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Research Paper

Method development and validation of levonorgesterel and ethinyl estradiol in bulk and its pharmaceuticals dosage forms

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ABSTRACT

HPLC is regarded as one of the most sophisticated and reliable analytical techniques currently available. The quantitative determination of Levonorgestrel and Ethinyl Estradiol was performed using an optimized HPLC method. The mobile phase comprised Methanol and Phosphate buffer (pH 3.0) in a ratio of 70:30 % v/v. Chromatographic separation was achieved on an Inertsil C18 column (4.6 × 150 mm, 5 µm) or an equivalent stationary phase chemically bonded to porous silica particles. Detection was carried out using a UV detector at 265 nm, with a constant flow rate of 0.7 mL/min.

The method demonstrated excellent linearity over the concentration ranges of 100–500 µg/mL for Levonorgestrel and 1–5 µg/mL for Ethinyl Estradiol, with correlation coefficients (R^2) greater than 0.999. The %RSD values for precision studies were below 2%, indicating satisfactory repeatability and accuracy. The percentage recoveries of Levonorgestrel and Ethinyl Estradiol were found to be within 98–102%, and both the limits of detection (LOD) and quantitation (LOQ) were within acceptable limits.

All validation parameters complied with ICH and USP guidelines, confirming the method's suitability for its intended purpose. The proposed HPLC method is simple, accurate, precise, and linear, and can be effectively employed for the routine quality control analysis of Levonorgestrel and Ethinyl Estradiol in pharmaceutical formulations.

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1. INTRODUCTION

Literature review reveals that there is no analytical method reported for the analysis of levonorgesterol and Ethinyl Estradiol by simultaneous estimation by RP-HPLC. Spectrophotometer, HPLC and HPTLC are the reported analytical methods for compounds either individually or in combination with other dosage form[1]. Hence, it was felt that, there is a need of new analytical method development for the simultaneous estimation of levonorgesterol and Ethinyl Estradiol in pharmaceutical dosage form. Present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method for the simultaneous analysis of levonorgesterol and Ethinyl Estradiol. The developed method will be validated according to ICH guidelines. The analytical method for the simultaneous estimation of levonorgesterol and Ethinyl Estradiol will be developed by RP-HPLC method by optimizing the chromatographic conditions.The developed method is validated according to ICH guidelines for various parameters specified in ICH guidelines, Q2 (R1).

2. METHODOLOGY

Preparation Sample solutions: For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 5mg of Levonorgestrel and 5.3mg of Ethinyl Estradiol working standard into a 10mL and 100 ml 0f clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock Solution). Further pipette 3 ml of Levonorgestrel & 0.3 ml of Ethinyl Estradiol of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent[2].

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 10 mg of Levonorgestrel and 10 mg of Ethinyl Estradiol working standard into a 10mL and 100 ml 0f clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock Solution).

Further pipette 3 ml of Levonorgestrel & 0.3 ml of Ethinyl Estradiol of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 150% solution (With respect to target Assay concentration):

Accurately weigh and transfer 14.4mg of Levonorgestrel and 14.5mg of Ethinyl Estradiol working standards into a 10mL and 100ml of clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 3 ml of Levonorgestrel & 0.3 ml of Ethinyl Estradiol of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent[3].

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Levonorgestrel and Ethinyl Estradiol and calculate the individual recovery and mean recovery values.

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.0%.

LINEARITY:

Preparation of stock solution:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Levonorgestrel and Ethinyl Estradiol (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Preparation of Level – I (100ppm of Levonorgestrel &1ppm of Ethinyl Estradiol):

1ml and 0.1 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – II (200ppm of Levonorgestrel &2ppm of Ethinyl Estradiol):

2ml and 0.2 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – III (300ppm of Levonorgestrel &3ppm of Ethinyl Estradiol):

3ml and 0.3 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – IV (400ppm of Levonorgestrel &4ppm of Ethinyl Estradiol):

4ml and 0.4 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent

Preparation of Level – V (500ppm of Levonorgestrel &5ppm of Ethinyl Estradiol)

5ml and 0.5 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent

Procedure:

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Acceptance Criteria:

Correlation coefficient should be not less than 0.999.

LIMIT OF DETECTION:

Limit of Detection: (For Levonorgestrel):

Preparation of 300µg/ml solution:

Accurately weigh and transfer 10 mg of Levonorgestrel working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.12µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.Pipette 0.4mL of 1µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank

Signal Obtained from LOD solution

S/N = 152/52 = 2.9

Acceptance Criteria:

S/N Ratio value Shall be 3 for LOD solution.

Limit Of Detection: (For Ethinyl Estradiol)

Preparation of 3µg/ml solution:

Accurately weigh and transfer 10mg of Ethinyl Estradiol working standard into a 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.015µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Further pipette 0.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank

Signal Obtained from LOD solution

S/N = 156/52 = 3.0

Acceptance Criteria:

S/N Ratio value shall be 3 for LOD solution.

LIMIT OF QUANTIFICATION:

Preparation of 300µg/ml solution:

Accurately weigh and transfer 10 mg of Levonorgestrel working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.42µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Pipette 1.0mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. Pipette 1.4 mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank

Signal Obtained from LOQ solution

S/N = 522/52 = 10.03

Acceptance Criteria:

S/N Ratio value shall be 10 for LOQ solution.

Limit Of Quantification:((for Ethinyl Estradiol)

Preparation of 3µg/ml solution:

Accurately weigh and transfer 10mg of Ethinyl Estradiol working standard into a 100mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.05µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Pipette 1.7mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to potassium dihydrogen phosphate with buffer (pH 3.0), Methanol in proportion 30: 70 v/v respectively[5].

Wave length selection:

UV spectrum of 10 µg / ml Norgestimate and Ethinyl Estradiol in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 265. At this wavelength both the drugs show good absorbance[4].

Optimization of Column:

The method was performed with various columns like C18 column, hypersil column, lichrosorb, and inertsil ODS column. Inertsil ODS (4.6 x 150mm, 5 m) was found to be ideal as it gave good peak shape and resolution at 0.7ml/min flow.

Chromatogram for Levonorgestrel and Ethinyl Estradiol

Column	:	Inertsil C18 (4.6 x 250mm, 5 m)
Buffer pH	:	3.0.
Mobile phase	:	30% buffer 70% Methanol
Flow rate	:	0.7 ml per min
Wavelength	:	265 nm
Temperature	:	ambient.
Run time	:	10min.

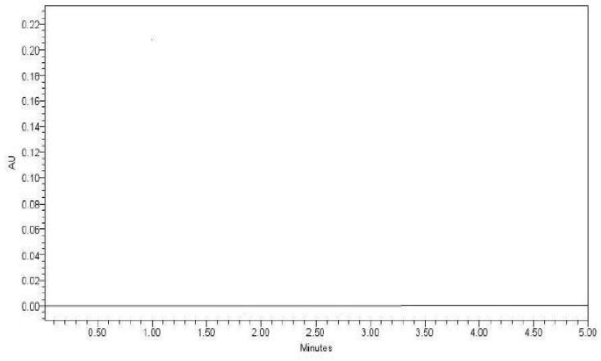


Figure 1 Chromatogram for blank

From the above chromatogram it was observed that there are no interferences.

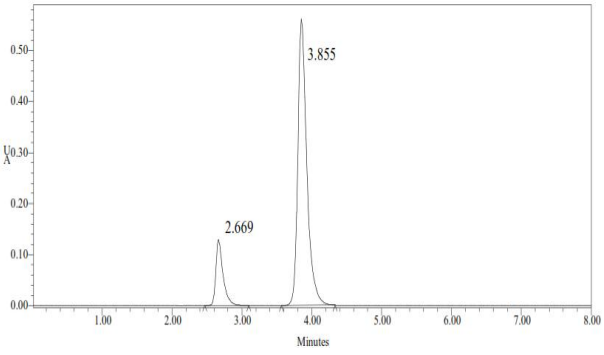


Figure 2 Chromatogram for Levonorgestrel and Ethinyl Estradiol sample Preparation

From the above chromatogram it was observed that the Norgestimate and Ethinyl Estradiol peaks are well separated

Retention time of Norgestimate – 2.669min

Retention time of Ethinyl Estradiol - 3.855 min.

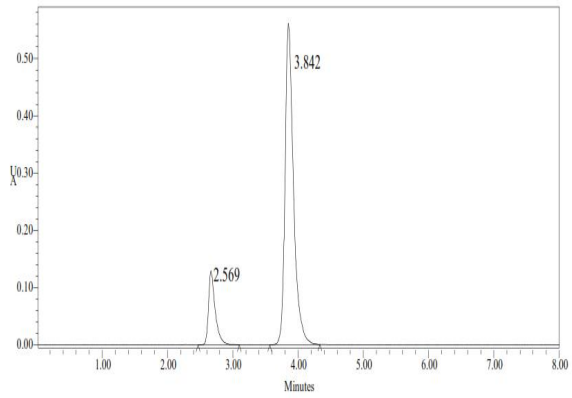


Figure 3 Chromatogram for Levonorgestrel and Ethinyl Estradiol Standard Preparation

Retention time of Norgestimate – 2.569 min

Retention time of Ethinyl Estradiol - 3.842 min.

SYSTEM SUITABILITY:

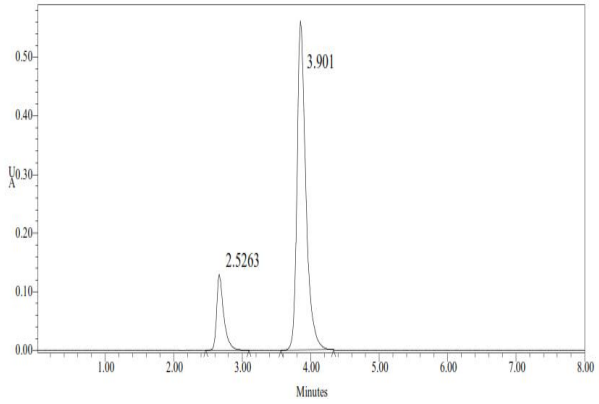


Figure 4 Chromatogram for system suitability

Organ-on-a-Chip technology represents a fundamental and necessary evolution in biomedical science. It provides a tangible solution to the crisis of translation that has long plagued drug development. By generating human-relevant data at the earliest stages of research, these systems have the potential to significantly de-risk the transition from the laboratory to the clinic.

However, the revolution is still maturing. Navigating the gauntlet of standardization, scalability, and regulation will require a concerted and collaborative effort. Based on the analysis within this review, the following recommendations are proposed:

For the Scientific and Research Community: Prioritize the development of standardized protocols and validation studies to build confidence in OoC-derived data. Foster deep, interdisciplinary collaboration between biologists, engineers, and data scientists to build more complex and predictive models.

For the Pharmaceutical and Biotechnology Industry: Strategically integrate OoC models into early-stage decision-making processes. Invest in automated, higher-throughput systems to enable larger-scale screening and toxicology studies[7].

For Regulatory Bodies and Policymakers: Continue to engage proactively with industry and academic experts to establish clear frameworks for the validation and acceptance of OoC data in regulatory submissions.

In conclusion, the journey of Organ-on-a-Chip technology is one of immense potential tempered by practical hurdles. By strategically addressing the field's core challenges, this technology is poised to become an indispensable tool in the creation of safer, more effective, and more personalized medicines[6].

3. RESULTS AND DISCUSSION

System Suitability Results:

1). Tailing factor Obtained from the standard injection is 1.3

2) Theoretical Plates Obtained from the standard injection is 4668.7

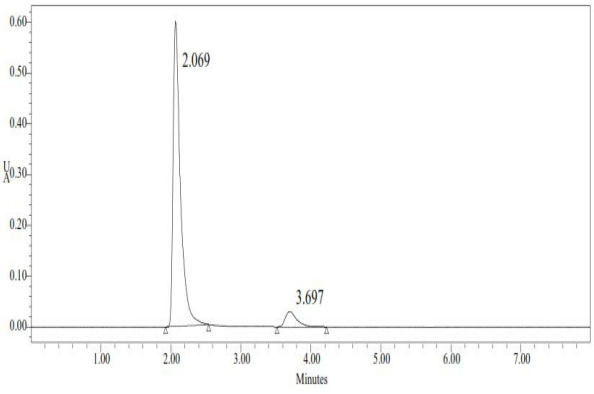
Table-1 Accuracy (recovery) data for Levonorgestrel

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	99.84%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	

Table-2 Accuracy (recovery) data for Ethinyl Estradiol

%Concentration(at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	100.51%
100%	1243353	10	10.10	100.01%	
150%	177940	14.2	14.45	99.68%	

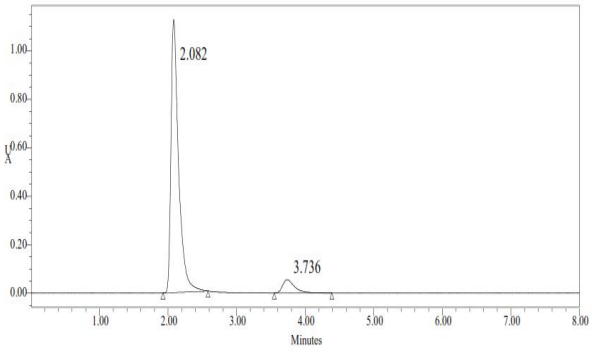
Acceptance Criteria:



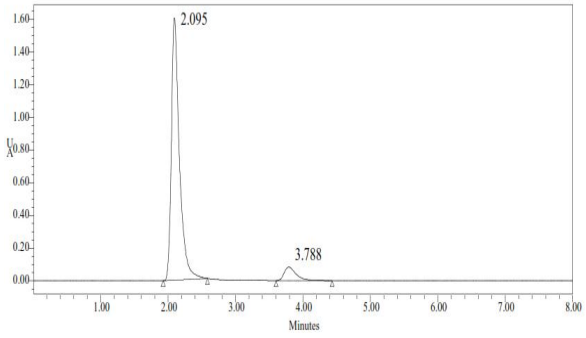
- The % Recovery for each level should be between 98.0 to 102.0%.
- The percentage recovery was found to be within the limit (97-103%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Chromatogram for linearity concentration-100µg/ml of Levonorgestrel & 5 µg/ml of Ethinyl Estradiol



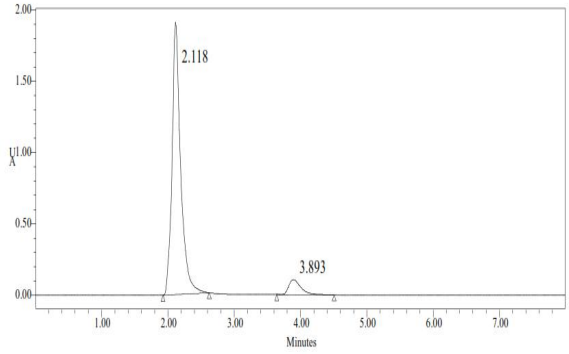
Chromatogram for linearity concentration-200 µg/ml of Levonorgestrel & 10 µg/ml of Ethinyl Estradiol



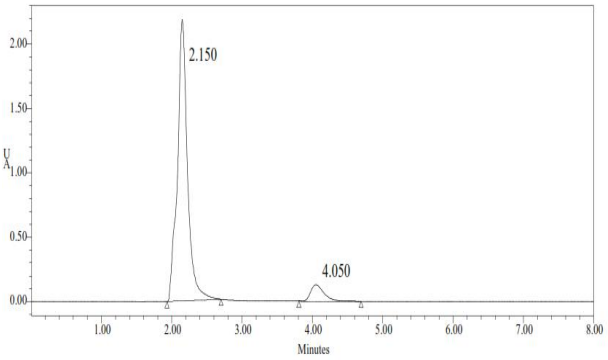
Chromatogram for linearity concentration--300 µg/ml of Levonorgestrel & 15 µg/ml of Ethinyl Estradiol

LINEARITY:

The linearity range was found to lie from 100µg/ml to 500µg/ml of Levonorgestrel, 5µg/ml to 25µg/ml 0f Ethinyl Estradiol and chromatograms are shown below.



Chromatogram for linearity concentration-400ppm of Levonorgestrel & 20ppm of Ethinyl Estradiol



Chromatogram for linearity concentration-500 µg/ml of Levonorgestrel & 25 µg/ml of Ethinyl Estradiol

Area of different concentration of Levonorgestrel

S.No.	Linearity Level	Concentration	Area
1	I	100ppm	668934
2	II	200ppm	956781
3	III	300ppm	1313873
4	IV	400ppm	1563458
5	V	500ppm	1867084
Correlation Coefficient			0.999

4. SUMMARY AND CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Levonorgestrel and Ethinyl Estradiol was done by RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5 m) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.7 ml/min. the linearity range of Levonorgestrel and Ethinyl Estradiol were found to be from 100-500 g/ml of and 1-5 g/ml of Ethinyl Estradiol . Linear regression coefficient was not more than 0.999.

The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Levonorgestrel and Ethinyl Estradiol. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

5.CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

6. ACKNOWLEDGEMENT

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