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Research Article

Cost Effective, Efficient and Stability indicating Method Development and Validation for determination of related substances for Levonorgestrel and Ethinyl Estradiol Tablets

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Abstract

The objective of this research was to develop and validate a simple, specific and accurate reverse phase of high performance of liquid chromatographic method for the determination of levonorgestrel (LVG) and ethinylestradiol (EE) in tablets. The chromatographic system included column Sun Fire ODS (150 mm × 4.6 mm i.d., particle size at 5 µm), mobile phase consisting of acetonitrile: methanol: aquabidest (60:15:25) with the flow rate of 1 mL/minute and effluents monitored at 230 nm. The validation of RP HPLC method for the simultaneous determination of LVG and EE was determined by accuracy, precision, linearity, and limit of detection (LOD) as well as the limit of quantitation (LOQ) parameters. The linearity range of both drugs was 1-70 µg/mL and 2-14 µg/mL for LVG and EE, respectively. The recoveries of LVG and EE were at 101.78% and 102.44% with the coefficients of variation of 0.94% and 1.92%, successively. The LOD of LVG and EE value were of 0.84 µg/mL and 0.03 µg/mL, and LOQ value were of 2.79 and 0.09µg/mL, respectively.

Keywords: Levonorgestrel (LVG), Ethinylestradiol, Method Validation, Method Validation, HPLC

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INTRODUCTION:

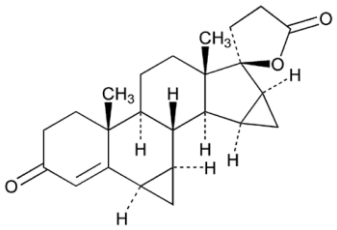
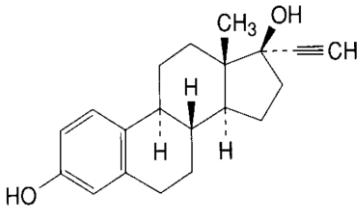
One method of contraception is the method of low-dose combined oral contraceptives (COCs), a combination of low-dose COCs containing synthetic estrogens such as ethinyl estradiol and synthetic progestogens, such as levonorgestrel or Norethisterone. Examples of contraceptive drugs, including COCs, are a combination of oral contraceptives of ethinyl estradiol and levonorgestrel. This combination of drugs works synergistically by suppressing gonadotropin and inhibiting ovulation[1-3].

Levonorgestrel and Ethinyl Estradiol Tablets product is official in USP. But the Assay, Dissolution method is given in USP-32 but method for related substances test was not available in USP. Hence in-house method was developed for related substances test using HPLC with UV detector. The API sources used in the product are Levonorgestrel and Ethinylestadiol (Schering)[4-6]. For both the API's all the impurities given in API supplier COA and the potential degradant impurities mentioned in DMF are considered for method development. Different trials were taken to separate all impurities and it is discuss in details under RS development. All the impurities checked for stability indicating nature of the method and finalized the related substances method by HPLC with UV detector. The method was checked for adequacy as per the ICH requirement before implementation[7-9].

List of Abbreviations:

% w/w	Percentage weight by weight
w.r.t	With respect to
EE	Ethinylestradiol
Dil Std	Diluted Standard
RT	Retention time
RRT	Relative Retention time
SD	Standard Deviation
RSD	Relative Standard Deviation
NLT	Not Less Than
NMT	Not More Than
PPM	parts per million
PA	Purity Angle
PT	Purity Threshold
MP	Method precision
IP	Intermediate precision

Drug Substance and Drug Product Information[10]

Name of Drug Product	:	Levonorgestrel and Ethinyl Estradiol Tablets (0.1 mg/ 0.02 mg)	
Description of Drug Product	:	Light yellow colored, round, flat tablets debossed with "105" on one side and other side plain.	
Name of Drug Substance	:	Levonorgestrel USP	Ethinyl Estradiol USP
Description of Drug Substance	:	Levonorgestrel is White to off-white powder.	Ethinyl Estradiol is a white to faintly yellowish white crystalline powder.
Chemical Name	:	17-Hydroxy-6β,7β:15β,16β-dimethylene-3-oxo-17α-pregn-4-ene-21-carboxylic acid, ϒ -lactone	19-Nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol
Structure	:		
Molecular Formula	:	$C_{24}H_{30}O_3$	$C_{20}H_{24}O_2$
Molecular Weight	:	366.49	296.40
CAS No.	:	67392-87-4	57-63-6

METHDOLOGY:

Reagents and Solvents:

Sr.No	Reagent/Solvent	Grade	Make
1	Water	HPLC grade	NA
2	Acetonitrile	HPLC grade	J.T. Baker or equivalent
3	Methanol	HPLC grade	J.T. Baker or equivalent
4	Conc. Hydrochloric Acid	AR grade	Rankem or equivalent
5	Sodium Hydroxide Pellets	AR grade	Merck or equivalent
6	Hydrogen Peroxide)	AR grade	Merck or equivalent

Sample Details:

Sr. No	Name	Batch No.	Strength
1	Levonorgestrel +Ethinylestradiol Tablets	LUTE/US/026	0.1 mg / 0.02 mg
2	Plain Placebo	NA	NA
3	Placebo+ Levonorgestrel	NA	NA
4	Placebo + Ethinylestradiol	NA	NA

Working Standard Details:

Sr.No	Standard Name	Batch No.	WS No.	% Purity	Retest Date
1	Levonorgestrel WS	M1482M	AR/WRS/024/00	99.7	28-11-2010
2	Ethinylestradiol WS	L00030258	AR/WRS/022/00	99.4	15-11-2010

Levonorgestrel Impurities

Sr. No	Name of the Impurity	Reason for selecting he impurity
1.	6-β Hydroxy Levonorgestrel	Potential degradant Impurity as per DMF
2.	6-Keto Levonorgestrel	Potential degradant Impurity as per DMF
3.	Δ 8(14) Levonorgestrel	Process Impurity as per DMF
4	Δ 6 Levonorgestrel	Potential degradant Impurity as per DMF
5	18-Methylnanodroloene	Process Impurity as per DMF
6	* 4-5 Dihydro(5)α-Methoxy Levonorgestrel (210nm)	Process Impurity as per DMF
7	* 8-α-Δ5(10) Levonorgestrel (210nm)	Process Impurity as per DMF
8	Levonorgestrel 3-Methyldienolether	Process Impurity as per DMF

Ethinyl Estradiol Impurities

Sr. No	Name of the Impurity	Reason for selecting he impurity
1.	Δ-9(11)-Ethinyl Estradiol	Potential degradant mentioned in COA
2.	17-β-Ethinyl Estradiol	Process Impurity mentioned in COA
3.	Estrone	Process Impurity mentioned in COA
4	6-α-hydroxy Ethinyl Estradiol	Potential degradant available in other source API's
5.	6-β-hydroxy Ethinyl Estradiol	Potential degradant available in other source API's
6.	Δ-6-Ethinyl Estradiol	Potential degradant available in other source API's
7.	6-keto-Ethinyl Estradiol	Potential degradant available in other source API's
8.	1-methyl-Ethinyl Estradiol	Process Imp available in other source API's
9.	4-methyl-Ethinyl Estradiol	Process Imp available in other source API's

Limits for considered: As per the label claim of the product all known impurities fall in the < 10mg Qualification threshold of ICH guidelines, hence the limits taken for all impurities are Not More Than 1.0% as per ICH.

Preparation of Standard Stock Solution: Weigh accurately about 15.0 mg of Levonorgestrel working standard & about 3.0 mg of Ethinylestradiol working standard into 100 ml volumetric flask. Add to this 80ml diluent and sonicate to dissolve. Dilute up to the mark with diluent Solution C. (Concentration: 60 µg per mL).

Preparation of Diluted Standard Solution: Pipette out 1.0 ml of standard stock solution in 100ml volumetric flask and dilute up to mark with diluent. (Concentration of Levonorgestrel about 1.5 ppm & Ethinylestradiol about 0.3 ppm)

Preparation of Sensitivity Solution: Dilute 1 ml of the above diluted standard solution to 10 ml with diluent.

Preparation of Impurities Standard Stock Solution: Weigh accurately about 3.0 mg of 17 β Ethinylestradiol & 3.0 mg of Estrone into 100ml volumetric flask Add to this 80ml diluent and sonicate to dissolve. Dilute up to the mark with diluent.

Preparation of System Suitability Solution: Weigh accurately about 15.0 mg of Levonorgestrel working standard & about 3.0 mg of Ethinylestradiol working standard into 100ml volumetric flask. Add to this 80ml diluent and 1.0ml of impurities standard stock solution sonicate to dissolve. Dilute up to the mark with diluent.

Preparation Placebo solution I: (Without Levonorgestrel & Ethinylestradiol): Weigh & transfer 15 tablets of placebo or placebo powder blend equivalent to 15 tablets into a 10ml volumetric flask, add to this 5.0 ml of diluent, sonicate for 30 minutes with intermediate shakings, cool and make up to the volume with diluent. Centrifuge for 10 minutes at 3500 rpm, filer though 0.45 nylon filter and inject.

Preparation Placebo solution II: (With Levonorgestrel): Weigh & transfer 15 tablets of placebo or placebo powder blend equivalent to 15 tablets into a 10ml volumetric flask, add to this 5.0 ml of diluent, sonicate for 30 minutes with intermediate shakings, cool and make up to the volume with diluent. Centrifuge for 10 minutes at 3500 rpm, filer though 0.45 nylon filter and inject.

Preparation Placebo III: (With Ethinylestradiol): Weigh & transfer 15 tablets of placebo or placebo powder blend equivalent to 15 tablets into a 10ml volumetric flask, add to this 5.0 ml of diluent, sonicate for 30 minutes with intermediate shakings, cool and make up to the volume with diluent. Centrifuge for 10 minutes at 3500 rpm, filer though 0.45 nylon filter and inject.

Preparation Sample Solution: Weigh & transfer 15 tablets into a 10ml volumetric flask, add to this 5.0 ml of diluent, sonicate for 30 minutes with intermediate shakings, cool and make up to the volume with diluent. Centrifuge for 10 minutes at 3500 rpm, filer though 0.45 nylon filter and inject.

Procedure: Inject the specified volume of Diluent, Placebo solution-I, Placebo solution-II, Placebo solution-III and Sample solution into the chromatograph and record the chromatogram. Disregard peaks due to blank and placebo

solution. Calculate all known impurities at corresponding wavelength as mentioned in below table. Calculate unknown impurities of Ethinylestradiol at 210 nm, and calculate unknown impurities of Levonorgestrel at both the wavelengths. Calculate any other unknown impurities at 210 nm against diluted standard area of Ethinylestradiol at 210 nm

Table 2: showing RRT's for known impurities w.r.t Levonorgestrel:

Sr. No.	Known impurities		RRT	
1	Levonorgestrel	Ethinylestradiol	EE	Levo
2	Levonorgestrel	Ethinylestradiol	1.00	1.00
3	6-β Hydroxy Levonorgestrel	6-αHydroxy Ethinylestradiol	0.31	0.34
4	6-Keto Levonorgestrel	6- β Hydroxy Ethinylestradiol	0.41	0.50
5	Δ 8(14) Levonorgestrel	6-Keto Ethinylestradiol	0.51	0.87
6	Δ 6 Levonorgestrel	Δ 9,11 Ethinylestradiol	0.89	0.95
7	18-Methylnanodroloene	Δ 6 Ethinylestradiol	0.93	0.97
8	* 4-5 Dihydro(5)α-Methoxy Levonorgestrel	Estrone	0.95	1.05
9	* 8-α-Δ5(10) Levonorgestrel	1-Methyl Ethinylestradiol	1.12	1.18
10	Levonorgestrel 3 Methyldienolether	17 β Ethinylestradiol	1.20	1.50

Evaluation of System Suitability:

For Sensitivity Solution: Inject the specified volume of sensitivity solution and record the chromatogram at 210 nm & 254 nm. The area counts of Ethinylestradiol peak at 210 nm and Levonorgestrel peak at 254 nm & 210 nm in sensitivity solution should be in the range of 0.09 to 0.11 times of the average area counts of Ethinylestradiol peak and Levonorgestrel peak in the diluted solution at corresponding wavelengths.

For System Suitability Solution: Inject the specified volume of system suitability solution and record the chromatogram at 210 nm & 254 nm. Resolution between Estrone peak and Ethinylestradiol peak should not be less than 1.5 at 210 nm. Resolution between 17β-Ethinylestradiol peak and Levonorgestrel peak should not be less than 1.2 at 210 nm. The tailing factor for Ethinylestradiol and Levonorgestrel peak should not be more than 2.0 at both the wavelengths and Theoretical plate for Ethinylestradiol and Levonorgestrel peak should not be less than 25000 at both the wavelengths.

For Diluted Standard Solution: Inject the specified volume of diluted standard solution for 6 times, the relative standard deviation for Levonorgestrel at both the wavelengths and Ethinylestradiol peak at 210 nm the area counts from 6 replicate injections of standard solution should not be more than 5.0 %.

METHOD DEVELOPMENT

Experiment No. 1: Sample and Standard preparation: prepared sample and standard solution as above mentioned in methodology section.

Chromatographic Condition:

Column : C18, 150 x 4.6, 5µ

Column Make : Peerless,

Mobile Phase : A: Water B: Methanol: Acetonitrile (80:20) [(A+B) (42:58)]

Flow : 1.0 ml/min.

Column Temperature : 25°C

Injection Volume : 50µl

Wavelength : 215 nm for Ethinylestradiol & 240nm for Levonorgestrel

Run Time : 45 minute

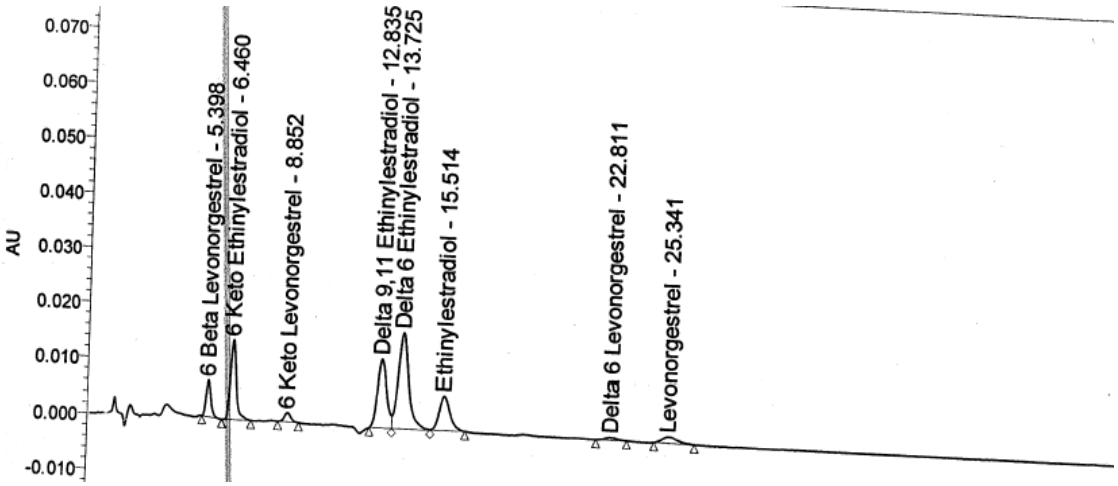


Figure 1: Reference Chromatogram of Experiment trail 1

Experiment No. 2: Sample and Standard preparation: prepared sample and standard solution as above mentioned in methodology section.

Chromatographic Condition:

Column : 250 x 4.6, 5µm

Column Make : YMC pack ODS-AM

Mobile Phase : A: Water B: Methanol

Flow : 1.0 ml/min.

Column Temperature : 25°C

Sample Temperature : 15°C

Injection Volume : 50µl

Wavelength : 210 nm for Ethinylestradiol & Levonorgestrel

Run Time : 95 minute

Gradient Program:

Time in min.	Flow ml/min	Mobile Phase A Water	Mobile Phase B Methanol
0	1.0	90	10
7	1.0	90	10
12	1.0	60	40
20	1.0	55	45
55	1.0	55	45
60	1.0	40	60
65	1.0	40	60
70	1.0	15	85
80	1.0	15	85
85	1.0	90	10
95	1.0	90	10

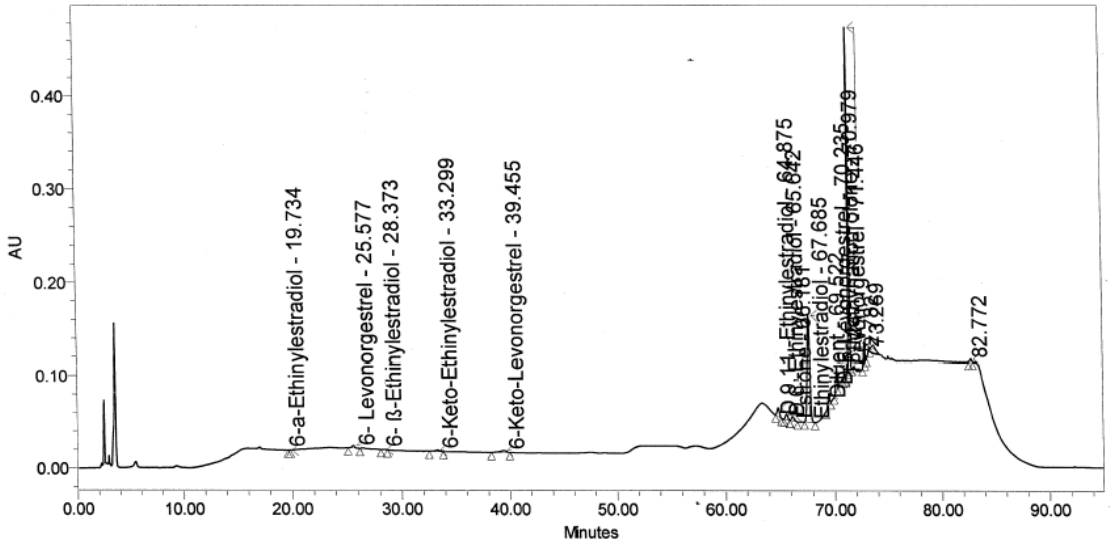


Figure 2: Reference Chromatogram of Experiment trail 2

Experiment No. 3: Sample and Standard preparation: prepared sample and standard solution as above mentioned in methodology section.

Chromatographic Condition:

Column : 250 x 4.6, 5µm

Column Make : YMC pack ODS-AM

Mobile Phase : A: Water B: Methanol C: Acetonitrile

Flow : 1.0 ml/min.

Column Temperature : 30°C

Sample Temperature : 15°C

Injection Volume : 50µl

Wavelength : 220 nm for Ethinylestradiol & Levonorgestrel

Run Time : 100 minute

Gradient Program:

Time in min.	Flow ml/min	(A) Water	(B) Methanol	(C) Acetonitrile
0	1.0	75	0	25
5	1.0	75	0	25
10	1.0	62	0	38
20	1.0	45	40	15
50	1.0	45	40	15
60	1.0	40	10	50
70	1.0	20	0	80
85	1.0	20	0	80
90	1.0	75	0	25
100	1.0	75	0	25

Conclusion of Experiment No 3: In this trial Ethinylestradiol & Estrone peak are not resolved & also 8-α (14) Levonorgestrel & 1-Methyl Ethinylestradiol impurities are not separated.

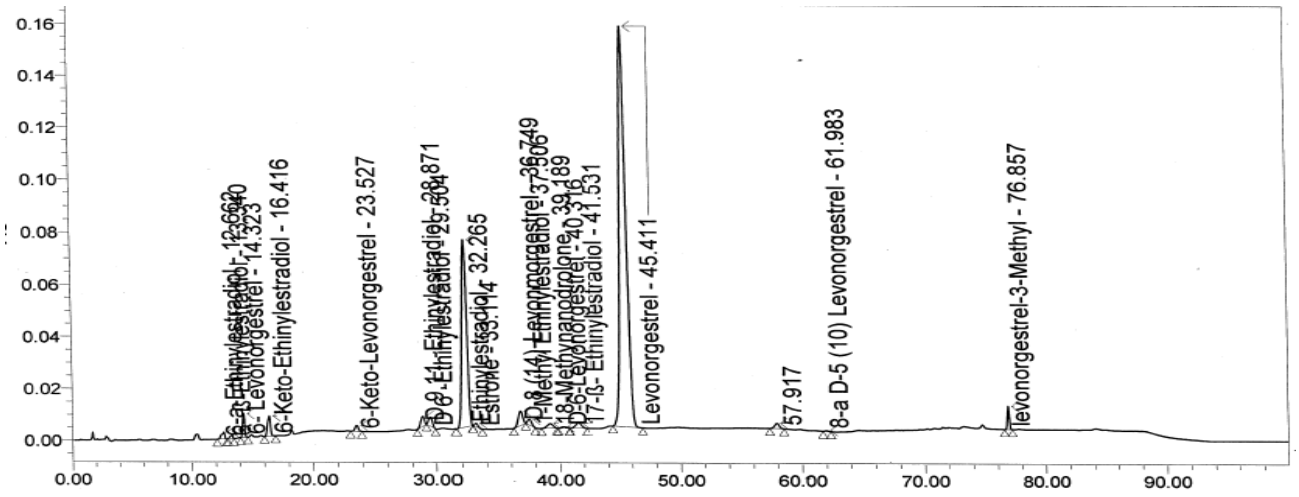


Figure 3: Reference Chromatogram of Experiment trail 3

Experiment No. 4: Sample and Standard preparation: prepared sample and standard solution as above mentioned in methodology section.

Chromatographic Condition:

Column : 250 x 4.6, 5µm

Column Make : YMC pack ODS-AM

Mobile Phase : A: Water B: Methanol: Acetonitrile (90:10)

Flow : 1.0 ml/min.

Column Temperature : 25°C

Sample Temperature : 15°C

Injection Volume : 50µl

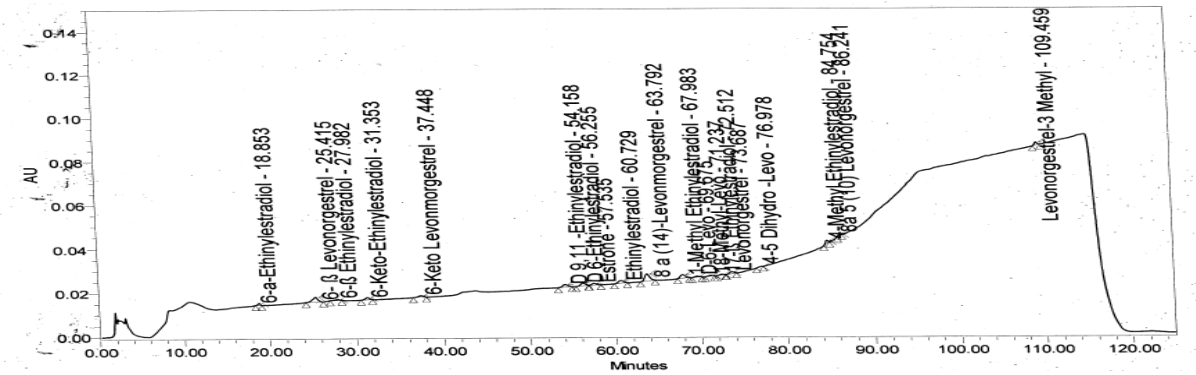
Wavelength : 210 nm for Ethinylestradiol & 254 nm for Levonorgestrel

Run Time : 125 minute

Gradient Program

Time in min.	Flow ml/min	(A) Water	(B) Methanol: ACN (90:10 v/v)
0	1.0	90	10
2	1.0	90	10
5	1.0	62	38
7	1.0	55	45
20	1.0	52	48
42	1.0	48	52
70	1.0	42	58
80	1.0	35	65
85	1.0	30	70
92	1.0	20	80
112	1.0	15	85
115	1.0	90	10
125	1.0	90	10

At 210n nm



At 254 nm

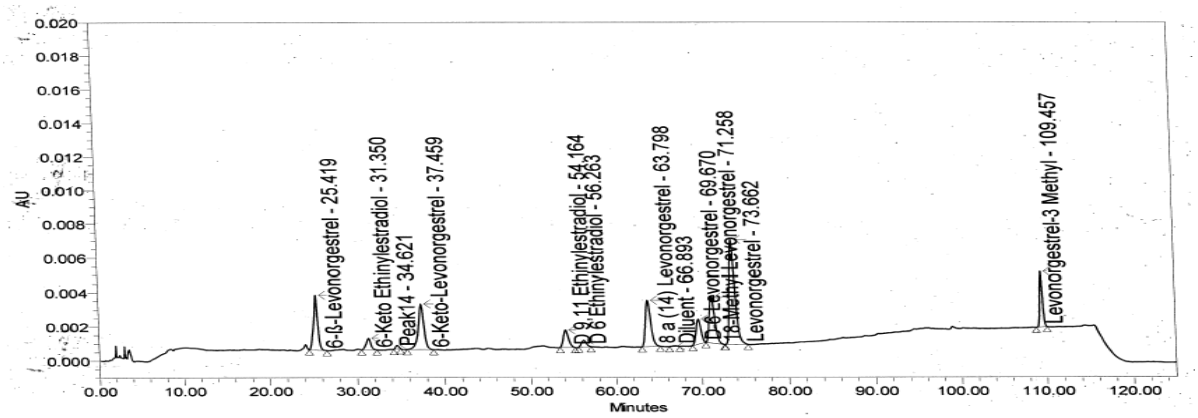


Figure 4: Reference Chromatogram of Experiment trail 4 at 210 nm and 254 nm

Method Finalization Summary: The Related substances method was finalized based on the below observations.

Injection Volume Selection: During the time of development study, 25µL and 50µL of 1% imp spike solution was injected separately. It was observed that in 50µL injection volume, the LOQ of all peak increased. Whereas in 25µL injection volume, the LOQ of all peak decreased. Hence an injection volume was finalized to 50 µL.

Diluent Selection: Following different diluents were tried for sample preparation,

1) Acetonitrile:Water (50:50)
2) Acetonitrile:Water (70:30)
3) Acetonitrile:Water (90:10)

The % recovery of Levonorgestrel and Ethinylestradiol in 50:50 compositions was lesser than other compositions, whereas hump was observed after Levonorgestrel peak in (90:10) diluent. Hence final (70:30) diluent was selected as a diluent for sample preparation.

Solvent Make Selection: By using two different make solvents, same method was run separately. A gradient induced hump was observed due to Rankem solvent just before Delta-9, 11 EE, which was not seen in J.T Baker solvents. So J.T. Baker solvents were finalized.

Wavelength Selection: Wavelength of Ethinylestradiol and Levonorgestrel was taken from API method of analysis (DMF), provided by API vendor. Also unknown impurities due to Levonorgestrel have average maxima at 254 nm whereas Ethinylestradiol impurities at 210 nm. Hence we finalized Wavelength of Ethinylestradiol at 210 nm and Levonorgestrel at 254 nm.

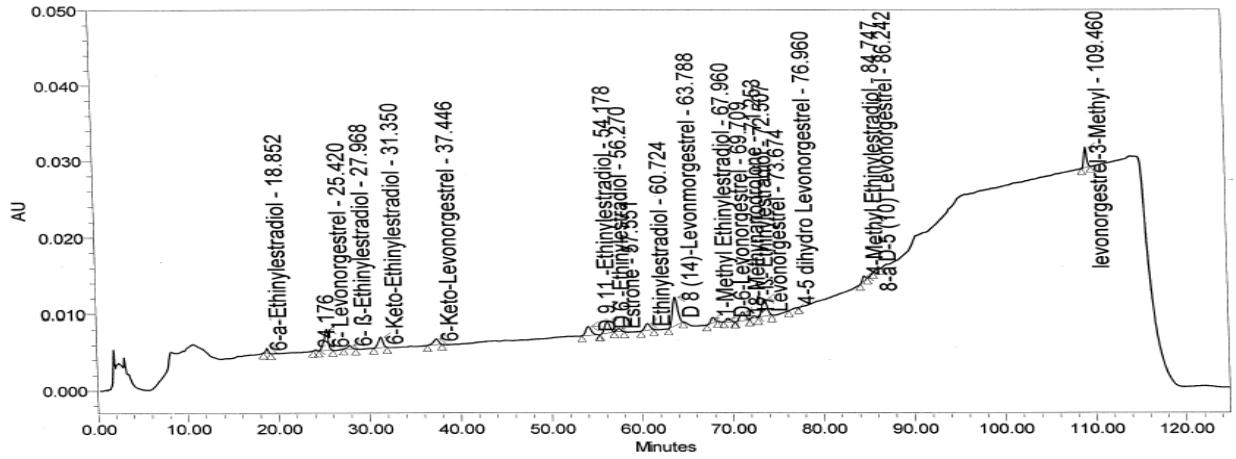


Figure 5: Reference Chromatogram Wavelength of Ethinylestradiol & Levonorgestrel (254 nm)

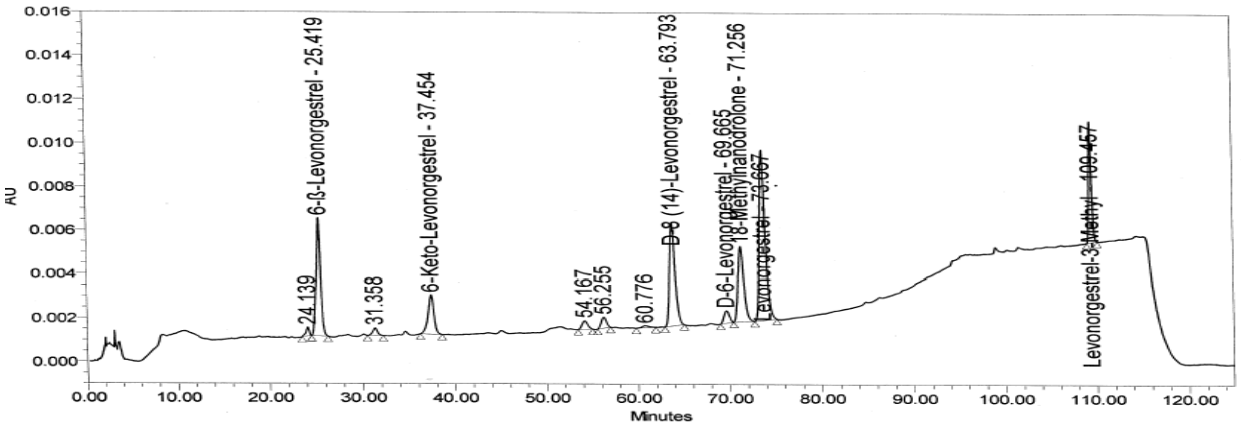


Figure 6: Reference Chromatogram Wavelength of Ethinylestradiol & Levonorgestrel (210 nm)

Conclusion of Development Trails:

Conclusion of experiment no 1: In this method resolution between δ 9, 11 Ethinylestradiol & δ 6 Ethinylestradiol are not resolved & also Estrone merge with δ 6 Ethinylestradiol. And also Levonorgestrel process impurities are not include.

Conclusion of experiment no 2: In this trial Levonorgestrel impurities are not separated.

Conclusion of Experiment No 3: In this trial all impurity of Levonorgestrel & Ethinylestradiol are resolved well. Based on the all experiment trails it its concluded that in experiment trail no 3 was separated each impurities very well and consider method trail no 3 for further method optimization/ method validation.

METHOD VALIDATION:

Method optimization of the given method was performed to check the stability indicating nature of the method. Validation parameters Specificity, Accuracy (Recovery), Limit of Detection and Limit of Quantitation, Forced Degradation study, Solution Stability, Precision, Filter Study were considered while an optimization of the Method.

Specificity:

Table 1: Specificity Study of Levonorgestrel and Ethinyl Estradiol

Parameter	Specification	Ethinyl Estradiol	Levonorgestrel
Specificity	**Identification** Results should be comparable with respect to Retention time.	**Identification** Results were comparable with respect to Retention time.	**Identification** Results were comparable with respect to Retention time.
	Placebo Interference Diluent and Placebo should not show any peak at the retention time of Active and its impurity peaks.	**Placebo Interference** Diluent and Placebo did not show any peak at the retention time of Active and its impurity peaks.	**Placebo Interference** Diluent and Placebo did not show any peak at the retention time of Active and its impurity peaks.
	Individual Active Ingredients Peak purity should pass. Main peaks should be pure and homogeneous and there should be no co-eluting peaks.	**Individual Active Ingredients** Peak purity passes. Main peaks were pure and homogeneous and there were no co-eluting peaks.	**Individual Active Ingredients** Peak purity passes. Main peaks were pure and homogeneous and there were no co-eluting peaks.

Accuracy (Recovery): Overall Mean recovery for all Ethinyl & Levo and their all impurities should be in the range of 90.0 % to 110.0 %. Table-2, shows Tentative Recovery of known impurities for Ethinylestradiol & Levonorgestrel.

Table 2: Recovery of known impurities for Ethinylestradiol & Levonorgestrel

Ethinyl	% Recovery	Levonorgestrel	% Recovery
6-αHydroxy Ethinylestradiol	102.2	6-β Hydroxy Levonorgestrel	91.4
6- β Hydroxy Ethinylestradiol	98.1	6-Keto Levonorgestrel	99.2
6-Keto Ethinylestradiol	100.6	Δ 8(14) Levonorgestrel	102.1
Δ 9,11 Ethinylestradiol	100.5	Δ 6 Levonorgestrel	96.0
Δ 6 Ethinylestradiol	97.6	18-Methylnanodroloene	103.6
Estrone	97.3	* 4-5 Dihydro(5)α-Methoxy Levonorgestrel	91.6
1-Methyl Ethinylestradiol	99.8	* 8-α-Δ5(10) Levonorgestrel	93.0
17 β Ethinylestradiol	94.3	Levonorgestrel 3-Methyldienolether	107.7
4-Methyl Ethinylestradiol	99.0		

Limit of Detection & Limit of Quantification: LOD Ethinyl & all impurities should be in the range of 0.1 % i.e. Test Concentration. Table 3, shows Tentative RF, LOQ and LOD for known impurities of Ethinylestradiol & Levonorgestrel.

Table 3: RF, LOQ and LOD for known impurities of Ethinylestradiol

Ethinylestradiol Impurities	% LOD	% LOQ	RF
6-αHydroxy Ethinylestradiol	0.033	0.099	2.45
6- β Hydroxy Ethinylestradiol	0.030	0.092	2.46
6-Keto Ethinylestradiol	0.034	0.104	1.07
Δ 9,11 Ethinylestradiol	0.049	0.102	0.92
Δ 6 Ethinylestradiol	0.026	0.078	0.77
Estrone	0.042	0.100	1.03
1-Methyl Ethinylestradiol	0.045	0.107	0.50
17 β Ethinylestradiol	0.048	0.145	1.52
4-Methyl Ethinylestradiol	0.045	0.102	1.16

Table 4: RF, LOQ and LOD for known impurities of Levonorgestrel.

Levonorgestrel Impurities	% LOD	% LOQ	RF
6-β Hydroxy Levonorgestrel	0.035	0.106	1.82
6-Keto Levonorgestrel	0.030	0.090	1.84
Δ 8(14) Levonorgestrel	0.025	0.075	1.61
Δ 6 Levonorgestrel	0.039	0.117	2.69
18-Methylnanodroloene	0.013	0.040	1.75
* 4-5 Dihydro(5)α-Methoxy Levonorgestrel	ND	0.50	--
* 8-α-Δ5(10) Levonorgestrel	0.035	0.106	1.41
Levonorgestrel 3- Methyldienolether	0.031	0.095	3.31

The forced degradation experiment condition: Forced degradation studies were carried out for related substances at following conditions and the degradants were well separated by this method and the peak was found to be spectrally pure for known impurities and main analyte peaks.

Table 5: Forced degradation Study

Condition	% Degradation Ethinyl Estradiol	% Degradation Levonorgestrel	Peak purity
Initial sample	---	---	Passed
Fifteen tablets + 1 ml of 1M HCl solution in a 10 ml volumetric flask, add 1 ml of diluent and sonicate for 30 min. with intermediate shaking Neutralized by adding 1 ml of 1M NaOH solution and add 3 ml water shake & dilute up to mark with diluent.	18.4 %	15.1 %	Passed
Fifteen tablets + 1 ml of 0.5 M NaOH solution in a 10 ml volumetric flask add 1 ml of diluent and sonicate for 30 min. with intermediate shaking. Neutralized by adding 1 ml of 0.5 M HCL solution and add 3 ml water shake & dilute up to mark with diluent.	25.5 %	25.0 %	Passed
Fifteen tablets + 1 ml of Hydrogen peroxide (30 %) solution in a 10 ml volumetric flask, sonicate for 30 min, with intermediate shaking and add 3 ml water shake & dilute up to mark with diluent.	10.0 %	7.7 %	Passed
Sample exposed to heat at 105°C for 24 hours on open Petri dish. Fifteen tablets + 3 ml water in a 10 ml volumetric flask, sonicate for 30 min, with intermediate shaking shake & dilute up to mark with diluent	9.6 %	7.5 %	Passed
Sample exposed in photo stability chamber for 1.2 million lux hour. Fifteen tablets + 3 ml water in a 10 ml volumetric flask, sonicate for 30 min, with intermediate shaking shake & dilute up to mark with diluent	8.7 %	2.0 %	Passed

Solution Stability: Mean recovery for all Ethinyl & Levo and their all impurities should be in the range of 90.0 % to 110.0 %. Table 6 shows solution stability for 48 hrs for known impurities of Ethinylestradiol & Levonorgestrel.

Table 6: Solution stability for known impurities of Ethinylestradiol & Levonorgestrel

Ethinylestradiol	% Recovery	Levonorgestrel	% Recovery
6-αHydroxy Ethinylestradiol	92.3	6-β Hydroxy Levonorgestrel	92.8
6- β Hydroxy Ethinylestradiol	92.6	6-Keto Levonorgestrel	99.5
6-Keto Ethinylestradiol	100.9	Δ 8(14) Levonorgestrel	98.6
Δ 9,11 Ethinylestradiol	98.5	Δ 6 Levonorgestrel	91.3
Δ 6 Ethinylestradiol	97.6	18-Methylnanodroloene	97.7
Estrone	105.5	4-5 Dihydro(5)α-Methoxy Levo	88.9
1-Methyl Ethinylestradiol	101.0	* 8-α-Δ5(10) Levonorgestrel	96.8
17 β Ethinylestradiol	91.2	Levonorgestrel 3-Methyldienolether	110.1
4-Methyl Ethinylestradiol	93.7	Levonorgestrel	100.20
Ethinylestradiol	100.8	NA	

Precision: RSD should not more than 15.0 %. Table 7, showing precision study for known impurities of Ethinylestradiol & Levonorgestrel.

Table 7: Precision study for known impurities of Ethinylestradiol & Levonorgestrel

Ethinylestradiol	% RSD	Levonorgestrel	% RSD
6-αHydroxy Ethinylestradiol	7.9	6-β Hydroxy Levonorgestrel	2.0
6- β Hydroxy Ethinylestradiol	12.8	6-Keto Levonorgestrel	1.0
6-Keto Ethinylestradiol	0.7	Δ 8(14) Levonorgestrel	1.6
Δ 9,11 Ethinylestradiol	3.2	Δ 6 Levonorgestrel	1.7
Δ 6 Ethinylestradiol	1.8	18-Methylnanodroloene	1.2
Estrone	2.0	* 4-5 Dihydro(5)α-Methoxy Levonorgestrel	4.1
1-Methyl Ethinylestradiol	1.8	* 8-α-Δ5(10) Levonorgestrel	7.5
17 β Ethinylestradiol	10.1	Levonorgestrel 3-Methyldienolether	3.7
4-Methyl Ethinylestradiol	4.1	Levonorgestrel	1.1
Ethinylestradiol	1.1	-	-

Filter compatibility: Should be between 90% - 110 %. Table 8, shows Filter study for known impurities of Ethinylestradiol & Levonorgestrel.

Table 8: Filter study for known impurities of Ethinylestradiol & Levonorgestrel

Ethinylestradiol	% Assay	Levonorgestrel	% Assay
6-αHydroxy Ethinylestradiol	108.7	Levonorgestrel	%
6- β Hydroxy Ethinylestradiol	107.6	6-β Hydroxy Levonorgestrel	103.0
6-Keto Ethinylestradiol	102.1	6-Keto Levonorgestrel	101.4
Δ 9,11 Ethinylestradiol	99.9	Δ 8(14) Levonorgestrel	102.2
Δ 6 Ethinylestradiol	100.4	Δ 6 Levonorgestrel	101.1
Estrone	98.3	18-Methylnanodroloene	101.8
1-Methyl Ethinylestradiol	100.3	4-5 Dihydro(5)α-Methoxy Levo	104.8
17 β Ethinylestradiol	103.0	8-α-Δ5(10) Levonorgestrel	104.1
4-Methyl Ethinylestradiol	101.7	Levonorgestrel 3-Methyldienolether	104.8

CONCLUSION:

The method is Specific, Linear, Precise and Accurate for Related substances, for Levonorgestrel 0.10 mg and Ethinyl Estradiol 0.02 mg Tablets. For one of the known impurity of Levonorgestrel, (4, 5-Dihydro-5-alpha-methoxy-levonorgestrel) LOQ is about 0.5%. This is a process impurity with limit specification of 0.3% in the API. Degradation for this impurity in the formulation will be confirmed by alternative method.

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