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Stability-Indicating Spectrophotometric Method for Determination of Dienogest in Pure form and Pharmaceutical Preparation

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Abstract : A simple, rapid, sensitive, accurate and precise stability-indicating spectrophotometric methods were developed for the determination of dienogest and its degradants in bulk powder and in a pharmaceutical preparation. The methods were validated over a linear range of 1 – 5 µg/mL and successfully applied to the determination of dienogest at 297 nm with an average percent recovery of 93.9 – 100.3. dienogest were subjected to stress degradation under different conditions recommended by ICH. The samples so generated were used for degradation studies using the developed method. The proposed methods were validated and can be used for analysis of formulation containing dienogest.
Keyword : Stability-indicating, dienogest, formulation, ICH.

Introduction:

Dienogest is also known as (17α)-17-Hydroxy-3-oxo-19-norpregna-4,9-diene-21-nitrile and is a semisynthetic progestogen which is orally-active and also possesses the properties of 17α-hydroxyprogesterone with a molecular formula of $C_{20}H_{25}NO_2$ and molecular mass of 311.42 gram/mol (**Fig. 1**). It has anti-androgenic properties and a derivative of 19-nortestosterone and it is commonly marketed as 2 mg tablets with a brand name of endoheal, endofit, dienofem, dinogest, dinofirst etc. It is primarily used as a contraceptive in combination with ethinyl estradiol, or in other combination form pills.

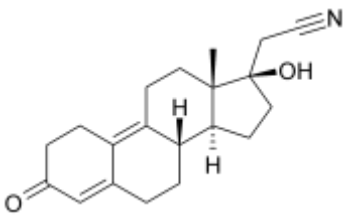

Fig. 1. Structure of dienogest

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A thorough literature review revealed that an RP-HPLC[1,2] method for estimation of dienogest has been reported and best to our knowledge an LC-MS/MS method has been reported for estimation of dienogest in human plasma[3]. Hence, our present work describes a highly sensitive stability-indicating UV-spectrometric method for the estimation of dienogest in commercial formulation.

Materials and methods

Instrumentation

A Shimadzu double beam UV-visible spectrophotometer 1700 with matched quartz cells corresponding to 1 cm path length. Sartorius single pan balance (BS R223S) was used for weighing the material and sonicator (Bandelin Electronics, Berlin) was used for dissolving.

Reagents and Chemicals

All the chemicals and solvents used were of AR grades. Double distilled water, Methanol and Whatman filter paper Grade-I was used throughout the experimental work. Working standards of dienogest (99.37%) was obtained from dienogest was obtained as a gift sample from IPC, New Delhi. Dienogest (2 mg) formulation has been procured from the local market, Bangalore.

Preparation of stock solution

An accurately weighed quantity of Dienogest (10 mg) was dissolved in methanol to make 10 ml solution (1000 µg/ml).

Working standard solution

A stock standard solution (1.0 ml) was transferred to avolumetric flask (10 ml) and the volume was made up to the mark with methanol so to obtain a concentrationof 100 µg/ml. The solution was further diluted to get a final concentration of 1 µg/ml. The solution was used for spectral studies.

Determination of λmax

The working standard solution was scanned in UV range (200-400nm) in 1.0 cm quartz cell against solvent blank. The 297 nm wavelength was selected for the further study. The UV spectra of drug show the spectrum wavelength selected for the estimation of the drug was 297 nm as λmaxof dienogest. At 297 nm, dienogest shows maximum absorbance (**Fig.2**)

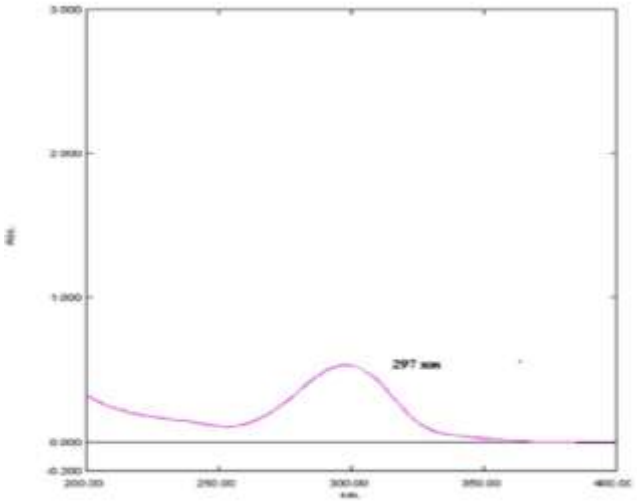

Fig. 2. UV spectrum of dienogest

Method Development

An accurately weighed quantity of tablet powder equivalent to 100 mg was taken in four 100 ml volumetric flasks. All these solutions were stored for 24 h under following different conditions.

- At room temperature after addition of 1.0 N HCl (acid) for 24 hrs.
- At room temperature after addition of 1.0 N NaOH (Alkali) for 24 hrs.
- At room temperature after addition of 1.0 ml of 3% H_2O_2 for 24 hrs
- Sunlight (UV) for 24 hr.

After 24 hrs methanol was added to each 10 ml sample solution, sonicated for 10 min and volume was made up to 100 ml (100 µg/ml). From each flask, the solution (0.3 ml) was diluted up to 10 ml of methanol to get 3 µg/ml of dienogest. The absorbance of each of the resulting solution was measured at 297 nm using solvent blank.

Method Validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The method was validated for different parameters like Linearity, Accuracy, Precision, Ruggedness, Limit Of Detection (LOD) and Limit Of Quantification (LOQ) as per the ICH (International Conference on Harmonization)[4].

Linearity

The linearity of the method was evaluated using five concentrations ranging from 1 – 5 µg/mL. The samples were scanned in UV-VIS Spectrophotometer using water as ablank. A calibration curve was found to be linear with a mean correlation coefficient of 0.9935 as shown in **Fig. 3**.

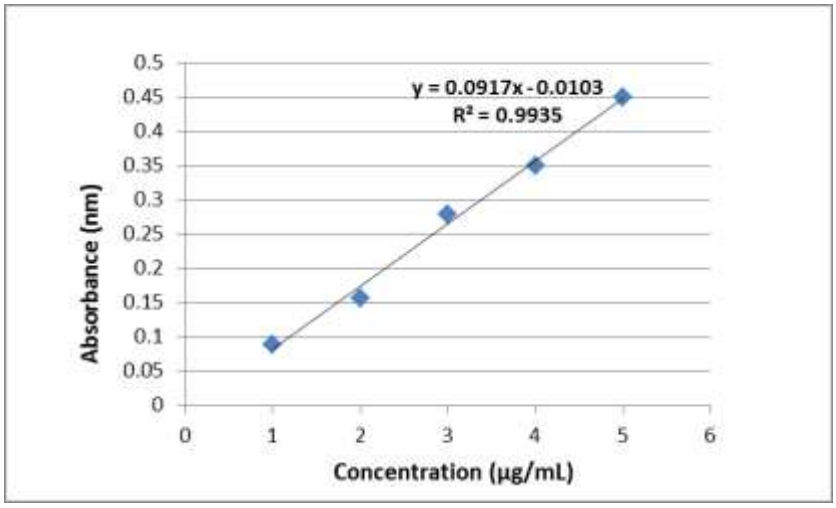

Fig. 3. Linearity range

Precision

The precision of the method was demonstrated by intraday and interday variation studies. In interday variation study, 3 different solutions of same concentration that is 3 µg/ml were prepared and analyzed two times on a day i.e. morning, evening and the absorbance was noted. The result was indicated by % RSD. In the intraday variation study, solutions of same concentration 3 µg/ml were prepared and analyzed three times for two consecutive days and the absorbancewas noted. The result was indicated by % RSD in table 1.

Table. 1. Precision studies

S.No	Days		Absorbance	Amount present	% Drug estimation	Mean	% RSD
1	Interday	Morning	0.279	3.00	100.0		
2		Evening	0.278	2.989	99.63		
3	Intraday	Day-2	0.278	2.989	99.63		
4		Day-3	0.276	2.967	98.39	98.96	1.032

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the method was determined by the recovery studies as shown in thetable. 2.

Table. 2. Accuracy studies

S.no	Weight of drug contributed by tablet (B) (µg/ml)	Amount of pure drug added (C) (µg/ml)	Absorption at 297 nm	Total drug estimated (A) (µg/ml)	% RSD	% Recovery
1	2	1	0.262	2.8172		93.9
2	2	1	0.280	3.0107		100.3
3	2	1	0.275	2.9569	2.83	98.5
4	2	1	0.269	2.8924		96.4

Limit of detection (LOD)

The limit of detection (LOD) was determined based on standard deviation of theresponse of the calibration curve. The standard deviation of absorbance of thecalibration curve and slope of the calibration curves was used. According to following formula was used to calculate the LOD. The limit of detection was found to be 0.5 µg/mL.

LOD=3.3 X S.D/S Where;
S.D=standard deviation
S=slope of absorbance of calibration curve

Limit of quantification (LOQ)

The limit of quantification (LOQ) was determined based on standard deviation of theresponse of the calibration curve. The standard deviation of absorbance of the calibration curve and slope of the calibration curves was used. According to following formula was used to calculate the LOQ. The limit of quantification was found to be 1 µg/mL.

LOQ=10 X S.D/S Where;
S.D=standard deviation
S=slope of absorbance of calibration curve

Assay method (Marketed tablet)

An accurately weighed quantity of tablet powder equivalent to 100 mg was taken in 100 ml volumetric flask containing methanol. The content in the volumetric flask was sonicated for 10 minutes. The volume was adjusted with methanol up to the mark (100 ml). Then the content was filtered through Whatman filter paper. From the filtrate, 1ml was diluted with methanol to get 100 µg/ml in a 10 ml volumetric flask. 3 ml of above solution was taken in a 10 ml volumetric flask and 5 ml of standard stock solution (10µg/ml) and added to the

flask and made up the volume with methanol (Table. 1). The recovery of dienogest was calculated by following formula.

% Recovery = $\frac{A}{B+C}$ **×100** Where,
A= Total drug estimated
B=Weight of drug contributed by tablet powder
C= Amount of pure drug added

Conclusion:

A simple, accurate, precise, robust and rapid UV visible spectrophotometric method has been developed for the estimation of dienogest in the pharmaceutical dosage form and validated as per ICH guidelines.

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