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HPLC method for simultaneous estimation of Drospirenone & Estetrol in Tablet Dosage Form

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Abstract

An efficient and accurate method was devised to simultaneously determine the levels of Drospirenone and Estetrol in Tablet dosage form. A chromatogram was performed using a Kromasil 150 column with dimensions of 4.1 x 150mm and a particle size of 2.1µm. A mobile phase consisting of a mixture of Buffer A and Kh2 in a ratio of 55:45 was passed through a column at a flow rate of 1.0ml/min. The buffer utilized in this procedure consisted of 0.1% OPA (ortho-phosphoric acid) and 0.1% Triethyl amine to achieve a pH of 4.5. The temperature was consistently maintained at 30°C. The wavelength selected for optimization was 221.0 nm. The retention time for Drospirenone was determined to be 2.408 minutes, while the retention time for Estetrol was discovered to be 3.163 minutes. The %RSD values for Drospirenone and Estetrol were calculated to be 0.2% and 1.1% respectively. Percentage. The recovery rates for Drospirenone and Estetrol were 100.58% and 99.43% respectively. The limits of detection (LOD) and limits of quantification (LOQ) for Drospirenone and Estetrol were determined to be 0.04 and 0.13, and 0.54 and 1.63, respectively, using regression equations. The regression equation for Drospirenone is y = 13263x + 345.0, while the regression equation for Estetrol is y = 14243x + 14243. The retention times and run time were reduced, making the devised method simple and cost-effective for routine use in quality control tests in industries.

Keywords: Drospirenone, Estetrol, HPLC

Introduction

In the absence of established protocols, novel approaches are devised for newly introduced products. Novel methods are devised to reduce expenses and time while enhancing accuracy and longevity for present-day commodities that are not regulated by pharmacopoeias. Experimental trials are conducted to validate and refine the process. In order to replace the current procedure with a different technique, it is important to have access to comparative laboratory data that outlines the advantages and disadvantages of the proposed alternative [1-3].

In pharmaceutical analysis, the validation of analytical procedures is vital as it is the only way to ensure the continued effectiveness and safety of each batch produced. The ability to control this quality is decided by the capacity of the analytical processes to produce reliable evidence of any deviation from the target requirements, when applied under specific conditions and with a predetermined level of sensitivity [4-5].

It is essential that the analytical methods developed for determining the level of purity and impurities have the capability to efficiently isolate all constituents, both desired and undesired, from the formulation matrix, without experiencing any interference [6-8].

The stability-indicating test approach should be used to accurately assess the active components, ensuring that there is no interference from excipients, degradation products, process pollutants, or any other potential impurities [9—12].

Drospirenone is a synthetic form of the hormone progesterone that is utilized in oral contraceptive pills to prevent conception and treat other medical ailments. Drospirenone is a synthetic progestin that is frequently present in the widely used oral contraceptive, Yaz, in conjunction with Ethinyl estradiol. Recently, it received approval from both Health Canada and the FDA for use as an oral contraceptive medication when combined with Estetrol. In addition to its contraceptive properties, drospirenone is employed in conjunction with estrogens to manage acne and premenstrual dysphoric disorder (PMDD) [13-15]. Estetrol is an estrogen that is used orally in combination with drospirenone for the purpose of contraception. Steroid estrogens, whether derived from natural sources or created artificially, offer a broad spectrum of therapeutic applications, including hormonal contraception and alleviating menopausal symptoms. Estetrol (E4) is an endogenous estrogen that is normally present during pregnancy, but can also be artificially produced from a botanical source and employed as a contraceptive. It possesses more potency and a higher level of safety compared to the synthetic estrogen ethinylestradiol (EE2) present in 97% of oral contraceptive pills. This reduces the build-up of undesired endocrine disrupting chemicals (EDCs) in the environment, which frequently result in detrimental epigenetic consequences [16-19].

The main objective of this study is to develop a rapid, precise, and sensitive analytical technique that can accurately assess both teneligliptin and remogliflozin in pharmaceutical formulations, while also being selective and capable of producing consistent results.

After conducting a comprehensive review of the existing literature and examining relevant patents, no method has been documented thus far for the utilization of this specific medication combination. Various spectrophotometric and chromatographic procedures were accessible for utilization with individual pharmaceuticals or in conjunction with additional medications. Hence, to measure these medications together in a combined dosage form, a highly accurate, precise, and uncomplicated HPLC approach is required. Hence, it was deemed intriguing to develop and validate a methodology for this purpose.

The primary objective of this project is to provide a highly reliable, exact, sensitive, specific, consistent, and fast analytical method for concurrently determining the quantities of Drospirenone and Estetrol (Pulmoclear) in both bulk and tablet forms.

Objective and Plan

The present effort aims to achieve the following objectives:

- The objective is to create a novel HPLC method that can accurately measure the levels of Drospirenone and Estetrol and simultaneously, while ensuring the stability of the compounds. Additionally, the method will be validated in accordance with the guidelines set by the International Council for Harmonisation (ICH).
- To utilize the verified technique for the concurrent determination of Drospirenone and Estetrolin in a pharmaceutical formulation.

Material and Methods

Materials

- The substances used in this study include Estetrol and Drospirenone in their pure form as active pharmaceutical ingredients (API). Additionally, a combination of Estetrol and Drospirenone in tablet form (Next stellis) was used. Other substances used include distilled water, acetonitrile, phosphate buffer, methanol, potassium dihydrogen ortho phosphate buffer, and ortho-phosphoric acid. All of the aforementioned chemicals and solvents are sourced from Rankem.

Methods

Diluent: The diluent was selected based on the solubility of the medicines. Acetonitrile and water were taken in a 50:50 ratio.

Preparation of Standard stock solutions: Precisely measured 14.2mg of Estetrol and 3mg of Drospirenone were placed into a 50ml volumetric flask. Then, 3/4 of the diluent was added to these flasks, and the mixture was subjected to sonication for a duration of 10 minutes. The flasks were prepared by combining diluents and labeled as the Standard stock solution. The concentration of Estetrol is 284µg/ml while the concentration of Drospirenone is 60µg/ml.

Preparation of Standard working solutions (100% solution): 1 milliliter was extracted from each stock solution and transferred into a 10 milliliter volumetric flask, which was then filled with diluent to the mark. The concentration of Estetrol is 28.4µg/ml while the concentration of Drospirenone is 6µg/ml.

Preparation of stock solutions for the samples: The weight of 5 tablets was measured and the average weight of each tablet was determined. Then, the weight of one tablet was transferred into a 100ml volumetric flask. 50ml of diluent was added to the flask and the mixture was sonicated for 25 minutes. The volume was then adjusted with diluent and filtered using HPLC filters. The resulting solution contained 142µg/ml of Estetrol and 30µg/ml of Drospirenone.

Preparation of Sample working solutions (100% solution): 2ml of the filtered sample stock solution was put into a 10ml volumetric flask. The flask was then filled with diluent, resulting in a concentration of 28.4µg/ml of Estetrol and 6µg/ml of Drospirenone.

Preparation of buffer:

0.1%OPA Buffer: The concentration of ortho phosphoric acid was reduced by diluting 1ml of it with 1000ml of HPLC grade water.

Validation:

System suitability parameters: The system suitability parameters were assessed by creating standard solutions of Estetrol (28.4 parts per million) and Drospirenone (6 parts per million). These solutions were injected six times, and metrics such as peak tailing, resolution, and USP plate count were determined.

The relative standard deviation (RSD) for the area of six standard injections should not exceed 2%.

Specificity: Assessment of potential interference in the optimal procedure. Interfering peaks should be absent in the blank and placebo samples at the specific retention durations of these medications as per the established technique. This strategy was described as being specific.

Precision:

Preparation of Standard stock solutions: Precisely measured 14.2mg of Estetrol and 3mg of Drospirenone were placed into a 50ml volumetric flask. Three-fourths of the diluent was added to these flasks, and the mixture was subjected to sonication for a duration of 10 minutes. The Flask was composed of diluents and designated as the Standard stock solution. The concentration of Estetrol is 284µg/ml while the concentration of Drospirenone is 60µg/ml.

Preparation of standard working solutions (100% solution): 1 milliliter was extracted from each stock solution and transferred into a 10 milliliter volumetric flask, which was then filled with diluent to the desired volume. The concentration of Estetrol is 28.4µg/ml, while the concentration of Drospirenone is 6µg/ml.

Preparation of stock solutions for the samples: Five tablets were weighed and the average weight of each tablet was determined. The weight corresponding to one tablet was then transferred into a 100ml volumetric flask. Next, 50ml of diluent was added and the mixture was sonicated for 25 minutes. The volume was then adjusted with diluent and filtered using HPLC filters. The resulting solution contained 142µg/ml of Estetrol and 30µg/ml of Drospirenone.

Process of preparing the sample working solutions: this process involved transferring 2ml of the filtered sample stock solution into a 10ml volumetric flask and then filling it up with diluent.The concentration of Estetrol is 28.4µg/ml, while the concentration of Drospirenone is 6µg/ml.

Linearity:

Preparation of Standard stock solutions: Precisely measured 14.2mg of Estetrol and 3mg of Drospirenone were placed into a 50ml volumetric flask. Then, 3/4 of the diluent was added to these flasks, and the mixture was subjected to sonication for a duration of 10 minutes. The flasks were prepared by combining diluents and labeled as the Standard stock solution. The concentration of Estetrol is 284µg/ml while the concentration of Drospirenone is 60µg/ml.

25% Standard solution: 0.25 milliliters were extracted from two standard stock solutions and combined to create a 10 milliliter solution. The resulting solution included 7.1 micrograms per milliliter of Estetrol and 1.5 micrograms per milliliter of Drospirenone.

50% Standard solution: A total of 0.5 milliliters was extracted from each of two standard stock solutions and then combined to create a final volume of 10 milliliters. The concentration of Estetrol is 14.2 micrograms per milliliter, and the concentration of Drospirenone is 3 micrograms per milliliter.

75% Standard solution: 0.75 milliliters were extracted from two standard stock solutions and combined to create a 10 milliliter solution. The resulting solution included 21.3 micrograms per milliliter of Estetrol and 4.5 micrograms per milliliter of Drospirenone.

100% Standard solution: 1.0ml aliquots were extracted from two standard stock solutions and diluted to a final volume of 10ml. The concentrations of Estetrol and Drospirenone in the final solution were 28.4µg/ml and 6µg/ml, respectively.

125% Standard solution: 1.25 milliliters were extracted from two standard stock solutions and combined to make a total volume of 10 milliliters. The resulting solution included 35.5 micrograms per milliliter of Estetroland and 7.5 micrograms per milliliter of Drospirenone.

150% Standard solution: A total of 1.5 milliliters was extracted from two standard stock solutions and then diluted to a final volume of 10 milliliters, resulting in a concentration of 42.6 micrograms per milliliter for Estetrol and 9 micrograms per milliliter for Drospirenone.

Accuracy:

Preparation of Standard stock solutions: Precisely measured 14.2mg of Estetrol and 3mg of Drospirenone were placed into a 50ml volumetric flask. Then, 3/4th of the diluent was added to these flasks, and the mixture was subjected to sonication for a duration of 10 minutes. The flasks were prepared by combining diluents and labeling them as the Standard stock solution, containing 284µg/ml of Estetrol and 60µg/ml of Drospirenone.

Preparation of 50% Spiked Solution: A 10ml volumetric flask was filled with 0.5ml of the sample stock solution. Then, 1.0ml was extracted from each standard stock solution and added to the flask. The flask was then filled to the mark with diluent.

Preparation of 100% Spiked Solution: A 10ml volumetric flask was filled with 1.0ml of the sample stock solution. Then, 1.0ml was taken from each standard stock solution and added to the flask. The flask was then filled with diluent until it reached the mark.

Preparation of 150% Spiked Solution: 1.5 milliliters of the original solution was transferred into a 10 milliliter flask. Then, 1.0 milliliter was collected from each standard solution and added to the flask. The remaining volume was filled with a diluent until the flask reached its mark.

Acceptance Criteria

The percentage of recovery for each stage should fall within the range of 98.0 to 102%.

Robustness: Minor intentional modifications in technique, such as adjusting the flow rate, mobile phase ratio, and temperature, were implemented. However, no significant impact on the outcome was seen, and the results remained within the acceptable range according to the ICH guidelines.

The robustness conditions, such as 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), were carefully maintained. The samples were injected in duplicate. The system suitability parameters were minimally affected and all the parameters were successfully met. The %RSD value fell under the specified limit.

LOD sample Preparation: 0.25 milliliters were extracted from each of two standard stock solutions and transferred to two individual 10 milliliter volumetric flasks. The flasks were then filled with diluents. For each of the solutions mentioned above, 0.1ml of Estetrol and Drospirenone were transferred into separate 10ml volumetric flasks. The flasks were then filled with the same diluents to make up the final volume.

LOQ sample Preparation: 0.25 milliliters were extracted from each of two standard stock solutions and placed into two individual 10 milliliter volumetric flasks. The flasks were then filled with diluent. 0.3ml of Estetrol solution and 0.3ml of Drospirenone solution were separately transferred into 10ml volumetric flasks. The flasks were then filled with the same diluent.

Degradation studies

Oxidation:

Separately, 1 ml of a 20% hydrogen peroxide (H2O2) solution was added to 1 ml of a stock solution containing Estetrol and Drospirenone. The solutions were maintained at a temperature of 600°C for a duration of 30 minutes. In the HPLC investigation, the solution that was obtained was diluted to achieve concentrations of 24.8 µg/ml and 6 µg/ml. Then, 10 µl of the solution were injected into the system and the resulting chromatograms were recorded in order to evaluate the stability of the sample.

Acid Degradation Studies:

1 ml of a stock solution containing Estetrol and Drospirenone was combined with 1 ml of 2N Hydrochloric acid. The mixture was then heated at 60 °C for 30 minutes using a reflux method. The resulting solution was diluted to achieve concentrations of 24.8µg/ml and 6µg/ml. Subsequently, 10µl of each solution were injected into the system and the resulting chromatograms were recorded in order to evaluate the stability of the sample.

Alkali Degradation Studies:

1 ml of Estetrol and Drospirenone stock solution was combined with 1 ml of 2N sodium hydroxide. The mixture was then heated to 60 °C and refluxed for 30 minutes. The resulting solution was diluted to achieve concentrations of 24.8 µg/ml and 6 µg/ml. Subsequently, 10 µl of each solution were injected into the system and the resulting chromatograms were recorded in order to evaluate the stability of the sample.

Dry Heat Degradation Studies:

The drug solution was subjected to dry heat degradation by placing it in an oven at a temperature of 105 °C for a duration of 1 hour. In the HPLC investigation, the solution obtained was diluted to concentrations of 24.8 µg/ml and 6 µg/ml. A volume of 10 µl from each solution was injected into the system, and the resulting chromatograms were recorded to evaluate the stability of the sample.

Photo Stability studies:

The drug's photochemical stability was assessed by subjecting samples with concentrations of 284µg/ml and 60µg/ml to UV light. This was done by placing the samples in a beaker within a UV chamber for a duration of 1 day or an exposure of 4000 Watt hours/m^2 in a photo stability chamber. In the HPLC investigation, the solution obtained was diluted to achieve concentrations of 24.8µg/ml and 6µg/ml. Then, 10µl of each solution were injected into the system and the resulting chromatograms were recorded in order to evaluate the stability of the sample.

Neutral Degradation Studies:

The study involved subjecting the medicine to stress testing by heating it in water at a temperature of 60ºC for 1 hour. In the HPLC investigation, the solution obtained was diluted to concentrations of 24.8 µg/ml and 6 µg/ml. A volume of 10 µl from each solution was injected into the system, and the resulting chromatograms were recorded in order to evaluate the stability of the sample.

Results and Discussion

Method development: The method development process involved altering several parameters such as mobile phase ratios and buffers.

Trial 1:

Chromatographic conditions:

Mobile phase	**:** Water: OPA (50:50 v/v)
Flow rate	**:** 1 ml/min
Column	**:** Agilent 150 (4.6 x 150mm, 5µm)
Detector wave length	**:** 221nm
Column temperature	**:** 30°C

Injection volume : 10 µL
Run time : 10 min
Diluent : Water and Acetonitrile in the ratio 50:50
Results : Only one analytical peak was detected in this trial.Therefore, a further investigation was conducted.

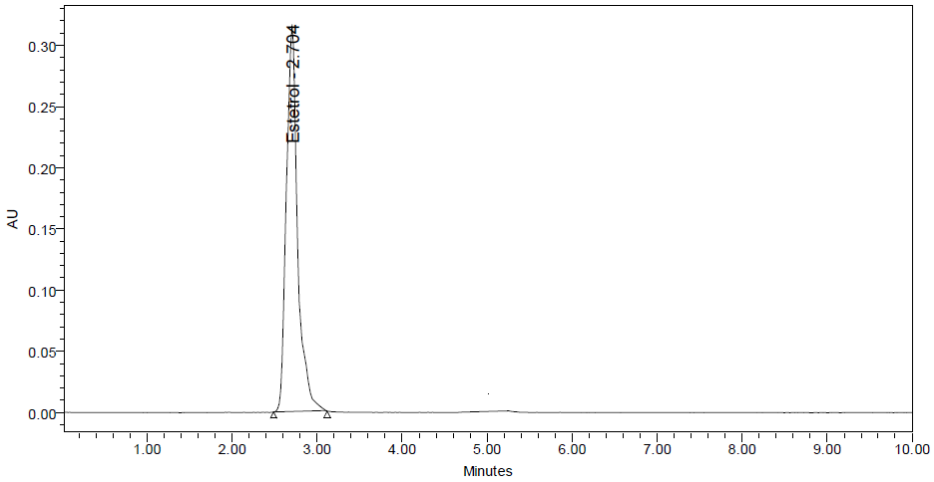

Figure 1: Trial chromatogram 1

Trial 2:
Chromatographic conditions:
Mobile phase : Methanol:OPA(50:50 v/v)
Flow rate : 1ml/min
Column : Kromasil 150 (4.1 x 150mm, 2.1µm)
Detector wave length : 221nm
Column temperature : 30°C
Injection volume : 10 µL
Run time : 10.0 min
Diluent : Water and Acetonitrile in the ratio (50:50)
Results : In this trail by change in the mobile phase both peaks were eluted but peak splitting was observed with Drospirenone. So, further trail was carried out.

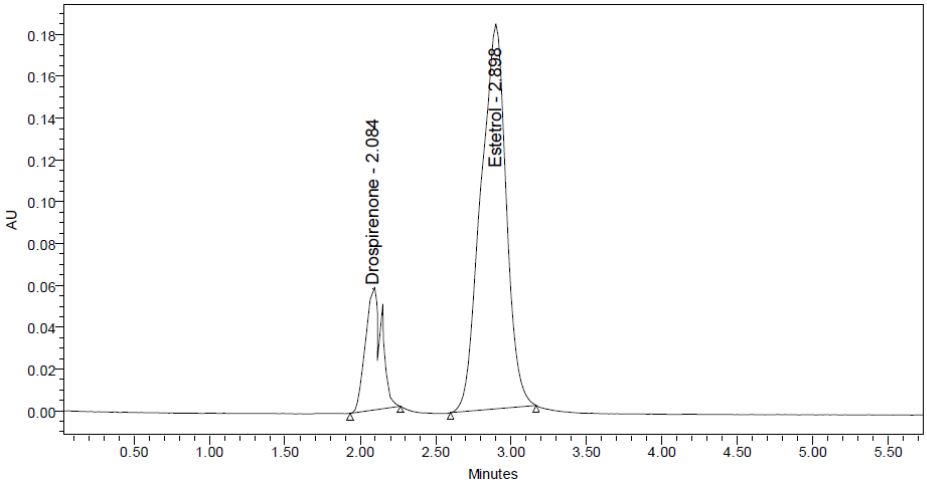

Figure 2: Trial chromatogram 2

Trial 3:

Chromatographic conditions:

Mobile phase	**:**	Acetonitrile: Kh2(50:50 v/v)
Flow rate	**:**	1ml/min
Column	**:**	Kromasil 150 (4.1 x 150mm, 2.1µm)
Detector wave length	**:**	221nm
Column temperature	**:**	30°C
Injection volume	**:**	10 µL
Run time	**:**	10.0min
Diluent	**:**	Water and Acetonitrile in the ratio 50:50
Results	**:**	In this trail by changing the Buffer and Organic Phase both peaks were eluted But Drospirenone peak eluted at void range. so, further trail was carried out.

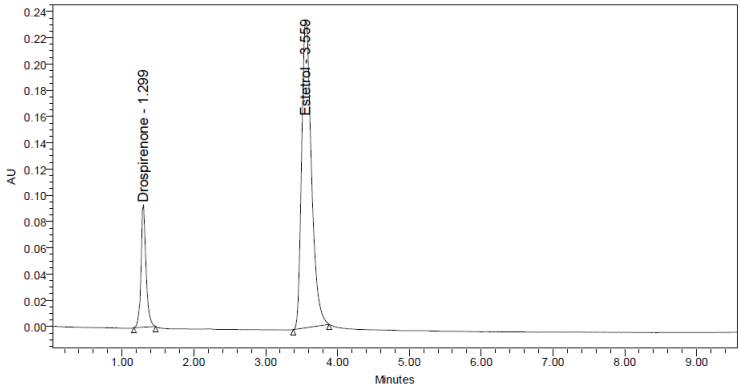

Figure 3: Trial chromatogram 3

Trial 4 :

Chromatographic conditions:

Mobile phase	**:**	Acetonitriile: Kh2 (70:30 v/v)
Flow rate	**:**	1 ml/min
Column	**:**	Kromasil 150 (4.1 x 150mm, 2.1µm)
Detector wave length	**:**	221nm
Column temperature	**:**	30°C
Injection volume	**:**	10 µL
Run time	**:**	6 min
Diluent	**:**	Water and Acetonitrile in the ratio 50:50
Results	:	In this trail also by changing the column both peaks were eluted but peak resolution was greater So, further trail was carried out.

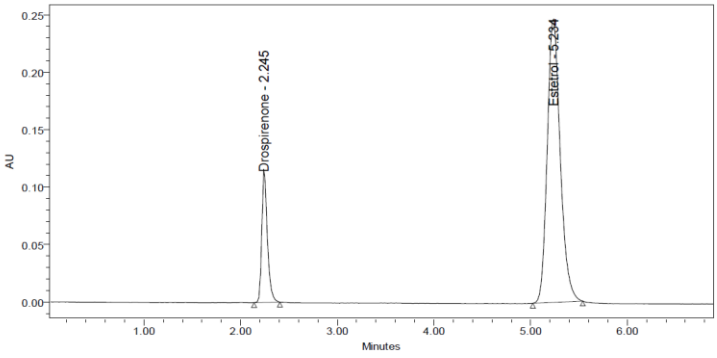

Figure 4: Trial chromatogram 4

Optimized method:

Chromatographic conditions:

Mobile phase	**:**	55% Acetonitrile: 45% Kh2
Flow rate	**:**	1 ml/min
Column	**:**	Kromasil 150 (4.1 x 150mm, 2.1µm)
Detector wave length	**:**	221nm
Column temperature	**:**	30°C
Injection volume	**:**	10 µL
Run time	**:**	6 min
Diluent	**:**	Acetonitrile And water in the ratio 50:50
Results	**:**	In this trial by changing the column and mobile phase both peaks were eluted and Both peaks have good resolution, tailing Factor, theoretical plate count and resolution.

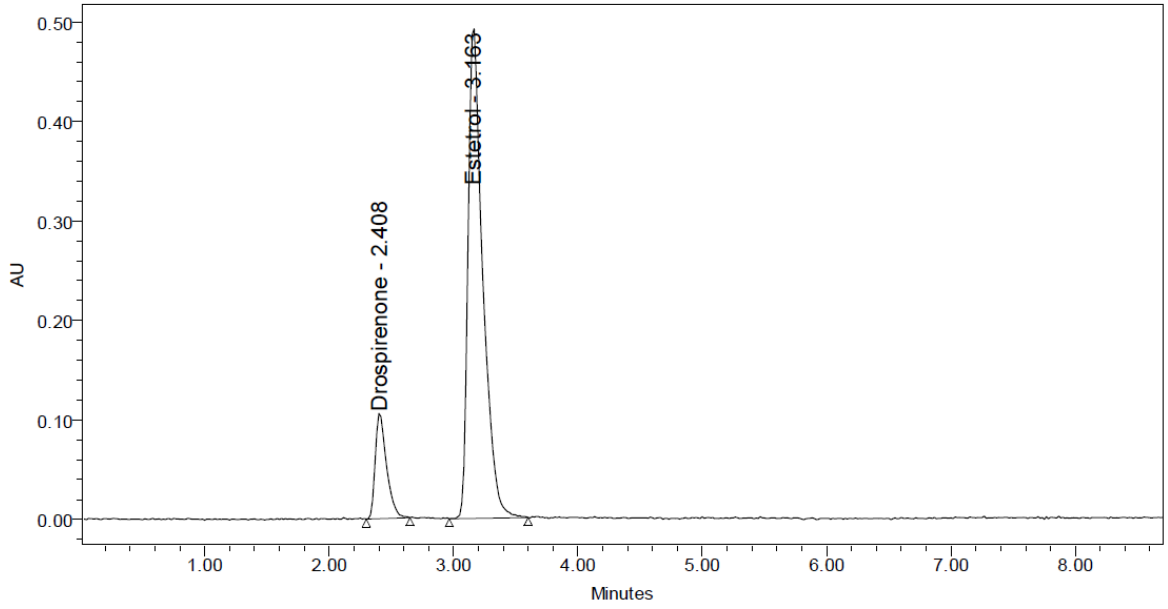

Figure 5: Optimized Method

Observation: Drospirenone and Estetrol were separated and detected at retention times of 2.408 min and 3.163 min, respectively, with excellent resolution. The plate count and tailing factor exhibited quite satisfactory results, thereby warranting the optimization and validation of this method.

System suitability: The system suitability metrics were all within the acceptable range and met the requirements outlined in the ICH recommendations.

Table 1: System suitability parameters for Drospirenone and Estetrol

S no	Drospirenone			Estetrol			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.404	3225	1.50	3.151	3397	1.58	3.6
2	2.412	3272	1.47	3.158	3311	1.60	3.6
3	2.415	3188	1.45	3.159	3326	1.63	3.7
4	2.415	3007	1.46	3.159	3346	1.62	3.6
5	2.416	3506	1.47	3.162	3424	1.59	3.7
6	2.417	3342	1.44	3.169	3404	1.58	3.7

Discussion: As per the ICH requirements, the plate count should exceed 2000, the tailing factor should be below 2, and the resolution must exceed 2. All the system-appropriate parameters were successfully met and remained within the specified ranges.

Validation:

Specificity:

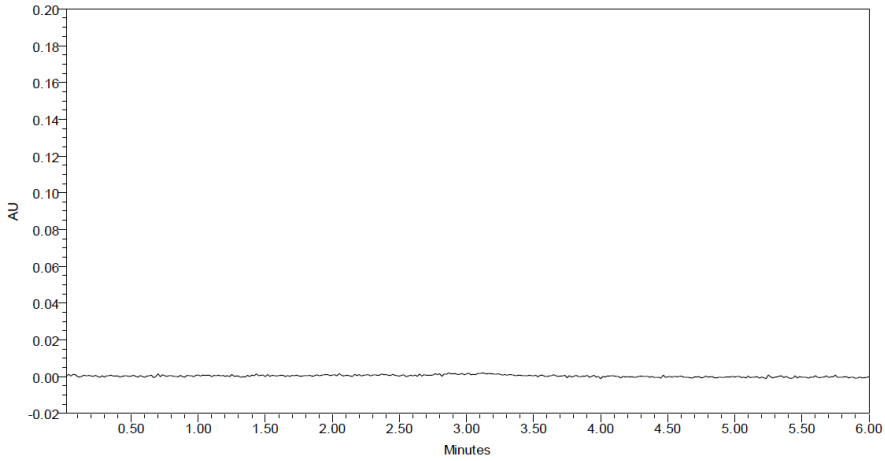

Figure 6: Chromatogram of blank

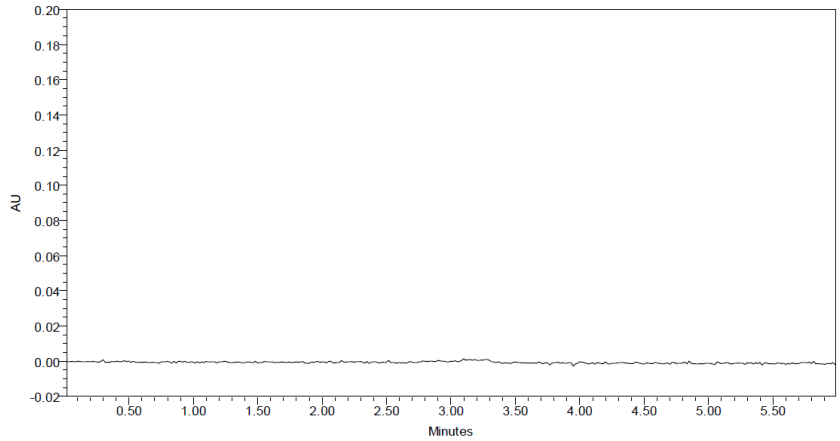

Figure 7: Chromatogram of placebo

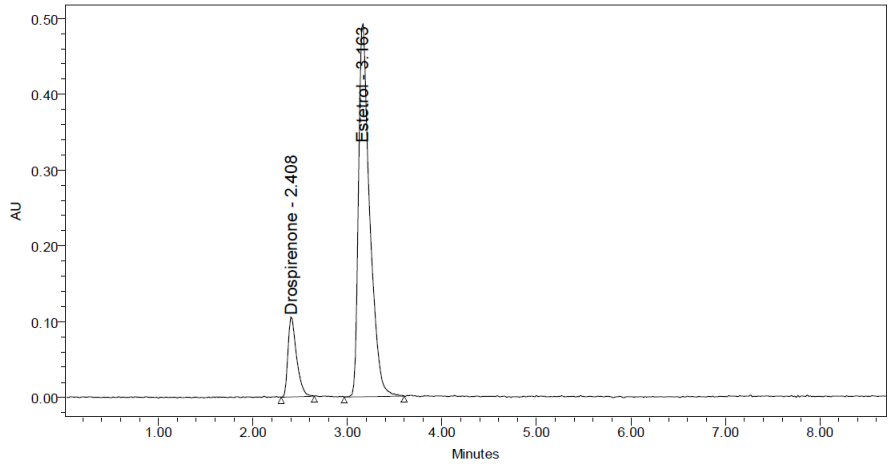

Figure 8: Typical Chromatogram

Discussion: The retention time for Drospirenone was 2.408 minutes, while the retention time for Estetrol was 3.163 minutes. No interfering peaks were detected in the blank and placebo samples at the retention times corresponding to the medicines analyzed in this approach. This strategy was described as being specific.

Linearity:

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	19971	7.1	103812
3	40782	14.2	205670
4.5	59854	21.3	305104
6	80769	28.4	403868
7.5	99465	35.5	514941
9	119355	42.6	603656

Discussion: Duplicate injections were performed with six linear doses of Drospirenone ranging from 1.5 to 9µg/ml, and Estetrol ranging from 7.1 to 42.6µg/ml. The previous discussion included the average areas, as well as the linearity equations derived for Drospirenone (y = 13263x + 345.0) and Estetrol (y = 14243x + 1925). The correlation coefficient obtained for the two medications was 0.999.

Precision:

System Precision:

Table 3: System precision table of Drospirenone and Estetrol

S. No	Area of Drospirenone	Area of Estetrol
1.	80385	406464
2.	80264	418286
3.	80674	406892
4.	80435	407212
5.	80589	409992
6.	80250	406835
Mean	80433	409280
S.D	171.3	4593.5
%RSD	0.2	1.1

Discussion: Using a single volumetric flask of the working standard solution, six injections were administered and the resulting areas were recorded above. The mean area, standard deviation, and relative standard deviation (RSD) were computed for two medications. The relative standard deviation (RSD) values obtained were 0.2% for Drospirenone and 1.1% for Estetrol. Since the precision limit was below "2", the system's precision exceeded this value in this procedure.

Repeatability:

Table 4: Repeatability table of Drospirenone and Estetrol

S. No	Area of Drospirenone	Area of Estetrol
1.	80960	404015
2.	80656	408262
3.	80425	404245
4.	80256	407265
5.	80541	407720
6.	81855	409743
Mean	80782	406875
S.D	576.4	2285.2
%RSD	0.7	0.6

Discussion: Multiple samplings were conducted from a stock solution, resulting in the preparation of six working sample solutions with identical concentrations. Each injection from these working sample solutions was administered, and the corresponding areas were recorded in the table above. The average area, standard deviation, and relative standard deviation (RSD) were calculated for two medications, Drospirenone and Estetrol. The values obtained were 0.7% and 0.6% respectively. Since the precision limit was below "2", the system's precision was exceeded in this manner.

Intermediate precision (Day_Day Precision):

Table 5: Intermediate precision table of Drospirenone and Estetrol

S. No	Area of Drospirenone	Area of Estetrol
1.	79494	381652
2.	79359	385334
3.	79234	383361
4.	78694	393458
5.	79771	389519
6.	79601	386494
Mean	79359	386636
S.D	375.3	4292.6
%RSD	0.5	1.1

Discussion: Multiple aliquots were taken from a stock solution, resulting in the preparation of six working sample solutions with identical concentrations. Each injection of the working sample solutions was performed on the day following their preparation, and the corresponding areas were recorded in the table above. The average area, standard deviation, and relative standard deviation (RSD) were calculated for two medications, Drospirenone and Estetrol. The values obtained were 0.5% and 1.1% respectively. Since the precision limit was below "2", the system exceeded the precision in this approach.

Accuracy:

Table 6: Accuracy table of Drospirenone

% Level	Amount Spiked(µg/mL)	Amount recovered(µg/mL)	% Recovery	%
	3	3.01108	100.37	
50%	3	3.02443	100.81	
	3	3.04380	101.46	
	6	6.06340	101.06	
100%	6	6.05948	100.99	100.58%
	6	6.00136	100.02	
	9	8.94579	99.40	
150%	9	9.04923	100.55	
	9	9.04968	100.55	

Table 7: Accuracy table of Estetrol

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	14.2	14.237576	100.26	99.43%
	14.2	14.067458	99.07	
	14.2	14.018100	98.72	
100%	28.4	28.224939	99.38	
	28.4	28.133595	99.06	
	28.4	28.080376	98.87	
150%	42.6	42.393723	99.52	
	42.6	42.720901	100.28	
	42.6	42.467724	99.69	

Discussion: Three levels of Accuracy samples were generated using the conventional addition procedure. Three injections were administered for each level of accuracy, resulting in mean %Recovery values of 100.58% for Drospirenone and 99.43% for Estetrol.

Sensitivity:

Table 8: Sensitivity table of Drospirenone and Estetrol

Molecule	LOD	LOQ
Drospirenone	0.04	0.13
Estetrol	0.54	1.63

Robustness:

Table 9: Robustness data for Drospirenone and Estetrol

S. No.	Condition	%RSD of Drospirenone	%RSD of Estetrol
1	Flow rate (-) 0.9ml/min	0.4	0.7
2	Flow rate (+) 1.1ml/min	0.3	0.9
3	Mobile phase (-) 65B:35A	0.6	0.9
4	Mobile phase (+) 55B:45A	0.3	0.3
5	Temperature (-) 25°C	0.6	0.4
6	Temperature (+) 35°C	0.5	0.9

Discussion: The robustness conditions, including a flow rate of 0.9 ml/min and 1.1 ml/min, a mobile phase composition of 50% B and 50% A, and a temperature range of 25°C to 35°C, were carefully maintained. The samples were injected in duplicate. The system suitability characteristics were minimally impacted and all metrics satisfied the required criteria. The %RSD value fell under the specified limit.

Assay: The product from Fourrts India is labeled as containing Drospirenone 3 mg and Estetrol 14.2 mg, marketed under the name Nextstellis. The assay was conducted using the aforementioned formulation. The average assay percentages for Drospirenone and Estetrol were 99.87% and 100.07% respectively.

Table 10: Assay Data of Drospirenone

S. No.	Standard Area	Sample area	% Assay
1	80385	80960	100.45
2	80264	80656	100.08
3	80674	80425	99.79
4	80435	80256	99.58
5	80589	80541	99.93
6	80250	81855	101.56
Avg	80433	80782	100.23
Stdev	171.3	576.4	0.72
%RSD	0.2	0.7	0.71

Table 11: Assay Data of Estetrol

S. no	Standard Area	Sample area	% Assay
1	406464	404015	98.52
2	418286	408262	99.55
3	406892	404245	98.57
4	407212	407265	99.31
5	409992	407720	99.42
6	406835	409743	99.91
Avg	409280	406875	99.21

Stdev	4593.5	2285.2	0.6
%RSD	1.1	0.6	0.6

6.8. Degradation data

Table 12: Degradation Data

Type of degradation	Drospirenone		Estetrol	
	% Recovered	% Degraded	% Recovered	% Degraded
Acid	93.87	6.13	93.83	6.17
Base	94.86	5.14	96.43	3.57
Peroxide	96.51	3.49	96.10	3.90
Thermal	97.93	2.07	98.78	1.22
Uv	96.87	3.13	98.45	1.55
Water	98.72	1.28	99.82	0.18

Summary and Conclusion

Table 13: Summary

Parameters		Drospirenone	Estetrol	Limit
Linearity		1.5-9 µg/ml	7.1-42.6 µg/ml	
Range(µg/ml)				
Regression coefficient		0.999	0.999	
Slope(m)		13263	14243	
Intercept(c)		345.0	1925	R< 1
Regression equation (Y=mx+c)		y = 13263x + 345.0.	y = 14243x + 14243.	
Assay (% mean assay)		100.23%	99.21%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.2	1.1	NMT 2.0%
Method precision %RSD		0.7	0.6	NMT 2.0%
Accuracy %recovery		100.58%	99.43%	98-102%
LOD		0.04	0.54	NMT 3
LOQ		0.13	1.63	NMT 10
Robustness	**FM**	0.4	0.7	
	FP	0.3	0.9	%RSD NMT
	MM	0.6	0.9	2.0
	MP	0.3	0.3	
	TM	0.6	0.4	
	TP	0.5	0.9	

Conclusion

A method was devised to accurately and precisely estimate the dosage of Drospirenone and Estetrolin Tablets together. The retention time for Drospirenone was determined to be 2.408 minutes, whereas the retention time for Estetrol was discovered to be 3.163 minutes. The percent relative standard deviation (%RSD) for Drospirenone was 0.2, while for Estetrol it was 1.1. Percentage. The recovery rates for Drospirenone and Estetrol were 100.58% and 99.43% respectively. The limits of detection (LOD) and limits of quantification (LOQ) for Drospirenone and Estetrol were determined to be 0.04 and 0.13, and 0.54 and 1.63, respectively, using regression equations. The regression equation for Drospirenone is y = 13263x + 345.0, while the regression equation for Estetrol is y = 14243x

+ 14243. The retention times and run time were reduced, making the new method simple and cost-effective for routine adoption in industrial quality control tests.

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