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Spectrophotometric Method for Analysis of Norethisterone in Pure and Marketed Formulations

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ABSTRACT: A new simple, sensitive, precise and economical spectrophotometric method of analysis for Norethisterone drug was developed and validated. The method employed methanol as solvent. The Keto group of Norethisterone is responsible for the development of condensation reactions with 2,4 DNPH. The drug was then estimated at 475 nm. The linear regression analysis data for the calibration plots showed good linear relationship with $r^2 = 0.998$ in the concentration range 2-12 $\mu\text{g/ml}$. The mean value of correlation coefficient, slope and intercept were 0.998, 0.061 and 0.013 respectively. The method was validated for precision, accuracy and recovery studies. LOD and LOQ for norethisterone was found to be 0.290 ($\mu\text{g/ml}$) and 0.890 ($\mu\text{g/ml}$) respectively. The method has been successfully applied in the analysis of marketed formulations.

KEY WORDS: Norethisterone, UV-Visible spectrophotometer, Keto group, Validation.

I. INTRODUCTION

Norethisterone (or Norethindrone) was the first orally highly active progestin to be synthesized. It was the progestin used in one of the first three oral contraceptives. Its tablets belong to a group of medicines called progestogen and may be used to prevent abnormal or unexpected bleeding from the uterus, the symptoms of pre-menstrual syndrome which occur before a period including breast pain, headache, migraine, water retention and mood disturbances, painful or heavy periods, endometriosis, certain types of breast cancer. The tablets can also be used to stop periods. The physical and chemical properties of the Norethisterone are presented below and its molecular structure was shown in Figure-1

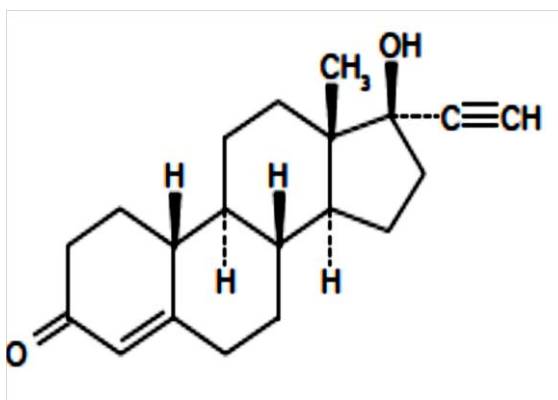


Figure-1: The molecular structure Norethisterone molecule

Drug profile

Nomenclature	: 17 β -Hydroxy-19-nor-17 α -pregn-4-en-20-yn-3-one
Molecular formula	: C ₂₀ H ₂₆ O ₂
Molecular weight	: 298.419
Category	: Contraceptive
Solubility	: Alcohol, water, acetonitrile and acetone
Water solubility	: 0.00704 g/L at 25 ^o C
Melting point	: 203 ^o C
Physical state	: White or yellowish-white odourless crystalline powder.
Formulations (Tablet) available	
PrimolutN (Zydus Cadila Healthcare)	: 5mg
Norethisterone Tablets (Symbolic Pharma)	: 5mg
Endotif (Proton Pharma Private Limited)	: 5mg

Very few visible spectrophotometric methods were available in the literature for quantitative determination of Norethisterone in bulk drug samples and tablets, therefore there may a scope for developing new method using chromogenic reagent, namely 2, 4 DNP to determine the quantity of the drug.

II. MATERIALS AND METHOD**UV- Visible spectrophotometer**

A Lab India model 3000 double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Win system software.

Preparation of drug standard solution

The stock solution (1mg/ml) of Norethisterone was prepared by dissolving 100 mg of Norethisterone in 50 ml of methanol, followed by dilution to 100 ml with distilled water. This solution was further diluted step wise with distilled water to obtain working standard solution of corresponding concentration 100 μ g/ml

Chemicals and chromogenic reagents

Analytical grade chemicals and reagents were used in the preparation of solutions and all the solutions were prepared in triply distilled water.

2, 4-dinitrophenyl hydrazine (2, 4 DNP) 0.1 % (w/v): A 0.1% w/v of the reagent solution was freshly prepared by dissolving 0.1 g of 2, 4 DNP in 2 ml of concentrated H₂SO₄ and diluting to 100 ml with water. The solution was freshly prepared and protected from light during the use.

10N Sodium hydroxide solution: About 40 g of sodium hydroxide was accurately weighed and transferred into a 100.0ml volumetric flask and made up to the mark with distilled water.

Potassium iodate 4% (w/v): Weighed and dissolved about 4 g of potassium iodate in a 100.0 ml volumetric flask and made up to the mark with distilled water.

III. GENERAL PROCEDURE

Aliquots of (0.2 to 1.2 ml, 100 μ g/ml) working standard solution of Norethistrone were transferred into a series of 10 ml volumetric flasks. To each of above-mentioned aliquots, 1.5 ml of 0.1% 2, 4 DNP, 1.5 ml of 4% potassium iodate and 1 ml of 10N sodium hydroxide was added. The mixture was then gently shaken until the appearance of colour chromogen. The contents were diluted up to 10 ml with distilled water. The absorption spectrum was recorded and the absorbance of remaining solutions was measured at 475nm. The calibration plot was as shown in Figure-2.

IV. VALIDATION OF THE METHOD

The proposed method was validated in terms of precision, accuracy, linearity, limit of detection, limit of quantification. The experimental details followed in validation were presented below.

Precision

Precision (reproducibility of measurements within a set of measurements) of the proposed method was determined from the absorbance values for six replicates of a fixed amount of Norethisterone within the Beer's law limits. The percent relative standard deviation was calculated.

Accuracy

Accuracy (the concordance between the measured value and the true) was determined at three different amounts of bulk samples of Norethisterone within the Beer's law limits, analyzed by the proposed method in triplicate. The amount of drug found was estimated and thus the percent of recovery of the drug was calculated. Mean percent of recovery, standard deviation and percent of relative standard deviation were calculated.

Linearity

Linear relationship between absorbance and concentration of the analyte was examined by developing coloured product, measuring absorbance at wavelength of maximum absorbance, and constructing the calibration graph. Linear least square regression analysis was carried out for getting the slope, intercept and correlation coefficient. Limit of detection and limit of quantification were evaluated. Wavelength of maximum absorbance, Beer's law limits, molar extinction coefficient, Sandall sensitivity, slope, intercept, correlation coefficient, standard deviation, %RSD, LOD, LOQ % assay and recovery of drug in the developed methods were presented in Table-1

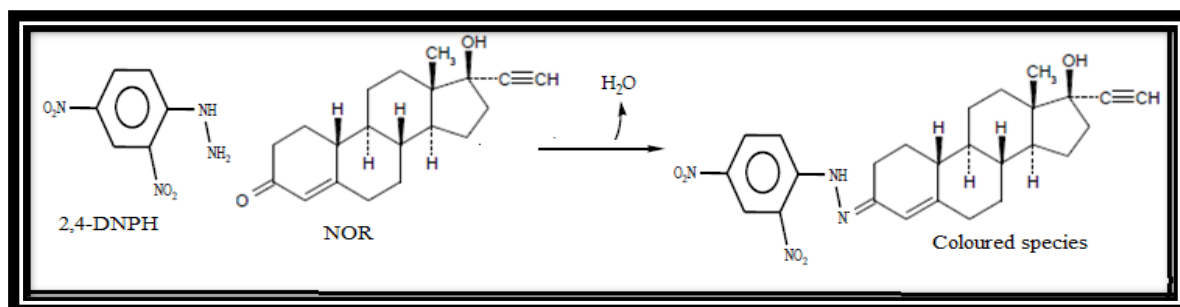
Assay of Pharmaceutical formulations

An accurately weighed portion of tablet content equivalent to about 100 mg of Norethisterone was transferred into a 100ml volumetric flask. Added about 80 ml of methanol and shaken well for about 20 min. The contents were diluted with methanol up to the mark and mixed thoroughly. The solution was filtered. The filtrate was evaporated to dryness. The residue was used for the preparation of formulation solution for method as given under standard solutions preparations.

The percent recoveries were determined by adding different known amounts of the standard drug to equal amount of test sample; reagents were added and diluted to the same volume. The absorption values were measured for every solution and results were plotted on a graph with the dependent variable (absorbance) on y – axis and the amount of the drug added on x- axis. Extrapolation of the straight line thus obtained to the point where the x-axis was cut provides a measure of drug in the test solution. The results of the recovery experiments by the proposed methods were listed in Table-1

V. CHEMISTRY OF COLOURED PRODUCT

In this proposed method condensation of Norethisterone with 2, 4 -DNPH results in formation of orange red colored hydrazone with removal of water molecule. The possible reaction is given in Scheme-1



Scheme:1 Colour reaction between Norethisterone and 2,4-DNPH

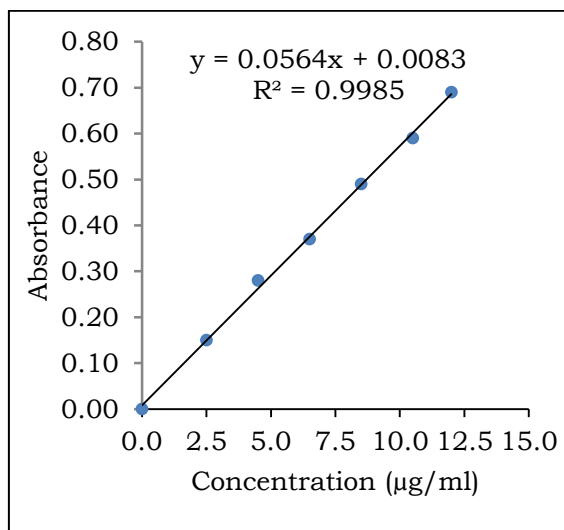


Figure:2 Calibration plot of Norethisterone

S.No	Parameter	Method
1	λ max (nm)	475
2	Beer's law range (µg/ml)	2-12
3	Molar extinction coefficient (Lit/mole/ cm)	1.82×10^4
4	Sandall's sensitivity (µg/cm ² /0.001)	0.0164
5	Regression equation (y = mx + b)	0.061
	Slope (m)	0.013
	Intercept (b)	
6	Correlation coefficient (r)	0.998
7	Standard deviation (SD)	0.005
8	Precision (%RSD)	1.418
10	LOD (µg/ml)	0.290
11	LOQ (µg/ml)	0.890
12	% of assay formulation	99.97
13	Average recovery	97.10

Table-1: Optical Characteristics of the proposed method

Pharmaceutical formulation	Labelled amount	*Amount found (mg) ± S.D	% Recovery ± R.S.D
Tablet-I	5 mg	4.89 ± 0.14	97.8 ± 0.11

Average of six determinations

Table-2: Assay of Norethisterone in Pharmaceutical Dosage Forms

VI. CONCLUSION

The proposed method exploits the functional group (Keto group) in Norethisterone. This method is simple, sensitive and reliable and can be used for routine determination of Norethisterone in bulk and pharmaceutical formulations. Statistical analysis of the results showed that the proposed procedure has good precision and accuracy. Results of the analysis of pharmaceutical formulations reveal that the proposed method is suitable for routine analysis with virtually no interference of the usual additives present in pharmaceutical formulations.

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