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DEVLOPMENT AND VALIDATION METHOD FOR QUANTIFICATION OF NORETHISTERONE IN FORMULATIONS BY USING RP-HPLC

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ABSTRACT

A simple, precise, rapid and accurate reverse phase HPLC method developed for the estimation of Norethisterone in pharmaceutical dosage form. A Spheris orb ODS II {4.6×250 mm i.d, particle size 5µm} with mobile phase consisting of acetonitrile and water in the ratio of 45:55 v/v was used. The flow rate was 2 ml/min and effluents were monitored at 245nm. The retention time was 6 min. The detector response was linear in the concentration of 0.01-2µg/ml. The respective linear regression equation being y= 18904 x + 2112.0 (R2=0.999) the limit of detection and limit of quantification was 0.028 µg/ml and 0.085µg/ml respectively. The percentage assay of Norethisterone was 89.7 The method was validated by determining its accuracy, precision, and system suitability. The results of the study showed that the proposed HPLC method is simple, rapid, precise and accurate which is useful for the routine determination of Norethisterone in bulk drug and in its pharmaceutical dosage forms.

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KEYWORDS : *Norethisterone, HPLC, estimation, tablets dosage.*

INTRODUCTION

Norethisterone or northindrone chemically is 19-nor-17α-ethynyltestosterone. It is a molecule used in some combined oral contraceptive pills[1]; progestogen only pills and is also available as a stand-alone drug. It is a progestogen and can be used to treat premenstrual syndrome, painful periods, abnormal heavy bleeding, irregular periods, menopausal syndrome (in combination with oestrogen), or to postpone a period. It is also commonly used to help prevent uterine hemorrhage in complicated non-surgical or pre-surgical gynecologic cases. There are several analytical methods are reported on the analysis of Norethisterone. UV spectrophotometric analysis[2,3] dissolution studies[4], determination in human plasma by HPLC[5], Tandem mass spectroscopy in plasma by hplc [6, 7] determinations of related substances[8] and gas chromatography were noticed from the literature. The developed analytical method is simple, specific, accurate, and precise for

the routine analysis of Norethisterone in bulk and pharmaceutical formulations.

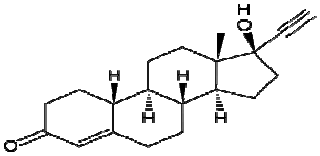

Figure 1 Norethisterone

INSTRUMENTATION

Quantitative HPLC was performed on LC waters separation, 2695 DAD dual absorbance detector module equipped with automatic injector with injection volume 200µl and 2487 pump. A Spheris orb ODS II {4.6×250 mm i.d particle size 5µm} was used. The HPLC system was equipped with empower software.

CHEMICAL AND SOLVENTS

Norethisterone was obtained as a gift sample from Wockhardt, DAMAN. Methanol and Acetonitrile were of HPLC grade and supplied by Merck, India ltd, Mumbai} commercially available Norethisterone tablets were procured from local market.

HPLC CONDITIONS

The contents of the mobile phase were acetonitrile and water in the ratio of 45:55 v/v was used. They were filtered before use through 0.45 µ membrane filters and pumped from the respective solvent reservoir to the column at a flow rate of 2 ml/min. The runtime was set at 10 min. and column temperature is ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluent were monitored at 245nm.

DILUENT: Methanol and water 80:20

PREPARATION OF STANDARD SOLUTION

A standard solution of the drug was prepared by accurately weigh 100 mg of Norethisterone in 100 ml volumetric flask. Dissolve in and dilute to volume with diluents. 1 ml of the above stock solution was taken in 100ml volumetric flask and thereafter makeup to 100ml with diluent {working standard solution

PREPARATION OF SAMPLE SOLUTION:

The sample Norethisterone equivalent to 100mg of is taken into a 100ml volumetric flask. Add about 50ml of diluents and sonicate to dissolve it completely and make volume up to the mark with diluents. Mix well and filter through 1.45µm filter. Further pipette 1 ml of the above stock solution into a 100ml volumetric flask and dilute up to the mark with diluents

RESULTS AND DISCUSSIONS

SYSTEM SUITABILITY

The working standard solution was prepared as per procedure and was injected 3 times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms. Tailing factor, retention time, theoretical plates and peak area are in table 1. The USP plate count is should be not less than 2000 and the USP tailing for the peaks should not be more than 2. These values were well within the limits as per ICH guide lines.

Table- 1: System suitability data

parameters	Norethisterone
Retention time	6.0
USP plate count	5766
USP tailing	1.09
Peak area	19862
%RSD	1.14
LOD	0.028
LOQ	0.085

* Mean of three determinations

METHOD VALIDATION

LINEARITY

Aliquots of standard Norethisterone stock solution were taken in different volumetric flasks and diluted up to the mark with the diluents such that the final concentrations of Norethisterone are in the range of 0.01-2.0µg/ml. Each of these drug solutions was injected 200µl into the column and the peak area and Retention time were recorded. Evaluation was performed with DAD detector at 245nm and a calibration graph was obtained by plotting peak area versus concentration of Norethisterone. The plot of peak area of each sample against respective concentration of Norethisterone was found to be linear in the range of 0.01-2.0µg/ml with correlation coefficient 0.9998. Linear regression least square fit data obtained from the measurements are given in table 2. The respective linear regression equation being Y= 18904 x + 2112.0

Table- 2: Linear regression data for calibration curve

Concentration{µg/ml}	Peak area
0.01	2492
0.02	4075
0.03	7995
0.04	8634
0.05	10412
0.1	20257
0.2	41753
0.3	60188
0.4	80656
0.5	97207
1.0	191918
2.0	378809
Concentration range	0.01-2.0 µg/ml
Slope	18904
Intercept	2112.0
Correlation coefficient	0.999

PRECISION

The precision of the method was determined in terms of repeatability {intraday} and intermediate {inter day} precision. The intraday and inter day variation in the peak area of drug solution was calculated in terms of %RSD. The standard solution was injected for five times and measured the area of all five injections. The %RSD was found to be within the specified limits {not be more than 2%} the intermediate precision {ruggedness} was performed on different day by using different make column of same dimensions. The %RSD was found to be within the specified limits {not be more than2%} Table: 3 precision and intermediate precision results

Table -3: precision and intermediate precision results

Concentration of Norethisterone. {30µg/ml}	Peak area	
	Intra day	Inter day
Injection 1	19589	19865
2	19803	19454
3	19821	19725
4	19753	19132
5	19621	19611
6	19771	19442
Average	19726	19538
S.D	97.33	256.32
%RSD	0.49	1.31

ACCURACY

The accuracy of the method was evaluated by determination of recovery of Febuxostat at 3 levels of concentrations. The sample was spiked with Febuxostat standard solutions corresponding to 50%, 100% and 150% of nominal analytical concentration. The results showed good recovery within limits {99.8% to 101.9%} the results are tabulated in Table 4

Table- 4: Accuracy studies results

%concentration	Amount added mg	Amount found mg	% recovery*	%RSD*	Mean Recovery
50%	0.05	0.0450	89.9%	1.2	89.7%
100%	0.1	0.0903	90.3%	1.4	
150%	0.15	0.138	91.7%	0.6	

*Mean of three determinations

LOD and LOQ

The limit of detection and limit of quantification of the developed method were determined by injecting progressively low concentration of the standard solutions found to be 0.028 µg/ml and 0.085µg/ml respectively. The LOD and LOQ values reveal that the developed method shows very good sensitivity.

ASSAY

Sample solution was injected under the same chromatographic conditions and the chromatogram was recorded in triplicate. The amount of Febuxostat present in tablet formulation was determined by comparing the peak area from the standard. The results are furnished in table 6. The % of recovery is 98.7%. No interference peaks were found in the chromatogram indicating that the determination of the drug content was free from interference by excipients.

Table- 6: assay results

Label claim in mg	Amount found in mg	% recovery
15	14.83	98.7

STABILITY OF SOLUTION

The stability of Norethisterone in analytical solution shall be established at room temperature by injecting a standard solution initially and at different time intervals up to 24 hours. The cumulative %RSD for the peak area counts of Norethisterone shall be determined. The results to be tabulated in table 7

Table-7: stability data

Time in hrs	Peak area	Cumulative mean	Cumulative % RSD
Initial	23809	0	0
1 hr	21050	22430	8.7
3hr	21387	21908	6.8
6hr	22654	22281	5.7
12hr	21903	22092	5.0
24hr	21794	21943	4.5

***Limit: NMT 10%**

CONCLUSION

The present method is precise, sensitive and accurate. The advantage of proposed method is its short analysis time and a simple procedure for sample preparation. The satisfying recoveries and the low coefficient of variation confirmed the suitability of proposed method for the routine analysis of Febuxostat in pharmaceuticals.

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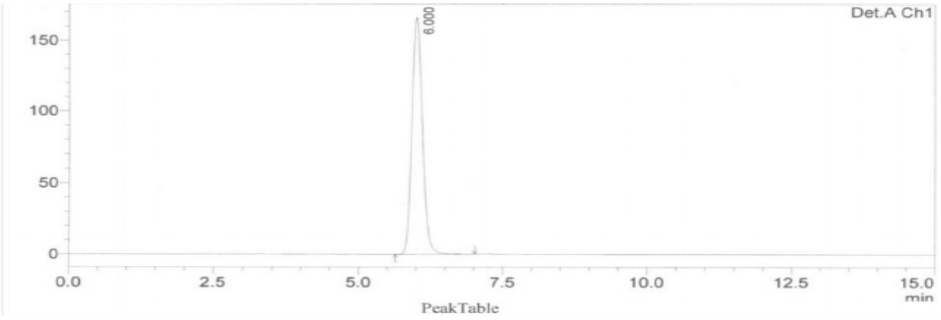
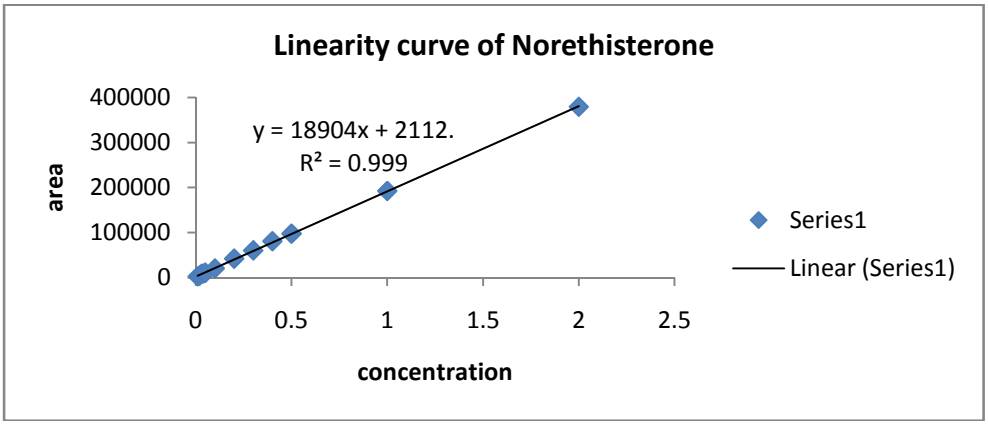
Figure- 2: chromatogram of Norethisterone





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