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RESEARCH ARTICLE

# VALIDATED RP - HPLC METHOD FOR THE ESTIMATION OF DROSPIRENONE IN FORMULATION

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Drospirenone in tablet dosage form. Isocratic elution at a flow rate of 1.0ml/min was employed on a symmetry C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of methanol: 1% Ortho phosphoric acid 54.5:45.5 (V/V). The UV detection wavelength was 252nm and 20µl sample was injected. The retention time for Drospirenone was 8.355 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Drospirenone in tablet dosage form.

KEYWORDS: *Drospirenone, RP-HPLC, UV detection, recovery, precise.*

## INTRODUCTION

Drospirenone is a synthetic progestin that is an analog to spironolactone, it is part of certain birth control formulations. The compound differs from other synthetic progestins in that its pharmacological profile in preclinical studies shows it to be closer to the natural progesterone. As such it has anti-mineralocorticoid properties, counteracts the estrogen-stimulated activity of the renin-angiotensin-aldosterone system, and is not androgenic. With its activities similar to spironolactone it may lead to less water retention and breast tenderness while improving skin appearance.

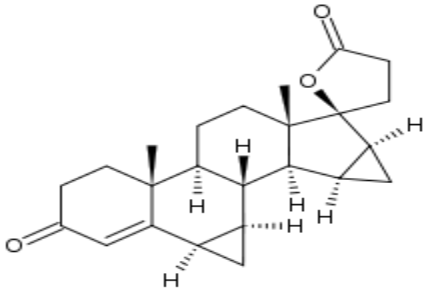

Figure-1: Chemical Structure of Drospirenone

Drospirenone Molecular formula $C_{24}H_{30}O_3$ Molecular weight 366.493 g/mol

IUPAC Name

(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'(5*H*)-furan]-3,5'(2*H*)-dione)

## EXPERIMENTAL

### Chemicals and reagents

HPLC grade ortho phosphoric acid and methanol were purchased from Merck Specialties Pvt. Ltd.

### Instrumentation and analytical conditions

The analysis of drug was carried out on a PEAK HPLC system equipped with a reverse phase C18 column (250x4.6mm, 5µm in particle size), a LC-P7000 isocratic pump, a 20µl injection loop and a LC-UV7000 absorbance detector and running on PEAK Chromatographic Software version 1.06. Isocratic elution with methanol: 1% ortho phosphoric acid 54.5:45.5(V/V) ($P^H$-4.0) was used at a flow rate of 1.0ml/min. The mobile phase was prepared freshly and degassed by sonicating for 5 min before use.

### Stock and Working standard solutions

Accurately weigh and transfer 10mg of Drospirenone working standard into a 10ml volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm nylon filter paper and finally 15ppm were prepared. The calibration curve was plotted with the five concentrations of the 3ppm – 15ppm working standard solutions. Calibration solutions were prepared daily and analyzed immediately after preparation.

### Assay of Drospirenone tablets

Weigh 20 Drospirenone (Loryna- 3mg) tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10mg of Drospirenone in to a 10ml volumetric flask. Add diluent and sonicate to dissolve it completely and make volume up to the mark with diluents. Mix well and filter through 0.45um filter. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to mark with diluents and finally 9ppm were prepared. Mix well and filter through 0.45um filter. An aliquot of this solution was injected into HPLC system. Peak area of Drospirenone was measured for the determination. The results are furnished in Table 3.

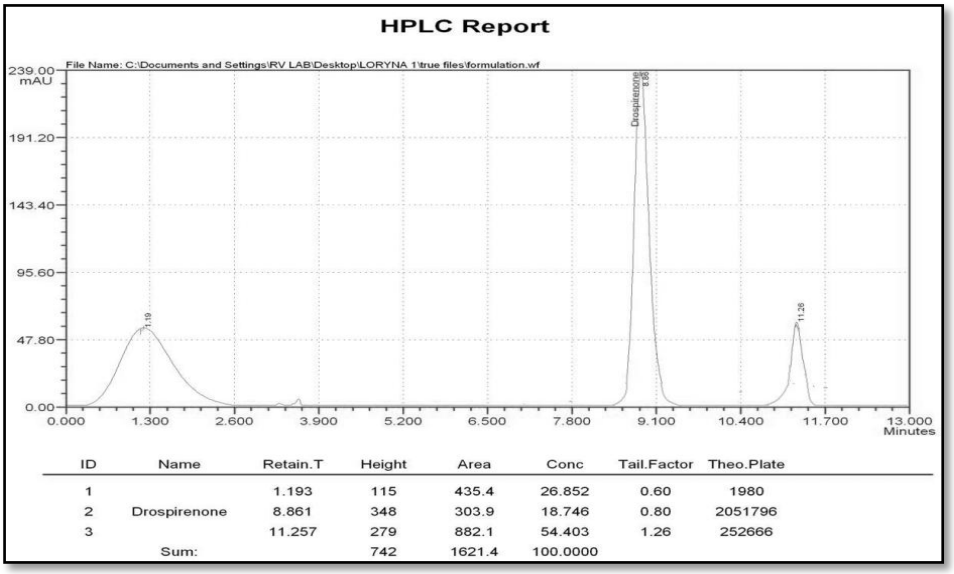


Fig 2: Typical chromatogram of Drospirenone Formulation

**Validation procedure**

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, specificity, stability and system suitability. Standard plots were constructed with five concentrations in the range of 3ppm to 15ppm prepared in triplicates to test linearity. The peak area of Drospirenone was plotted against the concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared Drospirenone test solution in the same equipment at a concentration value of 100% (9ppm) of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area of the Drospirenone was determined and precision was reported as %RSD.

Method accuracy was tested (% recovery and %RSD of individual measurements) by analyzing sample of Drospirenone at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of Drospirenone recovered in the samples. Sample solution short term stability was tested at ambient temperature (20±10ºC) for three days. In order to confirm the stability of both standard solutions at 100% level and tablet sample solutions, both solutions protected from light were re-injected after 24 and 48 hours at ambient temperature and compared with freshly prepared solutions.

**Table 1: Linearity of Drospirenone**

| S.No. | Linearity level | Concentration | Area |
|---|---|---|---|
| 1 | I | 3ppm | 62442.4 |
| 2 | II | 6ppm | 124322.3 |
| 3 | III | 9ppm | 180755.7 |
| 4 | IV | 12ppm | 238112.9 |
| 5 | V | 15ppm | 301407.7 |

**RESULT AND DISCUSSION**

**Optimization of the chromatographic conditions**

Proper selection of the stationary phase depends up on the nature of the sample, molecular weight and solubility. The drug Drospirenone in non-polar. Non-polar compounds preferably analyzed by reverse phase columns. Among C8 and C18, C18 column was selected. Non-polar compound is very attractive with reverse phase columns. So the elution of the compound from the column was influenced by polar mobile phase. Mixture of methanol and ortho phosphoric acid was selected as mobile phase and the effect of composition of mobile phase on the methanol and ortho phosphoric acid were optimized to give symmetric peak with short run time (Fig.3).

**VALIDATION OF METHOD**

**Linearity**

Five points graphs was constructed covering a concentration range 3-15ppm (Three independent determinations were performed at each concentration). Linear relationships between the peak area signal of Drospirenone the corresponding drug concentration was observed. The standard deviation of the slope and intercept were low. The statistical analysis of calibration is shown in Table 1.

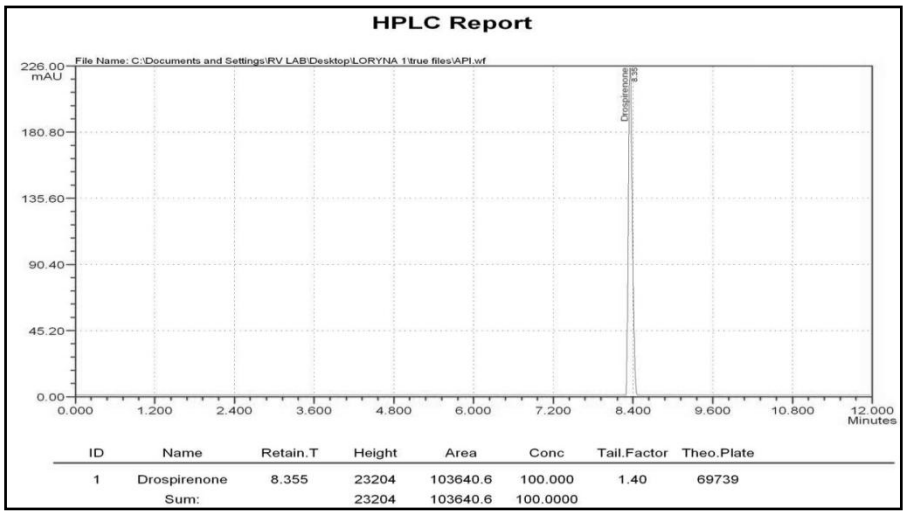


**Fig 3: Typical chromatogram of Drospirenone**

**Precision**

The validated method was applied for the assay of commercial tablets containing Drospirenone. Sample was analyzed for five times after extracting the drug as mentioned in assay sample preparation of the experimental section. The results presented good agreement with the labeled content. Low values of standard deviation denoted very good repeatability of the measurement. Thus it was showing that the equipment used for the study was correctly and hence the developed analytical method is highly repetitive. For the intermediate precision a study carried out by the same analyst working on the same day on two consecutive days indicated a RSD of 0.552. This indicates good method precision.

**Table 2: Recovery studies of Drospirenone**

| % Concentration | % Recovery | Mean Recovery |
|---|---|---|
| 50% | 99.73% | |
| 100% | 98.44% | 998.77% |
| 150% | 98.16% | |

**Table 3: System Stability Parameters**

| Parameters | Values |
|---|---|
| λ max (nm) | 252 |
| Beer's law limit (µg) | 3-15 |
| Correlation coefficient | 0.998 |
| Retention time | 8.355 |
| Theoretical plates | 69739.46 |
| Tailing factor | 1.40 |
| Limit of detection (µg) | 0.15 |
| Limit of quantification (µg) | 0.5 |
| Slope | 19724.04 |
| Intercept | 3891.84 |
| Accuracy | 99.22% |
| R.S.D. | 0.552 |
| % of Drospirenone in formulation | 4.2% |

**Stability**

The stability of Drospirenone in standard and sample solutions containing determined by storing the solutions at ambient temperature (20±10°C). The solutions were checked in triplicate after three

successive days of storage and the data were compared with freshly prepared samples. In each case, it could be noticed that solutions were stable for 48 hrs, as during this time the results did not decrease below 98%.

**Table 4: Assay**

| Formulation | Label claim (mg) | % Amount found |
|---|---|---|
| Loryna | 3mg | 4.2% |

**System suitability**

The system suitability parameter like capacity factor, asymmetry factor, tailing factor and number of theoretical plates were also calculated. It was observed that all the values are within the limits (Table.3). The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of Drospirenone in tablet formulation. The results are furnished in Table 3.

**Table 5: Chromatographic Condition**

| | |
|---|---|
| **Mobile phase** | 1% ortho phosphoric acid: methanol (45.5:54.5) |
| **PH** | 4.0 |
| **UV detection** | 252nm |
| **Analytical column** | C18 |
| **Flow rate** | 1.0ml/min |
| **Temperature** | ambient |
| **Injection volume** | 20µl |
| **Runtime** | 12min |
| **Retention time** | 8.355 min |

## CONCLUSION

A validated RP-HPLC method has been developed for the determination of Drospirenone in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 12 min allows the analysis of a large number of samples in short period of time.

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