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Research Article

Validated Stability Indicating RP-hplc Method for the Assay of Dienogest in Bulk and Tablet Dosage Form

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Abstract:
Dienogest is used for of treatment of endometriosis. A Stability indicating Reversed phase high Performance Liquid Chromatographic method has been developed for the determination of Dienogest in bulk as well as in innovator Product (Visanne Tablets 2mg). Various Critical issues regarding chromatographic conditions are resolved using Thermo Hypersil BDS C18 150 x 4.6mm, 5µm or equivalent column. 40% Acetonitrile is used as Mobile Phase for elution of very distinct Peak for the Dienogest. The developed Method has been validated as Per ICH Q2B to asses the method as simple, Precise, accurate and robust enough to withstand the changes in Method Parameters. The Peak of Dienogest is eluted near the Retention time of 4.5 Minutes

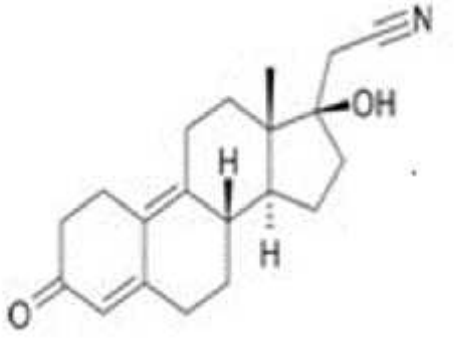
Keywords: Dienogest, Oral contraceptive, HPLC, Progestin

INTRODUCTION

Dienogest is an orally-active semi synthetic, steroidal progestogen (or progestin). It is available for use as an oral contraceptive in combination with Ethinylestradiol. It has antiandrogenic activity and as a result can improve androgenic symptoms. It is a non-ethinylated progestin which is structurally related to testosterone. Dienogest given in isolation is available for the treatment of endometriosis under the trade name Visanne. Dienogest was synthesized in 1979 in Jena, Germany under the leadership of Prof. Kurt Ponsold, was initially referred to as STS 557. It was found that its potency was 10 times that of Levonorgestrel.[9] The first product on the market to contain Dienogest as a contraceptive pill Valette in 1995 made by Jenapharm. It has been little used outside of Germany. The Present research work was aimed at developing a method which can qualitatively as well as quantitatively estimate the content of Dienogest in marketed formulations. It is having UV maxima of 305nm.

MATERIAL AND METHODS

Chemical and Reagents : Dienogest API was obtained from Crystal Pharma S.A.U. Boecillo (Valladolid, SPAIN), Milli Q water systems from the Millipore is used as a source of HPLC Grade water.(Millipore corporation Bioscience Division,Billerica ,Massachusetts, USA), All the reagents used were of AR grade. Acetonitrile and



Methanol used for the preparation of mobile phase and diluents is of HPLC grade. (Real Chemsys Product Pvt Ltd GHB).

Apparatus and Chromatographic Conditions: Chromatographic separations were performed on Shimadzu chromatographic systems equipped with a LC-2010AHT /2010CHT Quaternary Pump, equipped with PDA Detection module and Redone (7725i).

Mobile Phase: Measured accurately 60: 40% v/v (water: Acetonitrile), filter through 0.45µ filter paper and degassed it.

Diluents:

1. Acetonitrile
2. HPLC Grade water and methanol in the ratio of 50:50% v/v respectively.

Chromatographic conditions**:**

Column : Thermo Hypersil BDS C18 150 x 4.6mm, 5µm or equivalent

Column temperature : Ambient

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System Suitability

Injection	Response	Tailing factor	Theoretical plates
01	1510886	1.1	8462
02	1511687	1.1	8451
03	1511488	1.1	8438
04	1511617	1.1	8455
05	1511521	1.1	8452
06	1511460	1.1	8448
Mean	1511443	1.1	8451
SD	285.8	0.0	8.0
% RSD	0.0	0.0	0.1

Observation: In specification level

S.No	Name	Concentration (%)	Observation
01	DNG-I	0.15	Not Detected
02	DNG-II	0.15	Detected
03	DNG-III	0.15	Not Detected

For Identification Purpose:

S.No	**Name**	**Retention Time**
01	Dienogest	**4.508**
02	DNG-I	**24.719**
03	DNG-II	**7.032**
04	DNG-III	**13.902**

Stressed samples of drug product and Placebo were injected separately into the HPLC System equipped with PDA detector by using test method conditions

Stress Condition	Drug Product % degradation Dienogest	Peak Purity Index Dienogest
Refluxed with 1M HCl solution for 30min at 45ºC (Acid).	9.9	1.000
Refluxed with 1M NaOH solution for 15min at 45ºC (Base).	8.7	1.000
Refluxed with of 2.5% Hydrogen peroxide (H_2O_2) for 30min at 45ºC (Peroxide).	8.2	1.000
Refluxed with purified water for 3 hours at 45ºC (Aqueous).	2.2	1.000
Exposed to UV-Light for 200 Watts/m^2.	0.6	1.000
Exposed to Sun-Light for 1.2 Million Lux Hours.	0.8	1.000
Exposed to Dry heat at 105° C for about 15 hours.	2.5	1.000
Exposed to humidity at 25°C and 90% RH for about 7 days.	0.4	1.000

Flow rate : 1.0 mL / minute
Injection volume : 50 µl
Detector Wave length : 305 nm
Run Time : 8 minutes

Standard solution: Weigh accurately about 40mg of Dienogest working standard and transfer into a 200ml volumetric flask, then add 120ml of Acetonitrile sonicate for 5minutes to dissolve and make up the volume with Acetonitrile. Pipette out 10ml of this stock solution into a 25ml volumetric flask and make up the volume with diluent. Further Pipette out 5ml of this stock solution into a 50ml volumetric flask and make up the volume with diluent and mix well.

Test solution preparation: (Assay) Weigh accurately not less than 20 tablets and note down the weight. Then calculate the average weight. Crush the tablets into fine powder with mortar and pestle. Then weigh accurately tablets powder equivalent to 8mg of Dienogest and transfer into a 200ml volumetric flask, add 120mL of diluent, sonicate for 20minutes with occasional shaking

Linearity and range:

S. No.	Dienogest Concentration in µg/ml	Response
01	0.80	153415
02	2.00	380784
03	4.00	782939
04	6.00	1134008
05	8.00	1505203
06	10.00	1910266
07	12.00	2329047
Y intercept		-5284
Slope		192304
Coefficient of correlation (r^2)		0.9994

Range:

Level	Concentration in µg/ml	% RSD
Lower Level	0.80	0.0
Middle Level	8.00	0.1
Higher Level	12.00	0.0

Impurity interference: Established impurity interference

Method Precision (Repeatability)

No of Sample	Dienogest content in %
01	99.6
02	99.9
03	100.5
04	100.2
05	100.1
06	99.8
Mean	100.0
SD	0.3
% RSD	0.3
Confidence Interval	0.3

Intermediate Precision

Parameter	Analyst -1	Analyst -2
Analyst	Analyst 1	Analyst 2
Column	Thermo Hypersil BDS 150*4.6mm	Thermo Hypersil BDS 150*4.6mm
HPLC	Waters Alliance ,USA	Shimadzu,Japan

and make up the volume with diluent. Centrifuge the above solution at 2500 rpm for about 5 minutes. Pipette out 10ml of this centrifuged solution into a 50ml volumetric flask and make up the volume with diluent.

Method Validation

System Suitability: Developed method is validated as per ICH guidelines to attain method suitability for analysis Purposes. Six Replicate standards injected and calculated the standard deviation and Percent related Standard Deviation. System suitability test is performed, Results are depicted below.

Specificity

by spiking impurity mixed solution of Dienogest (DNG-I, DNG-II, DNG-III) at specification level and injected this solution into chromatographic system. Injected the higher concentration of the Impurities blend solution with diluent into chromatographic system for identification purpose.

Interference from Degradation products: A study was conducted to demonstrate the effective separation of degradants from Dienogest in Dienogest tablets 2mg assay method. Drug product, Placebo and Blank were

Level	No of Sample	Theoretical Dienogest content (mg)	Calculated Dienogest content (mg)	Recovery in %	Average recovery in %
10%	01	0.83	0.82	99.3	99.3
	02	0.82	0.81	98.7	
	03	0.81	0.81	100.0	
25%	01	2.04	2.03	99.5	99.6
	02	2.05	2.06	100.4	
	03	2.06	2.04	98.8	
50%	01	4.03	3.98	98.9	99.3
	02	4.02	3.99	99.3	
	03	4.00	3.99	99.8	
75%	01	6.01	5.98	99.4	100.0
	02	5.98	6.00	100.4	
	03	5.96	5.97	100.2	
100%	01	8.02	7.96	99.2	99.4
	02	8.00	7.97	99.6	
	03	8.01	7.96	99.4	
125%	01	10.02	10.05	100.3	100.0
	02	10.06	10.05	99.9	
	03	10.09	10.06	99.7	
150%	01	12.06	12.08	100.2	99.7
	02	12.09	12.04	100.2	
	03	12.12	12.03	99.3	
			Mean	99.6	
			SD	0.5	
			% RSD	0.5	
			Confidence Interval	0.2	

exposed to the following stress conditions to induce degradation.

A) Refluxed with 1M HCl solution for 30min at 45ºC (Acid).
B) Refluxed with 1M NaOH solution for 15min at 45ºC (Base).
C) Refluxed with of 2.5% Hydrogen peroxide (H_2O_2) for 30min at 45ºC (Peroxide).
D) Refluxed with purified water for 3 hours at 45ºC (Aqueous).
E) Exposed to Sun-Light for 1.2 Million Lux Hours.
F) Exposed to UV-Light for 200 Watts/m^2.
G) Exposed to Dry heat at 105° C for about 15 hours.
H) Exposed to humidity at 25°C and 90% RH for about 7 days.

Linearity was established by plotting a graph between concentration on X-axis and peak area on Y-axis and the correlation coefficient was determined. Seven different concentrations of Dienogest concentration ranging from about 10% to 150% with respect to working concentration were prepared and analyzed as per test method. The results are summarized in the table given below.

System to system variability

No of Sample	System-1	System-2
01	99.6	99.6
02	99.9	99.8
03	100.5	100.0
04	100.2	100.3
05	100.1	99.5
06	99.8	100.1
Mean	100.0	99.9
SD	0.3	0.3
% RSD	0.3	0.3
Confidence Interval	0.3	0.2

Column to column variability:

No of Sample	Column-1	Column-2
01	99.6	99.6
02	99.9	99.8
03	100.5	100.0
04	100.2	100.3
05	100.1	99.5
06	99.8	100.1
Mean	100.0	99.9
SD	0.3	0.3
%RSD	0.3	0.3
Confidence Interval	0.3	0.2

Effect of variation in Column Oven Temperature:

System Suitability Parameters	Observed value with Column Oven Temperature			Acceptance criteria
	20°C	25°C	30°C	
Tailing factor for Dienogest peak from first standard injection.	1.1	1.1	1.1	NMT 2.0
% RSD for peak areas of Dienogest peak from five replicate injections of standard solution.	0.0	0.0	0.1	NMT 2.0
Theoretical plate count for Dienogest peak from first standard injection	8290	8462	8496	NLT 2000

Precision:

Effect of variation in Column Oven Temperature:

System Suitability Parameters	Observed value with Column Oven Temperature			Acceptance criteria
	20°C	25°C	30°C	
Tailing factor for Dienogest peak from first standard injection.	1.1	1.1	1.1	NMT 2.0
% RSD for peak areas of Dienogest peak from five replicate injections of standard solution.	0.0	0.0	0.1	NMT 2.0
Theoretical plate count for Dienogest peak from first standard injection	8290	8462	8496	NLT 2000

Sample ID	Assay in %				
	Centrifuged	Sample filtered through Whatmann filter		Sample filtered through Nylon filter	
	% Assay	% Assay	% Difference	% Assay	% Difference
1.	100.4	98.1	2.3	99.7	0.7
2.	100.2	98.3	1.9	100.1	0.1

Intermediate Precision: The Intermediate Precision has been demonstrated by analyzing in six replicate the same samples of by mixing Dienogest drug substance and placebo as per composition given in the manufacturing formula as per the test procedure on two different days with different analysts and different lot of column. The individual values, the mean and the variance of method precision and intermediate precision are reported. The homogeneity of the variances has been verified by‘t’ test.

Accuracy:The Accuracy is demonstrated by preparing pharmaceutical preparations of the Dienogest content at various concentrations ranging from 10% to 150% of the target initial assay concentration (10%, 25%, 50%, 75%, 100%, 125% & 150%) and performed assay Dienogest analyzing the mixtures in triplicate. The individual values, the percent recovery, the mean value and the standard deviation and confidence interval results are reported. Summarize the results in the table given below.

Effect of variation in Flow Rate:

System Suitability Parameters	Observed value with Flow Rate			Acceptance criteria
	0.8 ml/min	1.0 ml/min	1.2 ml/min	
Tailing factor for Dienogest peak from first standard injection.	1.1	1.1	1.1	NMT 2.0
% RSD for peak areas of Dienogest peak from five replicate injections of standard solution.	0.1	0.0	0.0	NMT 2.0
Theoretical plate count for Dienogest peak from first standard injection.	9728	8462	7342	NLT 2000

Effect of variation in Mobile phase composition (Acetonitrile):

System Suitability Parameters	Observed value with Acetonitrile Composition			Acceptance criteria
	95%	100%	105%	
Tailing factor for Dienogest peak from first standard injection.	1.1	1.1	1.2	NMT 2.0
% RSD for peak areas of Dienogest peak from six replicate injections of standard solution.	0.0	0.0	0.2	NMT 2.0
Theoretical plate count for Dienogest peak from first standard injection.	9198	8462	8085	NLT 2000

Ruggedness:

System to system variability: The Ruggedness has been demonstrated by analyzing in six replicate the same samples by mixing Dienogest drug substance and placebo as per composition given in the manufacturing formula as per the test procedure on two different days with different analysts and instruments (minimum two different instruments). The individual values, the mean and the variance for each of the two days are reported.

Robustness:

Effect of variation in Mobile phase composition (Acetonitrile): A study to establish the effect of variation in mobile phase composition was conducted. Two mobile phases were prepared with 95% and 105% of the method organic phase (Acetonitrile) composition. Standard solution prepared as per the test method and injected six replicate injections of standard solution into HPLC system. The System suitability parameters were evaluated with mobile phases containing 95% and 105% of the method organic phase (Acetonitrile).

Effect of variation in Flow Rate: A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method and injected six replicate injections of standard solution into HPLC system with flow rate 0.8 ml/min. to 1.2 ml/min. The system suitability parameters were evaluated as per test method.

Effect of variation in Column Oven Temperature: A study was conducted to determine the effect of variation in column oven temperature. Standard solution prepared as per the test method and injected six replicate injections of standard solution into HPLC system at 20°C to 30°C of column oven temperatures. The system suitability parameters were evaluated as per the test method.

Effect of variation in Wavelength: A study was conducted to determine the effect of variation in wavelength. Standard solution prepared as per the test method and injected six replicate injections of standard solution into HPLC system at 303nm and at 307nm of wavelength.

Effect of variation in Column Oven Temperature: A study was conducted to determine the effect of variation in column oven temperature. Standard solution prepared as per the test method and injected six replicate injections of standard solution into HPLC system at 20°C to 30°C of column oven temperatures. The system suitability parameters were evaluated as per the test method.

A study to establish the suitability of filters was conducted using two different filters namely, Whatmann No 42 and 0.45 µm Nylon filters. Prepare the standard solution as per the test method and similarly prepare the test solution in duplicate.

Filtered the test solution through individual filters and Injected unfiltered standard solution and filtered test solution into the HPLC system as per method. Difference in % Assay of filtered test solution against unfiltered standard. Tabulate the results in the table given below.

Filter Validation:

Filter Description	Filters SY25NN(Nylon)	Whatmann No. 42
Manufacturer Name	Advance microdivices Pvt .Ltd	Whatmann International Ltd
Lot No.	SN1770	1442 125
Size	0.45µm	42

CONCLUSION

System Suitability and System Precision is established as mentioned in the test method. The test method is specific for the estimation of Dienogest in Dienogest Tablets 2 mg. There is no interference or co-elution of degradants in quantifying the Dienogest in Dienogest Tablets 2 mg. The detector response was found linear with a correlation coefficient of 0.9994 and Range is found within the limit. The test method is meeting the Method Precision and Intermediate Precision acceptance criteria. The method performance at lower to higher levels (10% to 150%) is Linear, Precise and Accurate. The test method is rugged for system to system, column to column variability. Standard Solutions and test Solution are stable at bench top for a period of 2 days 48 Hours (2 Days). Mobile Phase used in the assay of Dienogest tablets 2 mg, is stable for a period of 2 days at Bench top. The allowable variation in organic phase composition in mobile phase (Acetonitrile) is from 95% to 105% of the method organic phase composition (Acetonitrile). The allowable variation in flow rate is from 0.8 ml/min to 1.2 ml/min. The allowable variation in column temperature is from 20°C to 30°C. The allowable variation in wavelength of Dienogest Tablets 2mg is from 303nm to 307nm of the method wavelength (305nm). The centrifugation and 0.45 µm Nylon filter are suitable for performing filtration.

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