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Development and Validation of method for the determination of related substances of Norethindrone in Norethindrone Tablets and Degradation studies

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Abstract: A gradient, reversed-phase liquid chromatographic (RP-LC) method was developed for the quantitative determination of Norethindrone in Norethindrone tablets, used to treat drug for contraception and hormone replacement therapy. The gradient LC method employs solutions A and B as mobile phase. The solution A contains a mixture Milli Q water, acetonitrile and tetrahydrofuran in the ratio of 40:30:30 v/v/v and solution B contains a mixture Milli Q water, acetonitrile and tetrahydrofuran in the ratio of 40:30:30 v/v/v. The Chromatography was performed on a Supelco Ascentis Express C-18, 4.6 x 150mm, 2.7µ and detector of UV at 256 nm.0.9 mL/min as a Flow rate, 100 µL as an Injection volume. The chromatogram of Norethindrone its impurities namely NE-H (Impurity-H), NE-B (Impurity-B), NE-C (Impurity-C) and NE-D (Impurity-D) were found. Accuracy satisfactory by % recovery obtained in the range of 94.9 – 105.9, the linearity results for Norethindrone and impurities in the specified concentration Calibration curves were linear with a coefficient of variation (r) not less than 0.99. An accelerated degradation study on Norethindrone Tablets as following conditions Hydrolytic and Oxidative degradation, Thermal degradation, Humidity degradation, Photolytic degradation, Forced degradation studies (Acid, Alkali , Peroxide Thermal, Humidity and Photolytic).The proposed method was found to be specificity, linearity, and precision, intermediate precision, and accuracy, stability in analytical solution and robustness. The validation was performed according to the current requirements as laid down in the ICH guidelines.

Key words: Norethindrone tablets; Forced degradation; RP- High performance liquid chromatography; method validation.

Introduction:

Norethisterone (or norethindrone) (or 19-nor-17α-ethynyltestosterone) is a commonly used drug for contraception and hormone replacement therapy, whose carcinogenic potential is still controversial.All progestins currently used in oral contraceptives are derivatives of 19-nortestosterone and differ from testosterone by the elimination of the C19 methyl group.[1] The early progestins, termed estranes, consist of norethindrone 17R-ethynylnortestosterone [2],and its derivatives norethindrone acetate, norethynodrel, and ethynodiol diacetate. The activity of norethindrone derivatives depends on in vivo conversion to norethindrone itself. The 17R-ethynyl group of norethindrone resulted in increased oral bioavailability with surprisingly little effect on binding to PR. [3, 4]. Clearly, steroid receptors can tolerate an increase in ligand size in this region of the binding

pocket.Although norethindrone binds to the AR in vitro [5], clinical data indicate that when norethindrone or norethindrone acetate is combined with estrogen in monophasic oral contraceptives, it has minimal androgenic activity [6–12].

It is common to study percutaneous steroid permeation by comparing drug fluxes across the skin [13 - 16]. Thus only limited information can be obtained on the transport kinetics across the skin. However, by simultaneously evaluating the percutaneous drug flux and the intraepidermal drug distribution in the same skin samples as a function of time, it is possible to clarify the relationship between the kinetics of percutaneous transport and the kinetics of intracutaneous accumulation, distribution and binding. Furthermore, this approach permits visualization of the actual pathways of transport.

Experimental:

Reagents and chemicals:

HPLC grade Acetonitrile, Tetrahydrofuran and Methanol were obtained from Rankem, Ranbaxy Fine Chemical Limited, New Delhi, India. All other chemical of analytical grade were procured from local sources unless specified.

Instrumentation and Chromatographic Conditions:

The instrument used was a Waters Model Alliance 2695 seperation module equipped with auto sampler, Waters 2998 PDA Detector and the data recorded using empower software. The mobile Phase consisted of acetonitrile and tetrahydrofuran by using the column Supelco Ascentis Express C-18, 4.6 x 150mm, 2.7µ and detector of UV at 256 nm.

Results and Discussion:

Reversed phase HPLC was proposed as a suitable method for Norethindrone in Norethindrone Tablets.Chromatographic system consisted of a Waters Model Alliance 2695 seperation module equipped with auto sampler, by using the column Supelco Ascentis Express C-18, 4.6 x 150mm, 2.7µ and detector of UV at 256 nm.0.9 mL/min as a Flow rate, 100 µL as a Injection volume, at 40°C Column temperature, 80.0 minutes run time and 16.5 minutes Appr. RT of Norethindrone.

Standard preparation:

Weigh and transfer accurately about 35.0mg of Norethindrone WS into 50mL volumetric flask. Add to it 30mL of acetonitrile and sonicate to dissolve it completely and dilute to volume with the acetonitrile and mix. Dilute 2mL of above solution to 50mL volumetric flask, dilute to volume with diluent and mix. Again dilute 3mL of above solution to 50mL with diluent.

Procedure for Test preparation:

Weigh 20 tablets and calculate average weight. Weigh and transfer 10 tablets to into a 20mL volumetric flask. Add to it 15mL diluent and sonicate for 15 minutes with shaking in cool water. Dilute up to the mark with diluent. Centrifuge for 10 minutes at 3000 rpm and filter through 0.2µ nylon membrane filter. Procedure for Placebo preparation, weigh placebo powder accurately equivalent to 3.5mg of Norethindrone into a 20mL volumetric flask. Add to it 15mL diluent and sonicate for 15 minutes with shaking in cool water. Dilute up to the mark with diluent. Centrifuge for 10 minutes at 3000 rpm and filter through 0.2µ nylon membrane filter.

Procedure: Inject diluent in single, standard in duplicate and test, placebo in single. Calculate the system suitability parameters from standard chromatogram and system suitability solution as mentioned below. Theoretical plates (N): Not less than 3000 (for diluted standard), Tailing factor (T): Not more than 2.0 (for diluted standard), R.S.D. Not more than 5.0 % (For duplicate injections of diluted standard).

Method development:

Precision:

System precision: Six replicate injections of standard solution of Norethindrone were injected into the HPLC system and analyzed as per the proposed method. The areas of response of the analyte along with % RSD are 0.03.The % RSD observed on the replicates indicates the reproducibility and hence the precision of the system. Acceptance criteria: % RSD not more than 5.0.

Method precision: Six samples of Norethindrone Tablets were analyzed as per the method. Each named impurity and total impurities were calculated on these replicates. The % RSD observed 0.68 these results comply with the acceptance criteria and indicating acceptable precision of the system % RSD for each named impurity >0.1% not more than 15 and for total impurities not more than 10 The % RSD observed in the calculation of known impurities and total impurities indicate the precision of the method.

Specificity:

The related substances of Norethindrone Tablets were chromatographed individually and as a mixture added to sample and to placebo, to examine the interference with each other. Representative chromatograms of system suitability, standard, diluent, placebo, sample, and sample spiked with impurities, placebo spiked with impurities and individual impurity solutions as shown in fig 1.The results Peak is found to be homogeneous and there are no co-eluting peaks and relative retention time matches with method, hence indicating specificity of the method.

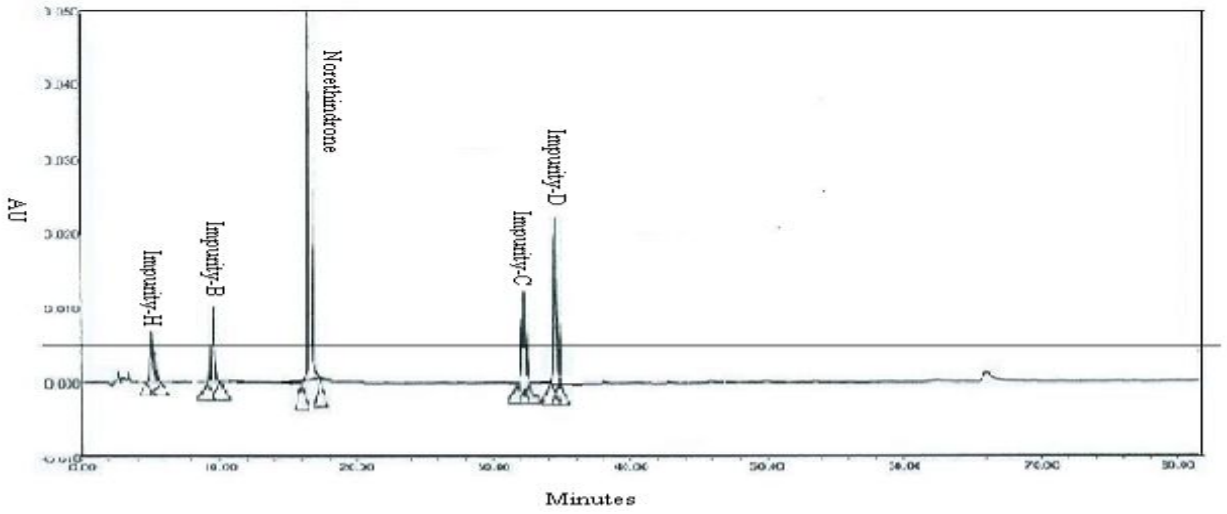

Figure 1) Chromotogram of Norethindrone in Norethhindrone tablets and spiked with impurities.

Limit of detection and Limit of quantification:
Six replicate injections of impurity solutions at previously calculated LOD and LOQ concentration level were injected into the HPLC system as per the proposed method. The area responses of the analyte along with % RSD are tabulated in Table 1 and Table 2 .The Acceptance criteria % RSD not more than 33 for limit of detection level and not more than 10 or limit of quantitation level.

Table 1: Limit of Detection level.

Injection no.	NE-H	NE-B	NE-C	NE-D
1	5344	6936	799	628
2	5363	6923	724	784
3	5376	6902	722	973
4	5343	6993	793	755
5	5325	6967	745	893
6	5381	6969	640	858
Mean	5355	6948	737	815
% RSD	0.40	0.49	7.87	14.76

Linearity:
The linearity of response for each known impurity was determined in the concentration range of limit of quantitation to about 150% of specification limit for each known impurity. Acceptance criteria squared correlation coefficient not less than 0.99. The Correlation coefficient as shown in Table 3.

Table 2: Limit of Quantitation level.

Injection no.	NE-H	NE-B	NE-C	NE-D
1	15957	21458	2621	2144
2	16370	21075	2576	2140
3	15945	21217	2519	2007
4	16214	21052	2717	2134
5	16047	21244	2757	2198
6	16137	21027	2684	2011
Mean	16112	21179	2646	2106

Accuracy:
A known amount of Norethindrone Tablets was taken into volumetric flask and spiked with known quantities of each named impurity at LOQ, 50%, 100% and 150% in triplicates.% Accuracy should be in the range of a) 80-120% for LOQ level and impurity less than 0.05% b) 85-115% for impurities between 0.05 – 0.50% c) 90-110% for impurities between 0.51 – 2.0% d) 95-105% for impurities more than 2.0%.

Table 3: Linearity method.

Drug	Slope	Y-intercept	Coefficient of correlation
Linearity of Impurity NE-H	200345.80356	-639.54834	0.99998
Linearity of Impurity NE-B	355119.96559	-6111.482869	0.99977
Linearity of Impurity NE-C	23404.48522	3599.10464	0.99606
Linearity of Impurity NE-D	77155.83239	2332.75134	0.99949

Intermediate precision:
On Method: The ruggedness of the method was determined by analyzing a sample prepared as per the method and different columns on different days six samples of Norethindrone Tablets were analysed as per the method. Each named impurity and total impurities were calculated on these samples. The Acceptance criteria % RSD for each named impurity >0.1% not more than 15 and for total impurities not more than 10 was observed.
At LOQ Level: The ruggedness of the method at LOQ level was determined by analyzing a solution prepared at LOQ level and different columns on different days, tailing factor 1.05 and % RSD 0.38 was observed. The Acceptance criteria % RSD for each named impurity not more than 10.
Intermediate precision at LOQ Level:
The % RSD observed in the calculation of known impurities at LOQ level indicates the tailing factor 1.06 and % RSD 0.03 was observed. The Acceptance criteria% RSD not more than 10 precision of the method.

Robustness:
To determine the robustness of the developed method experimental conditions were purposely altered and Theoretical plates for Norethindrone peak from first chromatogram of standard not less than 3000,Tailing factor for Norethindrone peak from chromatogram of standard not more than 2.0 and % RSD for replicate standard injections not more than 5.0.

System suitability:
Theoretical plates for Norethindrone peak from first chromatogram of standard should be not less than 3000, Tailing factor for Norethindrone peak from first chromatogram of standard not more than 2.0 and % RSD for replicate standard injections not more than 5.0.

Solution Stability:
Solution stability was checked and Sample solution spiked with impurities is found to be stable up to 1440 minutes at 10°C.% difference of response from initial for each known impurity >0.1% not more than 15 and total impurities not more than10.

Degradation studies:

An accelerated degradation study was carried out on Norethindrone Tablets according to the following conditions.
Hydrolytic and Oxidative degradation: Acid degradation: Weigh 10 tablets of Norethindrone Tablets into a 20mL volumetric flask, add about 10mL diluent,sonicate for about 15 minutes in cool water then add 1mL, 5N HCl solution and heat in a water bath for 30 minutes at 60°C. Keep at room temperature for 15 minutes. After specified time neutralize the solution by adding 1mL, 1N NaOH solution. Cool the solution at room temperature and then dilute up to the mark with diluent. Centrifuge at 3000 rpm for 10 minutes, Filter through 0.2µ nylon membrane filter. An equivalent amount of placebo was treated in the similar conditions mentioned above and a Noised as per the proposed method.
Alkali degradation: Weigh 10 tablets of Norethindrone Tablets into a 20mL volumetric flask, add about 10mL diluent,sonicate for about 15 minutes in cool water then add 3mL, 5N NaOH solution and heat in a water bath for 30 minutes at 60°C. Keep at room temperature for 15 minutes. After specified time neutralize the solution by adding 3mL, 5N HCl solution. Cool the solution at room temperature and then dilute up to the mark with diluent. Centrifuge at 3000 rpm for 10 minutes, Filter through 0.2µ nylon membrane filter an equivalent amount of placebo was treated in the similar conditions mentioned above and analyzed as per the proposed method.
Peroxide degradation: Weigh 10 tablets of Norethindrone Tablets into a 20mL volumetric flask, add about 10mL diluent, sonicate for about 15 minutes in cool water then add 1mL, 30% H2O2 and heat in a water bath for 30 minutes at 60°C. Keep at room temperature for 15 minutes. After specified time cool the solution at room temperature and then dilute up to the mark with diluent. Centrifuge at 3000 rpm for 10 minutes, Filter through 0.2µ nylon membrane filter. An equivalent amount of placebo was treated in the similar conditions mentioned above and analysed as

per the proposed method.

Thermal degradation: Norethindrone Tablets was spread in a petri dish and kept in an oven at 60°C. The sample after exposure for 7 and 21 days was removed and analysed as per the methodology. An equivalent amount of placebo was treated in a similar manner in the condition mentioned above and analysed as per the methodology.

Humidity degradation: Norethindrone Tablets was spread in a petri dish and kept in a humidity chamber of 40°C/75%RH. The sample after exposure for 7 and 21 days was removed from the chamber and analysed as per the methodology. An equivalent amount of placebo was treated in a similar manner in the condition mentioned above and analysed as per methodology.

Photolytic degradation: Norethindrone Tablets was spread in a petri dish and kept in Sun test instrument chamber to achieve light intensity 1.2 million lux, removed from the chamber and analysed as per the methodology. An equivalent amount of placebo was treated in a similar manner in the condition mentioned above and analysed as per methodology. Using peak purity test, the purity of Norethindrone Acetate, Ethinyl Estradiol and known impurity peaks were checked at every stage of the above study.

Conclusion:

A high performance liquid chromatography method was developed and validated for the determination of the related substances for Norethindrone in Norethindrone Tablets. The proposed method was found to be good results for specificity, linearity, precision, intermediate precision, and accuracy, stability in analytical solution, robustness and degradation studies. Therefore the method is suitable for its intended use for commercialization.

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