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Research Article

Analytical Method Development and Validation for Simultaneous Estimation of Ethinyl Estradiol and Drospirenone By HPLC in Combined Dosage Form

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ABSTRACT

A simple, accurate, rapid and precise isocratic High performance liquid chromatographic (HPLC) method was developed and validated for the determination of Ethinyl estradiol and drospirenone in tablet formulation. The method employs Waters HPLC system on Prontosil C18ace-EPS, 30cmX3.0 mm; followed in series by chromalith RP-18E C18, 10cm x 4.6 mm, 3µm and flow rate of 0.5 ml/min and injection volume 20 µL. The detection was carried out at 222 nm for drospirenone and for Ethinyl estradiol Fluorescence Detector: Excitation at 215nm and Emission at 315nm Fluorescence Detector: Excitation at 215nm and Emission at 344nm. Retention Time of Ethinyl estradiol and drospirenone were found to be 47 minutes and 34 minutes and For Ethinyl Estradiol Related compound B 41 minutes. This developed method was successfully utilized for the quantitative estimation of Ethinyl estradiol and drospirenone in pharmaceutical dosage forms. This method was validated for accuracy, precision, linearity and Robustness as per ICH guidelines.

Key words: HPLC; ICH; Ethinyl Estradiol ; Drospirenone ; Validation

1. INTRODUCTION

Ethinyl estradiol is also known as ethynyl estradiol (EE) which is a derivative of 17β – estradiol. It is the first orally active semi synthetic steroidal estrogen that is used for the management of menopausal symptoms and female hypogonadism. Ethinyl estradiol is an orally bioactive estrogen used in almost all modern formulations of combined oral contraceptive pills. Chemically it is 19-Nor-17α-pregna-1, 3, 5(10)-trien-20-yne-3, 17-diol [1,2]. Drospirenone is an analogue of the antimineralocorticoid spironolactone that is synthesized from androstenone. Unlike other progestogens, drospirenone, an analogue of spironolactone, has biochemical and pharmacologic profiles similar to endogenous progesterone, especially regarding antimineralocorticoid and antiandrogenic activities. As a combination, oral contraceptive, drospirenone with Ethinyl estradiol, is effective and has positive effects on weight and lipid levels[3,4]. So far to our present knowledge, HPLC methods were available in the literature for analyzing Ethinyl estradiol and drospirenone with other drug combinations in pharmaceutical dosage forms[5]. It felt necessary to develop a simple, precise and rapid spectrophotometric method for the quantitative determination of Ethinyl estradiol and drospirenone in combined dosage form. These are hormonal preparation used for reversible suppression of fertility. Because of our alarming population trends, antifertility drugs are the need of the day. In developing countries particularly, the mortality rate declined and birth rate has increased due to urbanization. In the earlier part of 20th century, methods of contraception used (condoms, diaphragms, spermicidal creams,

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foam tablets etc.) were intimately related to sexual intercourse, therefore, despised by most couples. These also have higher failure rate. Rock and Pincus announced the successful use of an oral progestin for contraception in 1955. Hormonal contraception refers to birth control methods that act on the endocrine system. Almost all methods are composed of steroid hormones. This type of birth control contains either progestin, estrogen or both. The original hormonal method the combined oral contraceptive pill was first marketed as a contraceptive in 1960[6,7].

2. MATERIALS AND METHODS

2.1 Materials and instruments

Working standards used:

Pharmaceutical grade Ethinyl estradiol and drospirenone working standards, sample and impurities were kindly supplied as a gift sample by Alpa Laboratories Private Limited, Sector 3, Pithampur, Madhya Pradesh, India.

Reagents and solvents used:

Water (HPLC grade, Milli Q or equivalent), Acetonitrile (HPLC grade, JT Baker or equivalent) Methanol (HPLC grade, JT Baker or equivalent)

Apparatus and instruments used in experiment are listed in table 1.

Table 1: Apparatus / Instruments used

S. No.	Instruments	Make	Software	Detector/ Model No.	Separation Module
1	HPLC	Waters Alliance	Empower 2	UV/2996	2695
2	HPLC	Waters Alliance	Empower 2	PDA/2998	2695
3	Weight balance	Mettler Toledo	NA	ML204	NA
4	Sonicator	Lab India	NA	NA	NA

2.2 Methodology

2.2.1 Preparation of Diluent

Prepared a mixture of Acetonitrile: Methanol: Water in the ratio of (26:19:55, v/v).

2.2.2 Mobile Phase

Mobile Phase A - Acetonitrile: Methanol: Water (26: 19:55, v/v).

Mobile Phase B- Acetonitrile: Methanol: Water (76: 19: 5, v/v).

HPLC gradient programming and chromatographic conditions are shown in table 2 and 3.

Table 2: HPLC gradient programming

Time (min.)	Flow rate (mL)	Mobile phase A (%)	Mobile phase B (%)
0	0.5	90	10
40	0.5	90	10
53	0.5	0	100
59	0.5	0	100
60	0.5	90	10
70	0.5	90	10

2.2.3 Preparation of System suitability stock solution

Weighed accurately about 54.0 mg of Drospirenone working standard and transfered it into 100.00 mL volumetric flask. Added about 70 mL of Diluents and sonicate to dissolve. Allowed it to come to room temperature and diluted upto the mark with Diluents' and mixed.

2.2.4 Preparation of System suitability solution

Transfered 1.0 ml of System suitability stock Solution into 10.0ml volumetric flask, added 1.0ml of 0.1N HCl and heat for 30 mins in a 40 °C Water bath. Immediately added 1.0ml of 0.1 N NaOH and allowed reaching room temperature. Diluted upto the mark with diluent containing drospirenone and 17-epidrospirenone. (Note: NaOH must be added immediately after heating for the reaction to proceed properly. The Drospirenone to 17-epidrospirenone Ratio must be between 3:1 and 7:1.)

2.2.5 Preparation of Drospirenone standard stock solution (solution A)

Weighed accurately about 45.0 mg of Drospirenone working standard and transfered it into 50.00 mL volumetric flask. Added about 30 mL of diluent and sonicated to dissolve. Allowed it to come to room temperature and diluted upto the

mark with diluent and mixed. (Concentration of Drospirenone is 900 µg/mL)

2.2.6 Preparation of Ethinyl Estradiol standard stock solution (solution B)

Weighed accurately about 20.0 mg of Ethinyl Estradiol working standard and transfered it into 100.00 mL volumetric flask. Added about 30 mL of diluent and sonicated to dissolve. Allowed it to come to room temperature and diluted upto the mark with diluent and mixed. Pipette out 3.0ml of above solution into 50.0ml of volumetric flask, diluted upto the mark with diluent and mixed. (Solution B) (Concentration of Ethinyl Estradiol is 12 µg/mL)

2.2.7 Preparation of Ethinyl Estradiol Related compound B (solution C)

Weighed accurately about 2.0 mg of Ethinyl Estradiol Related compound B working standard and transfered it into10.00 mL volumetric flask. Added about 6 mL of diluent and sonicated to dissolve. Allowed it to come to room temperature and diluted upto the mark with diluent and mixed. Pipette out 3.0ml of above solution into 50.0ml of volumetric flask, diluted upto the mark with diluent and mixed**.** (Concentration of Ethinyl Estradiol Related compound B is 12 µg/mL)

2.2.8 Preparation of Standard solution

Transferred 5.0 ml of Solution A, 5.0 ml of Solution B and 5.0 ml of Solution C into 100.0ml volumetric flask, diluted upto the mark with diluent and mixed. (Concentration of Drospirenone 45 µg/mL, Ethinyl Estradiol 0.6 µg/mL and Ethinyl Estradiol Rel comp B is 0.6 µg/mL)

2.2.9 Preparation of Sensitivity solution

Transferred 2.00 ml of the above diluted standard solution into 20.00ml volumetric flask, diluted upto the mark with diluent and mixed. (Concentration of Drospirenone 4.5 µg/mL, Ethinyl Estradiol is 0.06µg/mL and Ethinyl Estradiol Related compound B is 0.06µg/mL)

Preparation of Placebo Solution: (Placebo without both Drospirenone and Ethinyl Estradiol)

Weighed accurately about placebo blend equivalent to 15 tablets or 15 intact placebo tablets, transferred into a 20 mL volumetric flask, added accurately 10.0 mL of diluent with the help of pipette, stopper well and sonicate for 5 minutes at room temperature with intermittent shakings and placed in an ice bath for 10 mins. Allowed it to come to room temperature and centrifuge the solution for 5 minutes at about 3000 rpm. Filtered the supernatant solution through 0.45µm nylon membrane filter/ PVDF filter, discard first 2 mL of the filtrate and injected.

2.2.10 Preparation of Sample Solution

Weighed accurately about 15 tablets, transfer into a 20 mL volumetric flask, added accurately 10.0 mL of diluent with the help of pipette, stopper well and sonicated for 5 minutes at room temperature with intermittent shakings and place in an ice bath for 10 mins.. Allowed it to come to room temperature and centrifuge the solution for 5 minutes at about 3000 rpm. Filtered the supernatant solution through 0.45µm nylon membrane filter/PVDF filter, discard first 2 mL of the filtrate and injected. (Concentration of Drospirenone 4500 µg/mL, Ethinyl Estradiol is 30µg/mL)

2.2.11 Chromatographic Procedure

The chromatographic parameters used are summarized in table 3. Injected the specified volume of diluents', placebo solution, sensitivity solution, system suitability solution, diluted standard solution and sample solution into the chromatograph as mentioned in the Injection sequence table and recorded the chromatograms. Disregarded peaks due to diluent and placebo solution. Disregarded the impurity peaks below LOQ level for both Drospirenone and Ethinyl Estradiol. Calculated all known impurities showing response at wavelengths i.e. FLR (215nm/315nm) and FLR (215nm/344nm) was calculated at wavelength where its area response against Ethinyl Estradiol Standard area at respective wavelength. Disregarded the peaks due to placebo with the help of placebo solution. Calculated any unspecified impurity at 222nm against Drospirenone diluted standard area at 222 nm and calculated any unspecified impurity at FLR (215nm/315nm) against Ethinyl Estradiol diluted standard area at FLR (215nm/315nm)

Table 3: Chromatographic Conditions

Column	:	Prontosil C18 ace-EPS,30cmX3.0 mm, followed in series by chromalith RP-18E C18, 10cm x 4.6 mm, 3µm
Column Temperature	:	40°C
Sample Temperature		25°C
Wavelength a)Drospirenone b)Ethinyl Estradiol	: :	UV Detector: 222 nm Fluorescence Detector: Excitation at 215nm and Emission at 315nm Fluorescence Detector: Excitation at 215nm and Emission at 344nm
Injection Volume	:	20µL
Run time	:	70 minutes
Retention time of a)Drospirenone b)Ethinyl Estradiol c) Ethinyl Estradiol Related Compound B	: : :	About 34 minutes About 47 minutes About 41 minutes

3. RESULTS AND DISCUSSION

3.1 Specificity

Blank (diluent), sensitivity solution, system suitability solution, placebo solution, diluted standard solution, all known impurity solutions individually, sample solution and sample solution spiked with all known impurities at specification level were prepared and injected into the HPLC equipped with a photodiode array detector and analyzed as per methodology. The retention time of the peak of Drospirenone and Ethinyl Estradiol in sample solution was obtained at 35.04 min. and 48.6 min. respectively. The retention time of the peak of Drospirenone and Ethinyl Estradiol in diluted standard solution was obtained at 35.5 min. and 48.6 min. respectively. The retention time of all the known impurities spiked in sample solution were found to be comparable with those injected individually. Results are tabulated in Table 4.

Table 4: Retention Time of Known Impurities in Spike Sample

Impurities/Active	Individual Injection	Spiked Sample	
	Retention Time (min)	Retention Time (min)	RRT(Relative Retention Time)
Drospirenone and its impurities			
17-Epi Drospirenone	ND	ND	ND
Drospirenone	35.7	34.8	1.00
Ethinyl Estradiol and its impurities			
6α Hydroxy Ethinyl Estradiol	12.5	12.3	0.27
6β Hydroxy Ethinyl Estradiol	13.3	13.1	0.28
6 Keto Ethinyl Estradiol	19.9	19.7	0.42
Ethinyl Estradiol Rel comp B	43.19	42.8	0.88
Ethinyl Estradiol	49.12	48.5	1.00

Table 5: Interference Study

Impurities/Active	Peak Purity			
	Individual Injection		Spiked Sample	
	Purity Angle	Purity Threshold	Purity Angle	Purity Threshold
Drospirenone and its impurities				
17-Epi Drospirenone	ND	ND	ND	ND
Drospirenone	0.042	0.235	9.342	10.835
Ethinyl Estradiol and its impurities				
6α Hydroxy Ethinyl Estradiol	4.018	90	4.345	90
6β Hydroxy Ethinyl Estradiol	4.18	90	2.404	90
6 Keto Ethinyl Estradiol	3.918	4.35	3.537	3.864
Ethinyl Estradiol Rel comp B	2.493	90	2.25	90
Ethinyl Estradiol	4.127	90	0.353	90

3.2 Interference Study

Blank (diluent), sensitivity solution, system suitability solution, placebo solution, diluted standard solution, all known impurity solutions individually, sample solution and sample solution spiked with all known impurities at specification level were prepared and injected into the HPLC equipped with a photodiode array detector and analyzed as per methodology. Blank (diluent) and placebo solution I did not show interference

at the retention time of Drospirenone, Ethinyl Estradiol and their impurities. Peak purity of Drospirenone, Ethinyl Estradiol and their impurities in spiked sample solution passed. Results are tabulated in table 5.

3.3 Precision

Percentage RSD (n=6) for peak area counts of Drospirenone and Ethinyl Estradiol and Ethinyl Estradiol Related compound B from six replicate injections of diluted standard solution was found within acceptance criteria. Results are shown in table 6.

3.3.1 Method precision (Repeatability)

Six sample solutions of Drospirenone and Ethinyl Estradiol Tablets (2.5 mg/0.012 mg) were prepared by spiking the known impurities at the specification level and analysed as per methodology. The results are tabulated in table no 7.

Table 6: System Precision

Injection	Peak Area counts		
	Drospirenone	Ethinyl Estradiol	Ethinyl Estradiol Rel comp B
1	547227	29399	34746
2	540878	29534	34983
3	544885	29488	34885
4	548189	29281	34733
5	544133	28135	34556
6	543433	27398	34874
Mean	544791	28873	34796
SD	2648.7	892	150.4
% RSD	0.49	3.09	0.43

Table 7: Method Precision for Single maximum unknown and total impurities for Ethinyl Estradiol

Sample	Single maximum Unknown Impurities at 222nm	Total Impurities at 222 nm	Single maximum Unknown Impurities at 315nm	Total Impurities at 315nm
	% w/w	% w/w	% w/w	% w/w
1	0.091	0.65	0.080	0.79
2	0.087	0.63	0.080	0.77
3	0.097	0.63	0.081	0.80
4	0.094	0.64	0.080	0.77
5	0.084	0.63	0.080	0.75
6	0.098	0.64	0.081	0.81
Mean	0.092	0.64	0.080	0.78
SD	0.01	0.007	0.000	0.05
% RSD	7.79	1.15	0.47	5.84

3.3.2 Intermediate Precision (Ruggedness)

% RSD (n=6) for % of known impurities in six sample solutions and overall % RSD (n=12) for % of known impurities from Method Precision and Intermediate Precision results was found within acceptance criteria. Results are shown in table 8.

Table 8: Cumulative results of Method and Intermediate Precision (Ethinyl Estradiol impurities)

Sample	6α Hydroxy Ethinyl Estradiol		6β Hydroxy Ethinyl Estradiol		6 Keto Ethinyl Estradiol		Ethinyl Estradiol Rel comp B	
	M.P.	I.P.	M.P.	I.P.	M.P.	I.P.	M.P.	I.P.
	(%w/w)	(%w/w)	(%w/w)	(%w/w)	(%w/w)	(%w/w)	(%w/w)	(%w/w)
1	0.35	0.32	0.36	0.35	0.55	0.50	1.21	1.15
2	0.34	0.33	0.35	0.34	0.54	0.49	1.12	1.15
3	0.35	0.32	0.37	0.35	0.53	0.48	1.20	1.13
4	0.34	0.31	0.35	0.35	0.54	0.49	1.18	1.14
5	0.31	0.32	0.36	0.33	0.54	0.50	1.16	1.15
6	0.35	0.32	0.38	0.36	0.54	0.50	1.16	1.18
Mean(n=12))	0.33		0.35		0.52		1.16	
SD	0.01		0.01		0.03		0.03	
%RSD	3.00		2.90		5.80		2.60	

3.4 Limit of Detection (LOD) and Quantitation (LOQ)

Limit of Quantitation (LOQ) was established by injecting six replicates of a solution containing Drospirenone, Ethinyl Estradiol and their known impurities having a concentration which is less than the reporting threshold (0.1%). Limit of Detection (LOD) was established by quantitatively diluting and injecting six replicates of the LOQ solution to obtain the % RSD (n=6) not more than 30.0. % RSD (n=6) of peak area counts of Drospirenone, Ethinyl Estradiol and their known impurities in predicted LOD and LOQ solutions was found within acceptance criteria. The results are tabulated in Table 9 and Table 10.

Table 9: Limit of Detection for Ethinyl Estradiol impurities

Sample	Peak Area Counts			
	6α Hydroxy Ethinyl Estradiol	6β Hydroxy Ethinyl Estradiol	6 Keto Ethinyl Estradiol	Ethinyl Estradiol Rel comp B
	0.03 % w/w	0.03 % w/w	0.03 % w/w	0.03 % w/w
1	211821	173423	1460	142383
2	217182	173482	1436	141885
3	212164	170353	1474	140897
4	213137	177070	1455	142847
5	211563	174632	1487	147575
6	217696	174744	1454	144570
Mean	213927	173951	1461	143360
SD	2776.9	2203.0	17.6	2395.4
%RSD	1.30	1.27	1.21	1.67

Table 10: Limit of Quantitation for Ethinyl Estradiol impurities

Sample	Peak Area Counts			
	6α Hydroxy Ethinyl Estradiol	6β Hydroxy Ethinyl Estradiol	6 Keto Ethinyl Estradiol	Ethinyl Estradiol Rel comp B
	0.1% w/w	0.1% w/w	0.1% w/w	0.1% w/w
1	513818	400878	5023	503493
2	513457	400538	5102	507613
3	517516	406797	5051	500612
4	512652	401802	5093	501381
5	512079	405430	5017	501481
6	511416	409424	5016	503680
Mean	513490	404145	5050	503043
SD	2159.3	3625.6	38.80	2553.6
%RSD	0.42	0.90	0.77	0.51

3.5 Stability of Analytical Solution

One sample solution (unspiked) was prepared as per methodology and other by spiking known impurities at specification levels. Injected diluted standard solution, sample solution (unspiked) and spiked sample solution initially and at different time intervals upto 48 hours by storing the sample solutions at 25°C. For each sample solution % w/w of known and total impurities were calculated and compared with the initial results. Absolute value of % difference between % w/w of each known impurity in initial sample and sample injected at each time interval with respect to initial value should be NMT 20.0 (for impurities below 1.0%) and NMT 10.0 (for impurities above 1.0%). Spiked sample solution was found stable up to 48 hours at room temperature. Results are tabulated in table 11-table 12.

Table 11: Solution stability of Ethinyl Estradiol impurities in spike sample

Time Interval	6α Hydroxy Ethinyl Estradiol		6β Hydroxy Ethinyl Estradiol	
	% w/w	Absolute value of % difference w.r.t. initial	% w/w	Absolute value of % difference w.r.t. Initial
Initial	0.36	0.0	0.40	0.0
8 Hrs.	0.35	3.06	0.40	0.36
16 Hrs.	0.36	1.67	0.40	1.05
24 Hrs.	0.36	0.17	0.40	0.08
32 Hrs.	0.36	0.99	0.40	0.38
40 Hrs.	0.35	2.45	0.40	0.43
48 Hrs.	0.35	1.75	0.38	4.67

Table 12: Solution stability of Ethinyl Estradiol impurities in spike sample

Time Interval	6 Keto Ethinyl Estradiol		Ethinyl Estradiol Related comp B	
	% w/w	Absolute value of % difference w.r.t. initial	% w/w	Absolute value of % difference w.r.t. Initial
Initial	0.54	0.00	0.97	0.0
8 Hrs.	0.55	0.58	0.97	0.73
16 Hrs.	0.56	3.75	0.97	0.04
24 Hrs.	0.54	0.47	0.97	0.66
32 Hrs.	0.55	2.00	0.97	0.03
40 Hrs.	0.56	2.70	0.98	0.38
48 Hrs.	0.56	2.76	0.92	6.69

3.6 System suitability solution: The results are tabulated in Table 13-15

Table 13: System suitability for Drospirenone

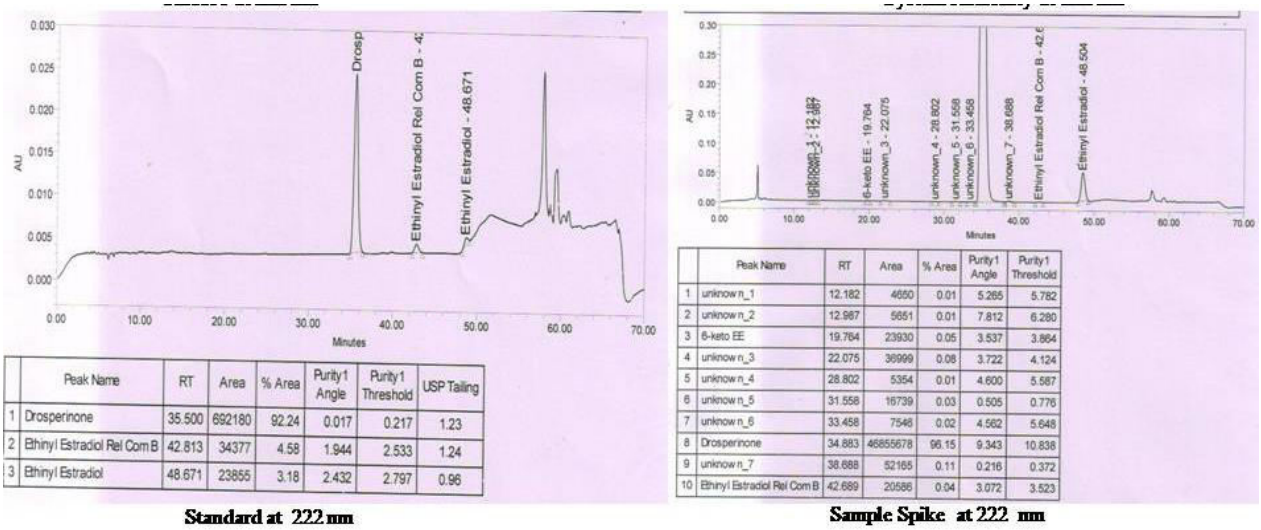
Parameter	Drospirenone			
	S/N ratio	System suitability	%RSD In diluted standard	Tailing factor
Specificity	642	3.87	0.31	1.23
Method Precision	296	3.89	0.49	1.19
Inter Precision	774	3.89	0.31	1.21
LOD/LOQ	484	3.87	0.5	1.19
Solution stability	728	3.88	0.22	1.24

Table 14: System suitability for Ethinyl Estradiol

Parameter	Ethinyl Estradiol			
	S/N ratio	System suitability	%RSD in diluted standard	Tailing factor
Specificity	17.9	N/A	1.45	0.96
Method Precision	14.7	N/A	3.7	0.97
Inter Precision	40.8	N/A	0.8	0.87
LOD/LOQ	23	N/A	1.2	0.95
Solution stability	21.9	N/A	0.5	0.80

Table 15: System suitability for Ethinyl Estradiol Related compound B

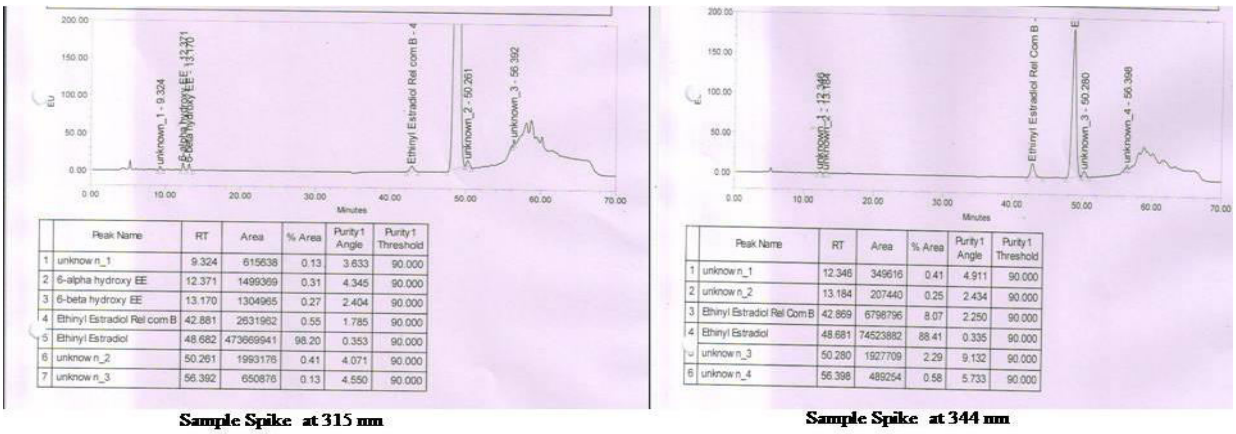
Parameter	Ethinyl Estradiol Related compound B			
	S/N ratio	System suitability	%RSD In diluted standard	Tailing factor
Specificity	27.3	N/A	0.63	1.24
Method Precision	15.8	N/A	0.6	1.02
Inter Precision	45.6	N/A	0.3	1.08
LOD/LOQ	25	N/A	0.7	1.14
Solution stability	40	N/A	0.22	1.14



	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	USP Tailing
1	Drosperinone	35.500	692180	92.24	0.017	0.217	1.23
2	Ethinyl Estradiol Rel Com B	42.813	34377	4.58	1.944	2.533	1.24
3	Ethinyl Estradiol	48.671	23855	3.18	2.432	2.797	0.96

	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold
1	unknown_1	12.182	4650	0.01	5.265	5.782
2	unknown_2	12.987	5651	0.01	7.812	6.280
3	6-keto EE	19.764	23930	0.05	3.537	3.864
4	unknown_3	22.075	36999	0.08	3.722	4.124
5	unknown_4	28.802	5354	0.01	4.600	5.587
6	unknown_5	31.558	16739	0.03	0.505	0.776
7	unknown_6	33.458	7546	0.02	4.562	5.648
8	Drosperinone	34.883	46855678	96.15	9.343	10.838
9	unknown_7	38.688	52165	0.11	0.216	0.372
10	Ethinyl Estradiol Rel Com B	42.689	20586	0.04	3.072	3.523

Figure 1: Chromatogram of spike sample at 222 nm.



	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold
1	unknown n_1	9.324	615638	0.13	3.633	90.000
2	6-alpha hydroxy EE	12.371	1499369	0.31	4.345	90.000
3	6-beta hydroxy EE	13.170	1304965	0.27	2.404	90.000
4	Ethinyl Estradiol Rel com B	42.881	2631982	0.55	1.785	90.000
5	Ethinyl Estradiol	48.682	473669941	98.20	0.353	90.000
6	unknown n_2	50.261	1993176	0.41	4.071	90.000
7	unknown n_3	56.392	650876	0.13	4.550	90.000

	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold
1	unknown n_1	12.346	349616	0.41	4.911	90.000
2	unknown n_2	13.184	207440	0.25	2.434	90.000
3	Ethinyl Estradiol Rel Com B	42.869	6798796	8.07	2.250	90.000
4	Ethinyl Estradiol	48.681	74523882	88.41	0.335	90.000
5	unknown n_3	50.280	1927709	2.29	9.132	90.000
6	unknown n_4	56.398	489254	0.58	5.733	90.000

Figure 2: Chromatogram spike sample at 315 and 344 nm.

4. CONCLUSION

The related substance method of analysis for Drospirenone and Ethinyl Estradiol Tablets (2.5 mg/ 0.012 mg) was validated for Specificity, LOD/LOQ Precision, Intermediate Precision (Ruggedness), and Stability of Analytical Solution .The method meets the acceptance criteria for all parameters. The validated method is Specific, Linear, Accurate, Precise, and Rugged for determination of related substances of Drospirenone and Ethinyl Estradiol in Drospirenone and Ethinyl Estradiol Tablets 2.5 mg/0.012 mg. Hence, this method can be used for routine analysis for the determination of related substances Drospirenone and Ethinyl Estradiol in Drospirenone and Ethinyl Estradiol Tablets 2.5 mg/0.012 mg.

5. LIST OF ABBREVIATIONS

HPLC	High performance Liquid Chromatography
% w/w	Percentage weight by weight
w.r.t	With respect to
Rel Com B	Related compounds B
Dil Std	Diluted Standard
RT	Retention time
RRT	Relative Retention time
SD	Standard Deviation
RSD	Relative Standard Deviation
NLT	Not Less Than
NMT	Not More Than
RPM	revolution per minute
PPM	parts per million
S/N	Signal to Noise
nm	nano meter

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REFERENCES

1. Martindale, I.e. (Drospirenone) . The complete drug reference. London Pharmaceutical Press; 2009.
2. G Babu Pradad, Ramana PS, M V . Validated RP-HPLC method for the estimation of drospirenone in formulation. Int J of Research Pharm and Bio Sci 2011;2(1):1341-1345.
3. M G Sagar Patel, G V. Development and validation of analytical method for simultaneous estimation of Ethinyl estradiol and cyproterone acetate in combined solid dosage form. Int J Uni Pharm and Life Sciences 2012;2(1):611-622.
4. N B Raju Babu, R R . Simultaneous analysis and validation of Risperidone and Drospirenone drugs in pharmaceutical dosage form. Int of Res Pharm and Bio Sci 2011;2(6):1638-1642.
5. B Deshpande Prabhakar, , S G . Simultaneous estimation of ethinylestradiol and levonorgestrel from transdermal patches by HPLC. Ind J Pharma Sci 1999;61(2):12-15.
6. A L Derral Philip,, Richard OM, W Y . Determination of norgostimate and ethinyl estradiol in tablets by high-performance liquid chromatography. J of Pharm Sci 1987;76(4):44-47.
7. J A Shek Gluck, , E . Determination of ethinylestradiol and norethisterone in an oral Contraceptive capsule by reversed-phase high performance liquid chromatography. J of Chromato Sci 1980;18(2):631-636. Available from: http://chromsci.oxfordjournals.org/cgi/doi/10.1093/chromsci/18.11.631 doi: 10.1093/chromsci/18.11.631.