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Review TDD

RP-HPLC Method for Simultaneous Determination of Drospirenone and Ethinylestradiol in Bulk and Their Pharmaceutical Dosage Form

Vipul T. Prajapati[1,*], Shantilal P. Padhiyar[1], Carol P. Macwan[2], Tejal G. Soni[3], B.N. Suhagia[4], Rohan V. Chauhan[1]

Abstract

Oral contraceptive is used to prevent the pregnancy which contains hormones to block the release of eggs from ovaries. Most of them contain estrogen and progesterone. Nowadays, usage of hormonal contraceptive is sharply enhancing; around 100 million of women worldwide are taking these drugs. With this drug fertility rate can be suppressed as long as they want or plan their activity along with these drugs provide efficacy, reliability, low cost, and overall safety of oral contraceptive. Currently, oral contraceptive is available in parenteral formulation. Contraceptive has two types: 1) Female contraceptive, and 2) Male contraceptive. Drospirenone and Ethylestradiol are decreasing follicle stimulating hormone (FSH) and luteinizing (LH) for preventing pregnancy by inhibiting ovulation. There isn't a published green method for RP-HPLC analysis of DRSP (drospirenone) and ETH (ethinylestradiol) that uses green analytical chemistry (GAC). The application of GAC in the creation and verification of a green RP-HPLC method is the main topic of the current work. Green solvents, specifically acetonitrile and acetate buffer (60:40, v/v, pH 6.0), were utilised as the mobile phase in RP-PLC. At 277 nm, the detection was done. The linearity, range, precision, accuracy, limit of detection, limit of quantification, sensitivity, and specificity of the method were all validated. It was shown to be linear in the range of 10–50 µg/mL for ETH and 300–1500 µg/mL for DRSP. In comparison to existing documented approaches, our approach showed exceptional environmental friendliness. To lessen the possible risks that organic solvents may have to the environment, the pharmaceutical industry should investigate these newly developed environmentally friendly green solutions.

Keywords: Oral contraceptive, FSH, LH, RP-HPLC, extreme greenness, method validation

***Author for Correspondence**
Vipul T. Prajapati
E-mail: vipulprajapati.ph@ddu.ac.in

[1]Assistant Professor, Department of Pharmaceutical Chemistry, Dharmsinh Desai University, Nadiad, Gujarat, India
[2]Assistant Professor, Department of Pharmacognosy, Dharmsinh Desai University, Nadiad, Gujarat, India
[3]Dean, Dharmsinh Desai University, Nadiad, Gujarat, India
[4]Professor, Faculty of Pharmacy, Dharmsinh Desai University, Nadiad, Gujarat, India

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INTRODUCTION

Oral Contraceptive

Oral contraceptive is used to prevent the pregnancy which contain hormones to block the release of eggs from ovaries. Progesterone and oestrogen are present in most of them. It is additionally recognised Progesterone and oestrogen are present in most of them. It goes under the name "birth control pill" as well like the pill for birth control [1–5].

Female Contraceptive

Female contraceptive is the pill which prevents the formation of estrogen and progesterone in ovaries.

There are three types of method which is below:

1. Oral.
2. Injectable.
3. Implants.

Oral

1. Combined pill.
2. Phased regimens.
3. Minipill.
4. Postcoital contraceptive.

Combined Pill

- The combined pill contains both progestin and oestrogen, which lowers the dosage of both hormones in the second-generation oral contraceptive pill without sacrificing effectiveness or increasing side effects or difficulties.
- Third-generation pills produce novel progestins, such as desogestrel, and have an enhanced profile of action [6–9].
- Ethinylestradiol acts on the body at 30 μg per day, but if progestin is taken in addition to an anti-ovulatory medication, the amount can be lowered to 20 μg per day.
- These drugs are inhibiting ovulation; included progestin ensures preventing bleeding at end of menstruation cycle and blocks the risk of developing endometrium carcinoma because of the estrogen.
- Take one pill every day for 21 days, commencing on the final day of your menstrual cycle. When bleeding starts after a 7-day break, the following dose will begin. The most effective approach is this one [10–15].

Phased Regimens

- These types of drugs are permit to reduction in total steroid dose without affect in efficacy. These are either biphasic or triphasic drug.
- The dose of estrogen is tried to keep constant, whilst dose of progestin is low in first phase and successively higher in the second and third phase.
- Only women above the age of 35 are advised to take these pills, unless there are other risk factors present.

Minipill

- It is devised to remove the estrogen from the body, because they have many long-term risk tend with this component.
- Progestin pill with the low dose must take continues without any disturbance of day.
- Twenty to thirty percent of women experience irregular cycles and ovulation, although other mechanisms function as a kind of contraception.

Postcoital Contraception

There are 3 regimens that are procurable: Take levonorgestrel 0.5 mg + ethinylestradiol 0.1 mg as soon as possible, but no more than 72 hours after the first dose, and again after 12 hours. The Yuzpe technique is a program that has gained popularity recently. Three out of every four pregnancies are avoided, but around half of the women experience nausea: only 20% of them vomit [16].

Injectable Contraceptive

- Injectable contraceptive is under investigational phase because tenacity of infertility for some months even though completing of treatment.
- This is a very convenient and reliable method as well as effective in preventing pharmacy also not needed like tablets.
- In the market Medroxyprogesterone acetate and Norethistone enanthate are available and irregularity in menstrual cycle is very common [17–19].

Mechanism of Action

- Different oral contraceptive methods have different effects on fertility; therefore, the significance of each method varies.
- Prevent the pituitary gland, situated at the base of the brain, from generating gonadotropin-releasing hormones. While the estrogen primarily reduced the production of follicular stimulating hormone, the progestin reduces the frequency of luteinizing (LH) hormone secretory pulses.
- When the combined drug intake both follicle stimulating hormone and LH hormone are reduced, and the menstrual cycle flow is eliminated. As a result of this drug, follicles do not develop in womb, due to this ovulation does not occur.

Adverse effect

- Oral contraceptive is used in certain cases otherwise healthy and young women might increase long term consequences assume significantly.
- The negative effects are dosage-dependent, meaning that the low dose preparation used now, which carries a minimal risk, cannot extend the evidence from earlier studies with high dose preparations [20].
- There are some side effects that occurred by combining oral pill which has been exaggerated used.

Non-Serious Side Effects

- These side effects generally show in between first 1 and 3 cycles and then disappear step by step.
- Nausea and vomiting.
- Headaches are generally mild.
- Breakthrough bleeding or spotting.
- Breast discomfort.

Serious Complications

- Leg vein and pulmonary thrombosis.
- Coronary and cerebral thrombosis.
- Rise in BP.
- Estrogen tends to raise plasma HDL/LDL ratio.
- Genital carcinoma.

Other Health Benefits

There are several other beneficial effects of oral contraceptive not only prevention of pregnancy but also which are below:

- Reduced likelihood of developing ovarian and endometrial cancer [21–23].
- Sometime, this contraceptive used for the reduced menstrual blood loss along with anemia, if cycle becomes an irregular and dysmenorrhea.
- Breast disease and ovarian cyst are reduced by using oral contraceptive pills.

MALE CONTRACEPTIVE

- The sole method of reducing male fertility through medication is to stop spermatogenesis.
- Considerable effort has made in one direction and effective drug found no acceptable measures.
- It is rather difficult to completely decrease spermatogenesis without damaging other tissues.

- Even if the medicine entirely inhibits spermatogenesis, it will still take a long time to cause infertility. Spermatogenesis takes 64 days.
- The inhibition of testosterone release resulting from gonadotropin suppression might lead to impotence and a decrease in libido [24–26].

MATERIALS

HPLC grade chemicals were all that were used. Acetonitrile is provided by High purity laboratory chemicals PVT. LTD and acetate buffer was prepared by self in laboratory, moreover both drugs Drospirenone and Ethinylestradiol were provided by west coast, Ahmedabad. All solutions were prepared in milli Q water which is available from the water purification system and used to prepare all the mixture in this water.

CHROMATOGRAPHIC CONDITIONS

- *Mode of Elution:* Isocratic.
- *Mobile phase:* Acetonitrile: Acetate buffer (pH 6.0) (60:40 v/v).
- *Stationary phase:* Phenomenex Luna C18 column (250 × 4.6 mm, 5 µ).
- *Flow rate:* 1 mL/min.
- *Injection volume:* 20 µL.
- *Detection wavelength:* 277 nm.
- *Run time:* 10 min.

EXPERIMENT

Preparation of Stock Solution

- *Preparation of standard solution of DRO:* DRO is weighed 10 mg accurately by using weighing machine and transfer into 10 ml volumetric flask, dissolved drug with mobile phase up to the mark of volumetric flask. This solution was prepared 1000 ppm and then did further dilution which prepared 100 ppm.
- *reparation of standard solution of ETH:* ETH is weighed 10 mg accurately by using weighing machine and transfer into 10 ml volumetric flask, dissolved drug with mobile phase up to the mark of volumetric flask. This solution was prepared 1000 ppm and then did further dilution which was prepared 100 ppm.
- *Preparation of standard solution:* As per the dose, weighed 300 mg DRO and 3 mg ETH in volumetric flask and diluted with mobile phase fill up to the mark of volumetric flask. This solution prepared 30000 ppm for DRO and 300 ppm for ETH after further dilution that makes 3000 ppm for DRO and 30 for ETH solution which was used in HPLC.
- *Preparation of test solution:* Twenty tablets were taken from the strips and weight individually, moreover, the mean weight of tablet was determined. All tablet was triturate with the mortar pestle and powder correspond to 10 coated tablet was weighed then used for preparation of stock solution. The powder was transferred into 25 ml volumetric flask and diluted in mobile phase. Then that mixture was sonicated for 20 min. Afterward solution was filtered through the 0.45µg with injection filter and then solution was diluted in Acetonitrile: Acetate buffer (60:40 %v/v) to obtain concentrations of Drospirenone (1200 µg/mL) and Ethinylestradiol (12 µg/mL).
- *Preparation of Acetate buffer (pH 6):* 1 gm ammonium acetate weighed and dissolved in 300 ml milli Q water then add 4.1 ml glacial acetic acid and adjusted pH with 10 M ammonia after that make up with 500 ml milli Q water.

METHOD DEVELOPMENT

Selection of Analytical Wavelength

- HPLC method depends on the UV because selection of the proper wavelength is necessary for maintaining the sensitivity of HPLC that is essential for great response of drug.

- In that study, prepare the standard solutions of DRO and ETH were performed on UV between the wavelength of 200–400 nm.
- These both drugs show the significance absorbance at 277 nm, therefore, simultaneous estimation of DRO and ETH in bulk and their pharmaceutical dosage form was performed at that wavelength by using HPLC.

Selection of Stationary Phase and Mobile Phase

- Resolution is essential criteria to achieve better method development. As for literature articles and some resources, RP-HPLC is suitable for analysis of DRO and ETH. There are many columns used in RP-HPLC among them Phenomenex Luna C18 column (250 × 4.6 mm, 5 µ) had given the better resolution so, that column was preferred to conduct further study.
- Moreover, there were many mobile phase compositions trying to identify suitable methods but according to pKa value and solubility of drug, that one chromatographic condition was optimized which is separated DRO and ETH along with the better resolution.
- That mobile phase was composed of the Acetonitrile and acetate buffer (pH 6) and the ratio is 60:40 v/v.
- At the flow rate 1 ml/min obtained satisfactory consequences for this method.

METHOD VALIDATION

The proposed method was validated as per the guideline of International Council of Harmonization (ICH) Q2 (R1).

Linearity

It is the vital parameter of the validation to find the range of concentration of the analyte sample. Concentration range 10–50 µg/ml and 300–1500 µg/ml for Ethinylestradiol and Drospirenone respectively took as a standard and 12 µg/ml for Ethinylestradiol and 1200 µg/ml for Drospirenone were taken as test sample (Figures 1-30). These ranges are performed on five concentrations samples. In addition, all samples were filtered by using 0.45 µm filter and injected in optimized chromatographic condition. Then after, linearity curve was evaluated by measuring the concentration versus peak area. By using linearity, least square regression equation was obtained (Figures 24–27) (Tables 3 and 4).

LOD and LOQ

Limit of Detection (LOD) is the analytical method to detect the lowest value of analyte, on the other hand Limit of Quantification (LOQ) is also an analytical method to determine the lowest possible concentration of analyte that can be quantified by this method (Table 6).

Precision

These studies were performed six times in a day and between a day. There are two types of precision Intraday precision and Interday precision.

- *Intraday precision:* It is the method to conduct in a short period. This method is performed on Drospirenone and Ethinylestradiol with prepared solution of 1200 µg/mL and 12 µg/mL respectively in a practical day.
- *Interday precision:* This validation parameter is performed on different days within laboratory variation, meanwhile diverse analysts, or equipment from same laboratory. Interday precision analyse with the higher, middle, and lower concentration of the analyte curve in three different days (Table 5).

Robustness

Robustness method is used for detecting small variation in method parameters. By using this condition, the method was developed by minor changes under these three chromatographic parameters: flow rate (±1 ml/min), Temperature (23 ± 1) and wavelength (277nm ± 1). The resolution between peaks was examined in this condition (Tables 7–9).

Accuracy

This analytical validation parameter was determined from the recovery studies. Recovery studies performed by the standard addition with the 50%, 100%, and 150% concentration of analyte (Tables 10–12).

Specificity

It is the process to identify the analyte accurately and specifically in the presence of other substances, which might be present in marketed formulation. The specificity method was performed by comparing the blank sample along with the tablet formulation and standard solution. Excipient might be detected in test sample (Figures 28 and 29).

Selectivity

One analytical technique that can be expected to be present in formulation is selectivity, which measures the analyte in the presence of a blank sample (Figures 30 and 31).

STABILITY STUDY

Acid Hydrolysis

- HCL is used to create acidic stress samples. By refluxing the drug in 0.1 N HCL, one can examine the hydrolytic breakdown of a novel medication in an acidic environment. Testing might end at this stage if a reasonable level of degradation is seen.
- Take 4.62 gm of HCL in volumetric flask and add distilled water and shake well after that make up the volume up to 250 ml mark (Figures 1–4 and Table 13).

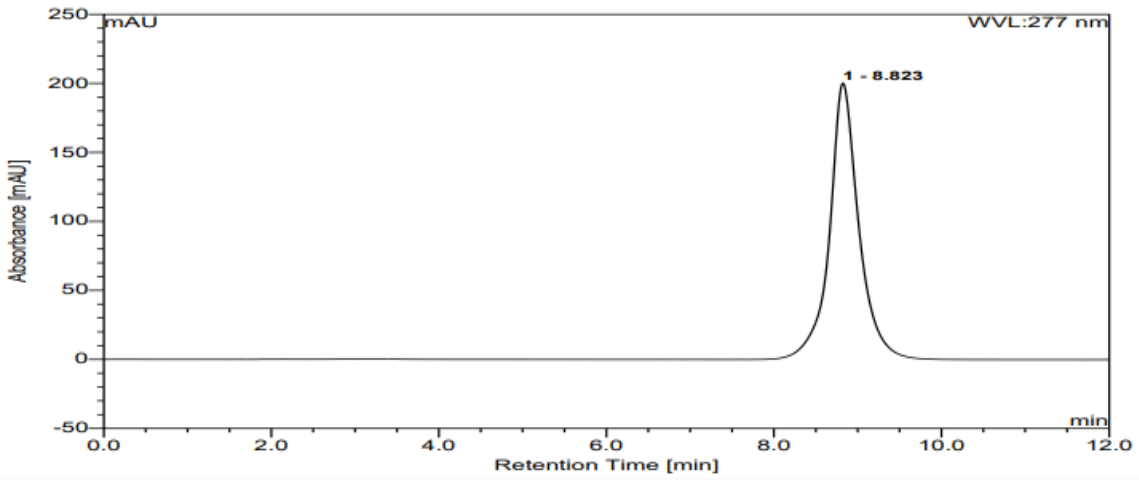

Figure 1. Chromatogram of Drospirenone (100 µg/ml).

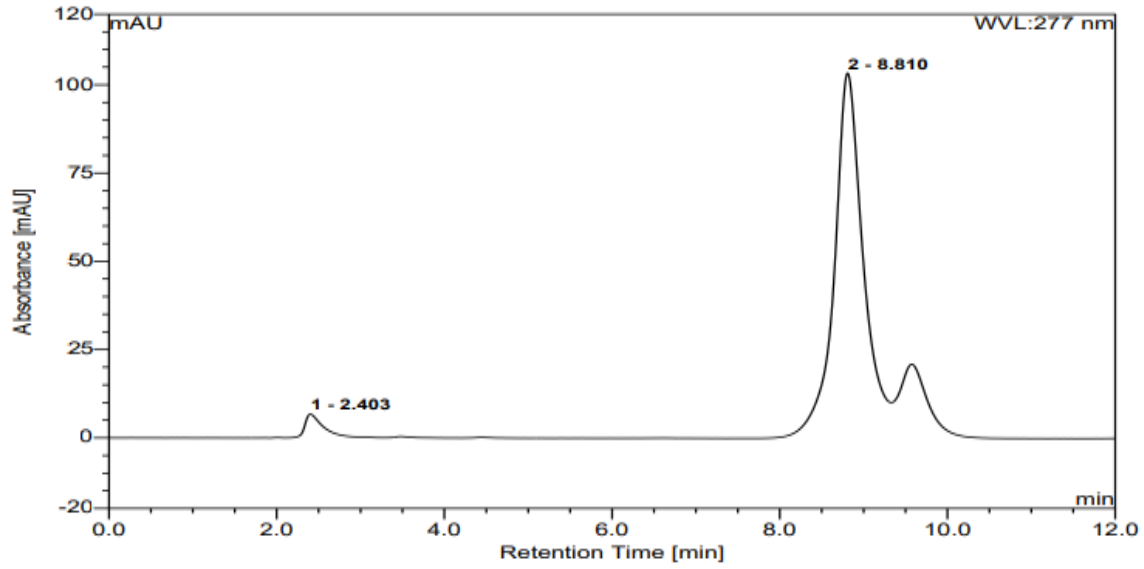

Figure 2. Chromatogram of Drospirenone in acid degradation (100 µg/ml).

Base Hydrolysis

- Hydrolysis is a common degradation process by the reaction of chemicals with water at different pH values. In forced degradation, the drug reacted with water at basic conditions. The concentration of base is selected according to the stability of drug substance.
- Take 2 g of NaOH in volumetric flask and add distilled water and shake well after that make up thew volume up to 250 ml mark (Figures 5–8).

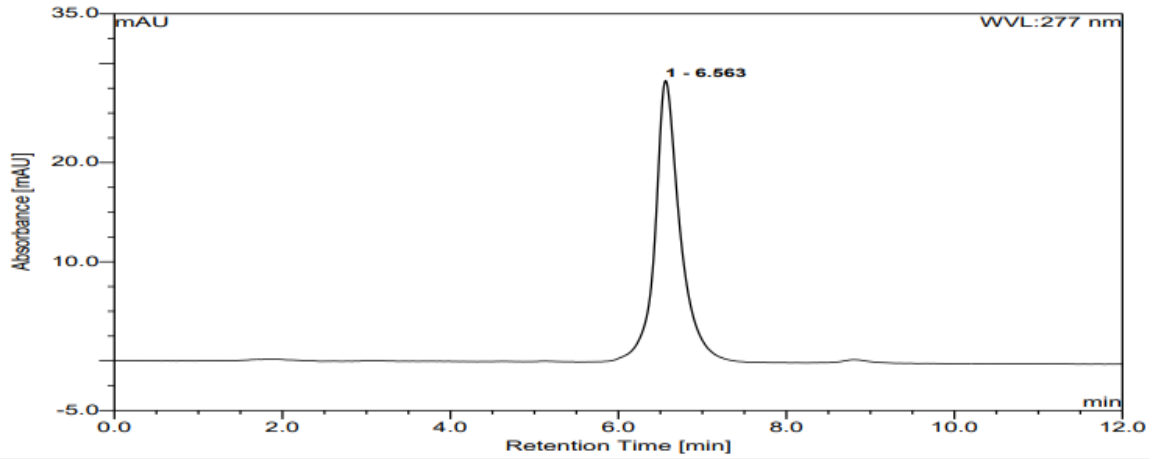

Figure 3. Chromatogram of Ethinylestradiol (100 µg/ml).

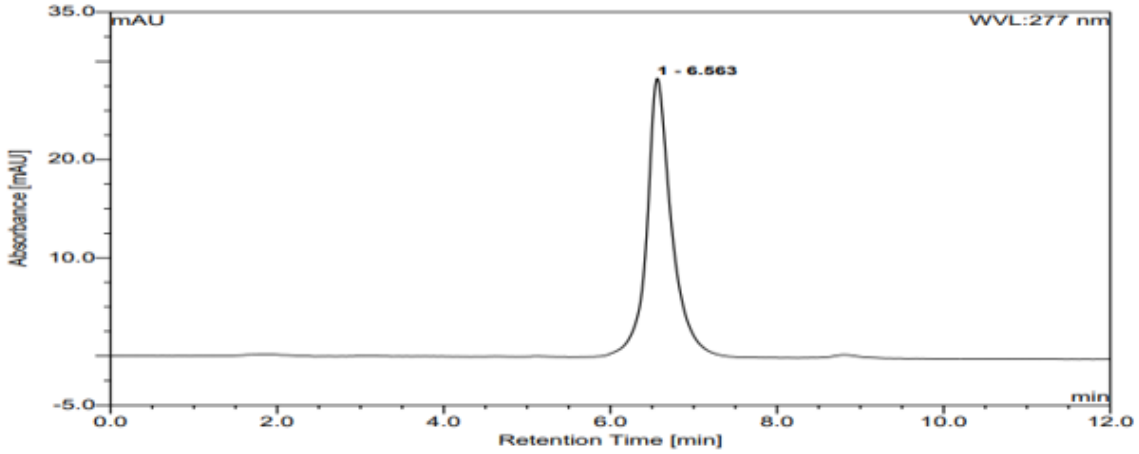

Figure 4. Chromatogram of Ethinylestradiol in acid degradation.

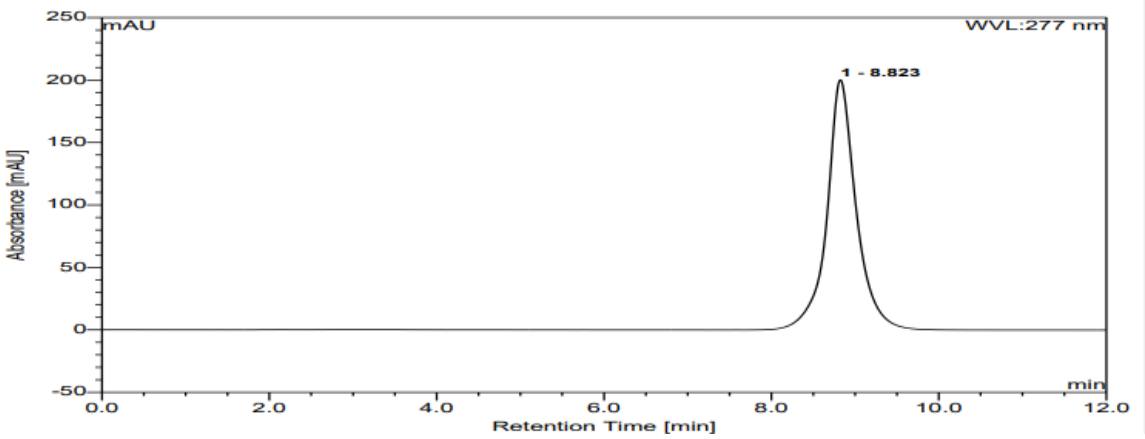

Figure 5. Chromatogram of Drospirenone (100 µg/ml).

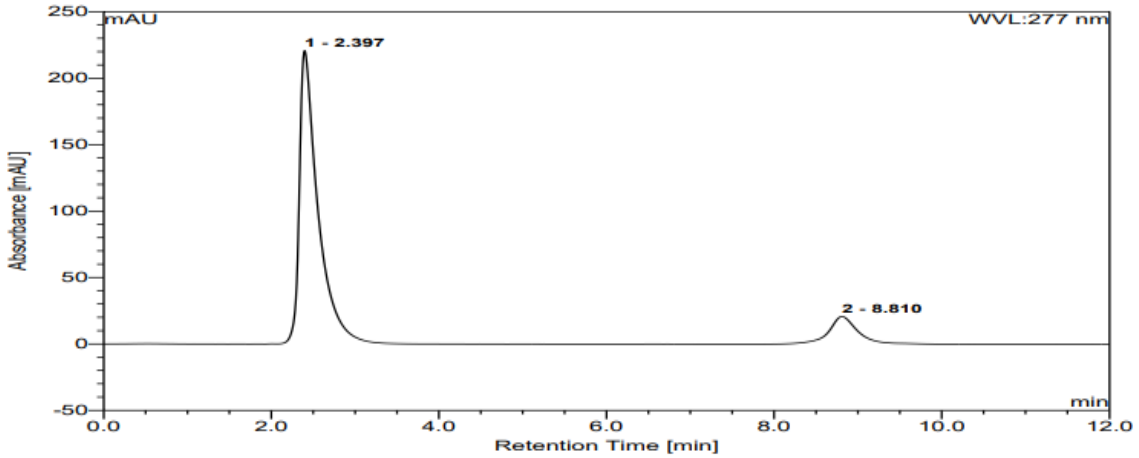

Figure 6. Chromatogram of Drospirenone in base degradation (100 µg/ml).

Photolytic Degradation

- The photo stability testing of drug substances must be evaluated to demonstrate that light exposure does not result in unacceptable change. Photo stability studies are performed to generate primary degradants of drug substance by exposure to UV or fluorescent conditions.
- Take 1 gm Drospirenone and Ethinylestradiol each drug in the butter paper and keep into the UV chamber for 1 hour. Then dissolved these drugs with the mobile phase of system and make a 100 µg/ml solution then observed peak in HPLC (Figures 9–12).

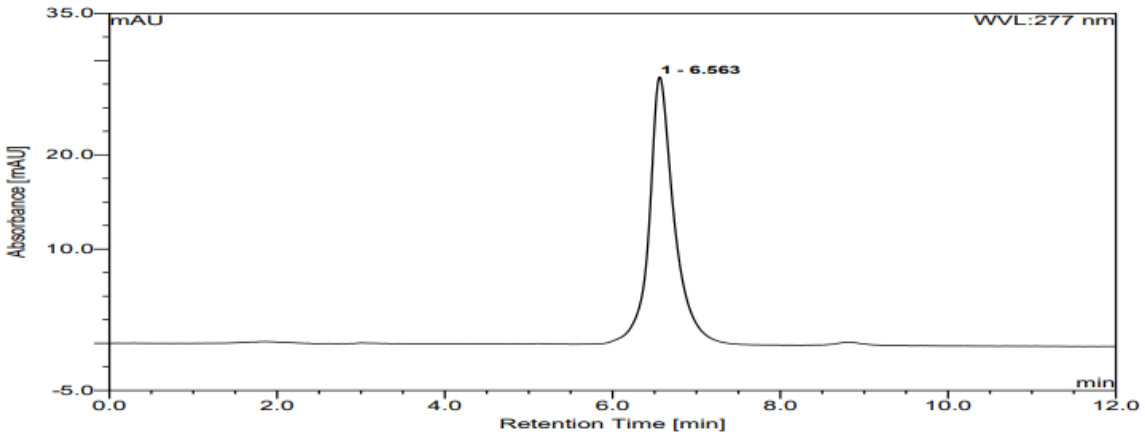

Figure 7. Chromatogram of Ethinylestradiol (100 µg/ml).

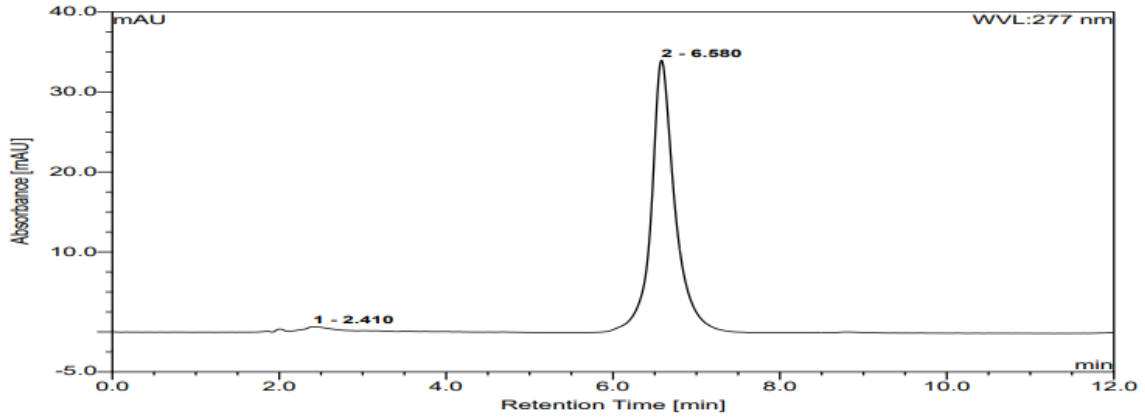

Figure 8. Chromatogram of Ethinylestradiol in base degradation (100 µg/ml).

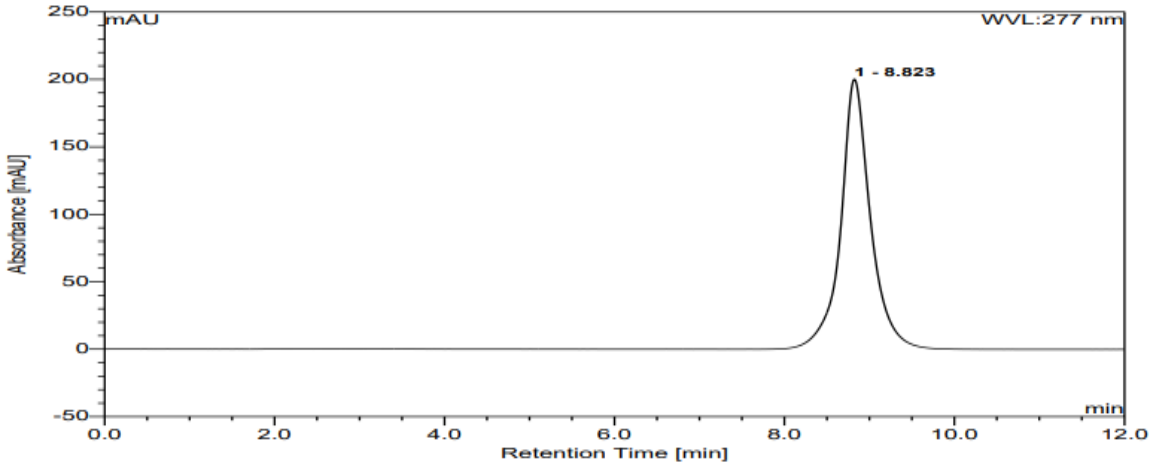

Figure 9. Chromatogram of Drospirenone (100 µg/ml).

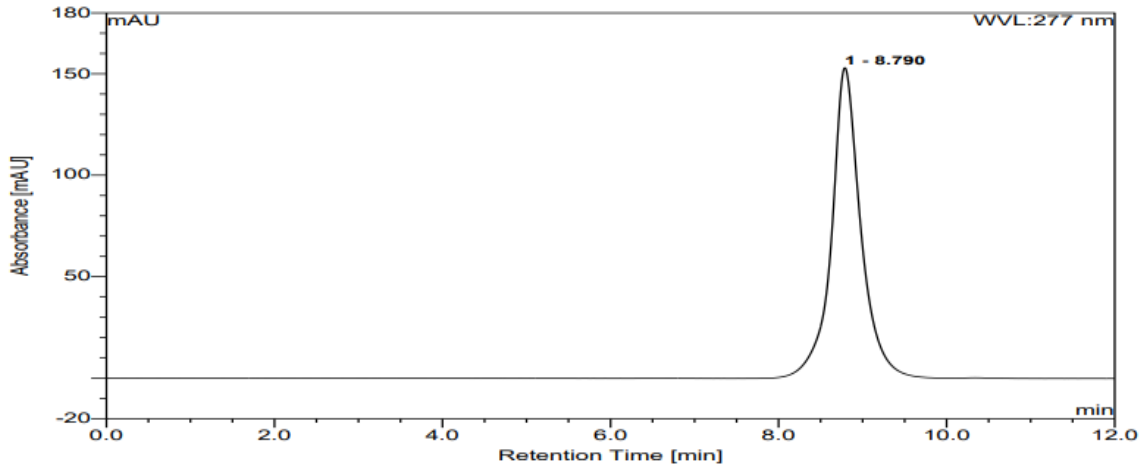

Figure 10. Chromatogram of Drospirenone in photolytic degradation (100 µg/ml).

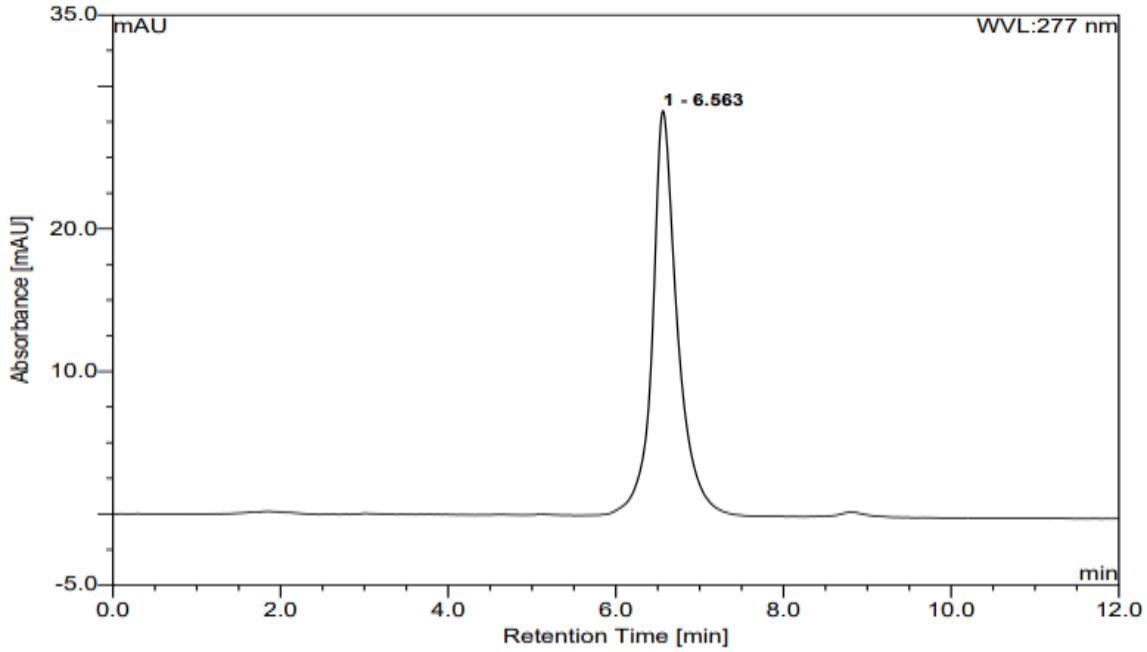

Figure 11. Chromatogram of Ethinylestradiol (100 µg/ml).

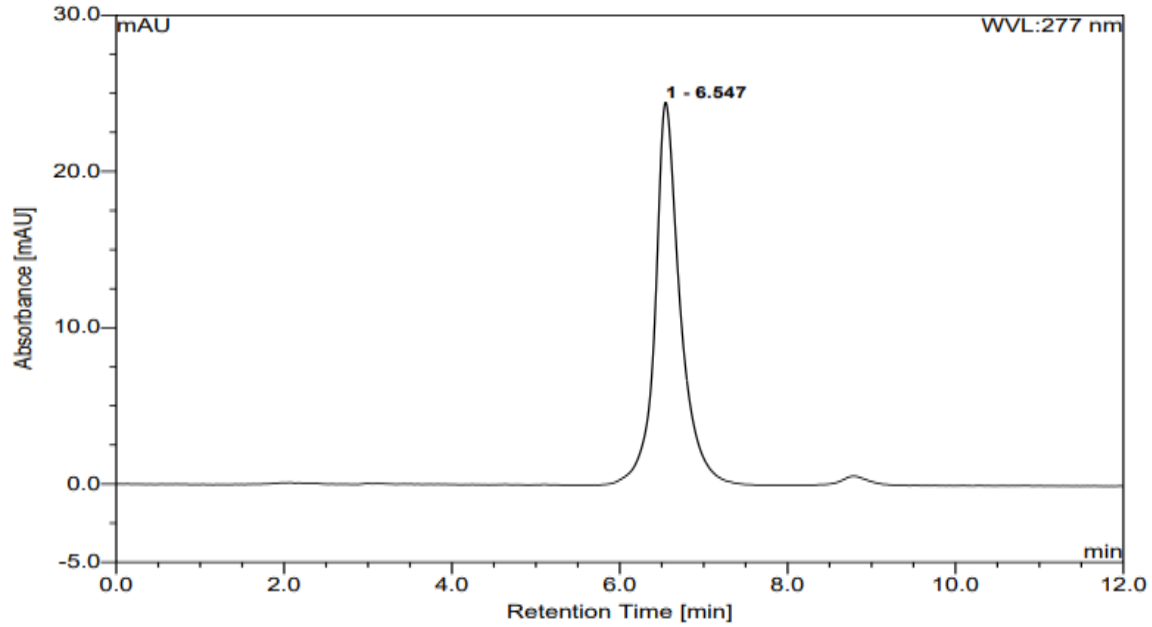

Figure 12. Chromatogram of Ethinylestradiol in photolytic degradation (100 µg/ml).

RESULT AND DISCUSSION

Method Development and Optimization

The method for Drospirenone and Ethinylestradiol analysis was optimized to ensure precise quantification, achieving retention times of 8.387 and 6.303 minutes, with % assay values of 98.39% and 100.25%, respectively (Tables 1 and 2).

Table 1. System suitability test.

Parameter	Drospirenon	Ethinylestradiol
Retention time	8.387	6.303
Peak area	1.05	1.17
Theoretical plate	3925	3084
Peak area	814.402	9.8168
%Assay	98.39	100.25
Resolution	3.32	

Selection of Mobile Phase

The selection of an appropriate mobile phase is crucial for optimizing the separation of analytes in liquid chromatography. In this study, we investigated the retention times of two key compounds: Drospirenone and Ethinylestradiol, using a mobile phase composed of Acetonitrile (ACN), water, Acetate buffer, and phosphate buffer (Figures 13–23).

Table 2. Optimized chromatographic condition.

Parameter	Chromatographic Condition
Column	Phenomenex Luna C18 column (250 × 4.6 mm, 5 µm).
Mobile phased	Acetonitrile: Acetate buffer (pH 6) (60:40 v/v).
Injection volume	20 µl.
Flow rate	1 ml/min.
Detector wavelength	277 nm.
Detector	PDA detector Photodiode array detector).
Run time	10 min.

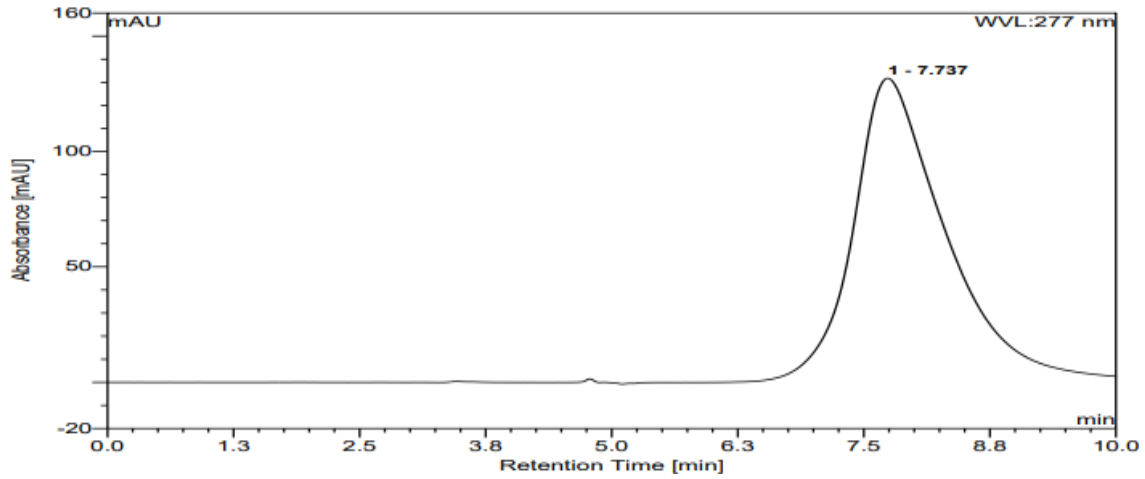

Figure 13. Drospirenone (100 µg/ml): 7.37 min ACN: Water (70:30).

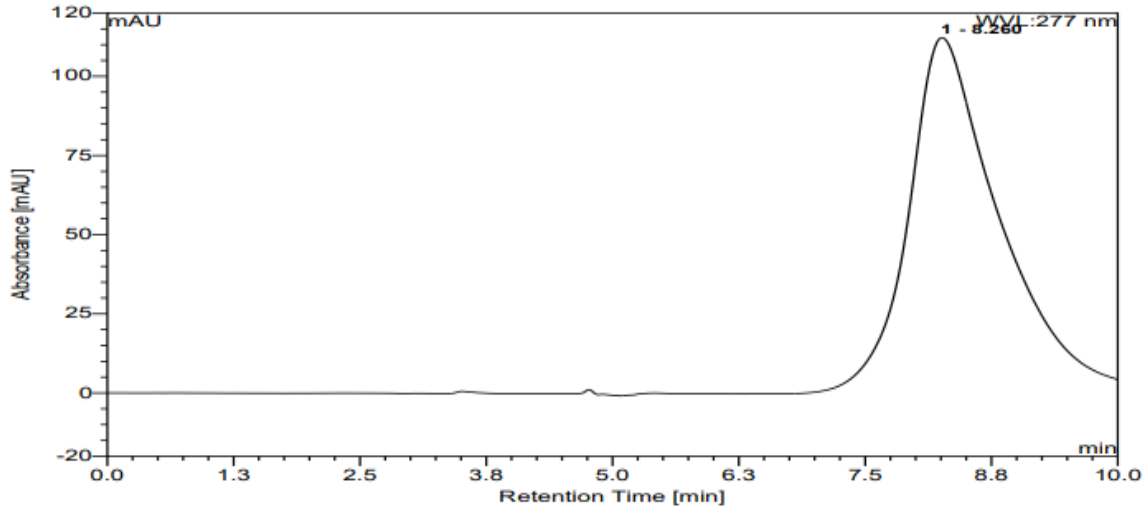

Figure 14. Ethinylestradiol (100 µg/ml): 8.260 min. ACN: water (70:30).

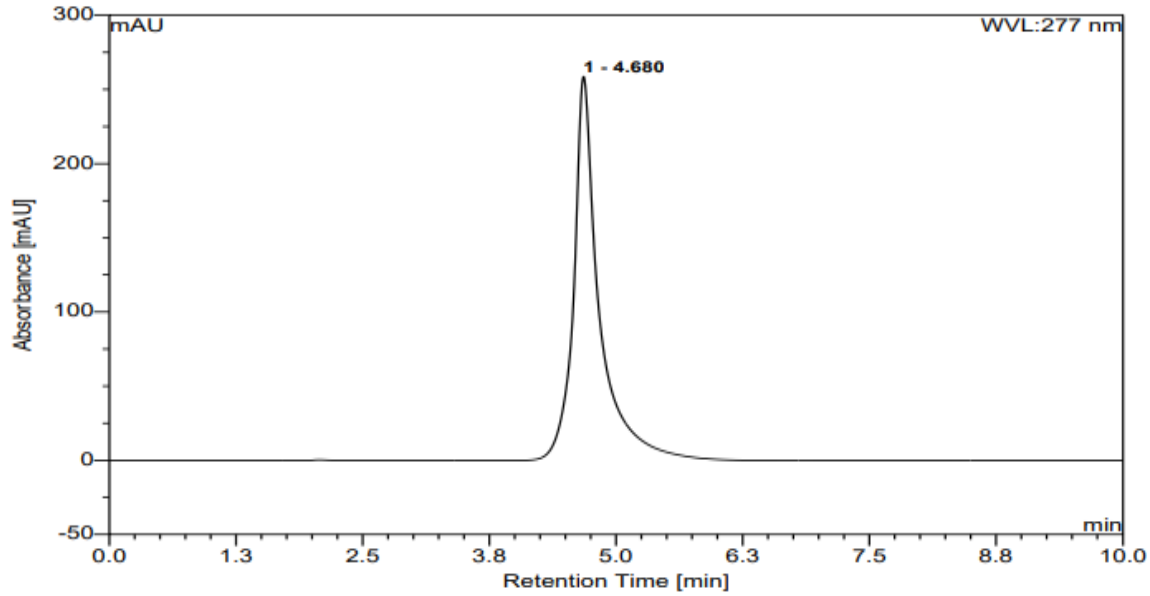

Figure 15. Drospirenone (100 µg/ml): 4.680 min. ACN: acetate buffer 3.7 (70:30).

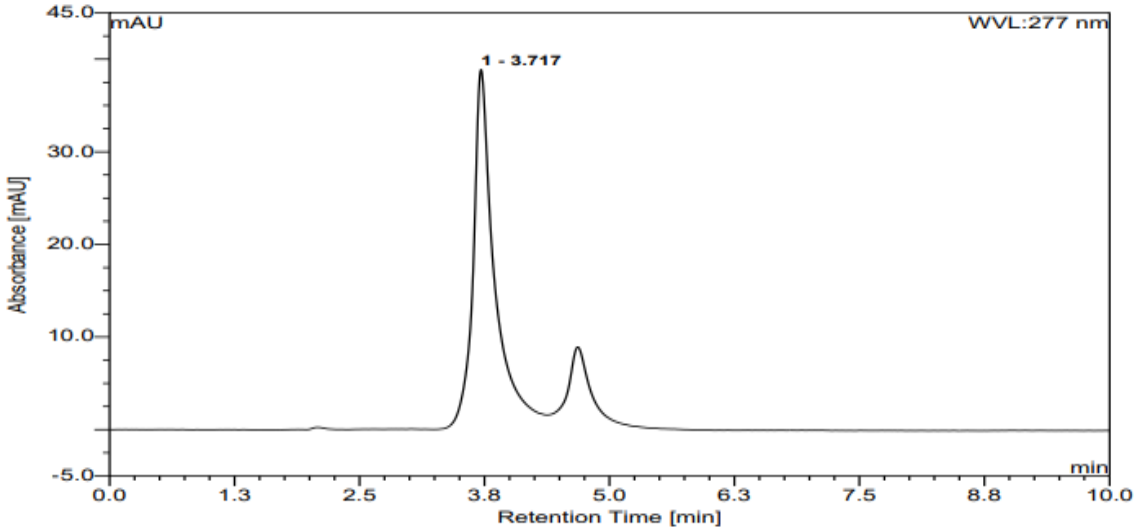

Figure 16. Ethinylestradiol (100 µg/ml): 3.717 min. ACN: Acetate buffer 3.7 (70:30).

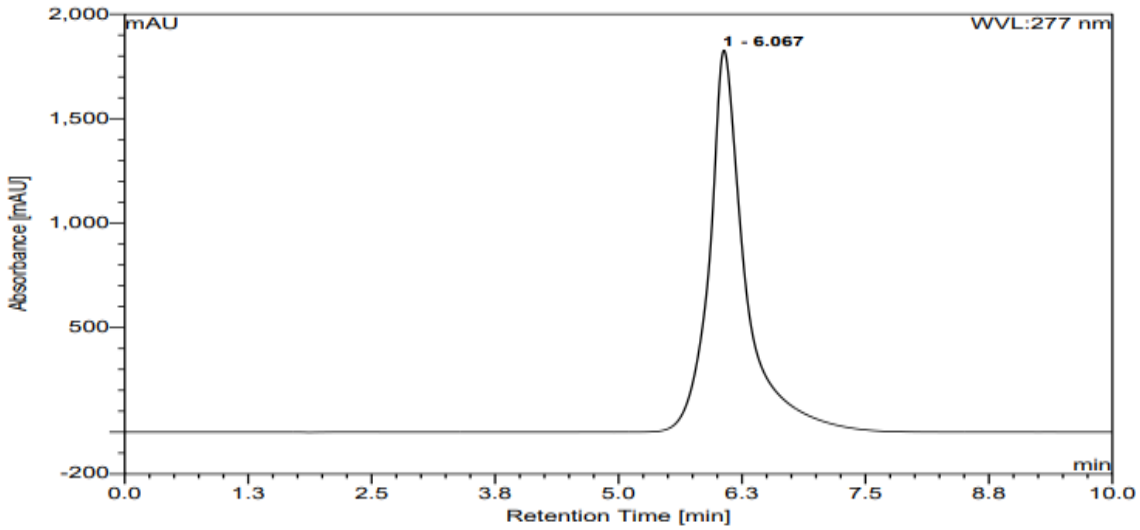

Figure 17. Drospirenone: 6.067 min. ACN: phosphate buffer 3.7 (70:30).

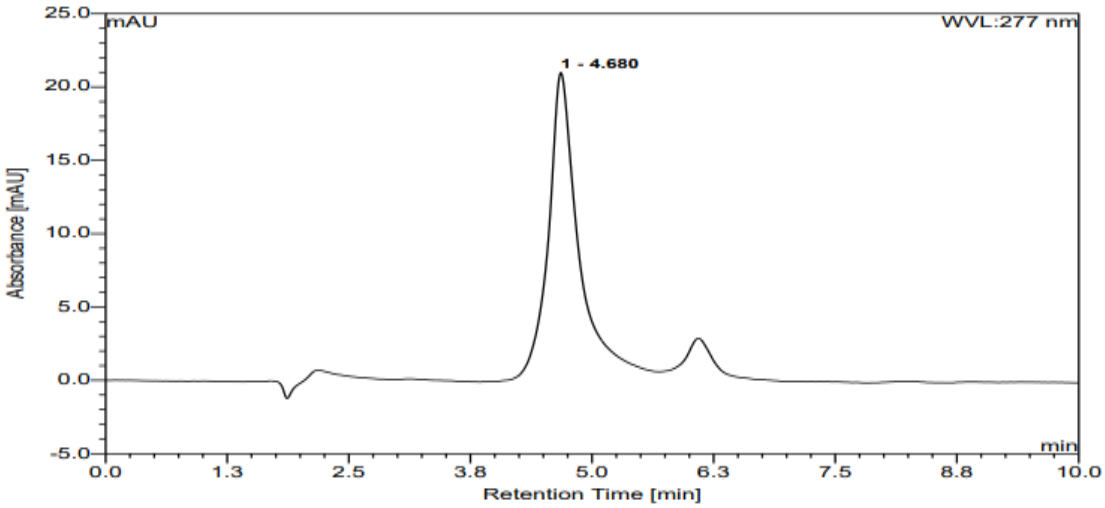

Figure 18. Ethinylestradiol (100 µg/ml): 4.680 min. ACN: phosphate buffer 3.7.

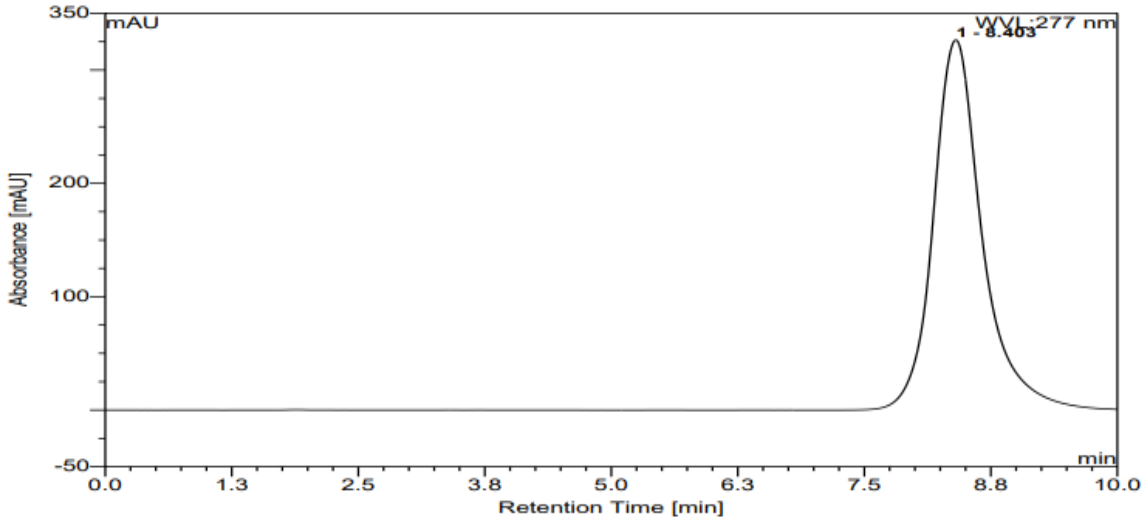

Figure 19. Drospirenone (100 µg/ml): 8.403 min. ACN: acetate buffer 6 (60:40).

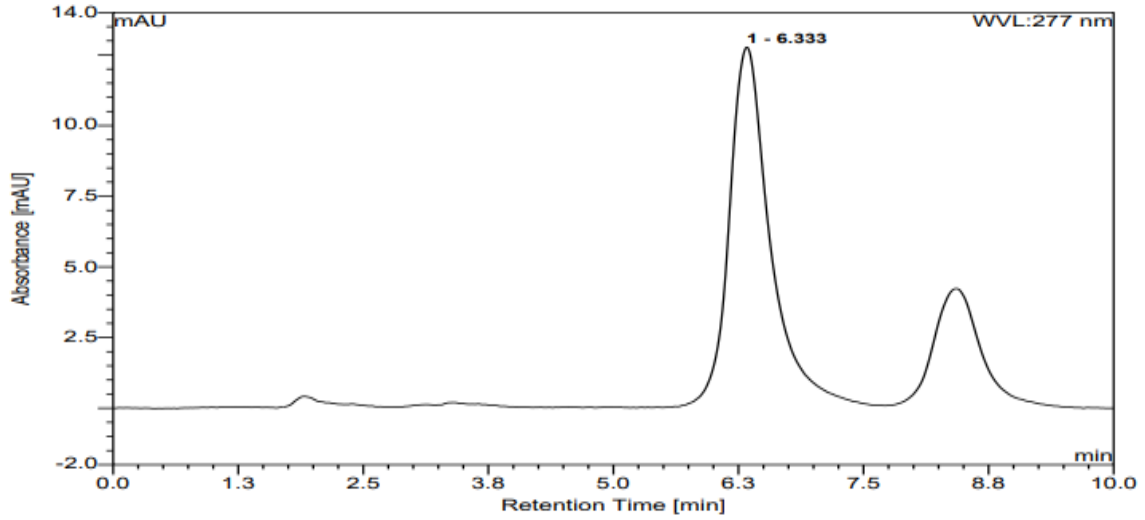

Figure 20. Ethinylestradiol (100 µg/ml): 6.333 min. ACN: Acetate buffer 6.

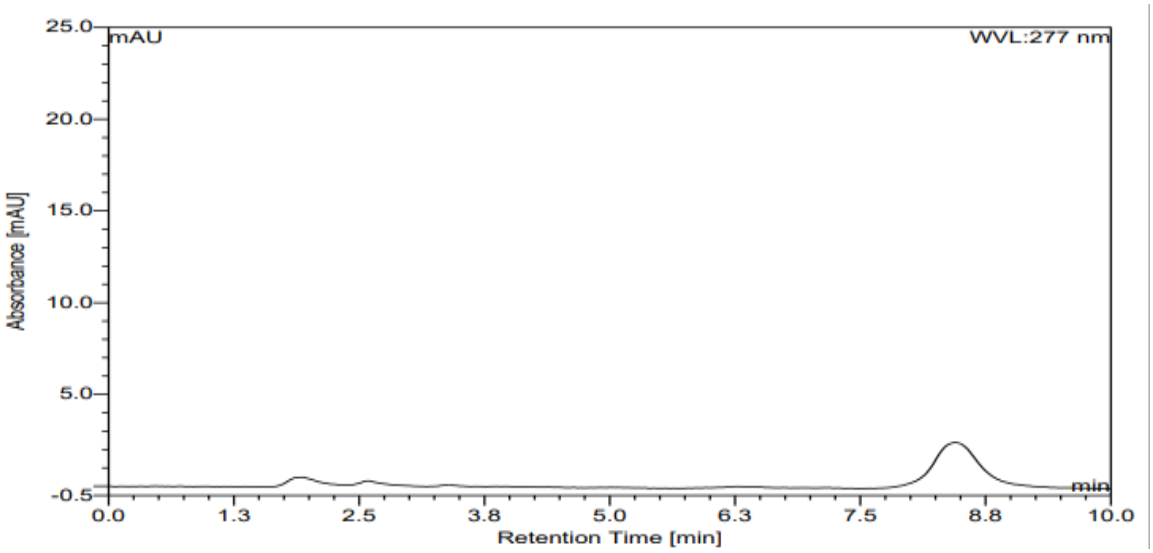

Figure 21. Blank of ACN: acetate buffer pH 6 (60:40).

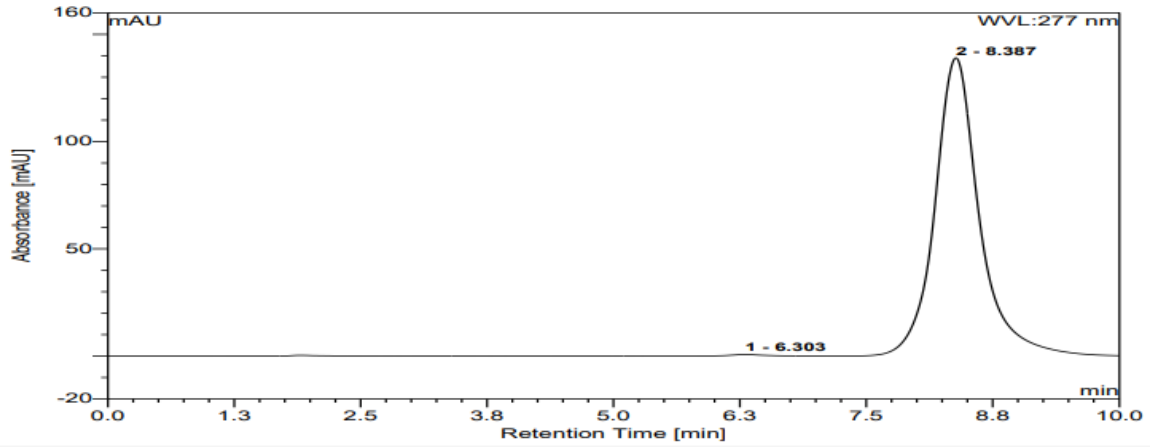

Figure 22. Drospirenone (300 µg/ml): 8.387 min and Ethinylestradiol (3 µg/ml): 6.303 min.

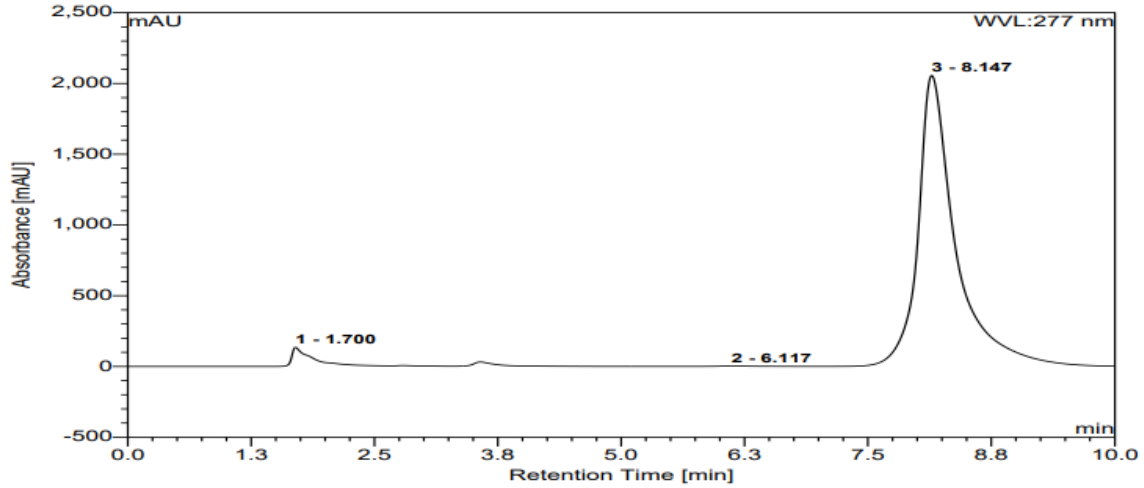

Figure 23. Drospirenone (1200 µg/ml): 8.147 min and Ethinylestradiol (12 µg/ml): 6.117 min tablet assay.

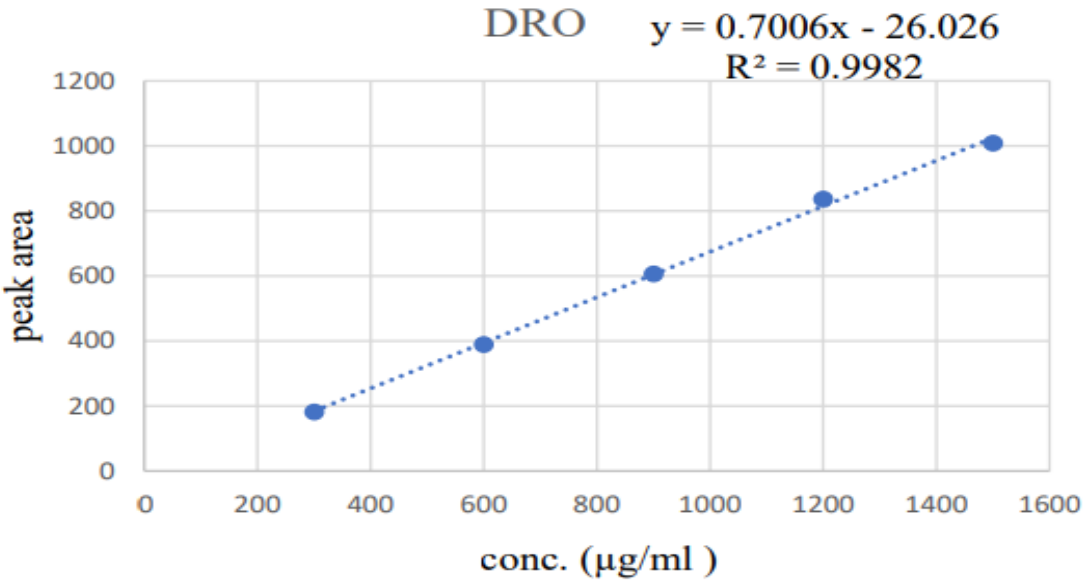

Figure 24. linearity graph of Drospirenone (300–1500 µg/ml).

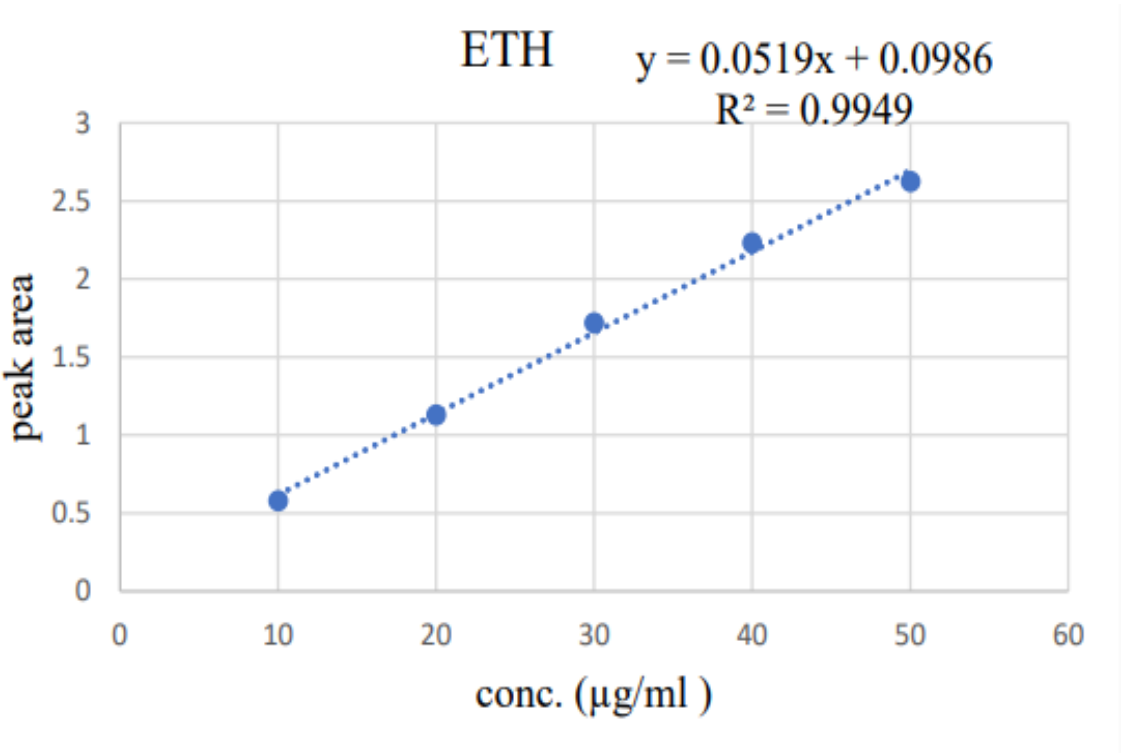

Figure 25. linearity graph of Ethinylestradiol (10–50 µg/ml).

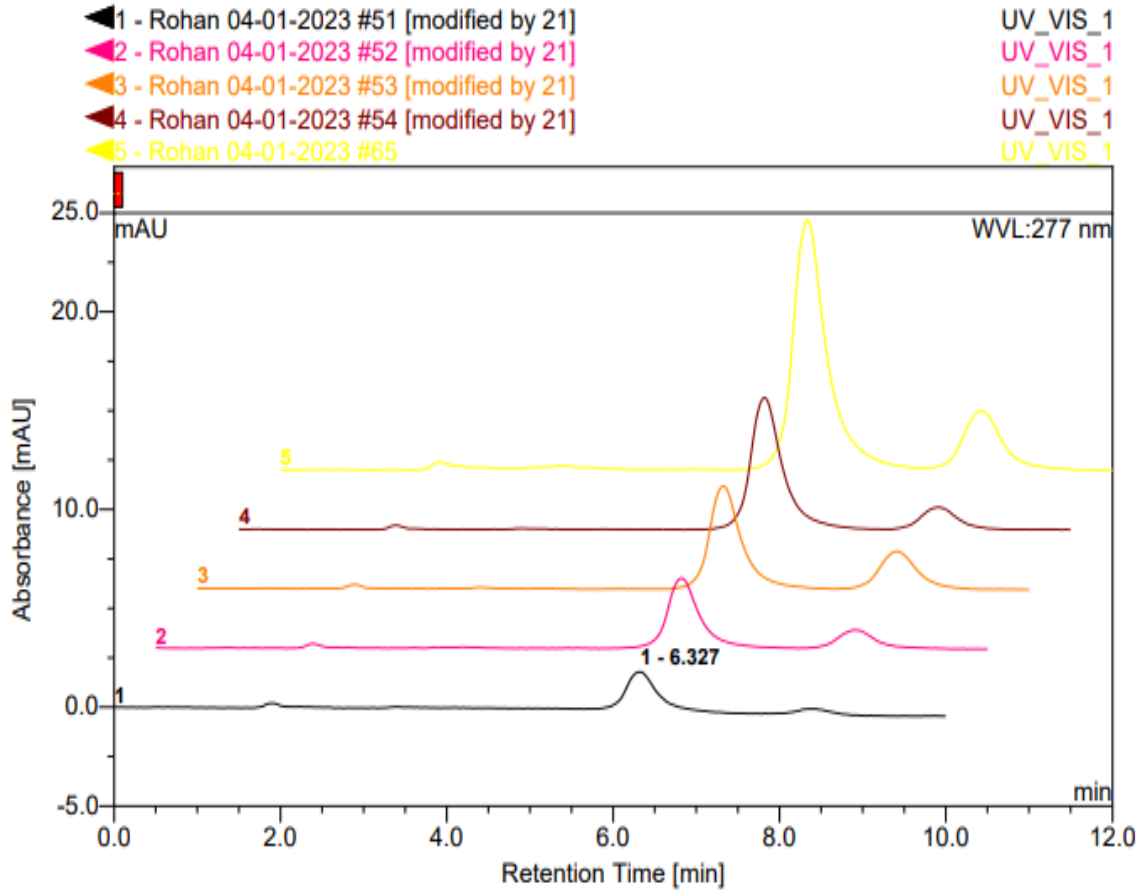

Figure 26. Chromatogram of Drospirenone (300–1500 µg/ml).

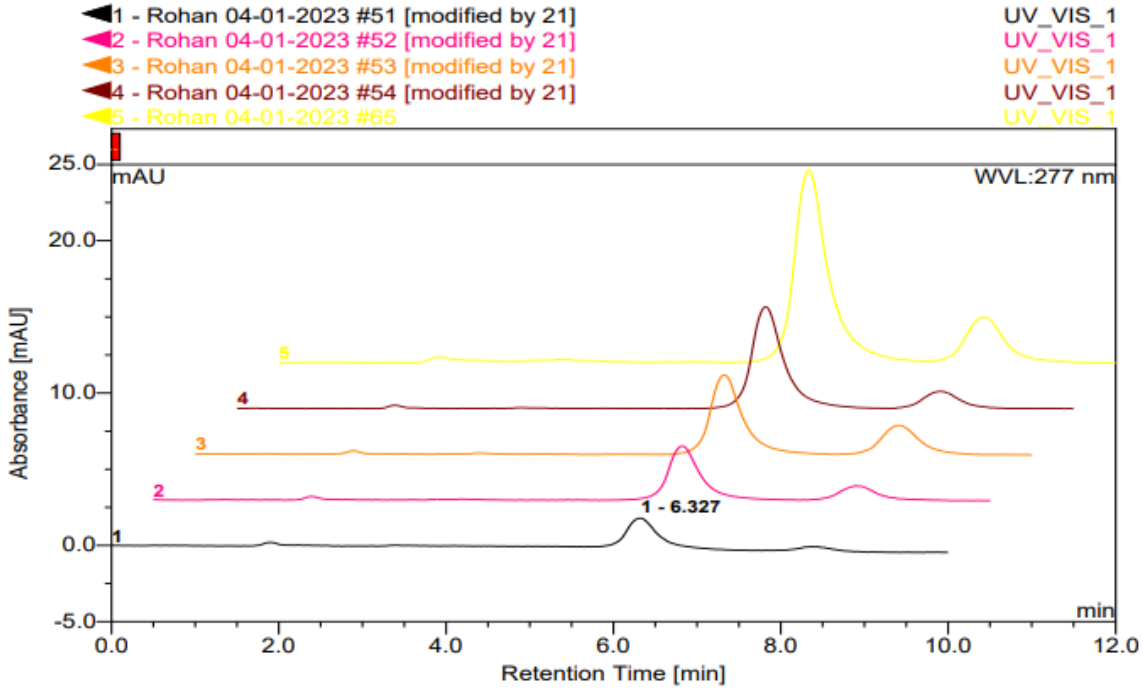

Figure 27. Chromatogram of Ethinylestradiol (10–50 µg/ml).

Table 3. Linearity of Drospirenone.

Drug	Conc. [µg/mL)	1	2	3	4	MEAN	SD	%RSD
DRO	300	182.09	181.75	182.00	181.84	181.84	0.209	0.115
	600	388.87	389.69	388.46	388.74	388.89	0.545	0.140
	900	605.93	605.96	605.45	605.98	606.16	0.544	0.089
	1200	836.45	837.56	837.65	837.54	836.83	0.606	0.072
	1500	1008.25	1008.45	1008.65	1008.45	1008.75	0.519	0.051

Table 4. Linearity of Ethinylestradiol.

Drug	Conc. [µg/mL)	1	2	3	4	MEAN	SD	%RSD
ETH	10	0.57	0.58	0.57	0.58	0.57	0.0037	0.6430
	20	1.10	1.15	1.12	1.13	1.12	0.0213	1.8821
	30	1.66	1.75	1.71	1.72	1.71	0.0275	1.6755
	40	2.22	2.24	2.23	2.21	2.23	0.0323	1.4617
	50	2.57	2.69	2.61	2.61	2.62	0.0507	1.9279

Table 5. Results of precision.

DRUG	PARAMETER	INTERDAY PRECISION CONCENTRATE [µg/ml) n=3			INTERDAY PRECISION CONCENTRATE [µg/ml) n=3		
		1	*2*	*3*	*1*	*2*	*3*
	MEAN	182.17	606.12	1008.15	182.011	606.40	1008.36
	SD	0.0822	0.7687	0.4420	0.2013	0.6614	0.5557
	%RSD	0.0451	0.1268	0.0438	0.1106	0.1090	0.0551

Table 6. LOD and LOQ.

Drug	LOD	LOQ
Drospirenone	2.0850	6.3182
Ethylinestradiol	0.5742	1.7400

Table 7. Results of robustness by changing of wavelength.

DRO	1	2	3	4	5	AVG	SD	%RSD
276	836.11	836.14	836.65	836.78	836.25	836.38	0.3092	0.0369
277	836.45	836.51	836.65	836.14	836.40	836.43	0.1864	0.0222
278	836.38	836.45	836.75	836.45	836.25	836.46	0.1821	0.0217

ETH	1	2	3	4	5	AVG	SD	%RSD
276	0.781	0.783	836.65	836.78	836.25	836.38	0.3092	0.0369
277	0.784	0.786	836.65	836.14	836.40	836.43	0.1864	0.0222
278	0.783	0.784	836.75	836.45	836.25	836.46	0.1821	0.0217

Table 8. Results of robustness by changing of flowrate.

DRO	1	2	3	4	5	AVG	SD	%RSD
0.8	837.14	837.25	837.75	837.89	837.35	837.40	0.2901	0.0346
1	836.45	836.54	836.32	836.14	836.36	836.36	0.1485	0.0177
1.2	835.12	835.48	835.45	836.42	835.34	835.56	0.4998	0.0598

ETH	1	2	3	4	5	AVG	SD	%RSD
0.8	0.794	0.796	0.800	0.798	0.799	0.797	0.0023	0.2957
1	0.784	0.787	0.772	0.778	0.781	0.780	0.0062	0.8010
1.2	0.771	0.775	0.774	0.776	0.779	0.775	0.0031	0.4075

Table 9. Result of robustness by changing of temperature of column.

DRO	1	2	3	4	5	AVG	SD	%RSD
22	834.14	834.23	834.45	834.14	834.36	834.26	0.1361	0.0163
23	836.45	836.54	837.32	837.44	836.36	836.82	0.5142	0.0614
24	835.12	835.35	836.14	836.22	835.47	835.66	0.4908	0.0587

ETH	1	2	3	4	5	AVG	SD	%RSD
22	0.774	0.778	0.781	0.775	0.779	0.777	0.0027	0.3582
23	0.784	0.787	0.784	0.786	0.781	0.784	0.0023	0.2977
24	0.781	0.785	0.781	0.781	0.786	0.783	0.0025	0.3237

Table 10. Result of repeatability.

CONC. (µg/mL)	Average	SD	%RSD
1200	837.03	0.6068	0.0725
12	0.781	0.0037	0.6430

Table 11. Results of Accuracy for Drospirenone.

%Level	Test conc. [µg/ml)	Std conc. [µg/ml)	Total conc. [µg/ml)	Peak Area	% Recovery	Mean	SD	%RSD
0%	1200	0	1200	834.83	102.39			
	1200	0	1200	834.74	102.384	102.39	0.058595	0.057226
	1200	0	1200	834.85	102.39			
50%	1200	600	1800	839.42	102.94			
	1200	600	1800	839.12	102.9	102.92	0.15695	0.152492
	1200	600	1800	839.35	102.93			
100%	1200	1200	2400	844.56	103.55			
	1200	1200	2400	844.96	103.6	103.36	0.471734	0.456399
	1200	1200	2400	844.02	103.48			
150%	1200	1800	3000	849.15	104.09			
	1200	1800	3000	849.26	104.11	104.11	0.168028	0.161394
	1200	1800	3000	849.48	104.13			

Table 12. Results of accuracy for Ethinylestradiol.

%Level	Test conc. [µg/ml)	Std conc. [µg/ml)	Total conc. [µg/ml)	Peak Area	% Recovery	Mean	SD	%RSD
0%	12	0	12	0.723	100.25			
	12	0	12	0.7229	100.24	100.27	0.000321	0.000321
	12	0	12	0.7235	100.33			
50%	12	6	18	0.7315	101.62			
	12	6	18	0.7398	102.56	102.24	0.004271	0.004177
	12	6	18	0.7374	102.56			
100%	12	12	24	0.7451	103.8			
	12	12	24	0.7454	103.85	103.79	0.060277	0.058074
	12	12	24	0.7465	103.73			
150%	12	18	30	0.7552	105.42			
	12	18	30	0.7546	105.33	105.46	0.153948	0.145978
	12	18	30	0.7565	105.63			

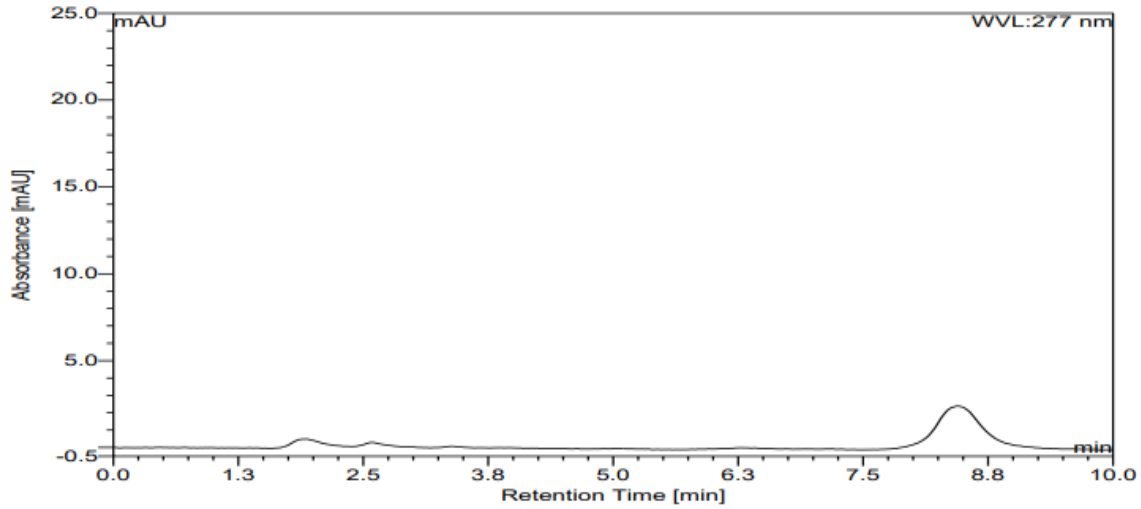

Figure 28. Blank solution.

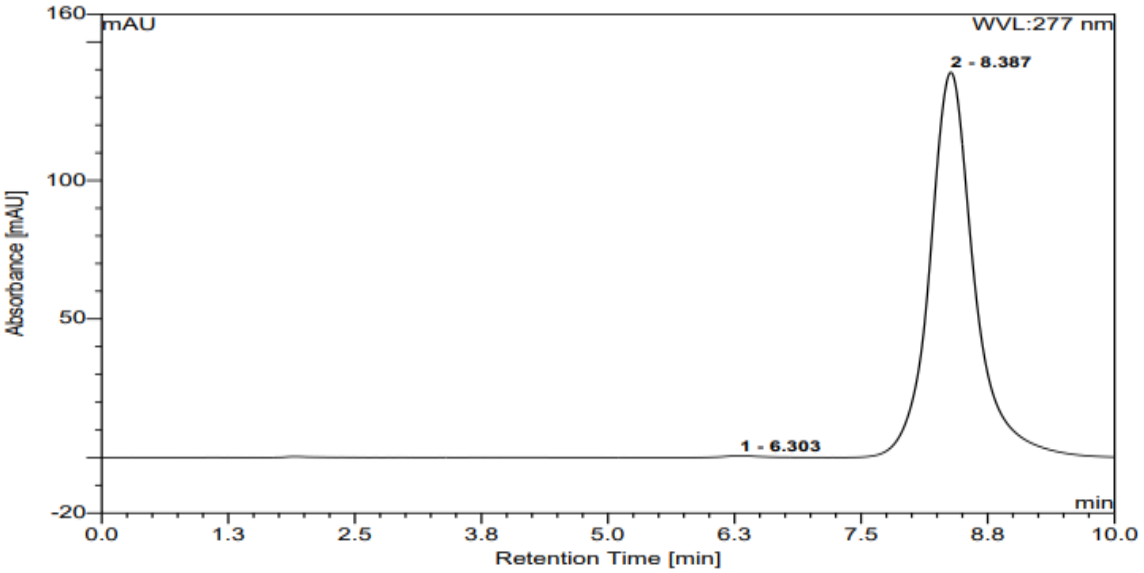

Figure 29. Chromatogram of standard drug Drospirenone (300 µg/ml), Ethinylestradiol (3 µg/ml).

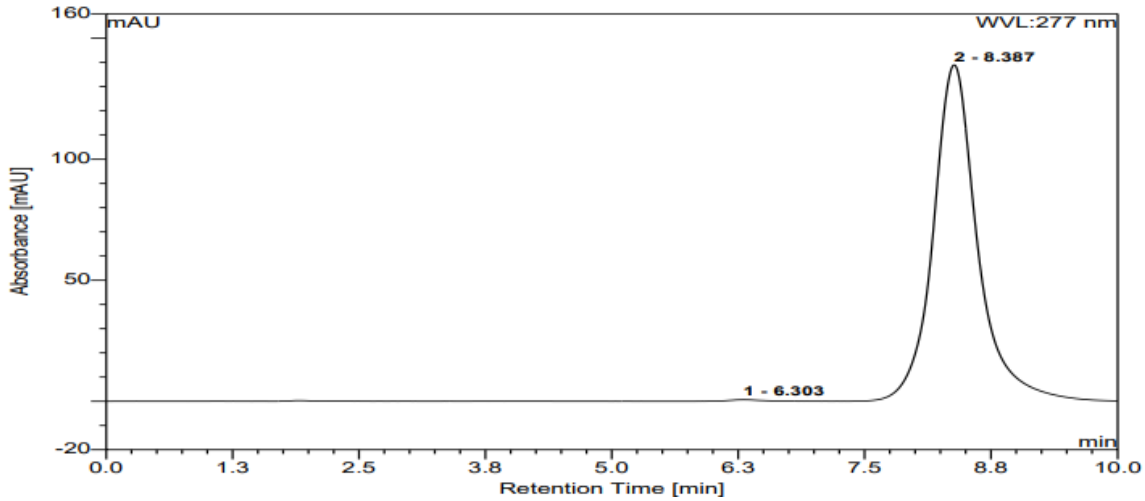

Figure 30. Chromatogram of standard sample Drospirenone (300 µg/ml), Ethinylestradiol (3 µg/ml).

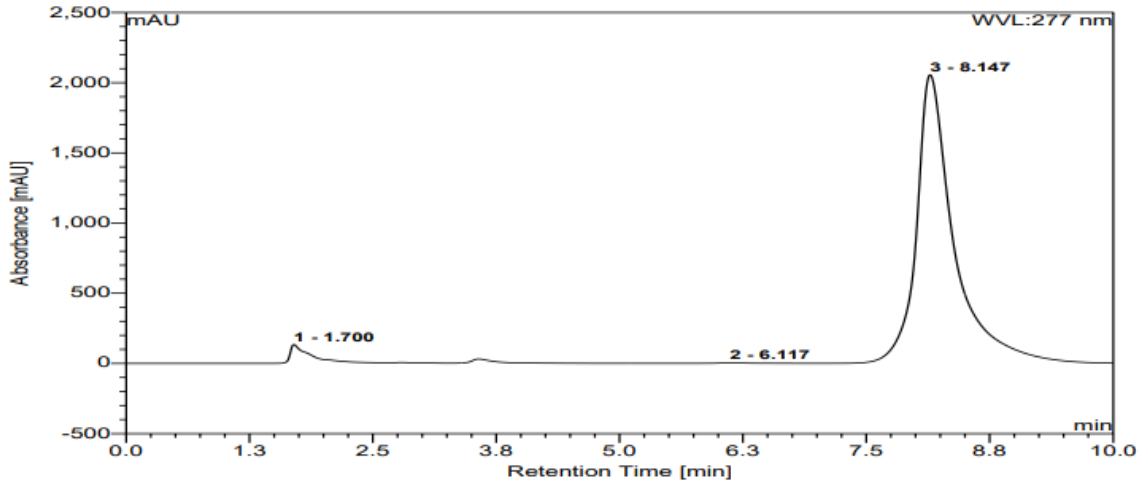

Figure 31. Chromatogram of test sample Drospirenone (1200 µg/ml), Ethinylestradiol (12 µg/ml).

Table 13. Results of stability study.

Sample Name	Drospirenone (Peak Area)	% Degradation	Ethinylestradiol (Peak Area)	%Degradation
	81.440	0%	10.120	0%
Acid (0.5 N)	34.844	57.22%	9.35	7.61%
Base (0.5 N)	3.751	95.39%	9.451	5.5%
Photolytic	62.060	23.8%	7.945	21.50%

Summary

Parameter	DRO	ETH
Wavelength	277nm	277nm
Concentration range (µg/mL)	300–1500	10–50
Slope	0.7006	0.5179
Intercept	–26.069	0.1009
r^2	0.9949	0.9982
% assay	98.39%	100.25%
LOD (µg/mL)	2.0850	0.5742
LOQ (µg/mL)	6.3182	1.7400

Precision (%RSD)	Intraday precision	0.0915	0.3462
	Interday precision	0.0719	0.4640
Robustness (%RSD)	Wavelength (±1)	0.0269	0.7062
	Flow rate (±2)	0.03706	0.5014
	Column temperature (±1)	0.04546	0.3265
Accuracy (%RECOVERY ± %RSD)	0%	102.39 ± 0.057226	100.27 ± 0.000321
	50%	102.92 ± 0.152492	102.24 ± 0.004177
	100%	103.36 ± 0.456399	103.79 ± 0.058074
	150%	104.11 ± 0.161394	105.46 ± 0.145978

CONCLUSIONS

- The detection and quantification of Drospirenone and Ethinylestradiol is extremely difficult due to the concentration ratio (100:1). The proposed RP-HPLC method was suitable for the Drospirenone and Ethinylestradiol for simultaneous determination in combine dosage form and these drugs has no interference between drug and excipient. It can be concluded that methods are more precise, accurate, convenient, reliable, robust, and economical than others and give high absorbance in that system. All the parameters of validation which were performed for this method was under the specific range of the ICH Q2(R1) and low value of % RSD describe the method as precise and accurate.
- Conduct the stability indicating method for Drospirenone and Ethinylestradiol to ensure the safety and efficacy of products therefore, to be concluded that drug is used under this condition and no further peak was detected before this condition so, drug can be used under these circumstances.

REFERENCES

1. Krattenmacher R. Drospirenone: Pharmacology and pharmacokinetics of a unique progestogen. Contraception. 2000;62(1):29–38. doi: 10.1016/s0010-7824(00)00133-5.
2. Sahu A, Tripathy P, Mohanty J, Nagy A. Doppler analysis of ovarian stromal blood flow changes after treatment with metformin versus ethinyl estradiol-cyproterone acetate in women with polycystic ovarian syndrome: A randomized controlled trial. J Gynecol Obstet Hum Reprod. 2019;48(5):335–339. doi: 10.1016/j.jogoh.2018.10.006.
3. Gupta V, Jain AD, Gill NS, Guptan K. Development and validation of HPLC method-a review. IJPRAS. 2012;2(4):17–25.
4. Kazakevich YV, Lobrutto R. HPLC for pharmaceutical scientists. John Wiley & Sons; 2007.16.
5. Ahuja S, Rasmussen H. Development for Pharmaceuticals, Vol.8 Separation Science and Technology. New York: Elsevier; 2007.
6. Rao BV, Sowjanya GN, Ajitha A, Rao VU. A review on stability indicating HPLC method development. WJPPS. 2015;4(8):405–423.
7. Charde MS, Welankiwar AS, Kumar J. Method development by liquid chromatography with validation. Int J Pharm Chem. 2014;4(02):57–61. doi: 10.7439/ijpc.v4i1.65.
8. Wu N, Lippert JA, Lee ML. Practical aspects of ultrahigh pressure capillary liquid chromatography. J Chromatogr A. 2001;911(1):1–12. doi: 10.1016/s0021-9673(00)01188-2.
9. Van Deemter JJ, Zuiderweg FJ, Klinkenberg AV. Longitudinal diffusion and resistance to mass transfer as causes of nonideality in chromatography. Chem Eng Sci. 1956;5(6):271–289. doi: 10.1016/0009-2509(56)80003-1.
10. Srivastava B, Sharma BK, Baghel US. UPLC: Chromatographic technique. Inter J Pharm Qual Assur. 2010;19–25.
11. Beckett A, Stenlake G. Practical Pharmaceutical Chemistry. Fourth Ed. New Delhi: CBS Publishers And Distributors; 2005.
12. Sharma Bk. Instrumental Methods of Chemical Analysis. Twenty Third Ed. Meerut: Goel Publishing House; 2004.
13. Praveen C, Ranganath MK, Divakar P. Method development and validation for simultaneous estimation of ethinyl estradiol and drospirenone and forced degradation behavior by HPLC in combined dosage form. Pharm Anal Acta. 2013;4:231.

14. Silva VB, Galdos AAG, Mothe CMA, Pallastrelli MB. Brazilian Journal of Pharmaceutical Sciences. 2013;49(3):1–8.
15. Galgatte UC, Kumbhar AB, Chaudhari PD. Development of in situ gel for nasal delivery: Design, optimization, in vitro and in vivo evaluation. Drug Deliv. 2014;21(1):62–73. doi: 10.3109/10717544.2013.849778.
16. Khurana S, Dubey N, Sharma D, Sharma S, Khadke P, Kumar A, et al. Highly sensitive and robust quantification method for ethinyl estradiol and drospirenone in plasma AB SCIEX Triple Quad™ 5500 System and UPLC chromatography.
17. Arsova-Sarafinovska Z, Pollozhani A, Aneta D. Determination of ethinylestradiol and drospirenone in oral contraceptives with HPLC method with UV and fluorescence detection. Arch Public Health. 2009;1(1):67–74.
18. Rajeswari B, Saritha N, Devanna N. RP-HPLC method development and validation for the simultaneous estimation of segesterone and ethinyl estradiol in bulk and its pharmaceutical dosage form. Pharma Innov. 2019;8(9):176–181.
19. Mohiuddin TM, Islam MD, Latif A, Hassan MM, Hasan MM, Haque P. Analytical method validation of RP-HPLC method for simultaneous estimation of Levonorgestrel and Ethinyl estradiol from combined drug product. Acta Chim Pharm Indica. 2018;8(2):128, 1–11.
20. Rao GK, Mandapalli PK, Manthri R, Reddy VP. Development and in vivo evaluation of gastroretentive delivery systems for cefuroxime axetil. Saudi Pharm J. 2013;21(1):53–59.
21. Laban A, Markovic S, Stankov M, Djurdjevic P. simultaneous determination of gestodene and ethinyl estradiol in contraceptive formulations by RP-HPLC. Anal Lett. 2004;37(2):273–282. doi: 10.1081/AL-120027792.
22. Reddy YN, Sreeramulu J, Balaswami B. A new stability-indicating RP-HPLC- PDA method for simultaneous estimation of segesterone acetate and ethinyl estradiol in tablet dosage form. Int j res anal rev. 2019;6(1):1–8.
23. Suchitra D, Satyanarayana B. Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of ethinyl estradiol and gestodene in bulk and pharmaceutical dosage forms. Int J Pharm Pharm Sci. 2021;13(7):49–58.
24. Hamida S, Cherradi B, El Gannour O, Terrada O, Raihani A, Ouajji H. New database of French computer science Words handwritten vocabulary. In: 2021 International Congress of Advanced Technology and Engineering (ICOTEN). IEEE; 2021. pp. 1–5. IEEE.
25. Tripathi KD. Essential Medical Pharmacology. Jaypee Publication. 6th Edition: pp. 311–317.
26. Seth SD. Textbook of Pharmacology. Reed Elsevier India Pvt Ltd, second edition: pp. 254–257.

