EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 2
277-281
Year: 2019

DEVELOPMENT AND VALIDATION OF RP-HPLC ASSAY METHOD FOR DETERMINATION OF LEVONORGESTREL IN TABLET FORM

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Article Received on 25/11/2018 **Article Revised on 15/12/2018** **Article Accepted on 05/01/2019**

ABSTRACT
A simple, sensitive and accurate isocratic reverse phase high performance liquid chromatography method was developed for determination of Levonorgestrel in tablets. The effective separation was achieved on Agilent Eclipsed XDB C18 4.6 X 150 mm with particle size 3.5µm. The mixture acetonitrile and DI water in the ratio 70:30 v/v used as a mobile phase. The flow rate of the mobile phase was 1.5 mL/min and the total elution time was 6 minutes. The UV detection wavelength has been carried at 245 nm and experiments were conducted at 40°C. The developed method was validated in terms of system suitability, selectivity, linearity, precision, accuracy, robustness and sample stability for Levonorgestrel following the ICH guidelines.

KEYWORDS: Levonorgestrel tablets, Method development, Eclipsed XDB C18 and RP-HPLC.

1. INTRODUCTION
Levonorgestrel is a hormonal medication which is used in a number of birth control methods. Chemically Levonorgestrel is (E)-4-[2-[3-(1, 1-Dimethylethoxy)-3-oxo-1-propenyl] phenyl]-1, 4-dihydro-2, 6-dimethyl-3,5 pyridine dicarboxylic acid diethyl ester (Figure 1) and molecular formula of $C_{21}H_{28}O_2$ (312.446 g/mol). It is white crystalline powder and water Soluble. Drug product available in pill form, sold under the brand name Plan B among others; it is useful within 120 hours as emergency birth control. It is also combined with an estrogen to make combined oral birth control pills. Levonorgestrel is a synthetic progesterone hormone with actions similar to those of progesterone and about twice as potent as it's racemic or (+-)-isomer (Norgestrel). It is used for contraception, control of menstrual disorders, and treatment of endometriosis.

Literature survey reveals that several analytical methods like LC-MS[2-5], Derivative Spectrophotometry method[6] and HPLC methods[7-10] have been reported for the estimation of Levonorgestrel in different forms. The aim of this study was to develop a RP-HPLC method, which could be employed for the routine analysis of the drug in pharmaceutical dosage forms using simple mobile phase composition with less run time. The present work describes a simple, isocratic RP-HPLC method for the determination of Levonorgestrel in tablets as per ICH guidelines.[11-13]

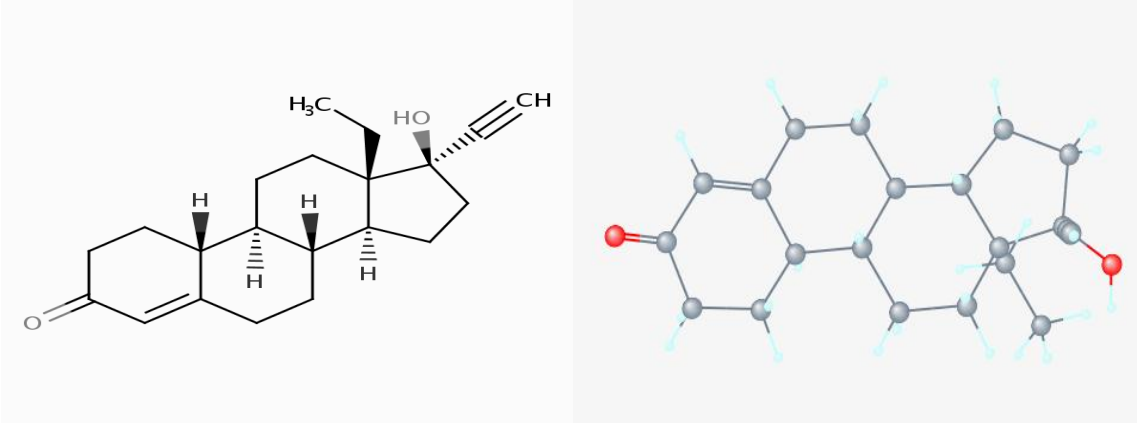

Fig. 1: Chemical structure of Levonorgestrel.

2. EXPERIMENTAL: MATERIALS AND REAGENTS

2.1 Instrumentation and software

A high performance liquid chromatography system manufactured by Waters which consist of PDA detector, sample manager, column heating compartment was used for assay determination of Levonorgestrel in tablets. HPLC instrument was controlled by empower software. Agilent Eclipsed XDB C18 4.6X150 mm, column with particle size of 3.5µm was used as stationary phase for chromatographic separation. Sartorius semi micro analytical balance was used for all weighing, and sonicator used to dissolve the solutions.

2.2 Preparation of mobile phase

Mixture of acetonitrile and DI water in the ratio of 70:30v/v was used as mobile phase.

2.3 Preparation of standard solutions

Weighed accurately and transferred 15 mg of Levonorgestrel standard into a 25 mL volumetric flask add about 15 mL of diluent (mobile phase), sonicate to dissolve the material completely, dilute to volume with diluent and mix well. Transfer 5 mL of above solution into 50mL volumetric flask and dilute to volume with diluent.

2.4 Preparation of sample solutions

Grind few number of tablets and weighed accurately and transferred equivalent to 30 mg of Levonorgestrel tablets powder into a 200 mL volumetric flask, add 45mL of DI water and disintegrate it. Add 105 mL of ACN and sonicate for 5 min and diluted to volume with diluent. Filter the solution through 0.45µm organic filter.

3. Method validation parameters

The system suitability was conducted using standard preparation and evaluated by injecting six replicate standard injections. Specificity is the ability of analytical method to assess un equivocally the analyte in the presence of component that may be expected to be present. Performed the specificity parameter of the method by injecting Diluent (Mobile Phase) and placebo into the chromatographic system and evaluated by show any peak at the retention time of analyte. Linearity has been performed in the range of 32 to 127% of specification limit. Recorded the area response for each level and calculated slope, intercept & correlation-coefficient. The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation of series of measurements. The system precision was conducted using Levonorgestrel and evaluated by six assay results using same sample. The Accuracy of the method by recoveries of Levonorgestrel sample solutions at different concentration levels ranging 50%-150% of sample concentration. The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

4. RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The following analytical parameters having major role during the method development and validation includes selection of appropriate chromatographic conditions/factors like detection wavelength, selection of stationary and mobile phases, column temperature and injection volume. The wavelength of 245 nm was selected due to it produces less noise, which minimizes problems that may exhibit around the active ingredient when attempting to quantify Levonorgestrel in tablets. Preliminary development trials were performed with various columns of different types and dimensions from different manufacturers were tested for the peak shape and tailing factor for specification concentrations. Finally by switching to Agilent Eclipsed XDB C18 4.6 X 150 mm, 3.5µm column with a significant improvement in the peak shapes with 0.8 tailing factor.

5. Method validation

5.1 System suitability

The RSD from six replicate injections of Levonorgestrel standard preparation was 0.2%. System suitability data is given in Table-1.

Table 1: System suitability results of Levonorgestrel.

Injection No.:	Levonorgestrel Peak Area	Tailing Factor
1	6516419	0.8
2	6499099	0.9
3	6489948	0.8
4	6489220	0.8
5	6499555	0.9
%RSD	**0.2**	

5.2 Selectivity

Performed the specificity parameter of the method by injecting diluent (mobile phase), standard preparation sample preparation into the chromatographic system and recorded the retention times. Specificity study of the method proved that no peak observed at retention time of Levonorgestrel. Specificity results have been tabulated below. The typical selectivity chromatograms are shown in Figure-2, 3 & 4.

Table 2: Specificity results.

S.No.	Compound	Retention time
1	Blank (Diluent)	--
2	Standard	3.993
3	Sample solution	3.991

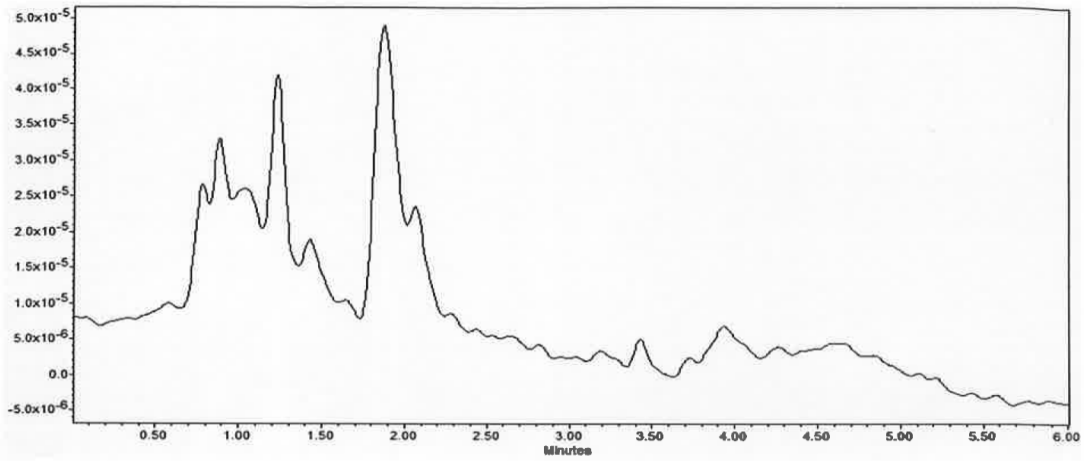

Fig. 2: Blank chromatogram.

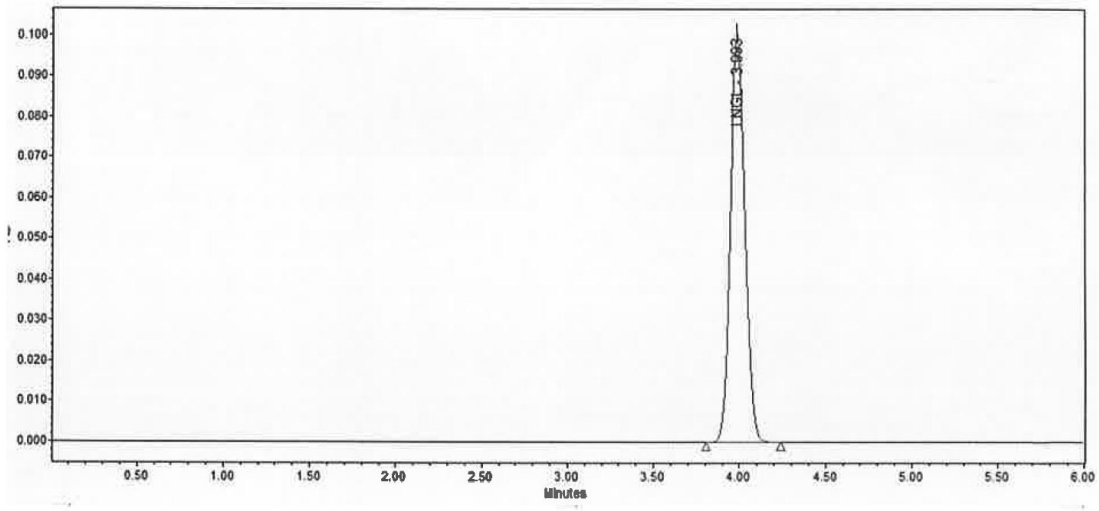

Fig. 3: Standard chromatogram.

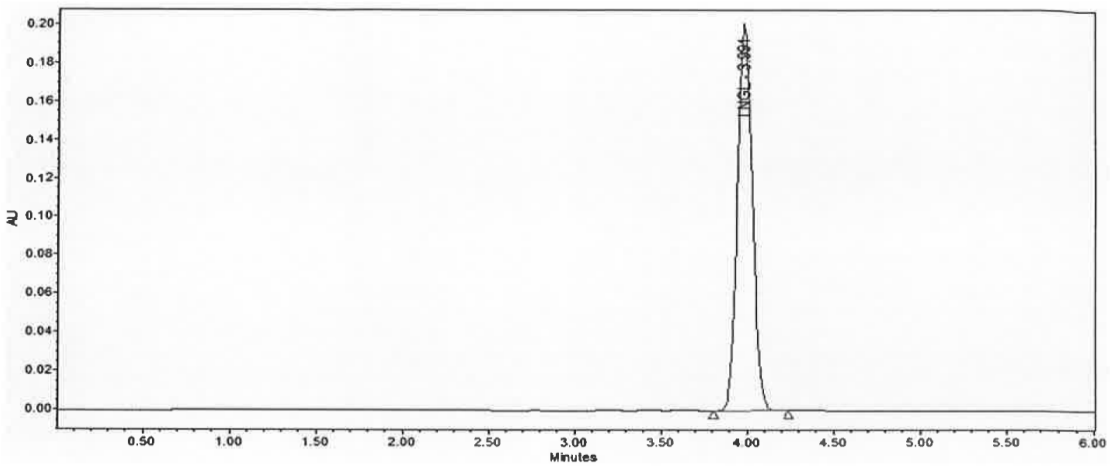

Fig. 4: Sample chromatogram.

5.3 Linearity

To demonstrate the linearity with Levonorgestrel in the range of 32 to 127% of specification limit. Correlation coefficient of Levonorgestrel is 1.000. The linearity results shown in the below Table -3. Linearity curves of Levonorgestrel shown in the Figure-3.

Table 3: Linearity results of Levonorgestrel.

S.No.	Levonorgestrel Concentration µg/ml	Concentration Level (%)	Peak Area
01	23.876	32	2595166
02	47.753	64	5164590
03	59.691	80	6428620
04	71.629	96	7684641
05	95.506	127	10189594
Correlation coefficient		**1.000**	
Slope		**1.8874**	
Intercept		**-1.2268**	

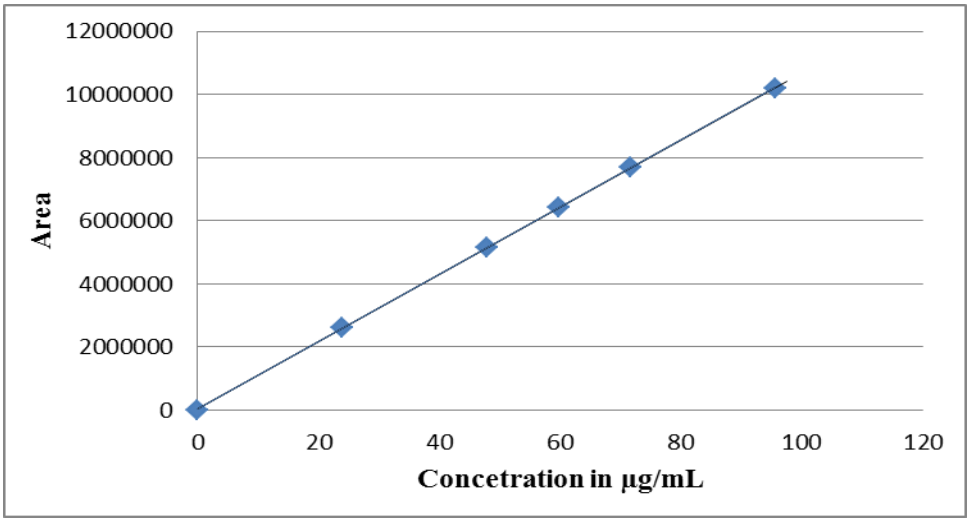

Figure 5: Linearity curves of Levonorgestrel.

5.4 Precision

The precision of test method was validated by relative standard deviation of individual % assay of Levonorgestrel from the six preparations. The precision results are given Table-4.

Table 4: Precision results.

Sample	% of Assay
Preparation-1	99.6
Preparation-2	99.2
Preparation-3	98.9
Preparation-4	99.5
Preparation-5	99.2
Preparation-6	100.2
Mean	**99.4**
%RSD	**0.5**

5.5 Accuracy

The accuracy study was performed at three levels 50%, 80%, 100%, 125% & 150% of sample concentration. The percentage recovery of Levonorgestrel in tablets ranged from 99.5 to 100.1 respectively. The accuracy results have been tabulated below.

Table 5: Accuracy results.

Sample	% Recovery	Mean
50%	99.8	99.7
	99.1	
	100.1	
80%	99.6	99.5
	99.8	
	99.2	
100%	99.8	100.0
	100.2	
	100.1	
125%	100.1	100.1
	99.9	
	100.3	
150%	99.7	99.7
	99.8	
	99.5	

5.6 Robustness

The method robustness was studied by injecting the system suitability solution at change in the Column temperature, Flow rate, Percentage of organic modifier (Acetonitrile) and Wavelength. The results were obtained as shown in the below Table-6.

Table 6: Robustness results.

Condition	Tailing factor (NMT: 2.0)	% RSD (NMT: 2.0)
Normal Condition (as such condition)	0.9	0.2
Column temperature 38°C	0.9	0.1
Column temperature 42°C	1.0	0.1
Flow rate 1.6 mL/min.	0.9	0.1
Flow rate 1.4 mL/min.	1.0	0.1
Change in organic component - 10%	1.0	0.1
Change in organic component + 10%	0.8	0.2
Wavelength 243	0.9	0.1
Wavelength 247	0.8	0.1

5.7 Solution Stability

The working standard and solution stability has been established by assaying it against the freshly prepared standard and sample. The stability results are tabulated below.

Table 7: Standard and sample results.

Condition	% of Recovery	Limit
Fresh Standard	100.2	98.0%-102.0%
Fresh Sample	100.3	
5 days Standard	100.6	
5 days sample	99.9	

6. CONCLUSION

A simple isocratic reverse phased HPLC method has been developed and validated for the determination of Levonorgestrel in tablets. The developed method has been found to be selective, sensitive, precise, linear and robust. The method can be directly adopted in quality control laboratories for routine analysis with respect to determination and quantification of Levonorgestrel with less run time.

ACKNOWLEDGEMENTS

I have thankful to department of chemistry, GITAM University, Hyderabad, India and Novast laboratories for encouragement and resources.

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