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A Newer validated and stability indicating UPLC Method for the Estimation of Nordette in Tablet Formulation

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ABSTRACT

A simple, selective, linear, precise and accurate UPLC Method was developed and validated for rapid assay of Nordette in tablet Formulation. Isocratic elution at a flow rate of 0.4ml/min was employed on C8 1.7 µm (2.1 mm x 100 mm) Column at ambient temperature40 °C. Injection Volume was found to be 5.0 µl. The mobile phase consisted of Acetonitrile : Water 60:40 v/v which is filter through a 0.2 µm filter The UV detection wavelength was 220nm and 2µl sample was injected. The retention time for Ethinylestradiol, Levonorgestrel is found to be ± 1.4 minutes and ± 2.1 minutes respectively. A linear regression curve was constructed, and the correlation coefficients (R^2) and assessment values calculated. The percentage RSD for both Ethinylestradiol, Levonorgestrel was found to be 1.5%.The Accuracy of method ranges between 97.0 – 102.8%. The method was validated as per the ICH guidelines. The method was successfully applied for routine quality control analysis of pharmaceutical formulation.

Keywords: Nordette, Ethinylestradiol, Levonorgestrel, UPLC, Recovery, Precise.

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INTRODUCTION

Levonorgestrel (or l-norgestrel or D-norgestrel) (Plan B, Next Choice, Postinor, and others[1] is a second generation synthetic progestogen used as an active ingredient in some hormonal contraceptives, including combined oral contraceptive pills, progestogen only pills, emergency contraceptive pills, intrauterine systems, contraceptive implants, and hormone replacement therapy. Ethinyl estradiol (EE) is a derivative of 17β-estradiol (E2),themaj or endogenous estrogen in humans. EE is an rally bioactive estrogen used in many formulations of combined oral contraceptive pills. It is one of the most commonly used medications for this purpose. According to Xiong Zet.al[2] developed A selective and sensitive ultra-performance liquid chromatography method with tandem mass spectrometric detection for simultaneous determination of gestodene and ethinyl estradiol (EE) in rat plasma was developed and validated. GES, EE and the internal standard, norgestrel, were extracted with ethyl acetate, and back-extracted into diethyl ether-hexane (2:1, v/v).

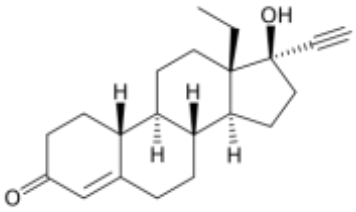


Figure:1 Levonorgestrel

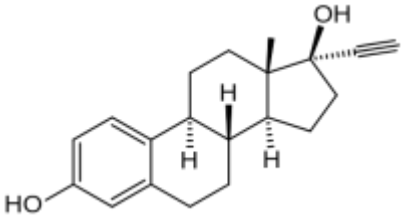


Figure:2 Ethinylestradiol

The separation was performed on an ACQUITY UPLC BEH C(18) column by using mobile phase acetonitrile and water (both containing 0.1% formic acid). The detection was carried out by electrospray ionization, mass spectrometry were linear (r(2) >or= 0.99), concentration ranges 1.59-159 and 0.196-78.4 ng/mL, respectively. The intra- and inter-day precisions were 6.9 and 12.9% for GES and 10.6 and 9.0% for EE. SzabolcsFeketea[3] developed An ultra performance liquid chromatographic method for simultaneous determination of active pharmaceutical ingredient residues. The UPLC method was validated using an UPLCTM BEH C18 column with a particle size of 1.7m (50 mm×2.1 mm) and acetonitrile–water (48:52, v/v) as mobile phase at a flow rate of 0.55 ml/min. Method development and method validation for cleaning control analysis are described. According to Navneet Kumar et.al[4]., This method was validated using an Acquity UPLC™ HSS T3 (100 × 2.1 mm2) 1.8 µm column with a isocratic mobile phase containing a mixture of 0.01 M potassium dihydrogen orthophosphate, pH adjusted to 3.0 with orthophosphoric acid and acetonitrile (60:40 v/v). The flow rate of the mobile phase was 0.4

ml/min with a column temperature of 40°C and detection wavelength at 230 nm. Cotton swabs, moisten with extraction solution (90% methanol and 10% water), were used to remove any residue of drug from stainless steel, glass and silica surfaces, and give recoveries >80% at four concentration levels. HPLC methods have been reported for the determination of Ethinylestradiol in combination with otherdrugs[5-8]. In this study we have developed a simple, accurate, sensitive and validated RP-HPLC method for simultaneous estimation of these compounds in bulk drug and in combined tablet dosage form and the method was applied for their identification by UPLC-MS

MATERIALS AND METHOD

Instrumentation:

PeakUPLC containing variable wavelength programmable UV-Visible detector and Rheodyne injector was employed for investigation. The chromatographic analysis was performed on a C8 1.7 µm (2.1 mm x 100 mm). Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. A Denwar Analytical balance was used for weighing the materials.

Chemicals and Solvents:

The reference sample of Nordette Yellow Tablets – Sugar Coated which consists of Ethinylestradiol 30 µg / tablet (27 – 33 µg / tablet) and Levonorgestrel 150 µg / tablet (135.0 – 165.0 µg / tablet) were obtained from Local Market. Methylene Chloride, alcohol. Acetonitrile and Water used was of HPLC grade and purchased from Merck Specialties Private Limited, Mumbai, India.

The mobile phase:

0.05 M Acetonitrile and Water 60:40 v/v was prepared and used as mobile phase.

SAMPLE (CAPSULE) SOLUTION:

Levonorgestrel Sample Preparation

Place 20 tablets in a 100 ml volumetric flask. Add 60 ml of solvent. Sonicate for 15 minutes with frequent shaking. Cool to room temperature and dilute to volume with solvent. Filter both sample and standard through 0.22 µm filter, discarding the first 5 ml of filtrate.

Ethinylestradiol Sample Preparation – Uniformity of Content

Place one tablet into each of ten 5 ml volumetric flask. About 3 ml of solvent.

Sonicate for 15 minutes with frequent shaking. Cool and dilute to volume with solvent.

Filter through a 0.22 µm, discard the first 1 ml of filtrate.

STANDARD SOLUTION OF THE DRUG:

Stock Standard Solution - Levonorgestrel

Accurately weigh 62.5 mg of Levonorgestrel into a 250 ml volumetric flask. Dissolve in 120 ml acetonitrile by sonicating for 10 minutes with frequent shaking. Cool to room temperature and dilute to the mark with water. Dilute 40 ml of this solution to 100 ml with solvent (Solution 1).

Stock Standard Solution – Ethinylestradiol

Accurately weigh 100 mg Ethinylestradiol into a 100 ml volumetric flask. Dissolve in 50 ml acetonitrile by sonicating for 10 minutes with frequent shaking. Cool to room temperature and dilute to the mark with water. Dilute 25 ml to 250 ml with solvent (Solution 2).

METHOD DEVELOPMENT

Detection wavelength:

The spectrum of 10ppm solution was recorded separately on UV/vis. spectrophotometer. The peak of maximum absorbance wavelength was observed. The spectra of the substance were showed maximum absorbance at 220nm.

Choice of stationary phase:

Preliminary trials have performed with different types, configurations and from different manufacturers. Finally the expected separation and peak shapes were obtained on chromosilC8 1.7 µm (2.1 mm x 100 mm).

Selection of the mobile phase:

To get low tailing factor, base line separation and sharp peak of the components, a number of trails were carried out by changing the composition of different solvents and flow rate. Indifferent combinations were tested as mobile phases on a C8 1.7 µm (2.1 mm x 100 mm).

Flow rate:

0.1 – 1.5 mL/min flow rates of the mobile phase were changed for optimum separation. It was found from the results, 0.40 ml/min flow rate was ideal for the successful elution of the analyte.

Optimization of chromatographic conditions:

Optimized chromatographic conditions were followed for the determination of nordettel in bulk samples and in its formulations.

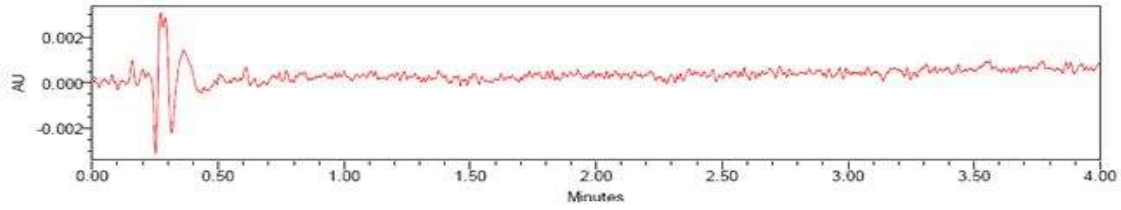
Validation of Proposed Method:

The analytical performance of the method of analysis was checked for Specificity, System suitability, Accuracy and Method Precision.

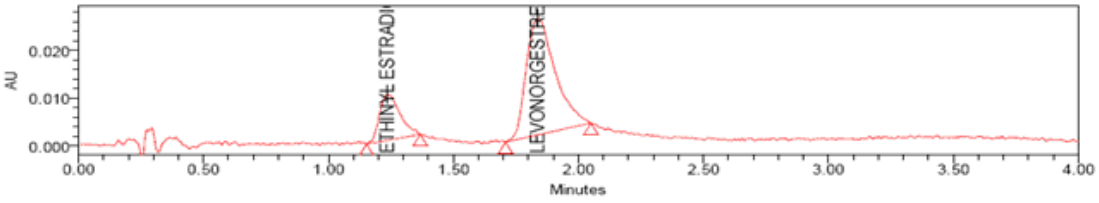
Specificity

Specificity of an analytical procedure is its ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The solvent and placebo solutions

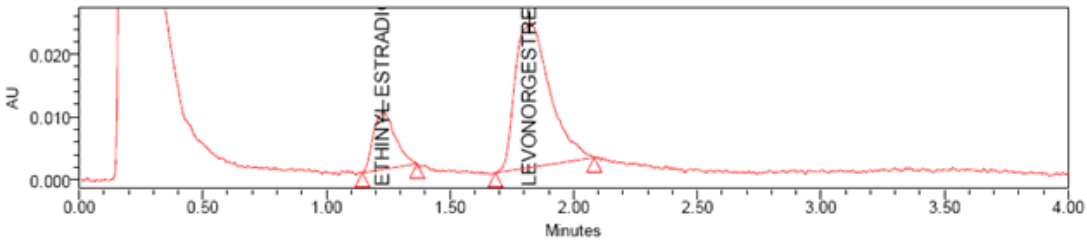
must contain no components, which co-elute with the Ethinyl estradiol and Levonorgestrel. The peak purity results from the photo diode-array analysis must show that the Ethinylestradiol and Levonorgestrel peak are pure – i.e. the purity angle (PA) must be less than the threshold angle (TH). The solutions listed below were injected using the conditions specified in the method of analysis.The following Chromatogram results were obtained. Chromatogram 1 represents the by taking the solvent no significant peak was detected. Chromatogram 2 represents about the drug activity – Peaks due to Ethinylestradiol and Levonorgestrel eluted at 1.4 and 2.1 minutes. Chromatogram 3 represents about the Placebo – No significant peak detected. Chromatogram 1.Ethinylestradiol and Levonorgestrel are stable under UV light exposure. No components are seen to co-elute with Ethinylestradiol and Levonorgestrel peak, and the peak purity results indicate that Ethinylestradiol and Levonorgestrel peak can therefore be considered spectrally pure.



Chromatogram 2

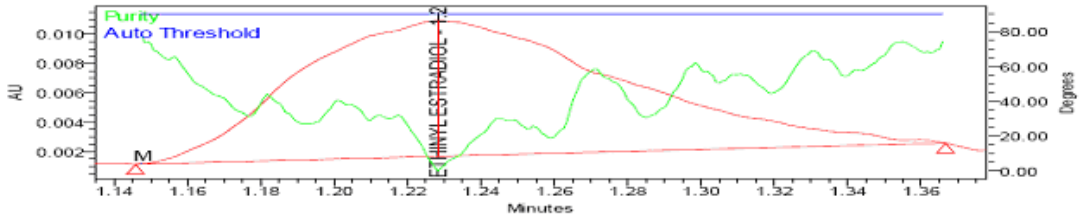


Chromatogram 3



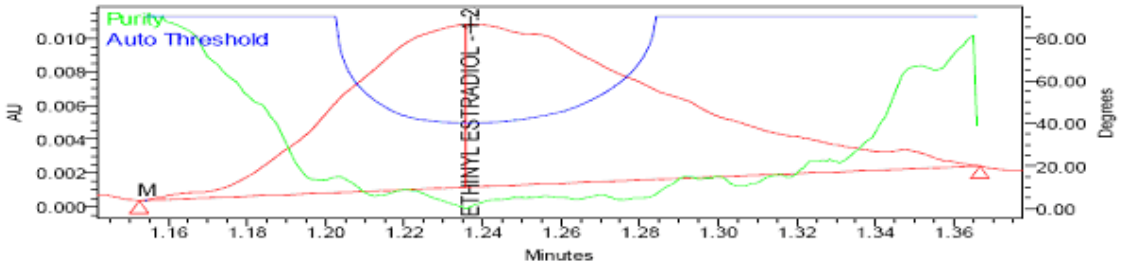
Peak purity 1:Ethinylestradiol

Purity angle(36.793)< Threshold(90.000)



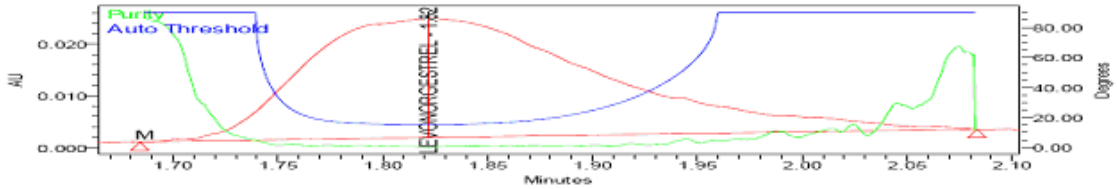
Peak purity 2:Ethinylestradiol

Purity angle(10.338)< Threshold(70.446)



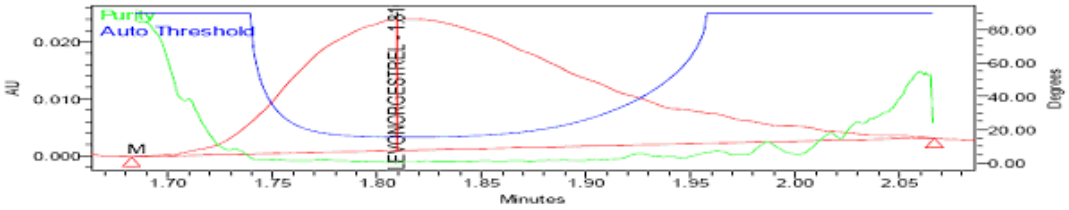
Peak purity 3:Levonorgestrel

Purity angle(1.830)< Threshold(23.366)



Peak purity 4: Levonorgestrel

Purity angle(2.029)< Threshold(23.522)



System Suitability

System suitability is a measure of the performance and chromatographic quality of the total analytical system – i.e. instrument and procedure. The requirements for system suitability for this method are: The % RSD of the peak responses due to Ethinylestradiol and Levonorgestrel for the six replicate injections must be less than or equal to 2.0 %.

Six replicate injections of working standard solution were injected according to the method of analysis.The percentage relative standard deviation (% RSD) for the peak responses was determined. The analytical system complies with the requirements specified by the system suitability. System Suitability results were tabulated in the

Table:1 Ethinylestradiol and Levonorgestrel Results

Sample	Ethinylestradiol Area	Levonorgestrel Area
1	69318	246331
2	68400	243393
3	71780	252600
4	70425	252802
5	70962	251364
6	70177	249329
Mean	70177	249303
% RSD	1.7	1.5

Linearity

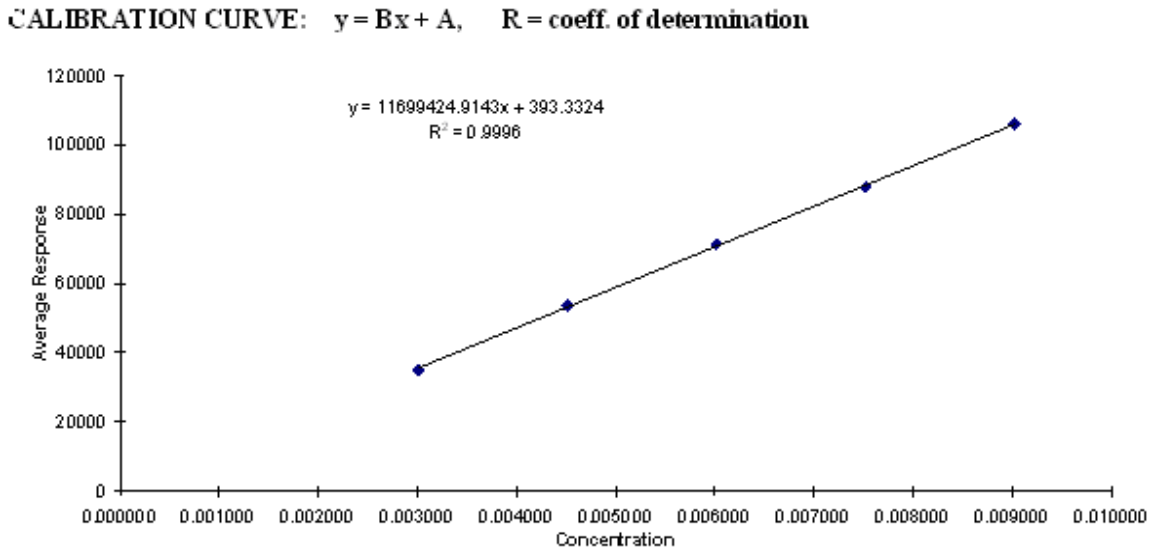
The linearity of an assay method is its ability to elicit test results, which are directly proportional to the concentrations of drug actives in samples in a given range.

Proof of linearity justifies the use of single-point calibrations. The correlation coefficient of the regression line for Ethinylestradiol and Levonorgestrel should be greater than or equal to 0.999. The Y-intercept of the line should not be significantly different from zero, i.e. the assessment value (z) falls within the specified limits only when + 5 > z > -5.

Five solutions containing 50, 75, 100, 125, and 150 % of Ethinylestradiol and Levonorgestrel, relative to the working concentrations, were prepared and injected according to the method of analysis. A linear regression curve due to Ethinylestradiol and Levonorgestrel were constructed, and the correlation coefficients (R^2) and assessment values calculated.

Calibration Curve for Ethinylestradiol

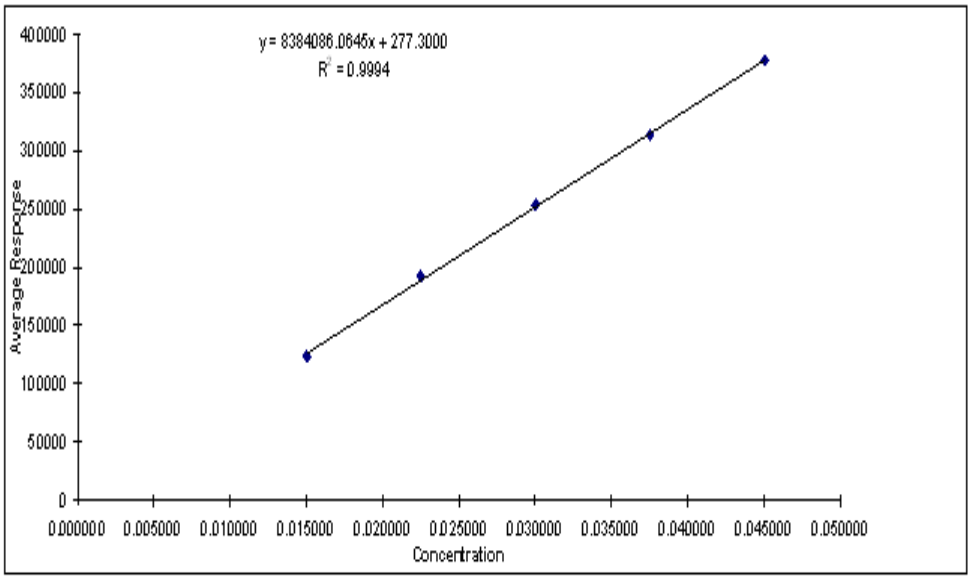
The correlation coefficient (R^2) for Ethinylestradiol is 1.000.The plot is a straight line, and the assessment value (z) is 1 for Ethinylestradiol.



Calibration Curve for Levonorgestrel :

The correlation coefficient (R^2) for Levonorgestrel is 0.999.The plot is a straight line, and the assessment value (z) is 0 for Levonorgestrel.

CALIBRATION CURVE: y = Bx + A, R = coeff. of determination



Accuracy

The accuracy of an analytical method expresses the closeness of test results obtained by that method to the true value. The percentage recovery of the active compounds, for each solution prepared, must be within 95.0 – 105.0 % of the actual amount.

Ethinylestradiol and Levonorgestrel

Sample solutions were spiked with known concentrations of Ethinylestradiol and Levonorgestrel to result in concentrations representing respectively 50, 75, 100,125, and 150% relative to the working concentrations.

Table 4: Ethinylestradiol

Sample	Theoretical	Actual	% Recovery	Average % Recovery
50 %	0.295	0.290	98.3	97.0
50 %	0.295	0.282	95.6	
75 %	0.442	0.433	98.0	98.3
75 %	0.442	0.436	98.6	
100 %	0.590	0.596	101.0	100.9
100 %	0.590	0.595	100.8	
125 %	0.737	0.724	98.2	99.7
125 %	0.737	0.745	101.1	
150 %	0.885	0.883	99.8	99.9
150%	0.885	0.884	99.9	

The above samples were injected in duplicate according to the method of analysis. From the accuracy results above, the percentage recovery values for Ethinylestradiol satisfy the acceptance criteria for accuracy across the range of 50 % - 150 %. From the accuracy results above, the percentage recovery values for Levonorgestrel satisfy the acceptance criteria for accuracy across the range of 50 % - 150 %. Accuracy results were tabulated in the

Table: 5: Levonorgestrel

Sample	Theoretical	Actual	% Recovery	Average % Recovery
50 %	1.474	1.452	98.5	98.8
50 %	1.474	1.460	99.1	
75 %	2.211	2.204	99.7	100.5
75 %	2.211	2.237	101.2	
100 %	2.948	3.047	103.4	102.8
100 %	2.948	3.009	102.1	
125 %	3.685	3.720	100.9	101.2
125 %	3.685	3.740	101.5	
150 %	4.422	4.489	101.5	101.3
150 %	4.422	4.471	101.1	

Method Precision

The precision of an analytical procedure expresses the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogenous sample.

Repeatability

This parameter determines the repeatability of assay results under the same operating conditions over a short period of time. The % RSD due to Ethinylestradiol and Levonorgestrel concentration for the six samples must be less than or equal to 2.0 %.

Table.6. Repeatability

Sample number	Results (µg/tab)	
	Ethinylestradiol	Levonorgestrel
1	28.88	144.8
2	29.25	145.4
3	29.72	147.6
4	29.14	146.2
5	29.05	145.8
6	29.28	146.5
Mean	29.22	146.0
% RSD	1.0	0.7

Table.7.Uniformity

Sample number	Results (µg/tab)	
	Ethinylestradiol	**Levonorgestrel**
1	104.3	104.5
2	104.7	104.9
3	93.5	93.6
4	92.2	93.7
5	104.1	104.1
6	91.6	92.3
7	95.7	98.2
8	90.5	89.7
9	96.5	96.5
10	103.4	101.3
Mean	97.7	97.9
% RSD	6.0	5.7

Six separate sample preparations of batch 245963 analyzed according to the method of analysis. The % RSD due to Ethinylestradiol and Levonorgestrel concentration for the assay meets the requirements for reproducibility at 0.7 and 1.0 % respectively. Results are tabulated in the Table.6.and uniformity results in the Table.7.

Intermediate Precision

Intermediate Precision of an analytical procedure expresses intra-laboratory variations of the repeatability test performed: by a different analyst, on a different day, and by using different reagents, mobile phases and solvents. The % RSD due to Ethinylestradiol and Levonorgestrel concentration for the six samples must be less than or equal to 2.0 %. The mean results obtained in the repeatability, and the intermediate precision must not differ by more than 3.0 %. Six separate sample preparations of batch 245963 were assayed according to the method of analysis. The % RSD for intermediate precision of Ethinylestradiol is 1.1 % and Levonorgestrel is 0.9 %. The intermediate precision and repeatability comply as they differ by 0.7 % for Ethinylestradiol and 2.8 % for Levonorgestrel. Results are tabulated in the Table: 8&9 respectively.

Table.8: Intermediate precision results

Sample	Results (µg/tab)	
	Ethinylestradiol	**Levonorgestrel**
1	29.47	151.4
2	29.03	150.1
3	29.74	153.9
4	29.21	151.1
5	29.67	152.7
6	29.85	151.7
Mean	29.50	151.8

% RSD	1.1	0.9

Table.9 Repeatability Values

Sample	Mean Results (µg/tab)	
	Ethinylestradiol	**Levonorgestrel**
Repeatability	29.22	146.0
Intermediate Precision	29.50	151.8
Mean	29.36	148.9
% RSD	0.7	2.8

Range

Range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. Based on the accuracy results, the range for the assay of Nordette Yellow Tablets is 15 – 45 µg/tab of Ethinylestradiol and 75.0 – 225.0 µg/tab Levonorgestrel, which represents

50 % - 150 % of the working concentration.

Declaration on the Validity of Method.

The method for the assay of Nordette Yellow Tablets – Sugar Coated complies with the requirements for Specificity, System suitability, Linearity, Accuracy and Method precision across the range of 50 % to 150 %. The method is therefore acceptable as valid and stability indicating. The rapid UPLC method is suitable for cleaning control assays within good manufacturing practices (GMP) of the pharmaceutical industry.

REFERENCES:

1. http://ec.princeton.edu/questions/dose.html#dose
2. 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(2):160-8. doi: 10.1002/bmc.1265.
3. Szabolcs Feketea,, Jeno Fekete, Katalin Ganzlera. Validated UPLC method for the fast and sensitive determination of steroid residues in support of cleaning validation in formulation area. J Pharma Biomedical Analysis 49 (2009) 833–838.
4. Navneet Kumar, D. Sangeetha, and P. Balakrishna. Development and validation of a UPLC method for the determination of duloxetine hydrochloride residues on pharmaceutical manufacturing equipment surfaces Pharm Methods. 2011 Jul-Sep; 2(3): 161–166. doi: 10.4103/2229-4708.90355

5. Prabhakar B, Deshpande SG. Simultaneous estimation of ethinyl estradiol& levonorgestrel from transdermal patches by HPLC. Indian J Pharma Sci 1999, 61(1), 12-15.
6. Durga Prasad, Changala Reddy G, Prasad P. S. S., Mukkanti, Simultaneous HPLC estimation of levonorgestrel and ethinylestradiol from tablets, Indian J Pharma Sci 2004, 66(2), 231-234.
7. Berzas JJ, Rodriguez J, Castaneda G. Simultaneous determination of ethinylestradiol and levonorgestrelin oral contraceptives by derivative spectroscopy. Analyst, 1997;122:41-44.
8. Denisova TA, Chistyakov VV, Sadchikova NP. Quantitative estimation of components of combined hormonal contraceptives by HPLC. Pharma Chemistry J 2008, 42, 291-293.