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Research Article

A VALIDATED SIMULTANEOUS RP-HPLC METHOD FOR DETERMINATION OF DESOGESTREL AND ETHINYL ESTRADIOL TABLETS

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ABSTRACT

A new RP-HPLC method was developed for selective and simultaneous determination of of Desogestrel and Ethinyl Estradiol Tablets. The chromatographic separation was achieved on a Zorbax SB C-18, 4.6 x 250mm, and 5µ column. The gradient LC method employs solutions A and B as mobile phase. The solution A milliQ water and acetonitrile in the ratio of 90:10 and degas and solution B contains a mixture of Milli Q water and acetonitrile in the ratio of 5:95 and degas. The flow rate was 1.0 ml/min and the detection wavelength was 225 nm. In the developed HPLC method, the resolution between Desogestrel, Ethinyl Estradiol and its potential impurities, namely Imp-1, Imp-2 and Imp-3 was found to be greater than 3. The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. Considerable degradation was found to occur in alkaline medium and oxidative stress conditions. Degradation product formed during base hydrolysis was found to be Imp-3. The stress samples were assayed against a qualified reference standard and the mass balance was found close to 99.5%. The developed RP-LC method was validated with respect to linearity, accuracy, precision and robustness.

Keywords: Desogestrel, Ethinyl Estradiol, HPLC, Method validation

INTRODUCTION

Ethinyl estradiol and desogestrel contains a combination of female hormones that prevent ovulation (the release of an egg from an ovary). This medication also causes changes in your cervical mucus and uterine lining, making it harder for sperm to reach the uterus and harder for a fertilized egg to attach to the uterus. Ethinyl estradiol and desogestrel are used as contraception to prevent pregnancy [1-3]. desogestrel and ethinyl estradiol Tablets provide an oral contraceptive regimen tablets each containing 0.15 mg desogestrel (13-ethyl-11-methylene-18, 19-dinor-17 alpha-pregn-4-en-20-yn-17-ol) and 0.03 mg ethinyl estradiol (19-nor-17 alpha-pregna-1, 3, 5 (10)-trien-20-yne-3, 17-diol). Inactive ingredients include vitamin E, corn starch, povidone, stearic acid, colloidal silicon dioxide, lactose, hydroxypropyl methyl-cellulose, polyethylene glycol, titanium dioxide, and talc. The molecular weights for desogestrel and ethinyl estradiol are 310.48 and 296.41, respectively. The structural formulas are as follows fig 1. The combination contains estrogen, ethinyl estradiol, and progesterone, desogestrel which prevent implantation and thus pregnancy. This ethinyl estradiol and desogestrel combination is associated with the same side effects as other combinations are. This specific combination does contain a lower dose of estrogen, ethinyl estradiol, and uses the progesterone, desogestrel, which may be associated with a lower risk of blood clots as with all combination oral contraceptives, broad spectrum antibiotics are able to reduce effectiveness. Smoking while using an oral contraceptive is linked to increase cardiovascular problems the combination has a unique dosing regime - 21 days of combination tablets, 2 days of no active ingredients, then 5 days of ethinyl estradiol. This regime provides a shorter hormone free period and is ideal for people who get migraines, dysmenorrhea, and other symptoms the week they are not taking the oral contraceptive [4-10].

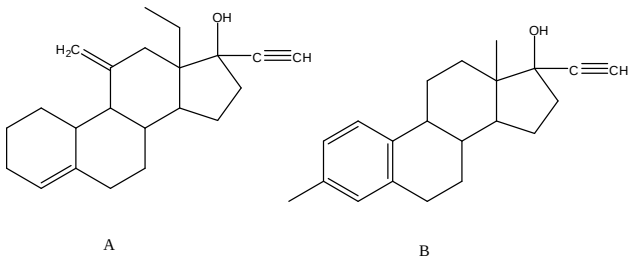

Fig. 1: Chemical structures of (A) Desogestrel (B) Ethinyl Estradiol

MATERIALS AND METHODS

Reagents and Chemicals

The solvents (acetonitrile, methanol and water) were of HPLC grade standard. The Standard samples of Desogestrel and Ethinyl Estradiol were obtained from Glenmark Pharmaceuticals Ltd., Nasik, India.

Chromatographic conditions

Chromatographic system consisted of a Waters Model Alliance 2695 separation module equipped with auto sampler Photodiode array ultraviolet (UV) detector. The data recorded using empower software. A gradient HPLC analytical column; Zorbax SB C-18, 4.6 x 250mm, particle size 5 µm; and detector of UV at 225 nm.

Preparation of standard solution

Weigh accurately 50mg of Ethinyl Estradiol into a 200mL volumetric flask. Add about 120mL of methanol. Sonicate to dissolve and dilute to volume with methanol and mix (EE Stock). Transfer 3mL of this solution to 50mL volumetric flask, dilute to volume with diluent and mix. Weigh accurately 38mg of Desogestrel into a 100mL volumetric flask. Add about 70mL of methanol. Sonicate to dissolve and dilute to volume with methanol and mix (Deso stock). Transfer 3mL of this solution to 25mL volumetric flask, dilute to volume with diluent and mix. Dilute 3mL of Ethinyl Estradiol solution and 5mL of Desogestrel solution to 100mL with diluent and mix.

Identification solution preparation

Separately weigh and transfer about 1.25mg each of Desogestrel Impurity C, Impurity D, and Impurity E into 100mL volumetric flask. Add to it 50mL methanol and sonicate to dissolve it completely and dilute to volume with the methanol and mix.

Separately weigh and transfer about 0.8mg each of Ethinyl Estradiol Impurity B and Impurity C into 100mL volumetric flask. Add to it 50mL methanol and sonicate to dissolve it completely and dilute to volume with the methanol and mix.

Dilute 3mL of each Desogestrel Impurity C, Impurity D, and Impurity E solution, 1mL of each Ethinyl Estradiol Impurity B and Impurity C, 10ml of Deso stock solution and 3mL of EE Stock into 50mL with water. (76ppm of Desogestrel, 15ppm of Ethinyl Estradiol, 0.75ppm of Desogestrel impurities and 0.16ppm of Ethinyl Estradiol impurities).

Test preparation (For 0.15/0.03mg)

Weigh 20 tablets and determine average weight and weigh and transfer 10 tablets to into a 20mL volumetric flask. Add to it 15mL diluent and sonicate for 15 minutes with shaking in cool water. Dilute up to the mark with diluent**.** Weigh 20 tablets and determine average weight and Weigh and transfer 15 tablets to into a 20mL volumetric flask. Add to it 15mL diluent and sonicate for 15 minutes with shaking in cool water. Dilute up to the mark with diluent**.**

Placebo preparation (For 0.15/0.02mg)

Weigh placebo powder accurately equivalent to 0.3mg of Ethinyl Estradiol into a 20mL volumetric flask. Add to it 15mL diluent and sonicate for 15 minutes with shaking in cool water. Dilute up to the mark with diluent. Weigh placebo powder accurately equivalent to 0.3mg of Ethinyl Estradiol into a 20mL volumetric flask. Add to it 15mL diluent and sonicate for 15 minutes with shaking in cool water. Dilute up to the mark with diluent.

Validation of HPLC Method

In order to confirm method suitability during routine quality control use, the proposed method was checked critically for the following validation characteristics as per ICH guidelines.

Linearity

Linearity for Desogestrel and Ethinyl Estradiol was determined in the concentration range 50 to 150 % of working concentration of standard. The peak area responses were plotted against the corresponding concentrations and the r^2 values were calculated.

Precision

System precision: Six replicate injections of mixed standard solution at the concentration of Desogestrel 0.15 mg/ml were injected into HPLC system. Similarly six replicate injections of mixed standard solution at the concentration of Ethinyl Estradiol 0.02 mg/ml were injected into HPLC system The percentage relative standard deviations (% RSD) in each case were calculated.

Method precision Six samples of Desogestrel and Ethinyl Estradiol Tablets were analyzed as per the method. Each named impurity and total impurities were calculated on these replicates.

Intermediate precision or inter-day precision

The intermediate or inter-day precision of the method was determined by six replicate analysis of Desogestrel and Ethinyl Estradiol from sample, as per the proposed method by different instruments and (Waters Alliance 2695 and Shimadzu), by same analyst on different days. The average drug content and the % RSD were calculated in each case.

Accuracy (recovery studies)

Recovery studies were performed by standard addition method at three levels i.e. 50%, 100% and 150%. Known amounts of standard Desogestrel and Ethinyl Estradiol were added to pre-analyzed samples and they were subjected to proposed HPLC method. Results of recovery studies are shown in Table 1.

Stability of analytical solution

A sample solution of Desogestrel and Ethinyl Estradiol Tablets was prepared as per the proposed method. To this sample all known impurities were quantitatively spiked at specification limit concentration and stored at 10°C. The sample was injected into the system initially and at various time intervals. Sample solution spiked with impurities was found to be stable up to 1600 minutes at 10°C.

Robustness (system suitability)

The robustness study was done by making small changes in the optimized method parameters as indicated in Table 2 and 3. Results are also shown there.

Ruggedness

The ruggedness study was done by the two analysts by using the proposed method by same instrument on same day. The % RSD for each analyst for each drug was calculated.

RESULTS AND DISCUSSION

In linearity study, The graphical representation of data proves that Desogestrel and linearity in the range of 0.02 mg to 0.15 mg with r^2 value 0.9999 and Ethinyl Estradiol linearity in the range of 0.02 mg to 0.15 mg with r2 value 0.9997. Linearity of graphs for Desogestrel and Ethinyl Estradiol in figure 2 and 3.

Table 1: Result of recovery studies.

Drugs	Recovery levels	Mean % of recovery	% RSD
Desogestrel	50 %	100.18	0.16
	100 %	101.90	0.15
	150 %	99.23	0.05
Ethinyl Estradiol	50 %	102.00	0.22
	100 %	101.56	0.11
	150 %	102.62	0.15

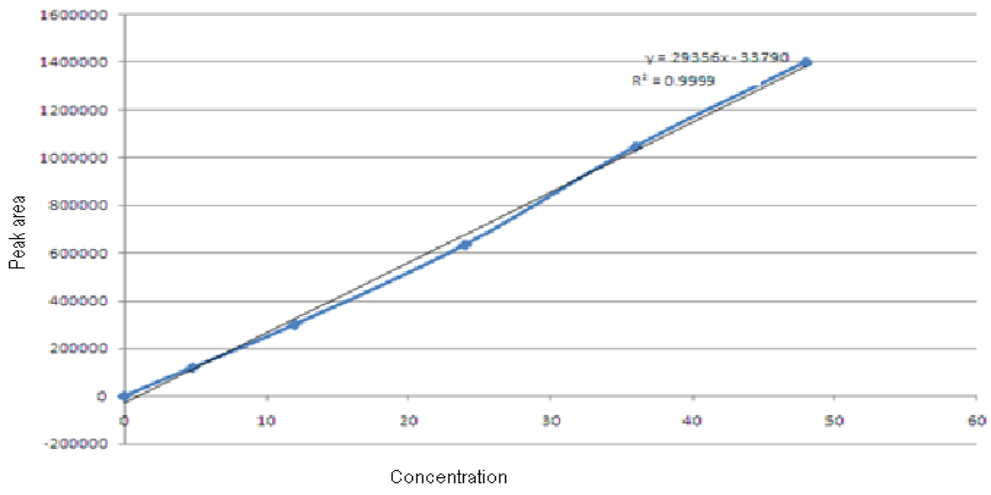

Fig. 2: Linearity of Desogestrel

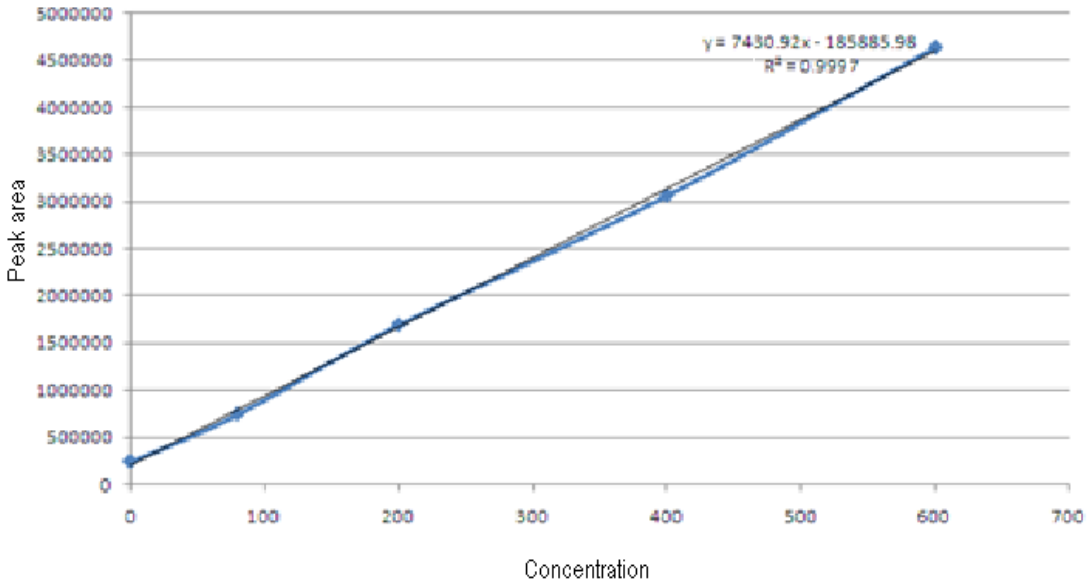

Fig. 3: Linearity of Ethinyl Estradiol

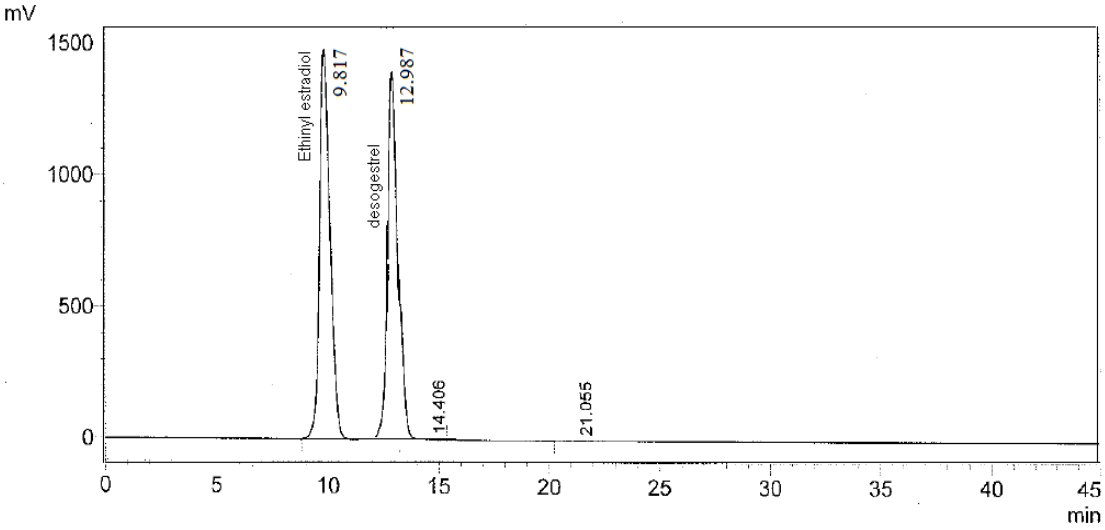

Fig. 4: Chromatographic separation of desogestrel and ethinyl estradiol

In system precision study, the % RSD for Desogestrel and Ethinyl Estradiol were found to be 0.36 and 0.58 respectively. The % RSD observed on the replicate indicates the precision of the system. The chromatography separation of Desogestrel and Ethinyl Estradiol as shown figure 4.

In method precision study, the mean % drug content for Desogestrel and Ethinyl Estradiol were found to be 103.56 % and 103.13 % respectively. The % RSD for Desogestrel and Ethinyl Estradiol were found to be 0.4 and 0.43. The results indicate that the method is validated for method precision. No interference form other components or excipient was found during determination.

In intermediate or inter-day precision study, the mean % drug content for Desogestrel and Ethinyl Estradiol were found to be 103.59 and 102.75 respectively. The % RSD for Desogestrel and Ethinyl Estradiol were found to be 3.04 and 3.8 respectively. There is no significant difference by same analyst by different instruments on different day. Therefore the intermediate or inter-day precision of the method can be considered to be acceptable.

In accuracy or recovery studies, the results are shown in Table 1. The overall % of recovery and % RSD for Desogestrel and Ethinyl Estradiol in marketed formulation indicated that there is no significant difference in percentage of recovery. Therefore, accuracy of the method considered acceptable as it was well within 99 to 102%.

A sample solution of Desogestrel and Ethinyl Estradiol Tablets was prepared as per the proposed method. To this sample all known impurities were quantitatively spiked at specification limit concentration and stored at 10°C. The sample was injected into the system initially and at various time intervals. Sample solution spiked with impurities was found to be stable up to 1600 minutes at 10°C.

In robustness or system suitability study, there was no significant impact on the % RSD and tailing factor. The results of the robustness study also indicated that the method is robust and is unaffected by small variations in the chromatographic conditions.

Table 2: Robustness for Desogestrel.

Parametrers	Theoretical plates	Tailing factor	% RSD of replicates
Wavelength: - 5 nm	297479	1.02	0.10
Wavelength: + 5 nm	298301	1.02	0.09
Column oven temperature: - 5°C	270947	1.01	0.11
Column oven temperature: + 5°C	336620	1.01	0.07
Flow rate: - 10%	278530	0.99	0.06
Flow rate: + 10%	326811	1.01	0.02
Organic composition (Mobile phase –A) - 2% absolute	280767	1.01	0.19
Organic composition (Mobile Phase –A) + 2% absolute	281627	1.01	0.13
Organic composition (Mobile phase –B) - 2% absolute	286735	1.05	0.14
Organic composition (Mobile Phase –B) + 2% absolute	274201	1.00	0.10
Gradient program time (- 2 minutes)	269347	1.01	0.15
Gradient program time (+ 2 minutes)	302145	1.01	0.04

Table 3: Robustness for Ethinyl Estradiol

Parametrers	Theoretical plates	Tailing factor	% RSD of replicates
Wavelength: - 5 nm	17674	1.06	0.10
Wavelength: + 5 nm	18036	1.06	0.04
Column oven temperature: - 5°C	18293	1.07	0.22
Column oven temperature: + 5°C	18181	1.07	0.14
Flow rate: - 10%	18607	1.07	0.09
Flow rate: + 10%	17368	1.07	0.09
Organic composition (Mobile phase –A) - 2% absolute	17946	1.07	0.04
Organic composition (Mobile phase –A) + 2% absolute	17345	1.08	0.16
Organic composition (Mobile phase –B) - 2% absolute	17330	1.06	0.14
Organic composition (Mobile phase –B) + 2% absolute	17514	1.03	0.33
Gradient program time (- 2 minutes)	17804	1.07	0.06
Gradient program time (+ 2 minutes)	17583	1.06	0.25

Degradation studies

An accelerated degradation study was carried out on Desogestrel and Ethinyl Estradiol Tablets according to the following conditions.

Hydrolytic and Oxidative degradation

Acid degradation

For 0.15/0.02 mg

Weigh 15 tablets of Desogestrel and Ethinyl Estradiol Tablets into a 20mL volumetric flask, add about 10mL diluent, sonicate for about 15 minutes in cool water then add 2mL, 5N HCl solution and heat in a water bath for 15 minutes at 60°C. Keep at room temperature for 15 minutes. After specified time neutralize the solution by adding 2mL, 5N NaOH solution. Cool the solution at room temperature and further analysed as per the methodology.

For 0.15/0.03 mg

Weigh 10 tablets of Desogestrel and Ethinyl Estradiol Tablets into a 20mL volumetric flask, add about 10mL diluent, Sonicate for about 15 minutes in cool water then add 2mL, 5N HCl solution and heat in a water bath for 15 minutes at 60°C. Keep at room temperature for 15 minutes. After specified time neutralize the solution by adding 2mL, 5N NaOH solution. Cool the solution at room temperature and further analysed as per the methodology. An equivalent amount of placebo was treated in the similar conditions mentioned above and analysed as per the proposed method.

Alkali degradation

For 0.15/0.02 mg

Weigh 15 tablets of Desogestrel and Ethinyl Estradiol Tablets into a 20mL volumetric flask, add about 10mL diluent, sonicate for about 15 minutes in cool water then add 2mL, 5N NaoH solution and heat in a water bath for 2 hours minutes at 60°C. Keep at room temperature for 15 minutes. After specified time neutralize the solution by adding 2mL, 5N HCl solution. Cool the solution at room temperature and further analysed as per the methodology.

For 0.15/0.03 mg

Weigh 10 tablets of Desogestrel and Ethinyl Estradiol Tablets into a 20mL volumetric flask, add about 10mL diluent, Sonicate for about 15 minutes in cool water then add 2mL, 5N NaoH solution and heat in a water bath for 2 hours at 60°C. Keep at room temperature for 15 minutes. After specified time neutralize the solution by adding 2mL, 5N HCl solution. Cool the solution at room temperature and further analysed as per the methodology. An equivalent amount of placebo was treated in the similar conditions mentioned above and analysed as per the proposed method.

Here alkaline oxidative stress condition one degraded product formed as given figure 5.

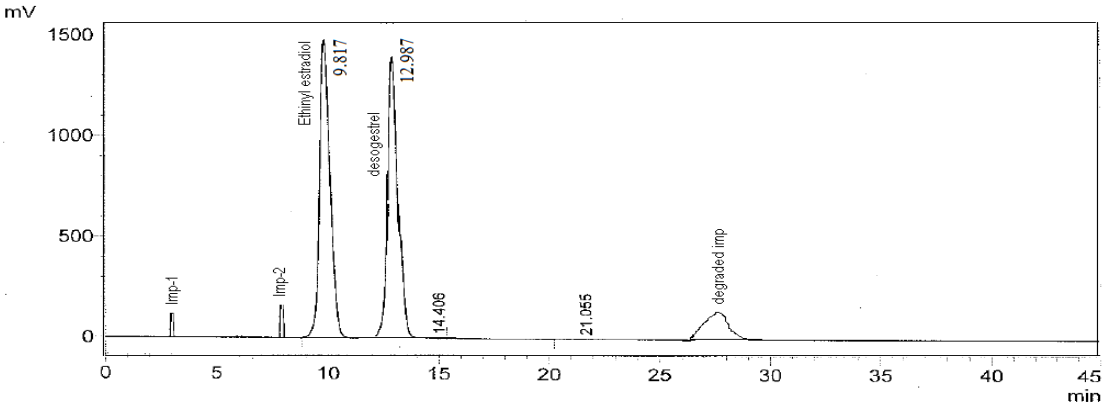

Fig. 5: Degradation product at oxidative stress condition

Peroxide degradation

For 0.15/0.03mg

Weigh 15 tablets of Desogestrel and Ethinyl Estradiol Tablets into a 20mL volumetric flask, add about 10mL diluent, sonicate for about 15 minutes in cool water then add 1mL, 30 % peroxide solution and heat in a water bath for 2 hours at 60°C. Keep at room temperature for 15 minutes. Cool the solution at room temperature and further analysed as per the methodology.

For 0.15/0.03mg

Weigh 15 tablets of Desogestrel and Ethinyl Estradiol Tablets into a 20mL volumetric flask, add about 10mL diluent, sonicate for about 15 minutes in cool water then add 1mL, 30 % peroxide solution and heat in a water bath for 2 hours at 60°C. Keep at room temperature for 15 minutes. Cool the solution at room temperature and further analysed as per the methodology. An equivalent amount of placebo was treated in the similar conditions mentioned above and analysed as per the proposed method.

Thermal degradation

Desogestrel and Ethinyl Estradiol Tablets was spread in a petri dish and kept in an oven at 60°C. The sample after exposure for 7 and 21 days was removed and analysed as per the methodology. An equivalent amount of placebo was treated in a similar manner in the condition mentioned above and analysed as per the methodology.

Humidity degradation

Desogestrel and Ethinyl Estradiol Tablets was spread in a petri dish and kept in a humidity chamber of 40°C/75%RH. The sample after exposure for 7 and 21 days was removed from the chamber and analysed as per the methodology. An equivalent amount of placebo was treated in a similar manner in the condition mentioned above and analysed as per methodology.

Photolytic degradation

Desogestrel and Ethinyl Estradiol Tablets was spread in a petri dish and kept in Sun test instrument chamber to achieve light intensity 1.2 million lux, removed from the chamber and analysed as per the methodology. An equivalent amount of placebo was treated in a similar manner in the condition mentioned above and analysed as per methodology. Using peak purity test, the purity of Desogestrel, Ethinyl Estradiol and known impurity peaks were checked at every stage of the above study.

CONCLUSION

Hence, it can be concluded that the newly developed RP-HPLC method was found to be simple, rapid, cost-effective, linear, accurate, precise and robust over the specified range; and selective for Desogestrel, Ethinyl Estradiol without any interference from other components or additives. This method can be employed conveniently, reliably and successfully for the estimation of Desogestrel, Ethinyl Estradiol for routine quality control and stability studies.

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