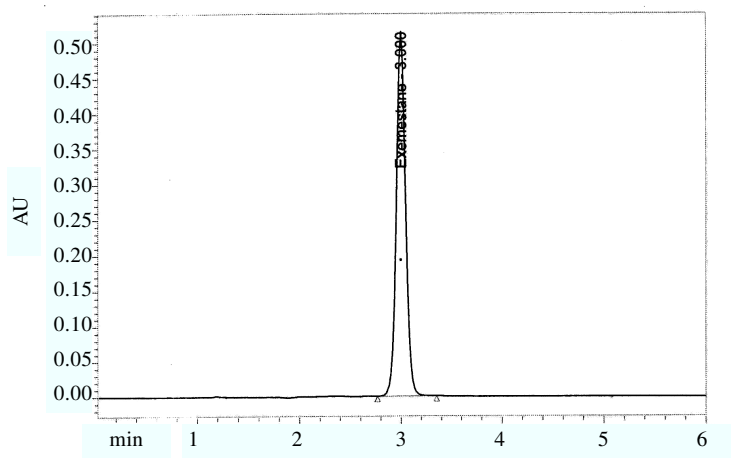

Fig. 1. Typical chromatogram showing separation of exemestane

Calibration plot: About 25 mg of exemestane was weighed accurately, transferred into a 100 mL volumetric flask and dissolved in 10 mL of acetonitrile. Then, 25 mL of a 50:50 v/v mixture of acetonitrile and water (diluent) was added to the flask. The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the diluent to get a 250 µg/mL solution. From this, a working standard solution of the drug (100 µg/mL) was prepared by diluting 4 mL of the above solution to 10 mL in a volumetric flask. Further dilutions ranging from 25 to 150 µg/mL were prepared from the solution in 10 mL volumetric flasks using the above diluent. 10 µL of each dilution was injected six times into the column at a flow rate of 1 mL/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against peak area was found to be linear in the concentration range of 25-150 µg/mL of the drug. The relevant data are furnished in Table-1. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of exemestane in tablets dosage forms.

Validation of the proposed method: The specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method for the determination of exemestane. Solutions containing 50, 100 and 150 µg/mL of exemestane

TABLE-1
CALIBRATION DATA OF THE METHOD

Concentration (μg/mL)	Mean peak area (n = 6)
25	818352
50	1622302
75	2470548
100	3242471
125	4017031
150	4906268

were subjected to the proposed HPLC analysis to check intra-day and inter-day variation of the method and the results are furnished in Table-2. The system suitability parameters are given in Table-3.

TABLE-2
PRECISION OF THE PROPOSED HPLC METHOD

Concentration of exemestane (μg/mL)	Measured concentration of exemestane (μg/mL)			
	Intra-day		Inter-day	
	Mean (n=3)	% C.V.	Mean (n=3)	%C.V.
50	49.84	0.28	50.53	0.19
100	100.12	0.16	100.27	0.05
150	150.23	0.33	150.45	0.42

TABLE-3
SYSTEM SUITABILITY PARAMETERS

Parameter	Result
Theoretical plates (N)	5743
Tailing factor	1.10
Peak asymmetry (A_S)	1.0
Limit of detection (μg/mL)	0.045
Limit of quantification (μg/mL)	0.150

Estimation of exemestane in tablet dosage forms: Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate exemestane in tablet formulations. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 25 mg of exemestane was transferred to a 100 mL volumetric flask and dissolved in 10 mL of acetonitrile. The contents of the flask were sonicated for 15 min and a further 25 mL of the diluent was added, the flask was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through a 0.45 μ membrane filter. From the filtrate, 4 mL of aliquot was taken in a separate 10 mL volumetric flask, the contents made up to the volume. The solution was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in

the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table-4.

TABLE-4
RECOVERY FROM DOSAGE FORMS

Formulation	Label claim (mg)	Amount found (mg) (n = 6)	% Amount found
Brand-1	25	24.65 ± 0.12	98.60
Brand-2	25	24.73 ± 0.10	98.92

RESULTS AND DISCUSSION

In the proposed method, the retention time of exemestane was found to be 3.0 min. Quantification was linear in the concentration range of 25-150 μg/mL. The regression equation of the linearity plot of concentration of exemestane over its peak area was found to be Y = 32452.217X + 6593 (r = 0.9998), where X is the concentration of exemestane (μg/mL) and Y is the corresponding peak area. The number of theoretical plates calculated was 5743, which indicates efficient performance of the column. The limit of detection and limit of quantitation were found to be 0.045 and 0.150 μg/mL respectively, which indicate the sensitivity of the method. The use of water and methanol in the ratio of 50:50 v/v resulted in peak with good shape and resolution. The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by proposed HPLC method.

Thus, the proposed HPLC method is rapid, sensitive, precise and accurate for the determination of exemestane and can be reliably adopted for routine quality control analysis of exemestane in its tablet dosage forms.

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