METHOD DEVELOPMENT AND VALIDATE A HPLC METHOD WITH PDA DETECTOR FOR THE ASSAY OF GEMIGLIPTIN TABLET TO BE EMPLOYED IN ROUTINE AND STABILITY TESTS

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Abstract: RP-HPLC method has been developed for the quantitative analysis of Gemigliptin in pharmaceutical dosage form. Chromatographic separation of Gemigliptin was achieved on Waters Alliance-e2695, by using Waters Symmetry C-18, 150mm x 4.6mm, 3.5µm, column and the mobile phase containing water pH-4 adjusted with OPA **& ACN** in the ratio of **30:70% v/v**. The flow rate was 1.0 ml/min; detection was carried out by absorption at **210nm** using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Gemigliptin was NLT 2000 and should not more than **2** respectively. %Relative standard deviation of peak area of all measurements always less than **2.0.**

Keywords: HPLC Gemigliptin.

1. Analytical method development of Gemigliptin Solubility study:

Trials	Column	Mobile Phase	Flow rate ml/min	Diluent	Observation
Trial-1	Agilent Eclipse C-18 150×4.6×3.5µ	water pH-4.0 adjusted with OPA : ACN 20:80	1ml/min	Mobile phase	Peak retention time is very low
Trial -2	Agilent Eclipse C-18 150×4.6×3.5µ	water pH-4.0 adjusted with OPA : ACN 30:70	1ml/min	Mobile phase	Peak retention time is very low
Trial -3	Waters X- Bridge RP 18 150×4.6×3.5µ	water pH-4.0 adjusted with OPA : ACN 20:80	1ml/min	Mobile phase	Base line is not sufficient
Trial -4	Waters X- Bridge RP 18 150mm x 4.6mm, 3.5µm	water pH-4.0 adjusted with OPA : ACN 30:70	1ml/min	Mobile phase	Peaks are not separated clearly
Trial -5	Waters Symmetry C-18, 150mm x 4.6mm, 3.5µm	water pH-4.0 adjusted with OPA : ACN 20:80	1ml/min	Mobile phase	Peak is splited into two peaks

Trial-6	Waters Symmetry C-18, 150mm x 4.6mm, 3.5µm	water pH-4.0 adjusted with OPA : ACN 30:70	1ml/min	Mobile phase	The peak Asymmetry factor was less than 2 for Gemigliptin. The efficiency was more than 2000 Gemigliptin

Results and Discussions:

2. Analytical Method Validation of Gemigliptin:

2.1 System suitability: Results for system suitability of Gemigliptin**:**

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)	Resolution
1	3.294	2045480	4949	1.09	-
2	3.297	2065941	4902	1.10	-
3	3.298	2047394	4919	1.10	-
4	3.299	2046571	4909	1.10	-
5	3.301	2043842	4923	1.10	-
6	3.301	2048229	4909	1.11	-
Mean		2049576			
SD		8162.04			
%RSD		0.40			

2.2 Linearity of Detector Response for Gemigliptin:

S.No.	Conc.(µg/ml) Gemigliptin	Area	Acceptance criteria
		Gemigliptin	
1	5	305045	Squared co relation coefficient should be not less than0.999.
2	12.5	628890	
3	25	1111639	
4	50	2184552	
5	62.5	2701235	
6	75	3185831	

2.3 Accuracy Data of Gemigliptin:

Recovery level	Accuracy Gemigliptin				
	Amount taken (mg)	Area	Ave Area	%Recovery	%RSD
50%	30	1110993	1112958	100.3	0.19
	30	1115236			
	30	1112646			
100%	60	2114892	2119120	100.1	0.39
	60	2113723			
	60	2128745			
150%	90	3159818	3171285	99.8	0.38
	90	3184011			
	90	3170026			

3.Limit of Detection (LOD) and Limit of Quantitation (LOQ):

S.No.	Sample name	LOD		LOQ	
		Conc.(µg/ml)	S/N	Conc. (µg/ml)	S/N
1.	Gemigliptin	0.0502	6	0.502	26

4. Stability: Sample was prepared and stability study was carried out at different time intervals upto 24 hours and the results were recorded.

Time period (hours)	Gemigliptin
	% Assay
Initial	101.7
6 Hrs	101.5
12 Hrs	101.4
18 Hrs	101.6
24 Hrs	101.7

5. Degradation Studies Data:

S.No	Degradation Parameters	%Recovery	%Degradation
		Gemigliptin	Gemigliptin
1	CONTROL	100.5	-1.5
2	ACID	81.2	16.1
3	ALKALI	76.6	14.5
4	PEROXIDE	72.8	13.2
5	REDUCTION	68.9	12.4
6	THERMAL	65	12.1

6. Conclusion: In conclusion a validated RP-HPLC method has been developed for determination of Gemigliptin the bulk and tablet dosage form. The results show that the method was found to be specific, simple, accurate, precise and sensitive. The method was successfully applied for the determination of Gemigliptin tablet dosage form.

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