Development and Validation of a High-Performance Liquid Chromatography Method for the Estimation of Desogestrel and Ethinyl Estradiol in Bulk & Dosage Forms

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Abstract

A new method was established for estimation of Tizanidine and Ibuprofen by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Tizanidine and Ibuprofen by using Intersil ODS C18 column (250×4.6mm) 5.0µm, flow rate was 1.0ml/min, mobile phase ratio was (40:60 v/v) Potassium di hydrogen Phosphate Buffer: Acetonitrile pH 3.2 (pH was adjusted with Ortho-phosphoric acid), detection wavelength was 269 nm. The instrument used was Shimadzu HPLC, UV detector 2450, Spinchrom -software version-2. The method shows linearity between the concentration range of 0.6-1.4µg / ml for Tizanidine and 120-280 for Ibuprofen. The % recovery of Tizanidine and Ibuprofen were found to be in the range of 98.0 % - 102.0 %.

***Keywords**: Intersil ODS C18 column, Tizanidine and Ibuprofen and RP-HPLC.*

INTRODUCTION

Desogestrel is chemically, 13-Ethyl-eleven-methylidene-18, 19-dinor17α-pregn-four-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 strong with a pKa of thirteen.04, barely soluble in acetone and ethanol (ninety-five %) It belongs to Contraceptive's category. The reproductive effects of desogestrel, including changes in the luteinizing hormone and follicle hormonal stimulation, decreases menstruation start and increases vaginal viscosity; and metabolic effects which include increasing insulin secretion and resistance, increasing lipase activity, and increasing fat deposition. The impact of desogestrel on lipids has been widely

investigated and the findings are inconsistent. Desogestrel is passive in the cell and operates by selective binding of the progesterone receptor and minimal androgenic action. The binding action of this factor is like a transcription factor and hence changes the production of mRNA.The terminal half-life of desogestrel is determined to be of 30 hours.

Ethinyloestradiol Chemically is nineteen-Nor-17α-pregna-1, three, five (10)-trien-20-yne-3, 17-diol having Molecular Formula and Molecular weight C20H24O2 and 296.40 respectively. It is White crystalline powder, freely soluble in alcohol; with pKa 17.59. It also belongs to Contraceptive's class. It is mainly used in hormone remedies for androgen structured disorders, pimples, hirsutism, seborrhea. Recently it's far shown that, the non-stop every day ovarian interest [1,2] and take away cyclic fluctuations in estradiol, progesterone, luteinizing hormone and follicle- stimulating hormone. The synthetic estrogenic substance is ethinylestradiol. The use of estrogens affects the body, including decreased bone density. Combined oral contraceptives eliminate ovulation by lowering gonadotrophic hormones, thickening cervical mucus in the process to prevent sperm from traveling and inhibiting endometrial changes necessary for fertilized egg implantation. Ethinylestradiol reduces the hormone luteinizing and lowers endometrial vascularity. It also boosts sex hormone globulin binding.

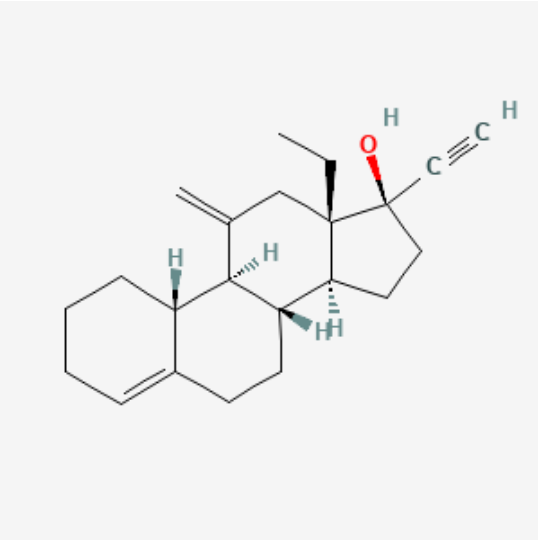

Figure: 1 Chemical structure of Desogestrel

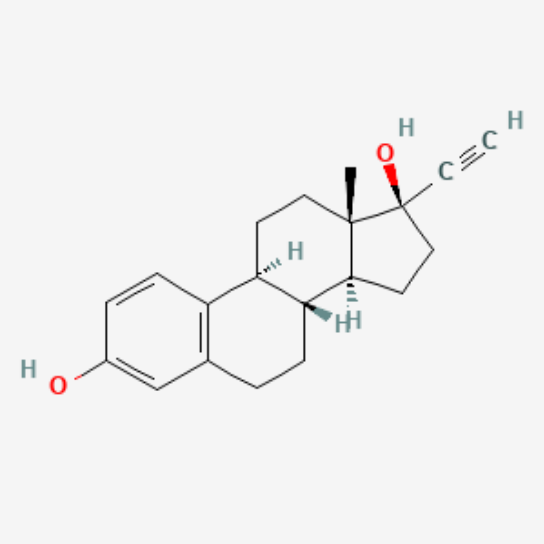

Figure: 2 Chemical structure of Ethinyl estradiol

Materials and Methods

HPLC instrument and chromatographic conditions

HPLC –Waters Model NO.2690/5 series Compact System Consisting of Inertsil-C18 ODS column. The mobile phase consisting of methanol and water was in a ratio of 45:55 v/v. Isocratic elution was carried out at ambient temperature. The flow rate was 1.0 ml/min and the injection volume was 20 µl. The UV detection wavelength was set at 210 nm.

Preparation of sample solution -A: Take 100mg Desogestrel in 100ml volumetric flask and add 70% methanol. Sonicate for 30 mins. After 30mins add remaining methanol up to mark. And sonicated to 10mins i.e. 1000ppm/1000gm-solution.

Preparation of sample solution –- B: Take 100mg Ethinyl estradiol in 100ml volumetric flask and add 70% methanol. Sonicate for 30 mins. After 30mins add remaining methanol up to mark. Andsonicated to 10mins i.e. 1000ppm/1000gm-solution.

Preparation of standard solution: Take 4ml of solution A and 4ml of solution B in 100ml volumetric flask and add up to mark with methanol. Sonicated to 20mins. i.e. 40ppm/40mg/ml.

Results and discussions

Method Development

Method development several trial runs were performed using C18 reversed phase columns, various mobile phase compositions and different flow rates for the separation of Desogestrel and Ethinyl estradiol with good chromatographic parameters such as resolution, theoretical plates, and tailing factor. A C18 column (250 mm × 4.6 mm, i.e., 5 µm) used as the stationary phase and a mobile phase consisting of methanol/water (45:55 v/v) at a flow rate of 1.0 ml/min and a UV detection wavelength of 210 nm for a run time of 12 min afforded the best separation with well-resolved and sharp peaks for both the drugs. The retention time Desogestrel and Ethinyl estradiol was 3.617 min and 5.013 min, respectively. The chromatogram of the optimized method is presented in Figure 3.

Preparation of pH 3.4 Phosphate buffer: 2.7218g of KH_2PO_4 was weighed and transferred into a 1000ml beaker, later it was dissolved and diluted to 1000ml with HPLC water and the pH was adjusted to 3.4 with orthophosporic acid.

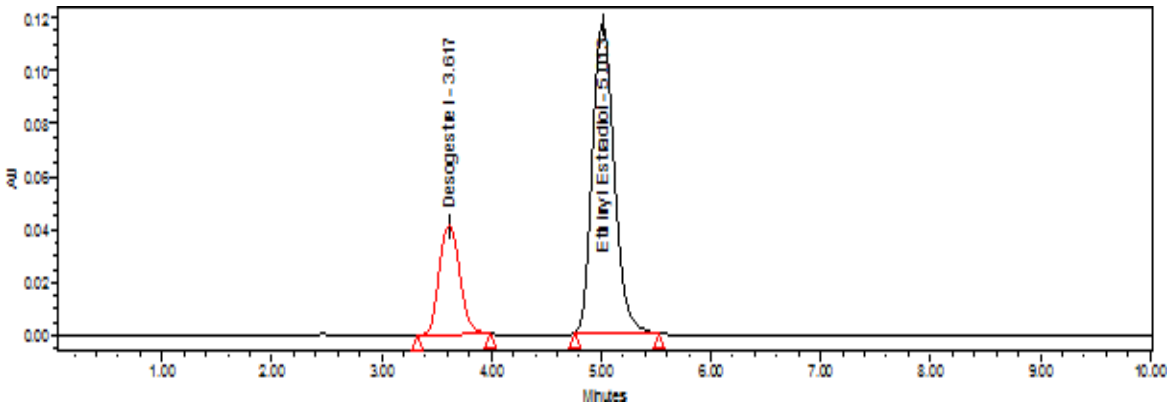

Figure 3: Standard chromatogram for optimised condition

Method Validation

The developed RP-HPLC method was validated in terms of the following parameters according to the International Conference on Harmonization (ICH) guidelines[3]. Linearity, Figure 1 and 2: Chemical structures of Desogestrel and Ethinyl estradiol. Reverse-phase high-performance liquid chromatography method for simultaneous estimation of Desogestrel and Ethinyl estradiol accuracy, precision, robustness, ruggedness, limit of detection (LOD), limit of quantitation (LOQ), specificity, and system suitability studies were carried out.

System suitability:

System suitability was determined by injecting six replicate injections of the standard solution of Desogestrel and Ethinyl estradiol. Results of system suitability parameters (resolution, theoretical plates, tailing factor, etc.) were found within the limit with %RSD values of parameters are represented in Table 1 and Table 2. In system suitability test, the method produced excellent separation of the analyte peaks with good resolution between the two analytes [Figure 4 and Figure 5]. Moreover, higher percentage of recovery and non interference of the formulation excipients[4] in Rt of the analytes exhibited the selectivity of the method for the simultaneous estimation of both the drugs in the combined formulation.

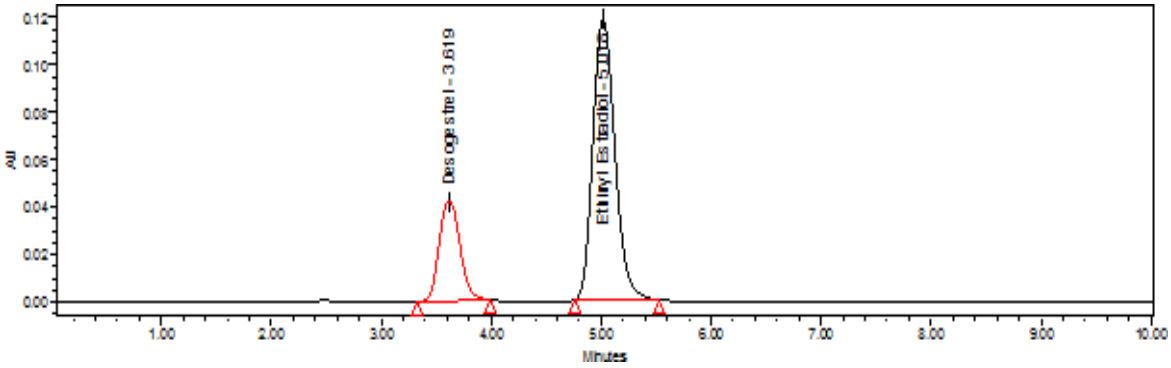

Figure 4: Standard Chromatogram System Suitability

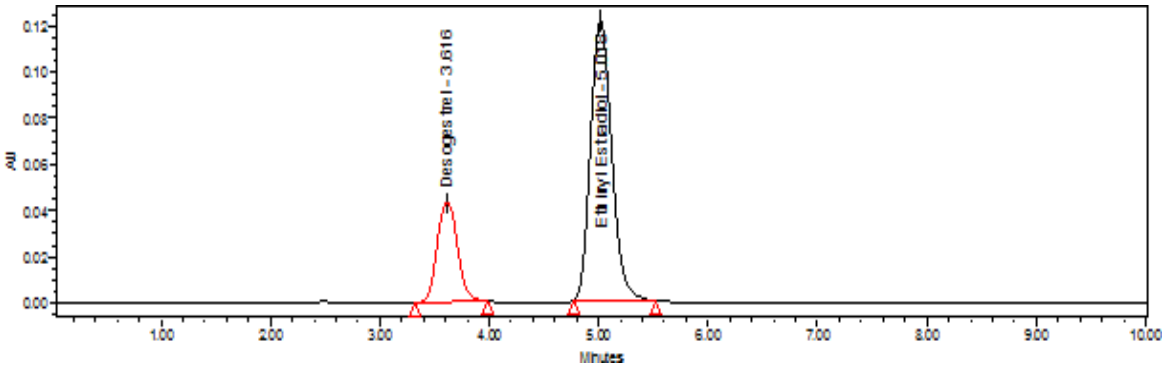

Figure 5: Sample Chromatogram System Suitability

Specificity:

The specificity of the method was demonstrated by the separation of the analytes of interest from other potential components such as excipients, impurities, and related active principles. A volume of 20 µl of sample solution was injected and the chromatogram was recorded. No

peaks were found in the chromatogram other than the peaks due to Desogestrel and Ethinyl estradiol with the Rt of 3.617 min and 5.013 min Results showed that the method was free from interference due to excipients, impurities, or other related components [Figure 6]. The proposed method is, therefore, claimed to be to be specific for the quantitative simultaneous determination of Desogestrel and Ethinyl estradiol in pharmaceutical formulations.

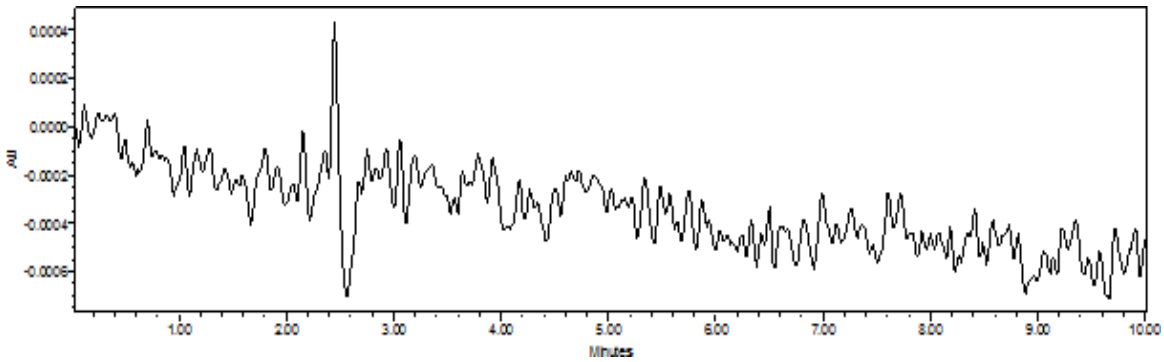

Figure 6 : Blank Chromatography of non -interferences

Precision:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate precision/ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day within the laboratory. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Table 1: Results of precision studies

Precision	**Repeatability (Intra-day)**	**Reproducibility (Inter-day)**
DG % Assay % RSD	 100.00±0.888 0.888	 100.02±0.898 0.616
EE % Assay % RSD	 99.91±0.35 0.35	 99.95±0.24 0.21

DG: Desogestrel, EE: Ethinyl Estradiol, RSD: Relative standard deviation. *mean±SD of six replicate observations (n=6)

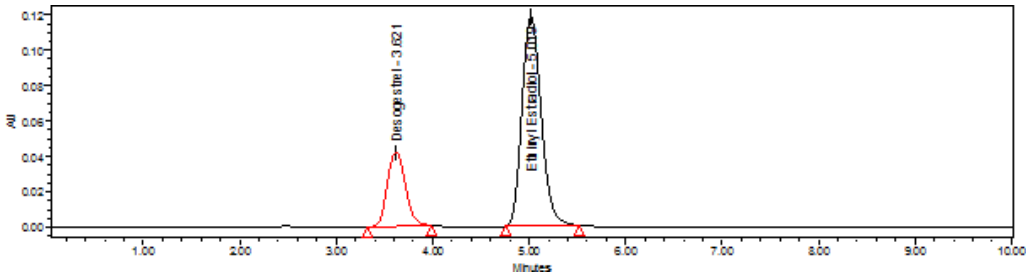

Figure 7: Precision chromatograph

Accuracy For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Table 2: Accuracy (% recovery) data for DZ and EE

Drug	Spiked level (µg/ml)	Concentration (µg/ml)		
		Amount Added	Amount Recovered	Mean %Recovery
DG	50	20		20.15
	100	99.69		
	150	40		39.88
		99.83		
EE	50	60		60.12
	100	99.97		
	150			
		20		20.04
		100.06		
		40		40.01
		100.04		
		60		60.08
		100.02		

DG: Desogestrel, EE: Ethinyl Estradiol; *mean of three replicate determinations (n=3)

Linearity: The linearity was evaluated by analyzing six (n = 6) standard solutions of Desogestrel and Ethinyl estradiol for a concentration range of 20–70 µg/ml. The calibration curve was constructed by plotting a graph between peak area and concentration. The straight-line equation was determined. The calibration plot was found linear in the range between 20 and 70 µg/ml for both Desogestrel and Ethinyl estradiol. The regression equations were obtained as follows: y =18600x + 276.23 (r2 =0.990) for Desogestrel [Figure 8], and y = 5140x+ 114.7 (r2 =0.999) for Ethinyl estradiol [Figure 9

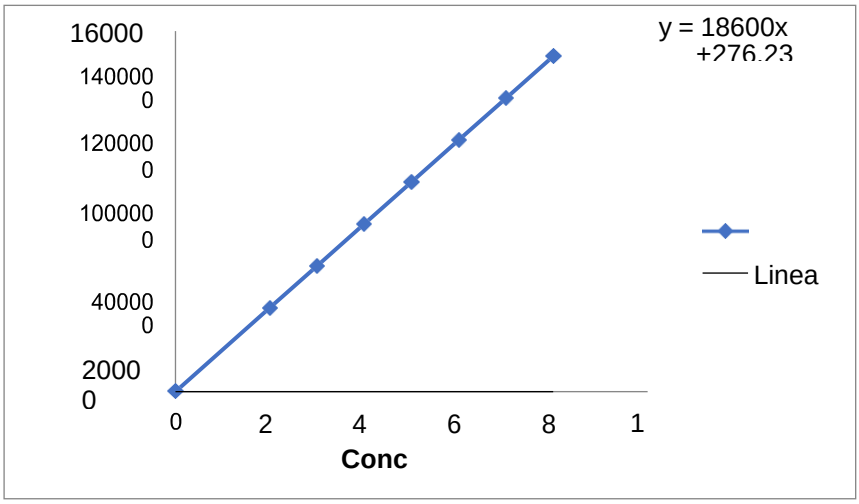

Figure 8: Desogestrel's Linearity Plot (concentration vs response)

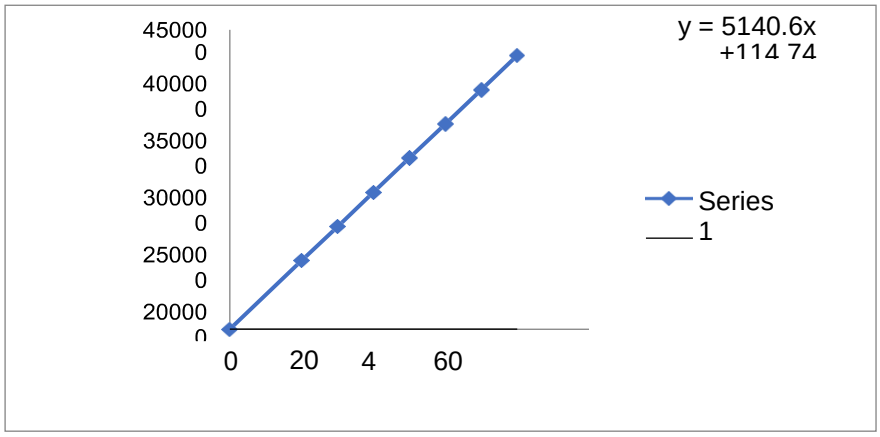

Figure 9: Linearity Plot Ethinyl Estradiol (concentration vs response)

Ruggedness:

Table 3: Results of Ruggedness

Ruggedness	**Analyst 1**	**Analyst 1**
DG % Assay % RSD	 98.64±0.12 0.12	 98.95±0.15 0.15
EE % Assay % RSD	 99.07±0.21 0.21	 99.09±0.22 0.22

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

TABLE: 4 Results of Robustness

Robustness	**Modification in Flow rates**		
	0.8 ml/min	1.0 ml/min	1.2 ml/min
DG			
Peak area	620425		634663
%RSD	6024440		
	0.086	0.184	0.09
EE			
Peak area	1273638	1205763	166277
%RSD	1.041	0.19	0.35

DG: Desogestrel, EE: Ethinyl Estradiol, RSD: Relative standard deviation. *mean±SD of six replicate observations (n=6)

Limit of Detection and Limit of Quantitation:

Desogestrel

From the linearity plot the LOD and LOQ are calculated:LOD = $\frac{3.3\ \sigma}{S}$

$$= \frac{3.3 \times 867.0705}{5140} = 0.56$$

$$LOQ = \frac{10\ \sigma}{S}$$

$$= \frac{10 \times 867.0705}{5140} = 1.69$$

Ethinyl Estradiol:

$$LOD = \frac{3.3\ \sigma}{S}$$

$$= \frac{3.3 \times 3244.904}{18600} = 0.57$$

$$LOQ = \frac{10\ \sigma}{S}$$

$$= \frac{10 \times 3244.904}{18600} = 1.74$$

Summary and Conclusion

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 241nm for Desogestrel and 254nm for Ethinyl Estradiol. Common wavelength will be 254nm and the peaks purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Inertsil C18, ODS chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area, satisfactory retention time and good resolution. Different ratios of mobile phase were studied, mobile phase with ratio of Methanol: Buffer (45:55) was fixed due to good symmetrical peaks and for good resolution. So this mobile phase was used for the proposed study.

The present recovery was found to be 98.0-101.50 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. Linearity study was, correlation coefficient and curve fitting was found to be. The analytical method was found linearity over the range of 20-70ppm of the target concentration for both the drugs. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

Bibliography

1. Rock Hill, MD: United States Pharmacopeia Agreement INC, 2010.Volume 31(4): 1092.
2. Indian Pharmacopoeia, Ministry of Health and Family Welfare, Government of India, 2007. List I and II: 740–742.

3. H.M. Stationary Office London, Medicinal and Medical Substances; 2012: Ph. Eur. Number 0931. British Pharmacopoeia Volume I & II Monographs: H.M. Stationary Office London, Medicinal and Medical Substances; 2012: Ph. Eur. Number 0931.

4. H.M. Stationary Office, London; 2012: Ph. Eur. Monograph dated 1524. British Pharmacopoeia Volume I & II Monographs: Prescription Substances in Medicinal and H.M. Stationary Office, London; 2012: Ph. Eu.