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Analytical Method Development and Validation for the Simultaneous Estimation of Drospirenone and Estetrol In Tablet and In Bulk Dosage Forms By RP-HPLC Technique

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ABSTRACT

A straightforward and accurate method was devised for simultaneously determining Drospirenone and Estetrol in tablet dosage form. The chromatogram was generated using a Kromosil C18 column (150 x 4.6 mm, 5.0 mm), with a mobile phase consisting of Buffer 0.01N KH2PO4: Methanol in an 80:20 ratio, flowing through the column at a rate of 1.0 ml/min. This method utilized OPA as the buffer, and the temperature was maintained at 30°C. The optimized wavelength for detection was set at 263.0 nm. The retention times for Drospirenone and Estetrol were determined to be 2.597 min and 2.1336 min, respectively, with %RSD values of 0.4 for both compounds. The %Recovery was calculated as 100.25% for Drospirenone and 100.09% for Estetrol. The LOD and LOQ values were obtained from the regression equations of Drospirenone and Estetrol, resulting in 0.01 and 0.02 for LOD, and 0.08 and 0.25 for LOQ, respectively. The regression equations for Drospirenone were found to be y = 361228x + 6065.6, and for Estetrol, y = 341114x + 46689. The method exhibited reduced retention times and overall run time, indicating its simplicity and cost-effectiveness. This makes it suitable for routine quality control testing in various industries.

Keywords: Drospirenone, Estetrol, RP-HPLC, Validation.

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INTRODUCTION

Drospirenone, a progestin, is utilized in oral contraceptive pills to prevent pregnancy and address other medical conditions. It functions as an analog of the diuretic spironolactone, exhibiting anti-mineralocorticoid activity by blocking aldosterone receptors, thereby enhancing sodium and water excretion. Drospirenone effectively inhibits the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), thereby preventing ovulation [1]. Estetrol, a synthetic estrogen, is employed in conjunction with drospirenone for oral contraception. It serves as a synthetic analogue of a naturally occurring estrogen observed during pregnancy, exhibiting specificity for both estrogen receptor-α (ER-α) and ER-β, effectively suppressing ovulation [2].NEXTSTELLIS [3] is a combination of drospirenone, a progestin, and estetrol, an estrogen, indicated for use by females of reproductive potential to prevent pregnancy

From the literature survey, it was found that determination of Estetrol and Drosperinone were estimated by analytical methods such as Spectrophotometry methods by simultaneous determination of Estetrol in combination with Drosperinone [4], by UPLC technique [5] Reverse-Phase HPLC method for quantitative determination of Drospirenone and Ethinylestradiol in tablet dosage form [6] **,** drospirenone and estetrol in tablet dosage form[7,8] **.**Simple, rapid spectrophotometric, and reverse-phase high-performance liquid chromatographic method for the concurrent analysis of 17-beta-estradiol (ESR) and drospirenone [9]

MATERIALS AND METHOD

Materials and reagents:

Drug samples were taken from Mayne Pharma. For the study, we obtained Estatrol and Drospirenone pure drugs (API), Combination Estetrol and Drospirenone(Nextstellis) tablets, Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen orthophosphate buffer, Ortho-phosphoric acid from Mayne Pharma [10]

INSTRUMENTATION:

Chromatography was performed on a WATERS 2695 HPLC column (waters corporation, Mildord, USA) [11]with an autosampler and Photo Diode Array detector with a spectral bandwidth of 2mm and 10mm. Components were detected using UV and that processing was achieved by Empower 2 software. A Ultrasonicator-BVK enterprises was used for thermal degradation of the samples and For measuring absorbances of Estetrol and Drospirenone solutions, a UV-VIS spectrophotometer (PG Instruments T60) with special bandwidths of 2mm and 10mm, along with matched quartz cells and Electronics Balance from Denver were used. This instrument was integrated with UV Win 6 Software.

CHROMATOGRAPHY CONDITIONS:

The chromatographic separation was performed on Kromosil C18 150 x 4.6 mm, 5.0mm. particle size) at an ambient column temperature. The samples were eluted using Buffer 0.01N Kh2Po4: Methanol was taken in the ratio 80:20 as the mobile phase at a flow rate of 1ml/min the mobile phase and Temperature was maintained at 30°C. The optimized wavelength selected was 263 nm. The buffer used in this method was OPA.

PREPARATION OF MOBILE PHASE:

0.01N Kh2PO4 Buffer: A 1.36-gram sample of KH2PO4 is dissolved in a 1000-milliliter volumetric flask. Approximately 900 milliliters of milli-Q water is added to the flask, and the solution is degassed to remove any dissolved air. The pH of the solution is then adjusted to 4.8 using a weak acid or base.

Diluent: Methanol and buffer (50:50)

PREPARATION OF STANDARD SOLUTION:

100% Solution: 1 ml of the solution was transferred to a 10 ml measuring vessel using a pipette and then adjusted to the desired concentration with an appropriate diluent.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions:

In this optimized method, Drospirenone and Estetrol were eluted with good resolution. The plate count and tailing factor were very satisfactory. An optimized chromatogram is given below in figure. Upon conducting an HPLC Chromatography scan, it was noted that Estetrol/Drospirenone displayed absorption maxima at 263 nm

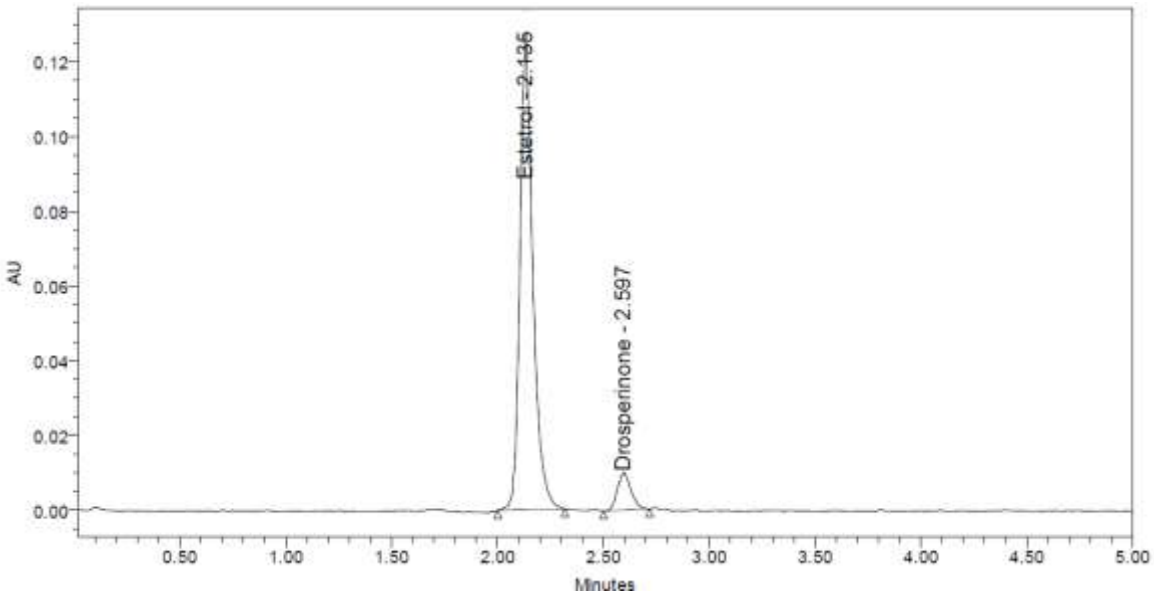
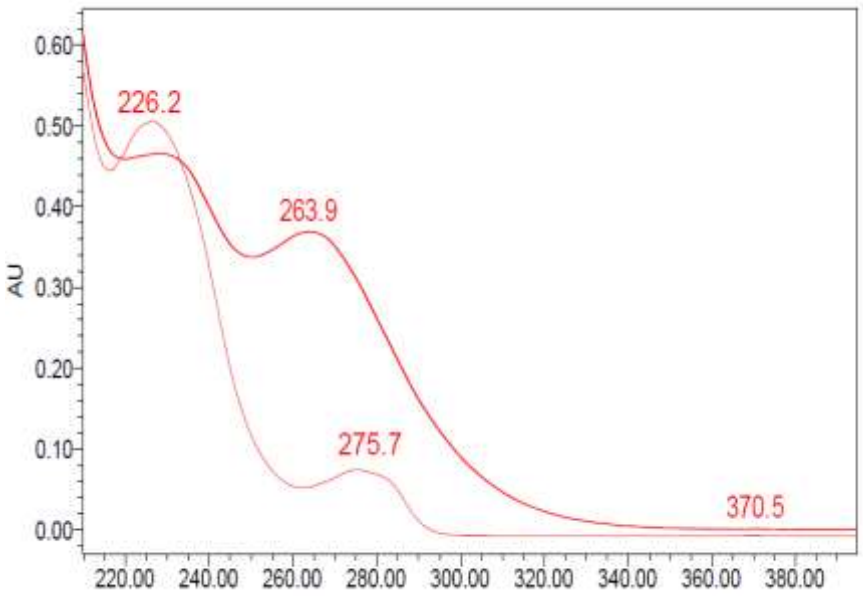


Figure 1: Optimized Chromatogram of Estetrol and Drospirenone



Validation of Method Developed:

The proposed method was validated according to the ICH guidelines for system suitability, specificity, recovery, precision, linearity, robustness, limit of detection (LOD) and limit of quantification (LOQ). Under the validation study, the following parameters were studied

System suitability test: The system suitability parameters were determined by preparing standard solutions of Estetrol (28.4ppm) and Drospirenone (6ppm) and the solutions were injected six times and parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

Specificity: The specificity of the method was carried out to check whether there is any interference of any impurities with the retention time of analyte peaks. The specificity was performed by injecting blank, Placebo and standard solutions of drugs.

Precision:

Preparation of Standard stock solutions: Accurately weighed 14.2mg of Estetrol, 3mg of Drospirenone and transferred to 50ml volumetric flask and 3/4 th of diluents was added to these flasks and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (284µg/ml of Estetrol and 60µg/ml of Drospirenone)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (28.4µg/ml of Estetrol and 6µg/ml of Drospirenone)

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to tablet was transferred into a 100ml volumetric

flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (142µg/ml of Estetrol and 30µg/ml of Drospirenone)

Preparation of Sample working solutions (100% solution): 2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent.(28.4µg/ml of Estetrol and 6µg/ml of Drospirenone)

Linearity:

Preparation of Standard stock solutions: Accurately weighed 14.2mg of Estetrol, 3mg of Drospirenone and transferred to 50ml volumetric flask and 3/4 th of diluents was added to these flasks and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (284µg/ml of Estetrol and 60µg/ml of Drospirenone)

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (7.1µg/ml of Estetrol and 1.5µg/ml of Drospirenone)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (14.2µg/ml of Estetrol and 3µg/ml of Drospirenone)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (21.3µg/ml of Estetrol and 4.5µg/ml of Drospirenone)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (28.4µg/ml of Estetrol and 6µg/ml of Drospirenone)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (35.5µg/ml of Estetrol and 7.5µg/ml of Drospirenone)

150% Standard solution: 1.5ml each from two standard stock solutions was pipettede out and made up to 10ml (42.6µg/ml of Estetrol and 9µg/ml of Drospirenone)

Accuracy:

Preparation of Standard stock solutions: Accurately weighed 14.2mg of Estetrol, 3mg of Drospirenone and transferred to 50ml volumetric flask and 3/4 th of diluents was added to these flasks and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (284µg/ml of Estetrol and 60µg/ml of Drospirenone)

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.2ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Estetrol and Drospirenone solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Estetrol, Drospirenone solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Degradation studies [12,13]

Oxidation: To 1ml of stock solution of Estetrol and Drospirenone, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 minutes at 60℃. For the HPLC study, the resultant solution was diluted to obtain 24.8µg/ml&6µg/ml solutionand10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Acid Degradation Studies:

To 1 ml of stock solution Estetrol and Drospirenone, 1 ml of 2N Hydrochloric acid was added and refluxed for 30 minutes at 60℃. The resultant solution was diluted to obtain 24.8µg/ml&6µg/ml solution and 10µl solutions were injected into the system and the chromatograms were recor
ded to assess the stability of the sample.

Alkali Degradation Studies: To 1 ml of stock solution Estetrol and Drospirenone, 1 ml of 2N sodium hydroxide was added and refluxed for 30 minutes at 60℃. The resultant solution was diluted to obtain 24.8µg/ml&6µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in an oven at 105°C for 1h to study dry heat degradation. For the HPLC study, the resultant solution was diluted to 24.8µg/ml&6µg/ml solution and10µl was injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the 284µg/ml & 60µg/ml to UV Light by keeping the beaker in a UV Chamber for 1 day or 4000 Watt hours/m2 in a photostability chamber. For the HPLC study, the resultant solution was diluted to obtain 24.8µg/ml&6µg/ml solutions and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxingthedruginwaterfor1hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 24.8µg/ml&6µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION:

System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines

Table 1: System Suitability Parameters of Estetrol and Drospirenone

Sno	ESTETROL			DROSPIRENONE			
Inj	RT(min)	No. of Theoretical plates	Tailing	RT(min)	No. of Theoretical plates	Tailing	Resolution
1	2.131	6355	1.23	2.591	7158	1.17	3.9
2	2.132	6241	1.27	2.592	6630	1.12	3.7
3	2.132	6071	1.23	2.593	8604	1.15	3.9
4	2.132	5977	1.22	2.595	7298	1.18	3.7
5	2.133	5794	1.26	2.596	7013	1.24	3.9
6	2.135	6280	1.26	2.597	7517	1.14	4

Specificity:

The retention times of drospirenone and estetrol were 2.597 minutes and 2.135 minutes, respectively, in this method. No interfering peaks were observed at the retention times of these drugs in blank and placebo samples

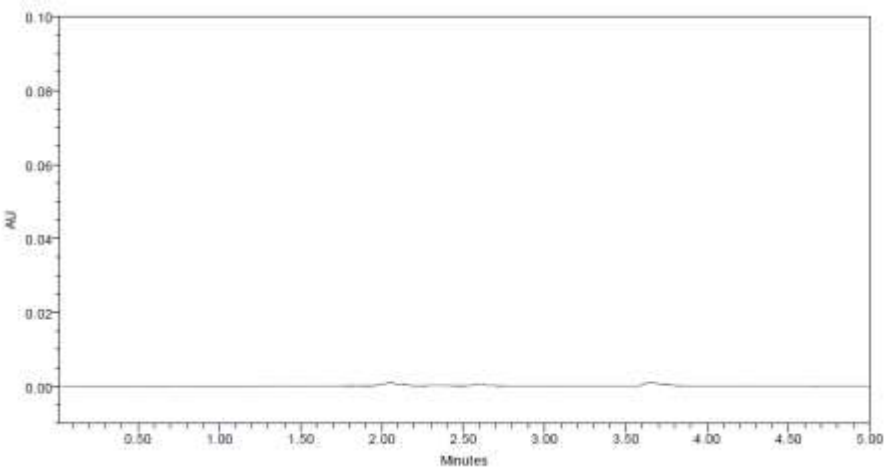


Figure 2: Chromatogram of blank.

Linearity table for Drospirenone and Estetrol: In duplicate injections, six different concentrations of Drospirenone (1.5-9µg/ml) and Estetrol (7.1-42.6µg/ml) were utilized. The average areas were provided, and the linearity equations for Drospirenone and Estetrol were determined as y = 361228x + 6065.6 and y = 341114x + 46689, respectively. The correlation coefficient for both drugs was found to be 0.999.

Table 2: Linearity table for Drospirenone and Estetrol

DROSPIRENONE		ESTETROL	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
1.5	556319	7.1	2420065
3	1084839	14.2	4942707
4.5	1650604	21.3	7360566
6	2155174	28.4	9856190
7.5	2708759	35.5	12082070
9	3265460	42.6	14525325

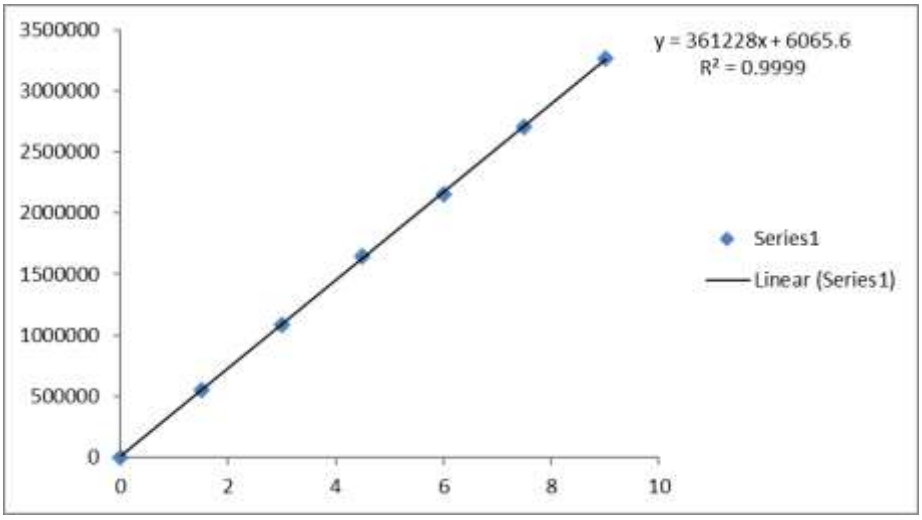


Figure 3: Calibration curve of Drospirenone

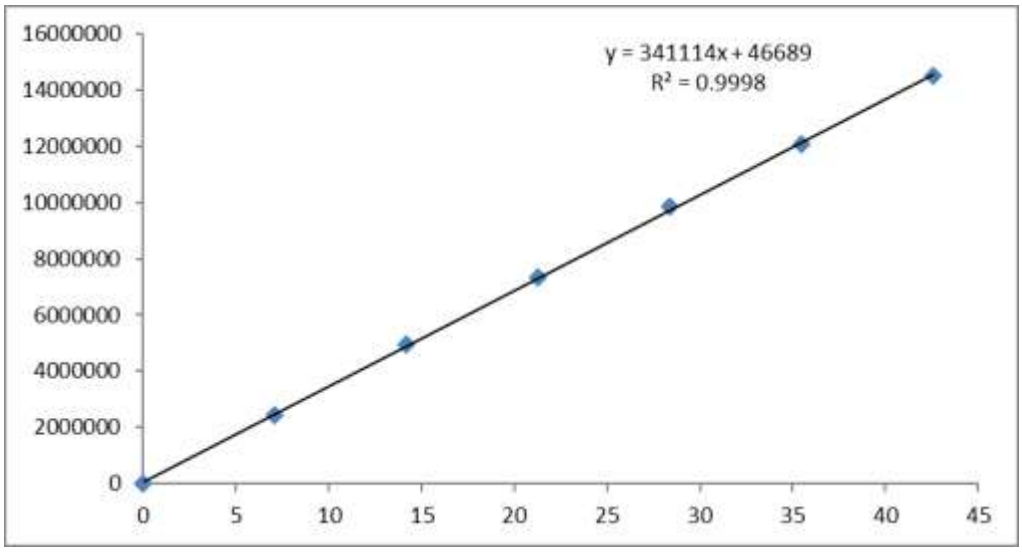


Figure 4: Calibration curve of Estetrol

Precision:

System Precision:

Six replicate injections of a known concentration of sample preparation of Drospirenone and Estetrol have been analyzed by injecting them into a HPLC column. From the results obtained, %RSD was calculated and was found to be within the limits (<2). The results of precision are given in Table .

Table 3: Linearity table for Drospirenone and Estetrol

S. No	Area of Drospirenone	Area of Estetrol
1.	2167484	9879846
2.	2174684	9823698
3.	2174775	9896544
4.	2176574	9858364
5.	2164658	9913784
6.	2153475	9801737
Mean	2168608	9862329
S.D	8766.8	43138.4
%RSD	0.4	0.4

Repeatability:

The normal range, standard deviation, and % RSD were calculated for two medications, resulting in values of 0.4% for both Drospirenone and Estetrol, respectively.

Table 4: Repeatability Table for Drospirenone and Estetrol

S. No	Area of Estetrol	Area of Drospirenone
1.	9787958	2164756
2.	xz9875606	2168464
3.	9803634	2175797
4.	9863844	2183649

5.	9898764	2183564
6.	9846774	2183649
Mean	9846097	2176647
S.D	42762.7	8425.9
%RSD	0.4	0.4

ACCURACY:

Samples with three levels of accuracy were prepared using the standard addition method. Triplicate injections were administered for each accuracy level, resulting in mean %Recovery values of 100.25% for Drospirenone and 100.09% for Estetrol, respectively.

Table 5: Accuracy table of Drospirenone

% Conc	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	% Mean
50%	3	3.02	100.69	100.25%
	3	2.99	99.76	
	3	3.02	100.72	
100%	6	6.02	100.34	
	6	6.02	100.40	
	6	5.95	99.17	
150%	9	9.08	100.86	
	9	9.02	100.24	
	9	9.01	100.09	

Table 6: Accuracy table of Estetrol

% Conc	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	% Mean
50%	14.2	14.20	99.97	100.09%
	14.2	14.28	100.60	
	14.2	14.13	99.49	
100%	28.4	28.43	100.09	
	28.4	28.53	100.47	
	28.4	28.59	100.67	
150%	42.6	42.70	100.23	
	42.6	42.61	100.03	
	42.6	42.33	99.25	

Sensitivity:

Table 7: Sensitivity of Drospirenone and Estetrol

Molecule	LOD	LOQ
Drospirenone	0.01	0.02
Estetrol	0.08	0.25

The limit of detection and limit of quantification were evaluated by serial dilutions of Drospirenone and .Estetrol The LOD value was found to be 0.01 µg/mL,0.08µg/mL respectively for *Drospirenone and .Estetrol and the corresponding LOQ values were found to be 0.02 µg/mL,0.025 µg/mL.*

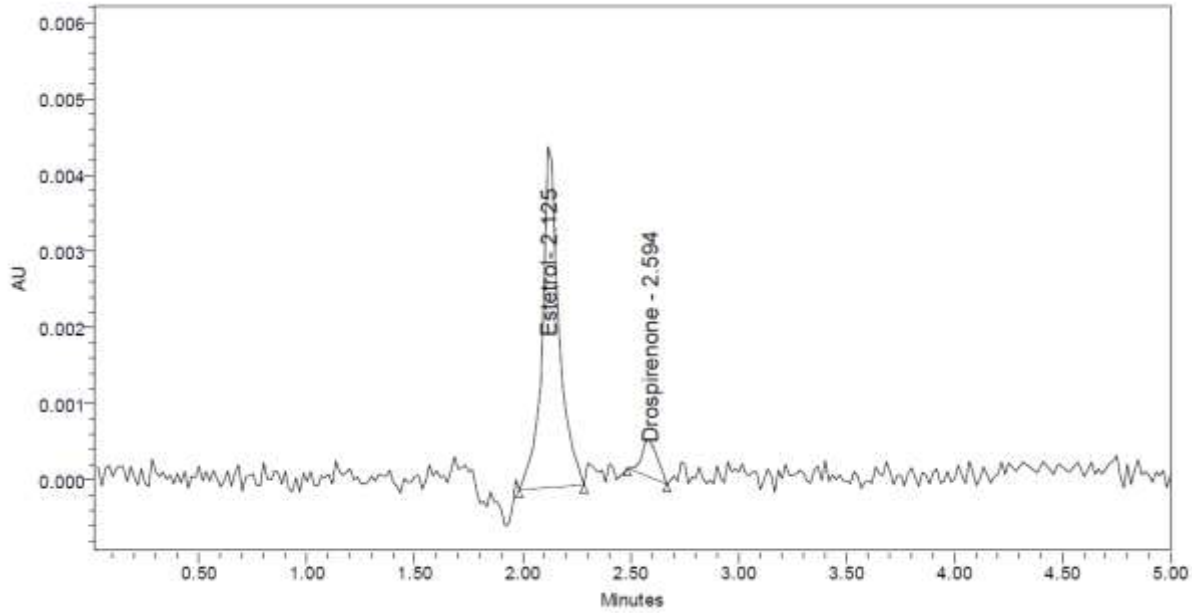


Figure 5: LOD Chromatogram of Estetrol and Drospirenone

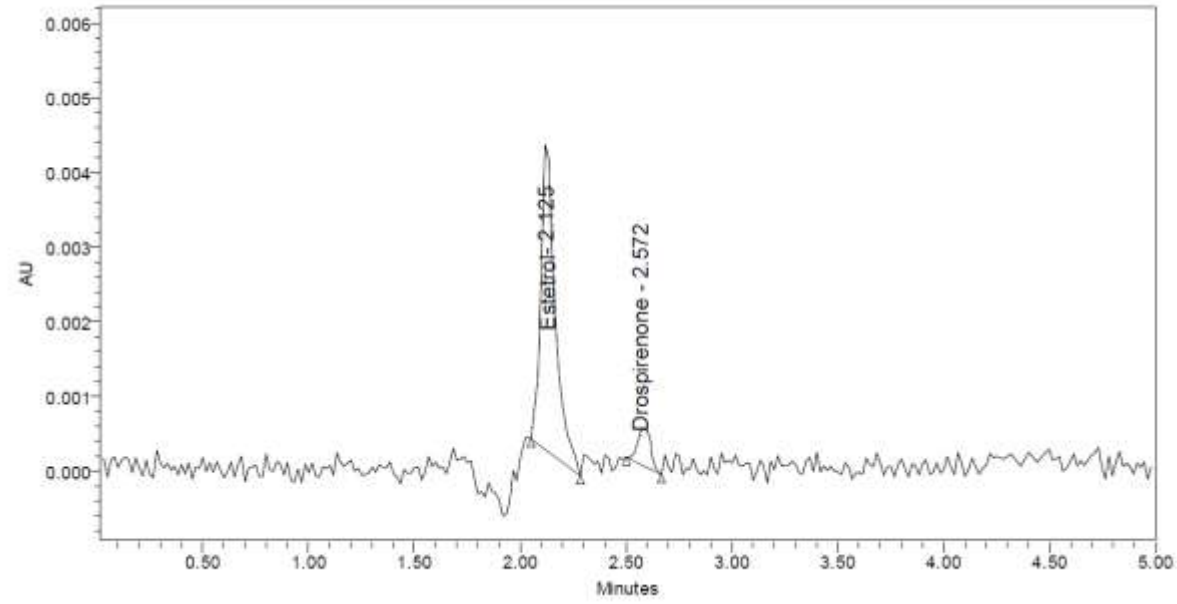


Figure 6: LOQ Chromatogram of Estetrol and Drospirenone

Robustness:

The suitability parameters for the system were minimally affected, and all criteria were successfully met, with %RSD remaining within the acceptable limit

Table 8: Robustness of Drospirenone and Estetrol

S.no	Condition	DROSPIRENONE	ESTETROL
1	FR (-) 0.9ml/min	1.3	0.2
2	FR (+) 1.1ml/min	0.5	0.5
3	MP (-) 75B:25A	0.8	0.4
4	MP (+) 85B:15A	1.3	0.5
5	Te(-) 25°C	0.7	0.6
6	Te (+) 35°C	1.1	0.5

ASSAY:

Nextstellis, containing the specified amounts of Drospirenone (3 mg) and Estetrol (14.2 mg), was subjected to examination. The average % assay results were found to be 100.17% for Drospirenone and 99.64% for Estetrol, respectively.

Table 9: Assay Chromatogram of Drospirenone

S.no	Standard Area	Sample area	% Assay
1	2167484	2164756	99.62
2	2174684	2168464	99.79
3	2174775	2175797	100.13
4	2176574	2183649	100.49
5	2164658	2183564	100.49
6	2153475	2183649	100.49
Avg	2168608	2176647	100.17
S.D	8766.8	8425.9	0.4
%RSD	0.4	0.4	0.4

Table 10: Assay Chromatogram of Estetrol

S.no	Standard Area	Sample area	% Assay
1	9879846	9787958	99.05
2	9823698	9875606	99.93
3	9896544	9803634	99.21
4	9858364	9863844	99.82
5	9913784	9898764	100.17
6	9801737	9846774	99.64
Avg	9862329	9846097	99.64
S.D	43138.4	42762.7	0.4
%RSD	0.4	0.4	0.4

Table 11: Forced Degradation Studies

Type of degradation	DROSPIRENONE		ESTETROL	
	% Recovered	% Degraded	% Recovered	% degraded
Acid	97.86	2.14	98.51	1.49
Base	97.72	2.28	97.89	2.11
Peroxide	93.77	6.23	93.49	6.51
Thermal	98.78	1.22	98.91	1.09
UV	98.89	1.11	98.41	1.59
Water	99.17	0.83	99.83	0.17

CONCLUSION

A precise and convenient method was developed for the simultaneous quantification of Drospirenone and Estetrol in capsule form. The retention times for Drospirenone and Estetrol were determined to be 2.597 minutes and 2.1336 seconds, respectively, with a consistent RSD of 0.4% for both drugs. The method's pH stability was ensured by OPA, while the temperature was maintained at 30°C. The λmax was identified at 263.0 nm, and the elution times were 2.597 minutes for Drospirenone and 2.1336 minutes for Estetrol. Both exhibited a comparable mean variation (RSD) of 0.4. The overall recovery rates for Drospirenone and Estetrol were assessed at 100.25% and 100.09%, respectively. The correlation formulas for Drospirenone and Estetrol resulted in LOD values of 0.01 and LOQ rates of 0.02, and so on. The method was successfully applied to the determination of Drospirenone and Estetrol in commercial capsule formulations.

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