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RP HPLC Validation of PREGABLIN in Bulk and Dosage Form

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Research Article

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ABSTRACT

In this article we are producing the results obtained checking the validation parameters System suitability, Specificity, Precision in this Method precision, Accuracy (recovery), Linearity of test method, Ruggedness, Robustness, Solution stability and showed that the results obtained all within the limits by using the method developed in trial and error method.

INTRODUCTION

All HPLC ways used for the event of prescription drugs and for the determination of their quality have to be compelled to be valid [1-6]. In cases whereby ways from the Pharmacopoeia's area unit used, it's not necessary to guage their quality, given that the analyses area unit conducted strictly per the methods' meant use [6-8].

The parameters tested throughout the tactic validation as outlined by the ICH, USP and government agency and different health organizations [9-13] area unit the following: Specificity or property, exactness (repeatability, intermediate exactness, reliableness or ruggedness), accuracy or exactitude or bias, dimensionality vary, limit of detection, limit of quantitation and strength. The terms property and specificity area unit usually used interchangeably [13-19]. The USP treatise defines property of associate analytical methodology as its ability to live accurately an analyte within the presence of interference, like artificial precursors, excipients, enantiomers and celebrated (or likely) degradation merchandise that may be gift within the sample matrix [20-25].

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- Recovery
- Response function
- Sensitivity
- Precision
- Accuracy
- Limit of detection
- Limit of quantization
- Ruggedness
- Robustness
- Stability
- System suitability.

METHOD VALIDATION

System suitability

Suitability delineates for the tactic of study is established by injecting 5 times with normal and double with standard value system [30-33]. Suitability parameters as per the take a look at procedure [34-39].

Purpose

To establish the system quality as per take a look at technique [40-43] **(Table 1)**.

Sequence

Table 1. System suitability sequences.

S. No.	Type of sample	No. of injections
1	Blank	01
2	Standard 1 solution	05
3	Standard 2 solution	02

Evaluate the subsequent system quality parameters [44-47]
%RSD, Theoretical plates & spatiality for traditional
Similarity issue between 2 standards

Acceptance criteria

- The sharp RSD for the retention times of principal peak from ten replicate injections of every normal answer ought to be less than 2.0% [47-50].
- The quantity of theoretical plates (N) for pregablin peaks is NLT 3000 [19].
- The Tailing issue (T) for pregablin peaks is NMT 2.0%
- Similarity issue between 2 standards ought to be zero.985 to 1.015 [39,45].

Observation

The similarities between 2 standards ought to be at intervals limits [51,52].

Specificity

Placebo interference

A study to ascertain the interference of placebo was conducted. Samples were ready in triplicate by taking the placebo such as concerning the burden in portion of take a look at preparation as per the take a look at technique [53,54]. Recording of placebo failed to show any additional peaks. This means that the excipients employed in the formulation don't interfere within the assay of pregablin tablets [55].

Acceptance criteria

No interference at the retention times of pregablin and analyte peak purity ought to be NLT0.99.

Observation

From Placebo chromatograms, it absolutely was finished that there was no interference with placebo as no peaks were determined at the retention times of pregablin peak [56-58].

Interference from degraded products

A study was conducted to demonstrate the effective separation of degradants from pregablin in capsules (150 mg). Separate parts of Drug product and Placebo were exposed to following stress conditions to induce degradation [59-61].

- Water degradation
- Acid degradation
- Base degradation
- Peroxide degradation
- Thermal degradation
- UV degradation
- Humidity degradation

Stressed samples were injected into the HPLC system with photograph diode array detector by following take a look at technique conditions [62,63]. All degrading peaks of pregablin within the chromatograms of all samples and placebo failed to show any substantial peaks beneath the higher than conditions [64-67]. The chromatograms of stressed samples were evaluated for peak purity of pregablin victimisation water's Empower software system [68]. For all forced degradation samples the degradants mustn't interference in quantitating the pregablin [69-71].

Precision

System precision

Standard resolution ready as per check methodology and injected 5 times [39].

Purpose

The purpose of this study is to determine the exactitude of the HPLC system being employed for the analysis [65] **(Table 2)**.

Sequence

Table 2. System precision sequences.

S. No.	Type of sample	No. of injections
1	Blank	01
2	Placebo solution	01
3	Standard solution	05

Acceptance criteria

The % relative variance of pregablin from the six units ought to be less than 2.0% [72].

Method precision

Prepared six sample preparations severally victimisation single batch of pregablin.

Purpose
To check the repeatability of check results obtained by this methodology [73-75] **(Table 3)**.

Sequence

Table 3. Method precision sequences.

S. No.	Sample	No. of injections
1	Diluent	1
2	placebo	1
3	Standard solution	5
4	Precision set 1	2

5	Precision set 2	2
6	Precision set 3	2
7	Precision set 4	2
8	Precision set 5	2
9	Precision set 6	2
10	Bracketing standard	1

Acceptance criteria

The sharp relative variance of pregablin from the six units ought to be less than 2.0% [41]. The assay of pregablin capsules 150 mg ought to be not but 90% and less than 110% [19].

Observation

Test results of pregablin in capsules (150 mg) area unit showing that the check methodology is precise. Refer **Table 3** for system exactitude and for methodology exactitude.

Accuracy (recovery)

Purpose

To establish methodology accuracy.

Study design

Demonstrate the accuracy of the check methodology by getting ready recovery samples (i.e., spiking placebo with is aware of quantities of standard) at the extent of fifty, 100% and one hundred and fiftieth of target concentration [76-79]. Prepare the recovery samples in triplicate and injecting duplicate at every level. The accuracy of the strategy shall is set by recovery experiments [80-83].

Procedure

The accuracy of the strategy shall is set by recovery experiments. The recovery is performed by adding pregablin commonplace to the placebo **(Tables 4 and 5)** (excipients mixture) within the vary of 50%-150% of check concentration [84,85].

Preparations

Table 4. Accuracy sequences.

Spike level (%)	Standard spiked (mg)	Weight of the placebo (mg)	Make up to the volume (ml)	Final concentration in mcg/ml
50%	15	340.0	20	750
100%	30	340.0	20	1500
150%	45	340.0	20	2250

Chromatograph the below samples and calculate the proportion recovery for the quantity value-added. Appraise the exactitude of the recovery at every level by computing the relative variance of triplicate recovery results [86].

Sequence

Table 5. Preparation of solutions to check accuracy.

S. No.	Sample	No. of injections
1	diluent	01
2	placebo	01
3	Standard solution	05
4	Recovery (50%) – Set 1	02
5	Recovery (50%) – Set 2	02
6	Recovery (50%) – Set 3	02
7	Recovery (100%) – Set 1	02
8	Recovery (100%) – Set 2	02
9	Recovery (100%) – Set 3	02
10	Recovery (150%) – Set 1	02
11	Recovery (150%) – Set 2	02
12	Recovery (150%) – Set 3	02
13	Bracketing standard	01

Acceptance criteria

The relative variance of assay mustn't be over 2.0% [39,87]. The typical recovery for every level must not be but 90% and not be over 110% [19].

Observation

The relative variance of assay mustn't be over a 2.0% [88]. The typical recovery for every level mustn't be but 90% and not be over 110% [22].

Linearity of test method

Purpose

To establish the dimensionality of analyte inside the required vary [89].

Study design

Demonstrate the dimensionality of analyte commonplace over the vary of 50%-150% of target concentration as mentioned below [90-92]. Preparation of dimensionality stock solution: Weigh accurately concerning 300 mg pregablin commonplace into 20 ml meter flask **(Tables 6 and 7)**, add 15 ml of thinner and sonicate to dissolve, further compose the amount with thinner (1500 ppm) [93-95].

Preparation of linearity solution

Table 6. Preparation of linearity solution.

Linearity level	Stock solution to be taken in ml	Make up to the volume (ml)	Final concentration in mcg/ml
50%	0.5	10	750

60%	0.6	10	900
80%	0.8	10	1200
100%	1.0	10	1500
120%	1.2	10	1800
140%	1.4	10	2100
150%	1.5	10	2250

Sequence

Table 7. Linearity sequences.

S. No.	Sample	No. of injections
1	diluent	1
2	Standard solution	5
3	Linearity level - 1 (50%)	3
4	Linearity level - 1 (60%)	3
5	Linearity level - 1 (80%)	3
6	Linearity level - 1 (100%)	3
7	Linearity level - 1 (120%)	3
8	Linearity level - 1 (140%)	3
9	Linearity level - 1 (150%)	3
10	Bracketing standard	1

Inject these solutions through HPLC and record the height space of dimensionality solutions [96]. Plot a graph of concentrations (in x-axis) vs. peak space (in y-axis) [35]. Evaluate the coefficient of correlation between concentration and peak space [7].

Acceptance criteria

Coefficient of correlation ought to be not but 0.9990 [97]. % RSD for level one and Level half-dozen ought to be less than 2.0% [65].

Observation

The coefficient of correlation was found to be 0.99958. From the higher than study it absolutely was established that the dimensionality of check methodology is from 50% to 150% of the target concentration.

Ruggedness

Purpose

To demonstrate the duplicability of check results obtained by this check methodology for the variability particularly System to system/Analyst to Analyst/column to Column variability study was conducted on totally different HPLC systems, columns and different analysts below similar conditions at different times [98,99].

Study design

Carry out exactitude study in six preparations of assay on one batch sample by 2 totally different analysts, on 2 totally different columns **(Table 8)** and on 2 totally different instruments [100].

Sequence

Table 8. Ruggedness sequences.

S. No.	Sample	No. of injections
1	Diluent	1
2	placebo	1
3	Standard solution	5
4	Precision set 1	2
5	Precision set 2	2
6	Precision set 3	2
7	Precision set 4	2
8	Precision set 5	2
9	Precision set 6	2
10	Bracketing standard	1

Comparison of each the results obtained on 2 totally different HPLC systems, column and different analysts shows that the assay check methodology is rugged for System to system/Analyst to Analyst/column to Column variability [25].

Acceptance criteria

The % relative variance of pregablin from the six sample preparations ought to be less than 2.0% [12]. All individual assays of pregablin capsules 150 mg should be between 90.0%-110.0% [19].

Observation

The % RSD was found with within the limits.

Robustness

Effect of variation of flow rate and column temperature

Purpose

To establish the lustiness of check methodology and to demonstrate its reliableness for minor changes in activity conditions [101] **(Table 9)**.

Sequence

Table 9. Robustness sequences.

S. No.	Sample	No. of injections
1	Diluent	1
2	Placebo	1
3	Standard solution	5
4	Test solution	2
5	Bracketing standard	1

Demonstrate the lustiness of check methodology by perform system quality and assay below traditional condition (i.e., methodology precision) and every of the altered conditions mentioned below [54,79].

Conditions

- Change in column temperature to 25 ±5°C [7].
- Amendment in rate of flow to 1.0 ± 0.2 ml [68].

Acceptance criteria

- System quality ought to be yielding [25].
- Merit RSD mustn't take issue over a pair of.0 from traditional condition study [19].

Observation

The tailing issue for pregablin was found to be with within the limits.

Filter variation

Purpose

To demonstrate the filter variation of assay methodology allotted on 2 totally different filters. Perform assay on pregablin capsules 150 mg as per the check methodology, draw sample through zero.45 µm Nylon filter and zero.45 µm PVDF filter. Calculate the distinction accountable for of assay between filtered parts.

Study design

Determine the assay of those samples with totally different filters and appraise distinction pending assay between filter parts **(Table 10)**.

Sequence

Table 10. Filter variation sequences.

S. No.	Sample	No. of injections
1	Diluent	1
2	Placebo	1
3	Standard solution	5
4	Sample set-1 (Nylon)	2
5	Sample set-1 (PVDF)	2
6	Bracketing standard	1

Acceptance criteria

The distinction between filtered sample solutions of various kinds of filter mustn't be over 2.0% [36].

Solution stability

Purpose

To demonstrate the steadiness of analytical solutions (i.e., commonplace and sample solution) at temperature (i.e., concerning 25°C) [58].

Study design

Prepare commonplace and sample solutions as per the check methodology and inject these solutions into HPLC system at regular intervals for minimum of 48 h, monitor the realm of each commonplace and sample solutions.

Acceptance criteria

Acceptable preference between initial and stability samples against recent commonplace isn't over 2.0%.

VALIDATION DATA

The validation data is shown from **Tables 11-18**.

Systems suitability

Table 11. System suitability of assay.

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	2.71	1500112	7605	1.24
2	2.74	1507818	5820	1.27
3	2.73	1507655	5651	1.08
4	2.71	1500123	7661	1.12
5	2.72	1501285	6125	1.06
6	2.73	1506996	5658	1.22
7	2.72	1503854	6412	1.09
Mean	2.72	1503977	6418	1.15
SD	0.010135	3261.219	---	---
% RSD			---	---

Specificity

Interference from degraded products

Table 12. Interference from degraded products.

Degradation mechanism/condition	Observation
Protected sample	No interference at RT of analyte peak
Water/Reflux – 30.0 min	No interference at RT of analyte peak
Acid degradation 0.1 N HCl Reflux – 30.0 min	No interference at RT of analyte peak
Base degradation 0.01 N NaOH Reflux 30.0 min	No interference at RT of analyte peak
Peroxide degradation 3.0% H_2O_2 Reflux – 30.0 min	No interference at RT of analyte peak
Thermal degradation At 105°C - 48 h	No interference at RT of analyte peak
Photolytic degradation At 254 nm - 24 h	No interference at RT of analyte peak
Accelerated degradation At 40°C/75% RH - 168 h	No interference at RT of analyte peak

Precision

System precision

Table 13. System Precision.

	Injection	Peak Areas of pregablin
Concentration 100%	1	1506996
	2	1508059
	3	1511449
	4	1510532
	5	1514515
Statistical **Analysis**	**Mean**	**1510310**
	SD	**2961**
	% RSD	**0.20**

Method precision

Table 14. Method precision.

Capsule ID	% Assay of pregablin	Statistical Analysis of pregablin	
1	100.0	Mean	**99.6**
2	100.0		
3	99.6	SD	**0.53**
4	99.4		
5	98.6	% RSD	**0.532**
6	100.0		

Accuracy (recovery)

Table 15. Accuracy of pregablin.

Concentration % of spiked level	Amount added (mcg/ml)	Amount found (mcg/ml)	% Recovery	Statistical Analysis of % Recovery	
50% Sample 1	748.72	745.97	99.65	**MEAN**	**99.3**
50% Sample 2	753.70	746.38	99.05	**SD**	**0.28**

50% Sample 3	751.21	745.17	99.2	%RSD	0.29
100 % Sample 1	1501.92	1495.755	99.6	MEAN	100.2
100% Sample 2	1496.94	1506.205	100.65	SD	0.48
100% Sample 3	1500.43	1504.65	100.3	%RSD	0.48
150% Sample 1	2250.64	2236.46	99.35	MEAN	99.3
150% Sample 2	2243.17	2244.93	100.10	SD	0.71
150% Sample 3	2247.15	2215.535	98.6	%RSD	0.72

Linearity

Table 16. Linearity for pregablin.

Linearity Level	Concentration ppm	Average area	% of RSD	Statistical Analysis of pregablin	
L1-50%	750.00	774302	0.19		
L2-60%	900.00	919946	0.27		
L3-80%	1200.00	1231232	0.10		
L4-100%	1500.00	1538305	0.18	Correlation Coefficient	0.999996
L5-120%	1800.00	1822882	0.21	r^2	0.999992
L6-150%	250.00	2264370	0.08		

Ruggedness

Table 17. Ruggedness for pregablin.

Capsule ID	% Assay of pregablin	Statistical analysis of pregablin	
1	99.1	Mean	99.1
2	99.1		
3	98.8	SD	0.2
4	99.2		
5	99.1	%RSD	0.21
6	99.4		

Robustness

Table 18. Robustness.

Parameters	Optimum range	Conditions in procedure	Remarks
Filter variation	Nylon PVDF	Ambient temp & 1 ml/min flow	

Flow rate ml/min	0.8-1.2	1.0	At lower flow rates the asymmetry factor was increased and at higher flow rates the relative retentions was decreased
Temperature	25-30ºC	Ambient	Beyond the optimum range peak shape and symmetry was lost

Solution stability

Table 19. Solution stability for pregablin.

Capsule sample ID	% Assay of pregablin		Statistical Analysis of pregablin		
	For 24 h	For 48 h		For 24 h	For 48 h
1	97.4	97.5	Mean	97.5	97.3
2	97.6	97.1	SD	8522	7977
			%RSD	0.50	0.48

CONCLUSION

The test method is validated for Specificity, Linearity, Precision, Accuracy, Range, Stability of solution, Ruggedness and Robustness and found to be meeting the predetermined acceptance criteria. The validated method is Specific, Linear, Precise, Accurate, Robust and Rugged for the assay of pregablin capsules 150 mg. Hence from the above data it is concluded that the method is stability indicating.

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