



RP-HPLC Method Development and Validation for Estimation of Drospirenone and Ethinyl Estradiol in Bulk and Combined Dosage Form

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ABSTRACT:

A precise and accurate reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for simultaneous estimation of Drospirenone (DP) and Ethinylestradiol (EE) from their combined pharmaceutical dosage form. The proposed RP-HPLC method utilizes a Phenomenex Luna C₁₈ (250mm x 4.6mm, 5 µm particle size) column at ambient temperature; the optimum mobile phase consists of acetonitrile:water (60:40,v/v); mobile phase flow rate of 1.5 ml/min; and UV detection at 280 nm. The retention time for DP and EE were 2.7 and 5.3 minutes respectively. The method was linear in the range of 80-400 µg/ml and 1.4-7.0 µg/ml and correlation coefficients were 0.997 and 0.996 for DP and EE respectively. Proposed method was validated as per ICH guidelines for precision, accuracy and linearity for estimation of DP and EE in commercially available tablet dosage form. Results of the validation were found satisfactory. The proposed method can be useful in the quality control of bulk manufacturing and pharmaceutical dosage forms.

Keywords: Drospirenone, Ethinylestradiol, RP-HPLC.

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INTRODUCTION

Drospirenone^(1,2) is (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*-dicyclopropa-6,7:15,16]cyclopenta[*a*]phenanthrene-17,2'(*5H*)-furan-3,5'(*2H*)-dione (Figure 1) and it is Soluble in water.

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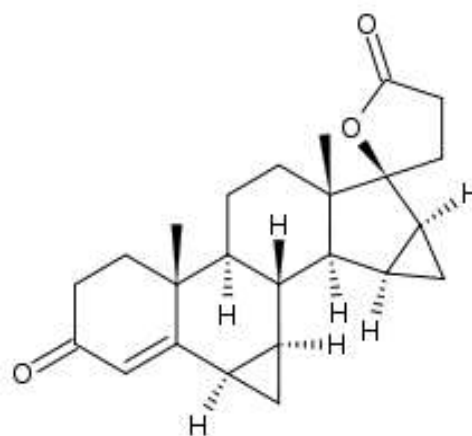
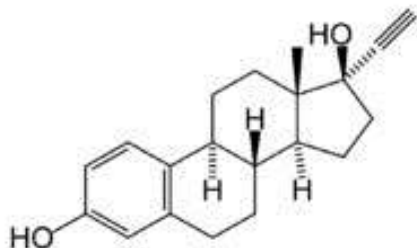


Figure: 1 Structure of Drospirenone

Figure: 2 Structure of Ethinyl Estaradiol



These are hormonal preparation used for reversible suppression of fertility^(3,4). 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, 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.

Literature review reveals that RP-HPLC, LCMS, HPTLC method have been reported for estimation of Drospirenone alone. Literature review reveals that HPLC, LCMS, HPTLC method have been reported for estimation of Ethinylestradiol alone⁽⁵⁻²²⁾. Literature review reveals there is no stability indicating HPLC methods have been reported for estimation of Drospirenone and Ethinylestradiol in combination.

In this report a developed and validated simple, rapid, sensitive and selective Stability Indicating RP-HPLC Method Development and Validation of Drospirenone and Ethinylestradiol in their combined pharmaceutical dosage form.

MATERIALS & METHODS:

RP-HPLC Method

Materials

Drospirenone and Ethinylestradiol (Supplied by Par Laboratories Ltd.Gozaria),Acetonitrile - HPLC grade, Methanol - HPLC grade, Water - HPLC grade (Finar chemicals Ltd. Ahmedabad)

Methods

Apparatus and Instrumentation

The HPLC system was of LC-2010HT (Shimadzu) with UV detector. The chromatographic analysis was performed using LC solution software on a Phenomenex Luna C₁₈ (250 mm x 4.6 mm), 5μ column. U.V. Spectrophotometer Pharmspec-1700 (Shimadzu).In addition, an electronic analytical balance (Mettler Toledo, model XP205), and a Sonicator (Ultrasonic cleaner, model OU-72SPL) were used in this study.

Chromatographic conditions

Stationary phase: Phenomenex Luna C₁₈ (250mm x 4.6mm, 5 μm particle size)

Mobile phase: Acetonitrile: Water (60:40, v/v)

Flow rate: 1.5 ml/min

Injection volume: 10 μl

Detection wavelength: 280 nm

PREPARATION OF SAMPLE AND STANDARD SOLUTIONS

Standard preparation

a) Preparation of Drospirenone (DP) stock solution

Accurately weighed 20 mg of quantity of DP reference standard was transferred into 50 ml volumetric flask and dissolved in 5ml mobile phase solution and sonicated for about 5 min with intermittent shaking and diluted up to the mark with mobile phase solution to give a stock solution having strength 400μg/ml.

b) Preparation of Ethinylestradiol stock solution (EE)

Accurately weighed 35 mg quantity of EE reference standard was transferred into 100 ml volumetric flask and dissolved in 50ml mobile phase and sonicated for about 5 min with intermittent shaking and diluted up to the mark with mobile phase to give a stock solution having strength 350 μg/ml.

Calibration curve of DP and EE

Aliquots of mixture of working standard solution of DP (80-400 μg/ml) and EE (1.4-7.0 μg/ml) were injected into the HPLC system The graph of area of peak obtained versus respective concentration was plotted. The mean area and its standard deviation were calculated.

Sample preparation

Twenty tablets of CRISANTA containing DP and EE in ratio of 3 mg: 0.03 mg respectively weighed and crushed to fine powder. Powder equivalent to 3 mg DP and 0.03 mg of EE was weighed and dissolved in 10 ml mobile phase, sonicated for 10 min and filtered through 0.45µm HVLP filter to obtain a solution with 300 µg/ml of DP and 3 µg/ml of EE.

Validation

The analytical method was validated according to International Conference on Harmonization (ICH) guidelines⁽²³⁾. The method was validated for accuracy, precision, specificity, detection limit, quantitation limit and robustness. The accuracy of the method was determined by calculating recoveries of Drospirenone and Ethinylestradiol by method of standard additions.

The instrument precision was evaluated by injecting the different five concentrations three times and peak area was measured.

The results are reported in terms of relative standard deviation.

The intra-day and inter-day precision study of Drospirenone and Ethinylestradiol was carried out by estimating the corresponding responses 3 times on the same day and on 3 different days and the results are reported in terms of relative standard deviation (RSD). Limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.601 µg/ml and 1.822µg/ml for Drospirenone while 0.049 µg/ml and 0.150 µg/ml for Ethinylestradiol. Robustness of the method was studied by deliberately

changing the experimental conditions like flow rate, temperature.

RESULTS AND DISCUSSION

Mobile phase and flow rate selection were based on peak parameters (symmetry, tailing), run time, ease of preparation, and cost. A typical chromatogram for Drospirenone and Ethinylestradiol using the Phenomenex Luna C₁₈ (250mm x 4.6mm, 5 µm particle size) with a mobile phase composition of Acetonitrile: Water (60:40, v/v) at a flow rate of 1.5 ml/min. Drospirenone and Ethinylestradiol produced a sharp and symmetric peak when chromatographed with these conditions. The UV spectrum of the drug shows an absorption band at 280 nm (Figure 3); therefore, the wavelength of detection was fixed at 280 nm. The use of mobile phase without buffers, which shortens column life, is the main advantage of the proposed LC method. Drospirenone and Ethinylestradiol were injected in HPLC system (Figure 4,5). The retention time observed allowed rapid determination of the drug, which is important for routine analysis. System suitability was performed and peaks of both drugs are separated with good resolution (Table 1). No interferences from the diluents, impurities, or excipients present in pharmaceutical formulations were observed at the detection wavelength.

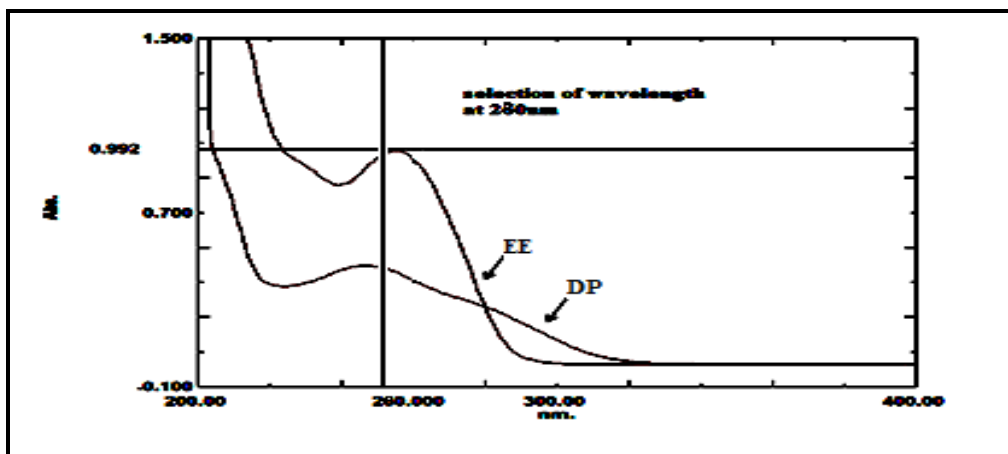


Figure 3: UV spectrum of Drospirenone and Ethinylestradiol standard solution

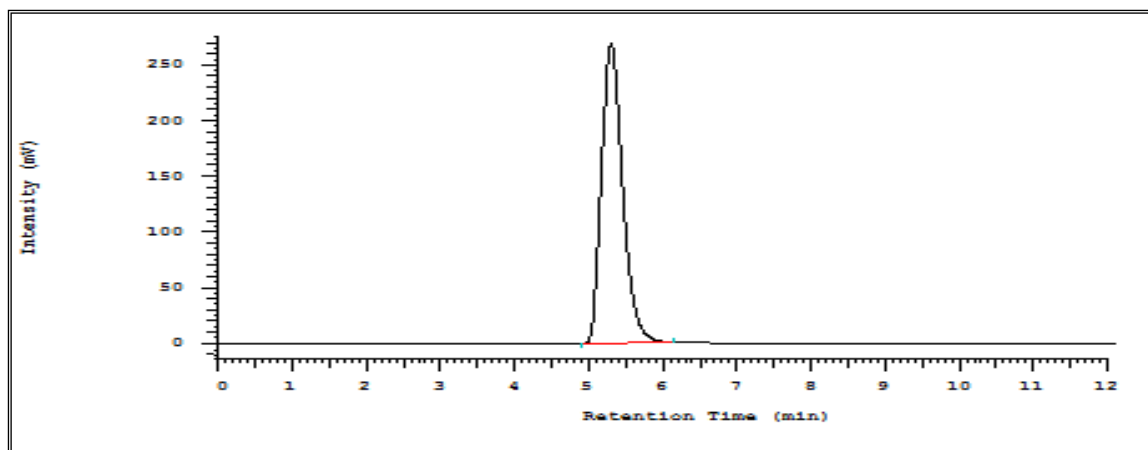


Figure 4: Chromatogram of standard solution of Drospirenone

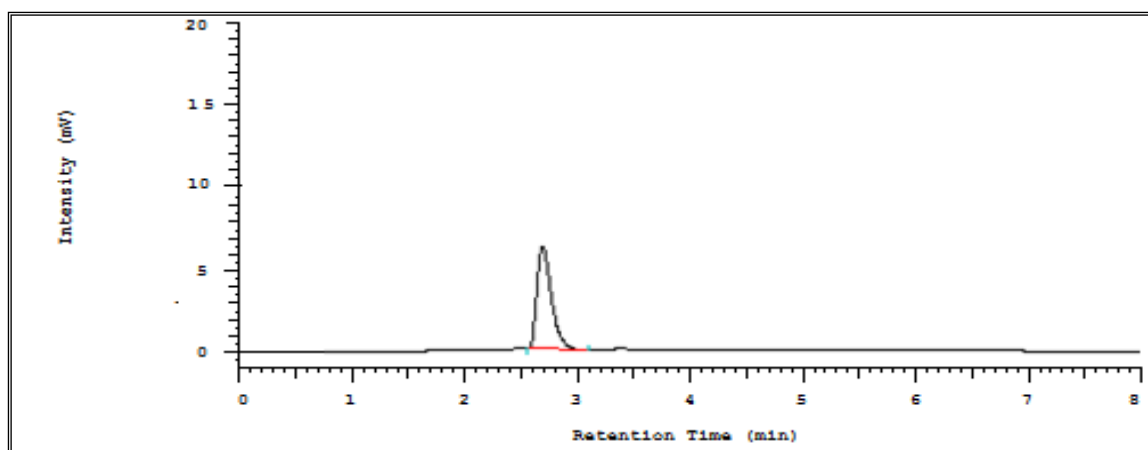


Figure 5: Chromatogram of standard solution of Ethinylestradiol

Applicability of the Method

Applicability of the proposed method was tested by analyzing the commercially available tablet formulation (Figure 6). The results are shown in Table 2.

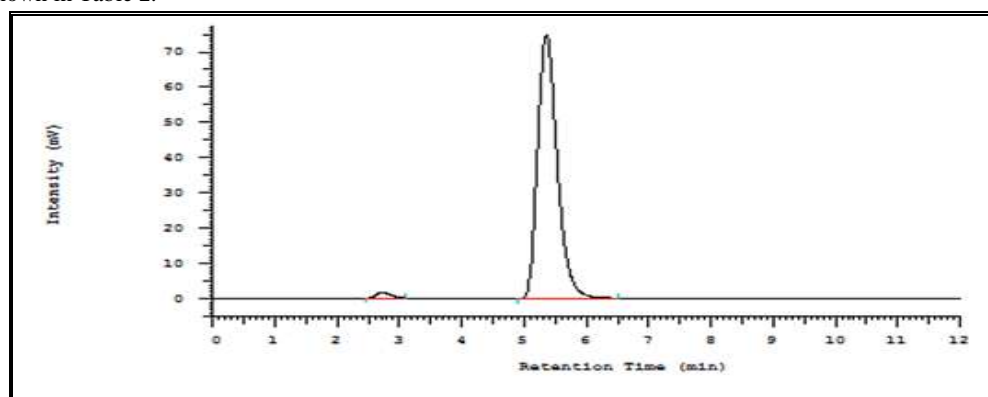


Figure 6: Chromatogram of sample solution

Table 2: Analysis of marketed formulation

Tablet	mg/tablet		Amount found (mg)		Assay (% of label claim)*	
CRISANTA	DP	EE	DP	EE	DP	EE
	3	0.030	2.94	0.0291	97.99±0.89	97.10±0.52

The linearity range for DP was found to be in the range of 80-400µg/ml and for EE it was 1.4-7.0 µg/ml. Range of Intraday %RSD was found to be 0.11-0.80% for DP and 0.18-0.93% for EE. Range of Interday %RSD was found to

be 0.19-0.63% for DP and 0.96-1.44% for EE. The recovery range for DP and EE were found to be 93.75-100.25% and 94.42-97.61%. All parameters are described in (Table 3).

Table 3: System suitability & Validation parameters

Parameter	DP	EE
Linearity range (µg/ml)	80-400	1.4-7.0
Correlation coefficients	0.997±0.02	0.996±0.01
Theoretical plate	5784	3492
Asymmetry factor	0.71	0.47
Retention time (min)	5.3	2.7
LOD (µg/ml)	0.601	0.049
LOQ (µg/ml)	1.82	0.150
Robustness	Robust	Robust
Specificity	No interferences found	No interferences found
Precision (%RSD)		
Intra-day (n=3)	0.11-0.80	0.18-0.93
Inter-day (n=3)	0.19-0.63	0.96-1.44
Accuracy (%recovery)	93.75-100.25%	94.42-97.61%.
Assay (%)	97.99±0.89	97.10±0.52

CONCLUSION:

The proposed LC method have the advantages of simplicity, precision, accuracy, and convenience. The developed method is fast and use simple reagents with minimal sample preparation procedure. It is suitable for the routine analysis in quality control of Drospirenone and Ethinylestradiol in combined tablet dosage form, such as assay and uniformity testing.

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