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Research Article

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Analytical method development and validation for simultaneous determination and quantification of Ethinyl Estradiol and Gestodene in combined tablet dosage form by RP-HPLC

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ABSTRACT

The present paper describes a simple isocratic reverse phase HPLC method for the simultaneous determination and Quantification of Gestodene and Ethinyl Estradiol in Pharmaceutical dosage forms. The Seperation and estimation was achieved with normal C_{18}(250 x4.6mm,5µ) columns using a mixture of acetonitrile,methanol and water(35:15:50) as the mobile phase at a flow rate of 2.0 ml/min and detection was carried out at 210 nm.The seperation was achieved within 18 mins and retention of Gestodene and Ethinyl Estradiol is 10.5 and 7.4 mins respectively. The developed method was validated and linearity of the proposed method was in the range of 1.88-11.33mg/mL(r-0.9994) for Gestodene, 0.755-4.531 mg/mL (0.99973) for Ethinyl Estradiol. The proposed method was suitable for simultaneous Determination and Quantification of Gestodene and Ethinyl Estradiol in combined pharmaceutical dosage forms.

Keywords: Development, Validation, Tablet, Assay, HPLC, Column

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INTRODUCTION

Gestodene is chemically (8R, 9S, 10R, 13S, 14S, 17R)-13-Ethyl-17-ethynyl-17-hydroxy-1-14-decahydro Cyclopenta[a]phenanthren-3-one[1] and Ethinyl Estradiol is 19-nor-17α -pregna-1, 3,5(10)-trien-20-yne-3, 17-diol[2] and both are progestogen hormonal. It's usually administered orally in the form of tablets. Most of Literatures were published for single dosage forms by spectrometric method[3-9] and few HPLC method was reported but technique adopted was not conventional and using special detection technique[10-14] and most reported method is by LC-MS[16-22].So sincere effort was made to develop an simple and easy Reverse HPLC method and method was validated[23] and statistical parameters were computed to prove the developed method was accurate and precise in nature. The method has been satisfactorily applied to the determination and quantification of Gestodene and Ethinyl Estradiol in pharmaceutical preparations.

EXPERIMENTAL SECTION

Materials and methods

Required Equipments like HPLC (Agilent and shimadzu) equipped with auto sampler and photodiode array detector. Column SB (250x4.6mm, 5µ), Millipore filtration kit, mobile phase reservoir, sample filtration assembly and glass wares were used throughout the experiment.

Chemicals and solvents

Acetonitrile, Methanol was from Merck HPLC grade and water filtered through Millipore was used for preparation of mobile phase and diluents. Pure sample of Gestodene and Ethinylestradiol was a obtained as a gift from local pharmaceutical industry. Commercial samples of tablets Femoden containing drug Gestodene and Ethinylestradiol were purchased and imported from pharmacy stores.

Chromatographic parameters
Equipment: HPLC with PDA detector
Column: SB C_{18} (250x4.6, 5µ)
Flow rate: 2.0 ml/min
Wavelength: 210 nm
Injection Volume: 100 µL
Column Temperature: 30°C
Run time: 18 mins
Auto sampler Temp: 10°C

Preparation of Mobile phase
Prepared a homogeneous mixture of 350 ml of Acetonitrile (35%), 150 ml of methanol (15%) and 500ml (50%) of Milli Q water and degas in ultrasonic water bath for 5 mins and filtered through 0.45 u filter under vacuum filtration

Diluent:
Mixed and degassed Milli Q water and acetonitrile in the ratio of 50:50

Preparation of solutions
***Stock Solutions*:**
Accurately weighed and transferred 47 mg of Gestodene working standard into 50 ml of volumetric flask. Dissolved and diluted to volume with methanol, then diluted 4 ml of above solution to 50 ml with diluent. Weighed about 50 mg of Ethinyl estradiol working standard into 100 ml volumetric flask and dissolved, diluted to volume with methanol, then diluted 3 ml of above solution to 50 ml with diluent.

Standard solution:
Pipetted out 5 ml of Gestodene stock solution and 5 ml of Ethinyl estradiol stock solution to 50 ml with diluent.

***Preparation of Test solution*:**
Accurately weighed 20 tablets and transferred into a 200 ml volumetric flask. Added 10 ml water and sonicated to disintegrate and then added another130 ml of diluent and sonicated for 20 minutes and made up to volume. Filtered the sample through 0.2µ membrane, discarded first few ml of sample and remaining used for analysis.

Method Validation
The proposed method was validated as per ICH guidelines. The preparations were adopted as mentioned in experiment section.

System suitability
System suitability was performed by injecting standard preparation, and measured the system suitability parameters like theoretical plates, tailing factor and % RSD were evaluated and all the results are well within the criteria and results obtained compiled in *Table-1* and representative chromatogram in *Figure-1,2&3.*

Figure 1: Typical Chromatogram of Blank preparation

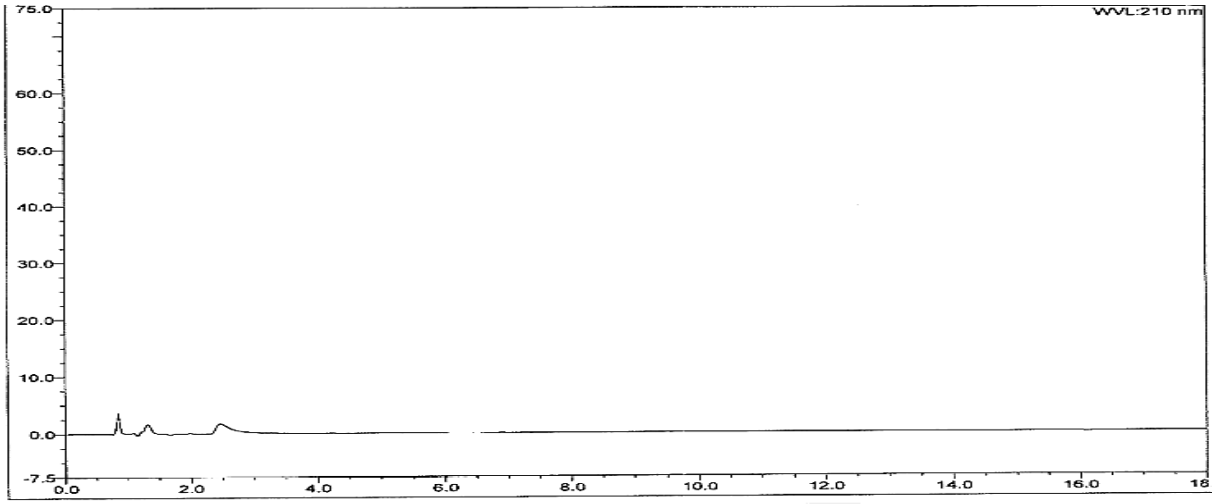


Figure 2:Typical chromatogram of standard solution (Gestodene and Ethinyl Estradiol)

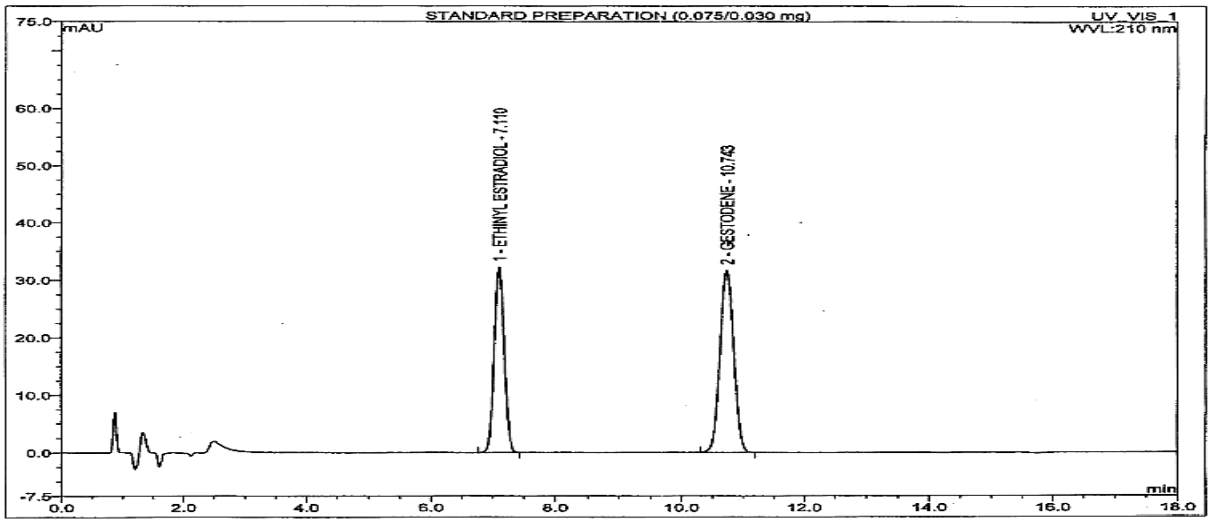


Figure 3: Typical chromatogram of Placebo solution

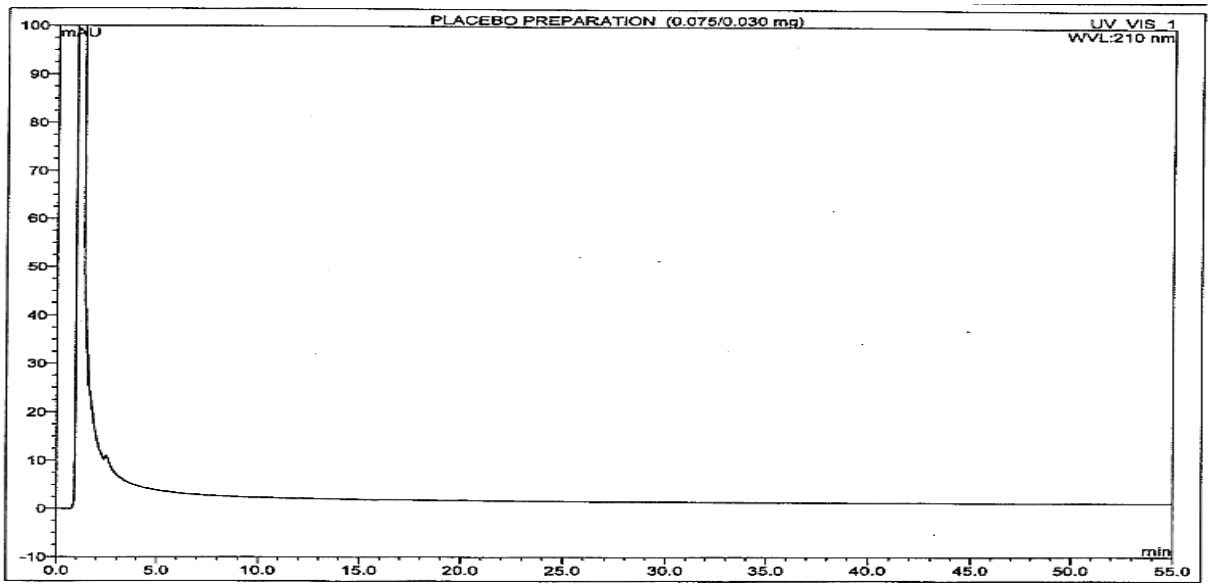


Table 1: Results of System Suitability Study

Parameters	Limits	Gestodene	Ethinyl Estradiol
Theoretical Plates	NLT 2000	11000	9100
Tailing factor	NMT 2.0%	0.98	1.2
%RSD	NMT 2.0%	0.54	0.70

Precision

System Precision:

System precision was performed by injecting standard solutions in six replicates, the area response and % RSD were calculated and results were compiled in *Table-2*.

Method precision:

This experiment was performed by injecting six sample preparations and percentage assay label claim was calculated and % RSD were calculated and results were compiled in *Table-2* and Figure-4, which indicates the developed method were precise.

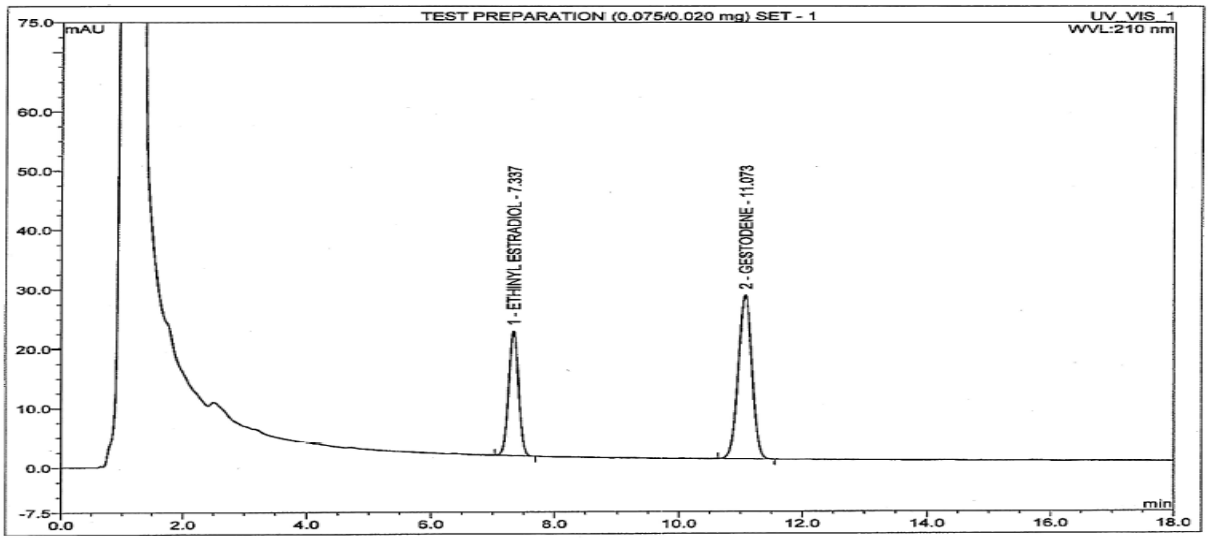
Intermediate Precision (Ruggedness):

This experiment was performed by injecting six sample preparations by Different analyst using different instrument and different column on different day and percentage assay of label claim was calculated along with %RSD and results were compiled in Table-2 and value indicates the precision of method.

Table 2: Results and values of System, Method and Intermediate Precision

S.No	Limits	Gestodene	Ethinyl Estradiol	% Assay(Gestodene)		% Assay (Ethinyl Estradiol)	
		System precision		Method Precision	Intermediate precision	Method Precision	Intermediate Precision
1	%RSD- NMT 2.0%	468.666	386.352	97.0	97.6	96.3	98.1
2		472.327	391.516	96.5	97.2	95.6	97.7
3		473.649	392.743	97.3	97.7	96.2	98.4
4		473.084	393.336	97.0	97.5	95.9	98.0
5		473.320	392.974	98.7	97.4	98.0	97.9
6		470.524	390.514	96.9	97.4	96.4	98.4
Mean		471.928	391.239	97.2	97.5	96.4	98.1
%RSD		0.41	0.67	0.79	0.18	0.87	0.28

Figure 4: Typical Chromatogram of Precision study



Linearity

Linearity study was performed by preparing standard solutions in the concentration range of 25% to 150% of working concentration. The area response was calculated and linearity graph derived and its slope, intercept and correlation coefficient was calculated and found well within the acceptance criteria of 0.995 which indicates the method was linear in nature and results were mentioned in *Table-3* and representative graphs and chromatograms were highlighted in *Figure-5,6 &7.*

Figure 5: Linearity graph of Gestodene

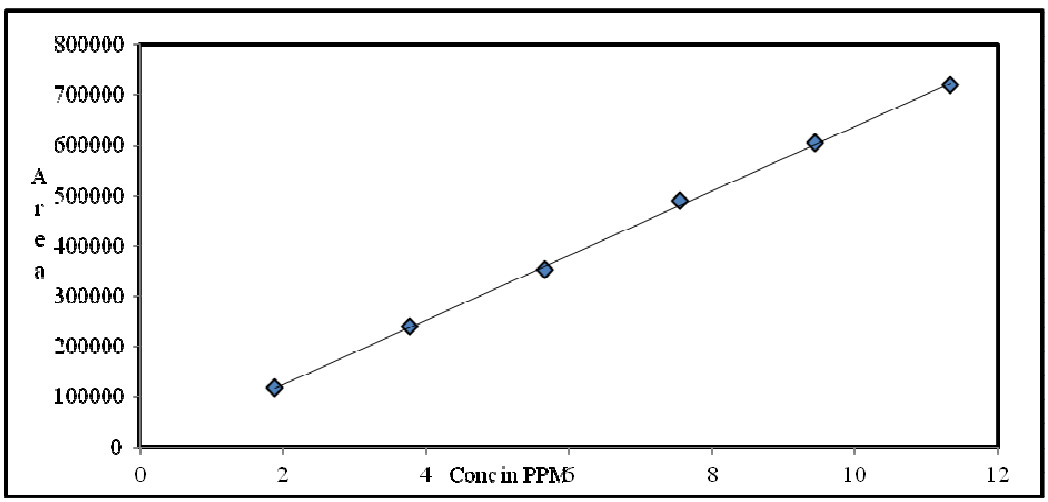


Table 3: Results of Linearity and Range study

	Gestodene	Ethinyl Estradiol
Concentration	1.88-11.33	0.755-4.531
Slope	64060.822	124214.495
Intercept	-5123.6618	-7716.2793
Corr .Coefficient	0.99948	0.99973
Residual Sum of squares	96046087	84393792

Figure 6:Linearity graph of Ethinyl estradiol

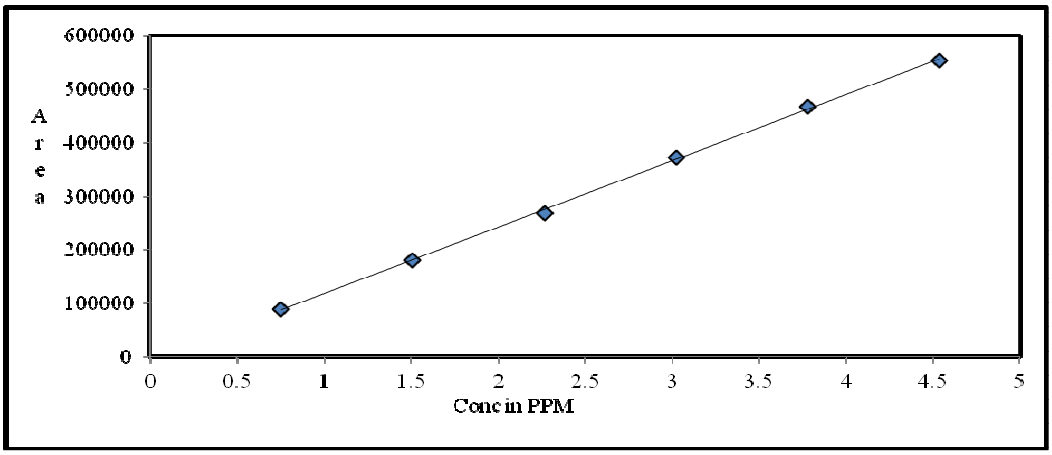


Figure 7:Typical chromatogram of Linearity

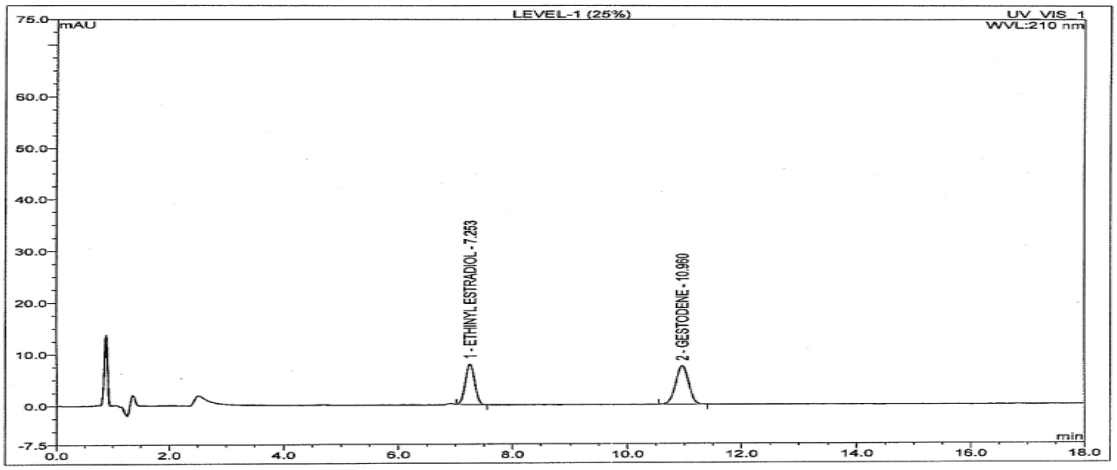


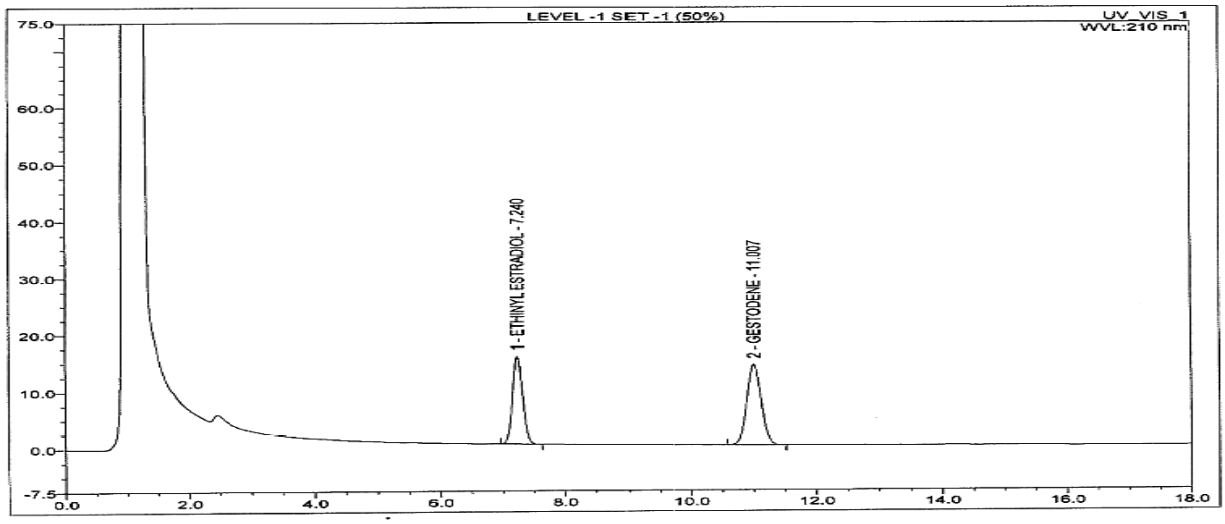
Table 4: Results of Accuracy (Recovery study)

% Level	Gestodene			Ethinyl Estradiol		
	Amount added(ppm)	Amount Recovered (ppm)	% Recovery	Amount added(ppm)	Amount Recovered (ppm)	% Recovery
50	3.7689	3.7258	98.9	1.5015	1.4900	99.2
	3.7689	3.7048	98.3	1.5015	1.4824	98.7
	3.7689	3.7162	98.6	1.5015	1.4841	98.8
	% Mean Recovery		**98.6**	**% Mean Recovery**		**98.9**
	%RSD		**0.30**	**%RSD**		**0.27**
100	7.5377	7.4797	99.2	3.0030	2.9678	98.8
	7.5377	7.4742	99.2	3.0030	2.9599	98.6
	7.5377	7.4186	98.4	3.0030	2.9444	98.0
	% Mean Recovery		**98.9**	**% Mean Recovery**		**98.5**
	%RSD		**0.47**	**%RSD**		**0.42**
150	11.3066	11.2932	99.9	4.5045	4.4438	98.7
	11.3066	11.2413	99.4	4.5045	4.4300	98.3
	11.3066	11.2336	99.4	4.5045	4.4272	98.3
	% Mean Recovery		**99.6**	**% Mean Recovery**		**98.4**
	%RSD		**0.29**	**%RSD**		**0.23**

Accuracy Study (Recovery Study)

Recovery study was performed by spiking known concentration (50%-150%) with placebo of Gestodene and Ethinyl estradiol in triplicate preparations and recovery was calculated and well within the acceptance criteria of 95% to 105% and shows the method was accurate and precise. The results of recovery study were compiled in Table-4 and representative chromatogram in Figure-8.

Figure 8: Chromatogram of Recovery (Gestodene and Ethinyl estradiol (100%level)



Stability of Analytical solution

Test and standard preparation was prepared and analysed initially and at various intervals which was stored at 10°C and % Area difference with respect to initial were monitored and found all were within the limit of ±2 of area difference and solution stability was established up to 12 hours and concluded that analytical solution was stable up to 12 hrs.

Robustness

The Robustness was performed by deliberately changing the chromatographic condition and impact on system suitability parameters and assay values were monitored and results were recorded and found that the values were well within the acceptance criteria and proved the robustness of the analytical method against the deliberate changes.

RESULTS AND DISCUSSION

The mobile phase consisting of mixture of Water: Methanol : Acetonitrile of definite ratio with isocratic elution, flow rate with 2ml/min was optimized for well Seperation of Gestodene and Ethinyl estradiol with shorter analysis time, UV absorption pattern shows the both the compound were absorbed appreciably at 210 nm, so wavelength was selected as a detection wavelength for analysis, to maintain the sharpness of eluent peaks ,the column temperature was maintained 30°C ,as analytical solution has stability variation on storage, so sampler temperature condition was kept as 10°C.The retention times for Ethinyl Estradiol and Gestodene were 7.0 min and 11.0 min respectively. The proposed method was successfully applied to identification and quantification of Ethinyl Estradiol and Gestodene in dosage forms.

The method validation was performed; specificity results were reflecting there was no interference of blank and its eluent peaks. Linearity and range for Ethinyl Estradiol and Gestodene was in the range of 0.75 – 4.53 ppm and 1.88 – 11.33 ppm respectively and correlation coefficient for both the eluent was 0.99973 and 0.99948 respectively. The precision study results was included as method precision which has results of 0.87 and 0.79% RSD for Ethinyl Estradiol and Gestodene respectively and intermediate precision result were 0.28 to 0.18% RSD were well within the acceptance criteria. The recovery of Ethinyl Estradiol and Gestodene was studied in spiking eluents in placebo on proposed method was studied and results were in the range 98.0-99.5% which indicates that the test method has acceptable level of accuracy and precision. The statistical results were compiled, proved that proposed method was specific, linear, accurate and precise in nature to quantify Ethinyl Estradiol and Gestodene in Drug dosage form.

CONCLUSION

The Developed reverse phase HPLC method was optimized based on shapes and resolutions of both Ethinyl Estradiol and Gestodene and method was validated, based on validation results and interpretation of values, it is concluded that this RP HPLC was simple, accurate and precise and method presented in this paper can be successfully used for routine monitoring and quantification of Ethinyl Estradiol and Gestodene in Dosage forms.

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