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VALIDATED STABILITY INDICATING HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF ESTETROL AND DROSPIRENONE IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple stability indicating high performance liquid chromatographic method has been developed for the simultaneous determination of Estetrol in combination with Dosperinone using reverse phase with Waters Symmetry C18 250x 4.6mm, 5µm with detection wavelength at 240.0 nm. The mobile phase consisting of 55:45 % (V/V) 0.01N Kh2po4: Acetonitrile and at a flow rate of 1.0mL/min with run time 6 minutues. The method was linear over the concentration range for Estetrol 3.55 – 21.3 µg/mL and for Dosperidone 0.75 – 4.5 µg/mL. The recoveries of active pharmaceutical ingredient (API) Estetrol and Drospirenone were found to be in the range of 100.17 % and 100.20%respectively. The method was validated and was successfully employed for the routine quantitative analysis of pharmaceutical formulations containing Estetrol and Drospirenone in combined tablet dosage form.

Keywords: Estetrol, Drospirenone HPLC and Validation.

INTRODUCTION

Estetrol is an estrogen used in combination with drospirenone for oral contraception. Naturally or synthetically produced steroid estrogens have a wide range of pharmaceutical uses ranging from hormonal contraception to the treatment of menopausal symptoms[1]. Estetrol (E4) is a native estrogen occurring naturally during pregnancy, but can be synthesized from a plant source and used for contraception[2]. Estetrol is a synthetic analogue of a naturally occurring estrogen present during pregnancy, demonstrating selectivity for both estrogen receptor-α (ER-α) and ER-β and suppressing ovulation[3]. Estetrol binds with a low to moderate affinity human estrogen receptor alpha (ER alpha) and ER beta with a preference for ER alpha[4].

Drospirenone is a progestin used in oral contraceptive pills for the prevention of pregnancy and other conditions. Drospirenone is a synthetic progestin commonly found in the popular oral contraceptive, Yaz in combination with Ethinyl estradiol[5]. Drospirenone has been the subject of widespread safety concern due to the possibility of an increased risk of venous thromboembolism associated with its use[6,7]. Most recently, it was approved by both Health Canada and the FDA in combination with Estetrol as an oral contraceptive therapy[8,9]. side from its contraceptive effects, drospirenone is used with estrogens to control acne and premenstrual dysphoric disorder (PMDD). Drospirenone and ethinyl estradiol in combination suppress the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH), preventing ovulation. Drospirenone is an analog of the diuretic spironolactone, which exerts anti-mineralocorticoid activity, blocking aldosterone receptors, which increases sodium and water excretion[10].

EXPERIMENTAL

Chemicals and reagents

- Methanol (HPLC grade, Merck Ltd),
- Milli-Q water,
- Drospirenone and Estetrol (Reference standard purchased from Alkemers, Dublin),
- Ortho phosphoric acid, Potassium dihydrogen phosphate (LR Grade, SD Fine Chem. Ltd).
- All other chemicals are of the highest grade commercially available unless otherwise specified.

Apparatus and chromatographic condition

The Analytical equipment consists of a WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sample injector. Data acquisition was done by using the software Empower 2.

The analytical C18 ace-EPS column (100 × 4.6 mm, 5µm) was used. The mobile phase consist of

40 parts of Acetonitrile is mixed with 60 parts of 0.1% Orthophosphoric acid to obtain 60:40 % (v/v) of 0.1% Orthophosphoric acid and Acetonitrile. The mixture is mixed well, sonicated in an ultrasonic bath for 20 min and then used for experiment. The solution is labeled and used within 7 days from the date of preparation. The mobile phase was prepared freshly, filtered, sonicated before use and delivered at a flow rate of 1 mL/min and the detector wavelength was set at 226nm. The injection volume was 10 µL. The mobile phase was used as diluent.

Preparation of Drospirenone and Estetrol diluent , standard & sample solution

Diluent solution preparation

50% of Acetonitrile and 50% of milli-Q water were mixed well to obtain 50:50 % (v/v) of Acetonitrile and water. This mixture was mixed well and it is sonicated in an ultrasonic bath for 20 minutes and then used for the analysis. The solution is labeled and it is used within 7 days from the preparation date.

Standard solution preparation

Accurately weighed 7.5mg of Drospirenone, 5mg of Estetrol and transferred to separate 50ml volumetric flasks, 3/4 th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluent (50% Acetonitrile and 50% milli-Q Water) and labeled as Standard stock solution (150µg/ml of Drospirenone and 100µg/ml Estetrol). 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (15µg/ml of Drospirenone and 10µg/ml of Estetrol).

Sample Solution Preparation

10 tablets were accurately weighed, powdered and then Weight equivalent to 1 tablet was transferred into a 100mL volumetric flask, 50mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered (150µg/ml of Drospirenone and 100µg/ml Estetrol). From the filtered solution 1 ml was pippeted out into a 10 ml volumetric flask and made upto 10ml with diluent (15µg/ml of Drospirenone and 10µg/ml of Estetrol).

Procedure

Preparation of Mobile Phase

45 parts of Acetonitrile is mixed with 55 parts of 0.01N Potassium dihydrogen phosphate to obtain 55:45 % (v/v) of 0.01N Potassium dihydrogen phosphate and Acetonitrile. The mixture is mixed well, sonicated in an ultrasonic bath for 20 min and then used for experiment. The solution is labeled and used within 7 days from the date of preparation.

RESULTS AND DISCUSSION

All of the analytical validation parameters for this proposed method were determined according to ICH guidelines[12]. Obtained validation parameters are presented in Table 1.

Specificity

Specificity is the ability to ascertain the analyte which is expected to have impurities, degradation products and matrix components in it. Specificity of the method can be demonstrated by placebo interference. No interference of drug peak with excipients was observed which means the method was specific. The chromatograms which show the overall separation for specificity were given in figures 3.4, and 3.5 for blank and sample containing both Estetrol and Drospirenone drugs respectively.

Linearity

The linearity of an analytical method can be known by obtaining the test results within the given range which are directly proportional to the concentration of the analyte in a sample. The linearity shall be established by evaluating serial dilutions of analyte that were prepared from the stock solutions ranging from 3.55 to 21.3 for Estetrol and 0.75 to 4.5 for Drospirenone respectively and were diluted with the mobile phase to get the series. The curves obtained are shown in figure 3.6 for Estetrol and figure 3.7 for Drospirenone. Linearity average area responses were presented in Table 3.6 and 3.7.

Accuracy
It is the closeness of an agreement between true value and measured value. It can be calculated by standard addition method. First of all solvent stock solutions of the drugs of known concentration were prepared. Spiked the known amount of the stock solution to the test sample solution, such there the concentration of the solvent will be in the specification limit (ICH). Same as spike the stock solvent solution to test solution at different levels example 50%, 75%, 125%, 150% of specification limit. The experiment was done, recovery of the product and % RSD were calculated. A recovery ranged from 98.00 - 102.00% has been obtained by the method indicates its accuracy. Results obtained for accuracy are illustrated in tables 3.9 and 3.10 for Estetrol and Drospirenone respectively

Precision
Precision of the method was studied in terms of the repeatability or reproducibility (Intraday assay) and intermediate precision (Inter-day assay). Precision is the degree of agreement among the individual test results. It is expressed in terms of standard deviation or relative standard deviation of a series of measurements. Performed the analysis on six determinations at 100% level of specification limit concentrations.

Repeatability
Six working standards solutions of 14.2µg/ml and 3µg/ml of Estetrol and Drospirenone are injected and the % Amount found was calculated and table 3.11 shows the %RSD of Estetrol and Drospirenone and chromatogram was shown in fig 3.17.

Reproducibility
Six working Sample solutions of 14.2µg/ml and 3µg/ml of Estetrol and Drospirenone are injected and the % Amount found was calculated and table 3.12 shows the %RSD of Estetrol and Drospirenone and chromatogram was shown in fig 3.18.

Intermediate precision
Six working sample solutions of 14.2µg/ml and 3µg/ml of Estetrol and Drospirenone are injected on the next day of the preparation of samples and the % Amount found was calculated and table 3.13 shows the %RSD of Estetrol and Drospirenone and chromatogram was shown in fig 3.19.

3.7.5 Limit of Detection and Limit of Quantification (LOD and LOQ)
LOD is the lowest concentration of the analyte in a sample which can be detected under the proposed experimental conditions where as LOQ is the lowest amount of the analyte in a sample that can be detected quantitatively with acceptable accuracy and precision. LOD and LOQ can be determined based on signal to noise ratio of 3 and 10 respectively. The signal to noise ratio can be calculated by comparing the signals obtained from the samples with known lowest concentrations of the analyte and blank sample The LOD and LOQ values for Estetrol and Drospirenone were illustrated in tables 3.14 and 3.15.

3.7.6 Robustness
It can be defined as the capacity or measure of the method that withstand by small, but deliberate variations in the method conditions. A method called robust if it is unaffected by small changes in its operating conditions. An analysis was done to check the effects obtained by small but deliberate changes in the optimized chromatographic conditions like composition of the mobile phase, flow rate value and Temperature..

Variations in flow rate
To find out the effect of change in flow rate of the method, an experiment was performed at the flow rate changes of 0.9 mL/min and 1.1 mL/min within acceptable limits. The results were within the acceptance criteria. The obtained data for the flow rate variations were given in tables 3.16 and 3.17.

Variations in condition of temperature
Study was conducted to determine the effect of variation in temperature. Analysis was done by varying the temperature from 27^{o}C and 33^{o}C. The results were within the acceptance criteria. The obtained data for the temperature variations were given in tables 3.20 and 3.21

Variations in composition of mobile phase
The effect of variation in percent organic content of mobile phase was evaluated by changing the composition of organic component in mobile phase (55:45). A little change was observed in the tailing factor and the number of theoretical plates in the variation of mobile phase from (60:40) and (50:50) and the results were tabulated in tables 3.18 and 3.19.

Variations in condition of temperature
Study was conducted to determine the effect of variation in temperature. Analysis was done by varying the temperature from 27^{o}C and 33^{o}C. The results were within the acceptance criteria. The obtained data for the temperature variations were given in tables 3.20 and 3.21.

3.7.7 System suitability

System suitability testing is an integral part of analytical procedures and is a pharmacopoeia requirement test. It is intended to verify the reproducibility of the system and its suitability for the analysis. The study was carried out by injecting six replicate injections of the standard solutions and test sample solutions of the respective drugs, followed by evaluating % RSD of the peak observed, area, number of theoretical plates and resolution. The data was given in the table 3.22 within the acceptable limits.

3.7.8 Stress degradation studies

Combined mixture of Estetrol and Drospirenone were allowed to subject for the stress degradation studies. The stress studies involve acid, alkaline, photolytic (UV) and oxidation stress conditions. Both the drugs were stable and specific in presence of all stress conditions within 95 - 105% to differentiate the degradation peaks.

Oxidative stress condition

To 1 ml of stock solution of (Estetrol and Drospirenone) 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600C. For HPLC study, the resultant solution was diluted to obtain (14.2µg/ml and 3µg/ml) solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid stress conditions

To 1 ml of stock solution Estetrol and Drospirenone 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0C. the resultant solution was diluted to obtain (14.2µg/ml and 3µg/ml) solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Base stress condition

To 1 ml of stock solution Estetrol and Drospirenone 1ml of 2 N sodium hydroxide was added and refluxed for 30mins at 60^0C. the resultant solution was diluted to obtain (14.2µg/ml and 3µg/ml) solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat stress condition

The standard drug solution was placed in oven at 105^0C for 6h to study dry heat degradation. For HPLC study, the resultant solution was diluted to obtain (14.2µg/ml and 3µg/ml) solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Photolytic stress condition

The photochemical stability of the drug was also studied by exposing the (142µg/ml and 30µg/ml) solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/min photo stability chamber. For HPLC study, the resultant solution was diluted to obtain (14.2µg/ml and 3µg/ml) solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Stress condition

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hrs at a temperature of 60ºc. For HPLC study, the resultant solution was diluted to obtain (14.2µg/ml and 3µg/ml) solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Control sample

10 tablets each of the respective drugs were taken, weighed and made into a fine powder by crushing with mortar and pestle. A small amount of crushed powder is taken and transferred into a 100 mL of volumetric flask and 20 mL of diluent is added. The mixture is sonicated for 20 min and then made up to the mark by using diluent. Now 10 µL of this solution is taken and injected to the HPLC system under fixed conditions for 6 times. The peak areas of the drug were calculated by observing the peaks, and the drug content in the formulation is calculated by regression equation. The % assay results are shown in the table 3.25.

CONCLUSION

This study presents a simple and validated stability indicating HPLC method for simultaneous estimation of Estetrol and Drosperinone in the presence of degradation products. The developed method is specific, accurate, precise and robust. The method was linear response in stated range and is accurate and precise. All the degradation products formed during forced decomposition studies were well separated from the analyte peaks demonstrating that the developed method was specific and stability indicating**.** The method could be applied with success even to the analysis of marketed products of Estetrol and Dosperidone combined tablet formulation, as no interference was observed due to excipients or other components present.

ACKNOWLEDGEMENTS
The authors are thankful to Aurobindo Pharma Ltd., Hyderabad, India for providing the gift samples of Estetrol and Dosperidone.

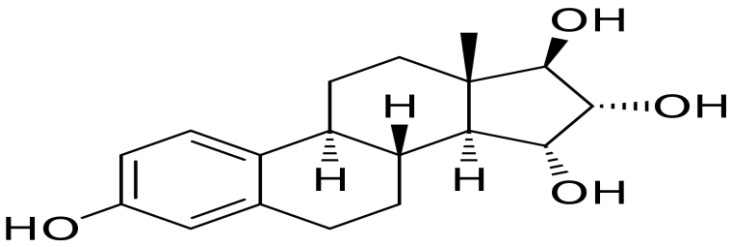


Fig. 1: Chemical structure of Estetrol

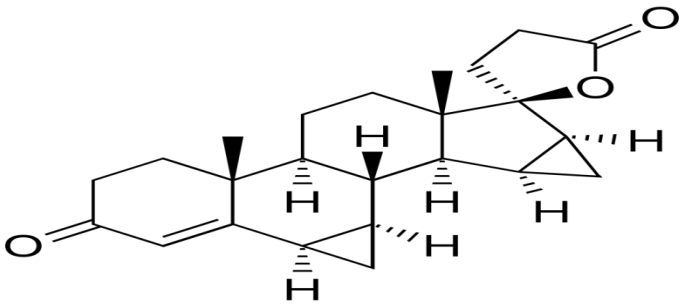
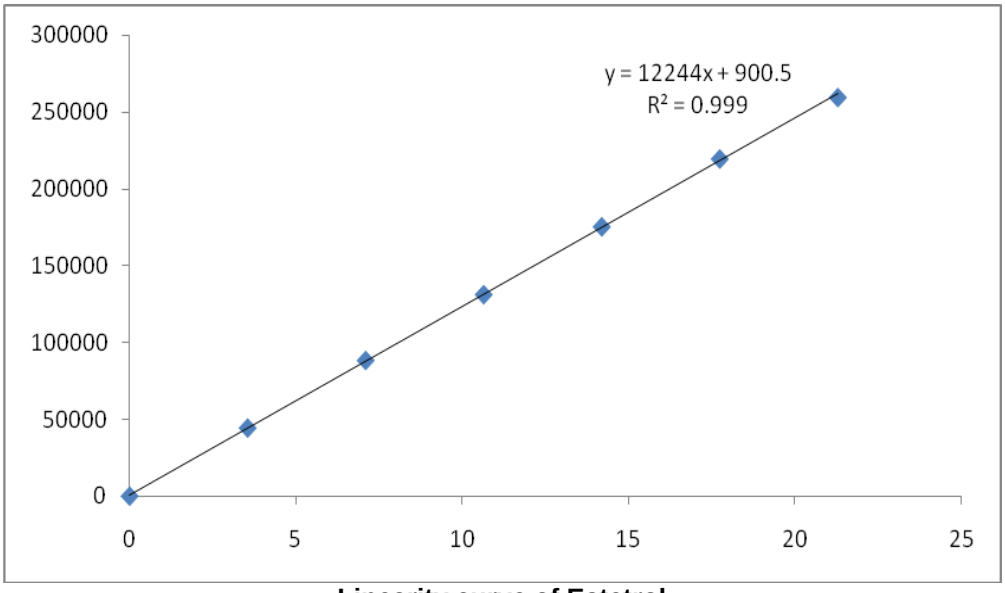
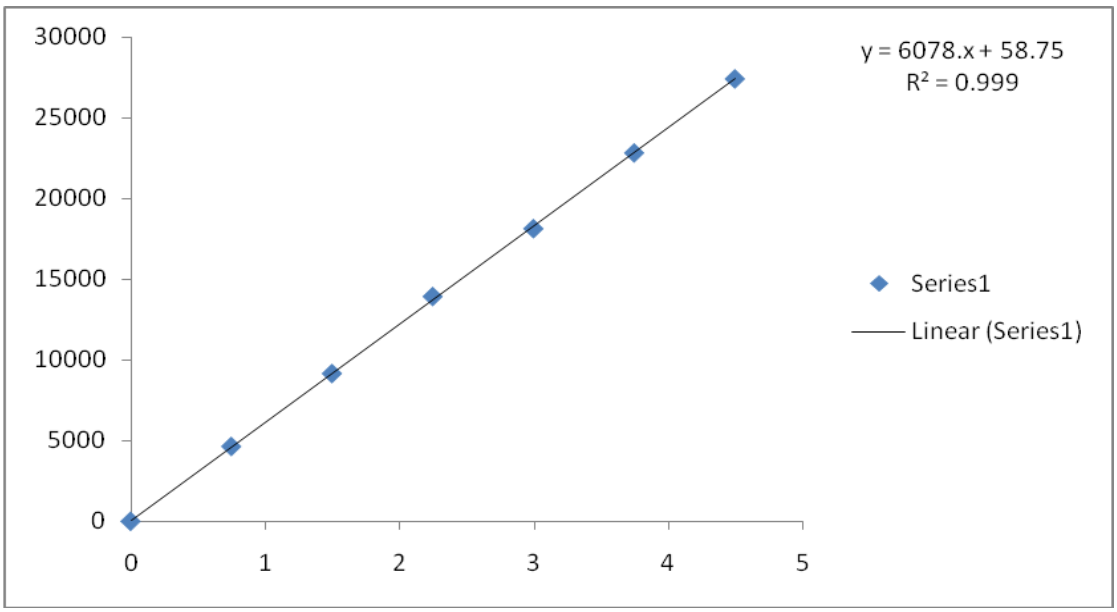


Fig. 2: Chemical structure of Dosperinone



Linearity curve of Estetrol



Linearity curve of Dosperinone

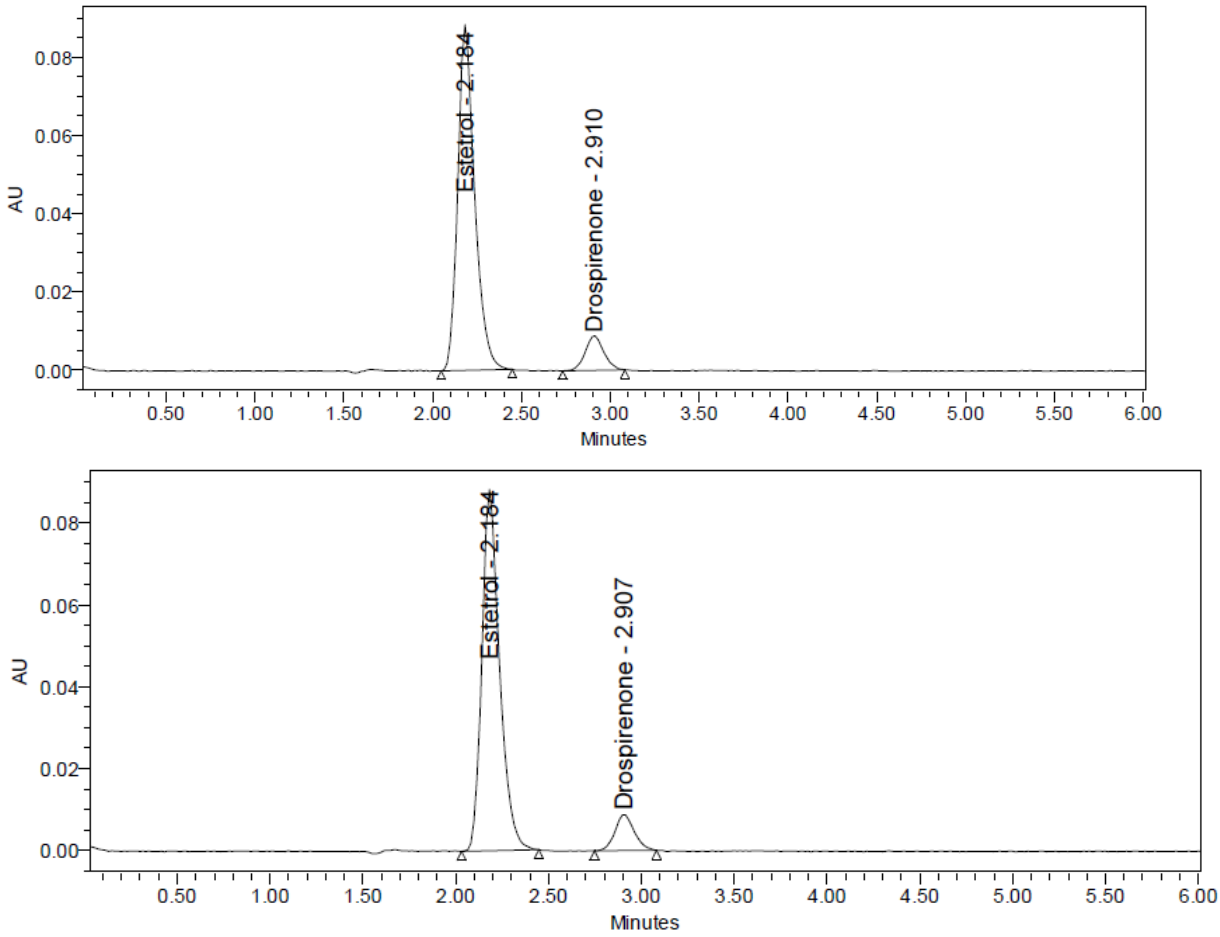


Fig. 3.8: Linearity 25% Chromatogram of Estetrol and Drospirenone

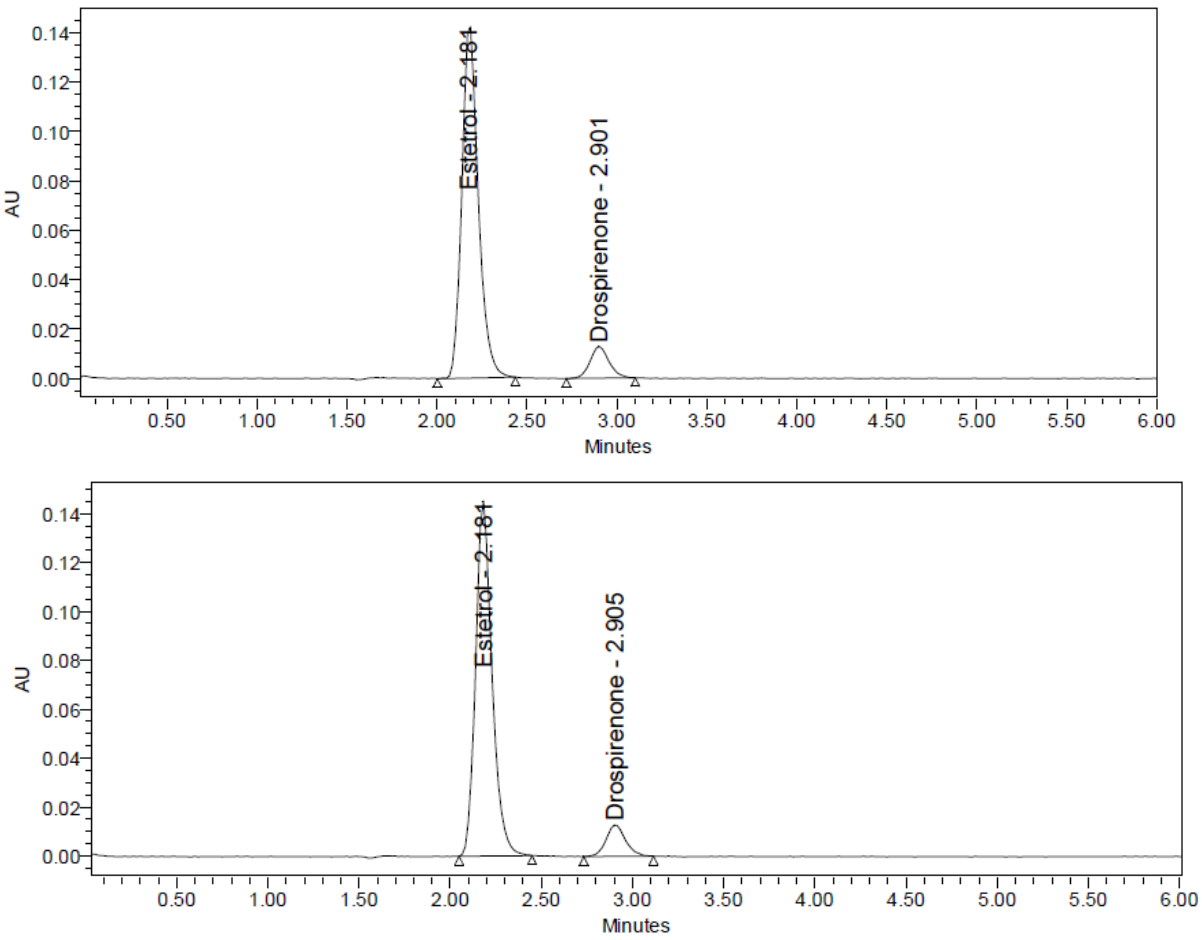
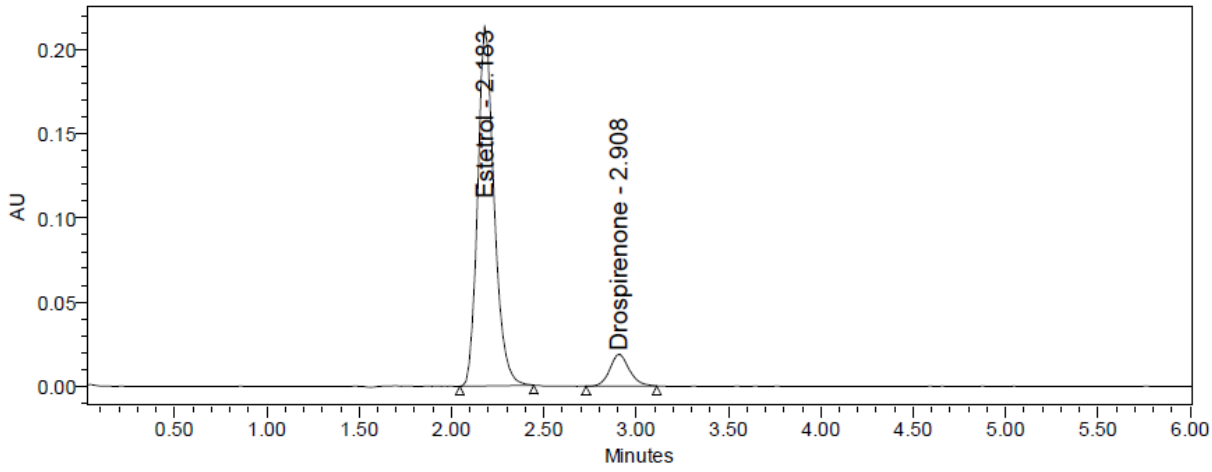


Fig. 3.9: Linearity 50% Chromatogram of Estetrol and Drospirenone



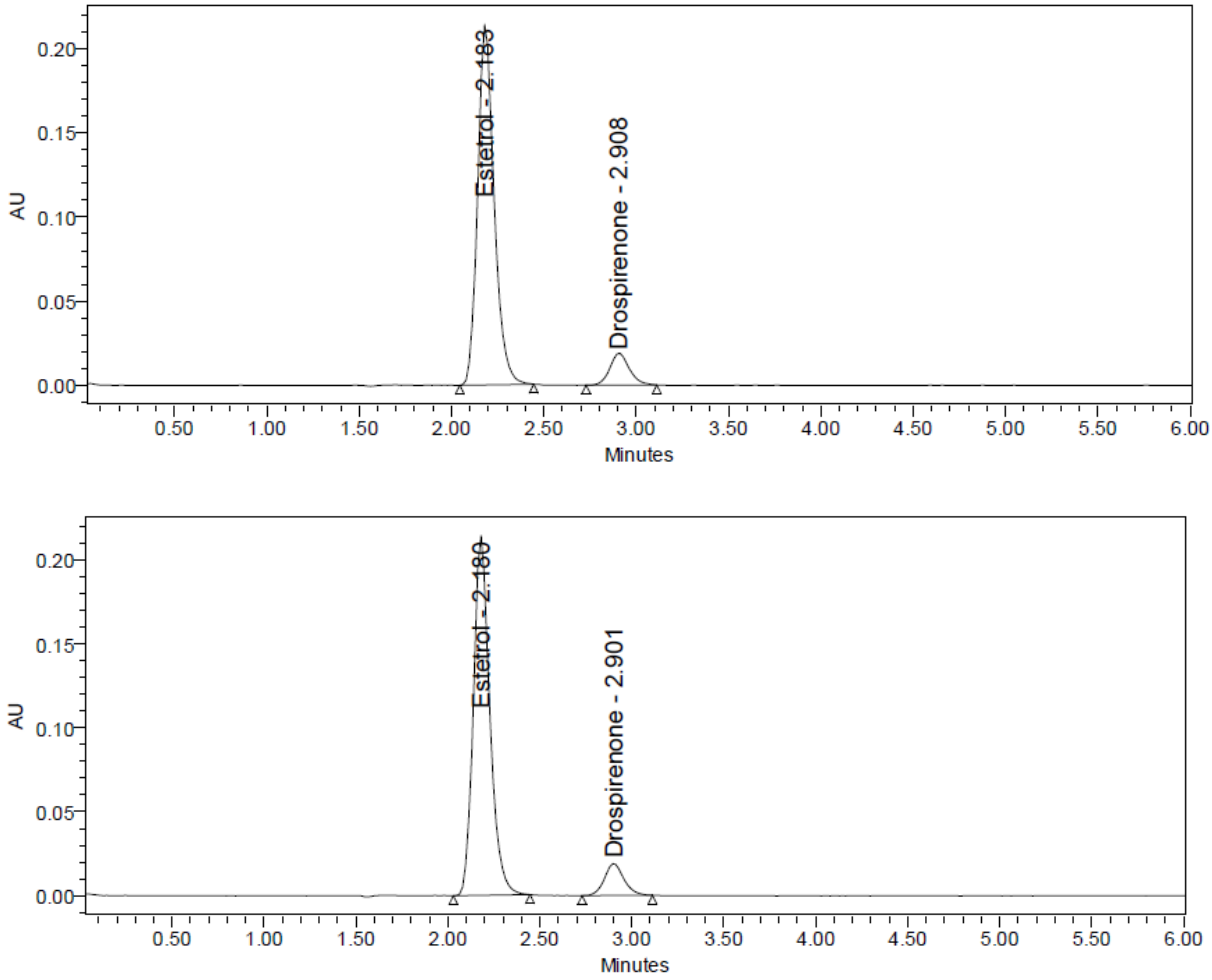
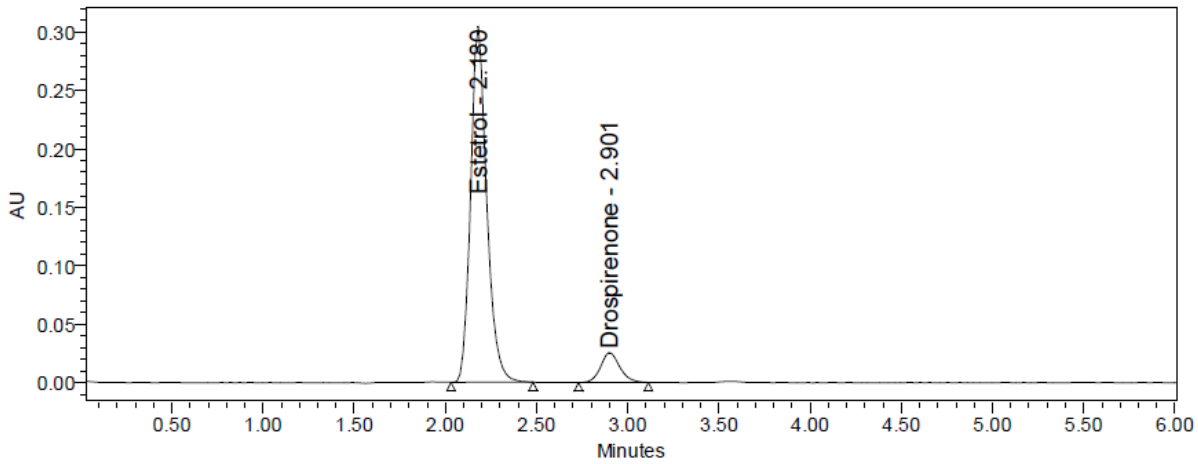


Fig. 3.10: Linearity 75% Chromatogram of Estetrol and Drospirenone



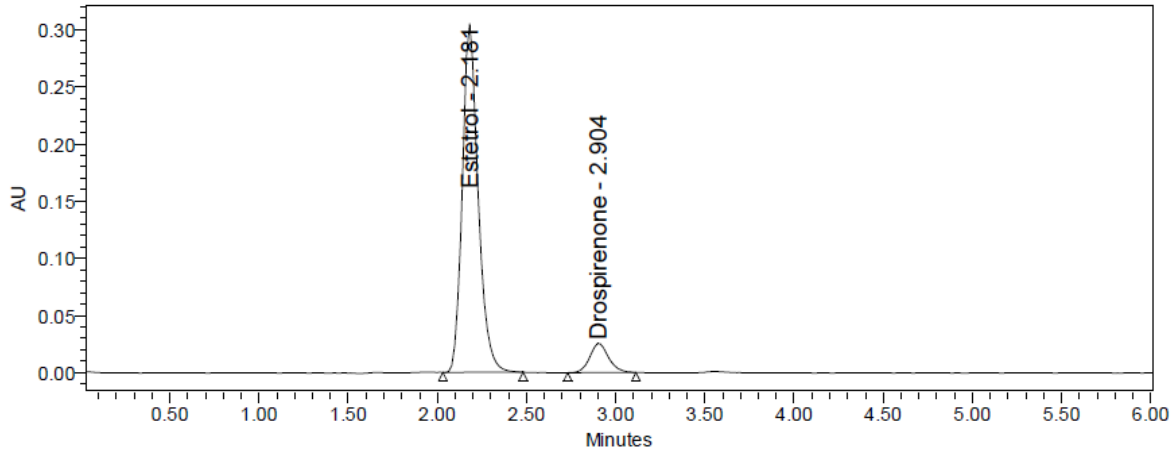
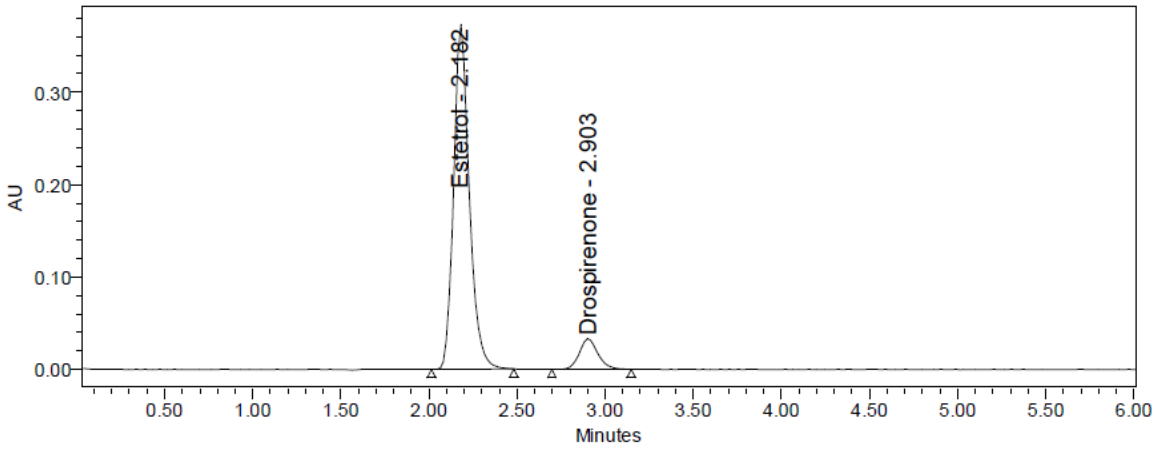


Fig. 3.11: Linearity 100% Chromatogram of Estetrol and Drospirenone



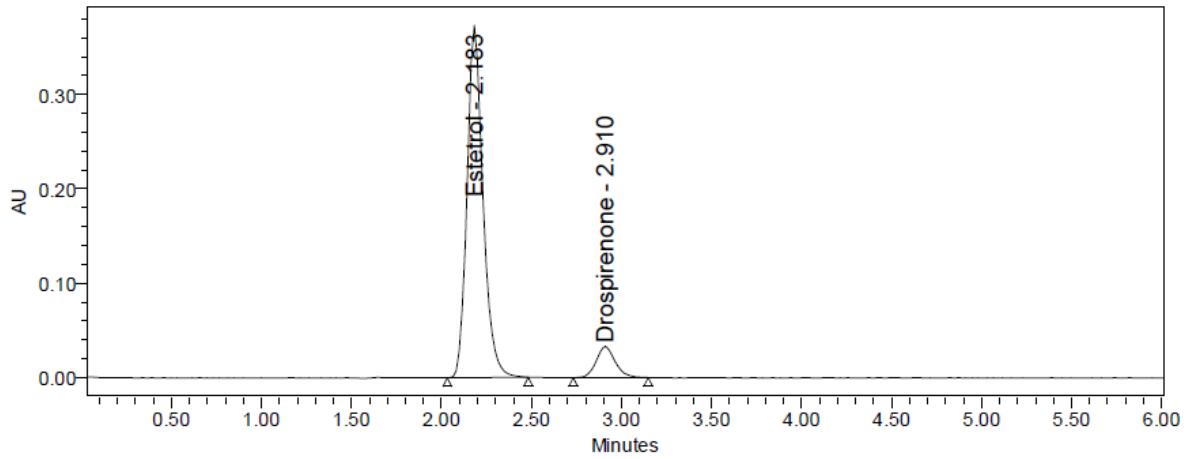


Fig. 3.12: Linearity 125% Chromatogram of Estetrol and Drospirenone

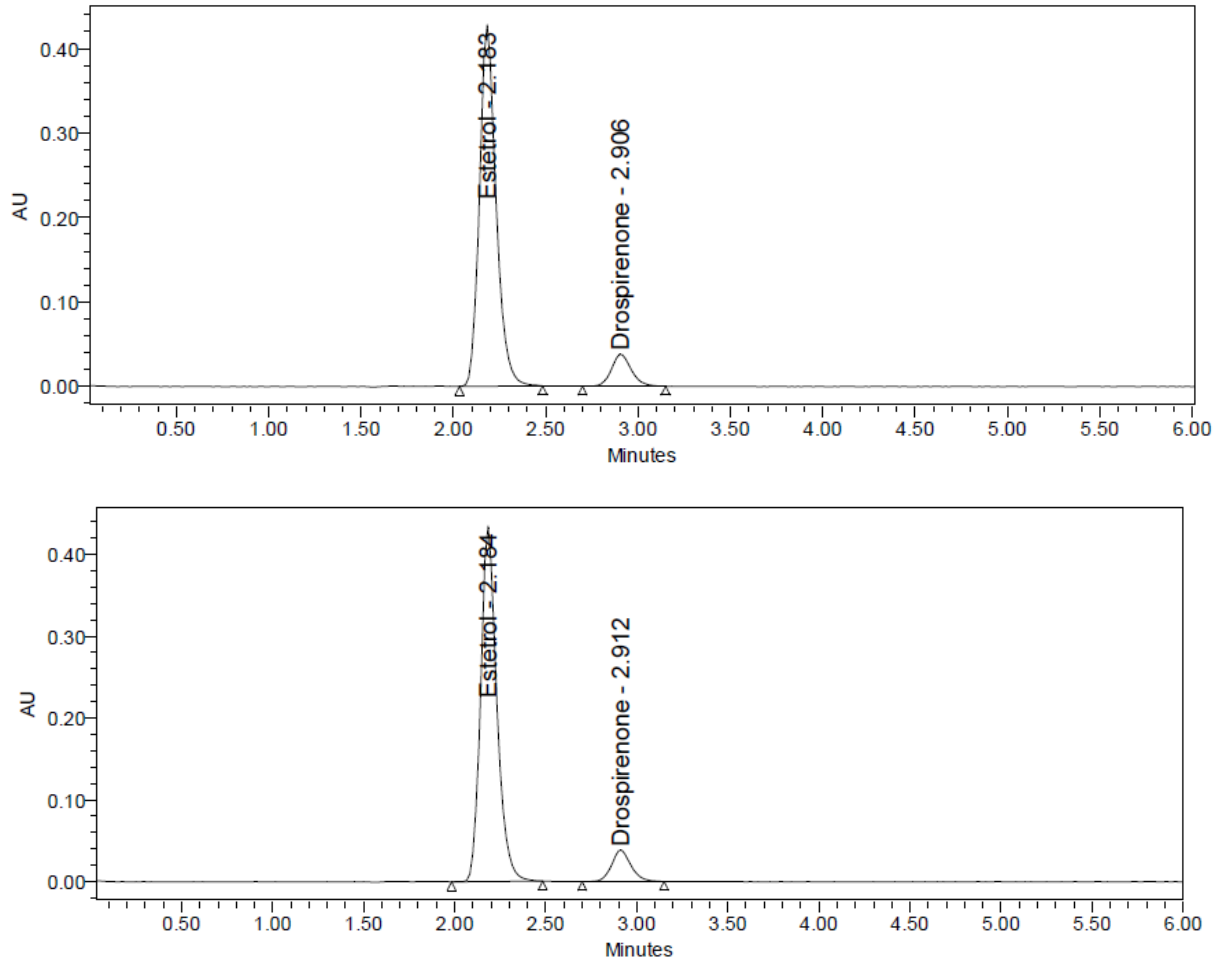
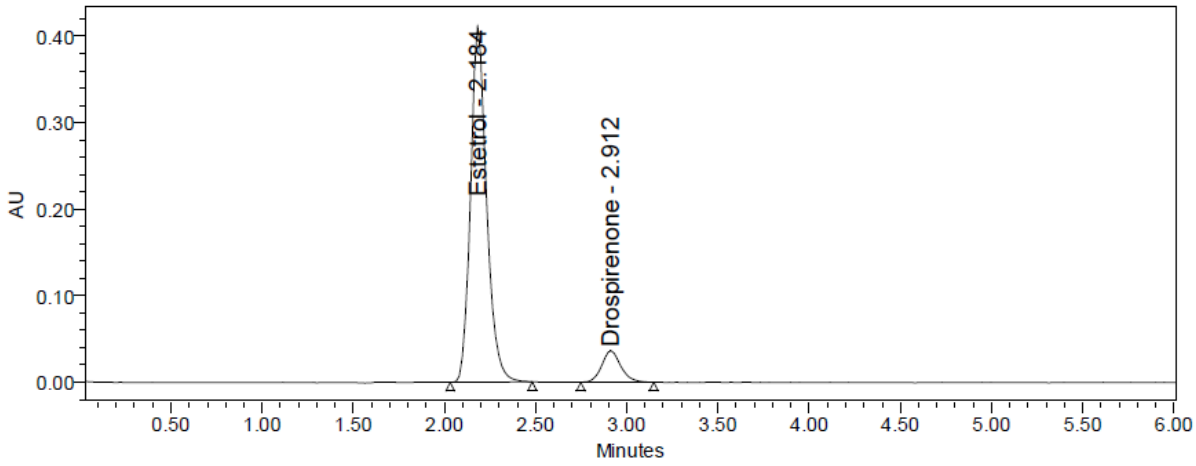


Fig. 3.13: Linearity 150% Chromatogram of Estetrol and Drospirenone



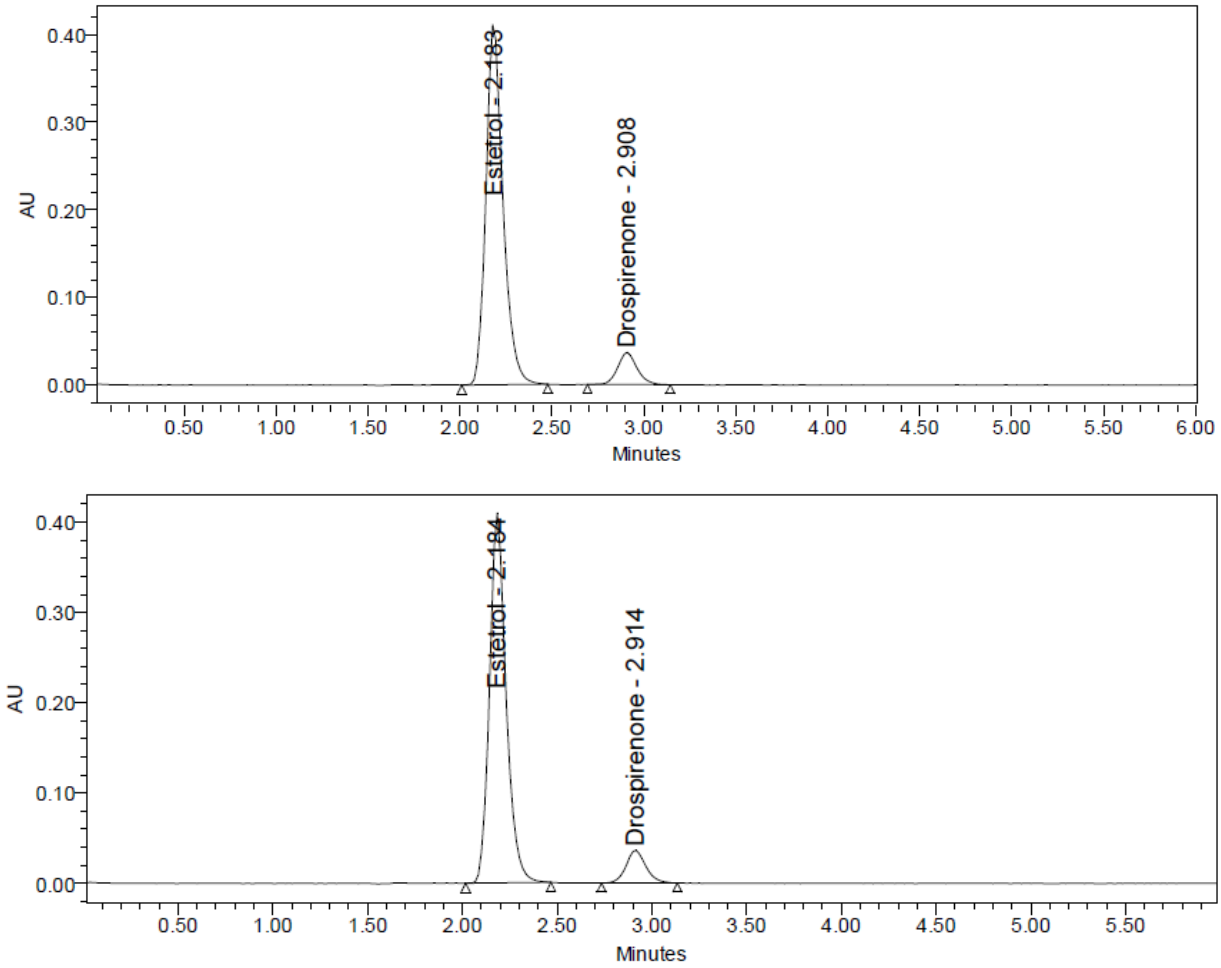
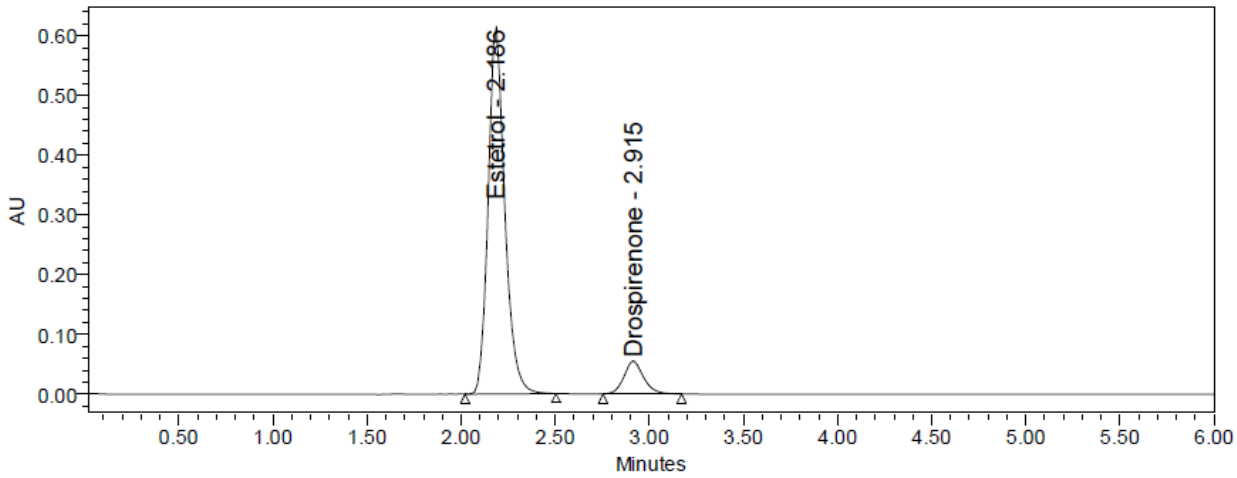


Fig. 3.14: Accuracy 50% Chromatogram Estetrol and Drospirenone



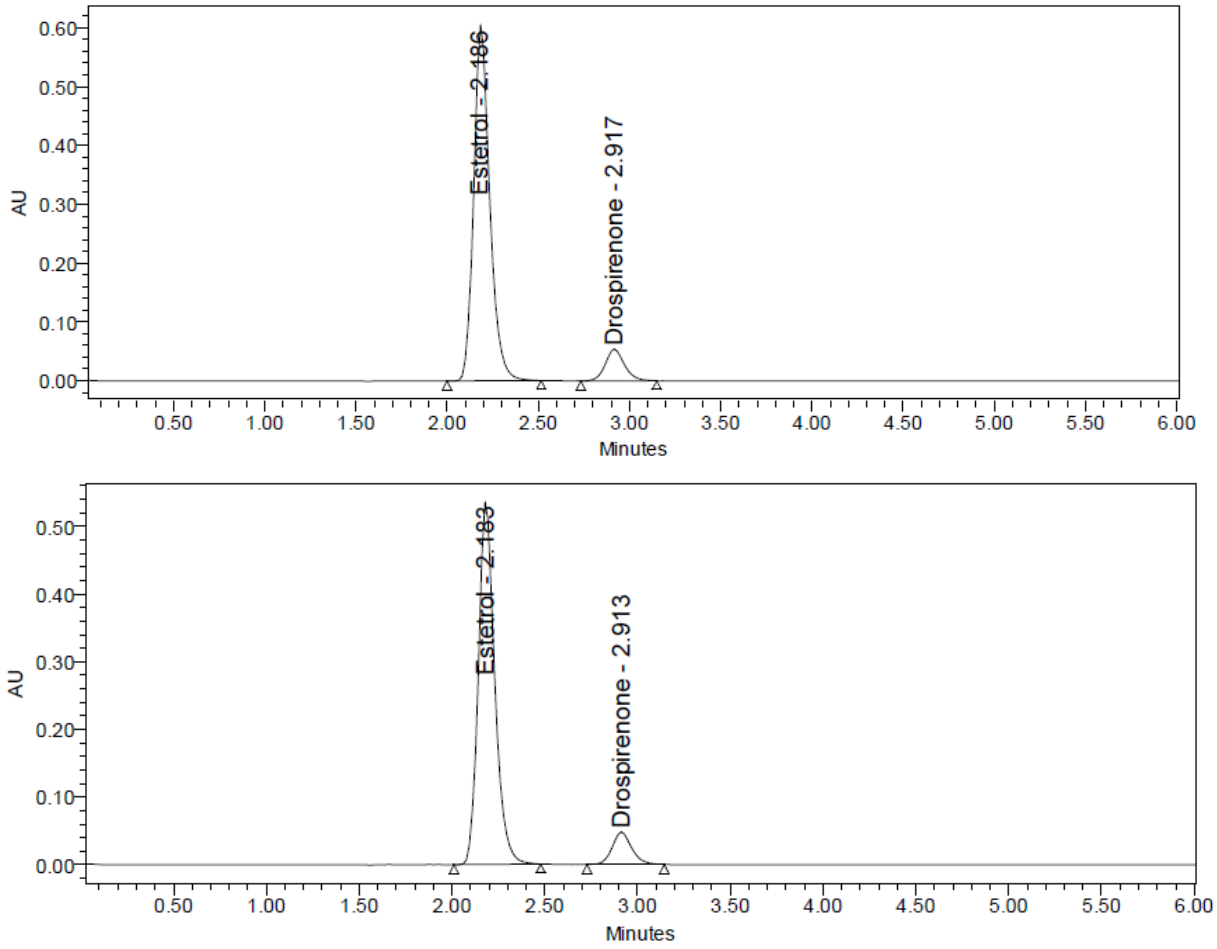
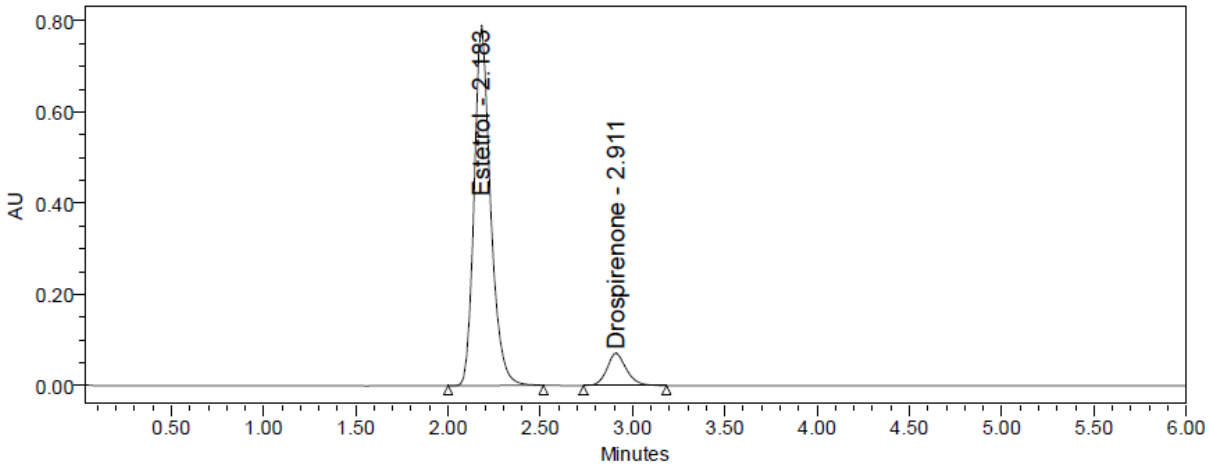


Fig. 3.15: Accuracy 100% Chromatogram of Estetrol and Drospirenone



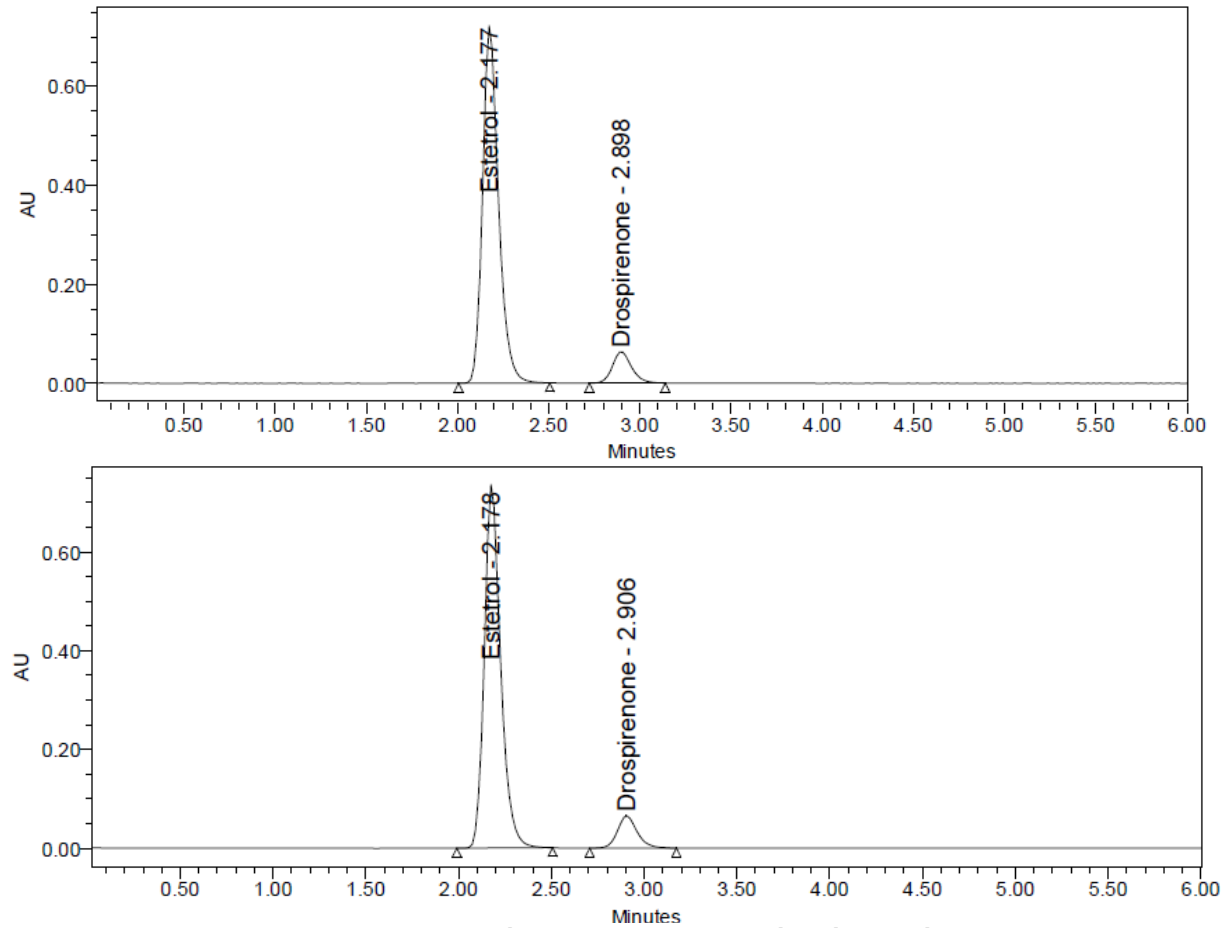
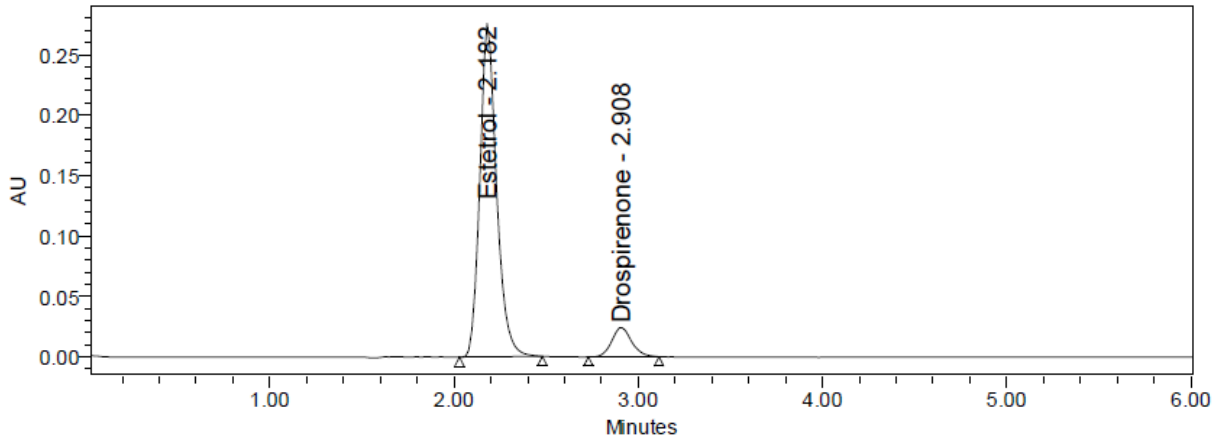
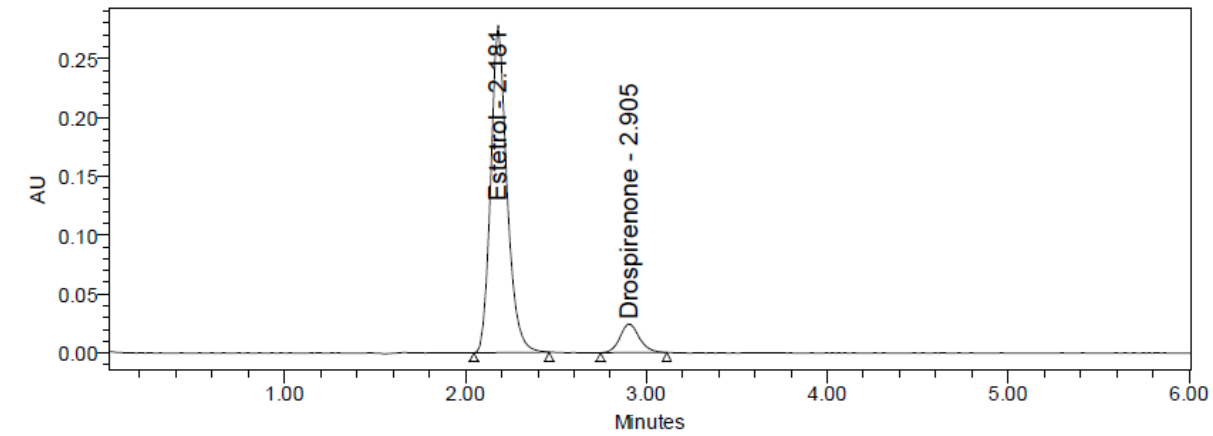
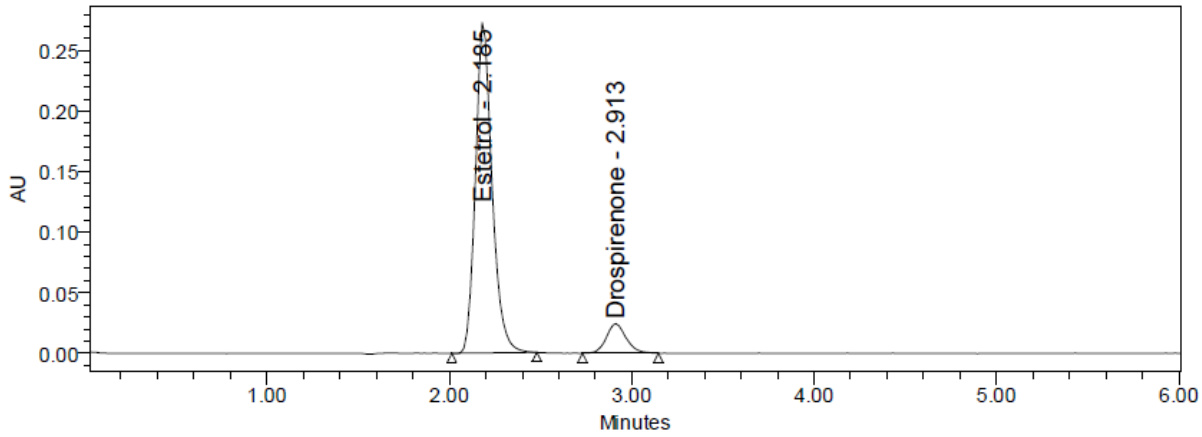


Fig. 3.16: Accuracy 150% Chromatogram Estetrol and Drospirenone




Estetrol - 2.181
Drospirenone - 2.905
AU
Minutes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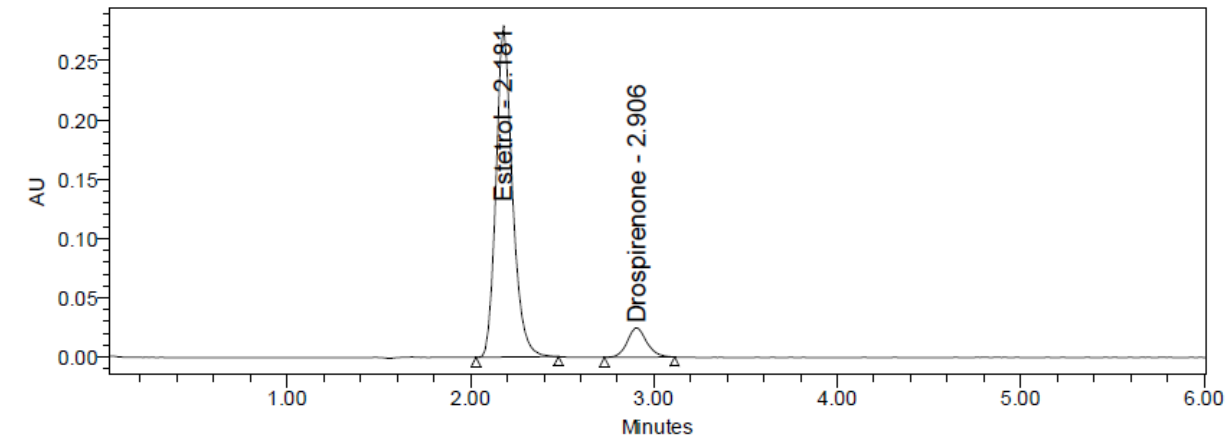
Drospirenone - 2.913
AU
Minutes


Estetrol - 2.181
Drospirenone - 2.906
AU
Minutes

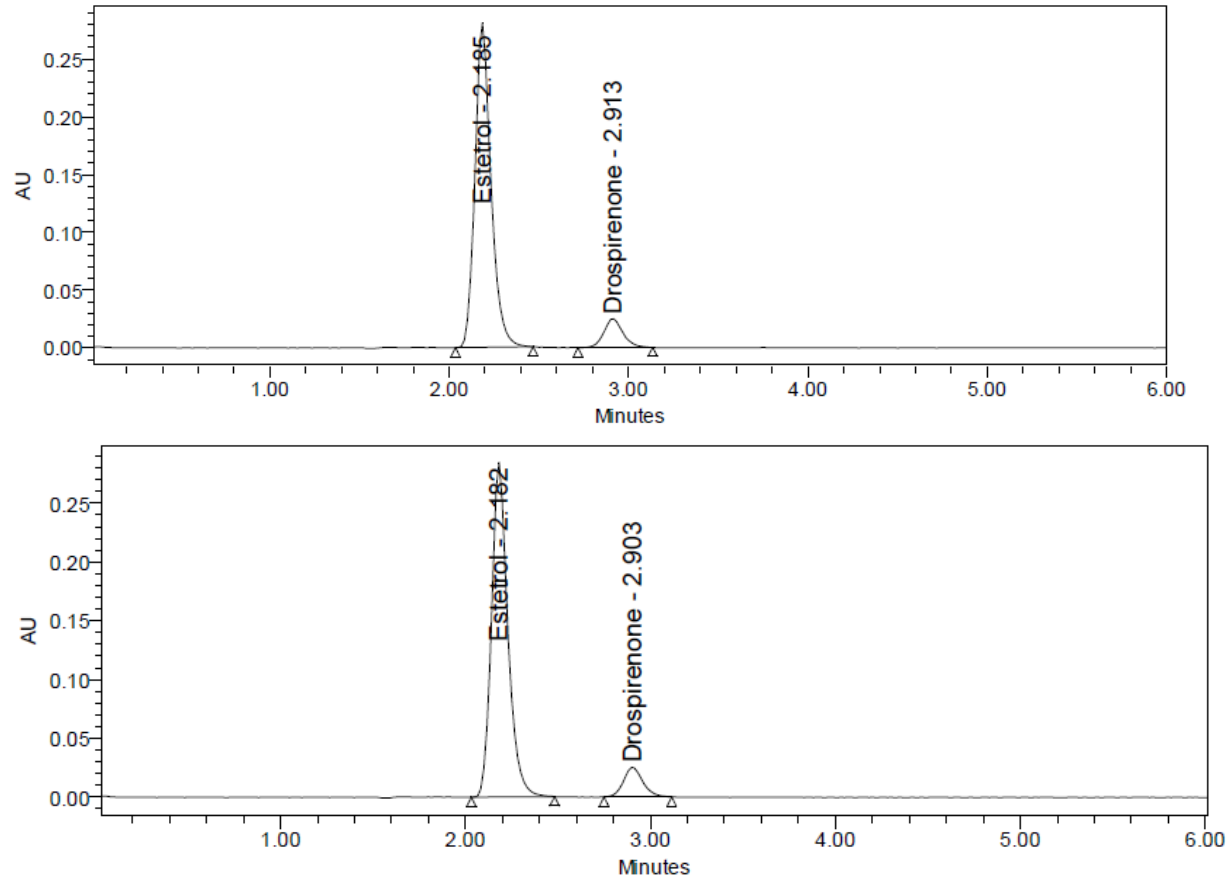
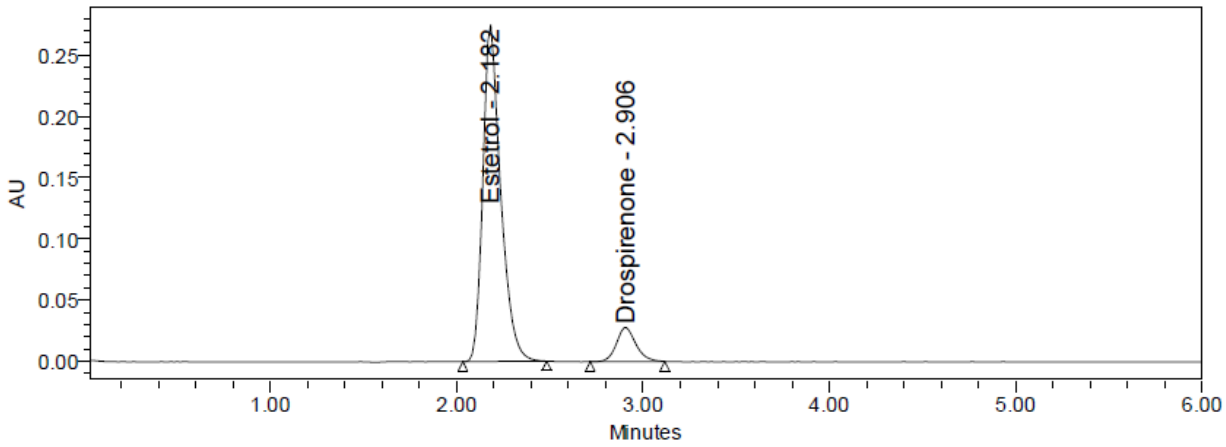
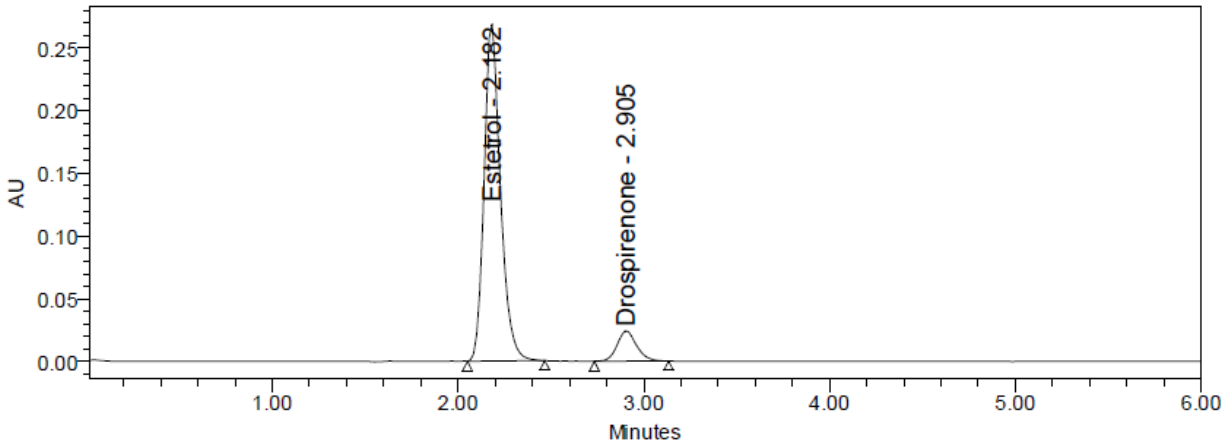


Fig. 3.17: Repeatability Chromatogram of Estetrol and Drospirenone




Estetrol - 2.182
Drospirenone - 2.905
AU
Minutes
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0.15
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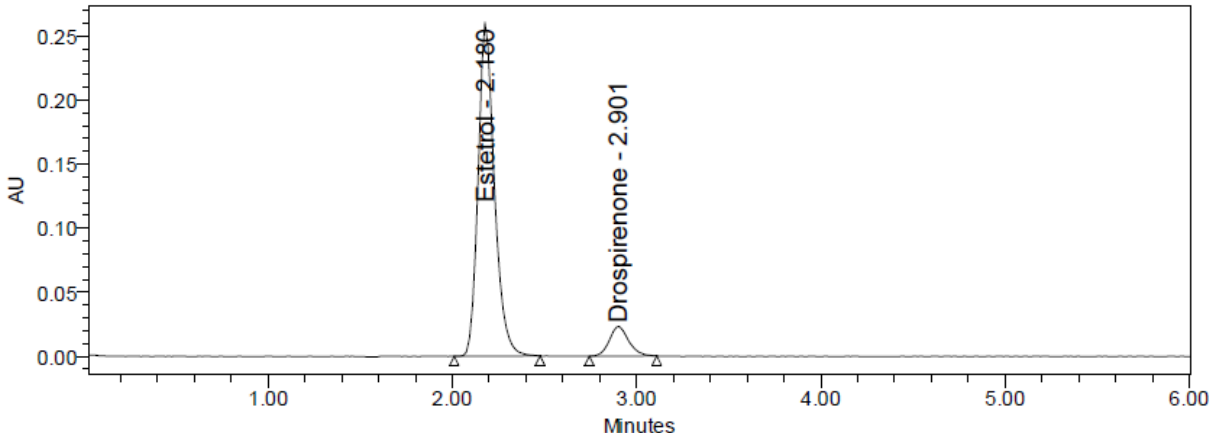
1.00
2.00
3.00
4.00
5.00
6.00


Estetrol - 2.180
Drospirenone - 2.901
AU
Minutes
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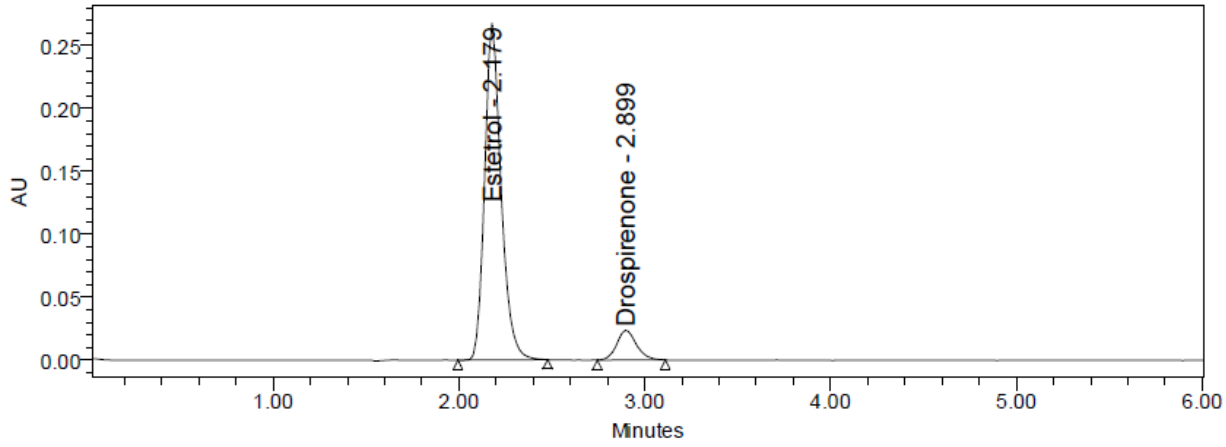
0.10
0.05
0.00
1.00
2.00
3.00
4.00
5.00
6.00


Estetrol - 2.179
Drospirenone - 2.899
AU
Minutes
0.25
0.20
0.15
0.10
0.05
0.00
1.00
2.00
3.00
4.00
5.00
6.00

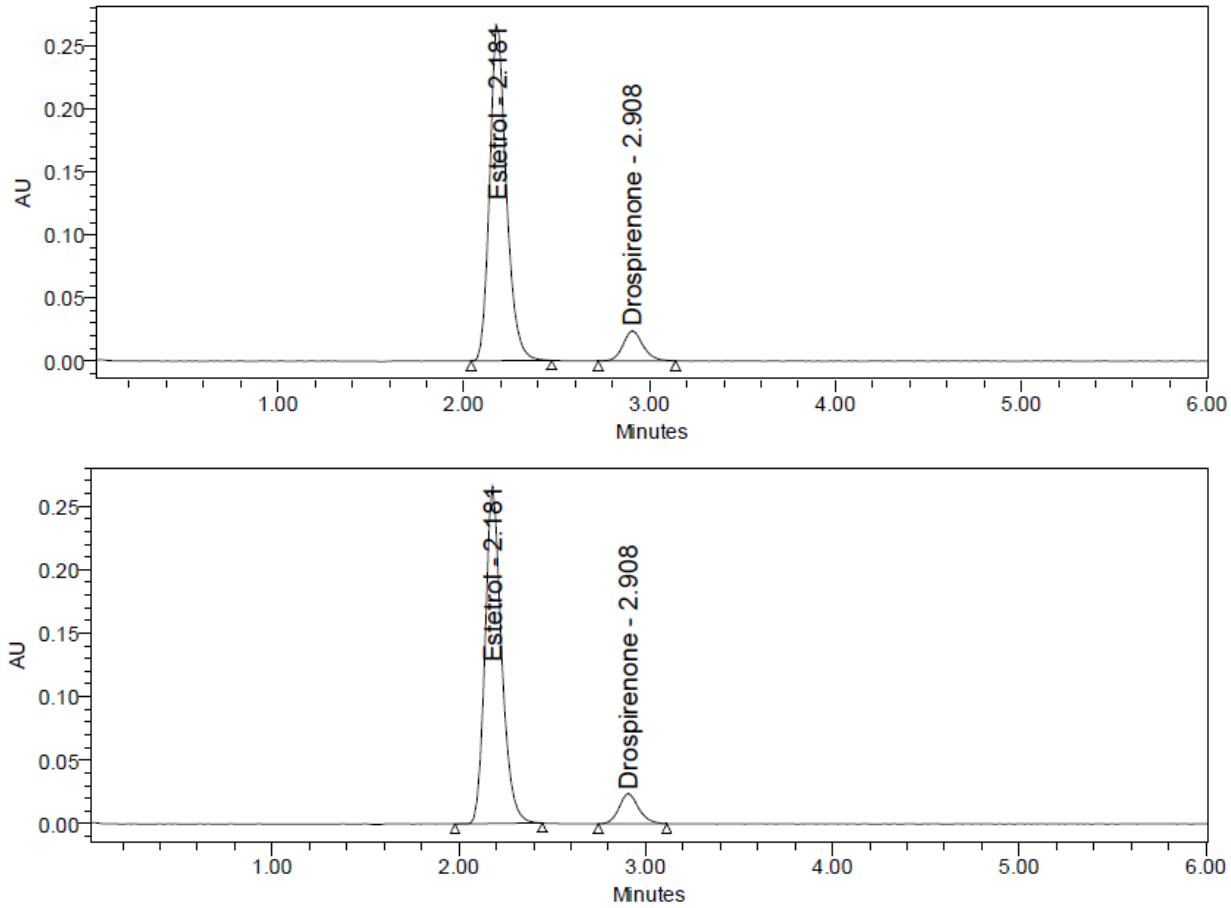
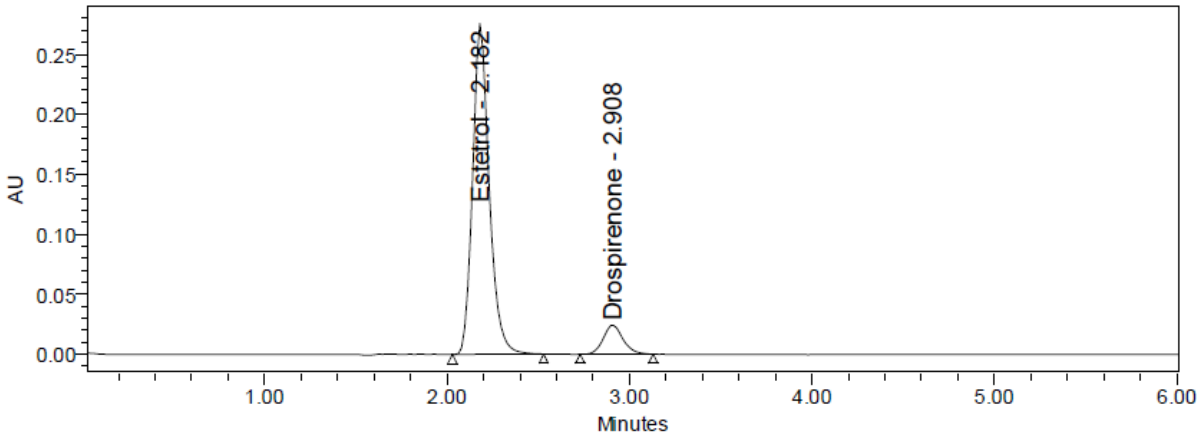
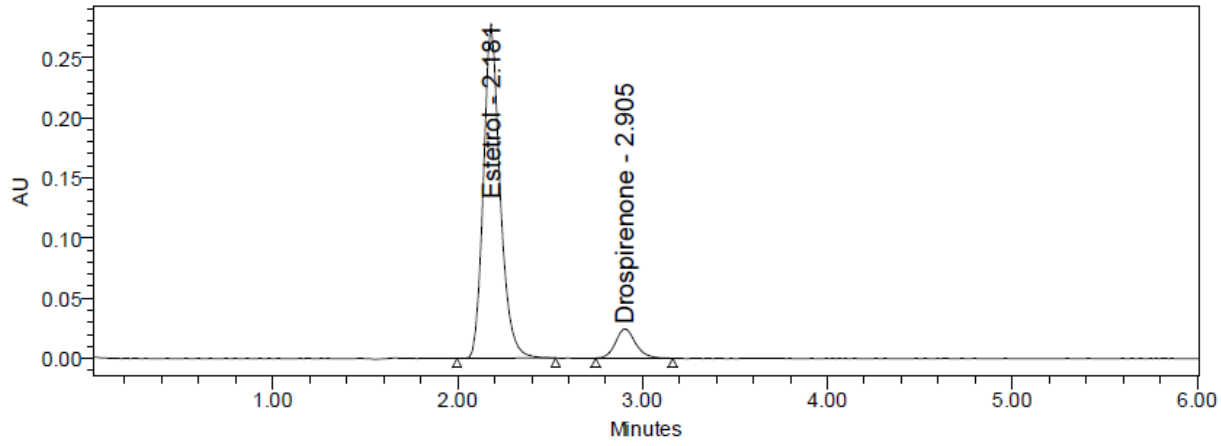
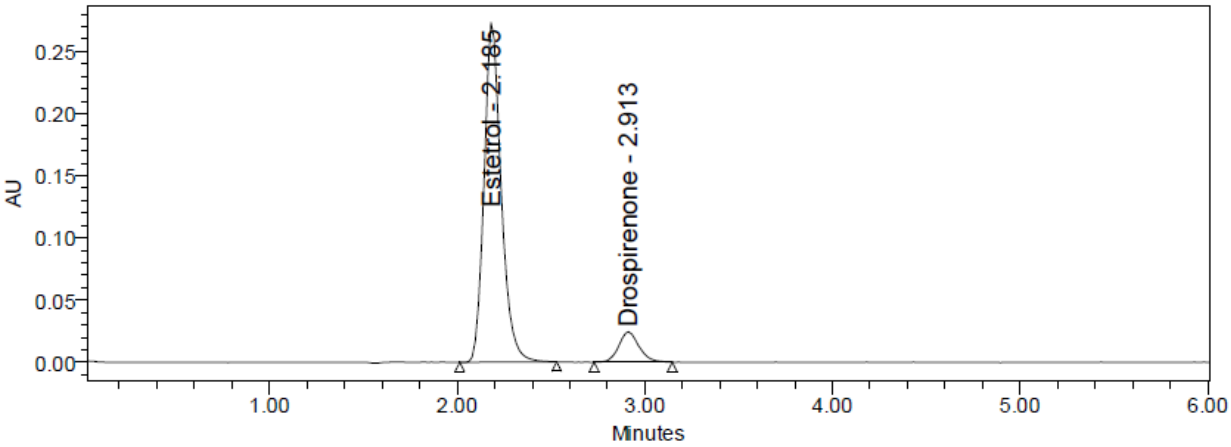
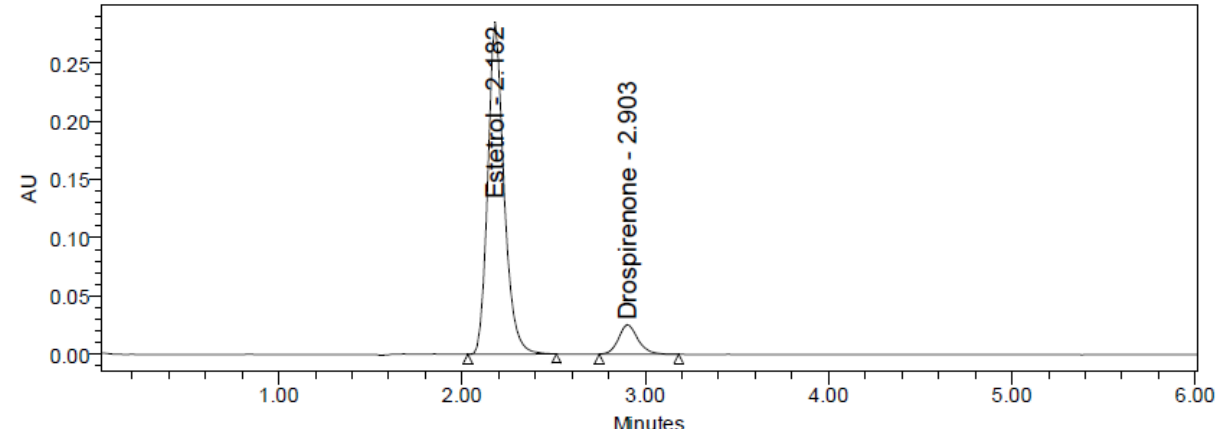


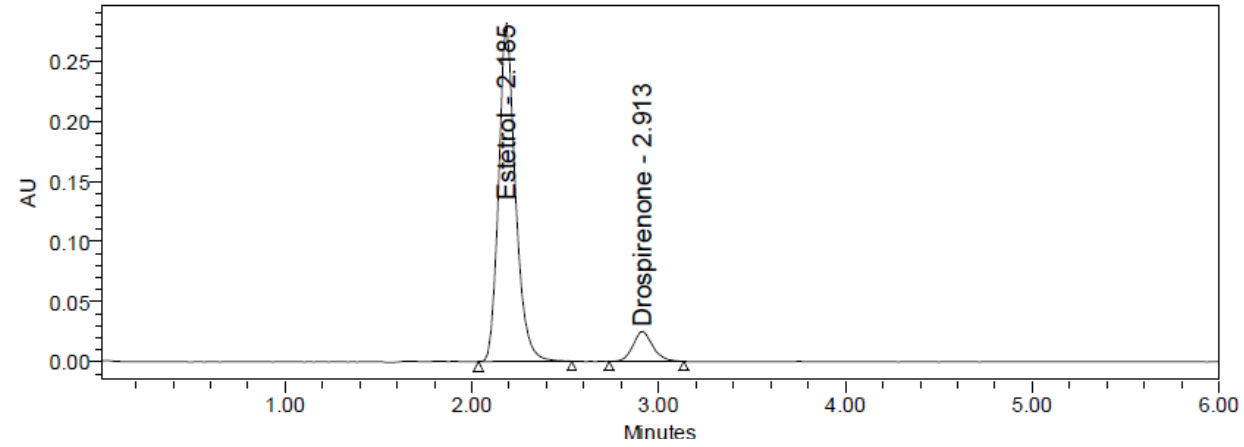
Fig. 3.18: Reproducibility Chromatogram of Estetrol and Drospirenone




Estetrol - 2.184
Drospirenone - 2.905
AU
Minutes


Estetrol - 2.185
Drospirenone - 2.913
AU
Minutes


Estetrol - 2.182
Drospirenone - 2.903
AU
Minutes


Estetrol - 2.185
Drospirenone - 2.913
AU
Minutes

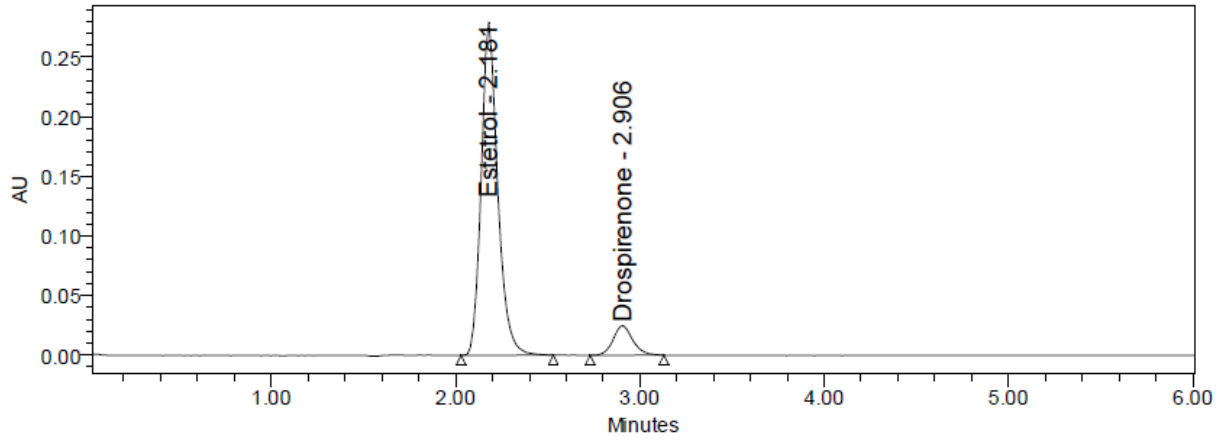


Fig. 3.19: Intermediate precision Chromatogram of Estetrol and Drospirenone

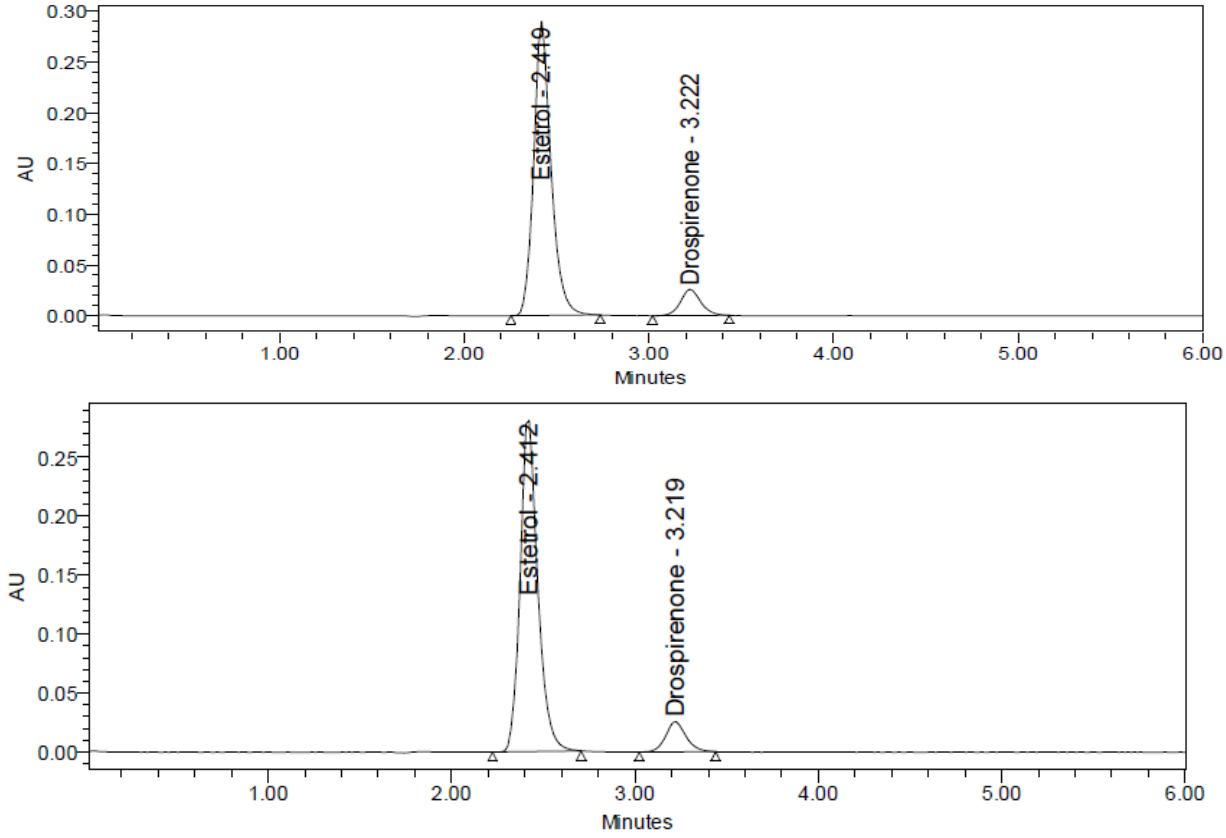


Fig. 3.20: Flow rate at 0.9 mL/min Chromatogram of Estetrol and Drospirenone

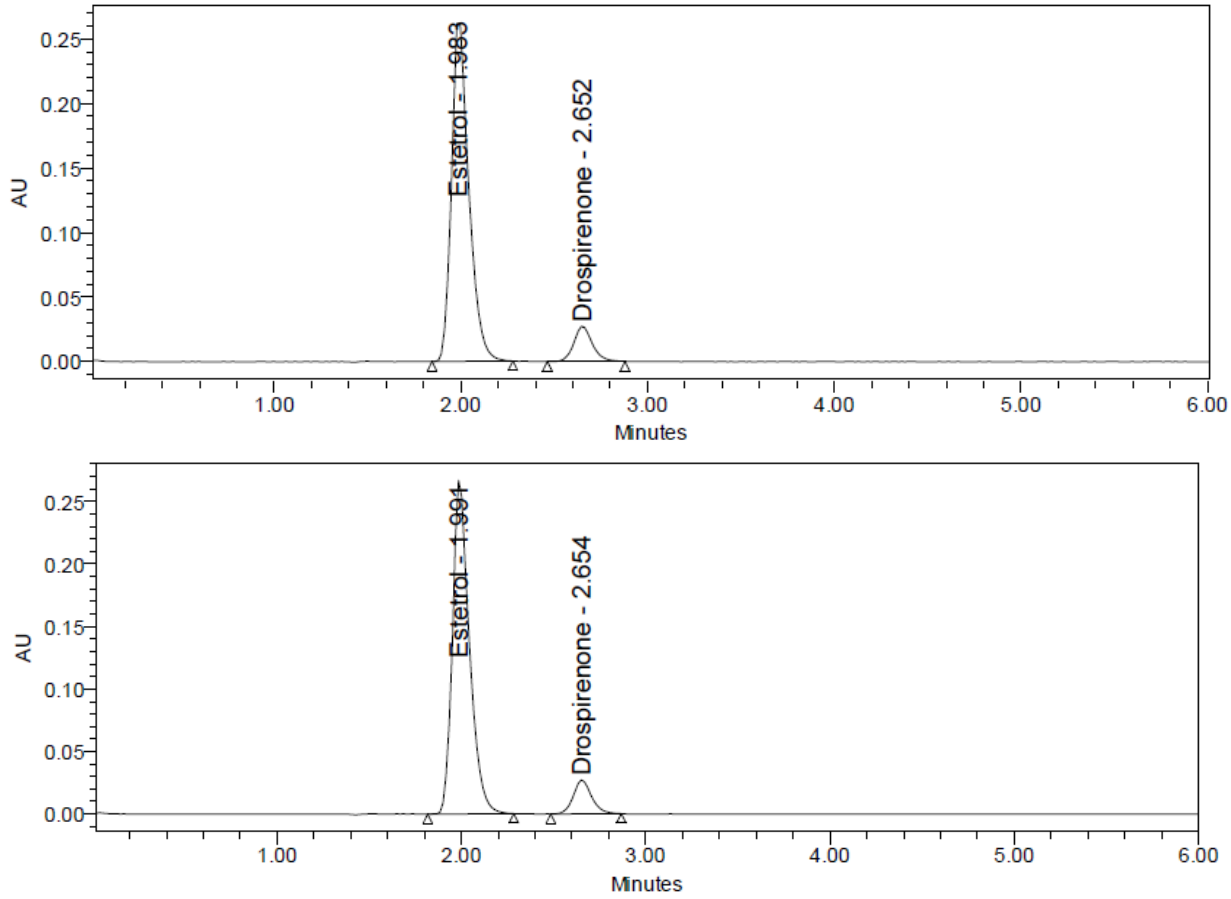


Fig. 3.21: Flow rate at 1.1 mL/min Chromatogram of Estetrol and Drospirenone

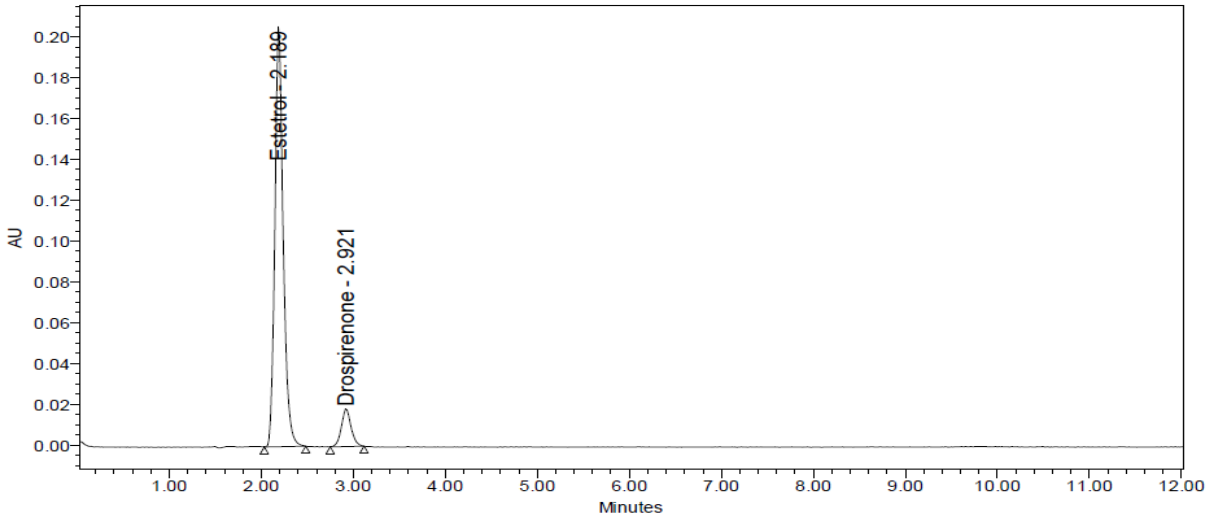


Fig. 3.26: Acid stress chromatogram of Estetrol and Drospirenone

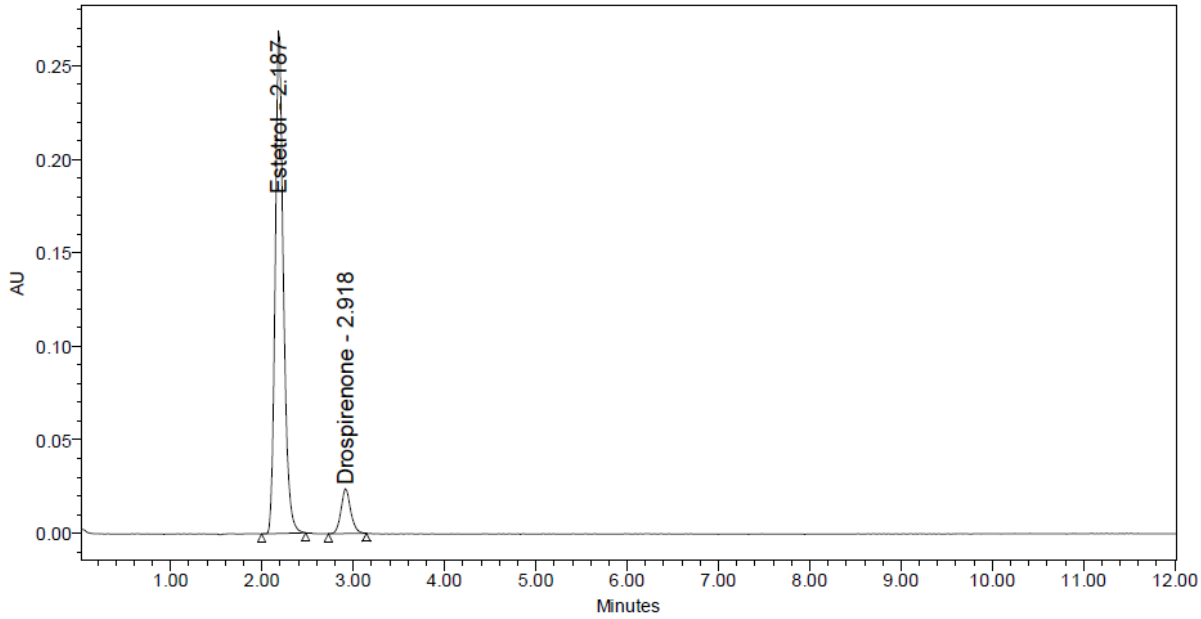


Fig. 3.27: Alkaline stress chromatogram of Estetrol and Drospirenone

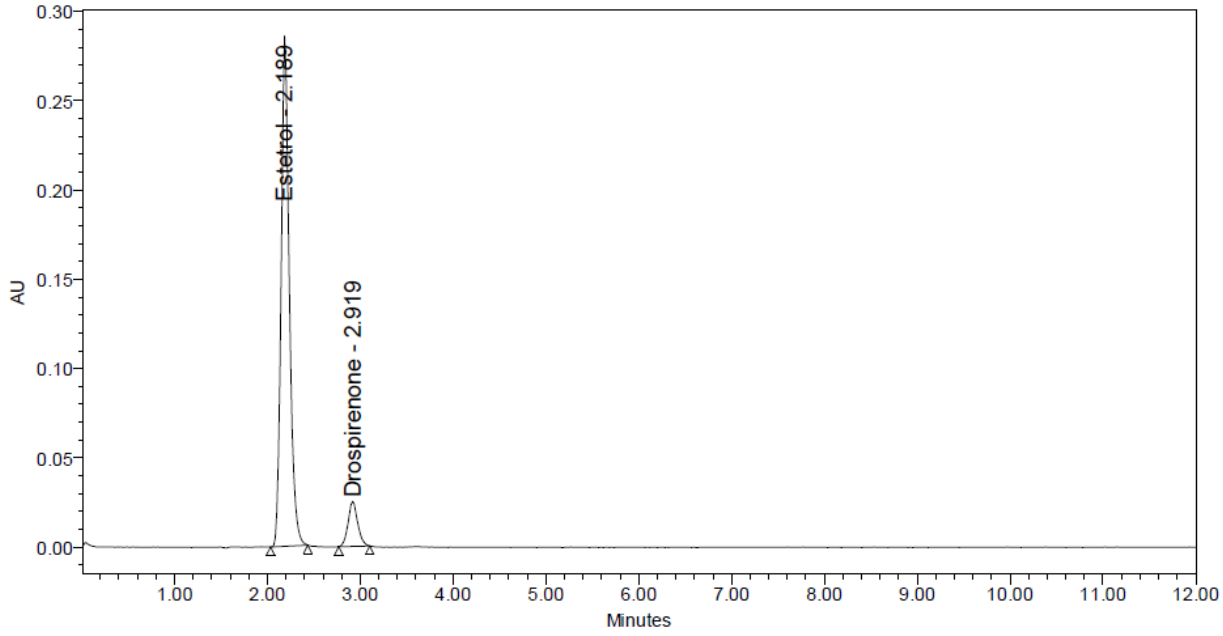


Fig. 3.28: Oxidative stress chromatogram of Estetrol and Drospirenone

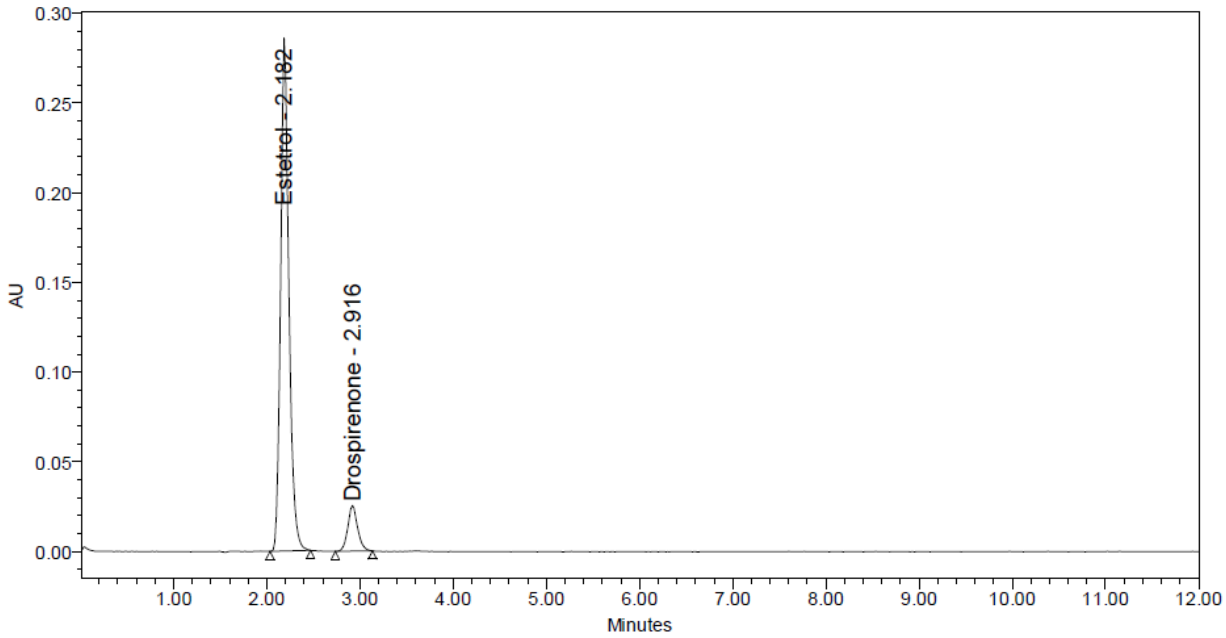


Fig. 3.29: Thermal stress chromatogram of Estetrol and Drospirenone

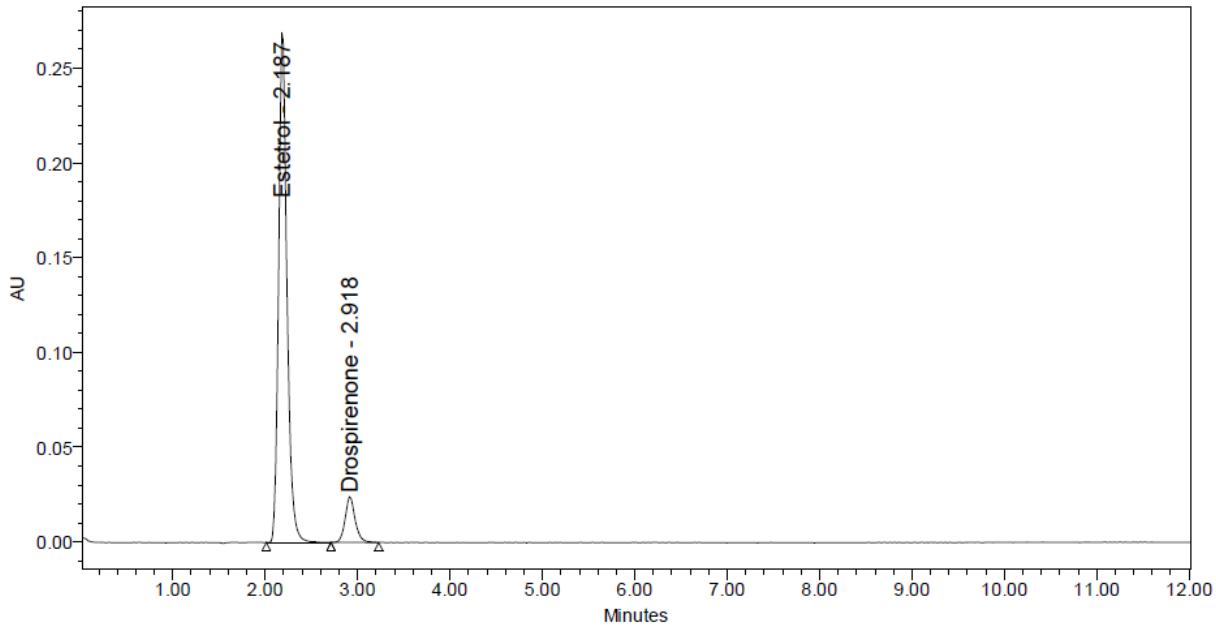


Fig. 3.30: Photolytic stress chromatogram of Estetrol and Drospirenone

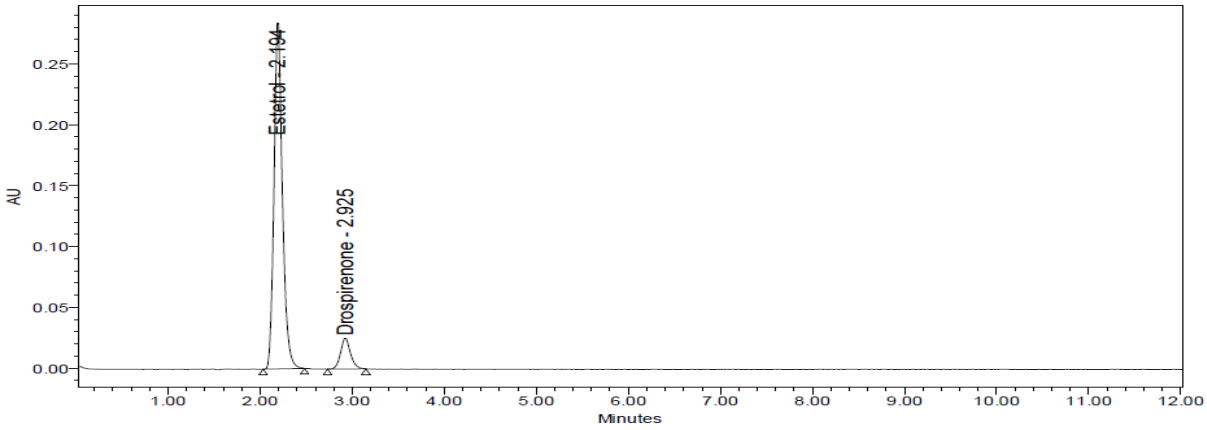


Fig. 3.31: Neutral stress chromatogram of Estetrol and Drospirenone

Table 3.6: (Table 1) Linearity results of Estetrol

%Level	Concentration Estetrol (µg/ml)	Peak Response
25	3.55	44375
50	7.1	88427
75	10.65	131287
100	14.2	175509
125	17.75	219739
150	21.3	259740

Table 3.7: Linearity results of Drospirenone

%Level	Concentration Drospirenone (µg/ml)	Peak Response
25	0.75	4642
50	1.5	9163
75	2.25	13938
100	3	18144
125	3.75	22837
150	4.5	27427

Table 3.8: Calibration parameters of Estetrol and Drospirenone

Best fit line parameters	Estetrol (Mean ± Std. Deviation)	Drospirenone (Mean ± Std. Deviation)
Slope	12244 ± 46.669	6078.6 ± 19.798
Intercept	900.52 ± 319.1	58.75 ± 34.2
Correlation coefficient (r^2)	0.999	0.999

Table 3.9: Accuracy obtained results of Estetrol

%Accuracy Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	7.1	7.01476	98.80	100.17%
	7.1	7.03093	99.03	
	7.1	7.11375	100.19	
100%	14.2	14.20489	100.03	
	14.2	14.33892	100.98	
	14.2	14.23527	100.25	
150%	21.3	21.49075	100.90	
	21.3	21.50390	100.96	
	21.3	21.38367	100.39	

Table 3.10: Accuracy obtained results of Drospirenone

%Accuracy Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	1.5	1.483917	98.93	100.20%
	1.5	1.527188	101.81	
	1.5	1.504648	100.31	
100%	3	3.039034	101.30	
	3	2.967629	98.92	
	3	2.991486	99.72	
150%	4.5	4.569143	101.54	
	4.5	4.530643	100.68	
	4.5	4.434888	98.55	

Table 2.11: Repeatability results of Estetrol and Drospirenone

S.No	Estetrol Peak Area	Drospirenone Peak Area
1	172847	18123
2	173123	18119
3	174699	18153
4	173082	18064
5	173475	18130
6	172682	18217
AVG	**173318**	**18134**
STDEV	**728.3**	**50.0**
%RSD	**0.4**	**0.3**

Table 3.14: LOD and LOQ results of Estetrol

Sample	Retention time	Peak area	S/N Ratio
LOD	2.182	54420	26.1
LOQ	2.182	64299	44.2

Table 3.15: LOD and LOQ results of Drospirenone

Sample	Retention time	Peak area	S/N Ratio
LOD	2.910	4610	13.5
LOQ	2.909	6140	18.9

Table 3.16: Robustness results of flow rate at 0.9 mL/min

S.No.	Estetrol			Drospirenone		
	Retention time	Peak Area	Tailing Factor	Retention time	Peak Area	Tailing Factor
1	2.412	186486	1.18	3.219	18461	1.10
2	2.419	185777	1.20	3.222	18225	1.09
Avg	**2.416**	**186132**	**1.19**	**3.221**	**18343**	**1.10**
Std dev.	**0.005**	**501.3**	**0.014**	**0.002**	**166.9**	**0.007**
%RSD	**0.2**	**0.3**	**1.2**	**0.07**	**0.9**	**0.6**

Table 3.17: Robustness results of flow rate at 1.1 mL/min

S.No.	Estetrol			Drospirenone		
	Retention time	Peak Area	Tailing Factor	Retention time	Peak Area	Tailing Factor
1	1.983	179266	1.30	2.652	18250	1.16
2	1.991	176149	1.28	2.654	18339	1.14
Avg	**1.987**	**177708**	**1.29**	**2.653**	**18295**	**1.15**
Std dev.	**0.006**	**2204.1**	**0.014**	**0.001**	**62.9**	**0.014**
%RSD	**0.3**	**1.2**	**1.1**	**0.05**	**0.3**	**1.2**

Table 3.18: Robustness results of mobile phase variation at (60:40)

S.No.	Estetrol			Drospirenone		
	Retention time	Peak Area	Tailing Factor	Retention time	Peak Area	Tailing Factor
1	2.127	178838	1.26	2.733	17974	1.15
2	2.131	182177	1.25	2.738	18090	1.17
Avg	**2.129**	**180508**	**1.26**	**2.736**	**18032**	**1.16**
Std dev.	**0.003**	**2361.0**	**0.007**	**0.004**	**82.0**	**0.014**
%RSD	**0.1**	**1.3**	**0.6**	**0.13**	**0.5**	**1.2**

Table 3.19: Robustness results of mobile phase variation at (50:50)

S.No.	Estetrol			Drospirenone		
	Retention time	Peak Area	Tailing Factor	Retention time	Peak Area	Tailing Factor
1	2.242	179959	1.21	3.117	18578	1.15
2	2.243	178380	1.23	3.124	18352	1.13
Avg	**2.243**	**179170**	**1.22**	**3.121**	**18465**	**1.14**
Std dev.	**0.001**	**1116.5**	**0.014**	**0.005**	**159.8**	**0.014**
%RSD	**0.03**	**0.6**	**1.2**	**0.16**	**0.9**	**1.2**

Table 3.20: Robustness results of Temperature variation at (27 °C)

S.No.	Estetrol			Drospirenone		
	Retention time	Peak Area	Tailing Factor	Retention time	Peak Area	Tailing Factor
1	2.240	182517	1.24	3.115	18389	1.15
2	2.246	183794	1.26	3.121	18521	1.12
Avg	**2.243**	**183156**	**1.25**	**3.118**	**18455**	**1.14**
Std dev.	**0.004**	**903.0**	**0.014**	**0.004**	**93.3**	**0.021**
%RSD	**0.19**	**0.5**	**1.1**	**0.14**	**0.5**	**1.9**

Table 3.21: Robustness results of Temperature variation at (33 °C)

S.No.	Estetrol			Drospirenone		
	Retention time	Peak Area	Tailing Factor	Retention time	Peak Area	Tailing Factor
1	2.127	184838	1.26	2.733	18125	1.15
2	2.131	184177	1.25	2.738	18337	1.17
Avg	**2.129**	**184508**	**1.26**	**2.736**	**18231**	**1.16**
Std dev.	**0.003**	**467.4**	**0.007**	**0.004**	**149.9**	**0.014**
%RSD	**0.13**	**0.3**	**0.6**	**0.13**	**0.8**	**1.2**

Table 3.22: System suitability results of Estetrol and Drospirenone

S.No.	Estetrol		Drospirenone	
	Peak Retention Time	Peak Area	Peak Retention Time	Peak Area
1	2.181	172847	2.903	18123
2	2.181	173123	2.905	18119
3	2.182	174699	2.906	18153
4	2.182	173082	2.908	18064
5	2.185	173475	2.913	18130
6	2.185	172682	2.913	18217
Mean	**2.183**	**173318**	**2.908**	**18134**
Std.dev	**0.002**	**728.3**	**0.004**	**50.0**
%RSD	**0.085**	**0.4**	**0.144**	**0.3**

Table 3.23: Degradation Data of Estetrol

S.NO	Degradation Condition	Retention time	Peak Area	% Drug Remained	% Drug Degraded
1	Acid	2.189	165516	95.31	4.69
2	Alkali	2.187	169256	97.46	2.54
3	Oxidation	2.189	170305	98.07	1.93
4	Thermal	2.182	171587	98.80	1.20
5	Photolytic	2.187	171869	98.97	1.03
6	Neutral	2.194	172565	99.37	0.63

Table 3.24: Degradation Data of Drospirenone

S.NO	Degradation Condition	Retention time	Peak Area	% Drug Remained	% Drug Degraded
1	Acid	2.921	17244	94.90	5.10
2	Alkali	2.918	17453	96.05	3.95
3	Oxidation	2.919	17498	96.30	3.70
4	Thermal	2.916	17905	98.54	1.46
5	Photolytic	2.918	17931	98.68	1.32
6	Neutral	2.925	18036	99.26	0.74

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