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A NOVEL STABILITY INDICATING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD FOR DETERMINATION OF NORETHISTERONE ACETATE AS BULK DRUG AND IN TABLET DOSAGE FORM

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ABSTRACT
A new simple, accurate, precise and selective stability-indicating high performance thin layer chromatographic (HPTLC) method for determination of Norethisterone acetate as bulk drug and in tablet dosage form has been developed and validated. Forced degradation studies were carried out under various stress conditions according to ICH guidelines to demonstrate the stability-indicating capability of the developed HPTLC method. Norethisterone acetate was found susceptible to all the analyzed stress conditions except photolysis. Chromatographic resolution of Norethisterone and its degradation products was achieved by using precoated silica gel 60 F_{254} aluminium plates as stationary phase and Toluene: Ethyl acetate (7.5: 2.5, v/v) as optimum mobile phase. Densitometric detection was carried out at 242 nm. The retention factor was found to be 0.47 ± 0.05. The developed method was validated with respect to linearity, accuracy, precision, limit of detection, limit of quantitation and robustness as per ICH guidelines. Results found to be linear in the concentration range of 300-1500 ng band^{-1}. The developed method has been applied successfully for the estimation of drug in tablet dosage form.

KEYWORDS: Norethisterone, HPTLC, Forced degradation, Tablet dosage form.

INTRODUCTION
Norethisterone acetate, also known as norethindrone acetate is a synthetic second-generation progestin used for contraception, prevention of endometrial hyperplasia in hormone replacement therapy, and in the treatment of other hormone-mediated illnesses such as endometriosis. The medication is available in both low-dose and high-dose formulations and both alone and in combination with an estrogen.[1] Norethisterone, chemically, [(8*R*,9*S*,10*R*,13*S*,14*S*,17*R*)-17-ethynyl-13-methyl-3-oxo-1,2,6,7,8,9,10,11,12,14,15,16 dodecahydrocyclopenta [a] phenanthren 17-yl] acetate is a synthetic estrane steroid and a derivative of testosterone which works by mimicking the effects of natural progesterone (female hormone) and helps in regulating the growth and shedding of the womb lining, thereby treating menstrual irregularities.[2]

Exhaustive literature analysis with regard to analytical methods revealed that Spectrophotometric methods for the estimation of Norethisterone as bulk and in tablet dosage form has been reported either as single drug or in combination with other drugs.[3-6] Reverse Phase High Performance Liquid Chromatography (RP-HPLC) methods were also available in the literature for estimation of Norethisterone either as single drug or in combination with other drugs in human plasma and/or in pharmaceutical dosage form.[7-14] Capillary gas chromatography method with mass-selective detection for quantitative determination of Norethisterone acetate in human plasma is also reported.[15]

To best of our information, no reports were found in the literature for determination of Norethisterone in tablet formulation by stability-indicating high performance thin layer chromatographic (HPTLC) method. This paper describes development and validation of simple, precise, accurate and selective stability indicating HPTLC procedure for determination of Norethisterone in accordance with International Conference on Harmonisation Guidelines.[16,17]

MATERIALS AND METHODS
Chemicals and Reagents
Analytically pure working standard Norethisterone acetate was obtained as gift sample from Johnlee Pharmaceuticals Pvt. Ltd. (Mumbai, India). The pharmaceutical tablet dosage form NORISTA-15 CR labeled to contain 15 mg of Norethisterone was used in this study and procured from the local pharmacy.

Toluene, Ethyl acetate, Methanol (all AR grade) was purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and Chromatographic conditions

A HPTLC (Camag) system including Camag Linomat V sample applicator, Hamilton syringe (100 µL), Camag TLC Scanner-III with winCAT software version 1.4.3, Camag twin- trough chamber (10×10 cm) (CAMAG, Muttenz, Switzerland) were used for the present work. Precoated silica gel 60 F_{254} (10 cm ×10 cm with 250 µm layer thickness) Merck TLC plates were used as stationary phase in order to achieve chromatographic separation of drug.

Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using mixture of Toluene: Ethyl acetate (7.5: 2.5, v/v) as optimum mobile phase. The mobile phase was saturated in chamber for 15 min. After development, TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on Camag TLC Scanner-III at 242 nm for all developments operated by winCATS software version 1.4.3.

Preparation of standard stock solution

Standard stock solution was prepared by dissolving 10 mg of drug in 10 mL of methanol to get working standard solution of concentration 1000 ng μL^{-1} from which 1.5 mL was further diluted to 10 mL to get solution of 150 ng μL^{-1}.

Selection of detection wavelength

After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that drug showed considerable absorbance at 242 nm. So, 242 nm was selected as the wavelength for detection.

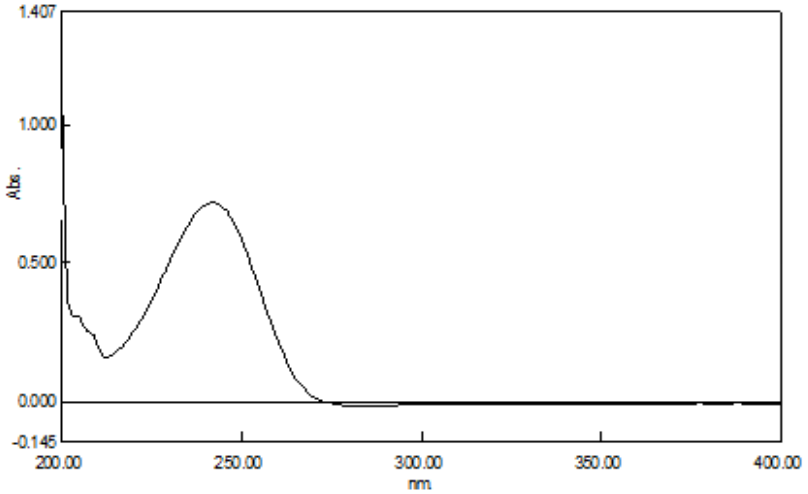

Fig. 1: Norethisterone spectra measured within 200 to 400 nm range.

Tablet formulation analysis

Commercial brand of tablets namely NORISTA-15 CR labeled to contain 15 mg of Norethisterone were selected for checking the appropriateness of the proposed method to estimate Norethisterone in tablet formulation. For this, 20 tablets were weighed and powdered. Tablet powder equivalent to 15 mg was transferred to 100 mL volumetric flask containing 50 mL of methanol and the contents were sonicated for 15 min. The solution was filtered using Whatman paper No. 41 and the volume was made up to the mark with methanol to obtain the final concentration of 150 ng $band^{-1}$. Four µL volume of this solution was applied on TLC plate to obtain final sample concentration of 600 ng $band^{-1}$. After chromatographic development peak areas of the bands were measured at 242 nm and the amount of drug present in sample was estimated from the respective calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Forced degradation study

Forced degradation studies were performed on bulk drug under hydrolytic, oxidative, thermolytic, and photolytic stress conditions in order to reveal the stability-indicating nature and selectivity of the proposed method. The study was carried out at concentration of 1500 µg μL^{-1}. The hydrolytic studies were performed by treating the stock solution of drug with 1N HCl and 1 N NaOH at room temperature for 1 h and 2 h, respectively. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 150 µg μL^{-1}. The oxidative degradation was carried out in 15 % H_2O_2 at room temperature for 1 h and sample was diluted with mobile phase to obtain 150 µg μL^{-1} solution. Thermal stress degradation was performed by keeping drug in oven at 60ºC for period of 8 h. Photolytic degradation studies were carried out by exposure of drug to UV light up to 200 watt h square $meter^{-1}$. Thermal and photolytic samples were diluted with mobile phase to obtain 150 µg μL^{-1} concentration.

RESULTS AND DISCUSSION

Method optimization

The intention of existing research work was to develop stability indicating HPLTC method which would be proficient to give the adequate resolution between Norethisterone and its degradation products. Varied solvent systems containing various ratios of benzene, chloroform, ethyl acetate, toluene, methanol were examined (data not shown) to separate and resolve spot of Norethisterone from its impurities and other excipients present in formulation. Finally, the mobile phase comprising of Toluene: Ethyl acetate (7.5: 2.5, v/v) was selected as optimum for attaining well defined and resolved peak. Densitometric evaluation was carried out at 242 nm. The retention factor was found to be 0.47 ± 0.05. Densitogram obtained from standard solution of Norethisterone is represented in Fig. 2.

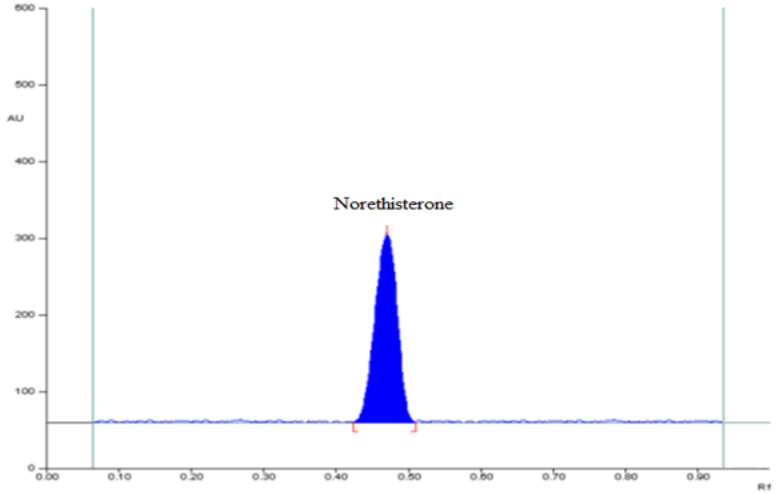

Fig. 2: Densitogram of standard solution of Norethisterone acetate (1200 ng band^{-1}, Rf= 0.47 ± 0.05).

The forced degradation results demonstrated susceptibility of drug to hydrolytic, oxidative and thermal stress conditions and stability under photolytic stress conditions. Significant degradation observed under acid hydrolysis with peak for degradation product at Rf 0.68 and oxidative degradation with peak for degradation product at Rf 0.32. The degradation products formed during acid and oxidative degradation were well resolved from the drug indicating specificity of the method. Figures 3-5 represents the densitograms of acid, alkali and oxidative degradation, while Figure 6 illustrates the densitograms of thermal degradation. The findings of degradation studies are represented in Table 1.

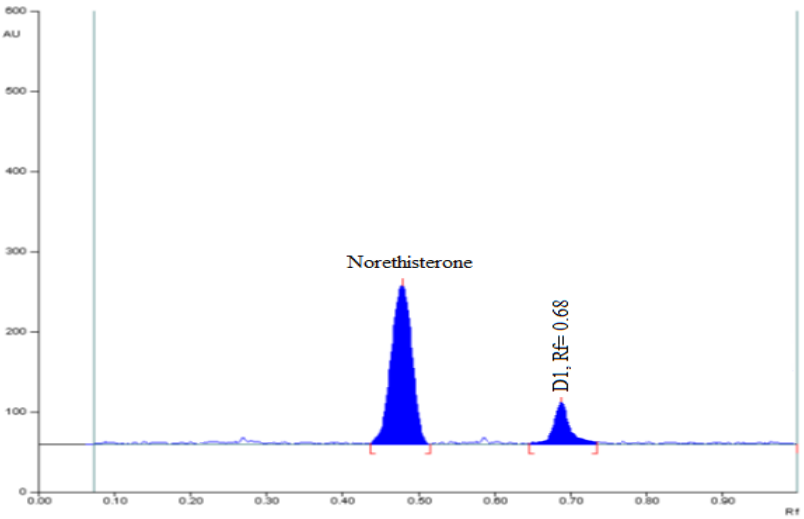

Fig. 3: Densitogram after treatment with 1N HCl with degradation product (D1, Rf = 0.68).

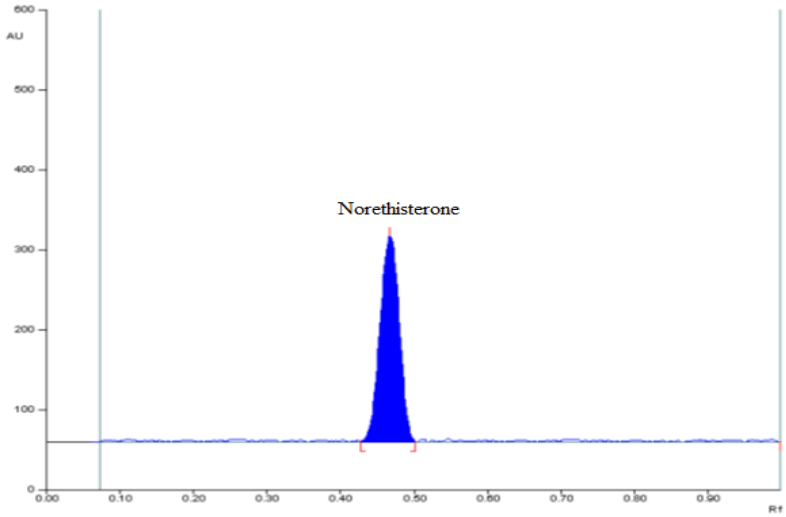

Fig. 4: Densitogram after treatment with 1N NaOH.

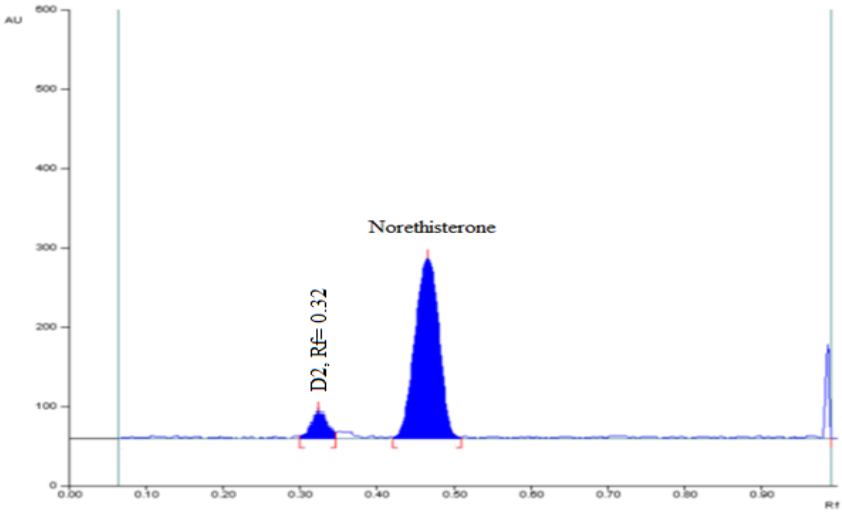

Fig. 5: Densitogram after treatment with 15% H_2O_2 with degradation product (D2, Rf = 0.32).

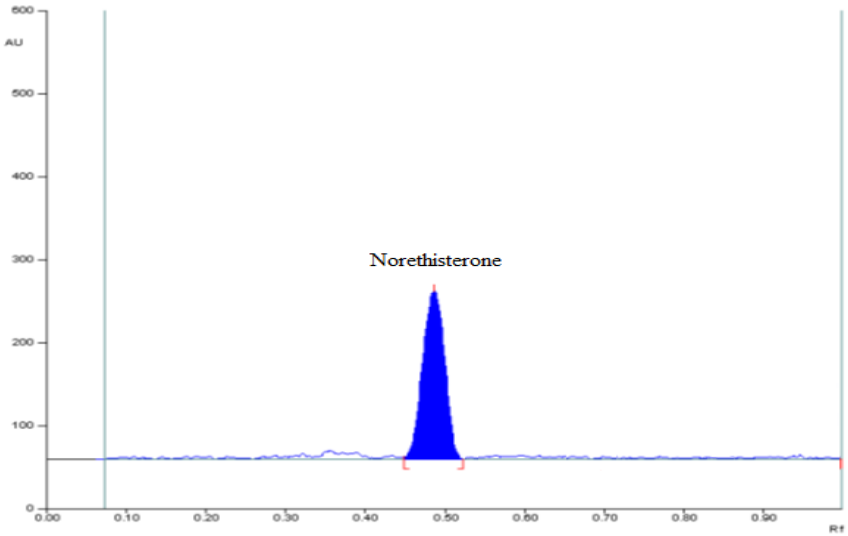

Fig. 6: Densitogram obtained after dry heat degradation at 60°C for 8 h.

Table 1: Summary of forced degradation studies.

Stress conditions/ duration	% Recovered	% Degradation
Acid / 1 N HCl/ Kept at RT for 1 h	83.68	16.32
Alkali /1 N NaOH/ Kept at RT for 2 h	87.89	12.11
Oxidative /15 % H_2O_2 / Kept at RT for 1 h	77.22	22.78
Dry heat/ 60ºC/ 8 h	79.55	20.45
Photolysis: UV light 200 watt h square meter^{-1}	98.62	----

Analytical method validation

The developed method was validated in terms of linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness, in accordance with ICH guidelines.

Linearity

Linearity of method was evaluated by spotting volumes 2, 4, 6, 8 and 10 µL of standard solution of Norethisterone (150 ng µL^{-1}) onto the TLC plates. The developed method was found to be linear in the concentration range 300-1500 ng band^{-1} with high correlation coefficient. Correlation coefficient closer to 1 for the drug also proved the linearity. The peak area was plotted against the corresponding concentrations to obtain the calibration curve as shown in Figure 7. The densitogram obtained for linearity is shown in Figure 8.

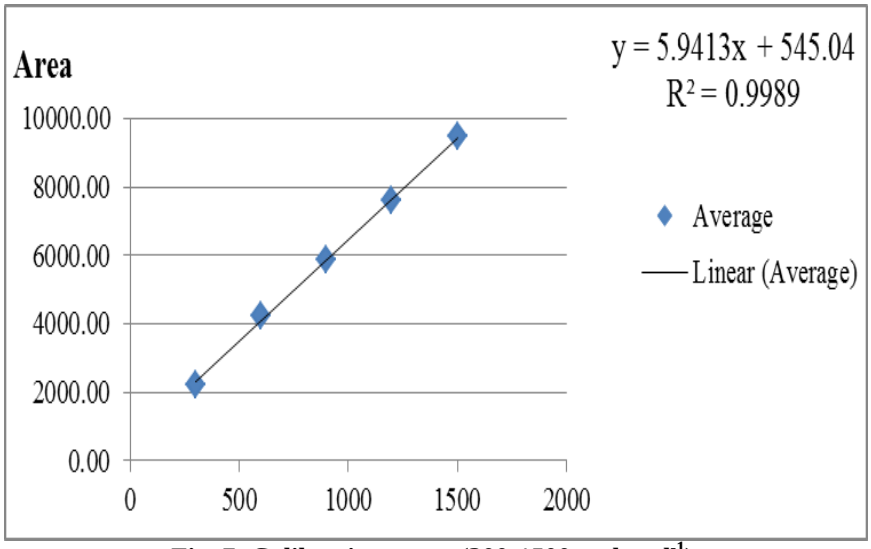

Fig. 7: Calibration curve (300-1500 ng band^{-1}).

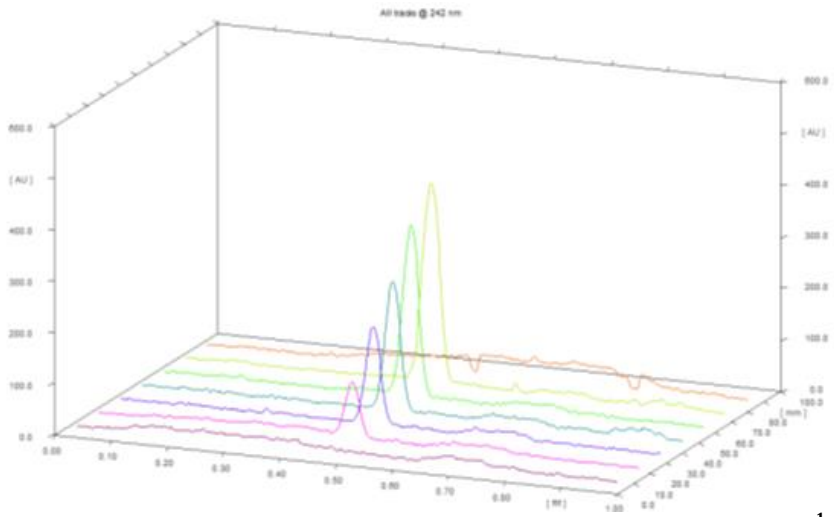

Fig. 8: 3 D Spectra of linearity in concentration range 300-1500 ng band^{-1}.

Precision

The precision of the method was demonstrated by intraday and interday variation studies in which three replicates of three concentrations within the linearity range were analyzed on the same day and on three consecutive days, respectively and percentage R.S.D. was calculated. The lower values of % R.S.D. (< 2) confirmed precision of method. The results obtained for

intraday and inter-day precision studies are shown in Table 2 and 3, respectively.

Table 2: Intraday precision studies.

Spotted Concentration (ng band^{-1})	Average Area	Recovered concentration (ng band^{-1})	% R.S.D.*
300	2325	299.65	1.52
600	4101	598.68	0.73
900	5900	901.44	0.38

* Average of three determinations

Table 3: Inter-day precision studies.

Spotted Concentration (ng band^{-1})	Average Area	Recovered concentration (ng band^{-1})	% R.S.D.*
300	2313	297.63	1.47
600	4116	601.10	0.75
900	5893	900.20	1.04

* Average of three determinations

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ were found to be 35.45 ng band^{-1} and 107.42 ng band^{-1}, respectively.

Accuracy

Accuracy of developed method was confirmed by performing recovery studies by standard addition method which involved addition standard drug solution to pre-analyzed sample solution at three different levels 50, 100 and 150 %. Basic concentration of sample chosen was 600 ng band^{-1} from tablet solution. The results of the recovery studies indicated accurateness of developed method for estimation of drug in tablet formulation (Table 4).

Table 4: Recovery studies.

Drug	Concentration taken (ng band^{-1})	Concentration added (ng band^{-1})	Concentration found (ng band^{-1})	% Recovery±R.S.D.*
Norethisterone acetate	600	300	901.87	100.21 ± 0.46
	600	600	1206.47	100.54 ± 1.34
	600	900	1504.75	100.32 ± 0.29

*Average of three determinations

Robustness

Robustness of the method was evaluated by introducing deliberate variations in the method parameters. The parameters varied were mobile phase composition (± 1 % ethyl acetate), wavelength (± 1 nm), chamber saturation time ((± 5 min) and the effect on the area of drug was noted. The areas of peaks of interest remained unaffected by small changes of the operational parameters which indicated robustness of the method.

CONCLUSION

Stability-indicating HPTLC technique with no any interference from the excipients or degradant products have been developed as well as validated for the estimation of Norethisterone acetate in tablet formulation. The method developed is simple, accurate, specific and reproducible. Stability indicating nature of method has been confirmed by forced degradation under different conditions viz. hydrolysis, oxidation, thermal and photolysis. The results of forced degradation studies indicated drug susceptibility to hydrolytic (acid and base), oxidative and thermal stress and stability under photolytic stress condition. The results obtained indicated that the method is highly specific and the degraded products were well separated from the drug.

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