Method Development and Validation for Desogestrel and Ethinylestradiol in Combined Pharmaceutical Dosage Form by RP-HPLC

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Abstract

A simple, rapid, sensitive RP-HPLC method for the simultaneous determination of Desogestrel and Ethinylestradiol in pharmaceutical dosage forms was developed. The analyte were resolved using KH₂PO₄ Buffer (0.02M): Acetonitrile (50:50), at a flow rate of 2.0 ml/min. on HPLC auto sampler system containing UV-visible and fluorescence detector with Empower software and Zorbax SB Phenyl C18 column (4.6×150 mm). Detector Fluorescence detector for Ethinylestradiol UV detector for Desogestrel. For the estimation the detection wavelength was taken as 310 nm and 285 nm excitation Ethinylestradiol and 210 nm for Desogestrel. Linearity for detector response was observed in the concentration range of 10-150% of test concentration. Correlation coefficient (r) for calibration curve was found to be 1.0. Retention times were found to be 2.4 min and 13.9 min for Ethinylestradiol and Desogestrel respectively. Percent recovery was found to be within the range of 98.0% to 102.0%. The percent RSD for the analyzed tablet and recovery studied was less than 2. The results of recovery studies were found to be linear in the range 50% to 150% of test concentration. Results of the analysis were validated statistically and by recovery studies. The developed method was found to be precise, selective and rapid for the simultaneous determination of Desogestrel and Ethinylestradiol in bulk and in pharmaceutical dosage form.

Keywords: Desogestrel; Ethinylestradiol; Reversed-phase HPLC; Validation

Introduction

Desogestrel is chemically, 13-Ethyl-11-methylened-18, 19-dinor-17α-pregn-4-en-20-yn-17-ol having Molecular Formula and Molecular Weight C₂₂H₃₀O and 310.5 respectively. It is white to off-white, crystalline solid with a pKa of 13.04, slightly soluble in acetone and ethanol (95%). It belongs to Contraceptives category [1].

Ethinylestradiol Chemically is 19-Nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol having Molecular Formula and Molecular weight C₂₀H₂₄O₂ and 296.40 respectively. It is a white crystalline powder, freely soluble in alcohol; with pKa 17.59. It also belongs to Contraceptives category. It is mainly used in hormone therapies for androgen dependent disorders, acne, hirsutism, seborrhea. Recently it is shown that, the continuous daily ovarian activity and eliminate cyclic fluctuations in estradiol [2,3], progesterone, luteinizing hormone and follicle-stimulating hormone [4]. In addition, the combination of these drugs was used as an oral contraceptive for female patients with androgenic symptoms [2]. In the present research the attempt has been made to use two detector linearly for both drugs (Figure 1).

Literature survey reveals that several methods were reported for estimation of like HPLC [3-11], RPHPLC [12,13], Derivative spectrophotometry [14], LC [15], HPLC-MS [16,17] Colorimetry [18].

Experimental Work

Instrumentation

The HPLC of Waters Alliance ID:-FCLS/Q/78 Detector UV 2487 and Fluorescence 2475 was used. The pH Meter, pH Tutor, Cyber Scan, Balance Sartorious & Mettler Toledo UV-Visible Spectrophotometer, Varian Single beam were other equipments used during the research work.
Determination of wavelength maxima and calibration curve by using ultraviolet visible spectroscopy

Stock Solution of Ethinylestradiol: Accurately weighed quantity (3.75 mg) of Ethinylestradiol was transferred to 50.0 ml volumetric flask, 30 ml of diluent was added and sonicated to dissolve the drug and diluted up to the mark with diluent (Concentration 125 mcg/ml).

Desogestrel stock standard solution:

Stock solution of desogestrel: Accurately weighed quantity (15 mg) of Desogestrel was transferred to 20 ml volumetric flask, 5 ml of diluent was added and sonicated to dissolve the drug. About 2 ml of Ethinylestradiol stock solution was added and mix well, diluted up to the mark with diluent. (Conc. of Desogestrel is about 6000 mcg/ml, conc. of Ethinylestradiol is about 125 mcg/ml).

Determination of wavelength maxima for Ethinylestradiol

The aliquot portions of stock standard solutions of Ethinylestradiol were diluted appropriately with solvent to obtain concentration 20 µg/mL of drug. The solutions were scanned in the range of 400-200 nm in 1 cm cell against blank. The UV absorbance spectrum of and Ethinylestradiol is shown in Figure 2. From the spectrum the wavelengths selected for estimation of Ethinylestradiol were 210 nm and 275 nm.

Determination of wavelength maxima for Desogestrel

The aliquot portions of stock standard solutions of Desogestrel were diluted appropriately with solvent to obtain concentration 20 µg/mL of drug. The solutions were scanned in the range of 400-200 nm in 1 cm cell against blank. The UV absorbance spectrum of and Desogestrel is shown in Figure 3. From the spectrum the wavelengths selected for estimation of Desogestrel were 210 nm and 275 nm.

Study of Beer- Lambert law

The aliquot portions of stock standard solutions of Desogestrel and Ethinylestradiol were diluted appropriately with solvent to get a series of concentration between 5-50 (µg/ml) of Desogestrel and Ethinylestradiol. Similarly aliquot portions of stock standard solutions were mixed and diluted to get series of concentration between 5-50 µg/ml. The absorbance of each solution was measured at 275 nm and absorbance of Ethinylestradiol only was measured 275 nm in 1 cm cell against solvent blank. The graphs plotted as concentration Vs absorbance at selected wavelengths are shown in Figure 4 and 5.

Selection of common solvent (Diluent): HPLC grade water and Acetonitrile of analytical reagent grade in the ratio of 50:50 v/v was selected as common solvent for developing Spectral characteristics of drug. The selection was made after assessing the solubility of both the drugs in different solvents.

Preparation of standard stock solution:

Stock Solution A: Accurately weighed 30.0 mg of Desogestrel working standard accurately weighed and was transferred to 100.00 ml volumetric flask, 70 ml of acetonitrile was added and sonicated till the
material completely dissolve. The volume was made with Acetonitrile and shaken. The 5.00 ml of resulting solution was transferred to 100.00 ml volumetric flask, to volume was made with diluents.

**Stock Solution B:** About 30.0 mg of Ethinylestradiol working standard accurately weighed and was transferred to 100.00 ml volumetric flask, about 70 ml of acetonitrile was added and sonicated till the material was completely dissolved. The volume was made up to mark with Acetonitrile and shaken. The 5 ml of resulting solution was transferred to 100.00 ml volumetric flask. The final volume was made by using diluents (Figure 6).

**Selection of chromatographic condition for estimation of drugs**

In this method development and validation for Desogestrel and Ethinylestradiol mixture. The detection of Desogestrel was done by using UV detector and Ethinylestradiol was done on fluorescence detector. The detection of both drugs was not possible on same detector because Ethinylestradiol quantity in the formulation was very less and it does not shows any peak on U.V. detector, that’s why we had used fluorescence detector for Ethinylestradiol detection which is linearly arranged with U.V. detector.

**Selection of mobile phase**

Standard stock solution A and B were appropriately diluted with diluents to obtain final concentration of Desogestrel and Ethinylestradiol, respectively. The standard solutions were injected into the HPLC system and run in different solvent systems. Mixture of different solvents were tried in order to determine optimum chromatographic conditions for effective separation of Desogestrel and Ethinylestradiol. After several permutation and combination, it was found that mixture of Buffer, 0.01 M potassium dihydrogen phosphate buffer and Acetonitrile (50:50) gives satisfactory results as compared to other mobile phases. Finally, the optimal composition of the mobile phase, Buffer: Acetonitrile (50:50 v/v), and flow rate 2.0 ml/min showed good resolution, peak shape and desired elution. Retention time of Desogestrel was 13.9 min and that of Ethinylestradiol was 2.4 min.

**Preparation of mobile phase**

The 500 ml of potassium dihydrogen phosphate buffer and 500 ml of acetonitrile was mixed in 1000 ml glass bottle and filtered through 0.45 μm membrane filter and degassed before use.

**Selection of analytical wavelength**

Standard stock solution A and B were injected separately to obtain extracted Chromatogram of Desogestrel and Ethinylestradiol. Each solution was scanned using PDA detector system and their Spectra were obtained. The wavelength selected was 210 nm as both the drugs showed significant absorbance at this wavelength.

**Optimized chromatographic conditions**

- Detector: Fluorescence detector for Ethinylestradiol UV detector for Desogestrel
- Wavelength: 310 nm Emission and 285 nm excitation for Ethinylestradiol 210 nm for Desogestrel
- Column: Zorbax SB Phenyl C18 column (4.6 x 150 mm).
- Flow Rate: 2.0 ml / minute
- Injection volume: 200 μl
- Run time: 20 minutes
- Retention Time: Ethinylestradiol about 2.4 minute Desogestrel about 13.9 minute
- Run Time: 20.0 min.
- Column Oven Temp: Ambient
- Sample Cooler Temp: Ambient

**Validation Program**

**System suitability**

Prepare the system suitability solution as per the proposed test method and inject into the HPLC system by following the instrumental condition as per the test method. Record the system suitability parameters observed into the following (Table 1) [19].

**Precision studies**

**System precision:** The standard solution was prepared as per test method and injected into the HPLC system in five replicates. The % RSD was evaluated and the observations (Table 2).

**Method precision:** Six assay sample preparations from a single lot of Desogestrel and Ethinylestradiol Tablets USP (0.15 mg / 0.03 mg) were made and analysed as per methodology. Content of Desogestrel and Ethinylestradiol in Desogestrel and Ethinylestradiol Tablets USP were calculated. L1 (Maximum allowed acceptance value) of Desogestrel and Ethinylestradiol in assay percentage of Desogestrel and Ethinylestradiol in six assay sample preparations was calculated.
and found within acceptance criteria. Analytical method meets the acceptance criteria for Method Precision. Hence, the method is precise (Table 3 and 4).

Ruggedness (Intermediate precision): Ruggedness of method was verified by preparing two sets of content uniformity test preparation and six assay sample preparations from a single lot of Desogestrel and Ethinyloestradiol Tablets USP (0.15 mg/0.03 mg) and analysed as per methodology by different analyst, by using different instrument (HPLC), different column, and different make of reagent and on a different day. Percentage of impurities was calculated. Content of Desogestrel and Ethinyloestradiol in Desogestrel and Ethinyloestradiol Tablets USP were calculated. Assay percentage of Desogestrel and Ethinyloestradiol in six assay sample preparations of Method Precision and Intermediate Precision was calculated and found within acceptance criteria as given in Table 5 and 6. Analytical method meets the acceptance criteria for Intermediate Precision (Ruggedness). Hence, method is precise and rugged.

<table>
<thead>
<tr>
<th>Observation No.</th>
<th>Assay of Desogestrel (%)</th>
<th>Assay of Ethinyloestradiol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>99.3</td>
<td>98.9</td>
</tr>
<tr>
<td>2.</td>
<td>99.5</td>
<td>98.4</td>
</tr>
<tr>
<td>3.</td>
<td>99.7</td>
<td>98.5</td>
</tr>
<tr>
<td>4.</td>
<td>99.3</td>
<td>98.7</td>
</tr>
<tr>
<td>5.</td>
<td>99.3</td>
<td>98.6</td>
</tr>
<tr>
<td>6.</td>
<td>98.7</td>
<td>99.7</td>
</tr>
</tbody>
</table>

Overall % RSD of results (N = 12) of two different sets

<table>
<thead>
<tr>
<th>Level</th>
<th>Desogestrel</th>
<th>Ethinyloestradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.3064</td>
<td>0.06036</td>
</tr>
<tr>
<td>75</td>
<td>0.4596</td>
<td>0.09054</td>
</tr>
<tr>
<td>100</td>
<td>0.6128</td>
<td>0.12072</td>
</tr>
<tr>
<td>125</td>
<td>0.7600</td>
<td>0.15090</td>
</tr>
<tr>
<td>150</td>
<td>0.9192</td>
<td>0.18108</td>
</tr>
<tr>
<td>200</td>
<td>1.2256</td>
<td>0.24144</td>
</tr>
</tbody>
</table>

Slope

Intercept

Correlation coefficient (r)

1.000

Table 5: Comparison between method precision and intermediate precision.

Table 6: Linearity of detector response.

Study of linearity and range

Preparation of stock solution and linearity level of Desogestrel: The 1500 mg of working standard of Desogestrel transferred in 30 ml of volumetric flask and diluted with 30 ml of diluent and sonicated to dissolve, volume was made with diluent. From above solution linearity level of 10-150% for Desogestrel were prepared. (Conc. of Desogestrel 30000 mcg/ml) as given in (Figure 7).

Preparation of stock solution and linearity level of Ethinyloestradiol: 75 mg of working standard of Ethinyloestradiol was transferred in 200 ml of volumetric flask and dilute with 100 ml of diluent and sonicated to dissolve, make up the volume with diluent. From above solution linearity level of 10-150% for Ethinyloestradiol were prepared. (Conc. of Ethinyloestradiol 375 mcg/ml) Then each solution (20 µl) was injected into the column and chromatographed using optimized chromatographic conditions. The corresponding chromatograms were recorded and area of each peaks for Desogestrel and Ethinyloestradiol were calculated and found within acceptance criteria as given in Table 5 and 6. Analytical method meets the acceptance criteria for Intermediate Precision (Ruggedness). Hence, method is precise and rugged.

Table 1: System suitability studies.

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>RT (min)</th>
<th>Area (µV*sec)</th>
<th>USP Tailing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desogestrel</td>
<td>13.510</td>
<td>75432</td>
<td>1.18</td>
</tr>
<tr>
<td>Ethinyloestradiol</td>
<td>2.445</td>
<td>469650</td>
<td>1.46</td>
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</table>

Table 2: System precision studies.

<table>
<thead>
<tr>
<th>Injection No.</th>
<th>Area Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Desogestrel</td>
</tr>
<tr>
<td>1</td>
<td>75432</td>
</tr>
<tr>
<td>2</td>
<td>76564</td>
</tr>
<tr>
<td>3</td>
<td>76188</td>
</tr>
<tr>
<td>4</td>
<td>76282</td>
</tr>
<tr>
<td>5</td>
<td>75821</td>
</tr>
<tr>
<td>Average</td>
<td>76058</td>
</tr>
<tr>
<td>SD</td>
<td>76496.48</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Table 3: Method precision area.

<table>
<thead>
<tr>
<th>Observation No.</th>
<th>Assay of Desogestrel (%)</th>
<th>Assay of Ethinyloestradiol (%)</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
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<td>2.</td>
<td>99.5</td>
<td>99.2</td>
</tr>
<tr>
<td>3.</td>
<td>99.7</td>
<td>99.7</td>
</tr>
<tr>
<td>4.</td>
<td>99.3</td>
<td>101.1</td>
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<td>5.</td>
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<td>100.6</td>
</tr>
<tr>
<td>6.</td>
<td>98.7</td>
<td>101.6</td>
</tr>
<tr>
<td>Assay</td>
<td>99.3</td>
<td>100.3</td>
</tr>
<tr>
<td>Minimum</td>
<td>98.7</td>
<td>101.6</td>
</tr>
<tr>
<td>Maximum</td>
<td>99.7</td>
<td>101.6</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.3</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Table 4: Method precision data.
and Ethinylestradiol were measured at 210.0 nm. Each sample solution was chromatographed in triplicate and the mean peak area for Desogestrel and Ethinylestradiol was calculated (Figure 8).

**Specificity**

**Placebo interference study:** Prepared the placebo solution by weighing equivalent amount of placebo present in the sample to be taken for assay preparation in triplicate, diluted it as per the test method and injected into the HPLC system. Evaluate the % interference from placebo and recorded the observation as given in Table 7.

**Accuracy**

The accuracy of method was determined by recovery experiments. The recovery studies were carried out using standard addition method at 50, 100 and 150% level; known amount of standards was added to reanalyzed sample and subjected them to the proposed HPLC method. Percentage recovery was calculated from the amount found and actual amount added result shows in Table 8 and 9.

**Standard stock solution (for Ethinylestradiol):** Accurately 37.5 mg of Ethinylestradiol WS was weighed and transferred into a 50 ml volumetric flask, add 30 ml of diluent and sonicated to dissolve, then diluted up to the mark with diluent and mixed well. Then make it up to the mark with diluent.

The column was equilibrated with the mobile phase with chromatographic condition for the proper baseline. First injected diluent as blank (one injection). Then injected standard preparation-I (one injection) and checked the system suitability parameter as given below (A). Then standard preparation-II was injected (one injection) and checks the similarity factor as given below (B). After getting the satisfactory result, standard preparation-II was injected (four injections), check relative standard deviation of five replicate injections of standard preparation-II as given below (C), diluents was injected as blank (one injection). Then proceed for duplicate injections of assay test preparation but inject one injection of standard preparation-II as given below (D), diluents was injected as blank (one injection) and checked the system suitability parameter as given below (A). Then standard preparation-II was injected (one injection) and checked the similarity factor as given below (B). After getting the satisfactory result, standard preparation-II was injected (four injections), check relative standard deviation of five replicate injections of standard preparation-II as given below (C), diluents was injected as blank (one injection). Then proceed for duplicate injections of assay test preparation but inject one injection of standard preparation-II as given below (D).


**Application of Proposed Method for Estimation of Desogestrel and Ethinylestradiol on Marketed Tablet Formulation**

**Test preparation**

The 20 tablets was weighed and transferred to 200.00 ml volumetric flask, 120 ml of diluents was added, the stopper was inserted and sonicated with intermittent shaking for 30 minutes. Volume was made with diluent and shaken. A portion of this solution was centrifuged at 3000 RPM for 10 minutes. 2.00 ml of resulting solution was transferred to 50.00 ml volumetric flask; the volume was made up to the mark with diluent and mixed well. Then make it up to the mark with diluent.
Conclusion

From the studies it can be concluded that RP-HPLC technique can be successfully used for the estimation of Desogestrel and Ethinylestradiol in their combined dosage Tablet formulations. The method shows good reproducibility compared to UV-spectrophotometric methods. The RP-HPLC method is accurate, precise, specific, reproducible and sensitive. No interference of additives, matrix etc. is encountered in these methods. Further studies on other pharmaceutical formulations would throw more light on these studies. The methods were found to be sensitive, reliable, reproducible, rapid and economic also.

References

18. Peinado A, Hammond J, Scott A (2011) Development, validation and transfer of a near infrared method to determine in-line the end point of a fluidised drying