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 bioactive estrogen used in almost all modern formulations of combined oral contraceptive pills. Chemically it is 19-Nor-17α-pregna-1, 3, 5(10)-tri-en-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         |
| 4    | Double distilled Water|       | Milli-QRO    |

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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 it for 10 minutes and make up with methanol (stock solution). Transfer 10 ml of stock solution into 50 ml of 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).

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

Table 1: Robustness results for ethinyl estradiol and drospirenone.

| Parameters | Ethinyl estradiol | Drospirenone |
|------------|-------------------|--------------|
| Flow 1     | RT: 1.805 USP Tailing: 3598 | RT: 1.202 USP Tailing: 8468 |
| Flow 2     | RT: 1.209 USP Tailing: 3540 | RT: 1.162 USP Tailing: 7074 |
| Temp 1     | RT: 1.450 USP Tailing: 3607 | RT: 1.186 USP Tailing: 7752 |
| Temp 2     | RT: 1.447 USP Tailing: 3590 | RT: 1.173 USP Tailing: 7891 |

Table 2: Results of forced degradation studies.

| Sample Name | Ethinyl estradiol | Drospirenone |
|-------------|-------------------|--------------|
| Acid        | RT: 1.452 Area: 2125132 | RT: 5.188 Area: 1507952 |
| Base        | RT: 1.450 Area: 2266435 | RT: 5.183 Area: 1591152 |
| Peroxide    | RT: 1.454 Area: 2267477 | RT: 5.192 Area: 1576856 |
| Water       | RT: 1.455 Area: 2227253 | RT: 5.194 Area: 1545941 |
| Light       | RT: 1.456 Area: 2281245 | RT: 5.195 Area: 1563567 |
| Mean        |                  |              |
| SD          |                  |              |
| %RSD        |                  |              |

Table 3: Linearity results for ethinyl estradiol and drospirenone.

| Drug | Conc.(µg/ml) | Equation of regression line | R²  |
|------|-------------|----------------------------|-----|
| ETH  | 0.06 – 0.18 | Y = 29020x + 42584       | 0.998|
| DRO  | 6 – 18      | Y = 20634x + 13023       | 0.999|

Table 4: Precision results for ethinyl estradiol and drospirenone.

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

Table 5: Accuracy results for 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        |        |
| 50%          | 625.40        | 1478424     | 0.059        | 0.06         | 100        |        |
| 50%          | 625.40        | 1462429     | 0.059        | 0.06         | 99         |        |
| 50%          | 625.40        | 149768      | 0.059        | 0.06         | 101        |        |
| 50%          | 625.40        | 1490425     | 0.059        | 0.06         | 101        |        |
| 100%         | 1250.80       | 2943921     | 0.119        | 0.12         | 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        |        |
| 150%         | 1876.20       | 4407875     | 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       | 4478268     | 0.178        | 0.18         | 100        |        |
| 150%         | 1876.20       | 4337840     | 0.178        | 0.18         | 98         |        |

Table 6: 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: 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

| 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             | R²=0.998 | R²=0.999 |
| Precision             | % RSD < 2 | % RSD < 2 |
| Recovery              | 99-101% | 96-102% |

(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.
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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