Pharmaceutica Analytica Acta
ISSN: 2153-2435

Bais et al., Pharm Anal Acta 2013, 4:7
DOI: 10.4172/2153-2435.1000262

Research Article Open Access

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

**Sanjay Bais[1]*, Anil Chandewar[2], Imran Popte[2], Indrajeet Singhvi[3] and Khemchand Gupta[3]**

[1]Research Scholar, PRIST University, Thanjavur-Tamilnadu, India
[2]P Wadhwani College of Pharmacy, Yavatmal-MS, India
[3]Pacific College of Pharmacy, Udaipur, Rajasthan, India

## Abstract

A simple, rapid, sensitive RP-HPLC method for the simultaneous determination of Desogestrel and Ethinyloestradiol in pharmaceutical dosage forms was developed the analyte were resolved using $KH_2PO_4$ Buffer (0.02M): Acetonitrile (50:50), at a flow rate of 2.0ml/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 Emission and 285 nm excitation Ethinyloestradiol 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 Ethinyloestradiol 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 Ethinyloestradiol in bulk and in pharmaceutical dosage form.

**Keywords:** Desogestrel; Ethinyloestradiol; Reversed-phase HPLC; Validation

## Introduction

Desogestrel is chemically, 13-Ethyl-11-methylidene-18, 19-dinor-17α-pregn-4-en-20-yn-17-ol having Molecular Formula and Molecular Weight $C_{22}H_{30}O$ 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].

Ethinyloestradiol Chemically is 19-Nor-17α-pregna-1, 3, 5(10)-trien-20-yne-3, 17-diol having Molecular Formula and Molecular weight $C_{20}H_{24}O_2$ and 296.40 respectively. It is 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).

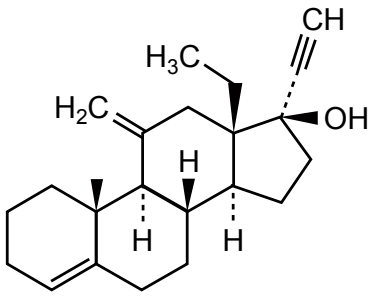


**Figure 1a:** Structure of Desogestrel.

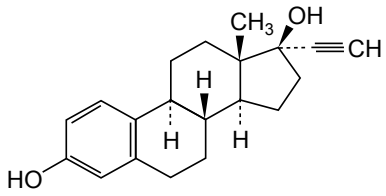


**Figure 1b:** Structure of Ethinylestradiol.

Literature survey reveals that several methods were reported for estimation of like HPLC [3-11], RPHPLC [12,13]. Derivative spectrophotmetry [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.

***Corresponding author:** Sanjay Bais, Research Scholar, PRIST University, Thanjavur-Tamilnadu, India, E-mail: sanjaybais@rediffmail.com

**Received** March 01, 2013; **Accepted** July 15, 2013; **Published** August 13, 2013

**Citation:** Bais S, Chandewar A, Popte I, Singhvi I, Gupta K (2013) Method Development and Validation for Desogestrel and Ethinylestradiol in Combined Pharmaceutical Dosage Form by RP-HPLC. Pharm Anal Acta 4: 262. doi:10.4172/2153-2435.1000262

## Determination of wavelength maxima and calibration curve by using ultraviolet visible spectroscopy

**Stock Solution of Ethinyloestradiol:** 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 Ethinyloestradiol 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 Ethinyloestradiol 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 275nm.

## Study of Beer- Lambert law

The aliquot portions of stock standard solutions of Desogestrel and Ethinyloestradiol were diluted appropriately with solvent to get a series of concentration between 5-50 (µg/ml) of Desogestrel and Ethinyloestradiol. 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 Ethinyloestradiol 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.

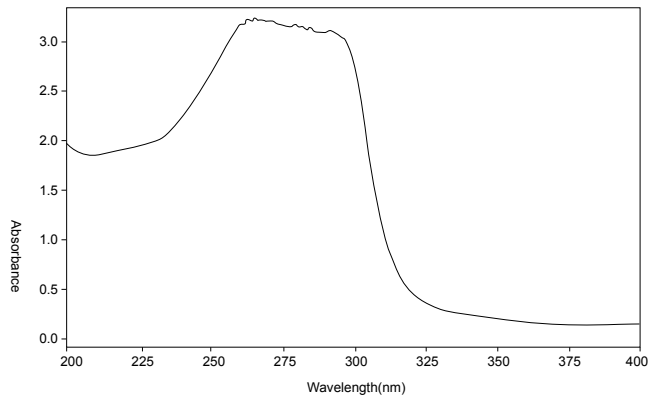


**Figure 2:** Spectra of Ethinyloestradiol.

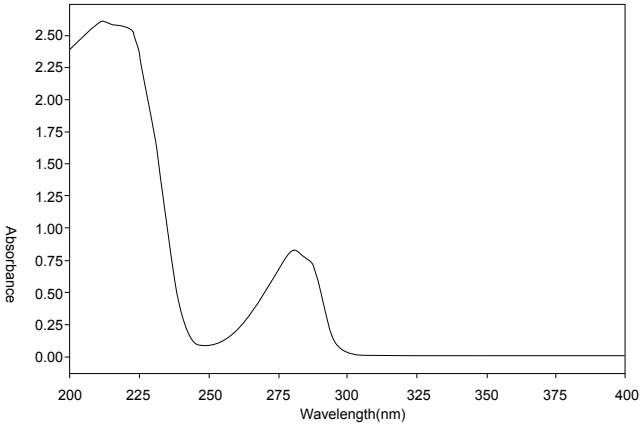


**Figure 3:** Spectra of Desogestrel.

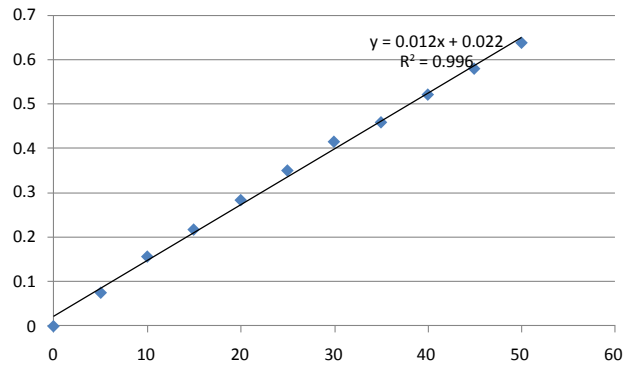


**Figure 4:** Plot of Beer-Lambert law forEthinyloestradiol at 275 nm.

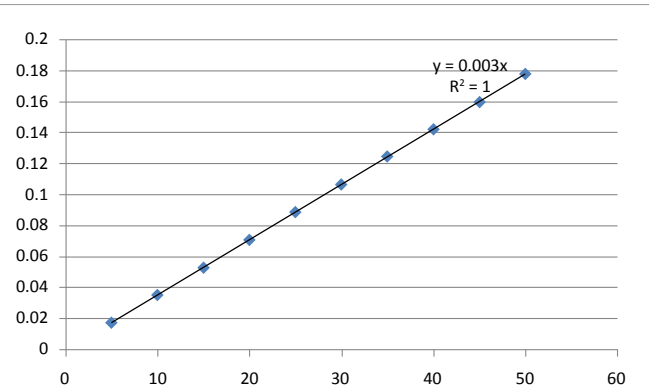


**Figure 5:** Plot of Beer-Lambert law for Desogestrel at 210 nm.

**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 Ethinyloestradiol 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 Ethinyloestradiol was done on fluorescence detector. The detection of both drug was not possible on same detector because Ethinyloestradiol 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 Ethinyloestradiol 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 Ethinyloestradiol. 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 Ethinyloestradiol 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: Ethinyloestradiol 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 Ethinyloestradiol 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

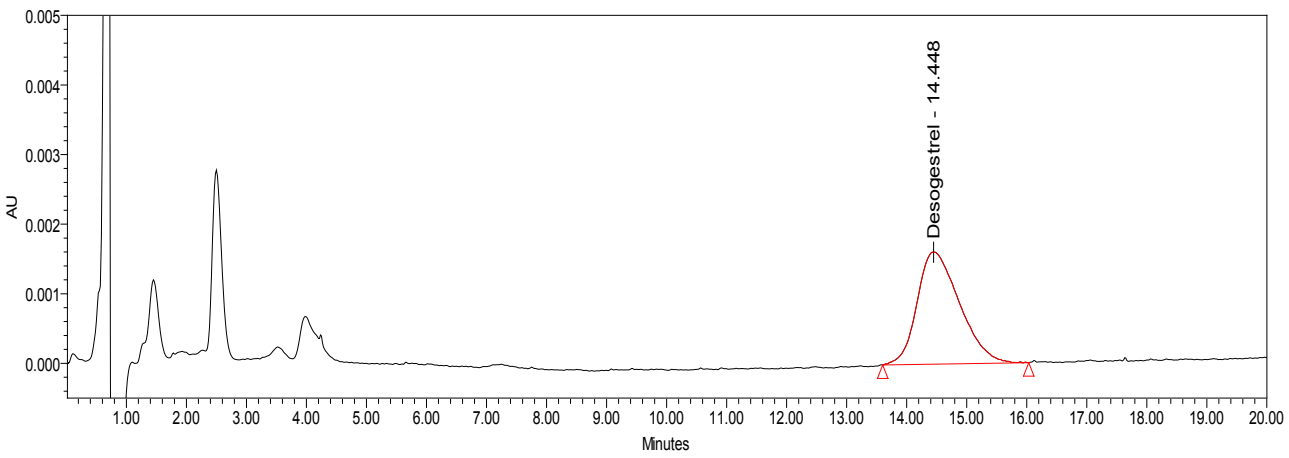


**Figure 6:** Chromatogram of Standard stock solution (UV detector).

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.

| | Peak Name | RT (min) | Area (µV*sec) | USP Tailing |
|---|---|---|---|---|
| 1 | Desogestrel | 13.510 | 75432 | 1.18 |
| 2 | Ethinyloestradiol | 2.445 | 469650 | 1.46 |

**Table 1:** System suitability studies.

| Injection No. | Area Response | |
|---|---|---|
| | **Desogestrel** | Ethinyloestradiol |
| **1** | 75432 | 469650 |
| **2** | 76564 | 468650 |
| **3** | 76188 | 474215 |
| **4** | 76282 | 485100 |
| **5** | 75821 | 486150 |
| **Average** | 76058 | 476753 |
| **SD** | 76496.48 | 485127.5 |
| **% RSD** | 0.6 | 1.8 |

**Table 2:** System precision studies.

| Injection No. | Area Response | |
|---|---|---|
| | **Desogestrel** | **Ethinyloestradiol** |
| **1** | 73829 | 447030 |
| **2** | 73723 | 447540 |
| **3** | 73809 | 447890 |
| **4** | 73629 | 447010 |
| **5** | 73525 | 447639 |
| **6** | 73660 | 447271 |
| **Average** | 73810 | 447400 |
| **SD** | 113.6981 | 318.3452 |
| **% RSD** | 0.15 | 0.071 |

**Table 3:** Method precision area.

| Observation No. | Assay of Desogestrel (%) | Assay of Ethinyloestradiol (%) |
|---|---|---|
| 1. | 99.3 | 99.5 |
| 2. | 99.5 | 99.2 |
| 3. | 99.7 | 99.7 |
| 4. | 99.3 | 101.1 |
| 5. | 99.3 | 100.6 |
| 6. | 98.7 | 101.6 |
| **Assay** | **99.3** | **100.3** |
| **Minimum** | **98.7** | **99.2** |
| **Maximum** | **99.7** | **101.6** |
| **% RSD** | **0.3** | **0.9** |

**Table 4:** Method precision data.

| Observation No. | Assay of Desogestrel (%) SET I | Assay of Desogestrel (%) SET II |
|---|---|---|
| 1. | 99.3 | 98.9 |
| 2. | 99.5 | 99.4 |
| 3. | 99.7 | 98.5 |
| 4. | 99.3 | 98.7 |
| 5. | 99.3 | 98.6 |
| 6. | 98.7 | 99.7 |
| **Assay** | **99.3** | **99.0** |
| **Minimum** | **98.7** | **98.5** |
| **Maximum** | **99.7** | **99.7** |
| **% RSD** | **0.3** | **0.5** |
| Overall % RSD of results (N = 12) of two different sets | **0.4** | |
| | **Ethinyloestradiol** | |
| | **SET I** | **SET II** |
| 1. | 99.5 | 100.4 |
| 2. | 99.2 | 99.5 |
| 3. | 99.7 | 99.3 |
| 4. | 101.1 | 101.5 |
| 5. | 100.6 | 99.7 |
| 6. | 101.6 | 99.9 |
| **Assay** | **100.3** | **100.1** |
| **Minimum** | **99.2** | **99.3** |
| **Maximum** | **101.6** | **101.5** |
| **% RSD** | **0.9** | **0.8** |
| Overall % RSD of results (N = 12) of two different sets | **0.9** | |

**Table 5:** Comparison between method precision and intermediate precision.

| Level | Desogestrel | | Ethinyl Estradiol | |
|---|---|---|---|---|
| | **Concentration (µg/mL)** | **Average Area response** | **Concentration (µg/mL)** | **Average Area response** |
| 50 | 0.3064 | 38983 | 0.06036 | 227772 |
| 75 | 0.4596 | 57156 | 0.09054 | 329650 |
| 100 | 0.6128 | 77449 | 0.12072 | 459720 |
| 125 | 0.7660 | 97777 | 0.15090 | 571145 |
| 150 | 0.9192 | 115030 | 0.18108 | 685265 |
| 200 | 1.2256 | 151600 | 0.24144 | 904195 |
| **Slope** | **123215** | | **3769899** | |
| **Intercept** | **1575.5** | | **-1328.1** | |
| **Correlation coefficient (r)** | **1.000** | | **1.000** | |

**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 50 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 Ethinylestradiol 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 measured at 210.0 nm. Each sample solution was chromatographed in triplicate and the mean peak area for Desogestrel and Ethinyloestradiol 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 Ethinyloestradiol):** Accurately 37.5 mg of Ethinyloestradiol 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.

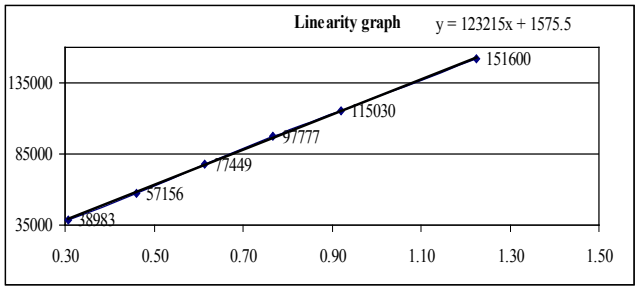


**Figure 7:** Linearity graph for Desogestrel.

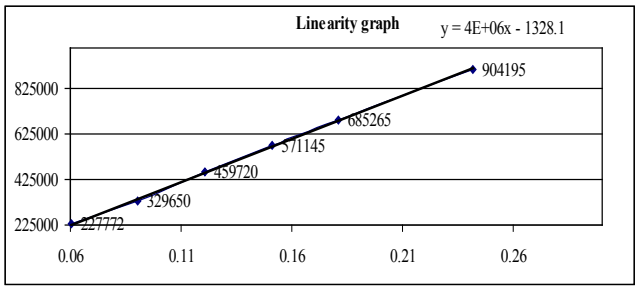


**Figure 8:** Linearity graph for Ethinyloestradiol.

| Sr. No. | Desogestrel | | Ethinyloestradiol | |
|---|---|---|---|---|
| | Assay (mg) | Assay % of LC | Assay (mg) | Assay % of LC |
| 1 | 74.41 | 99.2 | 0.783 | 104.5 |
| 2 | 74.72 | 99.6 | 0.798 | 106.5 |
| 3 | 74.62 | 99.7 | 0.789 | 105.2 |
| **Average** | 74.6 | 99.5 | 0.790 | 105.4 |
| **SD** | 0.1582 | 0.2646 | 0.0075 | 0.8544 |
| **% RSD** | 0.2 | 0.3 | 1.0 | 0.8 |

**Table 7:** Results and statistical data for estimation of DESO and ETHY in marketed formulation.

**Mixed standard preparation:** Weigh accurately 150 mg of Desogestrel working standard into a 20 ml volumetric flask, add 5 ml of diluent and sonicate to dissolve. The Ethinyloestradiol 2 ml stock solution was added and mixed well. The volume was made up to the mark with diluent.

## Sample preparation

**Accuracy:** The samples were prepared by adding active ingredients into the placebo at different concentrations or spiking the solution on placebo (50%, 100%, and 150%) each in triplicate. Injected each preparation into the HPLC system.

**Procedure:** The standard preparation and sample preparations were injected for recovery solutions into the chromatograph and the peak responses were measured for the major peaks. The system suitability was checked and the results were recorded.

# 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 with diluent and shake. Such two sample preparation was prepared. (Desogestrel 0.6 mcg/ml and Ethinyloestradiol 0.12 mcg/ml) as given in Table 8 and 9.

## Procedure and system suitability for content uniformity test

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 bracketing standard after each five injections of test preparation and check the relative standard deviation as given below (D).

A. Tailing factor for Desogestrel and Ethinyloestradiol should Not be more than 2.0

B. Similarity factor between area of standard preparation-I and standard preparation-II should be 0.98 to 1.02

$$\text{Similarity factor} \quad \frac{\text{Area of standard} -\text{I} \times \text{Weight of standard} -\text{II}}{\text{Area of first injection of standard} -\text{II} \times \text{Weight of standard} -\text{I}}$$

C. Relative standard deviation of 5 replicate injections of standard preparation-II should Not be more than 2.0%

D. Relative standard deviation between 5 replicate injections and bracketing standards should Not be more than 2.0%

| Recovery level | Desogestrel With Placebo | | | | |
|---|---|---|---|---|---|
| | Amount added (mcg / ppm) | Amount recovered (mcg / ppm) | % Recovery | Average recovery | % RSD |
| 70% | 0.4168 | 0.4220 | 101.2 | **101.3** | **0.3** |
| | 0.4168 | 0.4235 | 101.6 | | |
| | 0.4168 | 0.4208 | 101.0 | | |
| 100% | 0.5954 | 0.6041 | 101.5 | **100.8** | **0.6** |
| | 0.5954 | 0.6002 | 100.8 | | |
| | 0.5954 | 0.5963 | 100.2 | | |
| 130% | 0.7740 | 0.7718 | 99.7 | **99.7** | **0.1** |
| | 0.7740 | 0.7719 | 99.7 | | |
| | 0.7740 | 0.7728 | 99.8 | | |
| Overall | | | | 100.6 | 0.33 |

**Table 8:** Recovery data for desogestrel.

| Recovery level | Ethinyloestradiol With Placebo | | | | |
|---|---|---|---|---|---|
| | Amount added (mg / ppm) | Amount recovered (mg / ppm) | % Recovery | Average recovery | % RSD |
| 70% | 0.0835 | 0.0831 | 99.5 | **99.7** | **0.4** |
| | 0.0835 | 0.0836 | 100.1 | | |
| | 0.0835 | 0.0830 | 99.4 | | |
| 100% | 0.1192 | 0.1209 | 101.4 | **101.5** | **0.2** |
| | 0.1192 | 0.1210 | 101.5 | | |
| | 0.1192 | 0.1212 | 101.7 | | |
| 130% | 0.1550 | 0.1562 | 100.8 | **101.2** | **0.3** |
| | 0.1550 | 0.1568 | 101.2 | | |
| | 0.1550 | 0.1573 | 101.5 | | |
| Overall | | | | 100.8 | 0.33 |

**Table 9:** Recovery data for Ethinyloestradiol.

## 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

1. Maryadele J, Ann S, editors (2006) The Merck Index, An Encyclopedia Of Chemical, Drug's and Biologicals, (13thedn) ,White House Station.NJ: Merck and Co, Inc : 1603.
2. Farhad A, Ramin GM (2004) Determination of levonorgestrel and ethinyl estradiol in pharmaceutical formulations by H-point standard addi-tion method in nonaqueous solvent using simultaneous addition of both analytes, Journal of Reports in Pharmaceutical SciencesV 1: 6-14.
3. Szabó A (1977) [A technic for the determination of ethinyl estradiol and norgestrel]. Acta Pharm Hung 47: 24-28.
4. Strusiak SH, Hoogerheide JG, Gardner MS (1982) Determination of ethinyl estradiol in solid dosage forms by high-performance liquid chromatography. J Pharm Sci 71: 636-640.
5. Weston A, Brown PR (1997) High Performance Liquid Chromatography, Separations in High Performance Liquid Chromatography, Instrumentation for HPLC. In: HPLC and CE – Principles and practice. Academic Press, USA.
6. Prabhakar B, Deshpande SG (1999) Simultaneous estimation of Ethinyloestradiol and levonorgestrel from transdermal patches by HPLC. IJPS 61: 12-15.
7. Fakhari AR, Khorrami AR, Shamsipur M (2006) Stability-indicating high-performance thin-layer chromatographic determination of levonorgestrel and ethinyloestradiol in bulk drug and in low-dosage oral contraceptives. Anal Chim Acta 572: 237-242.
8. Durga PS, Changala R, Prasad PSS, Mukkanti K (2004) Simultaneous HPLC Estimation Of Levonorgestrel And Ethinyloestradiol From Tablets. Indian Journal of Pharmaceutical sciences 66: 231-234.
9. Matejícek D, Kubán V (2007) High performance liquid chromatography/ion-trap mass spectrometry for separation and simultaneous determination of ethynylestradiol, gestodene, levonorgestrel, cyproterone acetate and desogestrel. Anal Chim Acta 588: 304-315.
10. Van DR, Eickhoff WM, John KJ, Nicholas J (1987) Automated stability indicating high performance HPLC assay for Ethinyloestradiol and Levonorgestryl tablets. Pharmaceutical Research 4: 54-58.
11. Sarat M, Rambabu C (2012) A validated simultaneous RP-HPLC method for determination of Desogestrel and ethinyl estradiol tablets. International journal of pharmacy and pharmaceutical sciences 4:115-119.
12. Parmar AR, Raval TD, Bhakhar DN, Shah DK, Dobariya SD (2012) Development and validation of RP-HPLC method for estimation of drospirenone and Ethinyloestradiol in its tablet dosage forms. Inventi Rapid: Pharm Analysis & Quality Assurance.
13. Berzas JJ, Rodríguez J, Castañeda G (1997) Simultaneous determination of ethinylestradiol and levonorgestrel in oral contraceptives by derivative spectrophotometry. Analyst 122: 41-44.
14. Markopoulou CK, Koundourellis JE (2006) Development of a validated liquid chromatography method for the simultaneous determination of Ethinyloestradiol, cyproterone acetate, and norgestrel in breast milk following solid-phase extraction. J Liq Chromatogr Relat Technol 29: 685-700.
15. Li F, Hsieh Y, Korfmacher WA (2008) High-performance liquid chromatography-atmospheric pressure photoionization/tandem mass spectrometry for the detection of 17alpha-ethinylestradiol in hepatocytes. J Chromatogr B Analyt Technol Biomed Life Sci 870: 186-191.
16. Liu XF, Ding CG, Ge QH, Zhou Z, Zhi XJ (2010) [Simultaneous determination of gestodene, etonogestrel and ethinylestradiol in plasma by LC-MS/MS following derivatization]. Yao Xue Xue Bao 45: 87-92.
17. Eldawy MA, Tawfik AS, Elshabouri SR (1975) Rapid, sensitive colorimetric method for determination of ethinyl estradiol. J Pharm Sci 64: 1221-1223.
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

process for commercial production batches of an approved oral solid dose pharmaceutical product. J Pharm Biomed Anal 54: 13-20.

19. ICH (1994) Q2A, Text on Validation of Analytical Procedures. International Conference on Harmonization, Geneva.