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METHOD DEVELOPMENT AND VALIDATION OF NORETHINDRONE BY UV-VISIBLE SPECTROPHOTOMETER IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, accurate, precise, and economic method was developed for estimation of Norethindrone acetate in bulk and pharmaceutical dosage form. This is a first method for determination of Norethindrone by UV-Visible spectroscopy. Using methanol and water as solvent in 50:50 ratio spectrum was produced and maximum absorbance was found at 256nm. Then the method was optimized and validated for various parameters. By conducting accuracy percentage recovery was found to be 100.2%, from linearity studies correlation coefficient R^2 and range was found to be 0.9979, 25 to 100µg/mL. From precision and intermediated precision studies %RSD was found to be 0.22 and 0.88 respectively. LOD and LOQ were found to be 0.9µg/ml and 3µg/ml respectively. Assay was conducted by using Premoult-Nor marketed formulation and percentage purity was found to be 101.91%.

KEYWORDS: Norethindrone acetate, UV-Visible spectroscopy, methanol and water, method development, validation, assay.

INTRODUCTION

Norethindrone is in progesterone form of female hormone. It is used for replacement therapy. Norethindrone tablet is also used as an oral contraceptive product. Daily dose of 0.35mg Norethindrone provides a continuous oral contraceptive regimen.[1] IUPAC name of Norethindrone is (1S,2R,10R,11S,14R,15S)-14-ethynyl-15-methyl-5-oxotetracyclo [8.7.0.0^{2},^{7}.0^{11},^{15}] heptadec-6-en-14-yl acetate[2] Chemical structures of Norethindrone Acetate shown in fig. 1.[3]

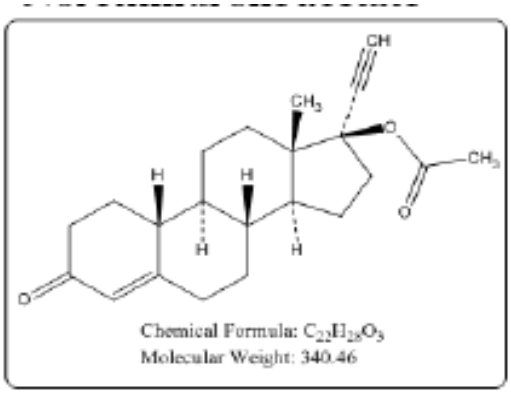

Fig. No. 1: Structure of Norethindrone Acetate.

No experiment was done for estimation of Norethindrone acetate by using UV spectroscopy. Few analytical methods have been reported for the assay Norethindrone and its combinations by using RP-HPLC, UPLC method in bulk and Pharmaceutical dosage forms.[4-9]

MATERIALS AND METHOD

Equipment: A Shimadzu UV–visible spectrophotometer was used for all absorbance measurements with matched quartz cells.

MATERIALS

All chemicals and reagents were of merk. Norethindrone acetate powder was provided by Hetero Pharmaceuticals Ltd. which was used as the reference standard. Aygestin tablet used as sample.

Standard Preparation

Weigh accurately about 10mg of Norethindrone acetate and dissolve in 10 ml of acetonitrile. Take 1ml solution from above and makeup the volume to 10ml with solvent system.

Solvent System

Methanol and water 50:50.

Determination of wavelength of maximum absorption:

A standard stock solution of norethindrone acetate (20 µg/mL) was prepared using diluents and 2 mL of norethindrone acetate was then diluted to 10 mL with solvent. An UV spectroscopic scanning (190– 400 nm) was carried out to determine the ʎmax for the detection using solvent system as blank.

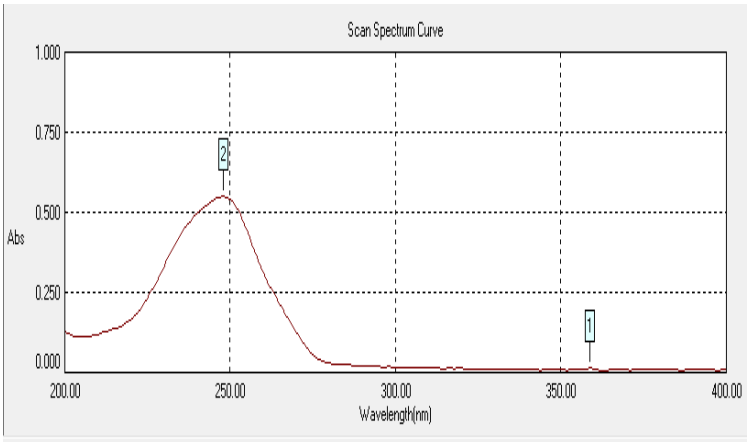

Fig. No. 2: Spectrum of Norethindrone Acetate.

Linearity and range

To establish linearity of the proposed method, five different sets of drug solution was prepared and analyzed. Standard curves were constructed in the concentration range of 25-150µg/ml of ritonavir (Fig.3). The correlation coefficient was determined as 0.9979 and the results were shown in Table no 5.

Table No. 1: Linearity Observation.

S. No.	Linearity Level	Concentration(µg/ml)	Absorbance
1	I	25	0.163
2	II	50	0.271
3	III	75	0.431
4	IV	100	0.564
5	V	125	0.713
6	VI	150	0.829
Correlation Coefficient			0.9979

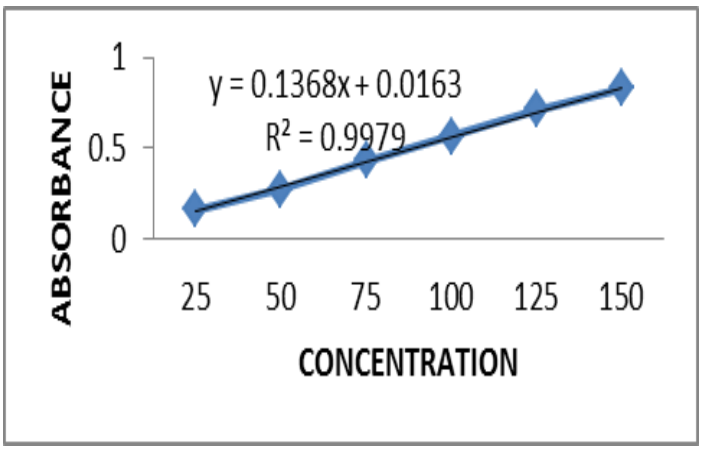

Fig. No. 3: Linearity.

Accuracy/recovery study

Accuracy of the method was demonstrated at three different concentration levels (50-150%) by spiking a known quantity of standard drugs into analyzed sample in triplicate. The percentage recovery was found to be 100.20%. The results of accuracy (Table 2) revealed that the method was more accurate.

Table No. 2: Accuracy Results.

%Concentration (at specification Level)	Absorbance	Amount Added(µg)	Amount Found(µg)	% Recovery	Mean Recovery
50%	0.281	50	49.82	99.64	100.20%
100%	0.565	100	100.17	100.17	
150%	0.853	150	151.2	100.8	

Precision

For the precision of the method, three replicate were injected into the system on same day and %RSD was calculated, i.e 0.223. Results of precision are given in Table 3, which indicated that the method is precise.

Table no. 3: Precision Results.

SOLUTION	ABSORBANCE
Standard Solution -1	0.564
Standard Solution -2	0.563
Standard Solution -3	0.566
Standard Solution -4	0.563
Standard Solution -5	0.563
Standard Solution -6	0.565
Avg	0.564
SD	0.001265
%RSD	0.224275

Intermediate Precision

For the precision of the method, three replicate were injected into the system on two different non consecutive days, in each case %RSD was 0.88. Results of precision are given in Table 4, which indicated that the method is precise.

Table no. 4: Observation of Intermediate System Precision.

SOLUTION	ABSORBANCE
Standard Solution -1	0.564
Standard Solution -2	0.561
Standard Solution -3	0.559
Standard Solution -4	0.556

Standard Solution -5	0.553
Standard Solution -6	0.551
Avg	0.557
SD	0.0049
%RSD	0.88

LOD and LOQ

LOD AND LOQ were calculated and results were shown in table no. 6.

Table No. 5: LOD&LOQ results.

Drug Name	LOD	LOQ
Ritonavir	0.9µg/ml	3µg/ml

Assay of Norethindrone

Primolut-Nor marketed formulation was selected, 10 tablets were randomly selected and analyzed using the newly developed and validated method. Take the tablets in mortar and pestle to crush in powder. Take 10mg equivalent drug powder and dissolve in acetone. Filter the solution and dilute the solution to 10ml. Sample and standard absorbance were measured and Drug percentage was calculated. The Percentage purity was found to be 101.91%.

CONCLUSION

A new method was developed and validated for the determination of Norethindrone Acetate in pharmaceutical dosage form using UV spectroscopy. The proposed method was found to be accurate, precise, simple, economic and rapid. The developed method can be applied for the assay of commercial tablets containing Norethindrone Acetate in routine quality control analysis.

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