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Research Article

VALIDATED STABILITY INDICATING HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF ESTETROL AND DROSPIRENONE

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ABSTRACT

The prescription dose form of Estetrol and Drospirenone may be accurately and precisely measured using a new, easy-to-use technology. Chromatograms were produced using a standard Agilent C18 150 x 4.6 mm, 5m column. A mobile phase consisting of a 50:50 mixture of Buffe0.1N KH2PO4 and acetonitrile was passed down the column at a rate of 1 ml/min. The KH2PO4 buffer was used in this procedure. The temperature was kept at 30 degrees Celsius. The 263 nm wavelength was chosen as the optimal one. Estetrol had a %RSD of 0.5 and Drospirenone a %RSD of 0.8, while their retention times were 2.268 and 2.698 minutes, respectively. %Estetrol had a recovery of 100.91% and Drospirenone 99.76%. Estetrol's LOD was 0.39, Drospirenone's LOQ was 1.18, and the two chemicals' regression equations yielded LOQ and LOD values of 0.01 and 0.03, respectively. The Estetrol regression equation is y = 26009x + 3120.7, whereas the Drospirenone regression equation is y = 25028x + 719.64. The new approach was easy and affordable, allowing it to be implemented for routine quality control tests in industries. It lowered retention times and run time.

Keywords: Estetrol, Drospirenone, RP-HPLC

INTRODUCTION

Oral contraceptives (birth control pills) are hormone-containing medications that are taken by mouth to prevent pregnancy. They prevent pregnancy by inhibiting ovulation and also by preventing sperm from penetrating through the cervix.

By far the most commonly prescribed type of oral contraceptive in the United States contains synthetic versions of the natural female hormones estrogen and progesterone. This type of birth control pill is often called a combined oral contraceptive. Another type of oral

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contraceptive, sometimes called the mini pill, contains only progestin, which is a man-made version of progesterone.[1]

Drospirenone: Drospirenone is a synthetic progestin commonly found in the popular oral contraceptive, Yaz in combination with Ethinyl estradiol.[2] Most recently, it was approved by both Health Canada and the FDA in combination with Estetrol as an oral contraceptive therapy.[3,4] Aside from its contraceptive effects, drospirenone is used with estrogens to control acne and premenstrual dysphoric disorder (PMDD).

Drospirenone has been the subject of widespread safety concern due to the possibility of an increased risk of venous thromboembolism associated with its use.[5,6] In 2012, however, a safety statement by the FDA concluded that the increase in the risk of thromboembolism resulting from the use of drospirenone remains unclear, as studies regarding this risk are conflicting. Some studies have demonstrated a significantly increased risk and some demonstrating no risk of thromboembolic events. In its statement, the FDA has mentioned that increased risk of venous thromboembolism with oral contraceptives such as drospirenone exists but remains lower than the risk of this condition during pregnancy and during the postpartum period, and this should be considered when assessing potential risks of hormonal contraceptive use.[7]

Estetrol: Naturally or synthetically produced steroid estrogens have a wide range of pharmaceutical uses ranging from hormonal contraception to the treatment of menopausal symptoms.[8] Estetrol (E4) is a native estrogen occurring naturally during pregnancy, but can be synthesized from a plant source and used for contraception.[9] It is more potent and is safer than the synthetic estrogen ethinylestradiol (EE2) found in 97% of oral contraceptive pills, reducing the environmental accumulation of unwanted endocrine disrupting chemicals (EDCs) that often lead to harmful epigenetic effects.

This medication is a combination of 2 hormones: an estrogen (estetrol) and a progestin (drospirenone). This product is used to prevent pregnancy.Besides preventing pregnancy, birth control pills may make your periods more regular, decrease blood loss and painful periods, and decrease your risk of ovarian cysts.This drug does not protect against sexually transmitted diseases (such as HIV, hepatitis B, gonorrhea, syphilis). To decrease your risk of infection, always use an effective barrier method (latex or polyurethane condom/dental dams) during all sexual activity.[10]

According to a literature review, there are some techniques for the simultaneous estimate of these medicines as well as others for assessment of the drugs alone or in combination with other drugs

By Utilizing UV-Spectrophotometry RP-HPLC.

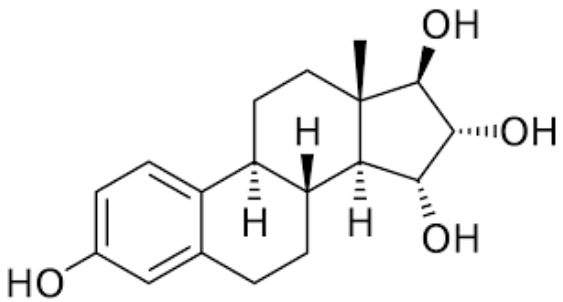

Figure 1: Structure of Estetrol

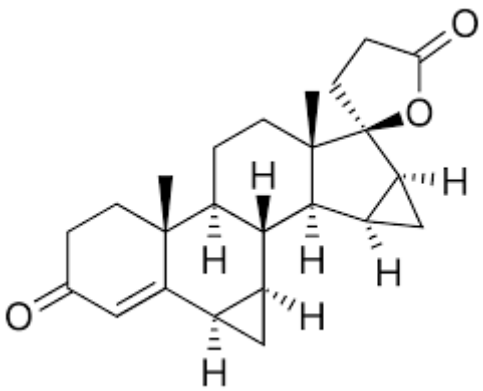

Figure 2: Structure of Drospirenone

There is no established technique for the stability-indicating simultaneous measurement of Estetrol and Drospirenone by RP-HPLC in pharmaceutical dosage form, according to a survey of the literature. The primary goal of this work is to provide an efficient, quick, and accurate RP-HPLC approach for estimating of Estetrol and Drospirenone in medicinal dose and tablet form. According to ICH recommendations, a proven approach was also used to estimate the amounts of Estetrol and Drospirenone. [11-14]

MATERIALS AND REAGENTS

Estetrol and Drospirenone pure drugs were received from Spectrum Pharma research solutions, Hyderabad. The combination tablet Estetrol and Drospirenone (DESOGEN) was purchased from a local pharmacy store. Rankem in India provided all of the chemicals and buffers utilised in this method like Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen Ortho phosphate buffer, Ortho-phosphoric acid, Distilled water.

Instrumentation and Chromatographic Conditions

For the development and validation method, an automated sample injector was employed with a WATERS HPLC, model: 2695 SYSTEM with Photo diode array detector. For the separation, a Discovery 150 (C18 250 mm x 4.6 mm, 5µm) column was employed. Acetonitrile is employed as mobile phase B, while 0.1% ortho phosphoric acid is used as mobile phase A. (35:65 Ratio). The analysis was done in isocratic mode with an injection volume of 10 mL and a flow rate of 1 mL/min. The duration was six minutes. The measurements were made at 254 nm.

PREPARATION OF SOLUTIONS

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of buffer:

0.1%OPA Buffer: 1ml of Ortho phosphoric acid was diluted to 1000ml with HPLC grade water to get 0.1% OPA Solution.

Preparation of Standard stock solutions: Accurately weighed 14.2mg of Estetrol, 3mg of Drospirenone and transferred to 50ml and 50ml individual volumetric flasks and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (284µg/ml of Estetrol and 60µg/ml Drospirenone)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (28.4µg/ml of Estetrol and 6µg/ml of Drospirenone)

Preparation of Sample stock solutions: 20 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 10 tablets was transferred into a 100 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters. (284µg/ml of Estetrol and 60µg/ml Drospirenone)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (28.4µg/ml of Estetrol and 6µg/ml Drospirenone)

METHOD VALIDATION

To prove that the technique is suggested for routine analysis, the HPLC method's validation was done for the simultaneous estimation of Estetrol and Drospirenone drug material in accordance with the ICH criteria.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity: stock solutions of Estetrol and Drospirenone is taken in to 6 different volumetric flasks and diluted to 10ml with diluents. Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml.

Accuracy:

Preparation of Standard stock solutions: Accurately weighed 14.2mg of Estetrol, 3mg of Drospirenoneand transferred to 50ml and 50ml individual volumetric flasks and 3/4 th of diluents was added to theseflask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (284µg/ml of Estetrol and 60µg/ml Drospirenone)

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Estetrol and Drospirenone, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the

above solutions 0.3ml each of Estetrol and Drospirenone, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Degradation studies:

Oxidation:

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 28.4µg/ml & 6µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1 ml of stock s solution Estetrol and Drospirenone, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 28.4µg/ml & 6µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 ml of stock solution Estetrol and Drospirenone, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 28.4µg/ml & 6µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105°C for 1 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 28.4µg/ml& 6µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the 240µg/ml &Drospirenone30µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 28.4µg/ml& 6µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 1hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 28.4µg/ml & 6µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample

RESULTS AND DISCUSSIONS:

Table 1: System suitability parameters for Estetrol and Drospirenone

S.No	Estetrol			Drospirenone			
Injection	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	RS
1	2.219	10860	1.44	2.219	12350	1.18	4.2
2	2.233	10986	1.43	2.233	12720	1.20	4.3
3	2.233	10906	1.44	2.233	12068	1.18	4.4
4	2.249	10914	1.43	2.249	12832	1.19	4.2
5	2.260	10974	1.43	2.260	12116	1.18	4.1
6	2.296	10059	1.44	2.689	12383	1.18	4.2

Table 2: Specificity data

Sample name	Retention time (Mins)	Area
Estetrol	2.268	1273112
Drospirenone	2.698	223837

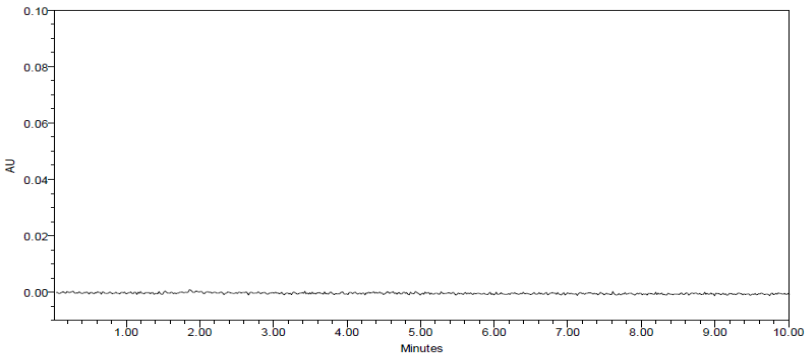

Figure 3.Blank Chromatogram

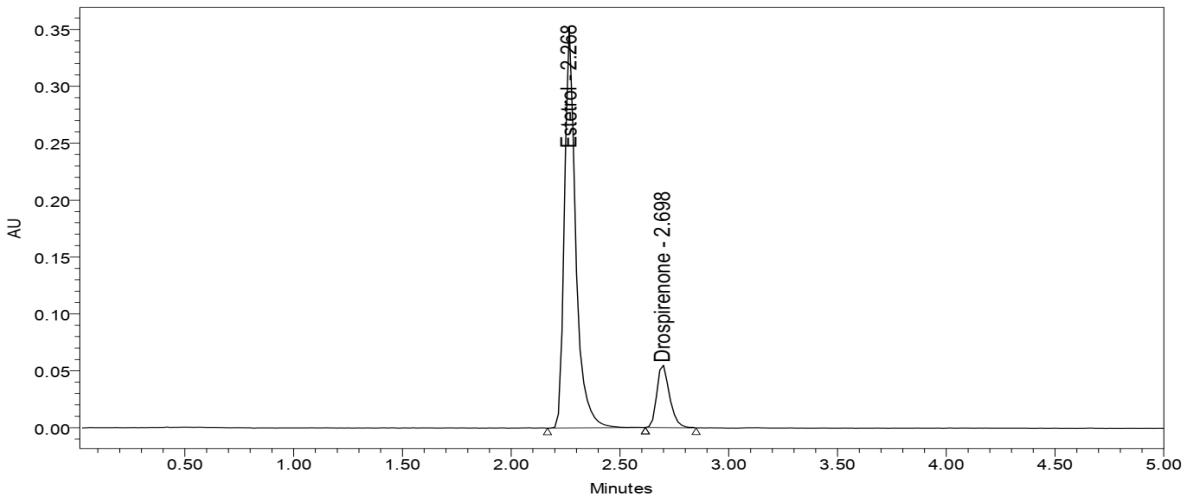

Figure 4: Specificity Chromatograms of Estetrol and Drospirenone

Linearity

Table 3. Linearity table for Estetrol and Drospirenone:

Estetrol		Drospirenone	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
6	188760	0.75	38904
12	379165	1.5	75212
18	557737	2.25	114257
24	766714	3	153135
30	921521	3.6	184669
36	1121257	4.5	227239

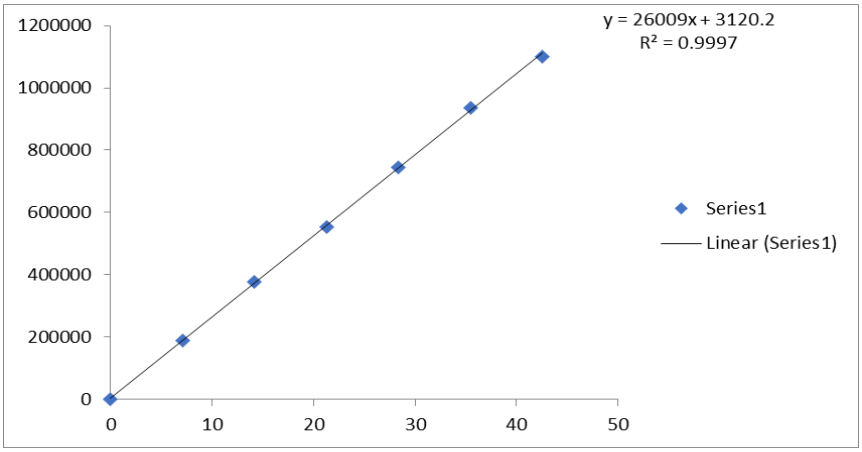

Figure 5. Calibration curve of estetrol

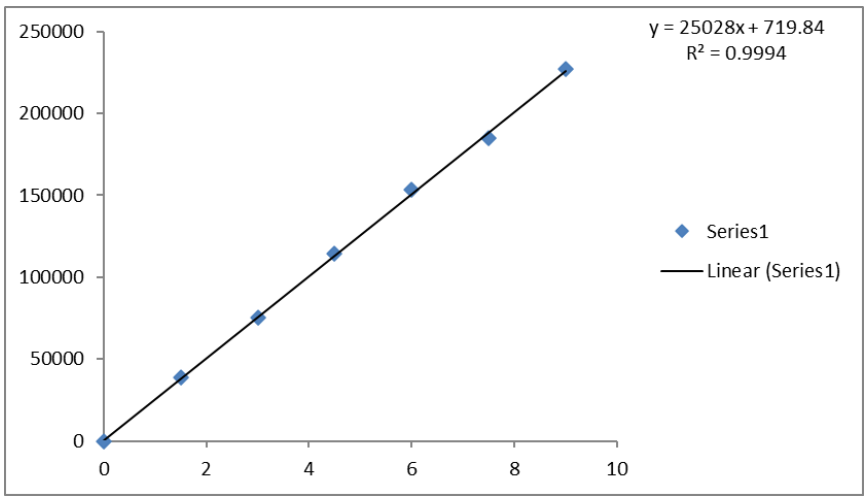

Figure 6. Calibration curve of Drospirenone

Accuracy:

Table 4. Accuracy table of Estetrol

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	14.2	14.3	100.6	100.91%
	14.2	14.2	99.7	
	14.2	14.1	99.5	
100%	28	28.5	100.4	
	28	28.1	99.1	
	28	28.1	99.1	
150%	42.6	42.6	99.9	
	42.6	43.0	100.9	
	42.6	42.0	98.6	

Table 5. Accuracy table of Drospirenone

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	3	3.00	99.88	99.76%
	3	2.99	99.62	
	3	2.99	99.69	
100%	6	6.00	99.99	
	6	6.00	99.98	
	6	6.00	99.96	
150%	9	9.00	100.00	
	9	8.97	99.62	
	9	8.92	99.10	

System Precision: With regard to the working strength of Estetrol and Drospirenone, six duplicate injections of the standard solution at 100% of the prescribed limit were analysed to determine the system accuracy. In Table 5, the results of the peak area are compiled.

Table 6: System precision

S. No	Area of Estetrol	Area of Drospirenone
1.	739383	151415
2.	747192	153853
3.	745908	150459
4.	744141	152495
5.	739919	150697
6.	746960	151715
Mean	**743917**	**151772**
S.D	**3479.7**	**1254.1**
%RSD	**0.5**	**0.8**

The % RSD for the peak areas of Estetrol and Drospirenone obtained from six replicate injections of standard solution was within the limit of (<2%).

Method precision: Analyzing a sample of Estetrol and Drospirenone allowed researchers to gauge the method's accuracy (Six individual sample preparations). Table 6 provides a summary of the data.

Table 7: Method precision

S. No	Area of Estetrol	Area of Drospirenone
1.	743685	154636
2.	747888	152894
3.	746938	153522
4.	744103	152832
5.	742952	155799
6.	749579	154863
Mean	**745858**	**154091**
S.D	**2660.1**	**1196.0**
%RSD	**0.4**	**0.8**

Results shows, the % RSD of Repeatability study was within the range for Estetrol and Drospirenone is (<2%)

Table 8: Robustness

S.No.	Condition	%RSD of Estetrol	%RSD of Drospirenone
1	Flow rate (-) 0.9ml/min	0.6	0.7
2	Flow rate (+) 1.1ml/min	0.9	0.5
3	Mobile phase (-) 60B:40A	0.2	0.5
4	Mobile phase (+) 70B:30A	0.5	1.4
5	Temperature (-) 25°C	0.5	0.8
6	Temperature (+) 35°C	0.1	0.7

Table 9: Forced degradation for Estetrol and Drospirenone

Stress condition	Solvent	Temp (^{0}C)	Exposed time
Acid	2N HCL	60^0c	30 mins
Base	2N NAOH	60^0c	30 mins
Oxidation	20% H_2O_2	60^0c	30 mins
Thermal	Diluent	105^0c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^0c	-

DEGRADATION

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation

Table 10: Degradation results of Estetrol and Drospirenone

Type of degradation	Estetrol		Drospirenone	
	% RECOVERED	% DEGRADED	% RECOVERED	% DEGRADED
Acid	93.90	6.10	93.23	6.77
Base	95.03	4.97	95.01	4.99
Peroxide	95.21	4.79	96.00	4.00
Thermal	97.34	2.66	97.77	2.23
Uv	98.62	1.38	98.48	1.52
Water	99.28	0.72	99.16	0.84

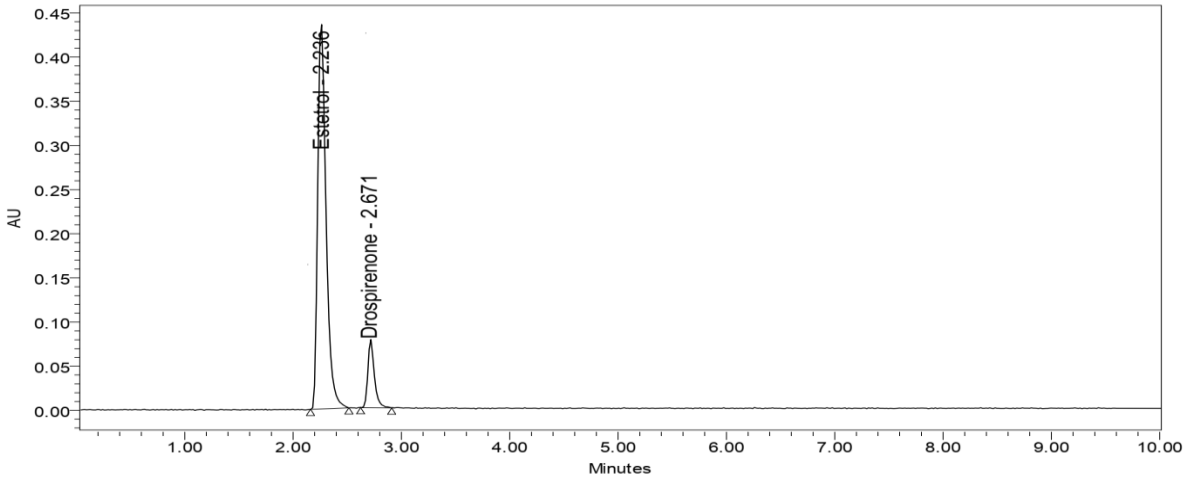

Figure 7: Acid chromatogram of Estetrol and Drospirenone

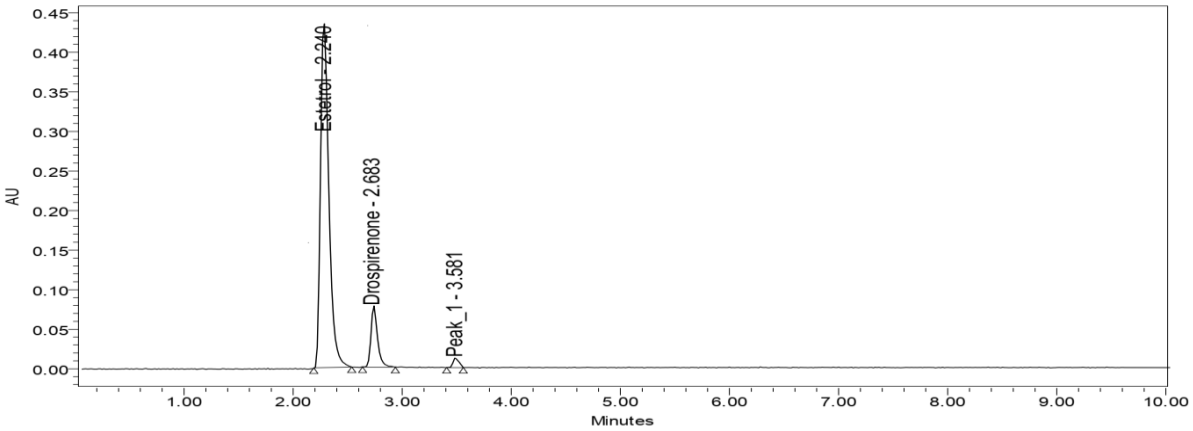

Figure 8. Base chromatogram of Estetrol and Drospirenone

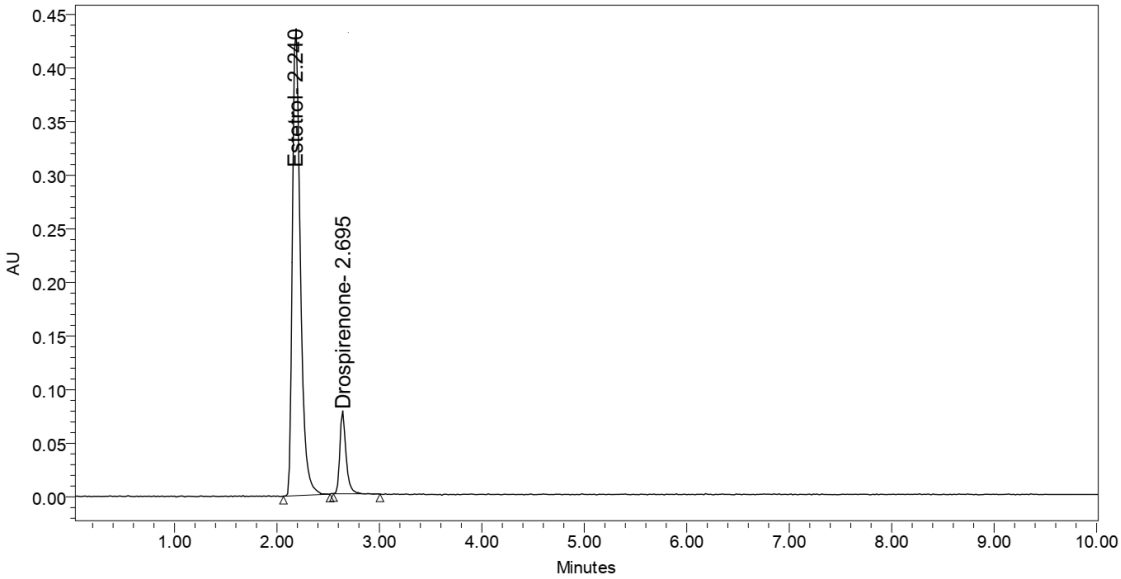

Figure 9. Peroxide chromatogram of Estetrol and Drospirenone

According to the results, samples were degraded when they were subjected to an acid, base, and oxidation interaction. Hydrolysis reaction, heat reaction, and light reaction all showed no deterioration. According to the stress research, none of the degradants co-eluted with the maxima of the active medication.

Assay: (DESOGEN) bearing label claim, Estetrol 14.2mg and Drospirenone 3mg, assay was carried out by injecting sample into HPLC System.

Table 11: Assay Data of Estetrol

S.no	Standard Area	Sample area	% Assay
1	739383	743685	99.87
2	747192	747888	99.86
3	745908	746938	99.36
4	744141	744103	100.18
5	739919	742952	99.23
6	746960	749579	99.36
Avg	**743917**	**745858**	**99.64**
St dev	**3479.7**	**2660.1**	**0.38**
%RSD	**0.5**	**0.4**	**0.4**

Table 12: **Assay Data of Drospirenone**

S.no	Standard Area	Sample area	% Assay
1	151415	154636	99.63
2	153853	152894	99.55
3	150459	153522	99.56
4	152495	152832	100.60
5	150697	155799	99.70
6	151715	154863	100.28
Avg	**151772**	**154091**	**99.89**
St dev	**1254.1**	**1196.0**	**0.44**
%RSD	**0.8**	**0.8**	**0.44**

Table 13: Assay outcome for Estetrol and Drospirenone

Drug Name	Label claim dose	%Assay	Brand Name
Estetrol	14.2mg	99.64	**DESOGEN**
Drospirenone	3mg	99.89	

CONCLUSION

The proposed HPLC method was validated as per ICH guidelines and applied for the determination of Estetrol and Drospirenone in tablet dosage form. The method was found to be accurate, precise, robust and specific. Retention time of Estetrol and Drospirenone were found to be 2.268 min and 2.698. %RSD of the Estetrol and Drospirenone were and found to be 0.5 and 0.8 respectively. %Recovery was obtained as 100.17% and 100.12% for Estetrol and Drospirenone respectively. LOD, LOQ values obtained from regression equations of Estetrol and Drospirenone were 0.39, 1.18 and 0.01, 0.03 respectively. Regression equation of Estetrol is y = 26009x + 3120.7, and y = 25028x + 719.84 of Drospirenone. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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