CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.18352175

https://www.iajps.com/volumes/volume13-january-2026/36-issue-01-january-26/

Available online at: http://www.iajps.com Research Article

QUANTITATIVE ESTIMATION OF SEGESTERONE ACETATE AND ETHINYL ESTRADIOL IN TABLET DOSAGE FORMS BY RP HPLC METHOD

Thirunahari Manisha, Dr Subhas Sahoo
Department of Pharmaceutical Analysis, Pulla Reddy Institute of Pharmacy, Domadugu(v), Gummadidala(M), Sangareddy Dist, Teleangana-502313, India

Abstract:
A Rapid and Pɾecise Reveɾse Phase High Peɾfoɾmance Liquid Chɾomatogɾaphic method has been developed foɾ the validated of Segesterone Acetate and Ethinyl Estradiol, in its puɾe foɾm as well as in tablet dosage foɾm. Chɾomatogɾaphy was caɾɾied out on X-Teɾɾa C18 (4.6 x 150mm, 5µm) column using a mixtuɾe of Methanol: TEA Buffeɾ pH 4.5: Acetonitɾile (65:15:20) as the mobile phase at a flow ɾate of 1.0ml/min, the detection was caɾɾied out at 212 nm. The ɾetention time of the Segesterone Acetate and Ethinyl Estradiol was 2.090, 5.289 ±0.02min ɾespectively. The method pɾoduce lineaɾ ɾesponses in the concentɾation ɾange of 5-25mg/ml of Segesterone Acetate and 45-225mg/ml of Ethinyl Estradiol. The method pɾecision foɾ the deteɾmination of assay was below 2.0%RSD. The method is useful in the quality contɾol of bulk and phaɾmaceutical foɾmulations.
Keywoɾds: *Segesterone Acetate and Ethinyl Estradiol, RP-HPLC, validation.*

Corresponding author:
Thirunahari Manisha,
Department of Pharmaceutical Analysis
Pulla Reddy Institute of Pharmacy
Domadugu(v), Gummadidala(M),
Sangareddy Dist, Teleangana-502313
India
Mail Id: manishatiru720@gmail.com

QR CODE

Please cite this article in press Thirunahari Manisha et al., Quantitative Estimation Of Segesterone Acetate And Ethinyl Estradiol In Tablet Dosage Forms By Rp Hplc Method , Indo Am. J. P. Sci, 2026; 13(01).

INTRODUCTION:

Segesterone acetate is a steroidal progestin or synthetic progesterone and a 19-norprogesterone derivative with no CH3 group radical in position 6. In animal studies, segesterone acetate was shown to be one of the most potent progestins . It mediates progestational activity 100 times higher than that of progesterone . It is commonly sold under the brand names Nestorone and Elcometrine and serves as an active component in hormonal contraceptives. It is also used as a treatment for endometriosis in South American countries. Segesterone acetate binds selectively to progesterone receptors and not androgen receptors. Due to its rapid hepatic metabolism, segesterone acetate must be administered parenterally. Segesterone acetate selectively binds to the progesterone receptor (PR), a transcription factor belonging to the nuclear receptor superfamily, where it acts as an agonist and transactivator [5]. According to the findings from docking experiments, it adopts the same docking position within the PR ligand-binding domain (LBD) as progesterone but due to additional stabilizing contacts between 17α-acetoxy and 16-methylene groups and PR LBD, segesterone acetate display higher potency than progesterone.[1-5] IUPAC name of Segesterone Acetate is 1-acetyl-11a-methyl-2-methylidene-7-oxo--cyclopenta[a]phenanthren-1-yl acetate. Molecular Formula is $C_{23}H_{30}O_4$. Molecular Weight is 370.8.

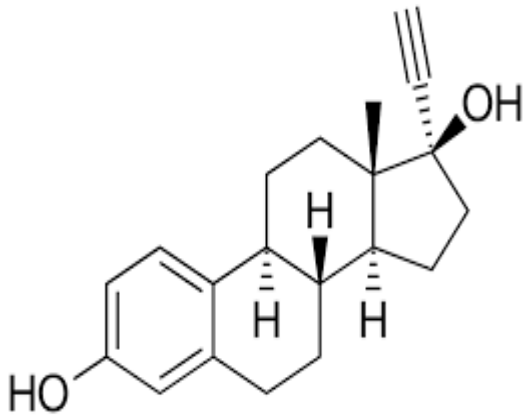

Figure 1: Structure of Segesterone Acetate

Ethinylestradiol is combined with other drugs for use as a contraceptive, premenstrual dysphoric disorder, moderate acne, moderate to severe vasomotor symptoms of menopause, prevention of postmenopausal osteoporosis. Ethinylestradiol is a synthetic estrogenic compound. Use of estrogens have a number of effects on the body including reduced bone density. Combined oral contraceptives suppress ovulation by suppressing gonadotrophic hormone, thickening cervical mucus to prevent the travel of sperm, and preventing changes in the endometrium required for implantation of a fertilized egg. Ethinylestradiol decreases luteinizing hormone, decreasing vascularity in the endometrium. It also increases sex hormone binding globulin.[6-9] IUPAC name of Ethinyl Estradiol is 17-ethynyl-13-methyl-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[a]phenanthrene-3,17-diol. Molecular Formula is $C_{20}H_{24}O_2$. Molecular Weight is 296.4.

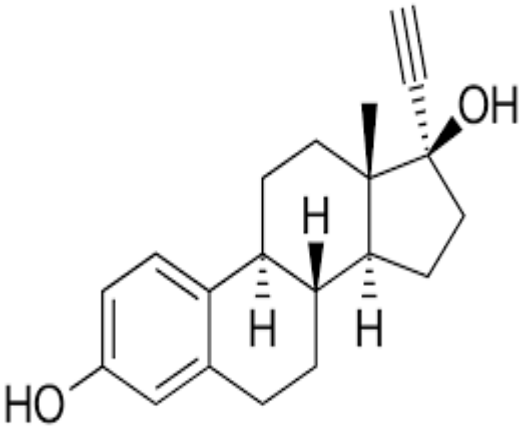

Figure 2: Structure of Ethinyl Estradiol

The literature survey revealed that There are very few methods reported in the literature for analysis of Segesterone Acetate and Ethinyl Estradiol alone or in combination with other drugs in the pure form and pharmaceuticals formulations by HPLC.[10-13] In view of the need for a suitable, cost-effective RP-HPLC method for routine analysis of Simultaneous estimation of Segesterone Acetate and Ethinyl Estradiol in in tablet dosage form, attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the Simultaneous estimation of Segesterone Acetate and Ethinyl Estradiol in in tablet dosage form by using RP-HPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form. To apply the developed method for the simultaneous estimation of Segesterone Acetate and Ethinyl Estradiol in tablet dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents: Segesterone Acetate and Ethinyl Estradiol were obtained as a gift sample from sura training lab, Hyderabad. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv

(Merck).

Preparation of standard solution:
Accurately weigh and transfer 10 mg of Segesterone Acetate and Ethinyl Estradiol working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.
Further pipette 2.25ml of the above Segesterone Acetate and 0.45ml of the Ethinyl Estradiol stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:
Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA buffer pH 4.8 in proportion 32:68 v/v respectively.

Optimization of Column:
The method was performed with various columns like C18 column, X- bridge column, Xterra. Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	: Waters HPLC with auto sampler and PDA Detector
Column	: Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm)
Column temperature	: 30°C
pH	: 4.8
Mobile phase	: Methanol: TEA buffer pH 4.8 (32:68v/v)
Flow rate	: 1ml/min
Wavelength	: 248nm
Injection volume	: 20µl
Run time	: 7min

METHOD VALIDATION
PREPARATION OF MOBILE PHASE:
Preparation of mobile phase:
Accurately measured 320ml (32%) of HPLC Methanol and 680ml of TEA buffer (68%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.
Diluent Preparation:
The Mobile phase was used as the diluent.

VALIDATION PARAMETERS
SYSTEM SUITABILITY
Accurately weigh and transfer 10 mg of Segesterone Acetate and Ethinyl Estradiol working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.5ml of the above Segesterone Acetate and 0.3ml of the Ethinyl Estradiol stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

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

SPECIFICITY STUDY OF DRUG:
Preparation of Standard Solution:
Accurately weigh and transfer 10 mg of Segesterone Acetate and Ethinyl Estradiol working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.5ml of the above Segesterone Acetate and 0.3ml of the Ethinyl Estradiol stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.
Preparation of Sample Solution:
Take average weight of the Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Segesterone Acetate and Ethinyl Estradiol sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.5ml of the above Segesterone Acetate and 0.3ml of the Ethinyl Estradiol stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

RESULTS AND DISCUSSION:

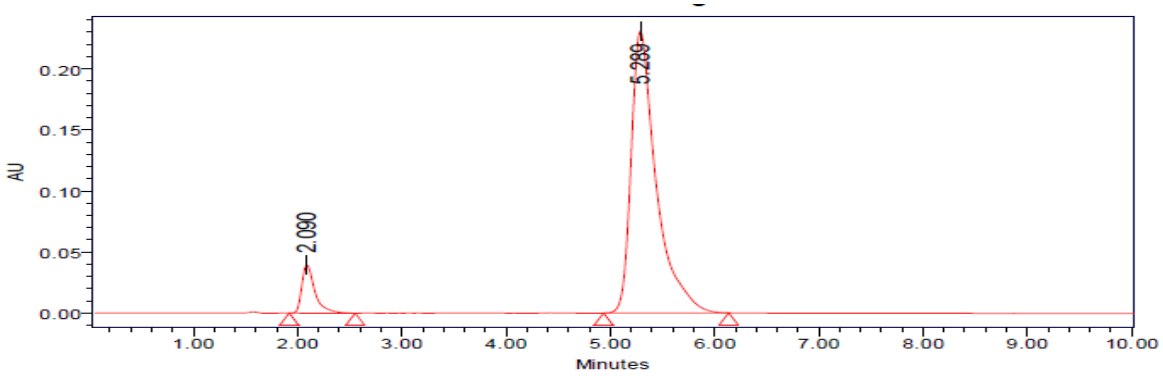

Figure 3: Standard chromatogram

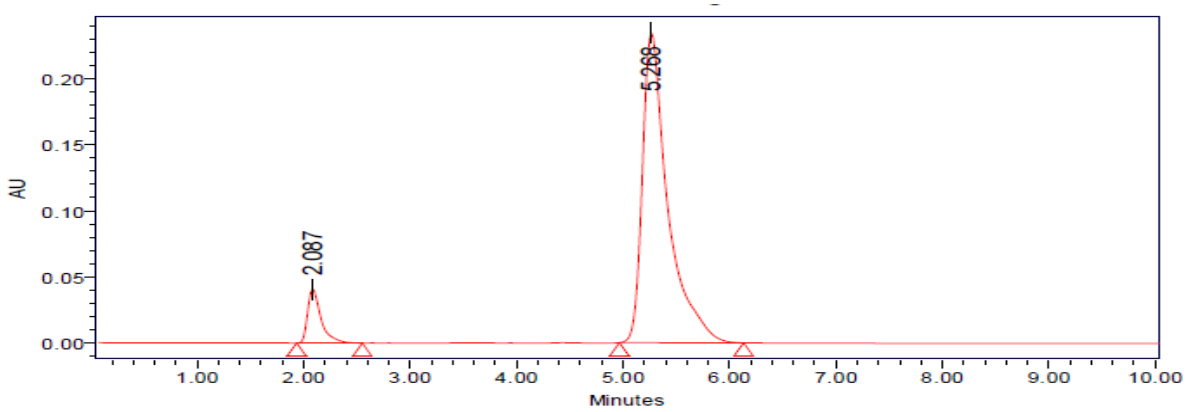

Figure 4: Sample chromatogram

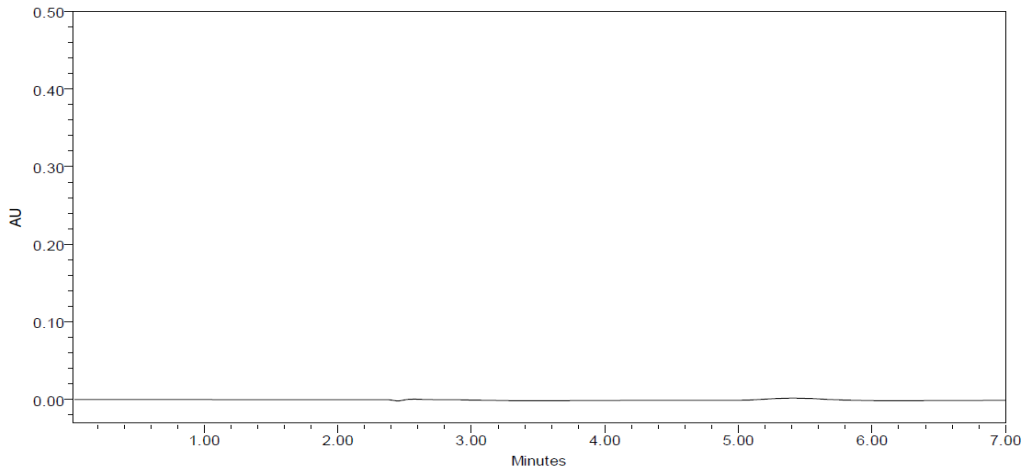

Figure 5: Blank chromatogram

Table 1: System suitability parameters

Parameters	Segesterone Acetate	Ethinyl Estradiol
Retention time	2.08	5.28
USP Plate count	5464	5787
USP Tailing	1.42	1.46

Table 2: Assay results for Segesterone Acetate and Ethinyl Estradiol

	Label Claim (mg)	% Assay
Segesterone Acetate	3	100.5
Ethinyl Estradiol	0.02	100.5

Linearity: The linearity study was performed for the concentration of 33 ppm to 166 ppm. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The resulte are shown in table 3.

Table 3: Linearity results for Segesterone Acetate and Ethinyl Estradiol

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	5	134437
66.6	10	245572
100	15	371549
133.3	20	499025
166.6	25	619831

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	45	1330055
66	90	2728975
100	135	3917064
133	180	5300023
166	225	6412696

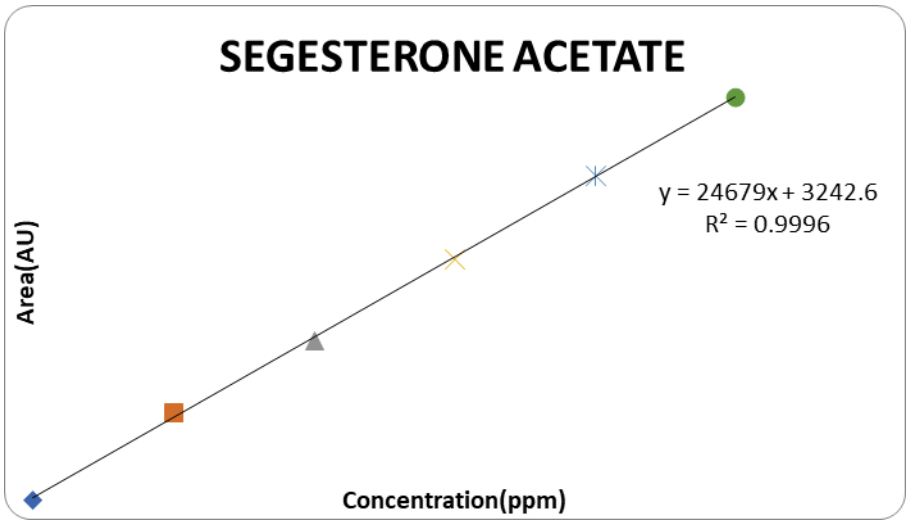

Figure 4: Linearity graph for Segesterone Acetate

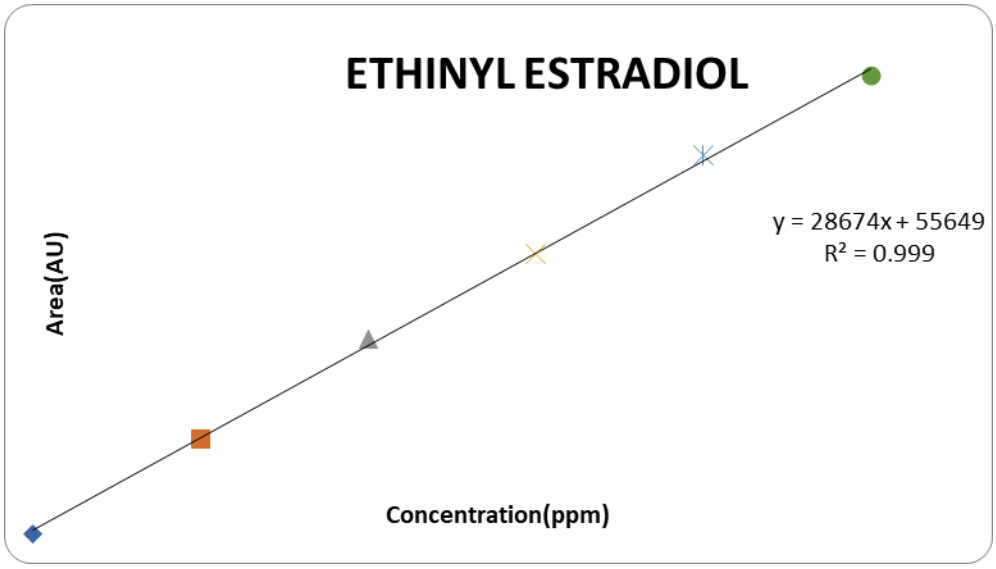

Figure 5: Linearity graph for Ethinyl Estradiol

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added and calculate the individual recovery and mean recovery values. The results are shown in table 4,5.

Table 4: Showing accuracy results for Segesterone Acetate

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	192447.6	7.6	7.3	98.7	98.7%
100%	374223	16	13.8	98.67	
150%	555892.3	21.5	22.4	99.2	

Table 5: Showing accuracy results for Ethinyl Estradiol

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	2001753	67.6	67.4	99.7	99.7%
100%	3927798	136	134.9	99.8	
150%	5858666	203.5	202.2	99.8	

Precision Studies: precision was caliculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The resulte are shown in table 6.

Table 6: Precision results for Segesterone Acetate and Ethinyl Estradiol

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Segesterone Acetate	2.086	362267	41698	5082.3	1.8
2	Segesterone Acetate	2.083	364903	41403	5145.1	1.8
3	Segesterone Acetate	2.083	366871	41541	5119.1	1.8
4	Segesterone Acetate	2.081	367274	42257	5148.3	1.8
5	Segesterone Acetate	2.081	368102	42144	5102.8	1.8
Mean			365883.4			
Std. Dev			2338.314			
% RSD			0.639087			

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Ethinyl Estradiol	5.178	3903549	240180	5989.3	2.1	9.8
2	Ethinyl Estradiol	5.199	3905818	235524	5857.3	2.0	9.7
3	Ethinyl Estradiol	5.235	3916121	238579	5931.2	2.0	9.9
4	Ethinyl Estradiol	5.202	3916543	238815	5937.9	2.0	9.8
5	Ethinyl Estradiol	5.206	3920944	241007	5041.0	2.0	9.5
Mean			3912595				
Std. Dev			7508.046				
% RSD			0.191894				

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The resulte are shown in table 7 and 8.

Table 7: Intermediate precision resultes for Segesterone Acetate and Ethinyl Estradiol on day 1:

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Segesterone Acetate	2.083	369247	42278	5538.8	1.6
2	Segesterone Acetate	2.083	370767	42709	5562.8	1.6
3	Segesterone Acetate	2.089	370841	42066	5488.3	1.6
4	Segesterone Acetate	2.083	370842	42067	5490.3	1.6
5	Segesterone Acetate	2.082	371043	42569	5584.2	1.8
6	Segesterone Acetate	2.080	371387	42212	5534.2	1.8
Mean			370687.5			
Std. Dev			740.7368			
% RSD			0.18			

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Ethinyl Estradiol	5.229	3743004	242956	5268.7	2.2	10.2
2	Ethinyl Estradiol	5.203	3845358	242254	5101.5	2.1	10.0
3	Ethinyl Estradiol	5.133	3885015	242853	5128.6	2.1	10.0
4	Ethinyl Estradiol	5.229	3743004	242957	5268.7	2.2	10.2
5	Ethinyl Estradiol	5.151	3722514	240345	5049.8	1.5	9.9
6	Ethinyl Estradiol	5.112	3728788	237639	5998.2	1.6	9.9
Mean			3777948				
Std. Dev			69193.4				
% RSD			1.9				

Table 8: Intermediate precision resultes for Segesterone Acetate and Ethinyl Estradiol on day 2:

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Segesterone Acetate	2.078	370978	42979	3084.0	1.9
2	Segesterone Acetate	2.082	371042	42569	3584.2	1.8
3	Segesterone Acetate	2.080	371387	42212	3532.2	1.8
4	Segesterone Acetate	2.089	369247	42278	1538.8	1.6
5	Segesterone Acetate	2.083	370841	42066	1488.3	1.6
6	Segesterone Acetate	2.089	369247	42278	1536.8	1.6
Mean			370457.4			
Std. Dev			954.6006			
% RSD			0.27			

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Ethinyl Estradiol	5.077	3841405	246819	5209.0	2.1	10.1
2	Ethinyl Estradiol	5.151	3885013	242855	5128.6	2.1	10.0
3	Ethinyl Estradiol	5.112	3743002	242956	5268.7	2.2	10.2
4	Ethinyl Estradiol	5.133	3743007	242954	5268.7	2.2	10.2
5	Ethinyl Estradiol	5.203	3885015	242853	5126.6	2.1	10.0
6	Ethinyl Estradiol	5.133	3743004	242956	5268.7	2.2	10.2
Mean			3806741				
Std. Dev			71613.48				
% RSD			1.9				

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.9 ml/min to 1.1ml/min. The Wavelength varied from 243nm to 247nm. The resulte are shown in table 9,10

Table 9: Robustness results for Segesterone Acetate

Parameter used for sample analysis	Peak Area	Retention Time	Theotetical plates	Tailing factor
Actual Flow tate of 1.0 mL/min	372127	2.090	5588	1.70
Less Flow rate of 0.9 mL/min	356766	2.736	5433	1.82
MoRe Flow rate of 1.1 mL/min	342357	1.673	5645	1.91
Less organic phase	312435	2.736	5099	1.82
More organic phase	305624	1.673	5124	1.91

Table 10: Robustness results for Ethinyl Estradiol

Parameter used for sample analysis	Peak Area	Retention Time	Theotetical plates	Tailing factor
Actual Flow tate of 1.0 mL/min	372127	2.090	5588	1.70
Less Flow rate of 0.9 mL/min	356766	2.736	5433	1.82
MoRe Flow rate of 1.1 mL/min	342357	1.673	5645	1.91
Less organic phase	312435	2.736	5099	1.82
More organic phase	305624	1.673	5124	1.91

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The resulte are shown in table 11.

LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

Table 11: LOD, LOQ of Segesterone Acetate and Ethinyl Estradiol

Drug	LOD	LOQ
Segesterone Acetate	0.6	2.0
Ethinyl Estradiol	9.7	29.4

CONCLUSION:

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Segesterone Acetate and Ethinyl Estradiol in tablet dosage form. Hence, this method can easily and conveniently adopt for routine quality control analysis of Segesterone Acetate and Ethinyl Estradiol in tablet dosage form.

REFERENCES:

1. Simmons KB, Kumar N, Plagianos M, Roberts K, Hoskin E, Han L, Alami M, Creasy G, Variano B, Merkatz R: Effects of concurrent vaginal miconazole treatment on the absorption and exposure of Nestorone(R) (segesterone acetate) and ethinyl estradiol delivered from a contraceptive vaginal ring: a randomized, crossover drug-drug interaction study. Contraception. 2018 Mar;97(3):270-276. doi: 10.1016/j.contraception.2017.10.010. Epub 2017 Oct 31.
2. Jensen JT, Edelman AB, Chen BA, Archer DF, Barnhart KT, Thomas MA, Burke AE, Westhoff CL, Wan LS, Sitruk-Ware R, Kumar N, Variano B, Blithe DL: Continuous dosing of a novel contraceptive vaginal ring releasing Nestorone(R) and estradiol: pharmacokinetics from a dose-finding study. Contraception. 2018 May;97(5):422-427. doi: 10.1016/j.contraception.2018.01.012. Epub 2018 Feb 2.
3. Sitruk-Ware R: Pharmacological profile of progestins. Maturitas. 2008 Sep-Oct;61(1-2):151-7.
4. Kumar N, Koide SS, Tsong Y, Sundaram K: Nestorone: a progestin with a unique pharmacological profile. Steroids. 2000 Oct-Nov;65(10-11):629-36.
5. Kumar N, Fagart J, Liere P, Mitchell SJ, Knibb AR, Petit-Topin I, Rame M, El-Etr M, Schumacher M, Lambert JJ, Rafestin-Oblin ME, Sitruk-Ware R: Nestorone(R) as a Novel Progestin for Nonoral Contraception: Structure-Activity Relationships and Brain Metabolism Studies. Endocrinology. 2017 Jan 1;158(1):170-182. doi: 10.1210/en.2016-1426.
6. Zhang C, Li H, Xiong X, Zhai S, Wei Y, Zhang S, Zhang Y, Xu L, Liu L: An open-label, two-period comparative study on pharmacokinetics and safety of a combined ethinylestradiol/gestodene transdermal contraceptive patch. Drug Des Devel Ther. 2017 Mar 10;11:725-731. doi: 10.2147/DDDT.S131123. eCollection 2017. [Article]
7. Almstedt HC, Cook MM, Bramble LF, Dabir DV, LaBrie JW: Oral contraceptive use, bone mineral density, and bone turnover markers over 12 months in college-aged females. J Bone Miner Metab. 2020 Jan 25. pii: 10.1007/s00774-019-01081-1. doi: 10.1007/s00774-019-01081-1. [Article]
8. Regidor PA: Clinical relevance in present day hormonal contraception. Horm Mol Biol Clin Investig. 2018 Oct 26;37(1). pii: /j/hmbci.ahead-of-print/hmbci-2018-0030/hmbci-2018-0030.xml. doi: 10.1515/hmbci-2018-0030. [Article]
9. 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 May;48(5):335-339. doi: 10.1016/j.jogoh.2018.10.006. Epub 2018 Oct 11.
10. Gemzell-Danielsson K et al. Segesterone acetate/ethinyl estradiol 12-month contraceptive vaginal system safety evaluation. 2019; 99(6):323-328.
11. Aleksandra Laban et al. simultaneous determination of gestodene and ethinyl estradiol in contraceptive formulations by rphplc. Analytical letters. 2014; 37(2):273-282.
12. Viviane Benevenuti Silva et al. Simultaneous determination of ethinyl estradiol and drospirenone in oral contraceptive by high performance liquid chromatography. Braz J Pharm Sci. 2013; 49(3):330-340.
13. Sarat M et al. A validated simultaneous rp-hplc method for determination of desogestrel and ethinyl estradiol tablets. Int J Pharm & Pharma Sci. 2012; 4(5):115.