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Development and Validation of a New Analytical Method for the Simultaneous Estimation Odrospirenone and Estetrol in Pharmaceutical Dosage Form

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Keywords: Drospirenone, Estetrol, RP-HPLC.

ABSTRACT

The simultaneous inference of drospirenone and estetrol in tablet dosage form was achieved by the development of a straightforward, accurate, and focused procedure. Ascentis C18 150 x 4.6 mm, 2.7m Chromatogram run. Buffer Ammonium Acetate-containing mobile phase: ACN was diluted 50:50 and pushed through the column at a flow rate of 1 ml/min. This approach employed ammonium acetate as a buffer. 30°C was kept as the temperature. The chosen optimized wavelength was 240.0 nm. Drospirenone and Estetrol had a respective retention duration of 2.514 min. and 3.180 min., and their %RSD values were 0.8 and 0.9, respectively. % For drospirenone and estetrol, recovery was 99.65% and 99.79%, respectively. LOD and LOQ values for drospirenone and estetrol, respectively, were 0.09, 0.28 and 0.19, 0.59 from regression models. Regression equation of Drospirenone is y = 57669x + 1713. and y = 61148x + 3213 of Estetrol. RT was decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

INTRODUCTION:

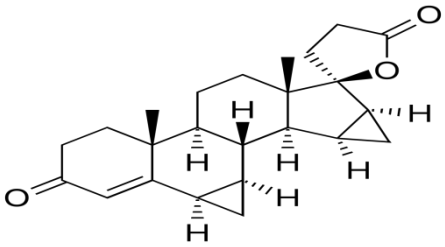
Combined oral contraceptives (COC) have been widely used, primarily due to COC's high oral bioavailability, long half-life, and high potency, which allow for the efficiency of low doses. However, there is a slight increase in the risk of cardiovascular complications due to EE's effects on the vascular endothelium and liver protein synthesis related to coagulation, fibrinolysis, and blood pressure.[1,2]. It includes estrogen and a progestin to prevent ovulation.[3]. Trials are currently in Phase 3 for an oral contraceptive that combines Drospirenone (DRSP) 3 mg and E4 15 mg.[4]. During pregnancy, human maternal plasma levels rise and peak at high quantities (1 ng/mL) at the end of gestation. It has been observed that at parturition, foetal plasma levels are almost 20 times greater than maternal plasma levels.[5].Different investigations from the 1970s and 1980s showed that E4 had less estrogenic action than E2, E3, and tamoxifen; this activity has been shown in the uterus.[6-8]

Estetrol (E4) is a natural oestrogen that is only created by the liver of the foetus in humans.[10].It has a different method of action from selective estrogen receptor modulators and works, unlike EE, selectively in tissues while having mixed agonist and antagonist estrogenic activity[9-12].

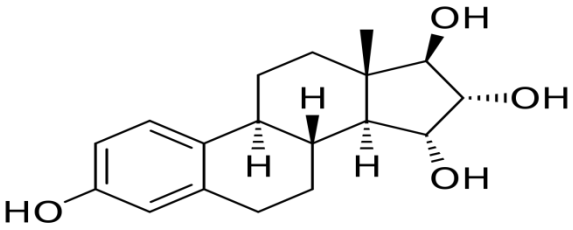
Drospirenone (DRSP), is a fourth-generation progestin with significant progestogenic, anti-mineralocorticoid, and antiandrogenic action. These properties set it apart from other progestogens found in the majority of other OCPs on the market today. This formulation has the added medical benefit of being a decent parallel therapy for mild acne and premenstrual dysphoric disorder in addition to being an efficient long-term OCP. We cover 3 mg DRSP/20 g EE-24/4's efficacy, tolerance, safety, and extra non-contraceptive advantages[13].

The combined permanent sterilization of women (27.7%) and vasectomies (10.9%) are more prevalent than oral contraceptives as a reversible form of contraception in America (26.9%). OCPs are used by 53% of US contraceptive users between the ages of 15 and 24 because they are highly effective, simple to use, have few adverse effects, and provide strong cycle control[14].

Despite their continuous necessity, sexually active, reproductive-age women may stop using effective hormonal contraception if the negative effects are intolerable. OCP users usually stop using it because to side effects such as irregular bleeding, nausea, headaches, and fluid retention-related symptoms like breast tenderness and bloating.[15].



1. Structure of Drospirenone



2. Structure of Estetrol

Figure-1, 2: Structures of Drospirenone and Estetrol

Several analytical approaches have been described; however, according to a thorough assessment of the literature[17-22]. No method for stability-indicating estimation has been reported. As a result, a simple, cost-effective stability-indicating simultaneous estimate of Esterol and Drospirenone pharmaceutical dosage form by RP-HPLC in pharmaceutical dosage form must be developed and validated by ICH (Q2 standard)[16].

MATERIALS AND REAGENTS

Pure medications Drospirenone and Estetrol were delivered by Akrivis Pharma Private Limited of Hyderabad. A local pharmacy provided the Drospirenone and Estetrol (Nextstellis) combination tablet. All of the chemicals and buffers used in this method were given by Rankem in India.

INSTRUMENTATION

WATERS HPLC, model: 2695 SYSTEM with Photodiode array detector was used for the development and method validation, with an automated sample injector with software Empower 2.

CHROMATOGRAPHIC CONDITIONS:

Flow rate: **1**ml/min

Column: Ascentis C8 (4.6 x 150mm, 2.7µm)

Mobile phase: 0.1% OPA : Acetonitrile

Detector: 240nm

Temperature: 30°C

Injection volume: 10.0µL

Run time: 10 mins

Diluent: Water and Acetonitrile in the ratio 50:50

PREPARATION OF SOLUTIONS

Preparation of 0.01NAmmonium Acetate Buffer: Add 0.77 g of ammonium acetate to the solution of 1000ml of distilled water in suitable container, sterilize the solution by passing it through a 0.22micron filter. Store the solution in tightly sealed bottles at 4^0C or at room temperature. Ammonium acetate decomposes in hot H2O and solutions containing it should not be autoclaved.

Preparation of Standard stock solutions: Accurately weighed 14.2mg of Estetrol, 3mg of Drospirenone and transferred to 50ml volumetric flask and3/4 th of diluents was added to these flasks and sonicated for 10 minutes. Flask was made up with diluents and labeled as a Standard stock solution. (284µg/ml of Estetrol and 60µg/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 the 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).

METHOD VALIDATION

The validation of the HPLC method was carried out by the ICH recommendations for the simultaneous estimation of Drospirenone and Estetrol drug material to show that the method is suitable for routine analysis.

System suitability:

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 the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injection results should not be more than 2%.

Specificity (Selectivity): Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.

Table 1: System suitability results

S no	**Drospirenone**			**Estetrol**			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.517	11545	1.19	3.097	9143	1.07	4.8
2	2.564	11286	1.19	3.151	9351	1.06	5.1
3	2.566	11029	1.18	3.152	9445	1.07	5.1
4	2.566	11228	1.15	3.153	9609	1.07	5.2
5	2.566	10969	1.17	3.154	9341	1.07	5.2
6	2.566	11198	1.15	3.156	9311	1.06	4.8

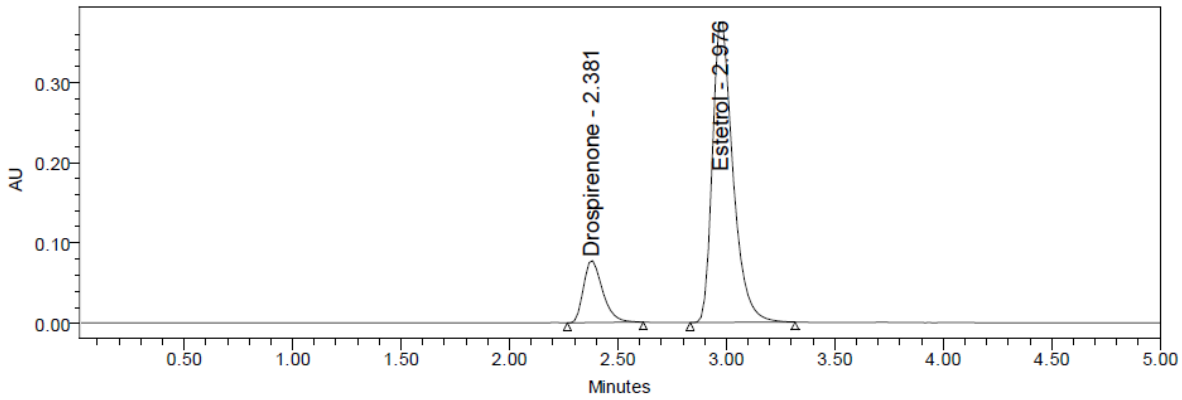
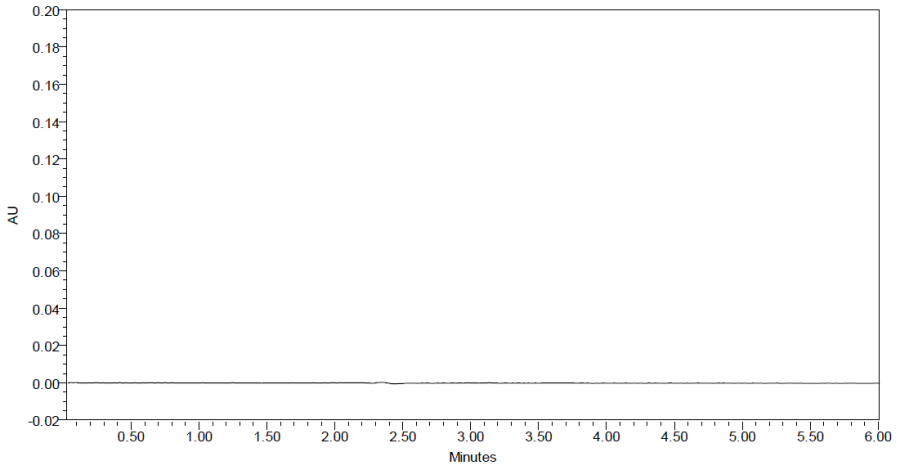


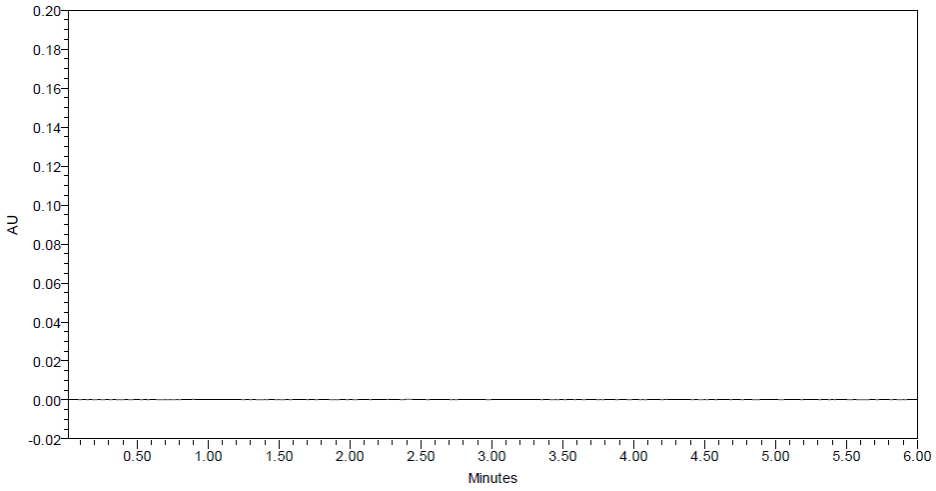
Figure 3: System suitability Chromatogram of Drospirenone and Estetrol.

Table 2: Specificity data

Sample name	Retention time(mins)	Area
Drospirenone	2.600	359290
Estetrol	3.200	1761279



Blank Chromatogram



Placebo Chromatogram

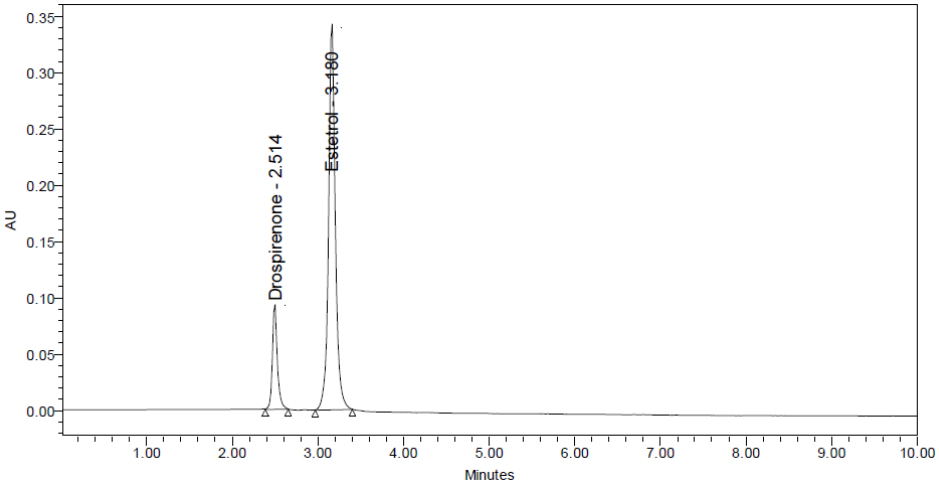


Figure 4: Specificity Chromatograms of Drospirenone and Estetrol.

Table 3: Drospirenone Linearity

% Level	CONC	Area
0	0	0
25%	1.5	89022
50%	3	174020
75%	4.5	262132
100%	6	350206
125%	7.5	435600
150%	9	517582
R^2 value		**0.999**

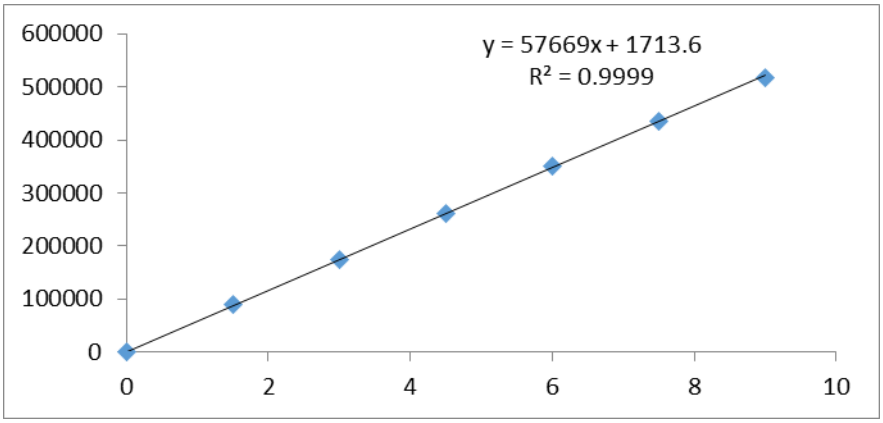


Figure 5: Drospirenone Calibration curve

Table 4: Estetrol Linearity

% Level	**CONC**	**Area**
0	0	0
25%	7.1	445716
50%	14.2	870520
75%	21.3	1285318
100%	28.4	1759463
125%	35.5	2177204
150%	42.6	2601428
R^2 value		**0.999**

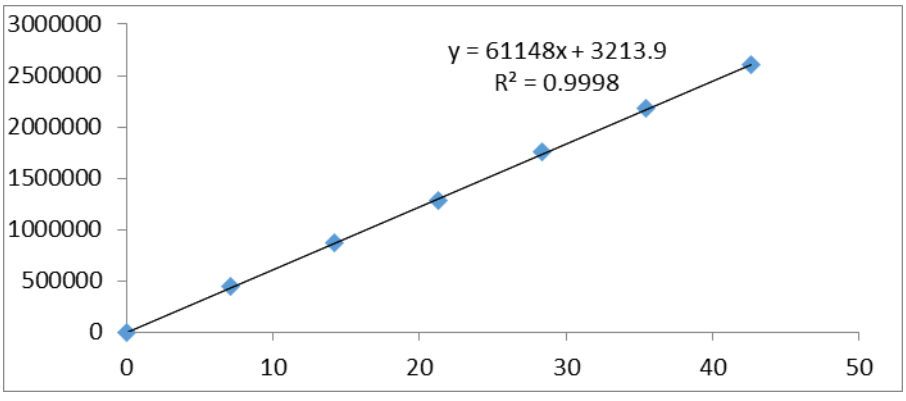


Figure 6: Estetrol Calibration curve

Table 5: Accuracy (%Recovery data)

%Level	**%Recovery**					
	Drospirenone			**Estetrol**		
	Amt added	Amt found	%Rec	Amt added	Amt found	%Rec
50% Level	3	3.00	100.00	14.2	14.2	100.3
	3	3.01	100.45	14.2	14.2	99.7
	3	2.97	99.04	14.2	14.1	99.6
100%Level	6	6.03	100.58	28.4	28.5	100.4
	6	5.98	99.65	28.4	28.4	100.1
	6	5.99	99.80	28.4	28.5	100.3
150%Level	9	8.92	99.12	42.6	42.2	99.1
	9	8.92	99.06	42.6	42.3	99.4
	9	8.92	99.15	42.6	42.3	99.2
Mean%			**99.65%**			**99.79%**

System Precision: The system precision was performed by analyzing six replicate injections of standard solution at 100% of the specified limit concerning the working strength of Drospirenone and Estetrol. Results of peak area are summarized in Table 7.

Table 6: System precision data

S. No	**Area of Drospirenone**	**Area ofEstetrol**
1.	357442	1783909
2.	360848	1778531
3.	359290	1761279
4.	358201	1777654
5.	352527	1803731
6.	355661	1802373
Mean	357328	1784580
S.D	2927.2	16194.4
%RSD	0.8	0.9

The % RSD for the peak areas of Drospirenone and Estetrol obtained from six replicate injections of standard solution was within the limit.

Method Precision: The precision of the method was determined by analyzing a sample of Drospirenone and Estetrol (Six individual sample preparations). Data obtained is summarized in Table 8.

Table 7: Method precision data

Injection	Drospirenone	Estetrol
1.	360929	1793038
2.	358499	1780921
3.	362290	1790340
4.	358260	1790090
5.	362119	1782608
6.	356605	1795645
Mean	359784	1788774
S.D	2329.1	5818.3
%RSD	0.6	0.3

From the above results, the % RSD of the method precision study was within the limit for Drospirenone and Estetrol.

Table 8: Robustness results

Chromatographic condition	Drospirenone (RSD)	Estetrol (RSD)
Flow rate (-) 0.9ml/min	0.2	0.3
Flow rate (+) 1.1ml/min	0.5	0.8
Mobile phase (-) 45B:55A	1	0.1
Mobile phase (+) 55B:45A	0.9	0.5
Temperature (-) 25°C	0	0
Temperature (+) 35°C	0.8	1

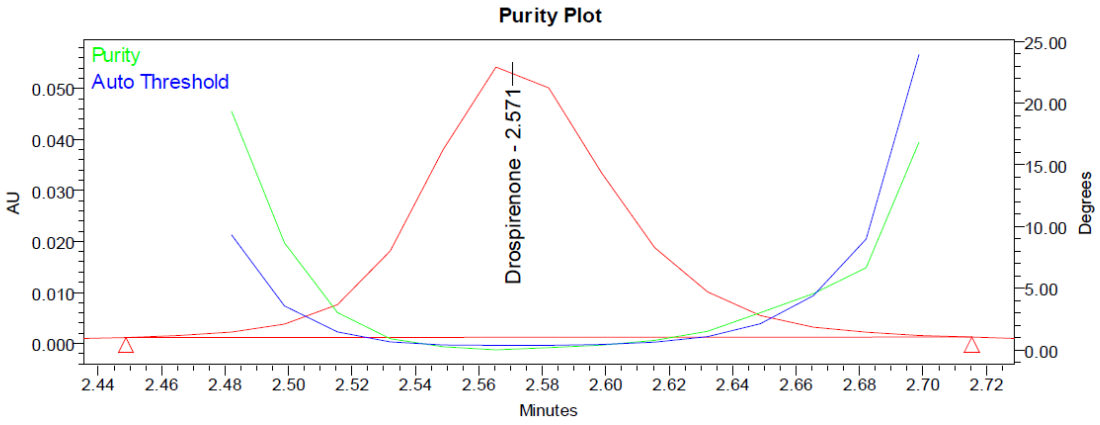
Table 9: Forced degradation conditions for Drospirenone and Estetrol

Stress condition	**Solvent**	**Temp(^{0}C)**	**Exposed time**
Acid	2N HCL	60^{0}C	30 mins
Base	2N NAOH	60^{0}C	30 mins
Oxidation	20% H_2O_2	60^{0}C	30 mins
Thermal	Diluent	105^{0}C	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}C	

From the results, no degradation was observed when the samples were exposed to acid, base, hydrolysis, thermal, light and water. According to the stress study, none of the degradant co-eluted with the active drug peaks formed.

Table 10: Degradation profile results

Degradation condition	**Drospirenone % Degraded**	**Estetrol % Degraded**
Acid	7.14	7.10
Base	4.86	5.53
Oxidation	6.24	4.63
Thermal	2.47	2.28
Photolytic	1.00	1.14
Hydrolytic	1.00	0.90



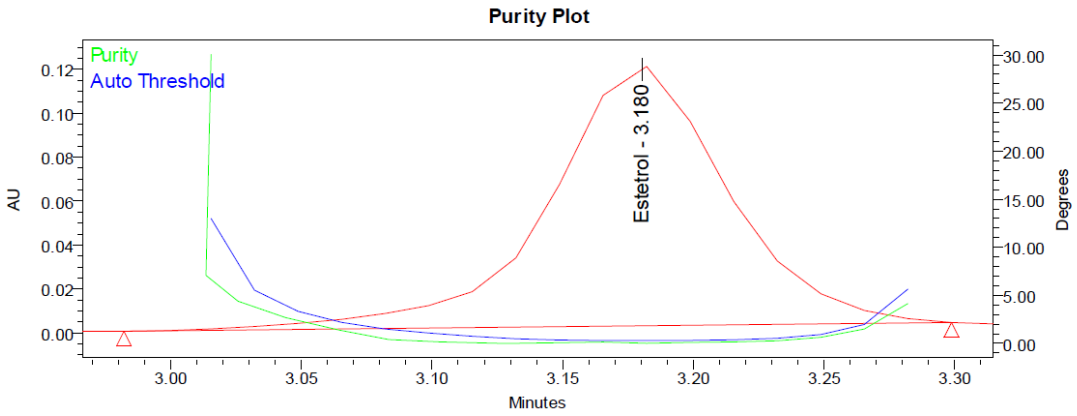


Fig no 7. Degradation purity plots

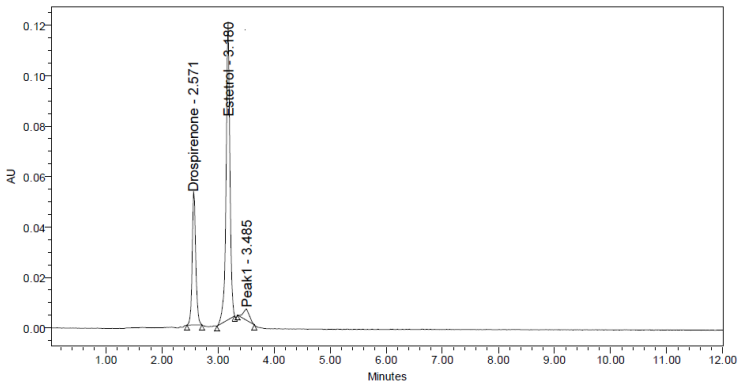


Fig no 8. Acid degradation chromatogram

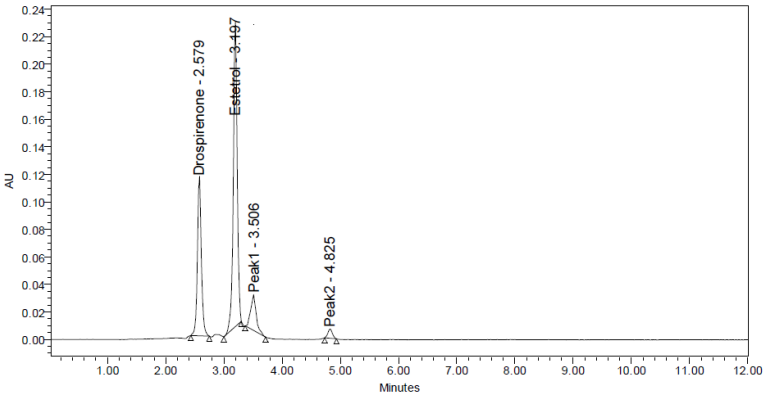


Fig no 9. Base degradation chromatogram

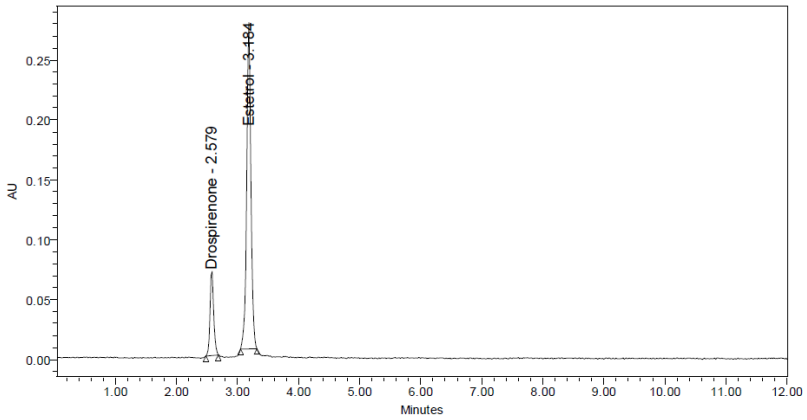


Fig no 10, Peroxide degradation chromatogram

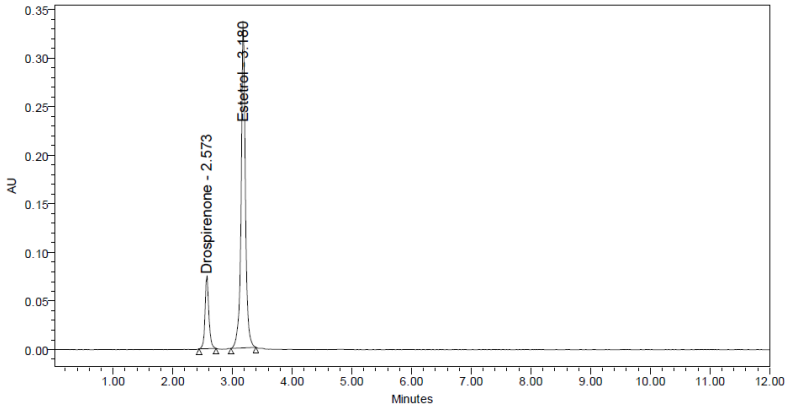


Fig no 11. Thermal degradation chromatogram

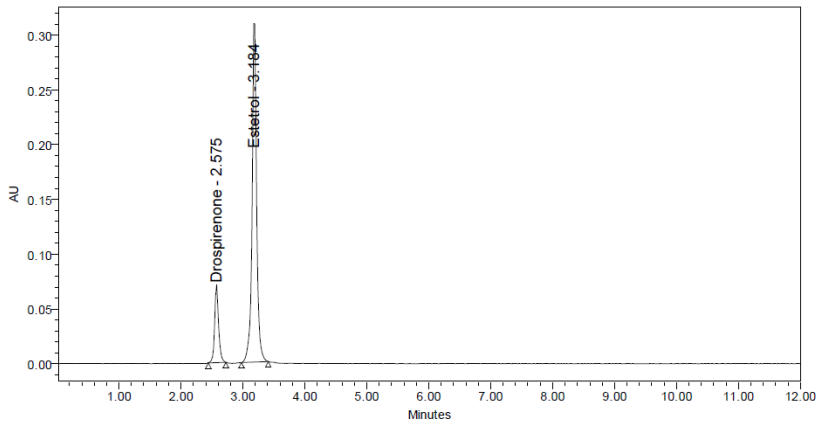


Fig no 12. UV degradation chromatogram

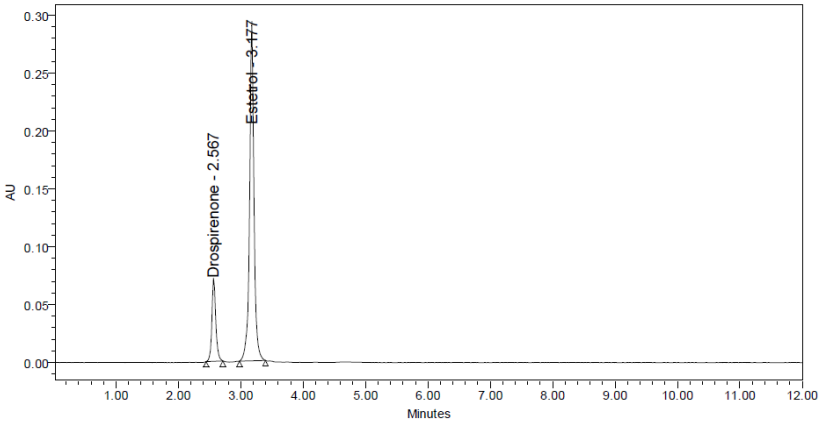


Fig no 13. Water degradation chromatogram

Table 11: Assay results for Drospirenone and Estetrol

Drug name	**Label claim dose**	**%Assay**	**Brand name**
Drospirenone	3mg	100.49%	***Nextstellis***
Estetrol	14.2mg	100.03%	

CONCLUSION

For the simultaneous estimate of the Drospirenone and Estetrolin Tablet dose form, an easy, accurate, and exact approach was established. Drospirenone and Estetrol had a respective retention duration of 2.514 min. and 3.180 min., and their %RSD values were 0.8 and 0.9, respectively. % For drospirenone and estetrol, recovery was 99.65% and 99.79%, respectively. LOD and LOQ values for drospirenone and estetrol, respectively, were 0.09, 0.28 and 0.19, 0.59 from regression models. Drospirenone's regression equation is y = 57669x + 1713, whereas Estetrol's is y = 61148x + 3213. As a result of shorter retention durations and shorter run times, the method was created to be straightforward and cost-effective, and it may be used for routine Quality Control Tests in Industries.

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