CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.22321315

Available online at: http://www.iajps.com Research Article

DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF DESOGESTREL AND ETHINYL ESTRADIOL IN BULK AND COMBINED FORM OF PHARMACEUTICAL TABLET DOSAGE FORMS BY USING RP-HPLC ANALYTICAL METHOD

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Abstract:

A novel, simple, specific, accurate, precise method development and validated for the simultaneous estimation of Desogestrel and Ethinyl Estradiol by RP-HPLC in bulk and marketed formulation. A High Performance Liquid Chromatography WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA Detector with Phenomenex Luna C18, 100A, 5µm, 250mmx4.6mm i.d. column, with mobile phase composition of Acetonitrile and Buffer pH-3.4 with OPA were taken in the ratio of 55:45% v/v/v was used. The flow rate of 1.0 ml min-1 and effluent was detected at 330 nm. The retention times of Desogestrel and Ethinyl Estradiol was 2.264 and 3.129 minutes. Linearity was observed over concentration range of 6-14µg/ml and 10µg/ml-30µg/ml for Desogestrel and Ethinyl Estradiol respectively. The Limit of detection and limit of quantification of Desogestrel and Ethinyl Estradiol was found to be 0.09ng ml-1 and 0.29ngml-1 & 0.1ng ml-1 and 0.3ngml-1 respectively. The accuracy of the proposed method was determined by recovery studies and found to be 98% to 102%. Then method was validated in terms of linearity, accuracy, precision, (repeatability, intermediate precision) specificity (by assay), robustness and system suitability. Thus, the validated method is can be successfully applied to routine analysis for regulate the quality. It also should be used for analytical research purpose.

Keywords: *Desogestrel and Ethinyl Estradiol, RP-HPLC, Method Development, Validation.*

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Please cite this article in press ***G. Vijaya Kumar*** *et al.,* ***Development And Validation For The Simultaneous Determination Of Desogestrel And Ethinyl Estradiol In Bulk And Combined Form Of Pharmaceutical Tablet Dosage Forms By Using Rp-Hplc Analytical Method.,****. Indo Am. J. P. Sci, 2026; 13(09).*

INTRODUCTION:

Desogestrel is a synthetic progestin used in contraception, often in combination with Ethinyl Estradiol. Oral Desogestrel is used in combination with Ethinylestradiol as a contraceptive agent for the prevention of pregnancy[1]. Desogestrel is part of the combined oral contraceptives that contain a mix of estrogen and progestin which inhibit ovulation. Desogestrel is primarily used as a hormonal contraceptive to prevent pregnancy. It works by preventing ovulation, inhibiting fertilization, and potentially altering the uterine lining[2]. Additionally, Desogestrel has secondary uses in managing endometriosis-associated pain, dysmenorrhea (painful periods), and premenstrual dysphoric disorder (PMDD). The IUPAC name of Desogestrel is (8S, 9S, 10R, 13S, 14S, 17R)-13-ethyl-17-ethynyl-11-methylidene-1, 2, 3, 6, 7, 8, 9, 10, 12, 14, 15, 16-dodecahydrocyclopenta [a] phenanthren-17-ol[3]. The Chemical Structure of Desogestrel is shown in following figure-1.

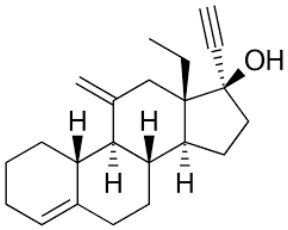

Fig-1: Chemical Structure of Desogestrel

Ethinylestradiol is an estradiol used as a contraceptive. Ethinylestradiol was first synthesized in 1938 by Hans Herloff Inhoffen and Walter Hohlweg at Schering. It was developed in an effort to create an estrogen with greater oral bioavailability[4]. These properties were achieved by the substitution of an Ethinyl group at carbon 17 of estradiol. Ethinylestradiol soon replaced mestranol in contraceptive pills. Ethinylestradiol is combined with other drugs for use as a contraceptive, premenstrual dysphoric disorder, moderate acne, moderate to severe vasomotor symptoms of menopause, prevention of postmenopausal osteoporosis[5]. Ethinylestradiol is a synthetic estrogen that decreases luteinizing hormone to decrease endometrial vascularization, and decreases gonadotrophic hormone to prevent ovulation. It has a long duration of action as it is taken once daily and a wide therapeutic index as overdoses are generally not associated with serious adverse effects[6]. Patients should be counselled regarding the risks of thrombotic events. The IUPAC Name of Ethinylestradiol is (8R, 9S, 13S, 14S, 17R)-17-ethynyl-13-methyl-7, 8, 9, 11, 12, 14, 15, 16-octahydro-6H-cyclopenta [a] phenanthrene-3, 17-diol. The Chemical Structure of Ethinylestradiol is shown in follows

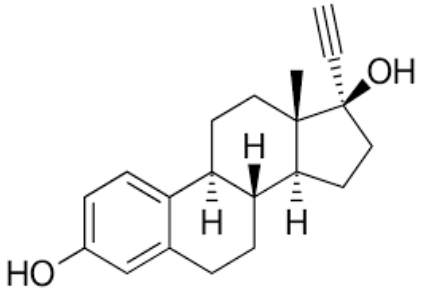

Fig-2: Chemical Structure of Ethinylestradiol

MATERIALS AND METHODS:

Table-1: Instruments Used

S.No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Table-2: Chemicals Used

S.No.	Chemical	Brand Names
1	Desogestrel	Synpharma Research Lab, Hyderabad
2	Ethinyl Estradiol	Synpharma Research Lab, Hyderabad
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Desogestrel and Ethinyl Estradiol working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1ml of the above Desogestrel and 0.2ml of the Ethinyl Estradiol stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines[35].

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: Phosphate Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Phosphate Buffer in proportion 55:45% (pH-3.4) v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, Symmetry and Zodiac column. Phenomenex Luna C18, 100A, 5µm, 250mmx4.6mm i.d. Column was found to be ideal as it gave good peak shape and resolution at 1.0ml/min flow[7].

Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-3.4):

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.4 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication[8].

Preparation of Mobile Phase:

Accurately measured 550 ml (55%) of Acetonitrile, 450 ml of Phosphate buffer (45%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

Method Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Desogestrel and 10mg of Ethinyl Estradiol working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Desogestrel and 0.2ml of the Ethinyl Estradiol stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent[9-12].

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity:

Preparation of Standard Solution:

Accurately weigh and transfer 10mg of Desogestrel and 10mg of Ethinyl Estradiol working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Desogestrel and 0.2ml of the Ethinyl Estradiol stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution:

Take average weight of one Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Desogestrel and Ethinyl Estradiol sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.1ml of the above Desogestrel and 0.2ml of the Ethinyl Estradiol stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The mean and percentage relative standard deviation were calculated from the peak areas[13].

Procedure:

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity:
Accurately weigh and transfer 10 mg of Desogestrel and 10mg of Ethinyl Estradiol working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent[14]. (Stock solution)

Preparation of Level – I (6ppm of Desogestrel & 10ppm of Ethinyl Estradiol):
Pipette out 0.06ml of Desogestrel and 0.1ml of Ethinyl Estradiol stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (8ppm of Desogestrel & 15ppm of Ethinyl Estradiol):
Pipette out 0.08ml of Desogestrel and 0.15ml of Ethinyl Estradiol stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (10ppm of Desogestrel & 20ppm of Ethinyl Estradiol):
Pipette out 0.1ml of Desogestrel and 0.2ml of Ethinyl Estradiol stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent[15-17].

Preparation of Level – IV (12ppm of Desogestrel & 25ppm of Ethinyl Estradiol):
Pipette out 0.12ml of Desogestrel and 0.25ml of Ethinyl Estradiol stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (14ppm of Desogestrel & 30ppm of Ethinyl Estradiol):
Pipette out 0.14ml of Desogestrel and 0.30ml of Ethinyl Estradiol stock solutions was take in a 10ml of volumetric flask 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[18-20].

Precision

Repeatability

Preparation of Desogestrel and Ethinyl Estradiol Product Solution for Precision:
Accurately weigh and transfer 10 mg of Desogestrel and 10mg of Ethinyl Estradiol working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.1ml of the above Desogestrel and 0.2ml of the Ethinyl Estradiol stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits[21].

Intermediate Precision:
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions[22].

Procedure:

Day 1:
The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

Day 2:
The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

Accuracy:

For preparation of 80% Standard stock solution:
Accurately weigh and transfer 10 mg of Desogestrel and 10mg of Ethinyl Estradiol working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.08ml of the above Desogestrel and 0.16ml of the Ethinyl Estradiol stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 100% Standard stock solution:
Accurately weigh and transfer 10 mg of Desogestrel and 10mg of Ethinyl Estradiol working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.1ml of the above Desogestrel and 0.2ml of the Ethinyl Estradiol stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 150% Standard stock solution:
Accurately weigh and transfer 10 mg of Desogestrel and 10mg of Ethinyl Estradiol working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.12ml of Desogestrel and 0.24ml of Ethinyl Estradiol from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:
Inject the Three replicate injections of individual concentrations (50%, 100% and 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses.

Calculate the Amount found and Amount added for Desogestrel and Ethinyl Estradiol and calculate the individual recovery and mean recovery values[23-25].

Robustness:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Desogestrel and 10mg of Ethinyl Estradiol working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Desogestrel and 0.2ml of the Ethinyl Estradiol stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of Flow Conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition:

The sample was analyzed by variation of mobile phase i.e. Acetonitrile: Phosphate Buffer was taken in the ratio and 60:40, 50:50 instead (55:45), remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded[26-28].

RESULTS AND DISCUSSION

Method Development:

Optimization of Method: The optimized chromatographic conditions were established after evaluating different chromatographic parameters to obtain satisfactory separation, peak symmetry, and reproducible results. Chromatographic separation was achieved using a Phenomenex Luna C18 column (250 mm × 4.6 mm i.d., 5 µm particle size, 100 Å pore size). The mobile phase consisted of Acetonitrile and buffer adjusted to pH 3.4 with orthophosphoric acid (OPA) in the ratio of 55:45 (v/v), which provided optimal peak shape and resolution. The mobile phase was delivered at a flow rate of 1.0 mL/min, and the analyte was detected at a wavelength of 330 nm using a UV/PDA detector. The injection volume was 20 µL, with both the column temperature and autosampler temperature maintained at ambient conditions. The total run time was 6 minutes, allowing rapid and efficient chromatographic analysis with satisfactory system suitability parameters.

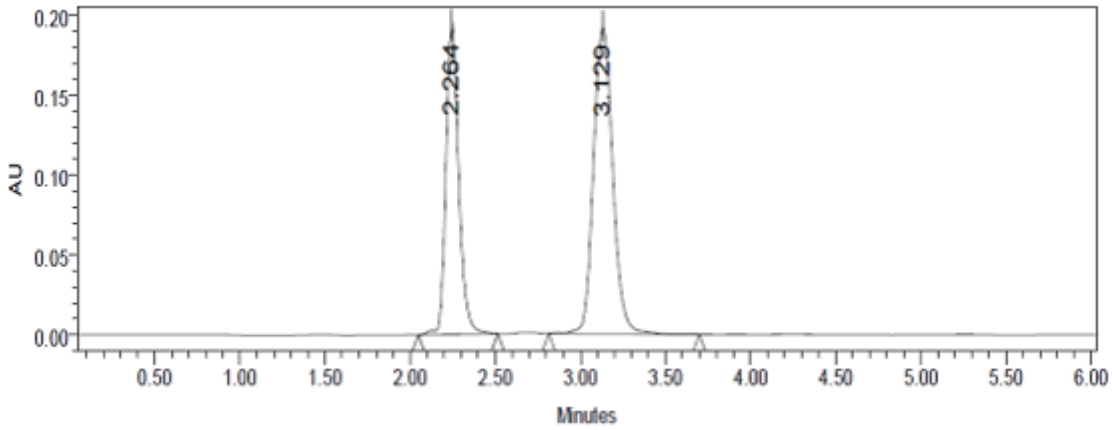

Fig-3: Optimized Chromatographic Condition

Method Validation:

1. Accuracy: The accuracy of the developed RP-HPLC method was evaluated by the recovery study using the standard addition technique at three concentration levels (typically 80%, 100%, and 120% of the target concentration). Each level was analyzed in triplicate, and the percentage recovery along with %RSD was calculated to assess the closeness of the measured values to the true value.

Recovery Study: Accuracy: For 80%

Table-3: Data of Recovery Studies for Desogestrel

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	8	8.039	348673	100.487	Mean= 100.633%
S_2 : 80 %	8	8.046	348945	100.575	S.D. = 0.182066
S_3 : 80 %	8	8.067	349745	100.837	% R.S.D.= 0.180921

Table-4: Data of Recovery Studies for Ethinyl Estradiol

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	16	15.991	989572	99.943	Mean= 100.1577%
S_2 : 80 %	16	16.143	998756	100.893	S.D. = 0.654939
S_3 : 80 %	16	15.942	986589	99.637	% R.S.D.= 0.653908

For 100%

Table-5: Data of Recovery Studies for Desogestrel

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 100 %	10	9.862	419823	98.62	Mean= 99.95%
S_2 : 100 %	10	9.993	424941	99.93	S.D. = 1.340112%
S_3 : 100 %	10	10.130	430295	101.3	R.S.D.= 1.340782

Table-6: Data of Recovery Studies for Ethinyl Estradiol

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 100 %	20	19.995	1231734	99.975	Mean= 100.795%
S_2 : 100 %	20	20.158	1241569	100.79	S.D. = 0.822511%
S_3 : 100 %	20	20.325	1251694	101.62	R.S.D.= 0.816024

FOR 120%

Table-7: Data of Recovery Studies for Desogestrel

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 120 %	12	12.115	507788	100.958	Mean= 100.9717%
S_2 : 120 %	12	12.179	510262	101.491	S.D. = 0.512637
S_3 : 120 %	12	12.056	505468	100.466	% R.S.D.= 0.507703

Table-8: Data of Recovery Studies for Ethinyl Estradiol

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 120 %	24	24.335	1494218	101.395	Mean= 100.805%
S_2 : 120 %	24	24.204	1486312	100.85	S.D. = 0.613739
S_3 : 120 %	24	24.041	1476398	100.170	% R.S.D.= 0.608837

Observation: The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

2. Precision

The precision of the analytical developed method was studied by analysis of multiple sampling of homogeneous (same) sample. The precision expressed as standard deviation (SD) or relative standard deviation (% RSD). The precision of the method can be analyzed by the intermediate precision. It includes the intra-day and inter-day variation[29].

2.1. Repeatability

The precision of each method was achieved separately from the peak areas obtained by actual estimation of 5 injections of fixed homogenous sample concentrations of Desogestrel & Ethinyl Estradiol. The % relative standard deviation for the Desogestrel and Ethinyl Estradiol was calculated.

Table-9: Results of Repeatability for Desogestrel and Ethinyl Estradiol

Concentration of Deso + Ethinyl in ppm	Rt of Deso	Peak area of Deso	Rt of Ethinyl	Peak area of Ethinyl
10 +10	2.264	3303800	3.132	951802
10 +10	2.246	3349883	3.132	958267
10 +10	2.264	3353514	3.129	954481
10 +10	2.246	3384162	3.113	952151
10 +10	2.280	3390496	3.113	952308
AVG	**2.26**	**3356371**	**3.1238**	**953801.8**
S.D.	**0.014353**	**34463.10324**	**0.009935**	**2709.017**
% RSD	**0.635075**	**1.026796598**	**312.38**	**0.284023**

Observation: The repeatability study which was conducted on the solution having the concentration of about 10 µg/ml for Desogestrel and 20 µg/ml for Ethinyl Estradiol (n =5) showed a RSD of 1.026796598 % for Desogestrel and 0.284023 % for Ethinyl Estradiol. It was concluded that the analytical technique showed good repeatability.

2.2. Intermediate Precision:

2.2.1. Intra-Assay & Inter-Assay: The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Desogestrel & Ethinyl Estradiol revealed that the proposed method is precise[30].

Table-10: Results of Intra-Assay & Inter-Assay

Conc. of Desogestrel (API) (µg/ml)	Observed Conc. of Desogestrel (µg/ml) by the Proposed Method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	8.09	0.97	8.03	0.96
10	10.05	0.45	10.04	0.47
12	11.98	0.37	11.90	0.12

Table-11: Data for Ethinyl Estradiol Intra-Assay & Inter-Assay Analysis

Conc. of Ethinyl Estradiol (API) (µg/ml)	Observed Conc. of Ethinyl Estradiol (µg/ml) by the Proposed Method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	7.97	0.27	8.09	0.59
10	10.14	1.29	9.95	0.64
12	12.08	0.61	11.94	0.26

Observation: The Intraday and interday related studies shows that the % RSD was found to be within limit i.e. (≤2%). So it is indicated that the developed is within the limits. Hence finally we concluded that the developed method was found to be precise.

3. Linearity and Range

Linearity range was found to be 0-14 µg/ml for Desogestrel and 0-30 µg/ml for Ethinyl Estradiol. The correlation coefficients were found to be 0.999 & 0.999, the slopes were found to be 39036 & 60481 and intercept were found to be 34828 & 22371 for Desogestrel and Ethinyl Estradiol respectively[31].

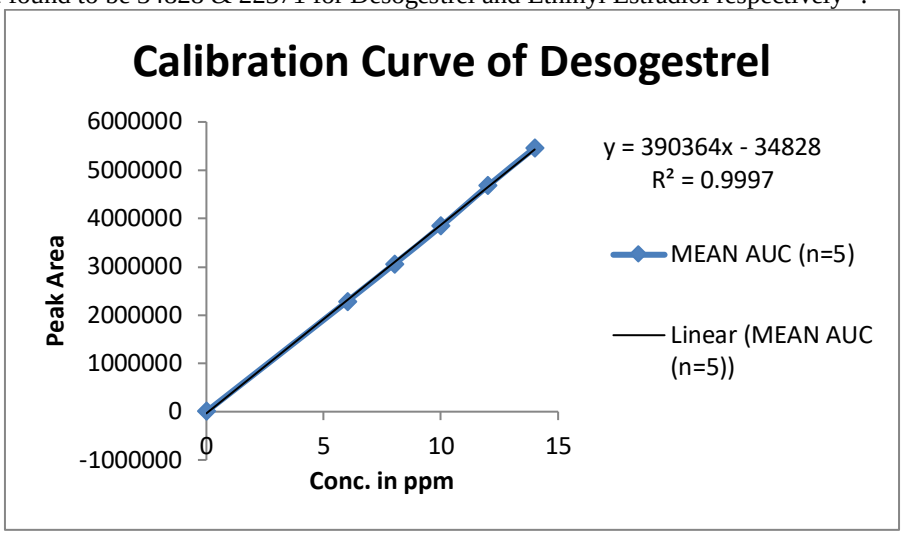

Fig-4: Standard Curve for Desogestrel

Table-12: Standard Curve for Desogestrel

CONC.(µg/ml)	MEAN AUC (n=5)
0	0
6	2281962
8	3053421
10	3837632
12	4673649
14	5462556

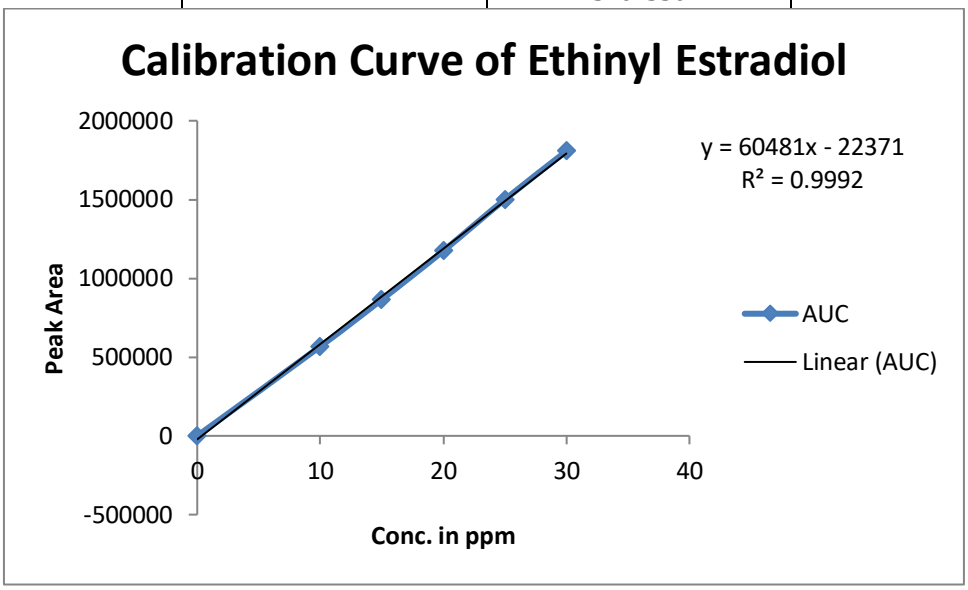

Fig-5: Standard Curve for Ethinyl Estradiol

Table-13: Standard Curve for Ethinyl Estradiol

CONC.	AUC
0	0
10	567458
15	865310
20	1174123
25	1500209
30	1806775

4. Method Robustness: Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-14, % RSD < 2%) the developed RP-HPLC method for the analysis of Ethinyl Estradiol (API)[32].

Table-14: Result of Method Robustness Test

Change in Parameter	% RSD
Flow (1.1 ml/min)	1.05
Flow (0.9 ml/min)	0.67
Temperature (27^{0}C)	0.58
Temperature (23^{0}C)	0.61
Wavelength of Detection (332 nm)	0.38
Wavelength of detection (328 nm)	0.17

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-15, % RSD <2%) the developed RP-HPLC method for the analysis of Ethinyl Estradiol (API).

Table-15: Result of Method Robustness Test

Change in Parameter	% RSD
Flow (1.1 ml/min)	0.09
Flow (0.9 ml/min)	0.07
Temperature (27^{0}C)	0.28
Temperature (23^{0}C)	0.74
Wavelength of Detection (332 nm)	0.86
Wavelength of detection (328 nm)	0.67

5. Limit of Detection (LOD) & Limit of Quantification (LOQ):

The detection limit (LOD) and quantization limit (LOQ) may be expressed as:

$$L.O.D. = 3.3 (SD/S).$$

$$L.O.Q. = 10 (SD/S)$$

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Observation: The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.09 & 0.29 µg/ml respectively for Desogestrel.

The LOD was found to be 0.1 µg/ml and LOQ was found to be 0.3 µg/ml for Ethinyl Estradiol which represents that sensitivity of the method is high[33].

System Suitability Parameter

It is an integral part of so many analytical procedures. The parameters are based on the idea that the equipment, electronics, analytical operations and the samples to be analyzed constitute as an integral system which can be examined. Finally system suitability test parameters are established. The obtained data is shown in the following table-16.

Table-16: Data of System Suitability Parameter

S. No.	Parameter	Desogestrel	Ethinyl Estradiol
1	Retention time	2.264	3.129
2	Theoretical plates	3154	3635
3	Tailing factor	1.02	1.25
4	Area	3061716	925851
5	Resolution	6.9	

The system suitability Parameters were found to be within the specified limits for the proposed method[34].

6. Assay of Desogestrel & Ethinyl Estradiol in Dosage Form:

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Finally the weighed tablets are powdered and triturated well by using mortar and pestle. A quantity of powder which is equivalent to the 100mg of drugs were transferred to a clean and dry 100ml of volumetric flask and add 70 ml of mobile phase and the resulted solution was sonicated for 15 minutes by using ultra sonicator, Then the final volume was make upto the mark with the mobile phase. The final solution was filtered through a selected membrane filter (0.45 µm) and in order to sonicate to degas the mobile phase (Solvent system). From this above stock solution (1 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system (Mobile phase).

The prepared solutions were injected in five replicates into the HPLC system and the observations were recorded.

A duplicate injection (Blank Solution) of the standard solution also injected into the HPLC system and the chromatograms and peak areas were recorded and calculated. The obtained data are shown in Table 17.

ASSAY:

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Average weight} = \text{mg/tab}$$

Where:

AT = Test Preparation Peak Area
AS = Standard preparation Peak Area
WS = Working standard weight taken in mg
WT = Sample weight taken in mg
DS = Standard solution dilution
DT = Sample solution dilution
P = Working standard percentage purity

The assay was performed as explained in the previous chapter (Above). The results which are obtained are following:

Table-17: Assay of DESOGESTREL & ETHINYL ESTRADIOL Tablets

Brand name of Tablets	Labelled amount of Drug (mg) Desogestrel/Ethinyl Estradiol	Mean (±SD) amount (mg) found by the Proposed Method (n=6)	Mean (± SD) Assay (n = 6)
Intimacy Plus 2 Tablet from Aristo Pharmaceuticals Pvt Ltd	0.15/0.02	0.1385 (± 0.368)/0.0168 (± 0.564)	99.685 (± 0.354) /99.748 (± 0.498)

STABILITY STUDIES

This is one type of accelerated stability studies that helps us determining the fate of the drug that is likely to happen after long time storage, within a very short time as compare to the real time or long term stability testing. The various degradation pathways studied are acid hydrolysis, basic hydrolysis, thermal degradation, photolytic degradation and oxidative degradation.

Results of Degradation Studies: The results of the stress studies indicated the **Specificity** of the method that has been developed. Desogestrel and Ethinyl Estradiol were stable only in photolytic stress conditions and little bit in thermal stress conditions. The results of forced degradation studies are given in the following Table-18.

Table-18: Results of Forced Degradation Studies of Desogestrel and Ethinyl Estradiol API.

Stress condition	Time (hours)	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	76.52	23.48	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	79.37	20.63	100.00
Thermal Degradation (50 ^{0}C)	24Hrs.	90.41	9.59	100.00
UV (254nm)	24Hrs.	99.21	0.79	100.00
3% Hydrogen peroxide	24Hrs.	81.62	18.38	100.00

SUMMARY AND CONCLUSION:

The analytical method was developed by studying different parameters. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Phenomenex Luna C18, 100A, 5µm, 250mmx4.6mm i.d. because it was giving good peak. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Acetonitrile and Buffer pH-3.4 with OPA was taken in the ratio of 55:45% v/v/v was fixed due to good symmetrical peak. So, this mobile phase was used for the proposed study. Methanol and water were selected because of maximum extraction sonication time was fixed to be 15min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 6.0min because analyze gave peak around 2.264min and 3.129min and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision were found to be accurate and well within range. The analytical method was found linearity over the range of 6-14µg/ml of the Desogestrel and 10-30µg/ml of the Ethinyl Estradiol target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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