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A Novel Validated High Performance liquid chromatographic stability indicating assay method for the chemical analytical quantification of Norethindrone Tablets and their degradation study

Rashid Azeez*, Masood Ahmed Siddiqui, Ziyaurrahman Azeez

Department of Clinical Biopharmacological Analysis & Quality Assurance Techniques, Royal college of Pharmaceutical education and Research (S.P. Pune University), Sayne, Malegaon (Nasik) ,423203 Maharashtra, India.

***Corresponding author:** Rashid Azeez , Department of Clinical Biopharmacological Analysis & Quality Assurance Techniques, Royal college of Pharmaceutical education and Research (S.P. Pune University), Sayne, Malegaon (Nasik) ,423203 Maharashtra, India; **E-mail:** drrashid.azeez@gmail.com*

ABSTRACT

Norethindrone is a synthetic chemical analogue of naturally occurring steroid whose structure is chemically based on a steroid nucleus-a hydrogenated cyclopentanoperhydrophenanthrene ring having 17 carbon atoms. This is new drug and vital in medicine. Tablet dosage form is new pharmaceutical formulation and used as oral contraceptive. They are low dose and have less label claim. The development and validation of assay method by RP-HPLC-DAD is challenging and not yet reported. The objective of this research was to develop and validate a precise, efficient, specific, accurate and stability indicating RP-HPLC-DAD assay method for the quantification of Norethindrone in Norethindrone tablets and their forced degradation studies. This HPLC method developed have a reverse phase Inertsil ODS-3V, 150 x 4.6mm, 5µ column, a mixture of amine buffer pH 2.5 and acetonitrile as mobile phase in isocratic mode with UV detection wavelength 240 nm. The method was validated and found to be precise, robust, accurate, linear in the range of 17 to 52 µg /mL (r2 = 0.9984) and specific for Norethindrone and degradation products ensuring suitability of the method for quantitative determination of Norethindrone. Solution stability evaluation indicated no evidence of degradation product, forced degradation study of Norethindrone shown that peak was pure and there was no co eluting peaks when samples were assayed against reference standard. Method was useful for routine analysis and quality check of Norethindrone formulations.

Keywords: Norethindrone, Assay, pharmaceutical analysis, HPLC method development, Validation, degradation study.

INTRODUCTION

Steroids are a group of naturally occurring or synthetic fat-soluble organic compounds, whose structure is chemically based on a steroid nucleus-a hydrogenated cyclopentanoperhydrophenanthrene ring system (17 carbon atoms arranged in 4 rings). Many hormones, body constituents and drugs are steroids. All the corticosteroid hormones of the adrenal cortex, all the sex hormones, (androgens, testosterones, estrogens and progesterone), all vitamin D group, some corticosteroid drugs like prednisone are steroids. Synthetic chemical analogues of many of the naturally occurring steroids are vital in medicine. Both natural and synthetic steroids are used to treat many disorders and play a vital role in the normal functioning of the body. The chemical name of Norethindrone is 19-Norpregn-4-en-20-yn-3-one-17-hydroxy-(17)-17-Hydroxy-19-nor-17-pregn-4-en-20-yn-3-one.

The method development of steroid hormone drug product analysis during the last 15 years can be characterized by the facts that HPLC undoubtedly became the most important routinely used pharmaceutical analytical method and the importance of hyphenated chromatographic spectroscopic techniques increasing and attract wide interest in steroid analysis [1]. Norethindrone is form of progesterone, a female hormone. Norethindrone tablet is used as oral contraceptive.

As per reported literature, a set of HPLC assay methods are presented to distinguish and qualitatively analyze nine steroid hormone drugs [2]. The force degradation study, specificity, solution stability parameters of validation are not yet reported. There is no fully validated, stability indicating analytical assay method available in literature with force degradation study by HPLC for determination of Norethindrone in Norethindrone Tablets 0.35mg [2-6]. There are some analytical methods available for determination of related substances by HPLC and UPLC [3,4]. According to the ICH stability testing guideline stress testing of the drug products is considered necessary to establish the stability of the molecule which is important to detect the degradation products and stability indicating nature of the analytical procedure [7,8]. Norethindrone tablets are official in USP Pharmacopoeia [9,10]. A successful related substances HPLC method and degradation studies their results and all analytical validation parameters data tabulated in work done by Rashidul et al. They did development and validation of RP-HPLC-DAD stability indicating related substances method for the estimation of impurities of Norethindrone tablets and their degradation products, comparison of those data with this work is done and found suitable [11]. In recent years FDA strictly asking mass balance in forced degradation of related compound method to prove specificity and capability to quantify degradation impurities. Considered the drug stability test guidance Q1A (R2) issued by ICH [12].

The prevalence of counterfeit drugs is a vast global problem, especially in developing countries. Counterfeit drugs may contain no active ingredient or the correct ingredient but lower or higher amount of active ingredient. Quick identification would be useful to combat these problems. Our goal in this work is to develop, optimize, and validate a simple, precise stability indicating assay method for determination of Norethindrone in Norethindrone Tablet 0.35mg by Reverse Phase High Performance liquid chromatography in combination with Diod array detector (RP-HPLC-DAD). Keeping track of adverse drug reactions in remote areas of the world, it is essential for the improvement of the quality of life at a global scale [13]. (Figure 1).

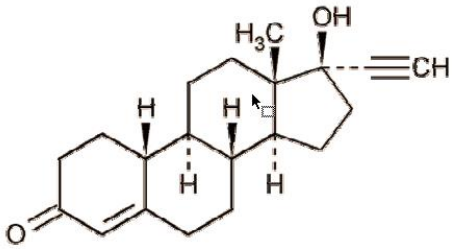

Figure 1: Structure of Norethindrone

MATERIALS AND METHODS

Chemicals and reagents

Triethylamine, methanol and acetonitrile were HPLC grade and purchased from Merck Speciality Pvt. Ltd. Water used in all experiments was passed through a Milli-Q (Millipore) water purification system (18 mΩ). All other chemicals taken are analytical grade. Reference standard Norethindrone and Norethindrone tablets 0.35mg were obtained as gift sample from Bayer, India.

Equipments

A waters HPLC system equipped with 2695 separation module PDA 2996, Waters 2487 dual wavelength detector with empower software, Inertsil ODS-3V, 150 x 4.6mm, 5µ, Digital ultra sonicator, pH meter of Thermo scientific, Mettler Toledo electronic analytical balance, Fisher scientific vacuum oven and humidity desiccator were used.

Analytical method development and optimization

The aim and objective of the present study was to develop a simple, precise, specific, accurate, stability indicating HPLC method for quantification of Norethindrone in Norethindrone tablets 0.35mg.

The pure drugs of Norethindrone were injected in to the isocratic HPLC system and run at different solvent systems. Different mobile phases like methanol and water, Acetonitrile and water, methanol and Acetonitrile were tried in order to find the best conditions for the elution. It was found that mixture of trimethylamine buffer (pH2.5) and acetonitrile in mobile phase illustrate good elution pattern and satisfactory results as compared to other mobile phases. The mobile phase system was tried with different proportions and using different flow rates. Several trials were taken by injecting standard solution of Norethindrone in several analytical column of C8 and C18, 150mm x4.6 length, column including Inertsil ODS-3V column 150 x 4.6mm, 5µ and Inertsil 150mm x 4.6mm, 5µ column. The good elution and peak shape observed at Inertsil ODS-3V, 150 x 4.6mm, 5µ column compared to other column. Standard solution was injected at 240nm in PDA detector to obtain chromatogram of Norethindrone, and the wavelength selected was 240 nm as the drug showed significant absorbance at this wavelength. Finally the optimum chromatographic condition achieved for best elution pattern are: composition of the mobile phase was determined to be mixture of triethylamine buffer pH2.5 adjusted with orthophosphoric acid and acetonitrile in the ratio of 60:40 v/v respectively at a flow rate of 2 ml/minute on Inertsil ODS-3V, 150 x 4.6mm, 5µ column at the detection wavelength of 240nm. Column temperature is 30°C, Injection volume is 20µl.The run time was set as 12 minutes. Retention time of Norethindrone peak is about 9.0 minutes.

Preparation of Buffer solution

Added 1.0 mL of triethylamine to 1000 mL of water. Adjusted pH to 2.5 ± 0.05 with Orthophosphoric acid.

Preparation of mobile phase

Used filtered and degassed mixture of buffer and acetonitrile in the ratio of 60:40 v/v.

Preparation of diluent

Mixed acetonitrile and water in the ratio of 60: 40 v/v.

Preparation of standard solution

Weighed accurately about 28 mg of Norethindrone standard into a 200 ml volumetric flask, added 150 ml of diluent, sonicated to dissolve and made up to the mark with diluent. Diluted 5 ml of this solution to 20 ml with diluent.

Preparation of sample solution

Transferred 10 tablets of Norethindrone 0.35mg strength in to a 100 ml volumetric flask, added 70 ml of diluent and sonicated for 15 minutes with intermittent shakings. Allowed to cool to room temperature. Diluted to volume with diluent. Mixed and allowed to settle for 5 minutes. Filtered through 0.45µ nylon filter, discarded first few mL of filtrate and injected the solution.

Evaluation of system suitability

Prior to perform method validation study, system suitability criteria was set forth as; inject the standard solution five times. The relative standard deviation of five replicate injections should not be more than 2.0%. The Tailing factor should not be more than 2.0 and number of theoretical plates should not be less than 2000. Separately inject equal volumes of blank and sample solution into the chromatograph and record the chromatograms. Measure the area counts for the Norethindrone Peak.

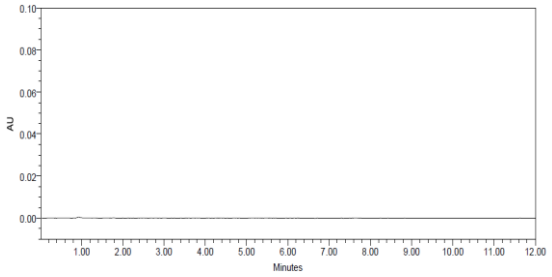

Figure 2: Diluent (blank) chromatogram

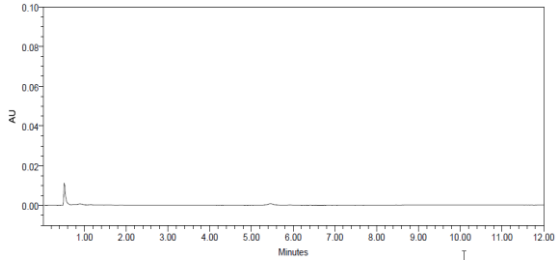

Figure 3: Placebo chromatogram

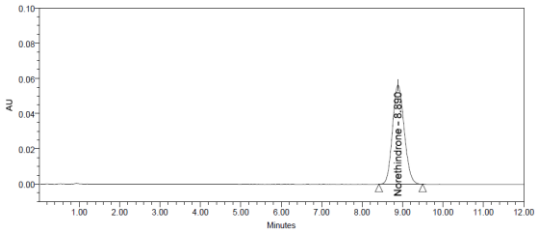

Figure 4: Norethindrone Standard chromatogram

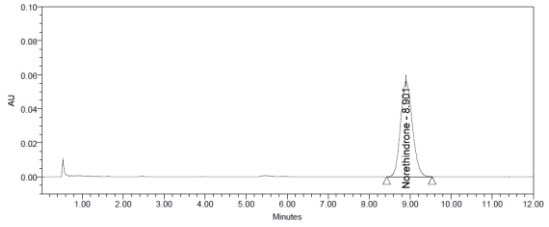

Figure 5: Norethindrone Control sample chromatogram

Analytical method validation

For validation of analytical method, the guidelines of the International Conference on the Harmonization [7-8] and the United States Pharmacopoeial Convention [9] recommend the accomplishment of linearity and range, accuracy, precision, LOD and LOQ and selectivity.
In Specificity once the forced degradation samples are generated, the next step is their evaluation for the degradation products formed under all the stress conditions. Studying the samples individually does this. Mostly samples are evaluated on chromatographic techniques, the most commonly used are HPLC, UPLC, UHPLC, and CE [1].

The major concern here is the successful separation of all the degradation products from the drug and from each other, viz development of stability-indicating method [1]. Hence, preference has to be given to a selective method, with emphasis on peak purity. Sensitivity of the developed analytical method should have well enough to detect impurities at low levels. During the method development it may happen that the drug peak may hide a degradation impurity peak that co-elutes with the drug. All known and unknown impurities should be well separated from main component peak and all unknown impurities should be well separated from known impurities. Sometime two merged unknown impurity may lead to out of specification results in real time stability therefore analytical method should have capability to resolve any unknown impurities higher than unknown impurity specification level from merging with each other.

Peak purity parameters are used to prove spectral uniqueness of compound. Peak purity of main component and known impurities in control and degradation samples should be established using PDA (Photo diode array) detector. There are some limitations in establishing of peak purity and cannot be calculated accurately if, UV detection response of main component at saturation level (nonlinear UV detection at higher absorbance values), Co-eluting peak having same UV spectra, co-eluting peak has no chromophoric functional group [6-7].

The forced degradation studies are performed to develop and validate a stability indicating method, to identify degradation products that may be generated during real time stability, to develop a method to quantify degradation impurities and separate excipients peaks (placebo) from impurities, to interpret possible degradation path-ways, to generate more stable formulations, to solve stability-related problems (e.g., mass balance).

In recent years FDA strictly asking mass balance in forced degradation of related compound method to prove specificity and capability to quantify degradation impurities. Mass balance establishes adequacy of a stability indicating method though it is not achievable in all circumstances. Lacking of mass balance lead a suspicion on capability of method to quantify all degradation products accurately. Mass balance is always challenging to evaluate accurately. The mass imbalance, apart from varying responses of analyte and degradation product peaks, may also happen due to potential loss of volatile degradation products, formation of non-chromophoric compounds, formation of early eluents, and retention of compounds in the column [6-7].

Specificity

The specificity is the ability to assess unequivocally the analyte of interest in the presence of those components which may be expected to be present in the sample matrix. Specificity/selectivity is the most crucial parameter of any analytical method used for stability and assay determination. The specificity of this method was determined by ensuring the absence of interference by any peaks in the blank diluent or placebo matrix, and resolving any possible degradation peaks from the peaks of interest.

For the forced degradation study, the sample and placebo excipient were all subjected to stress conditions (acidic, basic, oxidative, and thermal conditions). The stress conditions for the degradation study comprised of heat (105°C/72 hours), acid hydrolysis (5N hydrochloric acid/70°C/3 hours), base hydrolysis (2N sodium hydroxide/70°C/1hour), and oxidation (50% hydrogen peroxide/ 70°C/3 hours), humidity at 25°C/92%RH for 72 hrs., photolytic degradation sample was exposed to 1.2 million lux hours of light and analyzed. Acid and base hydrolysis samples were neutralized prior to diluting to the volume with diluent. Oxidation and thermal stress samples were diluted with diluent and all aliquots were filtered prior to do injection in HPLC. Percent assay recovery of each of the analytes and each degradant's % of the parent peak were calculated under each degradation condition.

Linearity and range

Linearity is the ability of a method to elicit test results that are directly proportional to the drug concentration within a given range. Five concentration levels used along with certain minimum specified ranges are required. The acceptance criterion for linearity is that the correlation coefficient (r2) should not be less than 0.990 for the least squares method of the analysis of the line [9]. To evaluate the linearity of the method, several concentrations of Norethindrone standard solutions were analyzed by RP-HPLC-DAD, and the peak area were used for plotting the curve as peak areas of the Norethindrone versus concentration (in ppm).

Accuracy

The accuracy of an analytical procedure is the closeness of test results obtained by that procedure to the true value. The accuracy of this method was verified by determining the recovery of a known amount of analyte added to the sample matrix (a spiked placebo).

Accuracy solutions were prepared by spiking in the appropriate amount of the analytes of interest into the sample matrix and assaying using a standard [9]. A stock solution of placebo was prepared and spiked by the known amount of Norethindrone. Accuracy samples were prepared by serial dilutions of the stock standard added to the placebo solutions at 80%, 100%, and 120% of the Norethindrone test concentrations. Three solutions of each active in each accuracy level were prepared by spiking in the appropriate amount of actives (separately) into the placebo. These solutions were prepared in triplicate for a total of 9 solutions and injected. The data were evaluated the amount prepared versus the amount recovered and expressed as a percentage recovery.

Precision

The precision of an analytical procedure is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample. Precision has been further divided into repeatability and intermediate precision (ruggedness). Repeatability is further divided into system precision and method precision (reproducibility). System precision is the ability of the system to show repeatable measurements of standard solution and method precision is reproducibility to show reproducible sample measurement. The system precision was evaluated by preparing single standard solution of norethindrone and injecting five times. Similarly, method precision was evaluated by preparing six samples of each Norethindrone. Method precision was repeated for Norethindrone tablet 0.35mg by a second analyst as a part of the intermediate precision evaluation. The second analyst prepared and assayed six samples on a different day, using a different HPLC instrument, and different column. Assay results of both analysts were combined (n=12) and the overall percent relative standard deviation was calculated [9].

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations to the procedure listed in the method and provides an indication of its reliability during normal usage. The robustness of this method was determined by assaying Norethindrone sample while deliberately adjusting the flow rate, the detector wavelength, and the column temperature. The system suitability parameters, %RSD of peak area response at the beginning and throughout the run, theoretical plates, and tailing factor were evaluated after every alteration to ensure system suitability meeting the criteria. Average assay results obtained with altered conditions were compared to results obtained using the original method, and the % recovery between the two was determined [9].

RESULTS AND DISCUSSION

System suitability

All parameters for system suitability were analyzed in each HPLC run for each parameter of method validation. The %RSD, number of theoretical plates, tailing factor and standard accuracy, all met the specific criteria set forth.

Specificity

Identification

The retention time of the Norethindrone peak in the chromatogram of the sample preparation corresponds to that of the Norethindrone peak in the chromatogram of the
standard preparation. Retention time of Norethindrone peak in standard solution is 8.90 minutes. Retention time of Norethindrone peak in sample solution is 8.91 minutes.

Placebo interference

Prepared representative placebo solution, standard solution and sample solution of Norethindrone tablets 0.35mg. Injected each of the blank, placebo solution, sample solution and standard solution into the HPLC connected with a photodiode array detector. No interference was observed from diluent and Placebo at the retention time of Norethindrone peaks (Figure 2-5).

Known impurity interference

Analyzed 3 samples of Norethindrone tablets 0.35mg by spiking with known related substances at 1% level and compared the assay results with control sample.

Table 1: Peak purity data of specificity for Norethindrone

	Peak Purity	
Name	**Purity Angle**	**Purity Threshold**
Standard solution	0.166	1.17
Control Sample	0.171	1.18
Spiked Sample	0.164	1.172

Average % Assay of control sample is 99.2% and spiked sample is 99.5%. Difference between these values is less than 1%. %RSD of 3 samples of un-spiked sample is 0.21 and spiked sample is 0.25. Norethindrone peak in control and spiked sample is pure. The purity angle is less than purity threshold, which indicates peak is pure. Hence no known impurity interference was seen. The peak purity data of Norethindrone peak in control sample and spiked sample indicates that the peak is homogeneous and has no co-eluting peaks indicating specificity of the method (Table1) and (Figure 5-9).

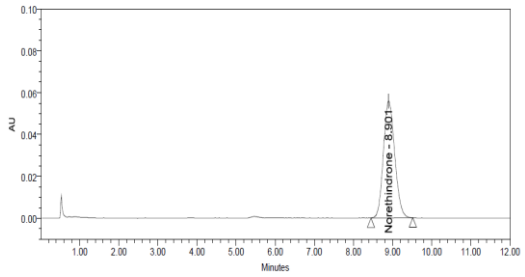

Figure 6: Norethindrone spiked sample chromatogram

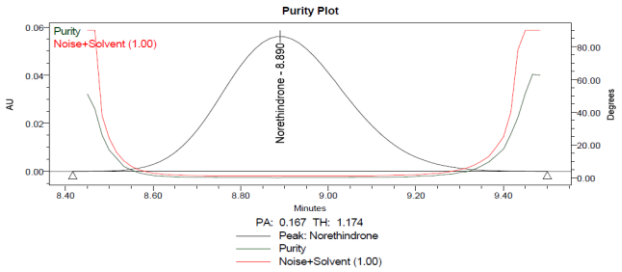

Figure 7: Peak purity of Norethindrone standard

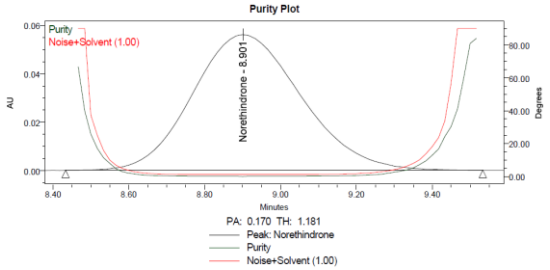

Figure 8: Peak purity of Norethindrone control sample

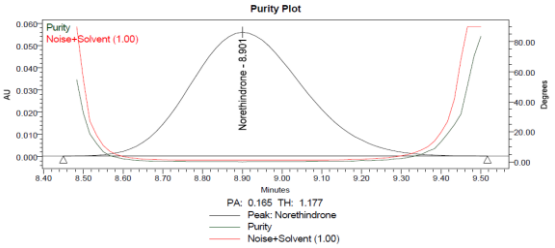

Figure 9: Peak purity of Norethindrone spiked sample

Forced degradation study

Forced degradation study is done in acid (4mL of 5N HCl/70°C/3hours), base (4mL of 2N NaOH/70°C/1hour), peroxide (4mL of 50% H2O2/70°C/3hours), thermal(105°C/72hours), photolytic(1.2 million lux hours of light) and humidity(25°C/92%RH/72hours) degradation. The peak purity data of Norethindrone peak in every degradation sample shows that the Norethindrone peak is homogeneous and there are no co-eluting peaks indicating that the method is stability indicating and specific (Table 2) and (Figure 10-17).

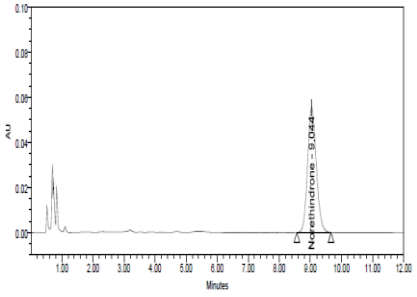

Figure 10: Acid stress

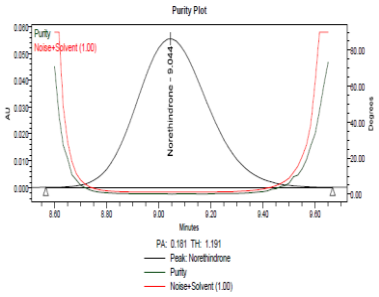

Figure 11: Acid stress purity

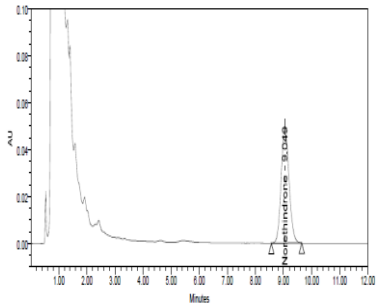

Figure 12: Base stress

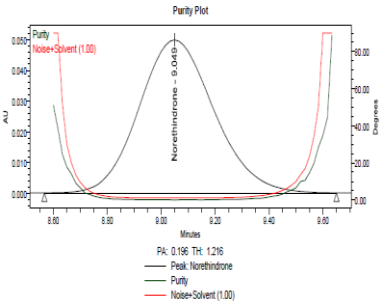

Figure 13: Base stress purity

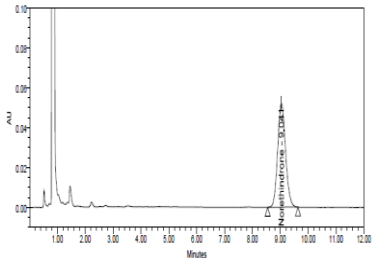

Figure 14: Peroxide stress

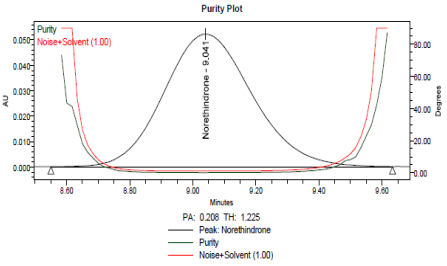

Figure 15: Peroxide stress purity

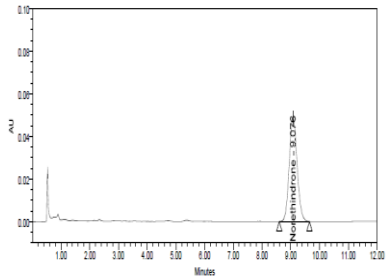

Figure 16: Thermal stress

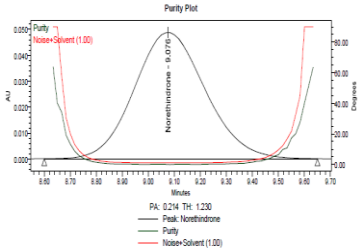

Figure 17: Thermal purity

Table 2: Forced degradation study data of Norethindrone

Sl no	Experiment	Degradation Condition	% Assay	% Degradation	Purity Angle	Purity Threshold
1	Control		98.9		0.17	1.181
2	Acid degradation	5N HCl/70°C/3hours	94.8	4.1	0.181	1.191
3	Base degradation	2N NaOH/70°C/1hour	84.7	14.2	0.196	1.214
4	Peroxide degradation	50% H2O2/70°C/3hours	89.7	9.2	0.208	1.223
5	Thermal degradation	105°C/72hours	82.4	16.6	0.214	1.229
6	Humidity degradation	25°C/92%RH/72hours	99.2	0	0.167	1.171
7	Photolytic degradation	1.2 million lux hours of light	98.7	0	0.199	1.181

Linearity and Range

A series of standard preparations of Norethindrone was prepared over a range of 50% to 150% of the working concentration of Norethindrone in Norethindrone Tablets. (Minimum five points in the range 80-120% of sample concentration for Assay). Since the working concentration is 12.5 µg per ml of Norethindrone the range proposed is about 6.25 µg per ml to 18.75 µg per ml of Norethindrone. Norethindrone solution is found linear from concentration range of 17.5 -52.5 µg per ml, Correlation coefficient is 0.998, hence method is linear (Table 3, Figure 18).

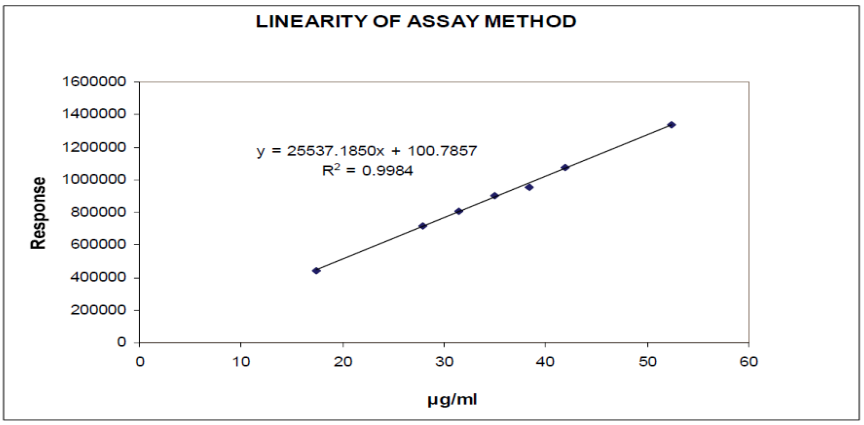

Figure 18: Linearity graph of Norethindrone

Table 3: Linearity of detector response for Norethindrone

% Concentration	Concentration(µg per mL)	Response (Area)		Statistical analysis
50%	17.46	444296	**Slope Intercept Correlation Coefficient**	25537.1
80%	27.936	716940		100.7
90%	27.936	808054		0.9984
100%	34.92	899616		
110%	38.412	956228		
120%	41.904	1077213		
150%	52.38	1340662		

Accuracy (Recovery)

Placebo of Norethindrone Tablets was spiked with Norethindrone drug substance at three different levels of 80%, 100% and 120% in triplicate (in total nine determinations). At the concentration level of 80% 100% and 120% mean recovery for Norethindrone is 100.0% & RSD is 0.19% and is within acceptance limit, reveals that HPLC assay method is accurate (Table 4).

Table 4: Accuracy results for Norethindrone (Acc: Accuracy, SD: Standard deviation, RSD: Relative standard deviation)

Sample No	Amount added (mg)	Amount recovered (mg)	% Recovery
Acc.80%-1	2.793	2.791	99.8
Acc.80%-1	2.793	2.789	99.8
Acc.80%-1	2.793	2.796	100
Acc.100%-1	3.491	3.49	99.9
Acc.100%-1	3.491	3.497	100.1
Acc.100%-1	3.491	3.496	100
Acc.120%-1	4.189	4.2	100.2
Acc.120%-1	4.189	4.205	100.3
Acc.120%-1	4.189	4.205	100.3
		Mean	**100**
		SD	0.194
		%RSD	0.19

Precision

Analytical repeatability: Five replicate injections of the standard preparation were injected into the HPLC. Six sample solutions of Norethindrone tablets 0.35 mg were prepared and injected into the HPLC. The %RSD of system precision is 0.14%, meeting the acceptance criteria hence the HPLC method is precise. The %RSD of method precision for Norethindrone is calculated and found 0.68% meeting the acceptance criteria hence the HPLC method developed is reproducible (Table 5 and 6).

Table 5: system precision for Norethindrone

Injection	Area
1	1110802
2	1109590
3	1109122
4	1109322
5	1106493
Mean	1109066
SD	1579.341
%RSD	0.14

Table 6: Method precision for Norethindrone

Sample	% Assay
1	100.1
2	100.2
3	98.4
4	99.3
5	99.7
6	100
Mean	99.6
SD	0.679
%RSD	0.68

Ruggedness (Intermediate Precision)

A second analyst repeated the method precision with the same samples that were quantified by the first analyst. Intermediate precision was performed on a different day, using a different instrument, and a different column lot number. Assay of Norethindrone tablet 0.35mg was done and met the % RSD criteria. Each assay performed by both analysts were compared, and the combined (n=12) %RSD calculated. The acceptance criteria for the intermediate precision were met. The %RSD is 0.79%, hence HPLC method is rugged (Table 7).

Table 7: Ruggedness (Intermediate precision)

Sample No	Analyst -1 % Assay	Analyst -2 % Assay
1	100.1	99.7
2	100.2	101
3	98.4	100.8
4	99.3	100.9
5	99.7	100.5
6	100	101.1
Mean	**99.6**	**100.7**
SD	**0.679**	**0.516**
%RSD	**0.68**	**0.51**
Overall Mean	**100.1**	
Overall SD	**0.795**	
Overall % RSD	**0.79**	

Robustness

Two samples of Norethindrone tablet 0.35mg were prepared and analyzed under both the varied conditions and the normal condition. Average % recovery of the assay values was obtained by comparing the assay results from varied conditions versus the normal condition. Deliberately changed parameters were column temperature (30°C), mobile phase flow rate (1.8 mL/min and 2.2 mL/min), detector wavelength (235nm and 245nm), as shown in the Table 8-10, all the % recovery assay results under the varied conditions were within 98-102% of the original method condition. System suitability meeting as per the test method at each variable condition. The deliberate changes in the method and operational conditions did not affect the chromatograms or the validity of the results; and hence the method is considered robust over that range of conditions.

Table 8: Robustness: Change in Flow rate (±0.2 mL/min)

Control		(+0.2 mL/min)	(-0.2 mL/min)
100.1	99.3	101.2	100.9
100.2	99.7	100.4	99.8
98.4	100	98.3	97.7
Cumulative Mean		**99.7**	**99.6**
Cumulative SD		**0.959**	**0.997**
Cumulative % RSD		**0.96**	**1**

Table 9: Robustness: Change in wavelength (± 5nm)

Control	(+5 nm)	(-5nm)
99.5	99.4	99.4
99.5	99.5	99.4
99.2	99.2	99.2
Cumulative Mean	**99.4**	**99.4**
Cumulative SD	**0.147**	**0.137**
Cumulative % RSD	**0.15**	**0.14**

Table 10: Robustness: Change in column temperature (± 5°C)

Control		(+0.2 mL/min)	(-0.2 mL/min)
100.1	99.3	102.5	103.0
100.2	99.7	100.6	100.5
98.4	100	98.3	98.4
Cumulative Mean		**99.9**	**100.0**
Cumulative SD		**1.271**	**1.383**
Cumulative % RSD		**1.27**	**1.38**

Stability of solutions

The sample and standard preparations were stored at room temperature and tested against freshly prepared standard preparations for 24 hours as per the developed method. Assay of old standard against freshly prepared was between 98.0 and 102.0%. Sample solution correlation of old sample solution against initial assay is between 98.0 and 102.0%. This experiment revealed that standard and sample solutions were stable for 24 hours at room temperature (Table 11).

Table 11: Stability of Norethindrone analytical solutions at Room Temperature

Sl. No.	Name	% Content	% Correlation
1	Standard solution - initial	--	--
2	Standard solution – 24 hours	100.5	100.5
3	Sample solution - initial	99.5	
4	Sample solution - 24 hours	99.6	100.1

Filter equivalency study

Sample prepared as per methodology and centrifuged in and filtered in triplicate through different membrane filters such as Nylon 0.45 , Glass filters discarding first few mL of the filtrate. Diluted 5 mL of the filtrate to 100 mL with diluent and mixed. Glass membrane filter and Nylon 0.45µ filter are suitable for filtration of samples when compared with centrifuged results. % correlation lies between 98-102%. (Table 12)

Table 12: Filter equivalency study

Sample No.	% Assay		
	Centrifuged	Nylon 0.45 µ	Glass membrane
1	100.3	100.6	99.6
2	100.6	99.6	99.6
3	100.8	100.2	99.8
Mean	**100.6**	**100.1**	**99.7**
RSD	**0.25**	**0.5**	**0.12**
% Correlation with centrifuged	**--**	**99.5**	**99.1**

Evaluation of Results

Once the forced degradation samples are generated, the next step is their evaluation for the degradation products formed under all the stress conditions. Studying the samples individually does this. Mostly samples are evaluated on chromatographic techniques, the most commonly used are HPLC, UPLC, UHPLC, and CE. The major concern here is the successful separation of all the degradation products from the drug and from each

other, viz development of stability-indicating method [11]. Hence, preference has to be given to a selective method, with emphasis on peak purity. Sensitivity of the developed analytical method should have well enough to detect impurities at low levels. During the method development it may happen that the drug peak may hide a degradation impurity peak that co-elutes with the drug. All known and unknown impurities should be well separated from main component peak and all unknown impurities should be well separated from known impurities. Sometime two merged unknown impurity may lead to out of specification results in real time stability therefore analytical method should have capability to resolve any unknown impurities higher than unknown impurity specification level from merging with each other[12].

Peak purity analysis

Peak purity parameters are used to prove spectral uniqueness of compound. Peak purity of main component and known impurities in control and degradation samples should be established using PDA (Photo diode array) detector. There are some limitations in establishing of peak purity and cannot be calculated accurately if,

1. UV detection response of main component at saturation level (Nonlinear UV detection at higher absorbance values).
2. Co-eluting peak having same UV spectra.
3. Co-eluting peak has no chromophoric functional group.

For further investigation other technique can be used to establish peak purity like LC-MS [12].

Mass balance

In recent years FDA strictly asking mass balance in forced degradation of related compound method to prove specificity and capability to quantify degradation impurities. Mass balance establishes adequacy of a stability indicating method though it is not achievable in all circumstances. Lacking of mass balance lead a suspicion on capability of method to quantify all degradation products accurately. Mass balance is always challenging to evaluate accurately. The mass imbalance, apart from varying responses of analyte and degradation product peaks, may also happen due to potential loss of volatile degradation products, formation of non-chromophoric compounds, formation of early eluents, and retention of compounds in the column. The known impurity standards can be used to calculate a mass balance more accurately, as their availability helps in the quantitative determination through corrected response factors. A popular formula used in industry for mass balance is;

[100-(water by KF + total volatiles + residue on ignition + % of non-chromophoric contents)] × HPLC purity/100 [12].

CONCLUSIONS

Norethindrone is a very interesting compound used as oral contraceptive. The proposed method provides a simple and effective means to determine Norethindrone in a diverse range of samples, enabling the direct analysis. Thus, the proposed method can be considered safe for both the operator and the environment. The proposed method is also useful in quality control of the whole industrial process to obtain the final pharmaceutical formulation. However, it is expected that the proposed method could be valid for the analysis of other tablet containing the same active compound in dose proportional. It is worth mentioning that a very important step in the manufacture of a new drugs is its stability when stored under normal conditions.

Forced degradation studies (stress testing) are very important tool in pharmaceutical research and development to develop stable formulation. It provides information about degradation pathways of drug substances and drug products, which can be used in excipient compatibility and provide help in early stage development. Degradation impurities generated in stress studies may or may not generate in real time stability study but this important step is used primarily to develop stability indicating analytical methods. It is strongly recommended that these studies should be started as early as possible to be able to provide valuable information that can be used to assess the inherent stability of a drug and to improve formulation and the manufacturing process.

The developed and validated assay method was found to be precise, selective efficient, specific, accurate and stability indicating RP-HPLC-DAD method for the quantification of Norethindrone in Norethindrone tablets 0.35mg. The force degradation study shows that there is no interference at retention time of analyte peak. Hence this method may be used for routine analysis of in process as well as final marketed dosage form of Norethindrone tablets.The known impurity standards can be used to calculate a mass balance more accurately, as their availability helps in the quantitative determination through corrected response factors.

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