SIMULTANEOUS ESTIMATION OF DROSPIRENONE AND ESTETROL AND FORCED DEGRADATION BEHAVIOUR BY RP-HPLC IN A COMBINED PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A new Reverse phase high performance liquid chromatography method has been developed for estimating Drospirenone and Estetrol in pure and pharmaceutical dosage form. The method uses a Waters SunFire C18 column for chromatographic separation and UV detection. The method is linear and has a 99%-100.4% recovery rate. The method was validated for accuracy, specificity, linearity, precision, robustness, and ruggedness, indicating its suitability for intended use.

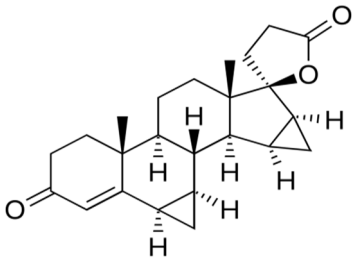
Keywords: Drospirenone, Estetrol, RP-HPLC, Validation.

1. INTRODUCTION

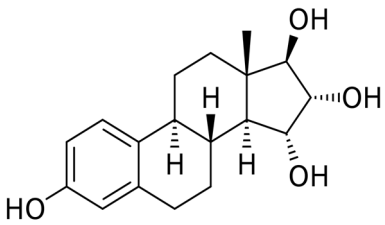
Drospirenone is a progestin used in oral contraceptive pills for the prevention of pregnancy and other conditions. It is a synthetic progestin commonly found in the popular oral contraceptive, Yaz in combination with Ethinyl estradiol. Most recently, it was approved by both Health Canada and the FDA in combination with Estetrol as an oral contraceptive therapy. Molecular formula $C_{24}H_{30}O_3$ Molecular Weight 366.4932 g/mol.

Estetrol (E4) is a native estrogen occurring naturally during pregnancy, but can be synthesized from a plant source and used for contraception. It is more potent and is safer than the synthetic estrogen ethinyl estradiol (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. Molecular formula $C_{18}H_{24}O_4$ Molecular Weight 304.3808 g/mol.

The aim of the current analytical study is to provide an RP-HPLC method for the combined tablet formulation of Drospirenone and Estetrol that is easy to use, accurate, precise, quick and affordable. And the method was validated as per ICH guidelines.



Drospirenone



Estetrol

Fig.1 The chemical structure of Drospirenone and Estetrol

2.MATERIALS AND METHOD

2.1 Reagents and chemicals

The API of Drospirenone and Estetrol was gifted from Rankem Chemicals. The pharmaceutical dosage form (Nextstellis) was purchased from pharmacy. The reagents used are of HPLC grade and are obtained from Research Laboratories.

2.2 Equipment

The HPLC used is of Shimadzu LC 20 AD with UV detector. The chromatographic conditions include, column used is Waters SunFire C18 column with mobile phase consist of Buffer 0.01N (NH4)3PO4: Acetonitrile taken in the ratio 70:30(v/v). The flow rate was adjusted to 1.0 mL/min. The injection volume was 20 µL. UV detection was achieved at 265nm.

2.3 Preparations of analytical solutions

Preparation of phosphate buffer:

Accurately weighed 1.32 g of Dibasic ammonium phosphate in a 1000 mL of Volumetric flask add about 900 mL of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 6.8 with dil. OPA.

Preparation of Mobile phase:

Mobile phase containing Buffer 0.01N (NH4)3PO4 : Acetonitrile taken in the ratio 70:30 and filtered through 0.45µm membrane filter and sonicated to degas. It is pumped through column at a flow of 1.2 mL/min.

Preparation of Standard Solution:

Weigh accurately 14.2mg of estetrol and 3 mg of drospirenone into 25 mL volumetric flask and 3/4th of diluents was added to these flasks and sonicated for 10 minutes. Flask was made up with diluents and labelled as Standard stock solution.

Preparation of Sample Solution:

10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to tablet was transferred into a 50 mL volumetric flask and until the tablets are

completely dispersed. Equilibrate to room temperature, 25 mL of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters.

3. METHOD DEVELOPMENT AND VALIDATION

The method development for simultaneous estimation of drospirenone and estetrol was developed to get the optimized RP-HPC method. From several trials final method is optimized with following conditions:

The mobile phase consists of Buffer 0.01N (NH4)3PO4: Acetonitrile taken in the ratio 70:30(v/v). The flow rate was adjusted to 1.0 mL/min., with injection volume 20µl. The UV detection was achieved at 265nm.

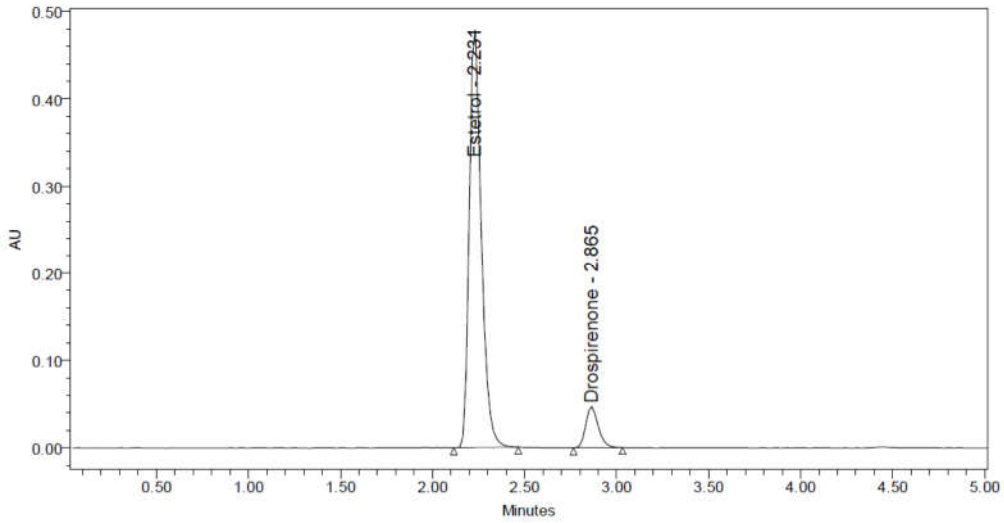


Fig. 2: Chromatogram of standard

3.1 SYSTEM SUITABILITY

Standard solutions of Drospirenone (12 µg/mL) and Estetrol (56.8 µg/mL) were made up and injected six times, and parameters such as peak tailing, resolution, and USP plate count were monitored.

Table 1: System Suitability results

Parameters	Drospirenone	Estetrol
Retention Time	2.8	2.2
Tailing factor	1.2	1.2
Theoretical plates	7860	6459

3.2 SPECIFICITY

The method's specificity was assessed via examination of the blank, standard, placebo, and sample solutions. Chromatograms were clearly separated from its excipients that there was no

interference of blank and placebo in standard and sample peaks. Hence, the method was found to be specific for determination of title analytes.

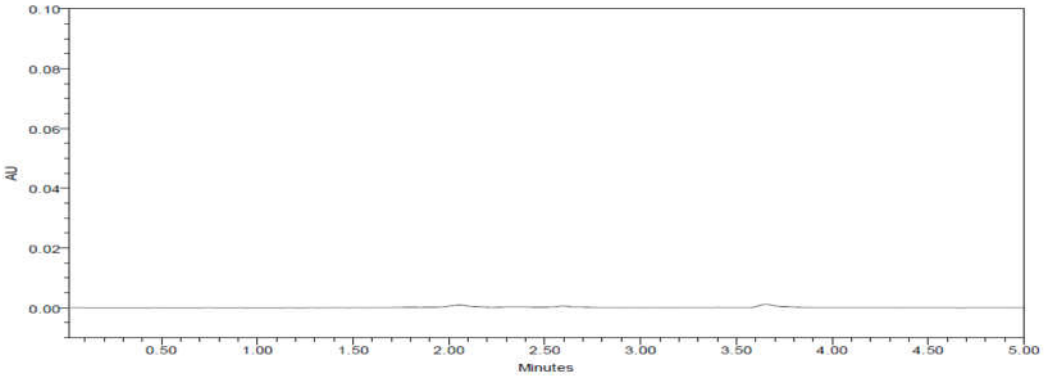


Fig. 3: Chromatogram of blank

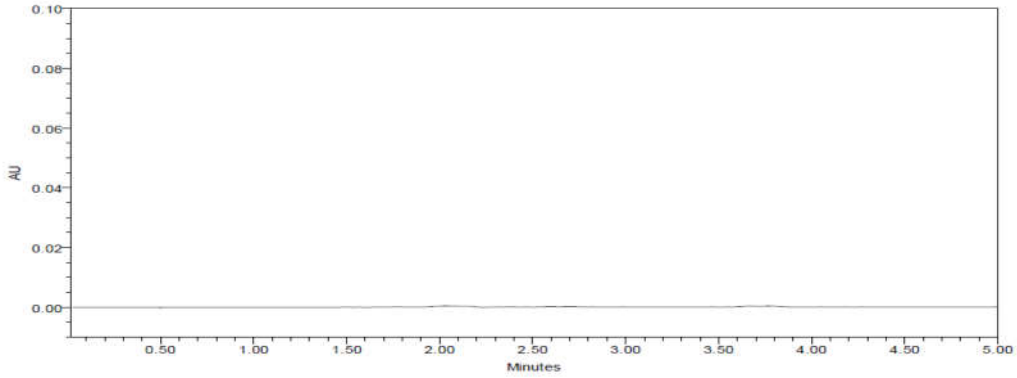


Fig. 4: Chromatogram of Placebo

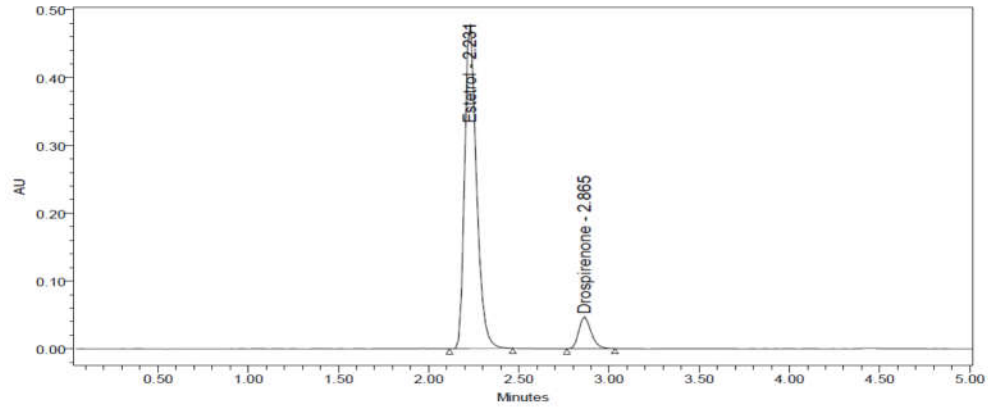


Fig. 5: Specificity chromatogram of Drospirenone and Estetrol

3.3 LINEARITY

Table 2: Linearity data of Drospirenone and Estetrol

S.No.	Drospirenone		Estetrol	
	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
1.	1.5	206605	14.2	982055
2.	3	420959	28.4	1959951
3.	4.5	628596	42.6	2995707
4.	6	841529	56.8	3861467
5.	7.5	1053610	71	4794596
6.	9	1233115	85.2	5871105

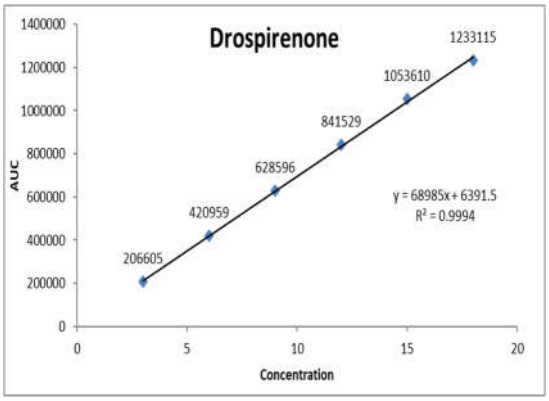


Fig. 6: Calibration curve of Drospirenone

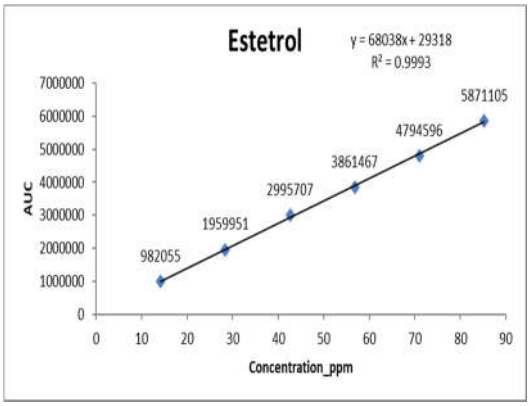


Fig. 7: Calibration curve of Estetrol

3.4 ACCURACY

Accuracy was performed by standard addition method. In this standard addition samples of 50%, 100%, 150%, was added to pre-analysed sample solution and injected into the HPLC system in triplicate and percentage recoveries were calculated.

Table 3: Accuracy (%Recovery data)

% Recovery	Drospirenone	Estetrol
1	100.1	100.3
2	99.5	100.19
3	100.7	100.6
Mean	100.15	100.39

3.5 PRECISION

System precision:

The system precision was established by injecting six replicate injections of standard solution into the chromatographic system by maintaining the optimized chromatographic conditions.

Table 4: System precision data

S. No	Area of Drospirenone	Area of Estetrol
1.	831353	3904664
2.	835565	3926464
3.	835767	3976556
4.	836565	3956566
5.	830667	3965654
6.	836757	3957464
Mean	834446	3947895
S.D	2708.3	26951.0
%RSD	0.3	0.7

Method precision:

Six standard samples concentration were prepared and injected into the chromatographic system. % RSD values of precision are within limits.

Table 5: System precision data

S. No	Area of Estetrol	Area of Drospirenone
1.	3936746	836464
2.	3957566	837564
3.	3916464	830876
4.	3904645	837565
5.	3926454	839677
6.	3954633	835465
Mean	3932751	836269
S.D	21002.1	2991.3
%RSD	0.5	0.4

3.6 ROBUSTENSS

Robustness was performed by evaluating small deliberate variations in flowrate and wavelength. The robustness was performed for flow rate variations for 1.0mL, 1.3mL and for temperature for 21°C, 31°C.

3.7 LIMIT OF DETECTION

LOD is determined by the analysis of samples with known concentration of analyte by establishing the minimum level at which the analyte can be detected.

The LOD was found to be 0.04 and 0.13 for Drospirenone and Estetrol.

3.8 LIMIT OF QUANTIFICATION

LOQ is determined by the analysis of samples with known concentration of analyte by establishing the minimum level at which the analyte can be quantified.

The LOQ was found to be 0.13 and 0.40 for Drospirenone and Estetrol.

Table 6: Degradation profile results

Type of degradation	Drospirenone		Estetrol	
	% Recovered	% Degraded	% Recovered	% Degraded
Acid	93.94	6.06	93.56	6.44
Base	95.85	4.15	95.49	4.51
Peroxide	95.86	4.14	95.74	4.26
Thermal	98.77	1.23	98.14	1.86
UV	98.58	1.42	98.41	1.59
Water	99.33	0.67	98.93	1.07

ASSAY

The proposed method was evaluated in the assay of commercially available tablets containing Drospirenone 3 mg and Estetrol 14.2 mg. The percentage assay of Drospirenone and Estetrol was found to be 100.2% and 99.42%.

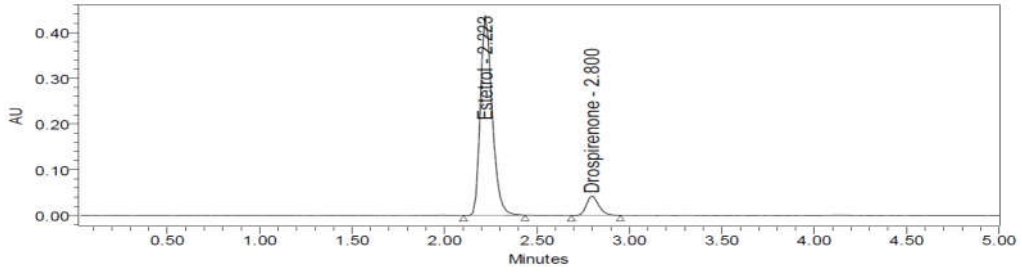


Fig. 8: Assay Chromatogram of Drospirenone and estetrol

SUMMARY TABLE:

S.No.	PARAMETERS	RESULT
1	Wavelength(nm)	265nm
2	Retention time	Drospirenone-2.8 minutes Estetrol-2.2 minutes
3	Linearity range (µg/ml)	Estetrol-14.2-85.2 µg/mL Drospirenone-1.5-9 µg/mL
4	%Recoveries	Drospirenone-100.15% Estetrol-100.39%
5	Theoretical plates	Drospirenone-7860 Estetrol-6459
6	Tailing factor	Drospirenone-1.2 Estetrol-1.2

CONCLUSION

A simple, reliable, and precise method has been established for the simultaneous estimation of drospirenone and estetrol in tablet dose form. The retention times for estetrol and dospirenone were found to be 2.231 and 2.865 minutes, accordingly. The percentage RSDs for estetrol and dospirenone were determined to be 0.7 and 0.2, correspondingly. % Drospirenone and estetrol showed recovery rates of 100.15% and 100.39%, respectively. Drospirenone and estetrol had respective LOD and LOQ values of 0.04, 0.13, and 0.13, 0.40. The developed technology was suited for use in routine quality control testing in industries since it was convenient to use and affordable.

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