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Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Tibolone in Tablet Dosage Form

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ABSTRACT:

A stability-indicating reversed-phase high performance liquid chromatography (RP-HPLC) method was developed for the determination of Tibolone, a drug used in the treatment of Hormone replacement therapy. The desired chromatographic separation was achieved on a Zorbex-Sb Phenyl (150*4.6)5 μ column, using isocratic elution at a 205 nm detector wavelength. The optimized mobile phase consisted of a ACN: Buffer 0.05 M(NH₄)HPO₄ (50 : 50) as solvent. The flow rate was 1.2 mL/min and the retention time of Tibolone was 5.7 min. The linearity for Tibolone was in the range of 12.5-37.5 μ g/mL. Recovery for Tibolone was calculated & The stability-indicating capability was established by forced degradation experiments .The developed RP-HPLC method was validated according to the International Conference on Harmonization (ICH) guidelines. This validated method was applied for the estimation of Tibolone in commercially available tablets.

Keywords : Tibolone, method validation, forced degradation, RP-HPLC, Zorbex-Sb Phenyl

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INTRODUCTION:

Tibolone (figure-1) is a Lipid lowering agent used in the treatment of Hormone replacement therapy.¹⁻⁸. Tibolone was approved for the treatment of Hormone replacement therapy as 2.5 mg and 5 mg tablets for oral administration. Few methods have been reported for the quantitative determination of Tibolone in tablet formulations by high performance liquid chromatography (HPLC) and spectrophotometric procedures. There is no official and reported stability indicating RP-HPLC method was found for estimation of Tibolone in tablet dosage form. The proposed assay is able to separate Tibolone from tablet ingredients and from unknown degradation products within 10 minutes. This assay was validated according to the International Conference on Harmonization (ICH) guidelines.

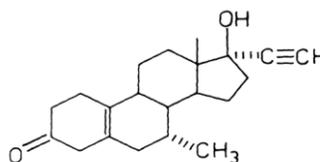


Figure-1 structure of Tibolone

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MATERIALS AND METHODS

Materials and reagents

Analytical pure Tibolone was procured from Famy care Ltd., Ahmedabad & HPLC grade methanol, Acetonitrile, water (Merck Laboratory, India) were used for the preparation of the mobile phase. TIBOMAX (2.5 mg) was purchased from local pharmacy.

Chromatographic conditions

Chromatographic separations was achieved by using an WATERS HPLCsystem comprising a pump, auto sampler, thermo stated column compartment, pdf detector. the column compartment temperature to 45 °C; 20 µL of sample was injected into the HPLC system.⁸⁻¹⁶ Separations were performed on the reversed-phase column Zorbex-Sb Phenyl (150*4.6)5µ. The isocratic mobile phase consisted ACN: Buffer 0.05M(NH₄)HPO₄ (50 : 50) The mobile phase was delivered at a flow rate of 1.2 mL/minute (min). Eluate was monitored at 205 nm.

❖ Preparation of Standard stock solution

Take 2.5 mg of tibolone reference standard and dilute it up to 100 ml with water and sonicate it for 15 minute. (25µg/ml)

❖ Preparation of test solution Tibolone (10 µg/ml):

Accurately Weight tablet powder equivalent to 2.5mg and dissolve it in diluent (Water: ACN) (25:75) firstly add 70ml of diluent and sonicate for 15 min. with continues shaking in cooled water. Make up the volume up to 100ml and filter it through 0.45µ nylon filter discarding 1st 5ml of filtrate.

Forced degradation study¹⁵⁻¹⁸

Control samples were used in each condition. A minimum 10% degradation of the initial concentration remaining was considered to indicate a significant loss in terms of stability.

❖ Acid degradation

Take powder tablet equivalent to 2.5mg in 100 ml volumetric flask and add 1 ml 0.2M HCL after making up the volume with diluent. Put the flask at R.T for 2 Hr. then neutralize with 0.1N NaOH and Filtered through 0.45µm membrane filter paper and injected in to HPLC system

❖ Base degradation

Take powder tablet equivalent to 2.5mg in 100 ml volumetric flask and add 1 ml 0.2M NaOH after making up the volume with diluent. Put the flask at R.T for 2 Hr. then neutralize with 0.1N HCL and Filtered through 0.45µm membrane filter paper and injected in to HPLC system

❖ Oxidative hydrolysis:

Take powder tablet equivalent to 2.5mg in 100 ml volumetric flask and add 1 ml 30% H₂O₂ after making up the volume with diluent. Put the flask at R.T for 2 Hr. Filtered through 0.45µm

membrane filter paper and injected in to HPLC system.

❖ Thermal Degradation

For dry heat degradation study, powder of Tibolone was spread over petri dish and exposed to dry heat (80°C) for 2 hour in an oven then from that powder Take powder weight equivalent to 2.5mg in 100 ml volumetric flask and after making up the volume with diluent. Put the flask at R.T for 2 Hr. Filtered through 0.45µm membrane filter paper and injected in to HPLC system.

• METHOD VALIDATION¹⁹⁻²¹

Calibration curve of standard of Tibolone

A calibration curve was plotted over a concentration range of 12.5 – 37.5 µg/ml. Accurately measured working standard stock solution (100 µg/ml) of Tibolone (2.5, 3.8, 5, 6 and 7.5ml) were transferred to a series of 20 ml volumetric flasks and the volume in each flask made up to the mark with Diluent to get concentration range of 12.5 – 37.5µg/ml. The resulting solution was injected into the column and the peak area obtained at retention time 5.71 minute at flow rate 1.2 ml/min was measured at 205 nm. Calibration curve was constructed by plotting peak area versus concentration at 205 nm.

A. Linearity

The linear response of Tibolone was determined by analyzing six independent levels of the calibration curve in the range of 12.5-37.5 µg/ml for Tibolone.

B. Precision

It provides an indication of random error. The precision of an analytical method is usually expressed as the standard deviation, relative standard deviation or coefficient of variance of a series of measurements.

1) Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision .

Repeatability of method was performed by preparing the test solution of Tibolone (25 µg/ml) for six times from tablet dosage form and analyzed as per the proposed method. Percentage relative standard deviation (RSD) should be less than 2% .

2) Intermediate Precision

It expresses within laboratory variations as on different days

analysis or equipment within the laboratory

a. Intra-day precision

Variation of results within same day is called Intra-day precision. The Intra-day precision for HPLC method was determined for three concentration of Tibolone solution for the three times on the same day.

b. Inter-day precision

Variation of results amongst days called Inter-day precision. The Inter-day precision for HPLC method was determined for Tibolone for three days.

C. Accuracy

Accuracy of the method was confirmed by recovery study from tablet sample solution at 3 level of standard Addition (50%, 100%, and 150%) of targeted solution (10 μ g/ml Tibolone) in triplicate.

D. Limit of Detection & Limit of Quantification

Limit of Detection

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantify under the stated experimental conditions.

Limit of detection can be calculated using following equation as per ICH guidelines

$$LOD = \frac{3.3 \sigma}{S}$$

Where, σ = Standard deviation of the y intercept

S = Slope of the calibration curve

Limit of Quantification

It is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. Limit of quantification can be calculated using following equation as per ICH guideline.

$$LOQ = \frac{10 \sigma}{S}$$

Where, σ = Standard deviation of the y intercept

S = Slope of the calibration curve

E. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Robustness of the method was studied by changing the flow rate; change in wavelength, change in pH, and composition of

mobile phase and change in response of drugs were studied.

F. Application of proposed Method to the pharmaceutical dosage form

The prepared sample solution was chromatographed for 10 minutes using mobile phase at a flow rate of 1.2 ml/min. From the peak area obtained in the chromatogram, the amount of the drug was calculated.

Results and Discussion:

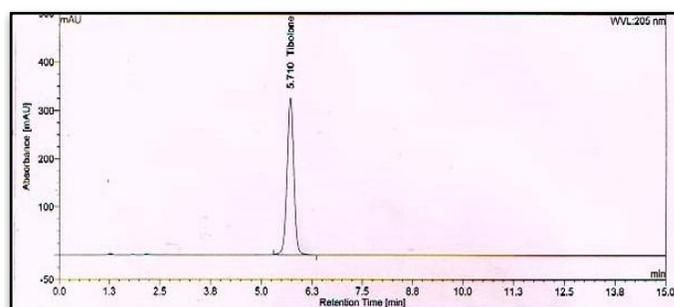


Figure-2 Chromatogram of Std Solution Of Tibolone (10 μ g/ml)

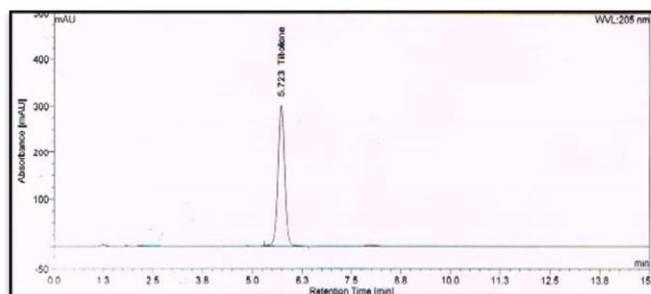


Figure-3 Chromatogram of test Solution of Tibolone (10 μ g/ml)

Forced degradation study

Forced degradation studies were performed to demonstrate selectivity and stability-indicating capability of the proposed RP-HPLC method

Result of Forced degradation study of Tibolone

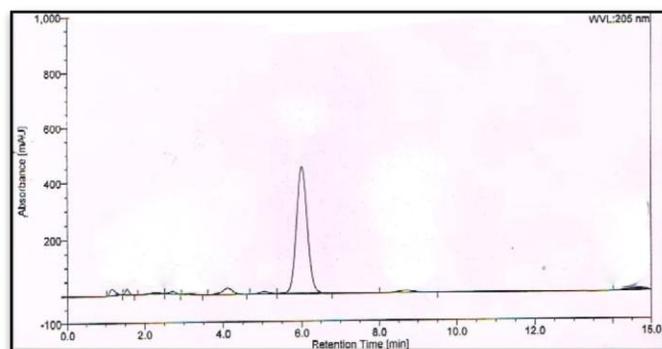


Figure-4 Chromatogram Of Tibolone Under Acid

Table No.-1 Result of Stability study of Tibolone

Condition of forced degradation	%Degradation of Tibolone	Condition of forced degradation
0.2 N HCl, 2hr,	8.12	0.2 N HCl, 2hr,
0.2 N NaOH, 2hr,	8.23	0.2 N NaOH, 2hr,
3% H2O2, 2hr,	15.78	3% H2O2, 2hr,
Thermal, 80°C, 2hr	7.97	Thermal, 80°C, 2hr

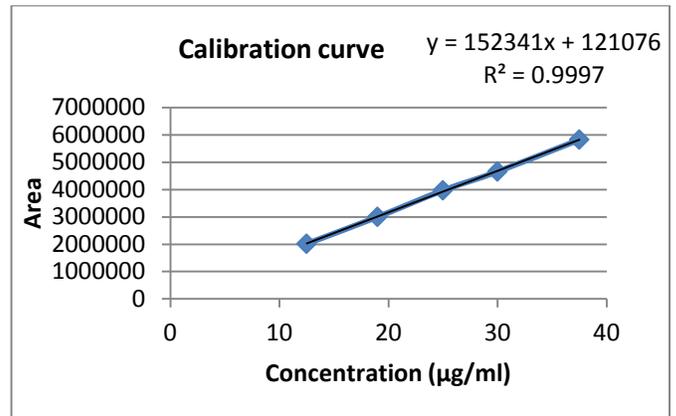


Figure-8 Calibration curve

Result of validation parameter

Table No.-2 Result of validation parameter

Parameter	TIBOLONE
Wavelength for estimation	205 nm
Linearity range (µg/ml)	12.5-37.5 µg/ml
Regression equation	$y = 152341x + 121076$
Correlation coefficient (r^2)	0.999
Retention time (min.)	5.71
Accuracy (n=3)	98.97 ± 18221.41
Precision	
Repeatability (n=6)	% Found 97.30 ± 0.38 % RSD 0.40
Intraday Precision (n=3)	% Found 100.55 ± 18267.37 % RSD 0.462377
Interday Precision (n=3)	% Found 99.5412 ± 0.548087 % RSD 100.35 ± 13400.3
Assay (n=6)	99.74 ± 0.75
%RSD	0.756061
LOD (µg/ml)	0.92
LOQ (µg/ml)	2.80

Application of the method to dosage forms

The present method is applied to the estimation of Tibolone in their commercially available tablets. The % recovery for

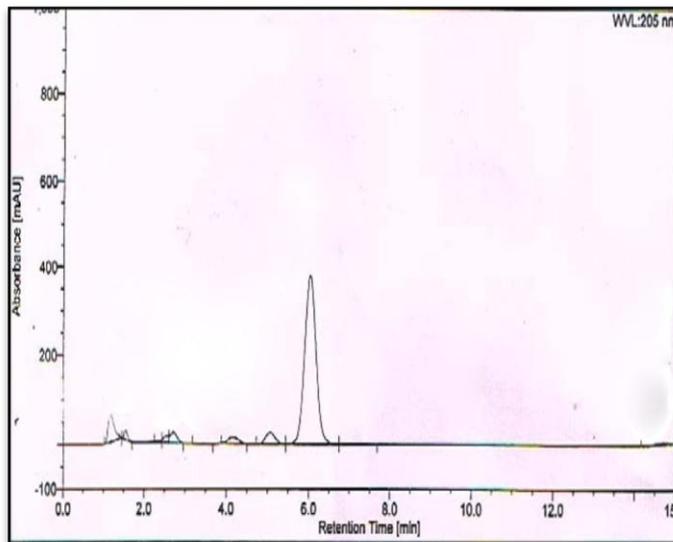


Figure-5 Chromatogram Of Tibolone Under base Degradation

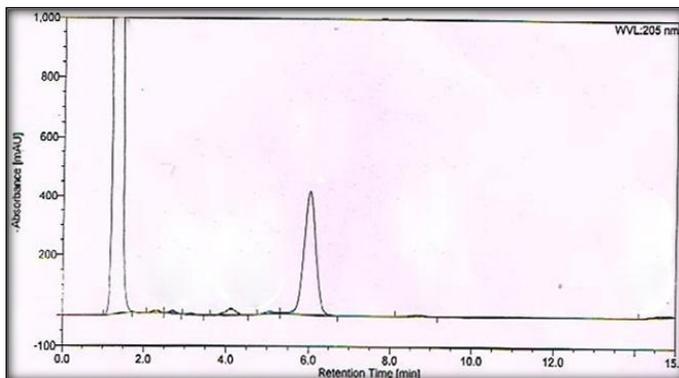


Figure-7 Chromatogram Of Tibolone Under Thermal Degradation

Validation of the method

Linearity

The linearity was determined by preparing standard solutions at 6 different concentrations levels ranging from 12.5-37.5 µg/mL. The regression equation of calibration curves was obtained as $y = 152341x + 121076$ with a correlation coefficient of 0.9999.

Tibolone was found to be 99.74 ± 0.75 (mean value \pm standard deviation) of three determinations, which was comparable to the corresponding labeled amounts.

Conclusion

A rapid, RP-HPLC method was successfully developed for the determination of Tibolone in the pharmaceutical tablets. The developed method is selective, precise, accurate, and linear. Forced degradation data proved that the method is specific for the analytes and free from the interference of blank and unknown degradation products. The run time (5.71 min) enables rapid determination of the tablet dosage form. Also, the results indicate the suitability of the method for acid, base, oxidation, and sunlight degradation studies. The method is suitable for the analysis of stability samples and the routine analysis of Tibolone in tablets.

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