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Research

Method validation for Separation Pregabalin impurity–B from Active pharmaceutical raw material by HPLC method by considering the validation parameter

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Abstract

Method validation for Separation Pregabalin impurity –B from Active pharmaceutical raw material by HPLC method by considering the validation parameter by HPLC. Verification of the method used for the analysis of Pregabalin USP active pharmaceutical raw material enantiometric purity for impurity–B by HPLC.This method was is development for alternate vendor source of the material to use multiple vendor during formulation. The monograph on pregabalin from the United States Pharmacopeia (USP) states different high performance liquid chromatography (HPLC) methods for the assay, the determination of organic impurities, and the determination of enantiomeric purity. In the United States Pharmacopeia (USP), the monograph on pregabalin states different high performance liquid chromatography (HPLC) methods for the assay and the determination of enantiomeric purity. This application note describes the performance of the assay as well as the determination of enantiomeric purity according to USP on the Agilent 1260 Infinity II Prime LC with a column selection valve. Both methods can be performed on one LC system with no manual intervention required. Furthermore, the assay and determination of enantiomeric purity for pregabalin are transferred to UHPLC conditions in accordance with USP guidelines, chapter 621, enabling time and solvent savings.

Keywords: Pregabalin, HPLC, Method validation parameter, impurity–B, Active pharmaceutical raw material

INTRODUCTION

Design of the study: Method validation for Separation Pregabalin impurity –B from Active pharmaceutical raw material by HPLC method by considering the different validation parameter. Analytical method validation for enantiometric purity for impurity –B test are carried out by considering following analytical parameter: System Suitability, Specificity, Linearity, and Range, Precision, System Precision, Method Precision, Percentage Recovery.

Drug Profile: *Pregabalin: (S)-3-(Aminomethyl)-5-methylhexanoic acid3-isobutyl GABA*

Molecular Formula: C8H17NO2, White to off-white crystalline solid, Melting Point : 176 - 178ºC, Freely soluble in water and both basic and acidic solutions

MATERIALS AND METHODS

Equipment and Instrument: All equipment and instrument used during method validation shall be qualify, validate and within calibration and preventive maintenances validity.

i. **HPLC:** Model : Waters, Instrument number : QC/HPLC/029
ii. **pH meter** Model : Mettler Instrument number : QC/pH/001
iii. **Analytical Balance**Model : Mettler, Instrument number : QC/BAL/026

Standards: Reference standards of Pregabalin which has purity 99.99 %
Solvent and Chemicals: Triethylamine, Orthophosphoric acid, Marfey'sreganetSodiumhydrogencarbonate,Hydrochloricacid, Acetonitrile water

Preparation of Buffer solution: Take 1000mL of water into a beaker, add 10 ml of Triethyl amine. Adjust PH to 2.0 with Orthophosphoric acid 75% and filter.
Preparation of mobile phase: Thoroughly mix Acetonitrile and Buffer solution in the ratio of 32.68v/v)
Preparation of Marfey's regent solution: Weigh and transfer about 500 mg of Marfey's regent in to a 10 mL volumetric flask. Dissolve in and dilute with Acetonitirile and mix.
Preparation of sodium hydrogen carbonate sodium: Weigh and transfer about 8.4 g of sodium hydrogen carbonate into a 100 Ml volumetric flask. Dissolve in and dilute to volume with water and mix.
Preparation of Hydrochloric acid solution: Take 8.7 ml of Hydrochloric acid into a 100ml volumetric flask, dilute to volume with water.
Dervatisation procedure: Transfer 500 µL of solution to a reaction vial. Add 500 µL of Marfey's regent solution and 50 µL of Sodium hydrogen carbonate solution. Seal the vial, mix and dervatise by maintaining the vial of 40 °C for 1 hour in a heating/ stirring module. Stop the reaction by adding about 50 µL of hydrochloric acid solution and mix well. Take 200 µL of dervatised solution and 800 µL of the mobile phase, Mix well and inject the solution into chromatograph.
Preparation of Blank solution: Dervatise the water as per dervatisation procedure.
Preparation of Test solution: Accurately weigh and transfer about 50 mg of sample into a 25ml volumetric flask, Dissolve in and dilute with water and mix. And derivatise this solution as per derivatisation procedure.
Impurity- B stock solution: Accurately weigh and transfer about the 5 mg Pregabalin Impurity –B into 100ml volumetric flask. Dissolve in and dilute to volume with and mix.
Reference solution preparation: Weigh and transfer about the 500 mg of Pregabalin working standard into a 25ml volumetric flask, add 0.5 ml of impurity-B stock solution. Dissolve in and dilute to volume with water and mix and derivatives this solution as per derivatisation procedure.

Chromatographic conditions

Column	: HYPERSIL BDS C-18, 250mm x 4.6 mm, 5µm or Equivalent
Wavelength	: 340mm
Flow rate Injection	: 2.0ml/minute
Volume	: 20µL
Column oven Temperature	: 30°C
Run time	: 50 minute

Procedure: Equilibrate the column for not less than 30 minute with mobile phase at flow rate of 2.0ml/minute. Inject 20 µL of blank solution into the Chromatographic system, record the Chromatogram. Program the data processor to inhibit the integration of peaks due to blank. Inject 20 µL of Reference solution into the Chromatographic system, record the Chromatogram and measure the peak response. Inject 20 µL of sample solution into the Chromatographic system, record the Chromatogram and measure the peak response. Inject 20 µL of Reference solution into the Chromatographic system, record the Chromatogram and measure the peak response. (Bracketing standard). Inject Bracketing standard after every 4 samples analysis and/or at the end of the sequence.

Evaluation of system suitability
The Resolution between peaks due to Pregabalin and Impurity –B derivatives in Reference solution is not be less than 5.

Impurities	RT	RRT
Pregabalin	About 8.0	0.999
Impurity- B	About 11	1.3

Integrate the Pregabalin and Impurity- B derivatives peaks in test solution and reference solution Chromatogram and report the area % of Impurity- B. (by area normalization method).

Specification

Enantiomeric purity by area no (By HPLC) (Ph. Eur.)	
Impurity- B	Not more than 0.15%

Design of Experiment

Experimental design is drawn to prove the test method is capable to reproduced results in the pre determine acceptance criteria. Acceptance criteria for the validation parameters are specified in the individual experimental designed.

The following typical performance characteristics are to be considered for the Analytical Method verification.

A. System Suitability
B. Specificity
C. Linearity and Range
D. Precision
   i. System Precision
   ii. Method Precision
E. Accuracy

System Suitability

Preparation of Buffer solution: Take 1000 mL of water into a beaker, add 10 ml of Triethyl amine . Adjust PH to 2.0 with Orthophosphoric acid 75% and filter.

Preparation of mobile phase: Thoroughly mix Acetonitrile and Buffer solution in the ratio of 32.68v/v)

Preparation of Marfey's regent solution: Weigh and transfer about 500 mg of Marfey's regent in to a 10 Ml volumetric flask. Dissolve in and dilute with Acetonitirile and mix.

Preparation of sodium hydrogen carbonate sodium: Weigh and transfer about 8.4 g of sodium hydrogen carbonate into a 100 Ml volumetric flask. Dissolve in and dilute to volume with water and mix.

Preparation of Hydrochloric acid solution: Take 8.7 ml of Hydrochloric acid into a 100ml volumetric flask, dilute to volume with water.

Dervatisation procedure: Transfer 500 µL of solution to a reaction vial. Add 500 µL of Marfey's regent solution and 50 µL of Sodium hydrogen carbonate solution. Seal the vial, mix and dervatise by maintaining the vial of 40 °C for 1 hour in a heating/ stirring module. Stop the reaction by adding about 50 µL of hydrochloric acid solution and mix well. Take 200 µL of dervatised solution and 800 µL of the mobile phase, Mix well and inject the solution into chromatograph.

Preparation of Blank solution: Dervatise the water as per dervatisation procedure.

Preparation of Test solution: Accurately weigh and transfer about 50 mg of sample into a 25ml volumetric flask, Dissolve in and dilute with water and mix. And derivatise this solution as per derivatisation procedure.

Impurity- B stock solution: Accurately weigh and transfer about the 5 mg Pregabalin Impurity –B into 100ml volumetric flask. Dissolve in and dilute to volume with and mix.

Reference solution preparation: Weigh and transfer about the 500 mg of Pregabalin working standard into a 25ml volumetric flask, add 0.5 ml of impurity-B stock solution. Dissolve in and dilute to volume with water and mix. And derivatives this solution as per derivatisation procedure.

Chromatographic conditions

Column : HYPERSIL BDS C-18, 250mm x 4.6 mm, 5µm orEquivalent
Wavelength : 340mm
Flow rate Injection : 2.0ml/minute
Volume : 20µL
Column oven : 30°C
Run time : 50 minute

Procedure: Equilibrate the column for not less than 30 minute with mobile phase at flow rate of 2.0ml/minute. Inject 20 µL of blank solution into the Chromatographic system, record the Chromatogram. Program the data processor to inhibit the integration of peaks due to blank. Inject 20 µL of Reference solution into the Chromatographic system, record the Chromatogram and measure the peak response. Inject 20 µL of sample solution into the Chromatographic system, record the Chromatogram and measure the peak response. Inject 20

µL of Reference solution into the Chromatographic system, record the Chromatogram and measure the peak response. (Bracketing standard).Inject Bracketing standard after every 4 samples analysis and/or at the end of the sequence.

Acceptance Criteria: The Resolution between peaks due to Pregabalin and Impurity –B derivatives in Reference solution is not be less than 5.

Impurities	RT	RRT
Pregabalin	About 8.0	0.999
Impurity- B	About 11	1.3

Calculations: Integrate the Pregabalin and Impurity- B derivatives peaks in test solution and reference solution Chromatogram and report the area % of Impurity- B. (by area normalization method)

Specification

Enantiomeric purity by area no (By HPLC) (Ph. Eur.)	
Impurity- B	Not more than 0.15%

Results

Parameters	Initial System Suitability	Bracketing System Suitabili
Resolution between peak due to Pregabalin and Impurity B	6.48	6.49
Acceptance Criteria: The Resolution between peaks due to Pregabalin and Impurity –B derivatives in Reference solution is not be less than 5.		

Specificity

Preparation of impurity- B specificity standard stock solution: Accurately weight and transfer about 5 mg of Impurity-B standard into 100 Ml volumetric flask, Dissolve and Dilute to volume with water and mix.

Preparation of impurity- B specificity standard solution: Transfer 1.5 ml of above solution into a25 ml volumetric flask dilute to volume with diluents and mix. And derivatise this solution as per derivatisation.

Preparation of sample solution: Accurately weigh and transfer about 50 mg of sample into a 25ml volumetric flask. Dissolve in and dilute to volume with diluent and mix. And derivatise this solution as per derivatisation.

Preparation of spiked sample solution: Accurately weigh and transfer about 50 mg of sample into a 25ml volumetric flask. Add 1.5 ml of Impurity –B specificity standard stock solution. Dissolve in and dilute to volume with diluent and mix. And derivatise this solution as per derivatisation procedure.

Procedure: Establish the system suitability as per method details and carry out the specificity study. Inject each specificity standard solution, sample solution and spiked sample solution into the system and record the Chromatograms.

Acceptance criteria: The retention time obtained from the Individual standard solution should be comparable with the retention times obtained from the spiked sample solution.

Results

Peak	Individual standard retention time	Spiked sample Retention time
Impurity B	11.207	11.194
Acceptance criteria: The retention time obtained from the Individual standard solution should be comparable with the retention times obtained from the spiked sample solution		
Conclusion: The test results shows that the specificity complies as the retention time obtained from the individual standard solution is comparable with retention times obtained from spike sample solution		

Linearity

Linearity is to validate the ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range.

Demonstrate the linearity of the analyte from 80% of target concentration to 120% of target concentration.

Preparation of Linearity Level-1 solution: Transfer 1.8 ml of the impurity-B specificity standard stock solution into a 100ml volumetric flask. Dilute to volume with diluent and mix. This solution contains limit of quantification for impurity-B of Pregabalin with respect to the specification level.

Preparation of Linearity Level-2 solution: Transfer 2.4 ml of the impurity-B specificity standard stock solution into a 50ml volumetric flask. Dilute to volume with diluent and mix. This solution contains about 80% for impurity-B of Pregabalin with respect to the specification level
Preparation of Linearity Level-3 solution: Transfer 3.0 ml of the impurity-B specificity standard stock solution into a 50ml volumetric flask. Dilute to volume with diluent and mix.This solution contains about 100% for impurity-B of Pregabalin with respect to the specification level.
Preparation of Linearity Level-4 solution: Transfer 3.6 ml of the impurity-B specificity standard stock solution into a 50ml volumetric flask. Dilute to volume with diluent and mix.This solution contains about 120 % for impurity-B of Pregabalin with respect to the specification level.
Preparation of Linearity Level-5 solution: Transfer 4.5 ml of the impurity-B specificity standard stock solution into a 50ml volumetric flask. Dilute to volume with diluent and mix. This solution contains about 150 % for impurity-B of pregabalin with respect to the specification level.
Procedure: System suitability details as per the method details and inject Level -1 and Level-5 solutions in replicates (6 times) and other levels in triplicate and record the chromatograms. Obtain a linearity curve by plotting the concentration of the pregabalin Ph. Eur (X-axis) against mean peak area responses of pregabalin ph. Eur (Y-axis).Determine the linearity correlation co-efficient.
Calculation: Linearity Correlation coefficient calculates using the Microsoft Excel.
Acceptance criteria: The Linearity Correlation coefficient should be more than 0.999.

Results

Parameters	Initial System Suitabilit	Bracketing System Suitability
Resolution between peak due to Pregabalin and Impurity B	6.48	6.49
Acceptance Criteria : The Resolution between peaks due to Pregabalin and Impurity –B derivatives in Reference solution is not be less than 4.		
Conclusion: The System suitability parameters complies as The Resolution between peaks due to Pregabalin and Impurity –B derivatives in Reference solution is not be less than 5		

Impurity B linearity standard studies

Level	Concentration-ppm	Area Average
1	**0.5**	**11254**
2	**0.13**	**28485**
3	**0.16**	**34202**
4	**0.19**	**41300**
5	**0.24**	**49875**
Linearity Correlation coefficient : 0.999.		
Acceptance criteria : The Linearity Correlation coefficient should be more than 0.999.		
Conclusion: The linearity complies as The Linearity Correlation coefficient should be more than 0.999.		

Range
Lower level: Reproduce the chromatograms from linearity level-1 and calculate % RSD for the main peak area response.
Preparation of Linearity Level-1 solution: Transfer 1.8 ml of the impurity-B specificity standard stock solution into a 100ml volumetric flask. Dilute to volume with diluent and mix.This solution contains limit of quantification for impurity-B of pregabalin with respect to the specification level.

Replicate injection	Concentration-ppm	Area Average
1	11414	The % RSD for peak area response of replicates at LQA level should not be more than 10.0 and at Linearity level -5 should not be more than 5.0
2	11038	
3	1156	
4	10860	
5	10659	
6	11987	
Mean	11254	
% RSD	4.4	
Acceptance criteria : The %RSD for peak are response of replicates at LOQ level should not be more than 10 and at Linearity level -5 should not be more than 5.		

Conclusion: The % RSD for peak area response of replicates at LQA level should not be more than 10.0 and at Linearity level -5 should not be more than 5.0

Upper level: Reproduce the chromatograms from linearity level-5 and calculate % RSD for the main peak area response.

Preparation of Linearity Level-5 solution Transfer 4.5 ml of the impurity-B specificity standard stock solution into a 50ml volumetric flask. Dilute to volume with diluent and mix. This solution contains about 150 % for impurity-B of pregabalin with respect to the specification level.

Replicate injection	Concentration-ppm	Area Average
1	49438	The % RSD for peak area response of replicates at LQA level should not be more than 10.0 and at Linearity level -5 should not be more than 5.0
2	50080	
3	50131	
4	49625	
5	49883	
6	50090	
Mean	49883	
% RSD	0.6	

Acceptance criteria : The %RSD for peak are response of replicates at LOQ level should not be more than 10 and at Linearity level -5 should not be more than 5.

Conclusion: The % RSD for peak area response of replicates at LQA level should not be more than 10.0 and at Linearity level -5 should not be more than 5.0

Precision

SystemPrecision: Prepare the standards solution as detailed in the method detailed and calculate the % RSD of peak response area of the standard solution. Reproduce the results from the system suitability.

Parameters	Initial System Suitability	Bracketing System Suitability
Resolution between peak due to Pregabali and Impurity B	6.48	6.49

Acceptance Criteria : The Resolution between peaks due to Pregabalin and Impurity –B derivatives in Reference solution is not be less than 4.

Conclusion: The System suitability parameters complies as The Resolution between peaks due to Pregabalin and Impurity –B derivatives in Reference solution is not be less than 5

Method Precision

Procedure: Prepare the six sample solution preparations for one batch and determine the components of each preparation as detailed in the method details calculate the % RSD of the component content values obtained for six samples preparation

Acceptance criteria: The % RSD of %w/w for known ,single maximum unknown impurity and shall not be more than 10.0 % and total impurities should not be more than10.0 %.

Replicate injectior	% Impurity B	Acceptance criteria
1	0.01	The % RSD of %w/w for known ,single maximum unknown impurity and shall not be more than 10.0 % and total impurities should not be more than10.0 %.
2	0.01	
3	0.01	
4	0.01	
5	0.01	
6	0.01	
Mean	0.01	
% RSD	0.00	

Conclusion: The % RSD is 0.00 and method is precise. The % RSD of %w/w for known ,single maximum unknown impurity and shall not be more than 10.0 % and total impurities should not be more than10.0 %.

Accuracy

Preparation of sample solution: Accurately weight and transfer about 50mg of sample into a 25 ml volumetric flask.Dissolved in and dilute to volume with diluent and mix. Add derivatives this solution as per derivatization procedure.

Preparation of accuracy level -1 solution:Accurately weigh and transfer about 50mg of sample into a 25ml volumetric flask.Add 1.2 ml of Impurity B .Specificity standard stock solution .Dissolve in and dilute to volume with dilute to volume with diluent and mix. Add derivatives this solution as per derivation procedure. This solution contains 100 % sample and about 80% of impurity –B of pregabalin with respect to sample solution concentration
Preparation of accuracy level -2 solution: Accurately weigh and transfer about 50mg of sample into a 25ml volumetric flask. Add 1.5 ml of Impurity B .Specificity standard stock solution .Dissolve in and dilute to volume with dilute to volume with diluent and mix. Add derivatives this solution as per derivation procedure. This solution contains 100 % sample and about 100% of impurity –B of pregabalin with respect to sample solution concentration
Preparation of accuracy level -3solution: Accurately weigh and transfer about 50mg of sample into a 25ml volumetric flask. Add 1.8 ml of Impurity B .Specificity standard stock solution .Dissolve in and dilute to volume with dilute to volume with diluent and mix. Add derivatives this solution as per derivation procedure. This solution contains 100 % sample and about 120% of impurity –B of Pregabalin with respect to sample solution concentration
Accuracy level 3 solution 1
Preparation of accuracy level -1 solution: Accurately weigh and transfer about 50mg of sample into a 25ml volumetric flask.Add 1.2 ml of Impurity B .Specificity standard stock solution .Dissolve in and dilute to volume with dilute to volume with diluent and mix. Add derivatives this solution as per derivation procedure. This solution contains 100 % sample and about 80% of impurity –B of pregabalin with respect to sample solution concentration
Preparation of accuracy level -3solution: Accurately weigh and transfer about 50mg of sample into a 25ml volumetric flask.Add 1.8 ml of Impurity B .Specificity standard stock solution .Dissolve in and dilute to volume with dilute to volume with diluent and mix. Add derivatives this solution as per derivation procedure. This solution contains 100 % sample and about 120% of impurity –B of pregabalin with respect to sample solution concentrationAccuracy level 3 solution 1
Procedure:Establish the system suitability as per method details and than carryout out the accuracy studyInject the test solution and use the contents of impurity-B for Accuracy calculationInject level-1, Level-2 and Level -3 triplicate preparationscalculate the % recovery of pregabalin impurities as below detailsImpurity B.

Injection	Level-1	Level -2	Level-3
1	105	105	105
2	104	104	104
3	103	103	103

% Recovery shall be not less than 80 % and not more than 120%

Parameters	Initial System Suitability	Bracketing System Suitabili
Resolution between peak due to Pregabalin and Impurity B	6.48	6.49
Acceptance Criteria : The Resolution between peaks due to Pregabalin and Impurity –B derivatives in Reference solution is not be less than 4.		
Conclusion: The System suitability parameters complies as The Resolution between peaks due to Pregabalin and Impurity –B derivatives in Reference solution is not be less than 5		

CONCLUSION

Cost-effective isolation methods were developed for the preparation of the process impurity of pregabalin on preparative HPLC, which was not possible to prepare by a synthetic route. The isolation parameters were optimized and the isolation conditions of different techniques were compared. The developed isolation methods can be tuned for the isolation of any other stability degradants.
The developed simple HPLC method for related substance and assay determination of Pregabalin is linear, precise, accurate and specific. The short run time of the developed method significantly saves lot of analysis time (~6 times faster) as well as the solvents cost (~3 times lesser). The results of the validation carried out for the method satisfied the ICH requirements. This method can be used for the detection and quantification of known, unknown and degradation impurities in the Pregabalin drug substance during routine analysis and also for stability studies in view of its capability to separate degradation impurities.

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