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Original Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ETHINYL ESTRADIOL AND GESTODENE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Objective: The principal objective of this study is to develop and validate a simple, new, fast, selective, precise, and economic stability-indicating the RP-HPLC method for the simultaneous estimation of Ethinyl estradiol and Gestodene in a bulk and pharmaceutical dosage form.

Methods: The present method was developed and validated on a Waters HPLC system using Phenomenex Gemini C18(250 mm × 4.6 mm i.d., 5 µm particle size) column and mobile phase composition of phosphate buffer: Acetonitrile (75:25 v/v) and the pH was adjusted to 3.6 using dilute orthophosphoric acid. The system was regulated at 1.0 ml/min flow rate at 237 nm UV detection.

Results: The two drugs Ethinyl Estradiol and Gestodene, were eluted at 1.788 min and 3.475 min retention time, respectively. The analytical parameters such as accuracy, precision, linearity, LOD, LOQ, ruggedness, and robustness were used for validating the developed method according to International Conference on Harmonisation [ICH] guidelines. Linearity was exhibited over the concentration range of 10-50µg/ml and 25-125µg/ml for Ethinyl Estradiol and Gestodene, respectively. The method revealed the Limit of Detection and Quantitation values for Ethinyl Estradiol and Gestodene were 1.399µg/ml, 3.909µg/ml and 4.24µg/ml, 11.85µg/ml, respectively. The stress testing was carried out to give rise to degradation products by exposing the drugs to acid, alkali, thermal, oxidative, photolytic, and hydrolytic degradation. The obtained data showed that the content of Active pharmaceutical ingredients and the degradation products were successfully separated without any interference, which confirmed the stability-indicating nature of the developed method.

Conclusion: The new, simple, rapid, selective, precise, and economic stability-indicating RP-HPLC method has been successfully developed and validated. It can be satisfactorily applied for the periodic laboratory quantitative estimation of Ethinyl Estradiol and Gestodene in formulations and active pharmaceutical ingredients.

Keywords: RP-HPLC, Method development, Method validation, Forced degradation studies

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INTRODUCTION

Ethinyl Estradiol is chemically (8*R*,9*S,* 13*S,* 14*S,* 17*R*)-17-ethynyl-13-methyl-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[a] phenanthrene -3,17-diol [fig. 1], an estrogen drug is used widely in birth control pills in combination with progestins. It is used to treat menopausal symptoms, gynecological disorders, and certain hormone-sensitive cancers [1]. Ethinyl estradiol binds to the estrogen receptor complex, enters the nucleus, and activates the DNA transcription process. It prevents ovulation by decreasing luteinizing hormone, which in turn decreases endometrial vascularization and decreases gonadotrophic hormone. In epididymal tissue, it lowers testosterone levels and prevents prostatic cancer by inhibiting the 5-alpha reductase enzyme. Along with cancer treatment, it is also used for osteoporosis [2].

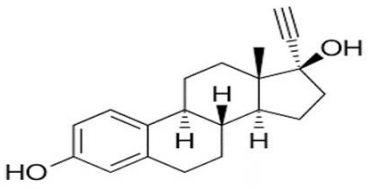

Fig. 1: Chemical structure of Ethinyl estradiol

Gestodene is chemically (8*R*,9*S*,10*R*,13*S*,14*S*,17*R*)-13-ethyl-17-ethynyl-17-hydroxy-1,2,6,7,8,9,10,11,12,14-decahydrocyclopenta[a]phenanthene-3-one [fig. 2], a progestogen hormonal contraceptive used in birth control pills and also used in menopausal hormone therapy [3]. It inhibits growth hormone release from the pituitary gland and suppresses ovulation. It also disrupts fertilization and inhibits implantation [4].

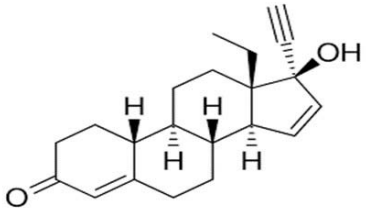

Fig. 2: Chemical structure of gestodene

Femovan tablet has 0.03 mg of Ethinyl estradiol and 0.075 mg of Gestodene as active pharmaceutical ingredients. This combination drug is used for contraception and in the treatment of irregular periods. This drug helps to prevent the release and fertilization of the egg. It is also used to treat ovarian cancer.

From the literature survey, it was revealed that several methods like RP-HPLC [5, 6], UPLC/MS-MS [7], and stability-indicating RP-HPLC [8] methods are described for the quantitative determination of Ethinyl estradiol and Gestodene and Ethinyl estradiol and

drospirenone [9] in combination form. But the published stability-indicating method's total analysis time was 65 min which takes more time for analysis and consumes more mobile phase thus becomes expensive. Therefore, it felt necessary to develop and validate a new, rapid, and economic stability-indicating RP-HPLC method, which can be successfully applied for the regular laboratory analysis of Ethinyl estradiol and Gestodene drugs.

MATERIALS AND METHODS

Chemicals and reagents

The standard drugs of Ethinyl estradiol and Gestodene were collected as gift samples from Sura labs, Hyderabad. The commercial tablet dosage form FEMOVAN containing 0.03 mg of Ethinyl estradiol and 0.075 mg of Gestodene, marketed by Bayer Zydus Pharma Ltd., was procured from a local pharmacy. Acetonitrile (HPLC grade), Methanol (HPLC grade), water (HPLC grade), and orthophosphoric acid used for the preparation of the mobile phase were a product of Merck.

Instrument used

The present method was quantitatively estimated on a Waters Alliance 2695 separation module HPLC system, and data processing was done using Empower 2 software. The eluates were monitored at 237 nm by 996 Photo-diode array detectors. Sonication's dissolution and degassing of the solvents and the mobile phase were achieved on Labman digital ultra sonicator. The pH of the solution was adjusted by using a Lab India pH meter.

Methods

Chromatographic conditions

The simultaneous estimation was achieved on Phenomenex Gemini C18(250 mm × 4.6 mm i. d, 5 µm particle size) column with mobile phase composition of phosphate buffer and acetonitrile (75:25 v/v, pH 3.6) adjusted to a flow rate of 1.0 ml/min for a total 8 min run time. The eluates were monitored at 237 nm by a Photo-diode array detector, and the two drugs Ethinyl Estradiol and Gestodene were eluted at 1.788 min and 3.475 min retention time, respectively.

Preparation of stock and working stock solutions

An accurately weighed 10 mg of Ethinyl Estradiol and Gestodene standard drugs were dissolved in 10 ml of mobile phase, sonicated, and filtered. Further prepared 20µg/ml and 50µg/ml concentration working stock solutions of Ethinyl Estradiol and Gestodene respectively, mixed thoroughly, sonicated, and filtered through 0.45µ membrane filter. Introduced the samples into the HPLC system, recorded the chromatograms, and noted the best-optimized conditions to proceed for validation as per ICH guidelines.

Preparation of sample solution

Femovan tablets (containing 0.03 mg Ethinyl estradiol and 0.075 mg Gestodene) were taken and crushed in a mortar using a pestle. An equivalent amount of 10 mg of tablet powder was weighed and dissolved in the diluent, diluted to volume, mixed thoroughly, sonicated, and filtered. Injected the sample in triplicates and calculated the % assay.

Method validation

As per ICH guidelines [10, 11], the parameters checked for method validation are Accuracy, precision, linearity, LOD, LOQ, specificity, and robustness [12-14].

System suitability

For evaluating the system suitability, the mixed working standard solution of Ethinyl Estradiol and Gestodene was injected five times into the HPLC system, recorded the chromatograms, measured the areas, and calculated the % RSD for all five injections in HPLC.

Linearity

The Linearity of the method was determined by plotting the standard curve in the concentration range of 10-50µg/ml and 25-125µg/ml for Ethinyl Estradiol and Gestodene, respectively. The peak areas were noted by injecting each level into the chromatographic system. Plotted a calibration curve of analyte response versus concentration, and by linear regression analysis, the correlation coefficient was calculated.

Accuracy

For evaluating the method's accuracy, added a pre-analyzed sample solution of 20µg/ml of Ethinyl estradiol and 50µg/ml of Gestodene to a known amount of standard solution of Ethinyl Estradiol (10, 20, and 30µg/ml) and Gestodene (25, 50 and 75µg/ml) respectively. All the solutions were prepared and injected in triplicates. Recorded the chromatograms and measured the peak responses. Calculated the Amount found and Amount added for Ethinyl Estradiol and Gestodene and calculated the individual recovery and mean recovery values.

Precision

The developed method's precision was evaluated by Intra-assay precision and Intermediate Precision.

Intra-day precision

The repeatability of the method was determined by introducing the standard solution containing 20µg/ml of Ethinyl Estradiol and 50µg/ml of Gestodene for five replicate injections, noted the areas on the same day under unchanged operating conditions over a short period and calculated the % RSD.

Intermediate precision

The intermediate precision of the method was evaluated by injecting the standard solution containing 20µg/ml and 50µg/ml of Ethinyl Estradiol and Gestodene, respectively, for five times. Measured the areas and calculated the %RSD for the five replicate injections on different days under unchanged operating conditions.

Robustness

The robustness of the developed method was evaluated by deliberate variations in flow rate and mobile phase organic composition.

Effect of the slight change in flow rate

Examined the solution at 0.9 ml/min and 1.1 ml/min rather than 1.0 ml/min, under unchanged operating conditions. 20 µl of the mixed standard solution was injected, recorded the chromatograms, and compared with an optimized chromatogram.

Effect of the slight change of percent organic mobile phase

The sample was analyzed by varying the percentage of organic mobile phase composition in the ratio of 70:30, 80:20 instead of 75:25, under identical conditions. Injected into the HPLC, recorded the chromatograms and compared with an optimized chromatogram.

Detection limit

The Ethinyl estradiol and Gestodene LOD values were quantitated by using the formula-

$$LOD = 3.3 \times \sigma / S$$

Where,

σ = Standard deviation of the intercept S = Slope of the calibration curve

Limit of quantitation

The quantitation limit of an analyte in the samples was quantified by using the formula-

$$LOQ = 10 \times \sigma / S$$

Where,

σ = Standard deviation of the response S = Slope of the calibration curve

Forced degradation studies

According to the ICH guidelines, stress testing was carried out upon exposure to extreme stress conditions of acid, base, peroxide,

thermal, UV, and hydrolytic degradation. Later studied the main peak of the drug for peak purity by calculating the percentage of degraded amount and percentage of the active amount.

Acid degradation

Added 1 ml of 2N HCl to 1 ml of Ethinyl estradiol and Gestodene stock solutions and refluxed at 60 °C for 30 min. Later 2N NaOH was added to neutralize the solutions and diluted to volume to obtain 20µg/ml and 50µg/ml solutions, respectively. Cooled the solutions to room temperature and filtered. Injected the sample into the HPLC system and recorded the chromatograms.

Alkaline degradation

Added 1 ml of 2N sodium hydroxide to 1 ml of Ethinylestradiol and Gestodene stock solutions, refluxed at 60 °C for 30 min. Later, 1 ml of 2N HCl was added for neutralization and diluted to volume to obtain 20µg/ml and 50µg/ml solutions, respectively. Cooled and then filtered through a 0.45µm membrane filter. Injected into the HPLC system and recorded the chromatograms.

Oxidation degradation

Added 1 ml of 20% Hydrogen peroxide to 1 ml of Ethinylestradiol and Gestodene stock solutions and kept for 30 min at 60 °C. Later diluted to volume to obtain 20µg/ml and 50µg/ml solution. Cooled and filtered using 0.45µm membrane filter. Injected the sample into the HPLC system and recorded the chromatograms.

Dry heat degradation

1 ml of standard drug solution was kept in an oven for six h at 60 °C later, diluted to final volume to obtain 20µg/ml and 50µg/ml solutions Ethinyl estradiol and Gestodene respectively. Cooled and filtered using 0.45µm membrane filter. Injected into the HPLC system and recorded the chromatograms.

Photodegradation

For this study, the stock solutions were exposed to UV light for 1d or 200Watt-hm-2 in a photostability chamber and later diluted to volume to obtain 20µg/ml and 50µg/ml solutions of Ethinyl estradiol and Gestodene, respectively, filtered through a 0.45µm membrane filter. Injected the solutions into the HPLC system and recorded the chromatograms.

Water degradation studies

Added 1 ml of distilled water to 1 ml of stock solution of Ethinyl estradiol and Gestodene and kept aside at 60 °C for 30 min. Later, diluted to volume to obtain 20µg/ml and 50µg/ml solutions of Ethinyl estradiol and Gestodene, respectively. Filtered the solutions and injected the mixed standard into the HPLC system, and recorded the chromatograms.

RESULTS AND DISCUSSION

Method development

For developing the present method, various columns like Symmetry and Zodiac columns were tried. But finally, Phenomenex Gemini C18(25×0.46 cm, 5 µm) was confirmed to be optimal since all the parameters are within the acceptance criteria like resolution, peak symmetry, and theoretical plates. Various mobile phases tried were water: methanol, Water: Acetonitrile, Phosphate buffer: Methanol, Phosphate buffer: Acetonitrile by varying proportions and at last, the Phosphate buffer: Acetonitrile (75:25 v/v) and the pH was adjusted to 3.6 using dilute orthophosphoric acid by maintaining the system at 1.0 ml/min flow rate at 237 nm UV detection was finalized as optimal. The optimized chromatogram of Ethinyl estradiol and Gestodene was displayed in fig. 3 and resulted in table 1.

In comparison to the previously reported methods [5, 8], the retention times of Ethinyl estradiol and Gestodene were observed to be more and required more analysis time for quantification. But the present established method requires lesser analysis time and consumes lesser solvents showing retention times of Ethinyl estradiol and Gestodene at 1.788 min and 3.475 min, which is more advantageous in pharmaceutical industries. This revealed that the developed method could be suitably applied for routine laboratory analysis.

Method validation

System suitability

All the efficiency parameters like theoretical plates were observed to be more than 7000 for Ethinyl estradiol and Gestodene drugs. The peak tailing was not more than 2.0. The %RSD for the five replicate injections was not more than 2.0 and ensured that the entire testing system and chemicals used could generate an accurate and precise result by showing all the efficiency parameters within the specified limits. Reported the results in Tables 2 and 3.

Linearity

The proposed method was confirmed to be linear in the concentration range of 10-50µg/ml and 25-125µg/ml for Ethinyl Estradiol and Gestodene, respectively, showing a correlation coefficient of 0.999, which was analyzed by linear regression analysis. The results proved that the analyte response is proportional to the analyte concentration in the selected concentration range. The calibration graphs of Ethinyl Estradiol and Gestodene are depicted in fig. 4 and fig. 5 and data in Tables 4 and 5, respectively.

Accuracy

The mean recovery values obtained at 50%, 100%, and 150% levels are 99.84% and 99.92% for Ethinyl estradiol and Gestodene, respectively, which are within the acceptance criteria. Thus, it confirmed the method's accuracy and reported the results in Tables 6 and 7.

Precision

The obtained %Relative standard deviation of intra-day precision and intermediate precision of Ethinyl estradiol and Gestodene was found to be not more than 2.0, which is within the acceptance criteria, indicating that the developed method is precise. Reported the measured results of intra-day precision in Tables 8,9 and intermediate precision in Tables 10, 11, 12, and 13.

Robustness

The results revealed that the method is robust upon slight changes in flow rate conditions and even by the ±5% organic phase for Ethinyl estradiol. For Gestodene, upon decreasing the %organic phase, the retention time was more, and retention time was less for more %organic phase. There was no significant change in resolution, asymmetry, and plate count. Reported observed results in Tables 14 and 15.

Limit of detection and limit of quantitation

Calculated the LOD and LOQ using the standard deviation of intercepts and slope of the calibration curve. The measured detection limit and quantitation limit was found to be 1.399µg/ml and 4.242µg/ml for Ethinyl estradiol and 3.909µg/ml and 11.848µg/ml for Gestodene, respectively. Thus, the present method is confirmed to be highly sensitive.

Assay determination of ethinyl estradiol and gestodene

The %Assay of Ethinyl estradiol and Gestodene in Femovan tablets is 100.6% and 98.63%, respectively, and are within the specified limits, which confirmed that the developed method could be successfully applied for the assay of pharmaceutical dosage forms, and reported the results in Tables 16, 17, 18, and 19.

Degradation studies

Upon degradation study, observed that Ethinyl estradiol and Gestodene had undergone degradation under all stress conditions. The calculated % degraded amount was within the acceptance criteria. It was observed that the drug was more susceptible to photolysis showing the highest degradation. The successful separation of the obtained degradation products from the active pharmaceutical ingredients without any interference confirmed the specificity. Thus, it proved the stable nature of the developed

method. Presented the acidic, basic, oxidative, thermal, photolytic, and hydrolytic degradation chromatograms in fig. 6, 7, 8, 9, 10, and 11, respectively. Reported the calculated results for Ethinyl estradiol and Gestodene in tables 20 and 21, respectively.

All the method validation parameters are within the acceptance criteria and assured sufficient precision and accuracy. A good linear relationship was observed in the concentration range of 10-50µg/ml and 25-125µg/ml for Ethinyl estradiol and Gestodene, respectively. The detection limit for Ethinyl estradiol was found to be 1.399µg/ml indicating the high sensitivity of the developed method compared to the reported method [6]. The recovery of the analyte was also found to be more than the reported methods [5, 8], indicating a high degree of accuracy. The non-interference of the degraded products with the active pharmaceutical ingredients revealed the stability-indicating nature of the developed method.

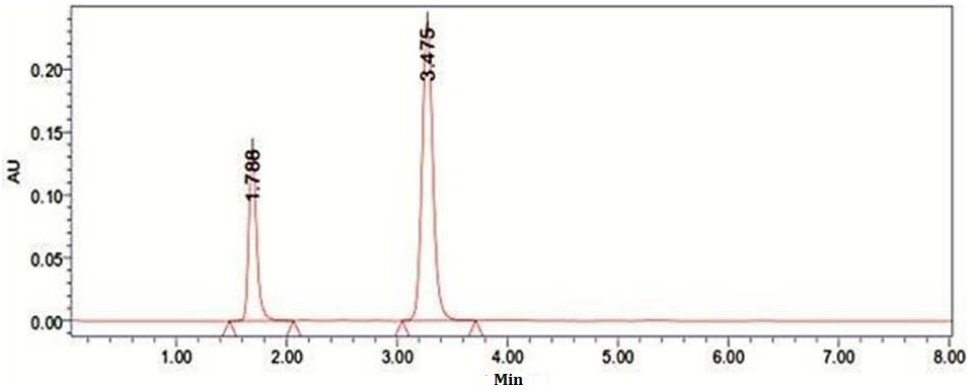

Fig. 3: Optimized chromatogram of ethinyl estradiol and gestodene

Table 1: Optimized chromatogram result

S. No.	Drug	Retention time (min)	Area of the peak	Height of the Peak	Resolution	Peak asymmetry	No. of theoretical plates
1	Ethinylestradiol	1.788	558647	7658		1.26	7854
2	Gestodene	3.475	7986585	48546	7.12	1.35	8865

Table 2: System suitability data of Ethinyl estradiol

Drug	S. No.	Retention time (min)	Analyte response	Theoretical plates(N)	Peak asymmetry
Ethinyl estradiol	1	1.788	558748	7854	1.26
	2	1.792	556985	7845	1.29
	3	1.793	557849	7896	1.28
	4	1.794	559865	7824	1.29
	5	1.791	558498	7869	1.27
	*Mean (n=5)		558389		
	±SD (n=5)		1070.298		
	% RSD (n=5)		0.191676		

*Mean of five determinations, SD: Standard Deviation, RSD: Relative Standard Deviation

Table 3: System suitability data of gestodene

Drug	S. No.	Retention time (min)	Area of the peak	No. of theoretical plates	Peak asymmetry	Resolution
Gestodene	1	3.438	7986952	8856	1.36	7.13
	2	3.446	7958484	8874	1.32	7.14
	3	3.444	7986958	8896	1.39	7.15
	4	3.440	7984874	8874	1.34	7.16
	5	3.442	7986989	8859	1.38	7.15
	*Mean (n=5)		7980851			
	±SD (n=5)		12536.55			
	% RSD (n=5)		0.157083			

*Mean of five determinations, SD: Standard Deviation, RSD: Relative Standard Deviation

Table 4: Linearity data of ethinylestradiol

Concentration µg/ml	Average peak Area
10	253898
20	501647
30	751256
40	985789
50	1235898

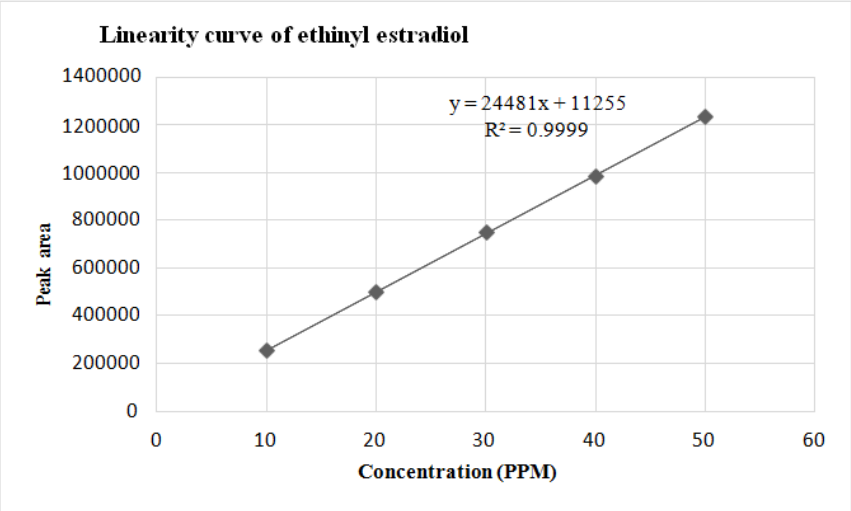

Fig. 4: Linearity plot of Ethinyl estradiol

Table 5: Linearity data of gestodene

Concentration µg/ml	Average peak area
25	3252897
50	6316585
75	9438787
100	12387436
125	15365874

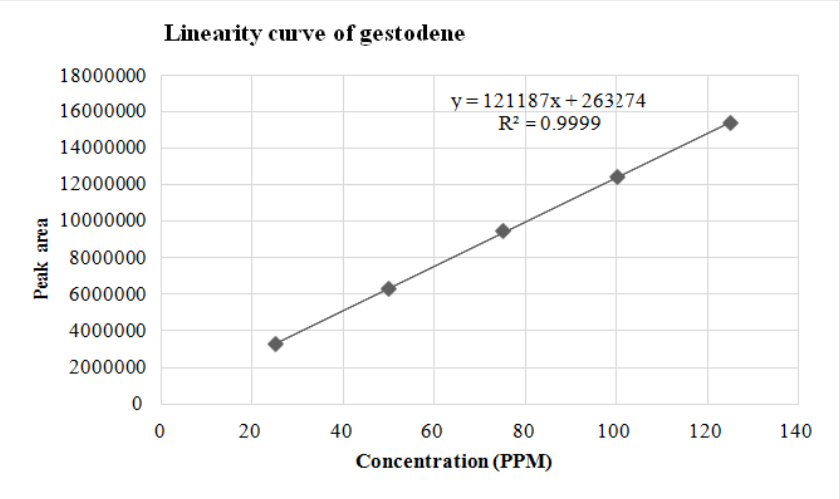

Fig. 5: Linearity plot of gestodene

Table 6: Ethinyl estradiol accuracy data

Drug	Spiking level	Peak area	*Average area (n=3)	Amount added (µg/ml)	Amount obtained (µg/ml)	*Percentage recovery	Average recovery (n=9)
Ethinyl estradiol	50%	253848 252856 253874	253526	10	9.89	98.9%	99.84%
	100%	501563 501689 502324	501858.67	20	20.04	100.2%	
	150%	748584 749897 748468	748983	30	30.13	100.43%	

*Mean of three determinations

Table 7: Gestodene accuracy data

Drug	Spiking level	Peak area	*Average area (n=3)	Amount added (µg/ml)	Amount found (µg/ml)	*Percentage recovery (n=3)	Average recovery (n=9)
Gestodene	50%	3314535 3312453 3318542	3315176.67	25	25.183	100.7%	99.92%
	100%	6287487 6279654 6286475	6284538.67	50	49.68	99.37%	
	150%	9328748 9315462 9325645	9323285	75	74.76	99.68%	

*Mean of three determinations

Table 8: Repeatability data of Ethinyl estradiol

Drug	Injection	Retention time (min)	Peak area	No. of theoretical plates	Peak asymmetry
Ethinyl estradiol	1	1.789	558748	7896	1.26
	2	1.780	558698	7845	1.28
	3	1.790	558475	7892	1.29
	4	1.791	558698	7849	1.27
	5	1.792	558265	7829	1.28
	*Mean (n=5)		558576.8		
	±SD (n=5)		203.8816		
	%RSD (n=5)		0.0365		

*Mean of five determinations, SD: Standard Deviation, %RSD: Relative Standard Deviation

Table 9: Repeatability data of gestodene

Drug	Injection	Retention time (min)	Peak area	No. of theoretical plates	Peak asymmetry
Gestodene	1	3.408	7986985	8856	1.36
	2	3.414	7985487	8849	1.37
	3	3.419	7985468	8874	1.39
	4	3.428	7968547	8957	1.38
	5	3.435	7982564	8965	1.37
	*Mean (n=5)		7981810		
	±SD (n=5)		7585.473		
	%RSD (n=5)		0.095034		

*Mean of five determinations, SD: Standard Deviation, %RSD: Relative Standard Deviation

Table 10: Day 1 Intermediate precision data of ethinylestradiol

Drug	Injection	Retention time (min)	Peak area	No. of theoretical plates	Peak asymmetry
Ethinyl estradiol	1	1.792	558965	7859	1.29
	2	1.789	558476	7895	1.28
	3	1.787	558947	7829	1.27
	*Mean (n=3)		558796		
	±SD (n=3)		277.2742		
	%RSD (n=3)		0.04962		

*Mean of three determinations, SD: Standard Deviation, %RSD: Relative Standard Deviation

Table 11: Day 1 Intermediate precision data of gestodene

Drug	Injection	Retention time (min)	Peak area	No. of theoretical plates	Peak asymmetry
Gestodene	1	3.435	7986986	8849	1.38
	2	3.477	7985985	8879	1.37
	3	3.482	7898654	8896	1.39
	*Mean (n=3)		7957208		
	±SD (n=3)		50712.01		
	%RSD(n=3)		0.637309		

*Mean of three determinations, SD: Standard Deviation, %RSD: Relative Standard Deviation

Table 12: Day 2 Intermediate precision data of ethinyl estradiol

Drug	Injection	Retention time (min)	Peak area	No. of theoretical plates	Peak asymmetry
Ethinyl estradiol	1	1.793	568965	7989	1.28
	2	1.789	569854	7986	1.29
	3	1.790	569878	7994	1.28
	*Mean (n=3)		569565.7		
	±SD (n=3)		520.331		
	%RSD (n=3)		0.091356		

*Mean of three determinations, SD: Standard Deviation, %RSD: Relative Standard Deviation

Table 13: Day 2 Intermediate precision data of gestodene

Drug	Injection	Retention time (min)	Peak area	No. of theoretical plates	Peak asymmetry
Gestodene	1	3.478	8045652	8987	1.38
	2	3.473	8065879	8959	1.39
	3	3.474	8075847	8937	1.37
	*Mean (n=3)		8062459		
	±SD (n=3)		15385.22		
	%RSD		0.190825		

*Mean of three determinations, SD: Standard Deviation, %RSD: Relative Standard Deviation

Table 14: Robustness data of ethinyl estradiol

Slightly changed parameters		Peak area	Retention time (min)	No. of theoretical plates	Peak asymmetry
Flow rate	1.0	558647	1.788	7854	1.26
(ml/min)	0.9	636589	1.867	7978	1.27
	1.1 More flow rate of 0.9 ml/min	535685	1.744	7576	1.39
% of	20	548576	1.831	7367	1.37
Acetonitrile	30	525874	1.874	7296	1.28

Table 15: Robustness study data of gestodene

Slightly changed parameters		Peak area	Retention time (min)	No. of theoretical plates	Peak asymmetry
Flow rate	1.0	7986585	3.475	8865	1.35
(ml/min)	0.9	8265847	3.724	9152	1.49
	1.1	7658745	3.097	8685	1.38
% Acetonitrile	20	7758498	6.242	8475	1.37
	30	7659854	2.402	8369	1.36

Table 16: Assay data of Ethinyl estradiol standard

Drug	Injection	Retention time (min)	Peak area	No. of theoretical plates	Peak asymmetry
Standard	1	1.791	558698	7854	1.26
Ethinyl	2	1.794	558674	7822	1.28
estradiol	3	1.793	558694	7895	1.29
	4	1.792	558748	7826	1.27
	5	1.788	558962	7849	1.26
	*Mean (n=5)0		558755.2		
	±SD (n=5)		118.7737		
	%RSD (n=5)		0.021257		

*Mean of five determinations, SD: Standard Deviation, %RSD: Relative Standard Deviation

Table 17: Assay data of gestodene standard

Drug	Injection	Retention time (min)	Peak area	No. of theoretical plates	Peak asymmetry	Resolution
Gestodene	1	3.442	7986852	8857	1.36	7.15
standard	2	3.440	7985685	8874	1.34	7.14
	3	3.444	7984573	8892	1.35	7.16
	4	3.446	7986365	8849	1.39	7.15
	5	3.438	7989856	8825	1.35	7.18
	*Mean (n=5)		7986666			
	±SD (n=5)		1977.644			
	%RSD (n=5)		0.024762			

*Mean of five determinations, SD: Standard Deviation, %RSD: Relative Standard Deviation

Table 18: Assay data of ethinyl estradiol sample

Drug	Injection	Retention time (min)	Peak area	No. of theoretical plates	Peak asymmetry
Ethinyl	1	1.791	562453	7965	1.28
estradiol	2	1.791	563124	7982	1.29
Sample	3	1.794	563256	7985	1.29
	*Mean (n=3)		562944.33		
	±SD (n=3)		430.59		
	%RSD (n=3)		0.076		

*Mean of three determinations, SD: Standard Deviation, %RSD: Relative Standard Deviation

Table 19: Assay data of gestodene sample

Drug	Injection	Retention time (min)	Peak area	No. of theoretical plates	Peak asymmetry
Gestodene sample	1	3.434	8012432	7264	1.39
	2	3.442	8023654	7285	1.38
	3	3.440	8012543	7293	1.37
	*Mean (n=3)		8016209.67		
	±SD (n=3)		6447.22		
	%RSD (n=3)		0.08		

*Mean of three determinations, SD: Standard Deviation, %RSD: Relative Standard Deviation

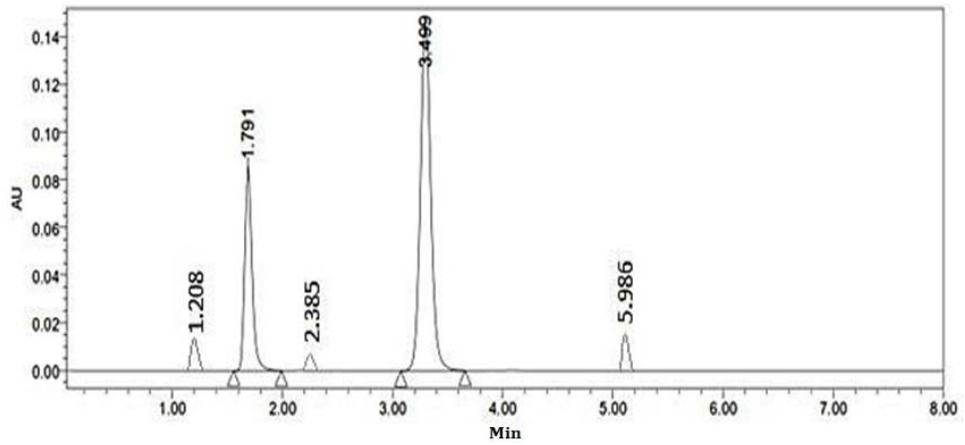

Fig. 6: Ethinylestradiol and Gestodene acidic degradation chromatogram

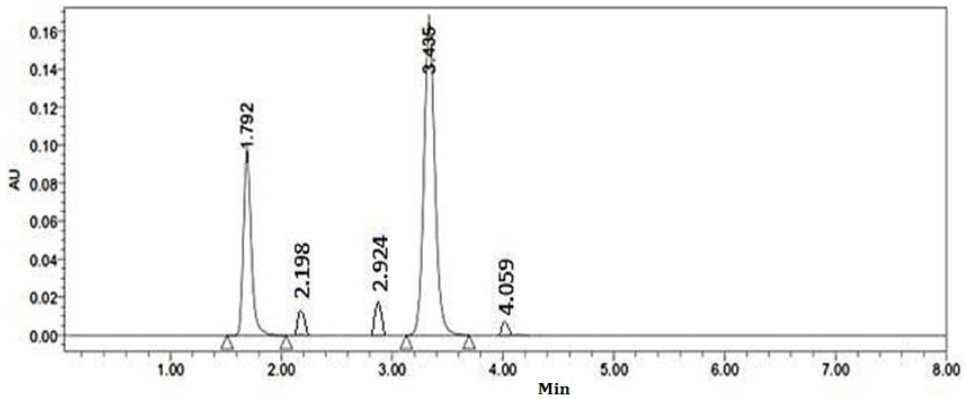

Fig. 7: Ethinyl estradiol and gestodene alkaline degradation chromatogram

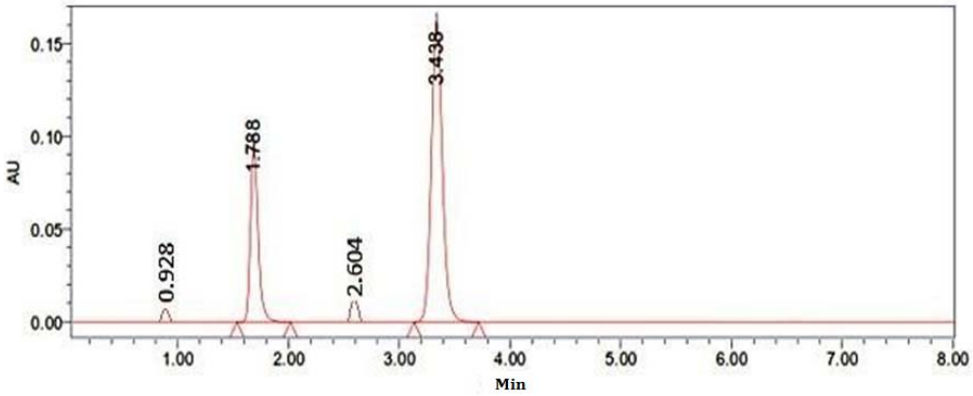

Fig. 8: Ethinyl estradiol and gestodene oxidative degradation chromatogram

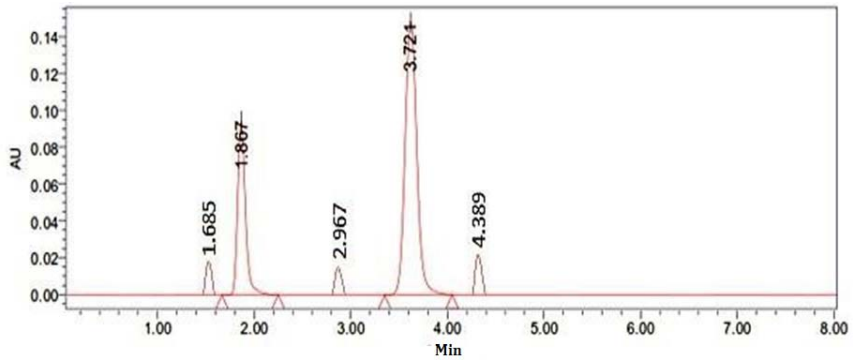

Fig. 9: Ethinylestradiol and gestodene thermal degradation chromatogram

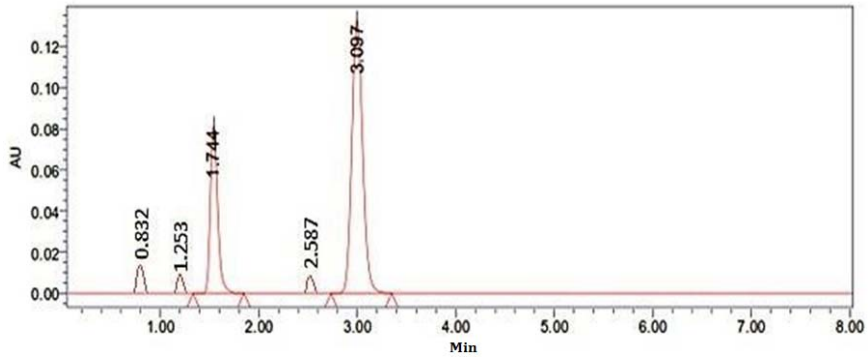

Fig. 10: Ethinyl estradiol and gestodene photolytic degradation chromatogram

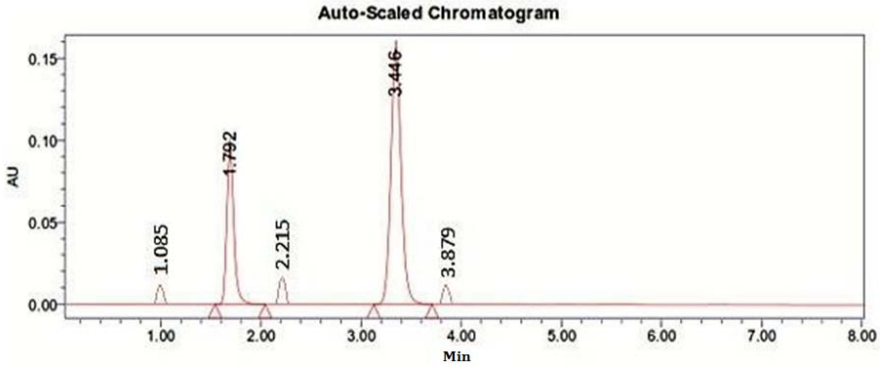

Fig. 11: Ethinyl estradiol and gestodene hydrolytic degradation chromatogram

Table 20: Forced degradation studies data for Ethinyl estradiol

S. No.	Stress condition	Peak area	% of degraded amount	% of active amount	Total % of amount
1	Standard	558647	0	100%	100%
2	Acidic	476693.48	14.67	85.33	100%
3	Basic	515351.85	7.75	92.25	100%
4	Oxidative	497307.55	10.98	89.02	100%
5	Thermal	486413.94	12.93	87.07	100%
6	Photolytic	395186.88	29.74	70.74	100%
7	Water	491832.81	11.96	88.04	100%

Table 21: Forced degradation studies data for gestodene

S. No.	Stress condition	Peak area	% of degraded amount	% of active amount	Total % of amount
1	Standard	7986585	0	100%	100%
2	Acidic	6733489.81	15.69	84.31	100%
3	Basic	7271785.64	8.95	91.05	100%
4	Oxidative	7032188.09	11.05	88.05	100%
5	Thermal	6880442.98	13.85	86.15	100%
6	Photolytic	5154541.96	35.46	64.54	100%
7	Hydrolysis	7108859.31	10.99	89.01	100%

CONCLUSION

The established present method revealed that it is simple, selective, specific, and can generate accurate and precise results. Moreover, the shorter duration of analysis time and lesser mobile phase consumption confirmed that the method is rapid and economical. The successful separation of the forced degradation products from the active pharmaceutical ingredients without any interference confirmed the stability-indicating nature of the developed method. Hereupon, the present method can be satisfactorily applied for the routine laboratory simultaneous estimation of Ethinyl estradiol and Gestodene.

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AUTHORS CONTRIBUTIONS

We declare that the author's present work was done and is an original work. D. Suchitra has carried out the research work. Professor Battu Satyanarayana guided, proofread the manuscript, suggested the necessary corrections, and helped in writing the manuscript.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. https://en.m.wikipedia.org/wiki/Ethinylestradiol [Last accessed on 10 Feb 2021]
2. https://pubchem.ncbi.nlm.nih.gov/compound/Ethinylestradiol [Last accessed on 10 Feb 2021]
3. https://en.m.wikipedia.org/wiki/Gestodene [Last accessed on 10 Feb 2021].
4. https://pubchem.ncbi.nlm.nih.gov/compound/Gestodene [Last accessed on 10 Feb 2021]
5. Kanchan Nautiyal, Ramakrishna K. Analytical method development and validation for simultaneous determination and quantification of ethinyl estradiol and gestodene in combined tablet dosage form by RP-HPLC. J Chem Pharm Sci 2014;6:74-80.
6. Aleksandra Laban, Slavko Markovic, Milena Stankov, Predrag Djurdjevic. Simultaneous determination of gestodene and ethinyl estradiol in contraceptive formulations by RP-HPLC. Anal Lett 2004;37:273-82.
7. Xiong Z, Sun X, Huo T, Li N, Zheng Y, Sun Y. Development and validation of UPLC-MS/MS method for simultaneous determination of gestodene and ethinyl estradiol in rat plasma. Biomed Chromatogr 2010;24:160-8.
8. Kishore Kumar I, Jayaprakash P, Sivannarayana Reddy V, Sriramulu J. A stability-indicating high-performance liquid chromatographic method for separation and quantification of impurities in ethinylestradiol and gestodene tablets. J Pharm Biol 2017;7:33-40.
9. 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 the combined dosage form. Pharm Anal Acta 2013;4:1-5.
10. ICH, Q2B-Validation of Analytical procedures; Methodology, International Conference on Harmonization; 1996.
11. International Conference on Harmonization, ICH Q1 A [R2]; Stability Testing of New Drug substances and products; 2003.
12. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC method development. 2nd ed. Hoboken: John Wiley and Sons, Inc; 1997.
13. Vikas Kumar Pal, Yogendra Pal. Analytical method development and method validation for determination assay and content uniformity of levonorgestrel by reversed-phase high-performance liquid chromatography. Asian J Pharm Clin Res 2020;13:101-7.
14. Majan Naim, Aejaz Ahmed, Khan GJ. Stability indicating reverse-phase High-Performance liquid chromatography method development and validation for simultaneous estimation of Telmisartan and Benidipine hydrochloride in the pharmaceutical dosage form. Asian J Pharm Clin Res 2018;11:342-50.