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Method Development and Validation for the Simultaneous Estimation of Drospirenone and Estetrol in Bulk and Pharmaceutical Dosage form by RP-HPLC

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ABSTRACT

For the simultaneous stimulation of drospirenone and estetrol in API and pharmaceutical dosage form, a simple, precise accurate and durable reverse phase RP-HPLC methods has been devises and validated. This approach uses a simple isocratic mobile phase of Ortho phosphoric acid and acetonitrile (55:45), HPLC system for chromatographic separation for chromatographic separation and PDA detection. Average retention time of drospirenone and estetrol was found to be 3.011 min and 2.403. The % assay for drospirenone and estetrol was determined to be 99.51% and 100.29%, indicating that the method is suitable for routine analysis. The linearity of drospirenone and estetrol was discovered to be linear with r^2 of 0.999 for all medications, indication that the approach can yield good sensitivity. The precision acceptance is that the % RSD should not exceed 2.0% and the method precision of 0.3 and 0.5 for drospirenone and estetrol respectively, indicating that the method is precise.

Key words: *Method development, validation, drospirenone, estetrol, linearity, RP-HPLC.*

INTRODUCTION

Drospirenone is a progestin used in oral contraceptive pills for the prevention of pregnancy and other conditions. Drospirenone is a synthetic progestin commonly found in the popular oral contraceptive, Yaz in combination with Ethinyl estradiol. Most recently, it was approved by both Health Canada and the FDA in combination with Estetrol as an oral contraceptive therapy. Aside from its contraceptive effects, drospirenone is used with estrogens to control acne and premenstrual dysphoric disorder (PMDD) [1]. Drospirenone is an analog of the diuretic spironolactone, which exerts anti-mineralocorticoid activity, blocking aldosterone receptors, which increases sodium and water excretion. Studies in animals have demonstrated that Drospirenone administration leads to anti-androgenic activity. This activity helps to oppose the effects of naturally occurring androgens, inhibiting the binding of dihydrotestosterone (DHT) to its receptor, and preventing androgen synthesis in the ovaries, helping to treat acne and hirsutism [2]. Drospirenone may also decrease the level of edema in sebaceous follicle during the second half of the menstrual cycle, when acne often appears. Drospirenone and ethinyl estradiol in combination suppress the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH), preventing ovulation. Other changes induced by this drug which may aid in the prevention of pregnancy include alterations in cervical mucus consistency, hindering sperm movement, and lowering the chance of embryo implantation. Drospirenone is sold under the brand name of Slynd [3,4].

Estetrol is an estrogen used in combination with drospirenone for oral contraception. Naturally or synthetically produced steroid estrogens have a wide range of pharmaceutical

uses ranging from hormonal contraception to the treatment of menopausal symptoms. Estetrol (E4) is a native estrogen occurring naturally during pregnancy, but can be synthesized from a plant source and used for contraception [5]. It is more potent and is safer than the synthetic estrogen ethinylestradiol (EE2) found in 97% of oral contraceptive pills, reducing the environmental accumulation of unwanted endocrine disrupting chemicals (EDCs) that often lead to harmful epigenetic effects. Estetrol is a synthetic analogue of a naturally occurring estrogen present during pregnancy, demonstrating selectivity for both estrogen receptor-α (ER-α) and ER-β and suppressing ovulation. Estetrol binds with a low to moderate affinity human estrogen receptor alpha (ER alpha) and ER beta with a preference for ER alpha. Estetrol demonstrates a unique mechanism of action via tissue selective activity, showing estrogen receptor agonist activity on the vagina, the uterus and the endometrium, and negative estrogenic activity on breast tissue. Estetrol is sold under brand name of Nextstellis [6-8].

The term "fixed dosage composition formulation" refers to the drugs that contain two or more medical medications together in single formulation dose. In combination Drospirenone and Estetrol both are used for the prevention of pregnancy [9].

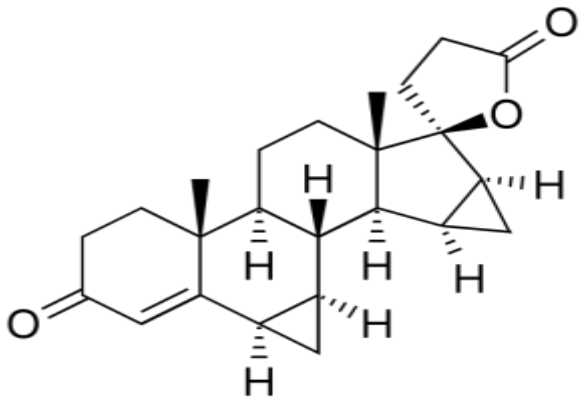

Fig.1: Structure of Drospirenone

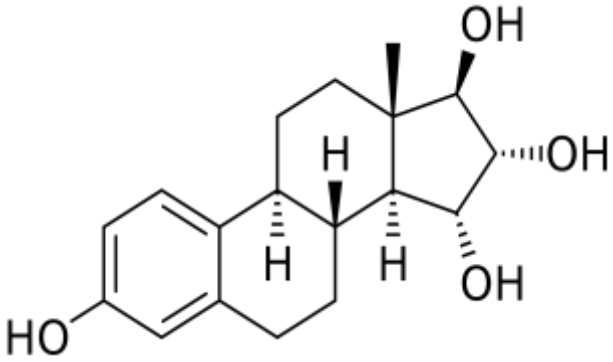

Fig. 2: Structure of Estetrol

Literature survey revealed that there are some methods reported for simultaneous estimation of these drugs, some methods for estimation of individual drugs include RP-HPLC and UV-Spectroscopy. The aim of this study is to develop a simple, accurate, and relatively sensitive and RP-HPLC technique for estimation of drospirenone and estetrol in bulk and tablet dosage form. Validated all these methods is applied for drospirenone and estetrol estimation as per the ICH guidelines.

MATERIALS AND METHODS

Chemicals

Sun Pharma Limited, Hyderabad has provided the drospirenone and estetrol pure API drugs. Renkem India provided all the chemicals and buffers utilized in this method. All the solvents used for work are HPLC grade chemicals.

Instrumentation and Chromatographic Condition

AGILENT HPLC, model G4-286b-HPLC system with photo diode array detector was used for the development and method validation with an automated sample injector. Aglient (150mm 4.5mm 3.5mm) column was used for the separation. Acetonitrile and water are used as mobile phase (50:50). Analysis was carried out in isocratic mode with flow rate of 1.0ml/min and injection volume were 10 µL. The column temperature was 30 °C; the run time was 6 min. The data acquired was at 240nm. The output signal was monitored and integrated using Empower2 software.

Preparations of Solutions

Diluent

Diluent used was acetonitrile and water in the ratio of 50:50.

Preparation of standard stock solution [10-12]

Accurately weighed 14.2mg of estetrol, 3mg of drospirenone and transferred to 50ml volumetric flask and 3/4th of diluents was added to these flasks and sonicated for 10 min. Flask was made up with diluents and labeled as Standard stock solution and from each stock solution 1ml was pipetted out and taken into a 10ml volumetric flask and made up with diluent (28.4µg/ml Estetrol of and 6µg/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 estetrol of and 6µg/ml of drospirenone).

Preparation of Sample 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 and from these 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).

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).

Preparation of Buffer [13]

0.1% OPA buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water and pH was adjusted.

Method Validation [14-15]

The method validation of HPLC was carried out for the simultaneous estimation of drospirenone and estetrol drug substance as per ICH guidelines to demonstrate that the method is proposed for routine analysis.

RESULTS AND DISCUSSION

System Suitability: The system suitability was performed for each validation parameters by injecting standard solutions containing drospirenone (6µg/ml) and estetrol (28.4µg/ml). The % RSD for the area of six standard injections results should not be more than 2%. System suitability parameters are shown in figure 2 and values and mentioned in Table 1.

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. Representative chromatogram is shown in Figure 3 and the experimental data is shown in Table 2.

Table 1: System Suitability Results

S. No.	Drospirenone			Estetrol			
Injection	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	3.013	5186	1.45	2.398	6132	1.05	4.2
2	3.016	5216	1.39	2.398	6253	1.06	4.2
3	3.016	4995	1.37	2.399	5990	1.04	4.1
4	3.016	5170	1.39	2.400	6170	1.06	4.2
5	3.017	5155	1.39	2.400	6420	1.05	4.2
6	3.018	5218	1.38	2.400	6122	1.06	4.1

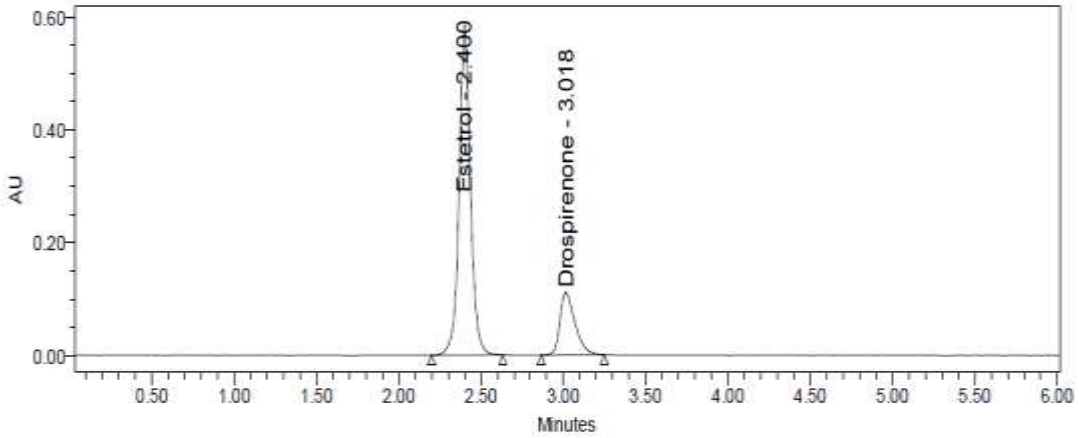

Fig. 3: System Suitability Chromatogram of Drospirenone and Estetrol

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

Table 2: Specificity Data

Sample Name	Retention Time (min)
Drospirenone	2.257
Estetrol	2.928

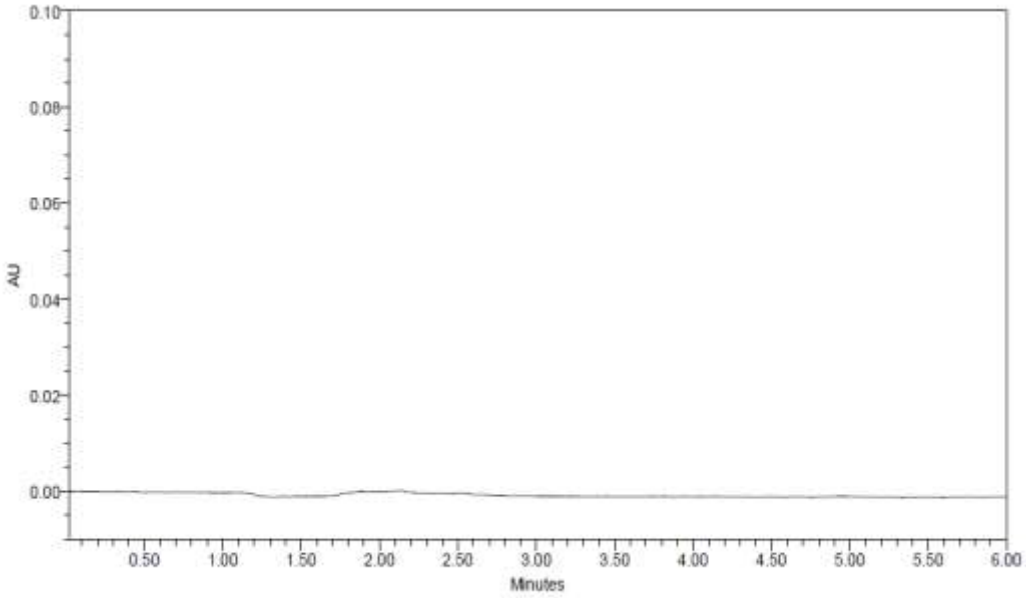

Fig. 4: Blank Chromatogram

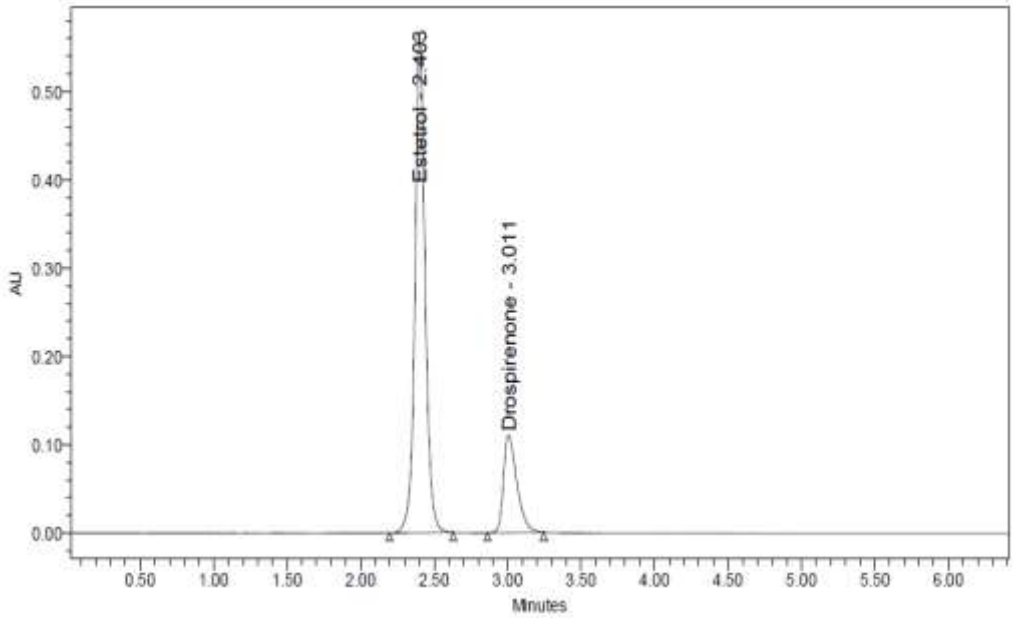

Fig. 5: Specificity Chromatogram of Drospirenone and Estetrol

Table 3: Linearity for Drospirenone and Estetrol

Drospirenone		**Estetrol**	
Conc. (µg/mL)	**Peak area**	**Conc. (µg/mL)**	**Peak area**
0	0	0	0
1.5	176517	7.1	699030
3	356514	14.2	1366524
4.5	524012	21.3	2032828
6	708968	28.4	2763528
7.5	890823	35.5	3487203
9	1056826	42.6	4045838

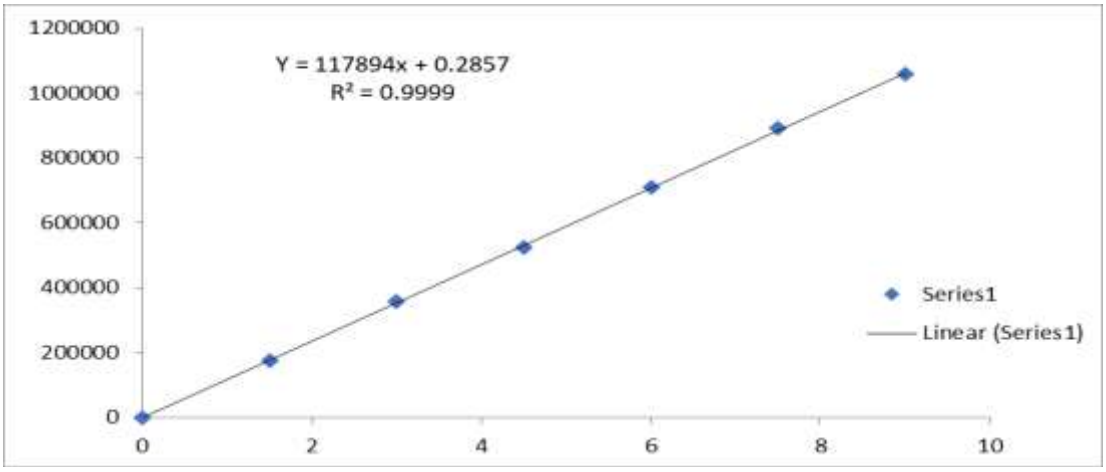

Fig. 6: Drospirenone Calibration Curve

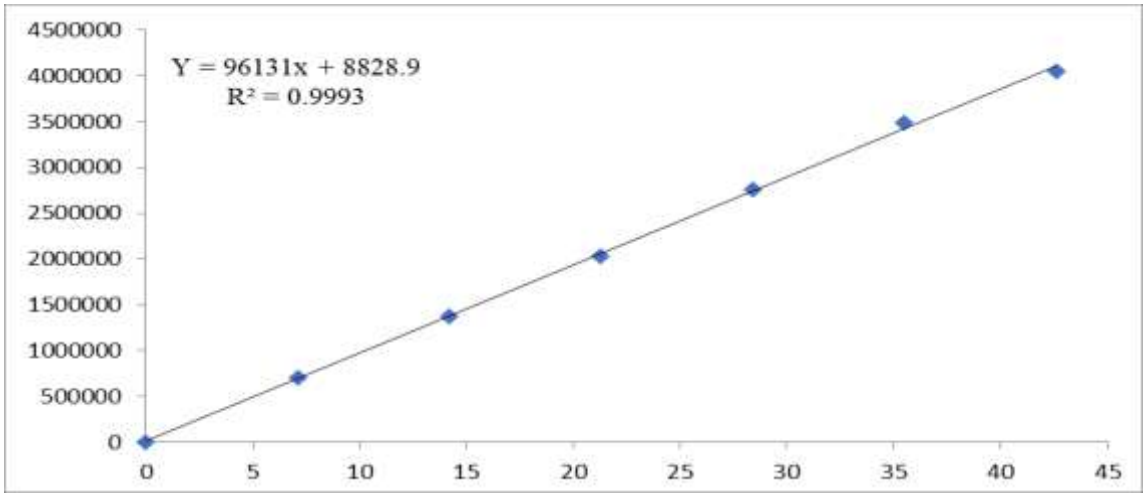

Fig.7: Estetrol calibration curve

Six linear concentrations of drospirenone (1.5-9µg/ml) and estetrol (7.1-42.6µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for drospirenone was y = 11789x + 0.285 and of estetrol was y = 96131x + 8828 Correlation coefficient obtained was 0.999 for the two drugs.

Table 4: Accuracy of Drospirenone

% Level	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % recovery
50%	3	2.975645	99.19	**99.51**
	3	2.983279	99.44	
	3	3.000490	100.02	
100%	6	5.961777	99.36	
	6	5.978707	99.65	
	6	5.964669	99.41	
150%	9	8.918416	99.09	
	9	8.934294	99.27	
	9	9.016470	100.18	

Table 5: Accuracy of Estetrol

% Level	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % recovery
50%	14.2	14.07753	99.14	**100.29**
	14.2	14.25013	100.35	
	14.2	14.15807	99.70	
100%	28.4	28.34553	99.81	
	28.4	28.44198	100.15	
	28.4	28.59101	100.67	
150%	42.6	42.97405	100.88	
	42.6	43.08970	101.15	
	42.6	42.91272	100.73	

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.51% and 100.29% for drospirenone and estetrol respectively.

Table 6: System Precision Data of Drospirenone and Estetrol

S. No.	Area of Drospirenone	Area of Estetrol
1	704641	2714902
2	703566	2759438
3	701621	2758808
4	702187	2739975
5	706500	2779526
6	707458	2754810
Mean	704329	2751243
S.D	2328.3	21838.8
% RSD	0.3	0.8

The Above %RSD for the peak areas of drospirenone and estetrol obtained from six replicate injections of standard solutions which was within range of limit (<2%).

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.3% and 0.8% respectively for drospirenone and estetrol. As the limit of Precision was less than “2” the system precision was passed in this method.

Table 7: 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

Robustness conditions like flow minus (0.9ml/min), flow plus (1.1ml/min), mobile phase minus (50B:50A), mobile phase plus (60B:40A), 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.

Table 8: % Assay of Drospirenone and Estetrol

Drug name	Label claim	% Assay	Brand name
Drospirenone	3mg	99.87	Fourrts India
Estetrol	14.3mg	100.07	Nextstellis

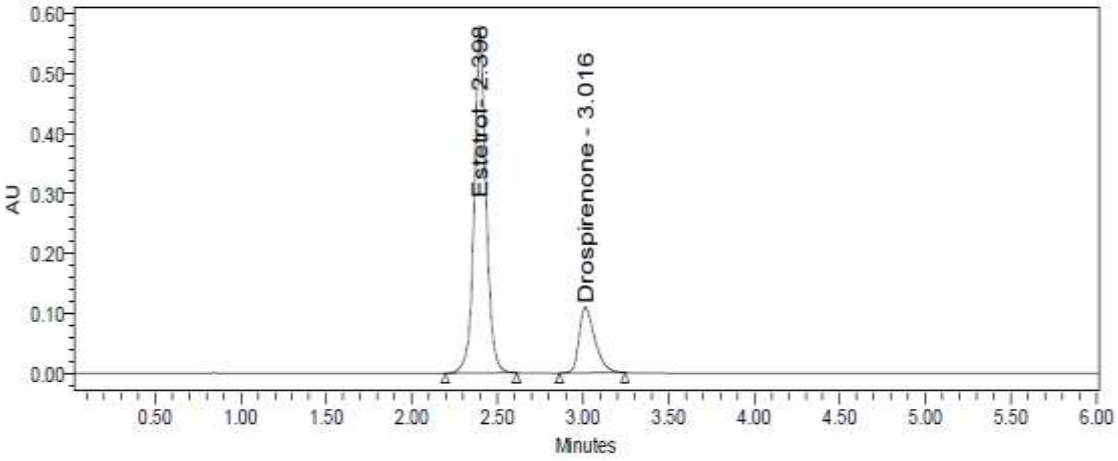

Fig. 8: % Assay of Drospirenone and Estetrol

Table 9: LOQ and LOD data

Drug	LOD	LOQ
Drospirenone	0.09	0.26
Estetrol	0.36	1.10

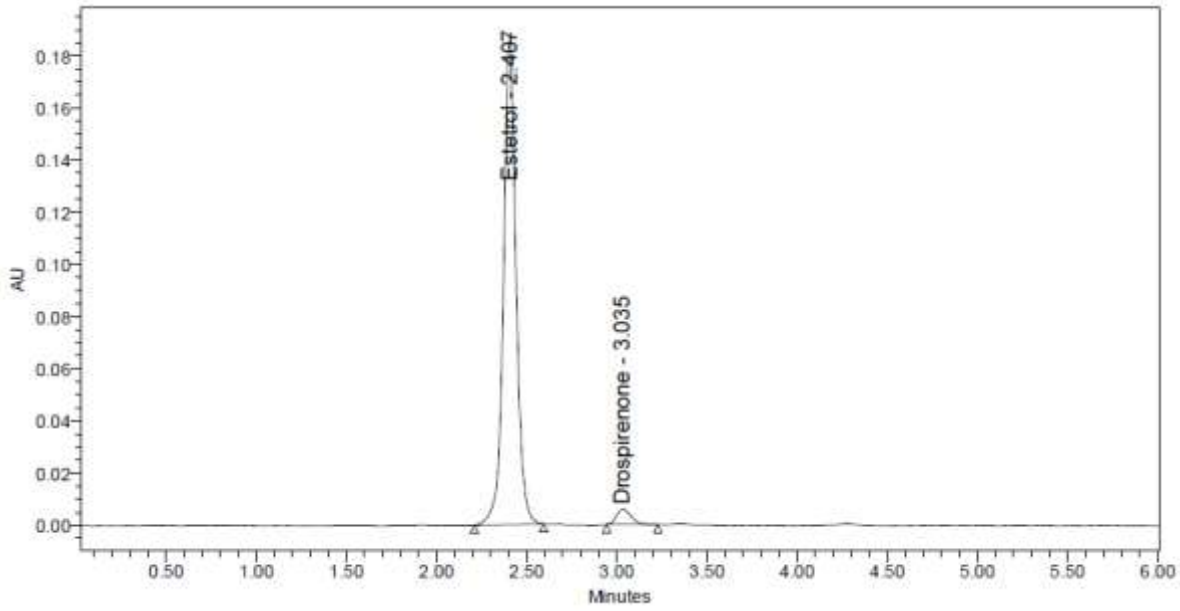

Fig.9: LOD Chromatogram of Standard

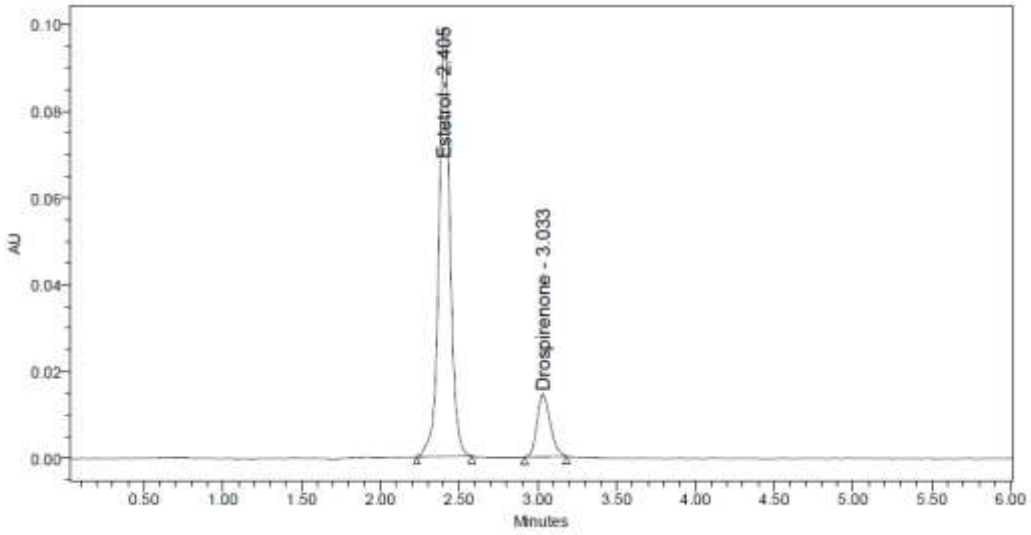

Fig.10: LOQ Chromatogram of Sample

A simple, accurate, precise method was developed for the simultaneous estimation of the drospirenone and estetrol in bulk and in tablet dosage form. Retention time of drospirenone and estetrol were found to be 3.011 min and 2.403. % RSD of the drospirenone and estetrol were and found to be 0.3 and 0.8 respectively. %Recovery was obtained as 99.51% and 100.29% for drospirenone and estetrol respectively. LOD, LOQ values obtained from regression equations of drospirenone and estetrol were 0.09, 0.26 and 0.36, 1.10 respectively. Regression equation of drospirenone is y = 11789x + 0.285. and y = 96131x + 8828 of estetrol. Retention times were 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.

CONCLUSION

A new stability indicating RP-HPLC technique was developed and validated for the simultaneous estimation of drospirenone and estetrol in bulk and pharmaceutical dosage form (tablets). The developed method was precise, accurate, higher resolutions, shorter retentions with various degradants and economical. Hence, this method can be used for in process evaluation in pharmaceutical firms and quality control of these in drug testing.

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ETHICAL APPROVAL

This study does not involve experiments on animals or human subjects.

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