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Validated RP-HPLC method for the determination of estradiol valerate in bulk and pharmaceutical formulations

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ABSTRACT

The main objective of the present work is to develop a specific validated HPLC method for the determination of Estradiol valerate in bulk and pharmaceutical dosage forms. A reverse phase HPLC method was developed using µ Bondapak Phenyl 5µm (3.9 mm x 30 mm) column and mobile phase of composition Acetonitrile : Water in the ratio of 80:20 v/v at a flow rate of 0.8 mL/min with UV detection at 220 nm for Estradiol Valerate. The retention time of the drug was 2.262 minutes. The developed method was validated for specificity, linearity, precision, accuracy and robustness as per ICH guidelines. Linearity was found in the range of 0.04-0.12mg/ml. The mean recovery of the drug was 80.0 %. The proposed method could be used for routine analysis of Estradiol valerate in their dosage forms. The proposed method is accurate, precise, simple, sensitive and rapid and can be applied successfully for the estimation of estradiol valerate in bulk and in pharmaceutical formulations without interference and with good sensitivity.

Keywords: Liquid Chromatography; Estradiol Valerate, dosage forms, determination, Validation

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INTRODUCTION

Drug Profile

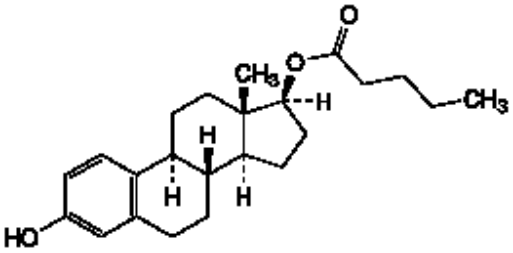


Fig 1: Estradiol Valerate

Estradiol valerate[1] [(17β)-3-hydroxyestra-1,3,5(10)-trien-17-yl valerate] is a synthetic ester, specifically the 17-pentanoyl ester, of the natural estrogen, 17β-estradiol.[3][4] Upon ingestion, regardless of the route of administration, estradiol valerate behaves as a prodrug, being cleaved byesterases in blood plasma and the liver into 17β-estradiol and valeric acid.[5][2] However, compared to estradiol itself, estradiol valerate is absorbed more slowly and possesses

a longer duration, especially when given in an oil solution viaintramuscular injection (in which it acts as a depot). As a result, it can be administered less frequently.[6][7][8] It is a female estrogen hormone. It works by replacing natural estrogens in a woman who can no longer produce enough estrogen. It works for advanced prostate cancer by antagonizing male hormones. The side effects mainly include nausea/vomiting, bloating, breast tenderness, headache, or weight changes may occur.

MATERIALS AND METHODS

Instrumentation:
Peak HPLC containing LC 20AT pump and variable wavelength programmable UV-Visible detector and Rheodyne injector was employed for investigation. The chromatographic analysis was performed on a µBondapak Phenyl 5µm (3.9 mm x 30 mm) column. 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 Estradiol Valerate was obtained from Cipla, Mumbai. The Formulation was procured from the local market. Water, Buffer, Methanol and acetonitrile used were of HPLC grade and purchased from Merck Specialties Private Limited, Mumbai, India.

The mobile phase
A mixture of Acetonitrile : water in the ratio of 80:20 v/v was prepared and used as mobile phase.

Preparation of solutions
Preparation of Norgestrel Stock Solution
Accurately weighed 62.5 mg Norgestrel Dissolved in 120 ml acetonitrile by sonicating for 1 minute with frequent shaking. Allowed to cool to room temperature and diluted to 250 ml with water.

Preparation of Estradiol Valerate Stock Solution
Accurately weighed 100 mg Estradiol Valerate into a 100 ml volumetric flask. Dissolved in 80 ml of acetonitrile by sonicating for 10 minutes with frequent shaking. Allowed it to Cool to room temperature and diluted to 100 ml with water.

Preparation of Working Standard Solution
Diluted 8 ml of Stock Standard Estradiol Valerate and 8 ml of Stock Standard Norgestrel to 100 ml with solvent .

Sample solution
Placed 10 tablets in a 250 ml volumetric flask. Added 50 ml of water. Sonicated for 15 minutes with frequent shaking. Diluted to volume with acetonitrile. Filtered through a 0.45 µm filter before use, discarding the first 5 ml of filtrate.

METHOD DEVELOPMENT
For developing the method [23-37], a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choice of stationary and mobile phases. The following studies were conducted for this purpose.

Detection of wavelength
The spectrum of 10 ppm solution of Estradiol Valerate was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength 220nm was observed. The graph was shown in the Fig:2.

Choice of stationary phase and mobile phase
Finally the expected separation and peak shapes were obtained on µBondapak Phenyl 5µm (3.9 mm x 30 mm) column.

Flow rate
Flow rates of the mobile phase were changed from 0.5-1.5 ml/min for optimum separation. It was found from experiments that 0.8 ml/min flow rate was ideal for elution of analyte.

Optimized chromatographic conditions
Chromatographic conditions as optimized above were shown in Table: 1 These optimized conditions were followed for the determination of Estradiol Valerate in bulk samples and in its Formulations.

VALIDATION OF PROPOSED METHOD AND REQUIREMENTS:
The proposed method [9-22] was validated as per ICH guidelines. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and solution stability.

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 Estradiol Valerate peaks. The peak purity results from the photo diode-array analysis must show that the Estradiol Valerate peak is pure – i.e. the purity angle (PA) must be less than the threshold angle (TH). The solutions Solvent, Placebo at working concentration, API at working concentration, Product at working concentration was injected using the conditions specified in the method of analysis. Significant peaks were not detected in the chromatogram 1and 6. Drug actives – Peak due to Norgestrel eluted at 1.730 min and Estradiol Valerate eluted at 2.272min. Results are shown in the Figure 4. Product – Peak due to Estradiol Valerate eluted at 2.262 min. Results are shown in the Figure 5. Actives – UV stress: Two peaks due to Norgestrel and Estradiol Valerate eluted at 1.734min and 2.326 min respectively. Results are shown in the Figure 6. Product – UV stress: Estradiol Valerate Peak eluted at 2.319 min. Results are shown in the Figure 7. Estradiol Valerate is very stable towards uv/vis exposure. No components are seen to co-elute with Estradiol Valerate peak, and the peak purity results indicate that Estradiol Valerate peak can therefore be considered spectrally pure. The method employed is specific for the assay Estradiol Valerate in the product. The Chromatogram results are shown from the Fig: 2 to Fig: 7 and the Peak purity results are shown from Fig: 8 to Fig: 13.

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 Estradiol Valerate 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 $+2 > z > -2$. Five solutions containing 50, 75, 100, 125 and 150 % of Estradiol Valerate, relative to the working concentrations of 0.08 mg/ml, were prepared and injected according to the method of analysis. A linear regression curve was constructed, and the correlation coefficients (R) and assessment values calculated. The correlation coefficient (R) for Estradiol Valerate is 1.000. The plot is a straight line, and the assessment value (z) falls within the specified limit at 0.3 for Estradiol Valerate. The method is therefore linear within the specified range. The linearity results are shown the table: 2 and the linearity curve is shown in Fig: 14.

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 Norgestrel and Estradiol Valerate for five injections must be less than or equal to 2%. The tailing factor of the peak due to Estradiol Valerate must not be more than 2.0 the peak resolution must be greater than 2.0. Five replicate injections of working standard solution were injected according to the method of analysis. The percentage relative standard deviation (% RSD) for the peak responses, the tailing factor, and resolution were determined. The analytical system complies with the requirements specified by the system suitability. System suitability results are tabulated in the Table: 3.

DETECTION LIMIT
The limit of detection by definition is a parameter of a limit test. It is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated under the stated experimental conditions. It merely substantiates

that analyte concentration is above or below a certain level. The Detection Limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliable detected. The maximum allowable carryover of Estradiol Valerateis 0.502 mg/swab as determined in the Cleaning Validation Matrix. Eight solutions containing 12.55, 1.255, 0.502, 0.251, 0.1255, and 0.06275 mg/swab of Mepyramine Maleate, relative to the working concentrations, were prepared and injected according to the method of analysis. A linear regression curve was constructed. The detection limit must be capable of detecting the API at 50% MAC. The method gives linear response from 12.55 – 0.0625 mg/swab of Estradiol Valerate. The Linearity curve for LOD is shown in the Fig: 15. The results are tabulated in the
Table: 4.

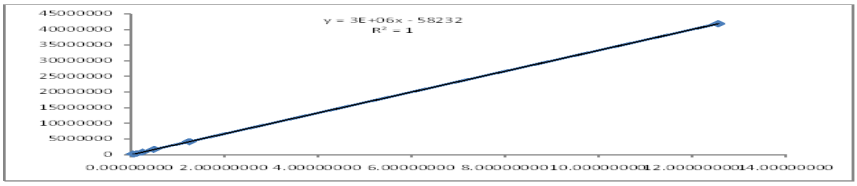


Fig: 15 Linearity curve for LOD

Table: 4 LOD Results

Conc. Mg/Slab	INJ 1	INJ 2	Average
0.06250000	125805	121735	123770
0.12550000	332705	333831	333268
0.25100000	765688	756402	761045
0.50200000	1675335	1671514	1673425
1.25500000	4155693	4144333	4150013
12.5500000	41786210	41862762	41824486

Percentage Relative Standard Deviation(R.S.D)
Inject the standard and sample preparation according to test the system suitability to the following criteria: The % recovery should be greater than or equal to 65 %. Results are tabulated in the Table: 5.

Table: 5 Percentage RSD Results

Sample	% Recovery	% Average Recovery
1	101	101
	100	
2	69	69
	69	
3	65	65
	65	
4	104	104
	104	
5	70	71
	71	
6	67	67
	67	
Mean		80

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 98 - 102% of the actual amount. Sample solutions were spiked with known concentrations of Estradiol Valerate to result in concentrations of 0.04mg/ml, 0.06 mg/ml, 0.08 mg/ml, 0. 1 mg/ml, and 0. 12 mg/ml representing respectively 50, 75, 100, 125 and 150% of Estradiol Valerate relative to the working concentration of 0.08 mg/ml. The above samples were injected in duplicate according to the method of analysis. The percentage recovery values for Estradiol Valerate satisfy the acceptance criteria for accuracy across the range of 50 % - 150 %. Following this, together with the precision and specificity results, it can be accepted that the method is accurate across the range of 50% - 150%. The Accuracy results are tabulated in the Table: 6.

Table: 6 Accuracy Results

Sample	Theoretical	Actual	%Recovery
50%	9.7330	9.8113	101
50%	9.7330	9.8173	101
75%	14.7330	14.9443	101
75%	14.7330	14.9487	101
100%	19.4660	19.7177	101
100%	19.4660	19.6806	101
125%	24.4660	24.2304	99
125%	24.4660	24.2039	99
150%	29.4660	28.9387	98
150%	29.4660	28.9140	98

Method Precision

The precision of an analytical procedure expresses the degree agreement among individual test results when the method is applied repeatedly to multiple samplings 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 Estradiol Valerate concentration for the six samples must be less than or equal to 2%. Six separate sample preparations of batch 226141 were assayed according to the method of analysis. The % RSD due to Estradiol Valerate concentration for the six samples meets the requirements for reproducibility at 1.0%. Repeatability results are shown in the table: 7.

Intermediate Precision

Intermediate Precision of an analytical procedure expresses intra-laboratory variations of the repeatability test performed by: by a different analyst, and on a different day, and on a different instrument, and using different columns, reagents, mobile phases and solvents. The % RSD due to Estradiol Valerate concentration for the six samples must be less than or equal to 2%. The mean results obtained in the repeatability, and the intermediate precision must not differ by more than 3%. Six separate sample preparations of batch 226141 were assayed according to the method of analysis. Specified parameters varied from the repeatability test. The % RSD for Estradiol Valerate for the six samples is 0.9 % respectively. The intermediate precision mean deviates from the mean obtained for repeatability by 0.6% for Estradiol Valerate. Results are tabulated in the table: 8 and 9.

Range

Range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity

RESULTS AND DISCUSSION

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of Acetonitril: Water 80:20 v/v and 0.8mL/min flow rate proved to be better than the other mixtures in terms of resolution and peak shape. The optimum wavelength for detection was set at 225nm at which much better detector responses for drug was obtained. As it was shown in Figure. The retention times for norgestrel and estradiol valerate were 1.73 min and 2.262min respectively. A system suitability test was applied to representative chromatograms for various parameters. The results obtained were within acceptable limits and are represented in Table. Thus, the system meets suitable criteria.

Table 1: Optimized chromatographic conditions for estimation Estradiol Valerate

Mobile phase	**Acetonitrile : Water 80:20v/v**
Pump mode	Isocratic
Diluent	Mobile phase
Column	µBondapak Phenyl 5µm (3.9 mm x 30 mm)30 mm) column 30 mm)
Column Temp	Ambient
Wavelength	220 nm
Injection Volume	20 µl
Flow rate	0.8 mL/min
Run time	5 min
Retention Time	± 2.262 minutes

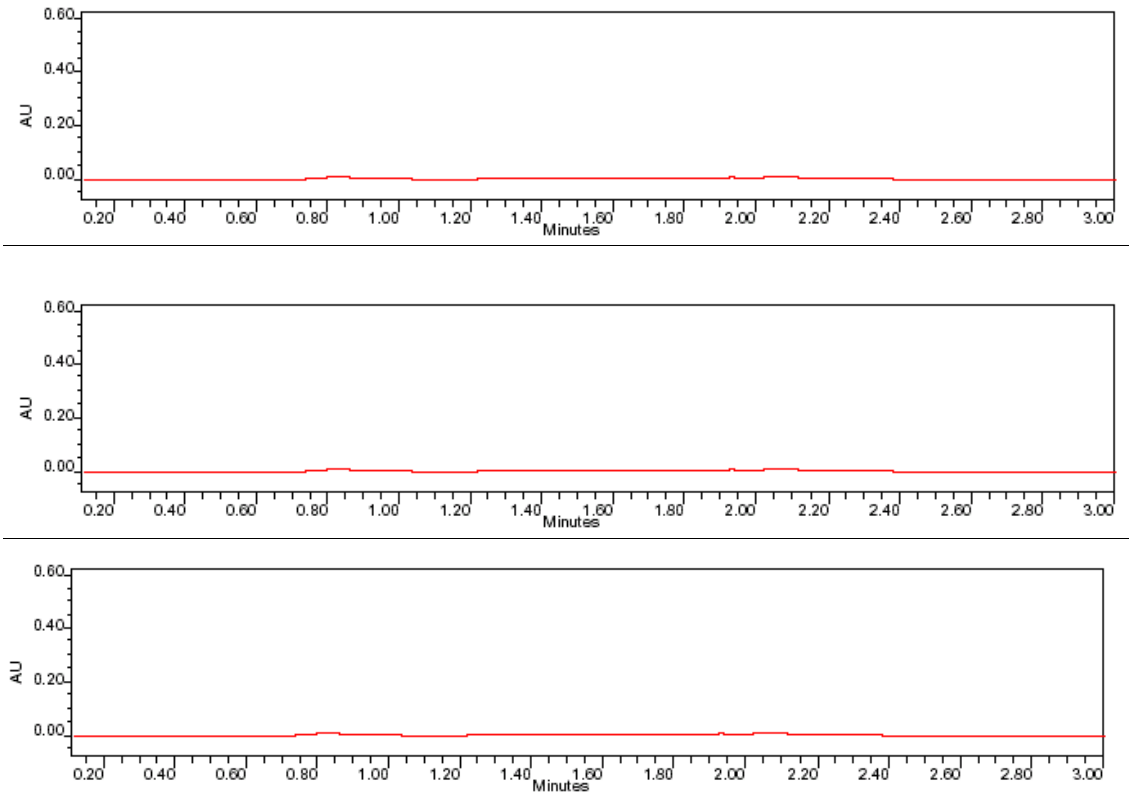


Fig: 2 No significant peak detected (solvent)

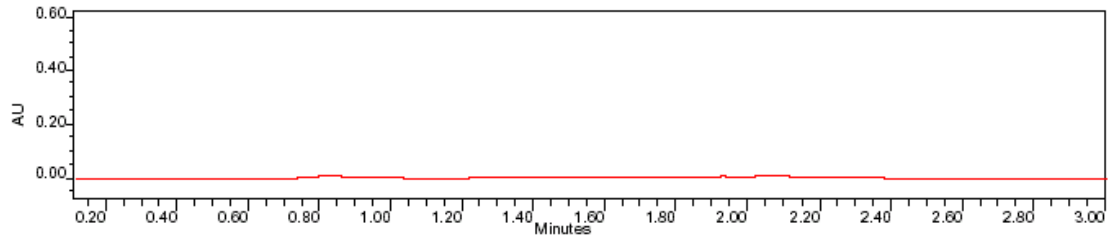
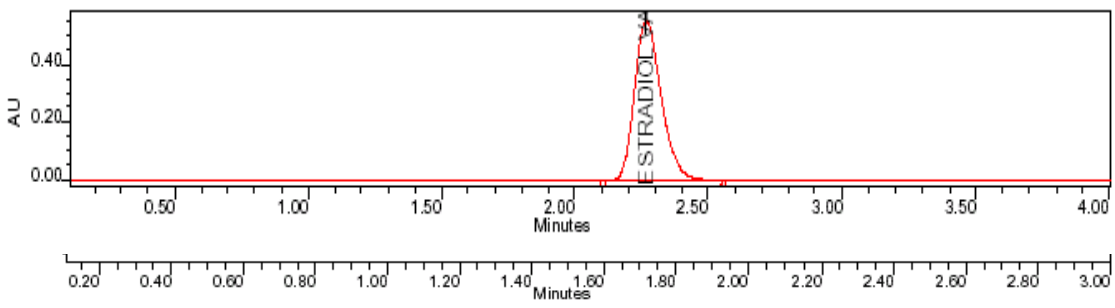


Fig: 3 No Significant peak (Placebo)



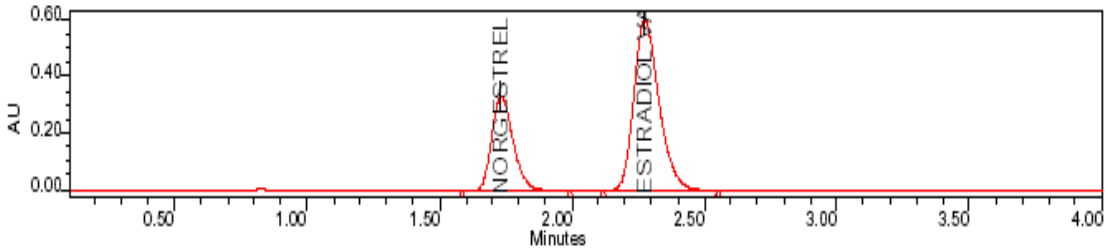


Fig: 4 Peak due to Norgestrel eluted at 1.730 min and Estradiol Valerate eluted at 2.272min

Fig: 5 Peak due to Estradiol Valerate

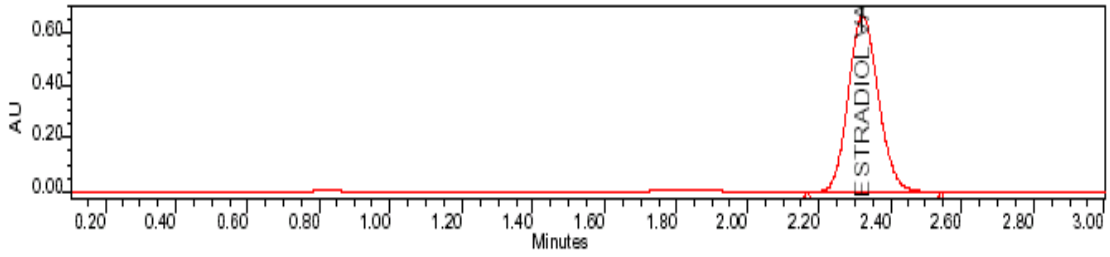


Fig: 6 Two peaks due to Norgestrel and Estradiol Valerate

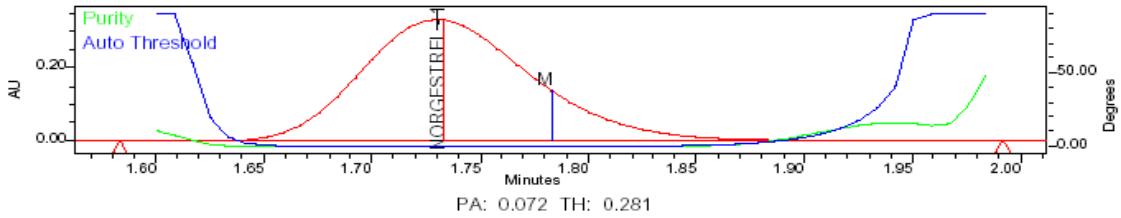


Fig: 7 Estradiol Valerate Peak

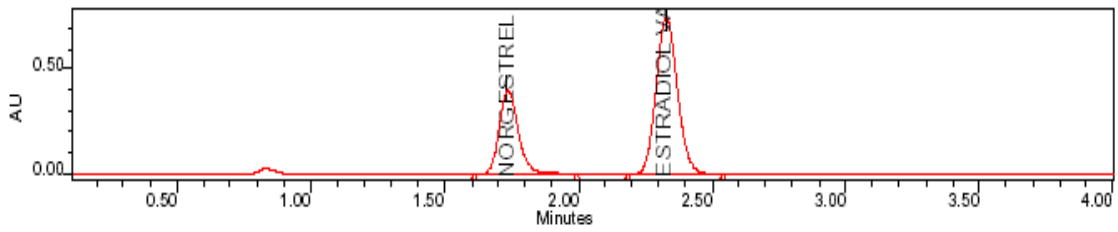


Fig: 8 Peak purity results: purity angle < Threshold angle: 0.072 < 0.346

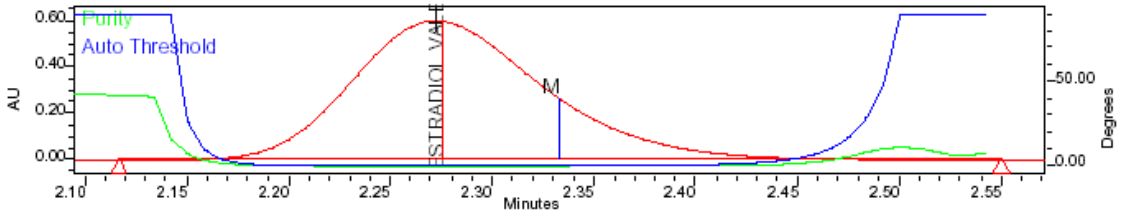


Fig: 9 Peak purity results: purity angle < Threshold angle: 0.132 < 0.346

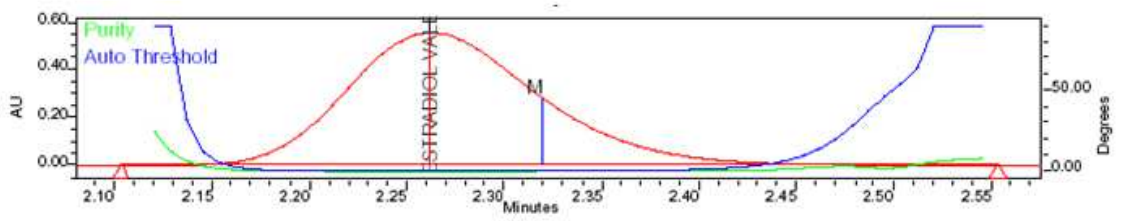


Fig: 10 Peak purity results: purity angle < Threshold angle: 0.166 < 0.379

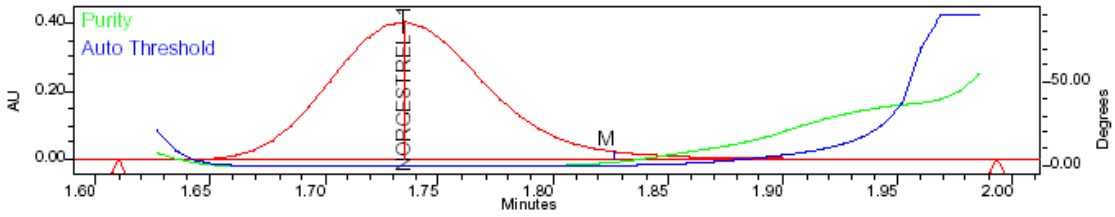


Fig: 11 Peak purity results: purity angle < Threshold angle: 0.141 < 0.269

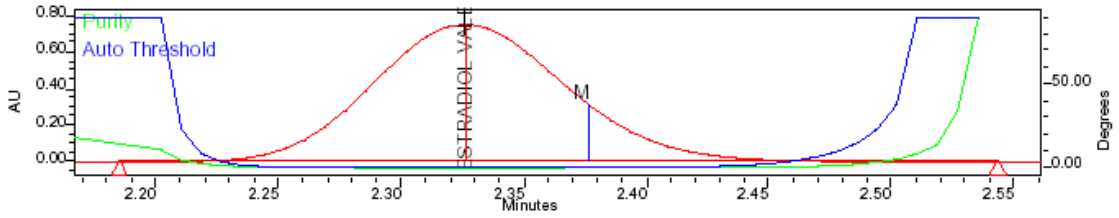


Fig: 12 Peak purity results: purity angle < Threshold angle: 0.177 < 0.380

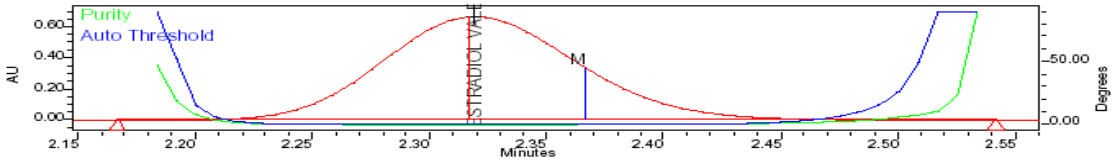


Fig: 13 Peak purity: purity angle < Threshold angle: 0.170 < 0.338

CALIBRATION CURVE: $y = Bx + A$, R^2 = coeff. of determination

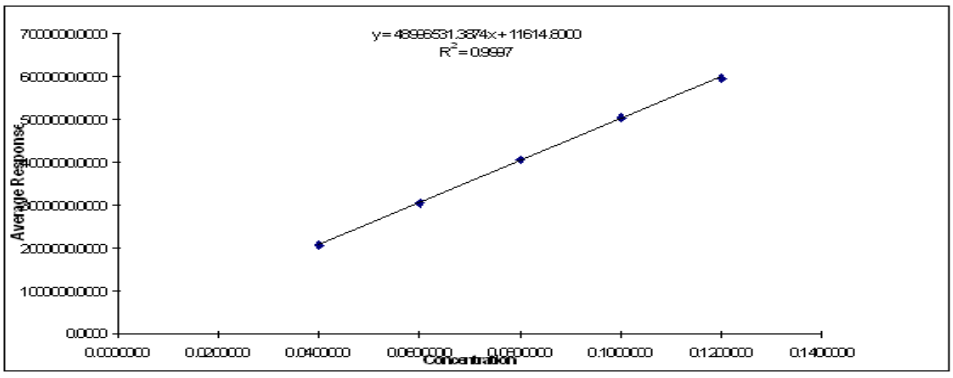


Figure: 14 Linearity Curve

The calibration curve was obtained for a series of concentration in the range of 0.04-0.12mg/ml and it was found to be linear. Seven point's graphs were constructed. The standard deviation of the slope and intercept were low. The data of regression analysis of the calibration curves are shown in Table 2.C.

Table: 2 Linearity results

Sample No.	Concentration	Response 1	Response 2	Average
1	0.0400160	20609620000	20598530000	20604075000
2	0.0600240	30536610000	30530040000	30533325000
3	0.0800320	40618800000	40648500000	40633650000
4	0.1000400	50419210000	50435560000	50427385000
5	0.1200480	59691060000	59655290000	59673175000

Table: 3 System Suitability results

Sample	Estradiol Valerate
1	3913406
2	3918697
3	3921196
4	3912303
5	3920651
Mean	3917251
% RSD	0.1
Tailing factor	1.3
Resolution, R,	3.5

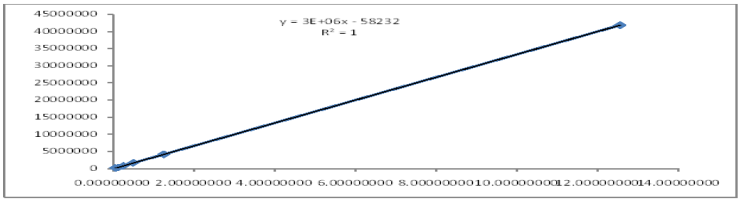


Fig: 15 Linearity curve for Limit of Detection (LOD)

Table: 4 Limit of Detection Results

Conc. Mg/Slab	**INJ 1**	**INJ 2**	**Average**
0.06250000	125805	121735	123770
0.12550000	332705	333831	333268
0.25100000	765688	756402	761045
0.50200000	1675335	1671514	1673425
1.25500000	4155693	4144333	4150013
12.5500000	41786210	41862762	41824486

The proposed method has been applied to the assay of the commercial tablets containing estradiol valerate. Sample was analyzed for five times after extracting the drug as mentioned in assay sample preparation of the experimental section. The results presented good agreement with the labeled content. Low values of standard deviation denoted very good repeatability of the measurement. Thus it was showing that the equipment used for the study was correctly and hence the developed analytical method is highly repetitive. For the intermediate precision a study carried out by the same author working on the same day on two consecutive days indicated a RSD of 1.910 & 1.963. This indicates good method precision.

Table: 5 Percentage RSD Results

Sample	% Recovery	% Average Recovery
1	101	101
	100	
2	69	69
	69	
3	65	65
	65	
4	104	104
	104	
5	70	71
	71	
6	67	67
	67	
Mean		80

Table: 6 Accuracy Results

Sample	Theoretical	Actual	%Recovery
50%	9.7330	9.8113	101
50%	9.7330	9.8173	101
75%	14.7330	14.9443	101
75%	14.7330	14.9487	101
100%	19.4660	19.7177	101
100%	19.4660	19.6806	101
125%	24.4660	24.2304	99
125%	24.4660	24.2039	99
150%	29.4660	28.9387	98
150%	29.4660	28.9140	98

Table: 7 Repeatability results

Sample number	Results [mg/tablet]
	Estradiol Valerate
1	1.9606
2	1.9461
3	1.9100
4	1.9379
5	1.9519
6	1.9630
Mean	1.9449
% RSD	1.0

Table: 8 Intermediate Precision

Sample number	Results [mg/tablet]
	Estradiol Valerate
1	1.9668
2	1.9905
3	1.9685
4	1.9405
5	1.9522
6	1.9489
Mean	1.9612
%RSD	0.9

Table: 9 Deviation of Repeatability mean from Intermediate Precision mean

Sample	Mean Results [mg/tablet]
	Estradiol Valerate
Repeatability	1.9449
Intermediate Precision	1.9612
Mean	1.9531
% RSD	0.6

The system suitability parameter like capacity factor, asymmetry factor, tailing factor and number of theoretical plates were also calculated. It was observed that all the values are within the limits. The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of estradiol valerate in tablet formulation.

All these factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive and rapid and can be applied successfully for the estimation of estradiol valerate in bulk and in pharmaceutical formulations without interference and with good sensitivity.

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