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Research Article

DEVELOPMENT AND VALIDATION OF A SIMPLE AND SENSITIVE RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DROSPIRENONE AND ETHINYLESTRADIOL IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

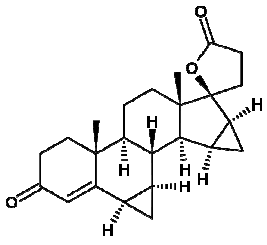
A simple, selective, suitable, rapid, precise and economical Reverse-Phase HPLC method has been developed and validated for quantitative determination of Drospirenone and Ethinylestradiol in tablet dosage form. The method was carried out with UV Spectrophotometer for the determination of absorption maxima using a SHIMADZU UV180, method development and validation was carried out by WATERS HPLC system consisting of 1525 Binary HPLC pump, 2489 UV/Visible Detector and BREEZE 2 software using 2707 Auto-sampler, equipped with a column of WATERS C18 (250 x 4.6 mm, 5µm,) and a guard column of the same type, at a flow rate of 1.0ml/min. Detection was carried out at 275 nm. The mobile phase consisted of Acetonitrile (solvent A) and10mM Formic Acid (solvent B) in the ratio of 70:30. The retention times of Drospirenone and Ethinylestradiol were 4.15 min and 2.25 min respectively. The method was developed and tested for linearity range of 50µg/ml to 150µg/ml. The developed method was validated in terms of suitability, linearity, accuracy, precision and ruggedness.

Keywords: HPLC, Drospirenone, Ethinylestradiol

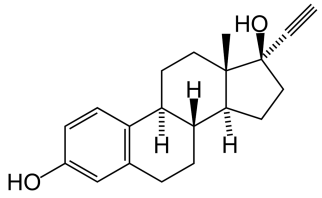
INTRODUCTION

Drospirenone, also known as 1,2-dihydrospirorenone, is chemically (6*R*,7*R*,8*R*,9*S*,10*R*,13*S*, 14*S*,15*S*,16*S*,17*S*) 1,3',4',6,6a, 7,8,9,10,11,12, 13,14,15,15a,16 - hexadecahydro-10,13-dimethylspiro-[17*H* icyclopropa-6,7:15,16] cyclopenta [a]phenanthrene-17,2'(5*H*)-furan]-3,5'(2*H*)-dione). Drospirenone is a synthetic steroidal progestin that is an analog to spironolactone, has biochemical and pharmacologic profiles similar to endogenous progesterone, especially regarding antimineralocorticoid and antiandrogenic activities. It is part of certain birth control formulations. As a combination oral contraceptive, drospirenone with ethinyl estradiol is effective and has positive effects on weight and lipid levels. The compound differs from other synthetic progestins in that its pharmacological profile in preclinical studies shows it to be closer to the natural progesterone. Its molecular formula is $C_{24}H30O3$ and mol. mass is 366.493 g/mol. It is sold under the brand names Yasmin, Yasminelle, Yaz, Beyaz, Ocella, Zarah, and Angeliq, all of which are combination products of drospirenone with an estrogen such as ethinylestradiol[1-3].

Ethinylestradiol is chemically, 19-Nor-17α-pregna-1,3,5 (10)-trien-20-yne-3,17- diol. Ethinylestradiol is a synthetic derivative of natural estrogen estradiol, having high estrogen receptor potency, used for treatment of vasomotor symptoms associated with menopause, female hypogonadism, prostatic carcinoma-palliative therapy, breast cancer, and oral and emergency contraceptive. Estradiol binds well to both estrogen receptors, ERα, and ERβ, in contrast to certain other estrogens, notably medications that preferentially act on one of these receptors. These medications are called selective estrogen receptor modulators or SERMs. Estradiol is the most potent naturally occurring estrogen. Its Molecular formula is $C_{18}H_{24}O_2$ and mol. mass is 296.40 g/mol[4-6].



(a)



(b)

Fig. 1: Chemical structure of (a) Drospirenone and (b) Ethinylestradiol

Literature survey of these two drugs shows that high performance liquid chromatographic (HPLC) methods for the determination of Drospirenone in human plasma and in pharmaceutical formulations either as a single and in combination with other drugs are available7-9. HPLC methods have been reported for the determination of Ethinylestradiol in combination with other drugs10-14. To the best of our knowledge no reports were found for the simultaneous estimation of the Drospirenone and Ethinylestradiol in combined tablet dosage form by RP-HPLC method. In this study we have developed a simple, accurate, sensitive and validated RP-HPLC method for simultaneous estimation of these compounds in bulk drug and in combined tablet dosage form and the method was applied for their identification by UPLC-MS. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines 15-16.

MATERIAL & METHODS

Chemicals and reagents

Working standards of pharmaceutical grade Drospirenone and Ethinylestradiol were obtained as generous gift from ONTOP Pharmaceuticals Ltd. (Bangalore, India) and was used as such without further purification. The pharmaceutical dosage form used in this study was "YAMINI" (Lupin pharmaceuticals Pvt. Ltd., Pune, India) labeled to contain 3 mg of Drospirenone and 0.03 mg of Ethinylestradiol was procured from the local market. Acetonitrile (HPLC grade) from Merk specialties Pvt. Ltd., Formic acid (GR grade) from Fluka Pvt. Ltd. and double distilled water (MILLI Q) from SG Series compact pre-treatment module were used in the analysis.

Instrumentation and Chromatographic conditions

WATERS HPLC system consisting of 1525 Binary HPLC pump, 2489 UV/Visible Detector and BREEZE 2 software was used for analysis. Separation was carried out on WATERS C18 (250 x 4.6 mm, 5µm,) column using Acetonitrile : 10mM Formic acid buffer in ratio of (70:30, v/v) as mobile phase at flow rate of 1 ml/min. Samples were injected using 2707 Auto-sampler with 30 µl loop and detection was carried out at 275 nm. All Weighing were done on Analytical Balance CB-50 CONTECH Instruments.

Preparation of standard solutions

The pure drug Drospirenone and Ethinylestradiol standard stock solutions were separately prepared in the concentration of 1mg/ml dissolving in Acetonitrile and 0.1% Formic acid buffer solution (70:30), which was ultrasonicated for 15 min and filtered through Whatman paper No. 4.1.

Preparation of sample solution

Twenty tablets were weighed accurately and powdered. Each tablet contains 3 mg of Drospirennone and 0.03 mg of Ethinylestradiol. Stock solution is prepared by dissolving 5 mg of tablet powder in 1ml mobile phase, which was ultrasonicated and filtered through a whatman paper No. 4.1 and further diluted to get solution of 100µg/ml of Drospirenone and 1µg/ml of Ethinylestradiol. The prepared solution was ultrasonicated for 5 min and filtered through Whatman paper No. 4.1.

Determination of Absorption Maxima

By appropriate dilution of standard drug solutions with mobile phase having 2 ug/ml, 4 ug/ml, 6 ug/ml, 8 ug/ml, 10 ug/ml concentration of Drospirenone and 2 ug/ml, 4 ug/ml, 6 ug/ml, 8 ug/ml, 10 ug/ml of Ethinylestradiol were scanned separately in the range of 200- 400 nm to determine the wavelength of maximum absorption for drugs. Drospirenone and Ethinylestradiol showed absorbance maxima at 254 nm, 275 nm, respectively.

System suitability

The system suitability parameters like number of theoretical plates, tailing factor, asymmetry factor, HETP (cm), resolution were also calculated. It was assessed by six replicate injections of the mixture containing 20 µg/ml of both the drugs. The values are given in Table 1.

Table 1: System suitability parameters for RP-HPLC method

Parameters	Drospirenone	Ethinylestradiol
1. Theoretical plates	2929	2089
2. Tailing Factor	1.07	1.14
3. Asymmetry Factor	1.14	1.34
4 Resolution	3.8	-
5. HETP	0.0853	0.119

Limit of Detection and Limit of Quantitation

LOD and LOQ were calculated using formula 3.3 σ /S and 10 σ /S respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Method Vaidation

The method was validated for linearity, accuracy, intraday and inter-day precision and robustness, in accordance with ICH guidelines.

Linearity

Five point graphs were constructed in the concentration range of 50% - 150% of both drugs in three replicates, linear relationship between the peak area signals of Drospirenone and Ethinylestradiol to the corresponding drug concentrations was observed. Fig.2 represents Drospirenone and Fig. 3 that of Ethinylestradiol. The statistical analysis of calibration is shown in Table 2.

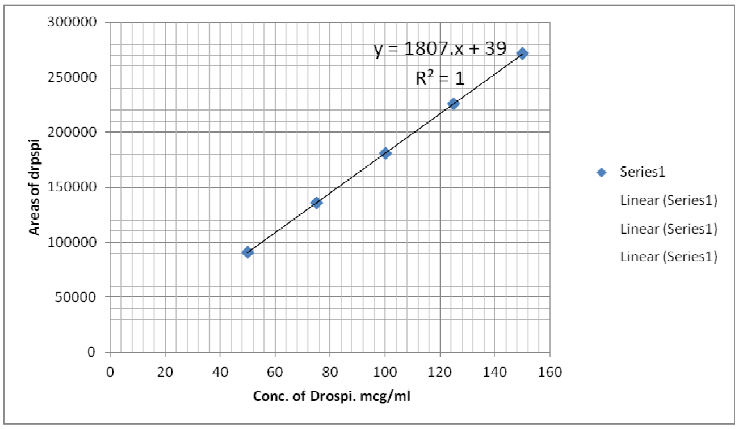


Fig. 2: Linearity of Drospirenone

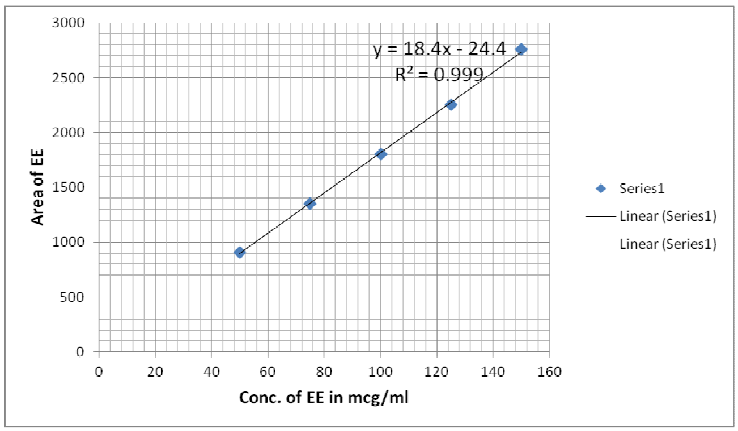


Fig. 3: Linearity of Ethinylestradiol

Table 2: Linearity and range: (Drospirenone and Ethinylestradiol)

Drospirenone Concentration µg/ml	Mean peak areas Drospirenone	Ethinylestradiol Concentration µg/ml	Mean peak areas Ethinyl estradiol
50%	90389	50%	907
75%	135659	75%	1355
100%	180785	100%	1803
125%	225981	125%	2277
150%	271177	150%	2756

Precision

Precision of method was demonstrated by Repeatability, Reproducibility and Intermediate precision[17]. In Repeatability six replicates of standard solution were injected into HPLC System. The mean, SD and %RSD for peak areas of Drospirenone and Ethinylestradiol were calculated. For reproducibility the six samples of a single batch were analyzed as per test method and % assay for Drospirenone and Ethinylestradiol. The percentage assay of Drospirenone and Ethinylestradiol were determined. Calculated % RSD for assay of Drospirenone and Ethinylestradiol in six replicates. The relative standard deviation values obtained for Drospirenone and Ethinylestradiol were 0.0068% and 1.224%, respectively.

Accuracy

Accuracy is in agreement with acceptable true value and actual result observed. The recovery studies were carried out using standard addition method at 50%, 100 %, 150% level; known amount of standards was added to pre-analyzed sample and subjected them to the proposed HPLC method. Sample solutions were prepared in triplicates for each spiked level. These solutions were analyzed by the HPLC method against 100% standard. Percentage recovery was calculated and results are shown in the Table 7 & 8.

Ruggedness

The studies of ruggedness were carried out under different conditions of the day and the analyst[18].

Interday (Different days)

The ruggedness of method was studied by using same procedure on different days. The % label claim was calculated using formula. Data obtained for day 1, day 2, and day 3 is shown in Table 9.

Intraday (within same day)

The ruggedness of method was checked by using same procedure at different intervals of time within a day. The percent label claim was calculated using formula. Results and statistical data are presented in Table 9.

Different Analyst

The sample solution was prepared by two different analysts and same procedure was followed as described earlier. The % label claim was calculated as done in marketed formulation estimation.

Table 3: Relative standard deviation for repeatability (Drospirenone)

	1	2	3	4	5	6	Mean	SD	%RSD
Std areas:	180486	180396	180409	180445	180356	180418.4	180418.4	31.6	0.017
RT :	4.16	4.13	4.18	4.16	4.15	4.12	4.15	$3.8X10^4$	1.38

Table 4: Precision Study (Drospirenone)

Condition	Sample Area of Drospirenone (µAU*sec)	Mg/tab	% L . C.	Mean	SD	% RSD
INITIAL 1	180461	2.991	99.66			
INITIAL 2	180264	2.988	99.56			
INITIAL 3	180193	2.986	99.49			
INITIAL 4	180376	2.990	99.62	99.62	0.0068	0.0068
INITIAL 5	180620	2.994	99.74			
INITIAL 6	180520	2.992	99.69			

Table 5: Relative standard deviation (Ethinylestradiol)

	1	2	3	4	5	6	Mean	SD	%RSD
Standard areas:	**1807**	1889	1865	1810	1848	1830	1804	35.30	1.95
RT :	2.26	2.22	2.19	2.24	2.20	2.18	2.21	0.034	1.5

Table 6: Precision study (Ethinylestradiol)

Condition	Sample Area of Ethinylestradiol (µAU*sec)	Mg/tab	% L . C	Mean	SD	%RSD
INITIAL 1	1807	0.0299	99.66			
INITIAL 2	1837	0.0304	101.33			
INITIAL 3	1863	0.0308	102	100.76	1.264	1.244
INITIAL 4	1799	0.0297	99			
INITIAL 5	1830	0.0303	101			
INITIAL 6	1820	0.0301	100.32			

Table 7: Recovery Studies (Drospirenone)

Accuracy Level	Wt. of Drospirenone mg/mL	Amt. of Drospirenone added in ppm	Area of Drospirenone	Amt. of Drospirenone Found in ppm	% Recovery
Level1 (50%)	0.187	3.74	90179	3.71	99.19
Level1 (50%)	0.184	3.68	90098	3.70	100.72
Level1 (50%)	0.182	3.79	91272	3.74	99.46
Level1 (100%)	0.372	7.44	180376	7.41	99.59
Level1 (100%)	0.375	7.48	180605	7.41	99.06
Level1 (100%)	0.378	7.55	181840	7.48	99.07
Level1 (150%)	0.561	11.22	270567	11.13	99.21
Level1 (150%)	0.564	11.25	270667	11.14	99.02
Level1 (150%)	0.551	11.02	264467	11.09	100.63

Table 8: Recovery Studies (Ethinylestradiol)

Accuracy Level	Wt. of EE found	Amt. of of EE added in ppm	Area mg/ml	Amt. of EE found in ppm	% Recovery
Level1 (50%)	0.00185	0.037	905	0.0372	100.74
Level1 (50%)	0.0019	0.038	917	0.0377	99.28
Level1 (50%)	0.0018	0.0352	868	0.0357	101.58
Level1 (100%)	0.0037	0.074	1785	0.0734	99.18
Level1 (100%)	0.0039	0.078	1921	0.0790	101.12
Level1 (100%)	0.0035	0. 070	1727	0.071	101.42
Level1 (150%)	0.0051	0.102	2413	0.101	99.01
Level1 (150%)	0.0054	0.108	2680	0.110	101.85
Level1 (150%)	0.0058	0.116	2870	0.118	101.72

Table 9: Result and statistical data for Interday and Intraday

S. No.	Interday		Intraday	
	Drospirenone	Ethinylestradiol	Drospirenone	Ethinylestradiol
1.	98.3	102.2	98.2	102.2
2.	98.4	102.4	98.4	102.4
3.	98.6	102.8	98.2	102.6
Mean	98.4	102.4	98.4	102.4
±S.D	0.02	0.39	0.02	0.39
RSD	0.02	0.38	0.02	0.38

RESULTS AND DISCUSSION

A RP-HPLC method was developed for Drospirenone and Ethinylestradiol which can be conveniently employed for routine estimation of them in tablet dosage form and in pure drug. The chromatographic conditions were optimized in order to provide a good performance.

The mobile phase for drug was selected based on its polarity. Different trials were taken and the final working mobile phase selected was acetonitrile : 10 mM formic acid buffer in ratio of (70:30, v/v).The Chromatograms of standard solution containing Drospirenone and Ethinylestradiol were observed at retention time 4.15 ± 0.00038 and 2.25 ± 0.034 min for Drospirenone and Ethinylestradiol respectively.

System suitability parameters like Theoretical plates, Tailing Factor, Asymmetry Factor, HETP and Resolution are in considerable limit. The similarity factor between two replicate(separately prepared and injected) standards was between 0.98 to 1.02, tailing factor ,asymmetry factor, for the Drospirenone and Ethinylestradiol peak was not more than 2.0 LOD & LOQ were also in limit. The LOD for Drospirenone and Ethinylestradiol were 0.3ppm and 1.02 ppm respectively. The LOQ for Drospirenone and Ethinylestradiol were 0.004ng and 0.016ng respectively.

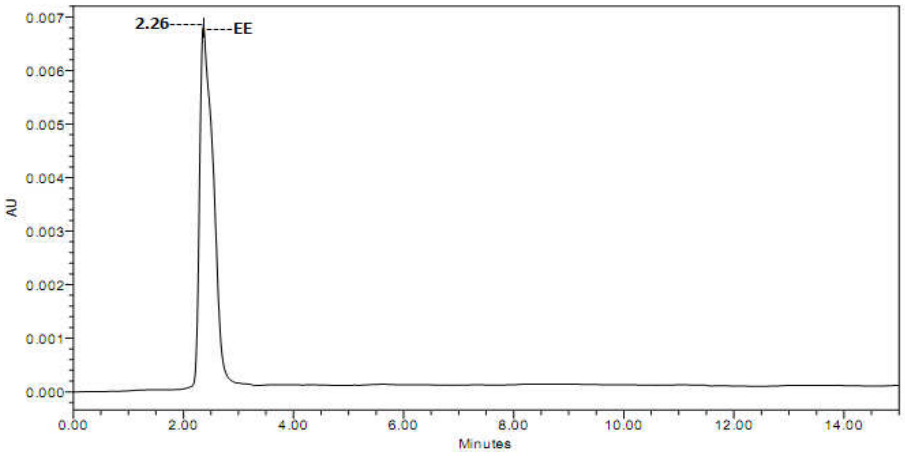


Fig. 4: Final trial Chromatogram of Ethinylestradiol

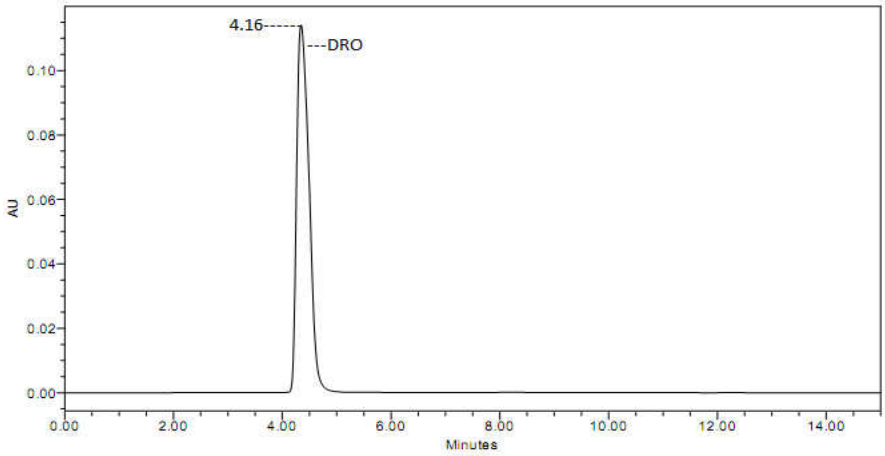


Fig. 5: Final trial Chromatogram of Drospirenone

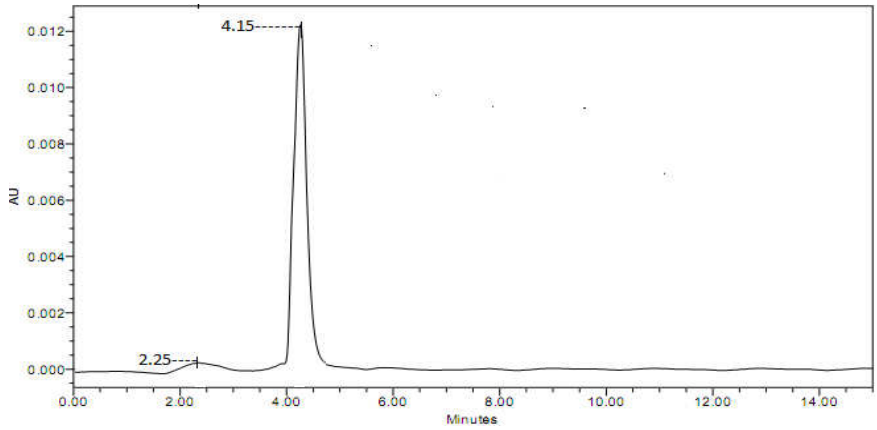


Fig. 6: Chromatogram of Ethinylestradiol (2.25 min) and Drospirenone (RT = 4.15min)

Linearity was observed in the concentration range of 50%, 75%, 125%, 100%, 150%, for Drospirenone and 50%, 75%, 125%, 100%, 150%, for Ethinylestradiol The correlation coefficients for the plots were 1.000 for Drospirenone and 0.999 for Ethinylestradiol. In case of accuracy % Recovery was found to be 99.97 ± 0.432 for Drospirenone and for Ethinylestradiol 99.55 ± 0.684 (mean ± S.D., n = 6). The method was found to be accurate and precise, as indicated by recovery studies and % RSD was not more than 2. The summary of validation parameters of proposed HPLC method is given in Table 10. In case of precision the relative standard deviation was 0.31 for Drospirenone and .0.99 for Ethinylestradiol. Different conditions are applied to study the ruggedness of the method like different day and different analyst, and observed RSD to be less than 2%.

Table 10: Validation Parameters

S. No.	Parameters	Results
1.	System Suitability	Pass
2.	Limit of detection	In limit
3.	Limit of Quantitation	In limit
4.	Linearity	Pass
5.	Accuracy	Pass
6.	Precision	Pass
7.	Ruggedness	Pass

Method application

The analytical method developed in this study was utilized for simultaneous identification of Drospirenone and Ethinylestradiol in tablet dosage form and also in pure drug form on an ESI-MS.

CONCLUSION

A validated RP-HPLC method has been developed for the quantitative analysis of Drospirenone and Ethinylestradiol in tablet dosage form. The proposed method is validate, which has shown that the method is simple, fast, rapid, accurate, precise, specific with good separation between two peaks and can be applied for identification of Drospirenone and Ethinylestradiol using LC-MS. Therefore, it is suitable for the routine analysis of Drospirenone and Ethinylestradiol in pharmaceutical dosage form.

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