Analytical Method Validation Report
Methycobalamin 750 mcg & Pregabalin 75 mg Tablets

ANALYTICAL METHOD VALIDATION REPORT FOR METHYCOBALAMIN 750 MCG & PREGABALIN 75 MG TABLETS

CONTRIBUTIONS:

This protocol is a team effort of Quality control Laboratory chemists to achieve the objective of validating the analytical methods carried out to estimate the contents of pharmaceutical products manufactured.

Analytical Method Validation Protocol Number		
Validation Frequency	Analytical Methods should be validated during and at the end of development process and after any significant change in analytical method.	
	Designation	**Name of the Person**
Prepared By	Executive QC	
Checked By	Asst. Manager QC	
Reviewed By	Manager QA	
Approved By	Operation Head	

What is Validation?

Validation is the evaluating of processes, products or analytical methods to ensure compliance with product or method requirements. One of the most popular definitions of Validation came from the 'US FDA' General Principle of Validation **"Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes."**

The term Validation & Qualification are often mixed up and there is also some overlap. Equipment Qualification means checking an instrument for compliance with previously defined functional and performance specifications. For Operational Qualification generic standards and analytical conditions are used rather than real sample conditions. Validation relates more to the entire but sample specific process including sample preparation, analysis, and data evaluation.

Validation efforts in the analytical laboratory should be broken down into separate components addressing the equipment and the analytical methods run on that equipment. After these have been

verified separately they should be checked together to confirm expected performance limits **(System-Suitability Testing),** and finally the sample analysis data collected on such a system should be authenticated with suitable validation checkouts. All methods / equipment that are used to create, modify, maintain, archive or distribute critical data for cGMP/GLP.

Analytical method should be validated prior to routine use and after changing method parameters. Peoples involved in Validation exercise should be qualified for their jobs. This includes education, training and/or experience.

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications.

Typical analytical performances characteristics that should be considered in the validation of the types of methods are as follows.

- **Accuracy**
- **Precision**
- **Specificity**
- **Detection Limit**
- **Quantitation Limit**
- **Linearity**
- **Range**
- **Robustness**

USP 40 in "(1225) Validation of compendial procedures" says Category I (Analytical methods for Quantization of major components of bulk drug substances or active ingredients including preservative in finished pharmaceutical products) should comply with **Accuracy, Precision, Specificity, Linearity, Robustness, & Range.**

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However after discussions with many experts & referring some of the IDAM – APA magazines, we have decided to at least comply with **Accuracy, Linearity, Precision, and Robustness.**

Validation Report

Once the method has been validated, a validation report should be prepared that includes.

- Objective & scope of the method (applicability, type).
- Summary of the methodology.
- Type of compound & matrix.
- All chemical, reagents, reference standards, detailed instruction on their preparation.
- Method parameters.
- Detailed condition on how the experiments were conducted including sample preparation. The report must be detailed enough to ensure that it can be reproduced by a competent technician with comparable equipment.
- Statistical procedures & representative calculations.
- Representative plots
- Performance data for acceptance limit
- Criteria for revalidation
- Summary & conclusions
- Approval with name, designations, date & signatures of those responsible for the review & approval of the analytical test procedure.

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Validation Analysis Records for Methycobalamin 750 mcg & Pregabalin 75 mg Tablets

OBJECTIVE: The efficacy & safety of a medicinal product can only be assured by analytical monitoring of its quality.

SCOPE: The scope of analytical validation is to ensure that the procedure under consideration is capable of giving reproducible and reliable results.

Product Name	Methycobalamin 750 mcg & Pregabalin 75 mg Tablets
Ingredient	Methycobalamin 750 mcg & Pregabalin 75 mg Tablets
Label Claim	Each film coated tablet contains:- Methycobalamin 750 mcg Pregabalin 75 mg Colour: Sunset Yellow FCF & Titanium Dioxide BP
(A)Test Method	By UV Spectrophotometer **Methylcobalamin**

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Specificity (Diluents Interference)

Placebo Preparation:

A placebo solution was prepared same as the formulation except for the addition of the active ingredients. Here, the product contains no inactive ingredients. So, here the diluents are used as the placebo solution. Absorbance at 352 nm, Observation Result: Nil

Conclusion for Specificity:

We observed that at wavelength 352 nm there is no significant Absorbance for placebo (Diluents) for Methylcobalamin assay method. Therefore specificity of the method considered acceptable.

System Accuracy:

The system precision of the above method was carried out by taking Absorbance for six times of the sample preparation.

Serial No.	Absorbance
1.	0.594
2.	0.593
3.	0.592
4.	0.595
5.	0.593
6.	0.594
Mean	0.5935
% RSD	**0.176**

Acceptance Criteria: RSD is not more than 2.0%.

Range:

Definition:

The Range of an analytical method is the interval between the upper & lower level of analyte that has been demonstrated with precision, accuracy & linearity using the method as written. The Range is

normally expressed in same units as test results e.g. Percent or Parts per million, obtained by the analytical method.

Assay:

For Methylcobalamin:-

Buffer: Add 3.4 ml of Orthophosphoric Acid in 1000 ml of water. Adjust the solution with Ammonia to a pH of 2.7.

Mobile phase: Acetonitrile : Buffer (215:800).

Reference Solution: Transfer 50 mg of Methylcobalamin RS in 100 ml volumetric flask and add 70 ml of mobile phase and shake for dissolving and sonicate and make up to volume with mobile phase. Dilute 5 ml of this solution to 50 ml with mobile phase.

Sample solution: Weigh powder equivalent to 5 mg of Methylcobalamin in 70 ml of the mobile phase, sonicate to dissolve and dilute to 100 ml with the mobile phase.

Chromatographic System:

Mode: LC

Detector: UV 352 nm

Flow Rate: 1 ml/min

Injection Volume: 20µl

Inject the test solution and reference solution.

Calculation:

$$\text{Assay in mg} = \frac{At}{As} \times \frac{Ws}{100} \times \frac{5}{50} \times \frac{100}{Wt} \times \frac{P}{100} \times AW = \text{-------mcg/Tab}$$

$$\%\ \text{Assay} = \frac{\text{Result in mcg}}{\text{Label claim}} \times 100 = \quad \%$$

Where,

At = Average area of test preparation of Methylcobalamin

As = Average area of standard preparation of Methylcobalamin

Ws = Weight of standard Methylcobalamin

P = Potency of Methylcobalamin Standard.

Wt = Weight of test sample

ANALYTICAL METHOD VALIDATION REPORT FOR METHYCOBALAMIN 750 MCG & PREGABALIN 75 MG TABLETS

AW = Average weight of the tablet

S.No.	Standards	Absorbance
1.	Standard-1	0.599
2.	Standard-2	0.599
3.	Standard-3	0.598
4.	Standard-4	0.599
5.	Standard-5	0.601
6.	Standard-6	0.599
	Mean	0.5992
	% RSD	**0.164**

S.No.	Sample absorbance	Mean	% Assay
1.	0.592	0.5925	99.23
2.	0.593		

Linearity/ Accuracy:

Definition:

The Linearity of an analytical method is its ability to elicit test results that are directly, or by a well defined mathematical transformation, proportional to the concentration of the analyte in samples within a given range. Linearity is usually expressed in terms of the variance around the Slope of the regression

line calculated according to an established mathematical relationship from test results obtained by the analysis of sample with varying concentration of analyte.

Standard solution :

Accurately weigh 750 mcg of Methylcobalamin WS in 100 ml volumetric flask and make up the Volume with 0.1M Methanolic HCl. Filter; take 2 ml and makeup to 100 ml with 0.1M Methanolic HCl.

Sample solution:

ANALYTICAL METHOD VALIDATION REPORT FOR METHYCOBALAMIN 750 MCG & PREGABALIN 75 MG TABLETS

Accurately weigh as required of the sample powdered quantity in 10 ml volumetric flask and make up the volume with methanol. Filter; take 2 ml and makeup to 100 ml with 0.1M Methanolic HCl. Take the absorbance of both the solution standard and test against 0.1M Methanolic HCl as blank at 352 nm:

Calculation:

$$\frac{\text{Sample abs.} \times \text{WS weight} \times \text{Potency of WS} \times \text{Average Weight} \times 100}{\text{Standard abs.} \times \text{Sample weight} \times 100 \times \text{Claim}}$$

= %

Sr. No.	Standards	Absorbance
1.	Standard-1	0.599
2.	Standard-2	0.599
3.	Standard-3	0.598
4.	Standard-4	0.599
5.	Standard-5	0.601
6.	Standard-6	0.599
	Mean	0.5992
	% RSD	**0.164**

Samples	Sample absorbance	Mean
Sample-A-01 80.0%	0.476	
Sample-A-02 80.0%	0.475	
Sample-A-03 80.0%	0.476	0.4753
Sample-B-01 90.0%	0.532	
Sample-B-02 90.0%	0.533	
Sample-B-03 90.0%	0.535	0.5333
Sample-C-01 100.0%	0.595	

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Sample-C-02 100.0%	0.593	
Sample-C-03 100.0%	0.593	0.5937
Sample-D-01 110.0%	0.655	
Sample-D-02 110.0%	0.656	
Sample-D-03 110.0%	0.654	0.655
Sample-E-01 120.0%	0.714	
Sample-E-02 120.0%	0.715	
Sample-D-03 120.0%	0.713	0.714

Data Collection:

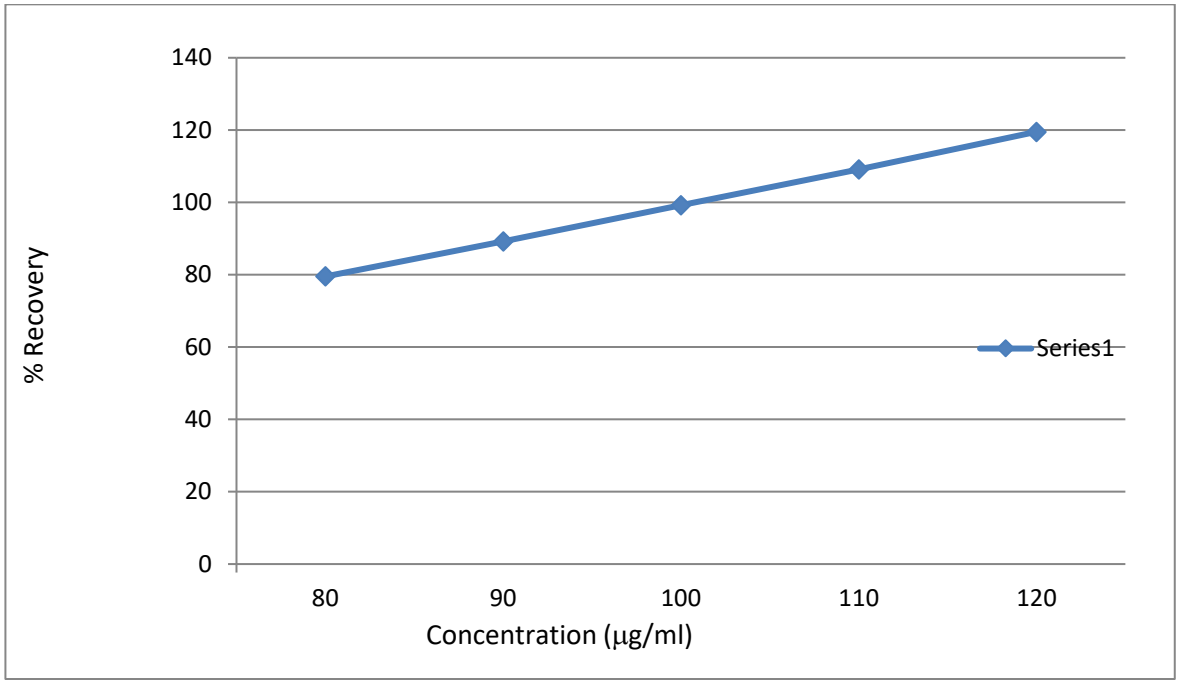
Concentration (µg/ml)	**Concentration in %**	**Corr. Coefficient**	**Sample Mean Abs**	**Recovery %**	**Corr. Coefficient**
16.0	80.0	1.0	0.4753	79.46	0.999986
18.0	90.0		0.5333	89.31	
20.0	100.0		0.5937	99.14	
22.0	110.0		0.6550	109.21	
24.0	120.0		0.7140	119.23	

From the above results, draw a curve.

Linearity plot for Methylcobalamin:

Concentration (µg/ml)	**% Recovery**
16.0	79.46
18.0	89.31
20.0	99.14
22.0	109.21
24.0	119.23

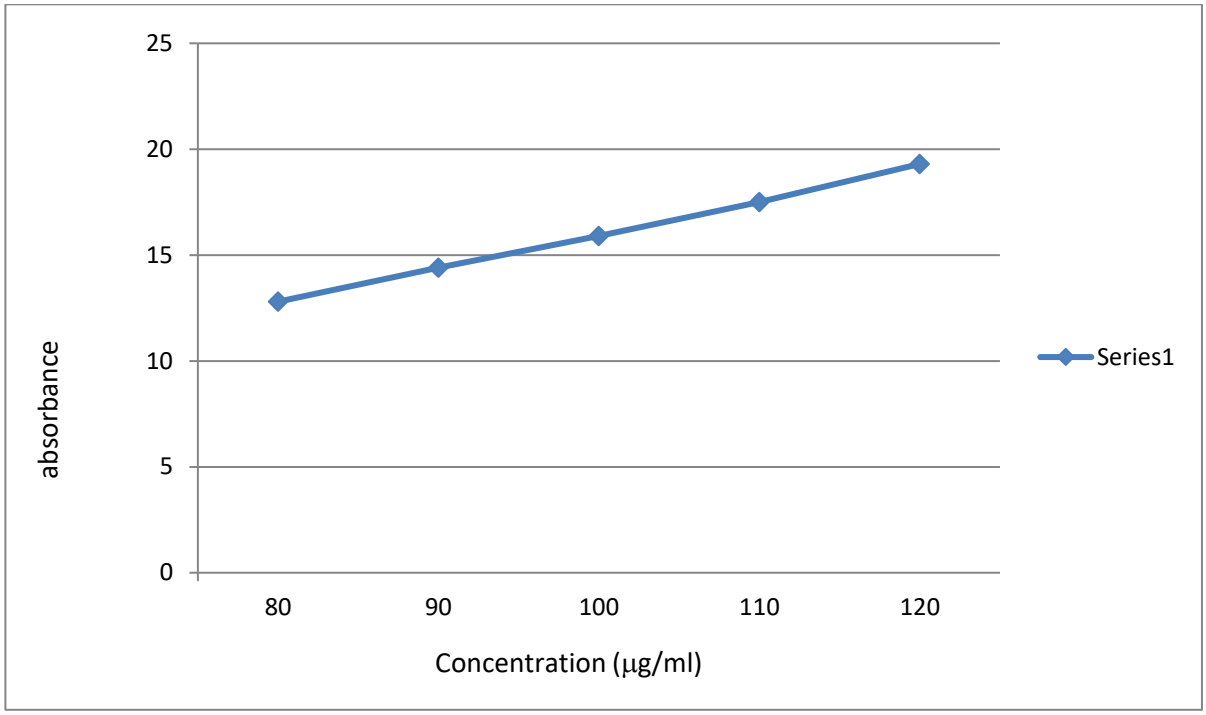
ANALYTICAL METHOD VALIDATION REPORT FOR METHYCOBALAMIN 750 MCG & PREGABALIN 75 MG TABLETS



Linearity plot for Methylcobalamin:

Concentration **(**µg/ml**)**	Absorbance
16.0	0.4753
18.0	0.5333
20.0	0.5937
22.0	0.6550
24.0	0.7140

ANALYTICAL METHOD VALIDATION REPORT FOR METHYCOBALAMIN 750 MCG & PREGABALIN 75 MG TABLETS



R-squared value (R^2)

The R-squared value, also known as the coefficient of determination, is an indicator that ranges in value from 0 to 1 and reveals how closely the estimated values for the trend line correspond to your actual data. A trend line is most reliable when its R-squared value is at or near 1.

Linearity Equation

Equations for calculating trend line

Calculates the least squares fit for a line represented by the following equation:

$$y = m x + b$$

Where m is the slope and b is the intercept.

x = concentration

y = Absorbance Value

Sample

Therefore, from Linearity Equation, **y = mx + b, m** ⟶0.999x

b ⟶0.163

$$\frac{\text{Sample area X WS weight X potency of WS X Average Weight X 100}}{\text{Standard area X Sample weight X 100 X claim}}$$

= %

Conclusion for Linearity:

The graphical representation & data collected during this exercise proves 0.99986 of Methylcobalamin in Methycobalamin 750 mcg & Pregabalin 75 mg Tablets for demonstrate linearity in the range of 80% to 120% when determined by Liquid Chromatographic method.

Precision:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogeneous sample. The precision of the analytical method is usually expressed as Standard deviation or relative standard deviation (coefficient of variation) of a series measurement. The precision may be measured of either the degree of reproducibility or of repeatability of the analytical method on the normal operating condition.

Precision – Method precision

Methylcobalamin: Label Claim 750 mcg/Tablets

(Limit: Not less than 90.0 % of the labeled amount).

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Standard solution :

Accurately weigh 750 mcg of Methylcobalamin WS in 100 ml volumetric flask and make up the volume with 0.1M Methanolic HCl. Filter; take 2 ml and makeup to 100 ml with 0.1M Methanolic HCl.

Sample solution:

Accurately weigh eq. to 100mg of Methylcobalamin of sample powdered in 100 ml volumetric flask and make up the volume with 0.1M Methanolic HCl. Filter; take 2 ml and makeup to 100 ml with 0.1M Methanolic HCl.

Sample Dilutions::

(A) Take 238.7mg of the sample and further proceed as above.

(B) Take 238.1mg of the sample and further proceed as above.

(C) Take 236.5mg of the sample and further proceed as above.

(D) Take 238.4mg of the sample and further proceed as above.

(E) Take 236.9mg of the sample and further proceed as above.

(F) Take 236.3mg of the sample and further proceed as above.

Calculation:

$$\frac{\text{Sample abs.} \times \text{WS weight} \times \text{Potency of WS} \times \text{Average Weight} \times 100}{\text{Standard abs.} \times \text{Sample weight} \times 100 \times \text{Claim}}$$

= %

ANALYTICAL METHOD VALIDATION REPORT FOR METHYCOBALAMIN 750 MCG & PREGABALIN 75 MG TABLETS

Test Data Collection:

S.No.	Standards	Absorbance
1.	Standard-1	0.599
2.	Standard-2	0.599
3.	Standard-3	0.598
4.	Standard-4	0.599
5.	Standard-5	0.601
6.	Standard-6	0.599
	Mean	0.5992
	% RSD	**0.164**

Samples		Sample Absorbance	Mean
Sample A	T1	0.594	0.5990
	T2	0.595	
Sample B	T1	0.592	0.5920
	T2	0.592	
Sample C	T1	0.589	0.5885
	T2	0.588	
Sample D	T1	0.592	0.5930
	T2	0.594	
Sample E	T1	0.587	0.5875
	T2	0.588	
Sample F	T1	0.588	0.589
	T2	0.590	

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Table for Six Replicate Assays

Sample Number	% Estimated Amount	Mean	Relative Standard Deviation (RSD)
Sample A	99.36	99.24%	0.174%
Sample B	99.19		
Sample C	99.27		
Sample D	99.23		
Sample E	98.94		
Sample F	99.44		

Acceptance Criteria: NMT 2% (% of Relative Standard Deviation)

Conclusion for precision: The overall % Relative standard deviation 0.174% for Methylcobalamin in Methycobalamin 750 mcg & Pregabalin 75 mg Tablets there is no significant difference. Therefore Repeatability of the method considered acceptable as it well within 2 % Relative Standard Deviation.

Intermediate Precision –

(Within laboratory variations such as different days, analyst & equipments):

Standard solution :

Accurately weigh 100.8 mg of Methylcobalamin WS in 100 ml volumetric flask and make up the volume with 0.1M Methanolic HCl. Filter; take 2 ml and makeup to 100 ml with 0.1M Methanolic HCl.

Sample solution:

Accurately weigh eq. to 100.0mg of Methylcobalamin of sample powdered in 100 ml volumetric flask and make up the volume with 0.1M Methanolic HCl. Filter; take 2 ml and makeup to 100 ml with 0.1M Methanolic HCl.

Sample Dilutions:

(A) Take 236.4mg of the sample and further proceed as above.

(B) Take 239.5mg of the sample and further proceed as above.

(C) Take 236.6mg of the sample and further proceed as above.

(D) Take 239.1mg of the sample and further proceed as above.

(E) Take 238.7mg of the sample and further proceed as above.

(F) Take 236.6mg of the sample and further proceed as above.

Test Data Collection:

Standards	Absorbance
Standard 1	0.599
Standard 2	0.598
Standard 3	0.601
Standard 4	0.602
Standard 5	0.599
Standard 6	0.600
Mean	0.5998
% RSD	**0.245**

Samples		Sample Absorbance	Mean
Sample A	T1	0.588	0.5890
	T2	0.590	
Sample B	T1	0.598	0.5975
	T2	0.597	
Sample C	T1	0.588	0.5885
	T2	0.589	
Sample D	T1	0.595	0.5960
	T2	0.597	
Sample E	T1	0.594	0.5935
	T2	0.593	
Sample F	T1	0.589	0.588
	T2	0.587	

Test Data analyst by "……."

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Sample Number	% Estimated Amount	Mean	Relative Standard Deviation (RSD)
Sample A	99.40	99.32%	0.155%
Sample B	99.53		
Sample C	99.23		
Sample D	99.44		
Sample E	99.19		
Sample F	99.15		

Acceptance Criteria: NMT 2 % (% of Relative Standard Deviation).

Conclusion for Intermediate Precision:

The overall % Relative standard deviation of two different analysts are 0.17% & 0.15% Methylcobalamin in Methycobalamin 750 mcg & Pregabalin 75 mg Tablets there is no significant difference between two analysts Within laboratory variations such as different days, analyst & equipments.

Therefore reproducibility of the method considered to be acceptable.

(B)Test Method

Pregabalin

Buffer: Weigh 2.72 g of KH_2PO_4 into a 1000 ml flask. Add containing 2 ml of Triethylamine and 900 ml of water. Dissolved it and dilute to volume with water. Adjust the solution with Orthophosphoric acid to a pH of 6.0.

Mobile phase: Acetonitrile : Buffer : Methanol (30:920:50).

Reference Solution: Transfer 100 mg of Pregabalin RS in 100 ml volumetric flask and add 70 ml of mobile phase and shake for dissolving and sonicate and make up to volume with mobile phase.

Sample solution: Weigh accurately powder equivalent to 100 mg of Pregabalin with 70 ml of the

mobile phase, sonicate to dissolve and dilute to 100 ml with the mobile phase.

Chromatographic System:

Mode: LC

Detector: UV 205 nm

Flow Rate: 1 ml/min

Injection Volume: 20µl

Inject the reference solution and the test solution.

Calculation:

$$\text{Assay in mg} = \frac{At}{As} \times \frac{Ws}{100} \times \frac{100}{Wt} \times \frac{P}{100} \times AW = \text{---------- mg/tab}$$

$$\text{\% Assay} = \frac{\text{Result in mg}}{\text{Label claim}} \times 100 = \text{\%}$$

Where,

At = Average area of test preparation of Pregabalin

As = Average area of standard preparation of Pregabalin

Ws = Weight of standard Pregabalin

P = Potency of Pregabalin Standard.

Wt = Weight of test sample

AW = Average weight of the tablet

ANALYTICAL METHOD VALIDATION REPORT FOR METHYCOBALAMIN 750 MCG & PREGABALIN 75 MG TABLETS

Test Method Liquid Chromatography.

Specificity (**Diluents Interference**)

Placebo Preparation: A placebo solution was prepared same as the formulation except for the addition of the active ingredients. Here, the product contains no active ingredients. So, here the mobile phase is used as the placebo solution. Area at 274 nm, Observation Result: Nil

Conclusion for Specificity: We observed that at wavelength 274 nm there is no significant area for placebo (Diluents) for **Pregabalin** in tablets assay method. Therefore specificity of the method considered acceptable.

System Accuracy

The system precision of the above method was carried out by taking area for six times of the sample preparation of exact weight.

Serial No.	Area of Pregabalin
1.	69010.7
2.	69125.1
3.	69245.8
4.	68956.2
5.	69124.5
6.	69245.6
Mean	69092.5
RSD	**0.163%**

Acceptance Criteria: RSD is not more than 2.0%.

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Linearity/ Accuracy:

Definition:

The Linearity of an analytical method is its ability to elicit test results that are directly, or by a well defined mathematical transformation, proportional to the concentration of the analyte in samples within a given range. Linearity is usually expressed in terms of the variance around the slope of the regression line calculated according to an established mathematical relationship from test results obtained by the analysis of sample with varying concentration of analyte.

Range:

Definition:

The Range of an analytical method is the interval between the upper & lower level of analyte that have been demonstrated with precision, accuracy & linearity using the method as written. The Range is normally expressed in same units as test results e.g. Percent or Parts per million, obtained by the analytical method.

Assay:

Limit: Pregabalin in Tablet.

Label Claim 75 mg/tablet

(Limit: 90.0 % to 110.0 % of the labeled amount).

Chromatographic conditions:-

Mode: LC

Detector: UV 205 nm

Flow Rate: 1 ml/min

Injection Volume: 20µl

Inject the reference solution and the test solution.

Standard Solution: Weigh accurately 25 mg of *Pregabalin WS* and transfer to a 25.0 ml volumetric flask, add 20.0 ml of solvent mixture and sonicate to dissolve. Make up the volume with solvent mixture

and mix. Dilute 1.0 ml of this solution to 25.0 ml with mobile phase and mix.

Test Solution: Weigh and powder 20 tablets. Disperse a quantity of the powder containing 75 mg Pregabalin in to 50.0 ml of volumetric flask add 40 ml of Solvent mixture and sonicate to dissolve. Make up the volume with solvent mixture and mix. Further dilute 1.0 ml of this solution to 10.0 ml with mobile phase.

Chromatographic system:

Solvent mixture. A mixture of 70 volumes of *acetonitrile* and 30 volumes of *water.*

Mobile phase: A mixture of 25 volumes of a buffer solution prepared by dissolving 2.72 g *of potassium dihydrogen orthophosphate* into 1000 ml of *water*, adjusted to pH 7.5 with *sodium hydroxide* solution, 75 volumes of *acetonitrile* and filter.

Procedure: Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency is not less than 2000 theoretical plates and the relative standard deviation for replicate injection is not more than 2.0 %.

Inject the reference solution and test solution.

Calculate the content of Pregabalin in the tablets.

$$\frac{\text{Sample area} \times \text{WS weight} \times \text{potency of WS} \times \text{Average Weight}}{\text{Standard area} \times \text{Sample weight} \times 100 \times \text{claim}} = \quad \%$$

ANALYTICAL METHOD VALIDATION REPORT FOR METHYCOBALAMIN 750 MCG & PREGABALIN 75 MG TABLETS

Sr no	Standards	Area
	Standard-1	69010.7
	Standard-2	69125.1
	Standard-3	69245.8
	Standard-4	68956.2
	Standard-5	69124.5
	Standard-6	69245.6
	Mean	69092.5
	RSD	**0.163%**

Samples	Sample Area	Mean
Sample-A-01 70mcg	48307.5	
Sample-A-02 70mcg	48285.2	48297.9
Sample-A-03 70mcg	48301.2	
Sample-B-01 80mcg	55045.2	
Sample-B-02 80mcg	55102.4	55065.3
Sample-B-03 80mcg	55048.5	
Sample-C-01 90mcg	62110.5	
Sample-C-02 90mcg	62045.6	62100.4
Sample-C-03 90mcg	62145.2	
Sample-D-01 100mcg	68945.5	
Sample-D-02 100 mcg	69012.4	68984.1
Sample-D-03 100 mcg	68994.5	

Data Collection:

Concentration (µg/ml)	Concentration in %	Sample Mean Area	Recovery %
14	70%	48297.9	69.88%

16	80%	55065.3	79.75%
18	90%	62100.4	90.0%
20	100%	68984.1	99.85%

From the above results, draw a curve.

Linearity plot for Pregabalin in tablets

Definition:

The Linearity of an analytical method is its ability to elicit test results that are directly, or by a well defined mathematical transformation, proportional to the concentration of the analyte in samples within a given range. Linearity is usually expressed in terms of the variance around the slope of the regression line calculated according to an established mathematical relationship from test results obtained by the analysis of sample with varying concentration of analyte.

Data Collection:-

Concentration in %	Corr. Coefficient	Sample consumed Volume (ml)	Recovery%	Corr. Coefficient
80.0	1.0	12.8	79.54	0.999931
90.0		14.4	89.26	
100.0		15.9	99.19	
110.0		17.5	109.13	
120.0		19.3	119.53	

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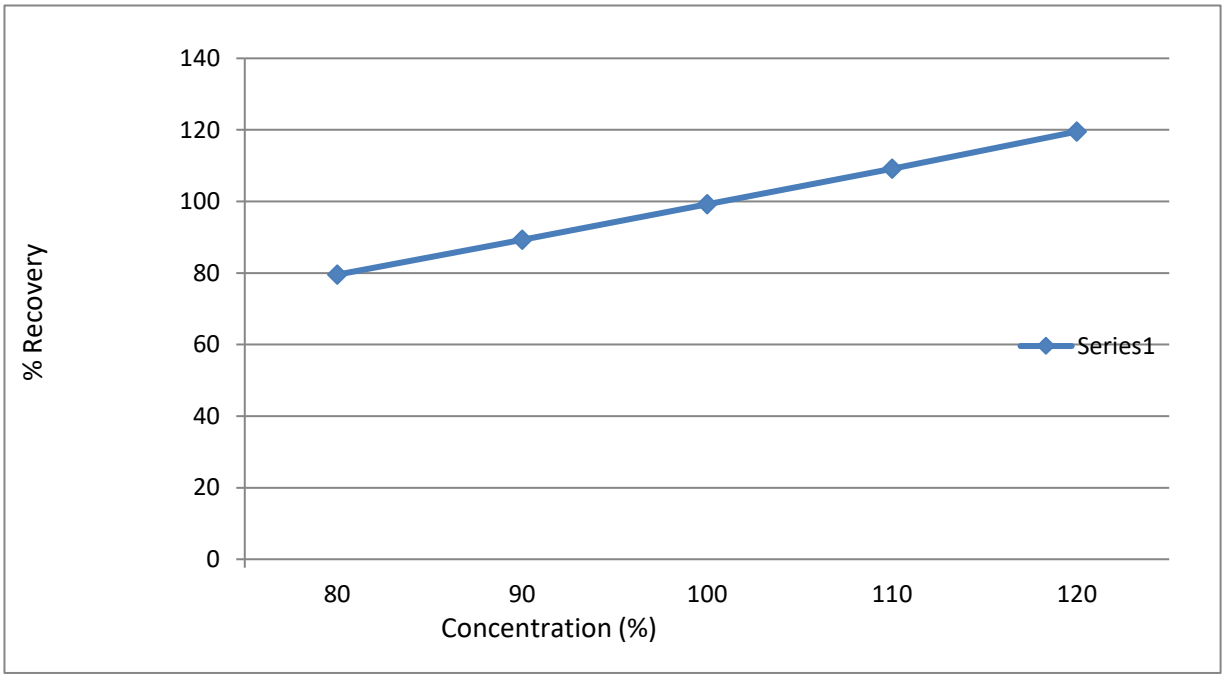
From the above results, draw a curve.

Linearity plot for Pregabalin:

Concentration (%)	% Recovery
80.0	79.54
90.0	89.26
100.0	99.19
110.0	109.13
120.0	119.53



Linearity plot for Pregabalin:

Concentration (µg/ml)	Area
14	48297.9
16	55065.3
18	62100.4
20	68984.1

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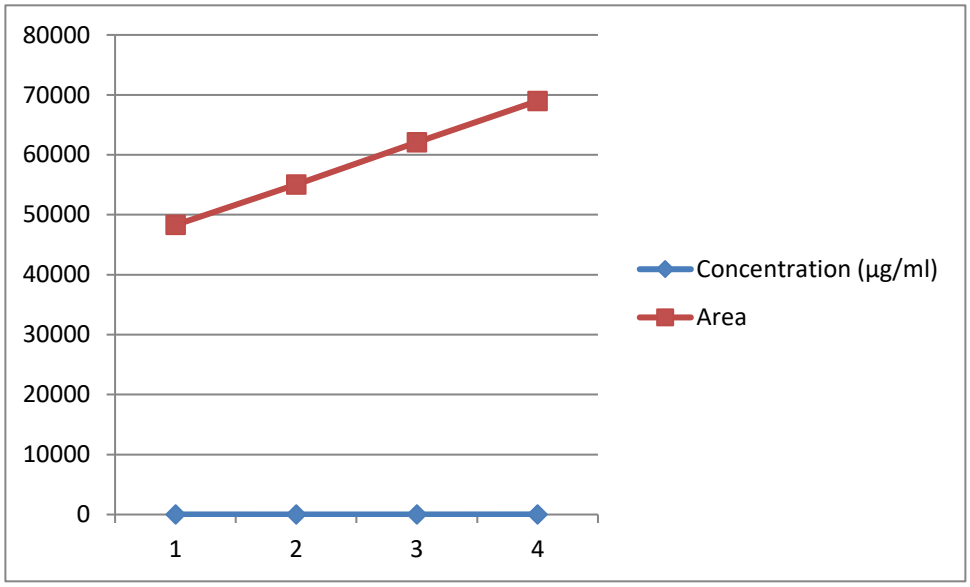

R-squared value ($R^{2)}$

The R-squared value, also known as the coefficient of determination, is an indicator that ranges in value from 0 to 1 and reveals how closely the estimated values for the trend line correspond to your actual data. A trend line is most reliable when its R-squared value is at or near 1.

Linearity Equation

Equations for calculating trend line

Calculates the least squares fit for a line represented by the following equation:

$$y = m x + b$$

Where m is the slope and b is the intercept.

x = concentration

y = Area Value

Sample

Therefore, from Linearity Equation, **y = mx + b, m** ⟶ 0.999x

b ⟶ 0.163

We can arrive sample concentration from the above equation is 100 mcg

$$\frac{\text{Sample area X WS weight X potency of WS X Average Weight}}{\text{Standard area X Sample weight X 100 X claim}}$$

= %

Conclusion for Linearity: The graphical representation & data collected during this exercise proves Pregabalin in Tablet for demonstrate linearity in the range of 70% to 100% when determined by Liquid Chromatographic method.

Precision:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogeneous sample. The precision of the analytical method is usually expressed as Standard deviation or relative standard deviation (coefficient of variation) of a series measurement. The precision may be measured of either the degree of reproducibility or of repeatability of the analytical method on the normal operating condition.

Precision – Method precision

Pregabalin in Tablet**.**

Label Claim 75 mg/tablet (Limit: 90.0 % to 110.0 % of the labeled amount).

ANALYTICAL METHOD VALIDATION REPORT FOR METHYCOBALAMIN 750 MCG & PREGABALIN 75 MG TABLETS

Chromatographic conditions:-

Mobile phase : a mixture of 25 volumes of a buffer solution prepared by dissolving 2.72 g *of potassium dihydrogen orthophosphate* into 1000 ml of *water,* adjusted to pH 7.5 with *sodium hydroxide* solution, 75 volumes of *acetonitrile* and filter.

Wavelength	: 215 nm
Flow Rate	: 2.0 ml/min
Column	: a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 pm),
Injection Volume	: 20 µl
Diluent	: Mobile phase

Mobile phase: a mixture of 25 volumes of a buffer solution prepared by dissolving 2.72 g *of potassium dihydrogen orthophosphate* into 1000 ml of *water,* adjusted to pH 7.5 with *sodium hydroxide* solution, 75 volumes of *acetonitrile* and filter.

Standard Solution: Weigh accurately 25 mg of *Pregabalin WS* and transfer to a 25.0 ml volumetric flask, add 20.0 ml of solvent mixture and sonicate to dissolve. Make up the volume with solvent mixture and mix. Dilute 1.0 ml of this solution to 25.0 ml with mobile phase and mix.

Test Solution: Weigh and powder 20 tablets. Disperse a quantity of the powder containing 75 mg Pregabalin in to 50.0 ml of volumetric flask add 40 ml of Solvent mixture and sonicate to dissolve. Make up the volume with solvent mixture and mix. Further dilute 1.0 ml of this solution to 10.0 ml with mobile phase.

Chromatographic system: Separately inject equal volumes of the standard solution and sample solution and measure the responses of the major peaks and calculate the content of Pregabalin.

Procedure: Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency is not less than 2000 theoretical plates and the relative standard deviation for

replicate injection is not more than 2.0 %.

Inject the reference solution and test solution.

Calculate the content of Pregabalin in the tablets.

$$\frac{\text{Sample Area X WS weight X potency of WS X Average Weight}}{\text{Standard Area X Sample weight X 100 X claim}} = \%$$

Standard Wt. taken: Weigh accurately 50 mg of Pregabalin WS into a 100 ml Volumetric flask, add 20 ml of methanol and sonicate to dissolve and make up to the volume with methanol. Take 2 ml of this solution and dilute to 50 ml make up to the volume with diluents.

Sample Dilutions: By "……"

Sample A 75.1 mg of sample into a 100 ml Volumetric flask, add 20 ml of methanol and sonicate to dissolve and make up to the volume with methanol. Take 2 ml of this solution and dilute to 50 ml make up to the volume with diluents.

Sample B 75.0 mg of sample into a 100 ml Volumetric flask, add 20 ml of methanol and sonicate to dissolve and make up to the volume with methanol. Take 2 ml of this solution and dilute to 50 ml make up to the volume with diluents.

Sample C 75.1 mg of sample into a 100 ml Volumetric flask, add 20 ml of methanol and sonicate to dissolve and make up to the volume with methanol. Take 2 ml of this solution and dilute to 50 ml make up to the volume with diluents.

Sample D 75.2 mg of sample into a 100 ml Volumetric flask, add 20 ml of methanol and sonicate to dissolve and make up to the volume with methanol. Take 2 ml of this solution and dilute to 50 ml make up to the volume with diluents.

Sample E 75.0 mg of sample into a 100 ml Volumetric flask, add 20 ml of methanol and sonicate to dissolve and make up to the volume with methanol. Take 2 ml of this solution and dilute to 50 ml make up to the volume with diluents.

ANALYTICAL METHOD VALIDATION REPORT FOR METHYCOBALAMIN 750 MCG & PREGABALIN 75 MG TABLETS

Sample F 75.1 mg of sample into a 100 ml Volumetric flask, add 20 ml of methanol and sonicate to dissolve and make up to the volume with methanol. Take 2 ml of this solution and dilute to 50 ml make up to the volume with diluents.

Test Data Collection

Standards	Area
Standard 1	69010.7
Standard 2	69125.1
Standard 3	69245.8
Standard 4	68956.2
Standard 5	69124.5
Standard 6	69245.6
Mean	69092.5
RSD	**0.163%**

Samples		Sample Area	Mean
Sample A	T1	68852.5	68904.5
	T2	68955.6	
Sample B	T1	68845.6	68917.6
	T2	68989.7	
Sample C	T1	69023.5	68984.5
	T2	68945.6	
Sample D	T1	68988.6	68971.9
	T2	68955.2	
Sample E	T1	68855.6	68800.1
	T2	68744.5	
Sample F	T1	68956.8	68962.5
	T2	68968.2	

ANALYTICAL METHOD VALIDATION REPORT FOR METHYCOBALAMIN 750 MCG & PREGABALIN 75 MG TABLETS

Estimated Amount

- Assay on % of Theory for sample A---- 99.70%
- Assay on % of Theory for sample B---- 99.80%
- Assay on % of Theory for sample C-----99.88%
- Assay on % of Theory for sample D-----99.84%
- Assay on % of Theory for sample E-----99.51%
- Assay on % of Theory for sample F-----99.69%

Table for Six Replicate Assays

Sample Number	Estimated Amount	Mean	Relative Standard Deviation (RSD)
Sample A	99.70%	99.74%	0.15%
Sample B	99.80%		
Sample C	99.88%		
Sample D	99.84%		
Sample E	99.51%		
Sample F	99.69%		

Acceptance Criteria: NMT 2% (% of Relative Standard Deviation)

Conclusion for precision: The overall % Relative standard deviation for Pregabalin in tablets there is no significant difference. Therefore Repeatability of the method considered acceptable as it well within 2 % Relative Standard Deviation.

Intermediate Precision – (Within laboratory variations such as different days, analyst & equipments):

Analyst: "……"

Standard Wt. taken: Weigh accurately 50 mg of Pregabalin WS into a 100 ml Volumetric flask,

add 20 ml of methanol and sonicate to dissolve and make up to the volume with methanol. Take 2 ml of this solution and dilute to 50 ml make up to the volume with diluents.

Sample A 75.0 mg of sample into a 100 ml Volumetric flask, add 20 ml of methanol and sonicate to dissolve and make up to the volume with methanol. Take 2 ml of this solution and dilute to 50 ml make up to the volume with diluents.

Sample B 75.2 mg of sample into a 100 ml Volumetric flask, add 20 ml of methanol and sonicate to dissolve and make up to the volume with methanol. Take 2 ml of this solution and dilute to 50 ml make up to the volume with diluents.

Sample C 75.1 mg of sample into a 100 ml Volumetric flask, add 20 ml of methanol and sonicate to dissolve and make up to the volume with methanol. Take 2 ml of this solution and dilute to 50 ml make up to the volume with diluents.

Sample D 75.0 mg of sample into a 100 ml Volumetric flask, add 20 ml of methanol and sonicate to dissolve and make up to the volume with methanol. Take 2 ml of this solution and dilute to 50 ml make up to the volume with diluents.

Sample E 75.1 mg of sample into a 100 ml Volumetric flask, add 20 ml of methanol and sonicate to dissolve and make up to the volume with methanol. Take 2 ml of this solution and dilute to 50 ml make up to the volume with diluents.

Sample F 75.1 mg of sample into a 100 ml Volumetric flask, add 20 ml of methanol and sonicate to dissolve and make up to the volume with methanol. Take 2 ml of this solution and dilute to 50 ml make up to the volume with diluents.

ANALYTICAL METHOD VALIDATION REPORT FOR METHYCOBALAMIN 750 MCG & PREGABALIN 75 MG TABLETS

Standards	Area
Standard 1	69085.6
Standard 2	68995.6
Standard 3	69105.5
Standard 4	68945.6
Standard 5	69045.2
Standard 6	69077.2
Mean	69035.5
RSD	0.094%

Samples		Sample Area	Mean
Sample A	T1	68856.2	68823.0
	T2	68789.8	
Sample B	T1	68856.8	68884.6
	T2	68912.5	
Sample C	T1	68824.5	68890.3
	T2	68956.2	
Sample D	T1	68875.6	68871.5
	T2	68867.5	
Sample E	T1	68945.1	68910.3
	T2	68875.6	
Sample F	T1	68910.2	68902.7
	T2	68895.3	

Calculation:

Pregabalin Content

$$\frac{\text{Sample area X WS weight X potency of WS X Average Weight}}{} = \%$$

ANALYTICAL METHOD VALIDATION REPORT FOR METHYCOBALAMIN 750 MCG & PREGABALIN 75 MG TABLETS

Standard area X Sample weight X 100 X claim

Estimated Amount analyst by "……"

- Assay on % of Theory for sample A ----99.70%
- Assay on % of Theory for sample B ----99.76%
- Assay on % of Theory for sample C ----99.85%
- Assay on % of Theory for sample D ----99.80%
- Assay on % of Theory for sample E ----99.65%
- Assay on % of Theory for sample F -----99.46%

Test Data analyst by "……..

Sample Number	Estimated Amount	Mean	Relative Standard Deviation (RSD)
Sample A	99.70%	99.75%	0.10%
Sample B	99.76%		
Sample C	99.85%		
Sample D	99.80%		
Sample E	99.65%		
Sample F	99.46%		

Test Data analyst by ""

Sample Number	Estimated Amount	Mean	Relative Standard Deviation (RSD)
Sample A	99.70%	99.74%	0.15%
Sample B	99.80%		
Sample C	99.88%		
Sample D	99.84%		

Sample E	99.51%		
Sample F	99.69%		

Acceptance Criteria: NMT 2 % (% of Relative Standard Deviation).

Conclusion for Intermediate Precision:

The overall % Relative standard deviation of two different analysts are 0.10% & 0.15% Pregabalin tablets there is no significant difference between two analysts Within laboratory variations such as different days, analyst & equipments.

Therefore reproducibility of the method considered to be acceptable.

2 Robustness:

To demonstrate the analytical method is capable to yield reproducibility results under;

Small but deliberate variations in method parameters during normal usage such as composition & Flow rate of mobile phase.

Procedure:

Perform the robustness study by injecting single of resolution solution & standard solution for six times for the following parameters.

- Change in ratio of the mobile phase. Record the observation in below observation table.
- Change in Flow rate of mobile phase. Record the observation in below observation table.

OBSERVATION TABLE:-

Change ratio in the mobile phase					
Mobile phase		Flow rate ml/min	System suitability		
Water	Methanol		Retention time	Theoretical plate	Tailing Factor
298ml	702ml	1.0 ml/min.	3.27	1568	1.5
300ml	700ml	1.0 ml/min.	3.29	1530	1.5
302ml	698ml	1.0 ml/min.	3.31	1598	1.4

ANALYTICAL METHOD VALIDATION REPORT FOR METHYCOBALAMIN 750 MCG & PREGABALIN 75 MG TABLETS

Change in flow rate.

Change in flow rate. At 274 nm				
Mobile phase		**System Suitability**		
Ratio of Mobile Phase (Water: Methanol)	**Change in flow rate**	**Retention Time**	**Theoretical Plates**	**Tailing Factor**
300:700	0.8 ml/min.	4.12	1610	1.5
300:700	1.0 ml/min.	3.30	1540	1.5
300:700	1.2 ml/min.	2.80	1605	1.4

Acceptance criteria:

Analytical method validation shall be robust (i.e. Theoretical Plates is not less than 1000 & tailing factor is not more than 2.0).

Conclusion for Robustness:

There is no significant difference for Pregabalin in tablets for different conditions**,** such as composition & Flow rate of mobile phase.

Therefore Robustness of the method considered acceptable.

CONCLUSION:

All the analytical parameter are checked as per the approved validation process and found well within specified acceptance criteria. Hence, It is concluded that ,this method is suitable for accurate & precise results for routine analysis**.**