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Method Development and Validation for Simultaneous Estimation of Ethinyl Estradiol and Drospirenone and Forced Degradation Behavior 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 Thermo Hypersil BDS C18 Column (4.6×250 mm and 5 µm) and flow rate of 1.0 ml/min with a load of 15 µl. Acetonitrile and ammonium acetate buffer was used as mobile phase in the composition of 30:70. The detection was carried out at 258 nm. Linearity ranges for ethinyl estradiol and drospirenone were 0.06-0.18 µg/ml, 6-18 µg/ml respectively. Retention Time of ethinyl estradiol and drospirenone were found to be 1.4 min, 5.3 min respectively. Percent Recovery study values of ethinyl estradiol and drospirenone were found to be within 97-103%. The combination product is exposed to acid/base, hydrolytic, photolytic and peroxide stress conditions and the stressed samples were analyzed. 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.

Keywords: Ethinyl estradiol; Drospirenone; RP HPLC; Forced degradation

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 bio-active 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.

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 (Figures 1 and 2) [1,2].

Earlier publications have described spectroscopic and chromatographic methods for the quantification of ethinyl estradiol and drospirenone individually. A high-performance liquid chromatography (HPLC) methodology useful for the quantification of drospirenone in tablet dosage form was reported [3].

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 [4,5]. 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.

Forced degradation studies were used in the development of this methodology as a stability indicating parameter. The devised method was found to be selective, reliable, faster and straight forward than other reported methods. Though no attempt was made to identify the degradation products, described method can be used as stability indicating method for the assay of ETH and DRO in their combined dosage form.

Materials and Methods

Apparatus / Instruments used

S.No	Name	Model	Manufacturer
1	HPLC	2695	Waters
2	Detector (PDA)	2998	Waters
3	UV-VIS Double Beam Spectrophotometer	3200	Labindia
4	Sonicator	-	Labindia
5	Weighing balance	Bsa224s-cw	Sartorius

S.No	Name	Grade	Manufacturer
1	Ammonium acetate	-	Fisher Scientific
2	Acetonitrile	HPLC	Merk
3	Methanol	HPLC	Merk
		HPLC Double	Milli-QRO
4	Double distilled Water		
		distilled	purification system

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Working standards

Pharmaceutical grade ethinyl estradiol and drospirenone were kindly supplied as a gift sample by Lara Drugs Private Limited, Hyderabad, Andhra Pradesh, India.

Method development

Preparation of mobile phase: The mobile phase was prepared by mixing ammonium acetate: acetonitrile in the ratio of (70:30) and was filtered and degassed.

Preparation of standard drug solutions: Accurately weigh and transfer 24 mg drospirenone and 0.24 mg ethinyl estradiol into a 50 ml volumetric flask. Add about 30 ml of methanol and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution). Further pipette out 5 ml of stock solution into a 10 ml volumetric flask and dilute up to the mark with methanol to get a solution of drospirenone (0.12 mcg/ml) and ethinyl estradiol (0.0012 mcg/ml).

Accurately weigh about 24 mg of the drospirenone and transfer into a 50 ml clean, dry standard volumetric flask, add 25 ml of methanol, sonicate for 30 minutes and make up with methanol (stock solution). Further pipette out 5 ml of stock solution into a 10 ml volumetric flask and dilute up to the mark with methanol to get a solution of drospirenone (0.12 mcg/ml).

Accurately weigh about 0.24 mg of the ethinyl estradiol and transfer into a 50 ml clean, dry standard volumetric flask, add 25 ml of methanol, Sonicate for 30 minutes and make up with methanol (stock solution). Further pipette out 5 ml of stock solution into a 10 ml volumetric flask and dilute up to the mark with methanol to get a solution of ethinyl estradiol (0.0012 mcg/ml).

Preparation of sample drug solution: Transfer 1250.8 mg (10 tablets) of marketed sample into a 50 ml of volumetric flask, add 25 ml of methanol, sonicate it for 10 minutes and make up with methanol (stock solution). Transfer 10 ml of stock solution into 50 ml of volumetric flask dilute to volume with methanol.

Chromatographic Run: Standard solution containing a mixture of drospirenone and ethinyl estradiol was loaded into the injector and HPLC parameters were entered as per table 7. This method was saved and then sample was injected and run for 7 min.

	Ethinyl estradiol			Drospirenone		
Parameters	**RT**	**USP Tailing**	**USP Plate Count**	**RT**	**USP Tailing**	**USP Plate Count**
FLOW1	1.805	1.706	3598	6.392	1.202	8468
FLOW2	1.209	1.750	3540	4.367	1.162	7074
TEMP1	1.450	1.704	3607	5.236	1.186	7752
TEMP2	1.447	1.753	3590	5.160	1.173	7891

Table 1: Robustness results for ethinyl estradiol and drospirenone.

Ethinyl estradiol			Drospirenone	
Sample Name	**RT**	**Area**	**RT**	**Area**
ACID	1.452	2125132	5.188	1507952
BASE	1.450	2266435	5.183	1591152
PEROXIDE	1.454	2263747	5.192	1578856
WATER	1.455	2227253	5.194	1545941
LIGHT	1.456	2281245	5.195	1583567
Mean		2232762		1561493
SD		63362		34558
%RSD		2.8		2.2

Table 2: Results of forced degradation studies.

Drug	Conc.(µg/ml)	Equation of regression line	R^2
ETH	0.06 – 0.18	Y= 29020x + 42584	0.998
DRO	6 – 18	Y= 20634x + 13023	0.999

Table 3: Linearity results for ethinyl estradiol and drospirenone.

Drug	%RSD (intra-day)	%RSD (inter-day)
ETH	0.717	0.91
DRO	1.414	0.57

Table 4: Precision results for ethinyl estradiol and drospirenone.

ETHINYL ESTRADIOL						
Spiked Level	**Sample Weight**	**Sample Area**	**mcg/ml added**	**mcg/ml found**	**% Recovery**	**% Mean**
50%	625.40	1484085	0.059	0.06	101	101
50%	625.40	1476424	0.059	0.06	100	
50%	625.40	1462429	0.059	0.06	99	
50%	625.40	1489768	0.059	0.06	101	
50%	625.40	1490425	0.059	0.06	101	
50%	625.40	1483474	0.059	0.06	101	
100%	1250.80	2943921	0.119	0.12	100	100
100%	1250.80	2915651	0.119	0.12	99	
100%	1250.80	2957407	0.119	0.12	100	
150%	1876.20	4423964	0.178	0.18	100	99
150%	1876.20	4409785	0.178	0.18	100	
150%	1876.20	4420727	0.178	0.18	100	
150%	1876.20	4302456	0.178	0.17	97	
150%	1876.20	4427862	0.178	0.18	100	
150%	1876.20	4338740	0.178	0.18	98	

Table 5: Accuracy results for ethinyl estradiol.

Chromatographic conditions for the optimized method

Parameters	Description
Column	C18 Thermo Hypersil BDS (250×4.6×5 mm)
Mobile phase	Ammonium acetate : Acetonitrile (70:30)
Injection volume	15 µl
Flow rate	1 ml/min
Detector Wavelength	PDA at 258 nm
Column Temperature	40°C
Auto Sampler Temperature	25°C
Run Time	7 min

Results and Discussion

Validation

The method was validated for accuracy, precision, linearity, limit of detection, limit of quantitation and robustness as per ICH guidelines (Figures 3 and 4).

Specificity and selectivity

It is the extent to which the procedure applies to analyte of interest and is checked by examining the formulation samples for any interfering peaks. The specificity of the method was evaluated with regard to interference due to presence of blank and any other excipients. The figure shows that drug was clearly separated from blank

and its excipients. Figure 2 shows chromatogram for the formulation show that the selected drugs were clearly separated. Thus the proposed HPLC method is selective.

Forced Degradation studies of the drug product were carried out under stress conditions. The drug product in solution state was conducted with 0.1N HCl for 30 min. Base hydrolysis of drug product was conducted by 0.1N NaOH for 30 min. For oxidative stress, sample solutions of drug product in 3% hydrogen peroxide were kept (Tables 1 and 2).

Linearity

The linearity of the method was determined at five concentration levels ranging from 0.06 - 0.18 µg/ml for ethinyl estradiol and 6 - 18 µg/ml for drospirenone. The regression equation of calibration curves

DROSPIRENONE						
Spiked Level	**Sample Weight**	**Sample Area**	**mcg/ml added**	**mcg/ml found**	**% Recovery**	**% Mean**
50%	625.40	1000737	6.056	5.96	98	
50%	625.40	997394	6.056	5.94	98	
50%	625.40	987911	6.056	5.89	97	
50%	625.40	1001888	6.056	5.97	99	
50%	625.40	1007424	6.056	6.00	99	
50%	625.40	1006174	6.056	6.00	99	98
100%	1250.80	2045007	12.120	12.19	101	
100%	1250.80	2034841	12.120	12.13	100	
100%	1250.80	2064497	12.120	12.30	102	101
150%	1876.20	3087552	18.178	18.40	101	
150%	1876.20	3086164	18.178	18.39	101	
150%	1876.20	3100722	18.178	18.48	102	
150%	1876.20	3112915	18.178	18.55	102	
150%	1876.20	3122673	18.178	18.61	102	
150%	1876.20	3130111	0.178	18.65	103	102

Table 6: Accuracy results for Drospirenone.

Validation Parameters	Ethinyl estradiol	Drospirenone
Mobile Phase	70:30 (Ammonium Acetate: ACN)	70:30 (Ammonium Acetate: ACN)
Flow Rate	1 ml/min	1 ml/min
Detection wave Length	PDA at 258 nm	PDA at 258 nm
Rt	1.438	5.321
Run Time	7 min	7 min
Asymmetry	1.693	1.188
Theoretical Plates	3636	7728
LOD	0.00026 ppm	0.0925 ppm
LOQ	0.00087 ppm	0.308 ppm
Linearity	R^2=0.998	R^2=0.999
Precision	% RSD < 2	% RSD < 2
Recovery	99-101%	98-102%

Table 7: System suitability parameters for HPLC.

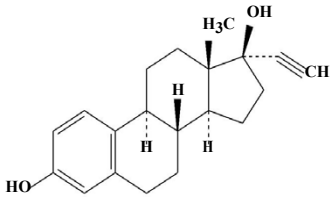

Figure 1: Ethinyl estradiol.

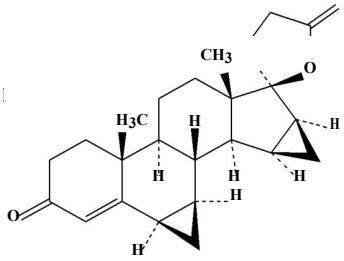

Figure 2: Drospirenone.

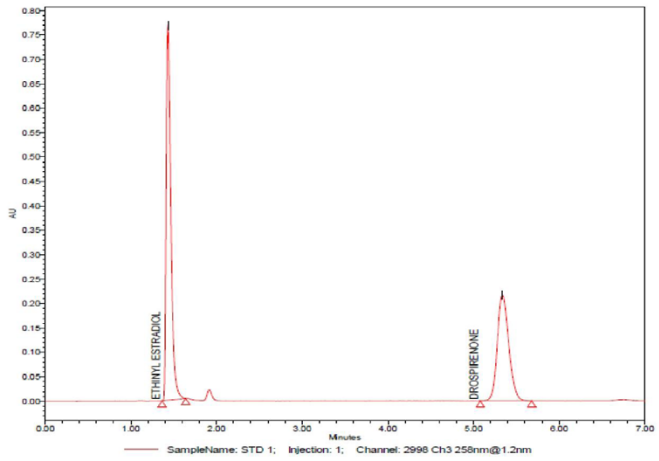

Figure 3: Chromatogram of standard solution of ethinyl estradiol and Drospirenone.

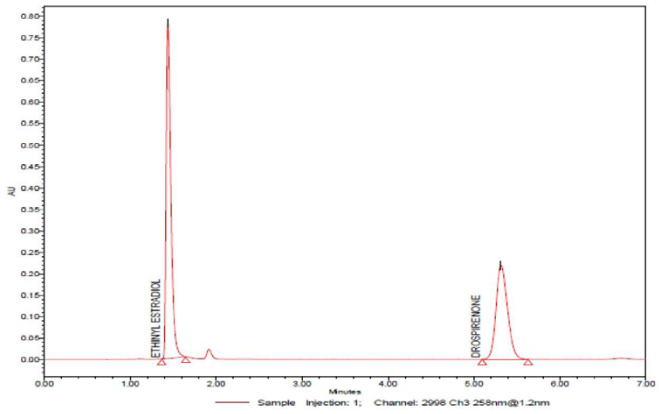

Figure 4: Chromatogram of DROSTAL™ (Tablet Formulation).

(Figures 3 and 6) were Y= 29020x + 42584 for ethinyl estradiol and Y= 20634x + 13023 for drospirenone and are summarized in table 3, Figures 5 and 6.

Precision

Precision of the method was studied as repeatability, intra-day and inter day variations. The intra-day precision was determined by analyzing ETH and DRO six times each on same day (intra-day study). This was repeated on the second day (inter-day study) and the results were shown in table 4, figures 7-10.

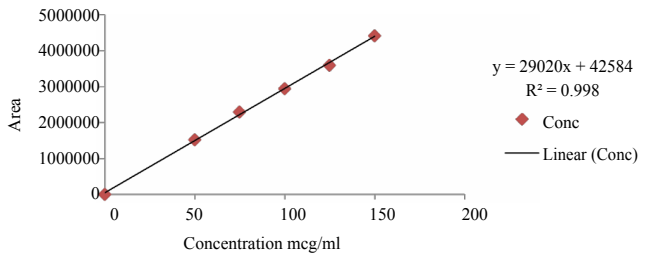

Figure 5: Linearity of Ethinyl estradiol.

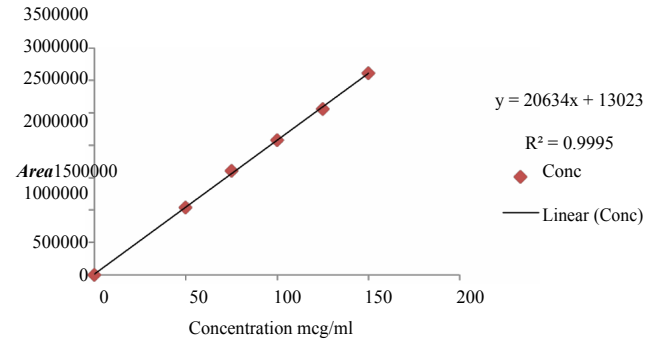

Figure 6: Linearity of Drospirenone.

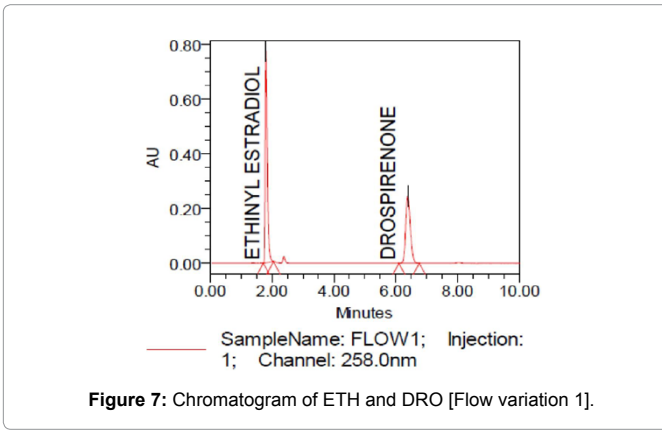

Figure 7: Chromatogram of ETH and DRO [Flow variation 1].

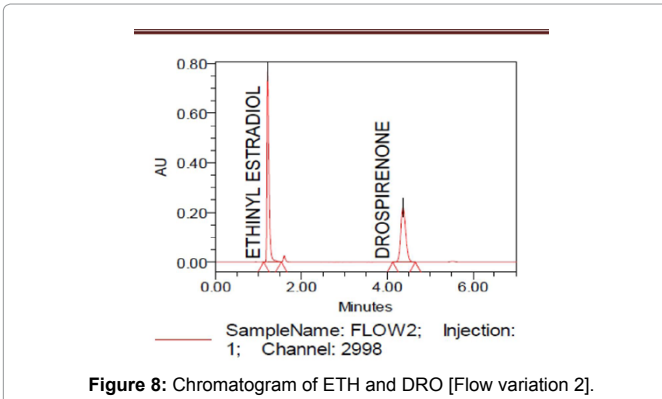

Figure 8: Chromatogram of ETH and DRO [Flow variation 2].

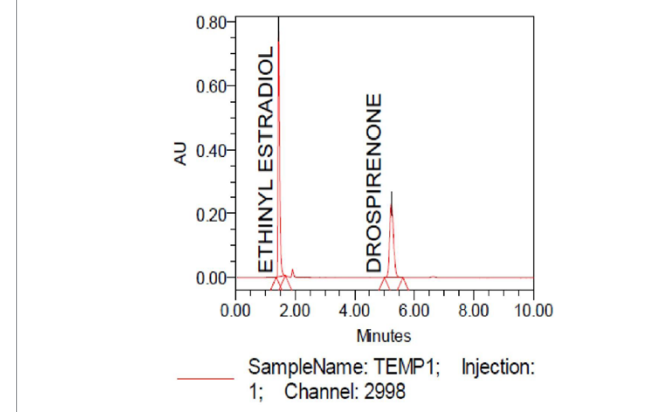

Figure 9: Chromatogram of ETH and DRO [Temperature variation 1].

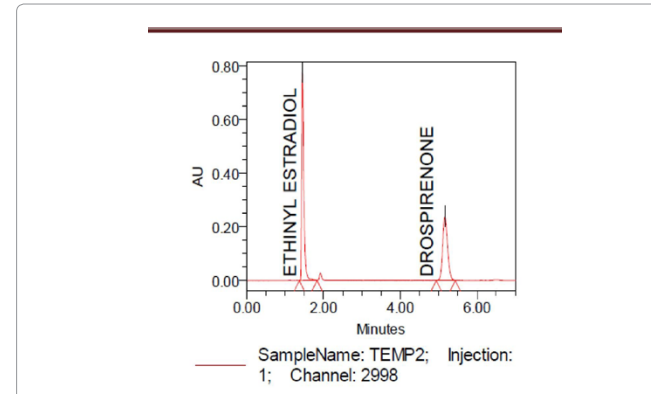

Figure 10: Chromatogram of ETH and DRO [Temperature variation 2].

Accuracy

The accuracy of the method was determined by recovery studies. The recovery studies were performed by standard addition method at 50% for six times, 100% for three times, 150% for six times and summarized in tables 5 and 6.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD is the ability of analytical method to detect the lowest concentration of the analyte. LOQ is the lowest concentration of the analyte with acceptable precision and accuracy. It can be calculated based on the signal to noise ratio. The LOD of ETH and DRO were 0.00026 ppm and 0.0925 ppm. The LOQ of ETH and DRO were 0.00087 ppm and 0.308 ppm respectively.

Robustness

Robustness of the method was determined by making slight changes in the flow rate and column temperature. It was observed that there were no marked changes in the retention time and area of the chromatograms which demonstrated that the RP HPLC method developed was robust and data are summarized in table 1.

Conclusion

The Proposed RP-HPLC and UV-Spectrophotometric method were suitable techniques for simultaneous determination of Ethinyl estradiol and Drospirenone in combined dosage combinations without any interferences form each other and excipients. All the parameters

for both the drugs had met the criteria of ICH guidelines for method validation. The low values of % RSD indicate the method is precise and accurate.

From the forced degradation studies it can be concluded that there is no other co-eluting peak with the main peaks and the method is specific for the estimation of Ethinyl estradiol and Drospirenone in presence of its degradation products and impurities. Result of validation parameter demonstrates that the analytical procedure is suitable for its intended purpose and meets the criteria defined in ICH Q2A/B.

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