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Development and Validation of RP-HPLC Method for the Estimation of Gemigliptin

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ABSTRACT

The RP-HPLC method has been developed for the estimation of Gemigliptin. The quantification was carried out C_{18} bonded phase i.e. Zorbax Eclipse XDB-C_{18} (4.6×250mm×5µ) with particle size 5 µm in an isocratic mode with a mobile phase consisting of Methanol: Water (20:80 % v/v). The detection was carried out using a UV detector at 233 nm. The solutions of Gemigliptin was chromatographed at a constant flow rate of 1 ml/min & the retention time of the drug was found to be 2.3 min. The linearity range of Gemigliptin was found to be from 1- 35 µg/ml. linear regression coefficient was 0.999. As per ICH guideline, the method was validated for recovery, Precision, ruggedness and linearity.

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INTRODUCTION

Gemigliptin is *Dipeptidyl Peptidase – 4* inhibitor class of anti-diabetic drug[1]. Its chemical formula is $C_{18}H_{19}F_8N_5O_2$ [2]. Gemigliptin adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus and can be taken with or without food. It is DPP-4 inhibitors which block the cleavage of the gliptins and thus lead to an increase insulin level and a reduced glucagon level in a glucose-dependent way[3]. The structure of Gemigliptin is as shown in figure 1.

Literature survey revealed that very few analytical methods have been reported for estimation of Gemigliptin. Rapid and sensitive RP-HPLC method for analysis of Gemigliptin and Metformin Hydrochloride was available[4, 5].

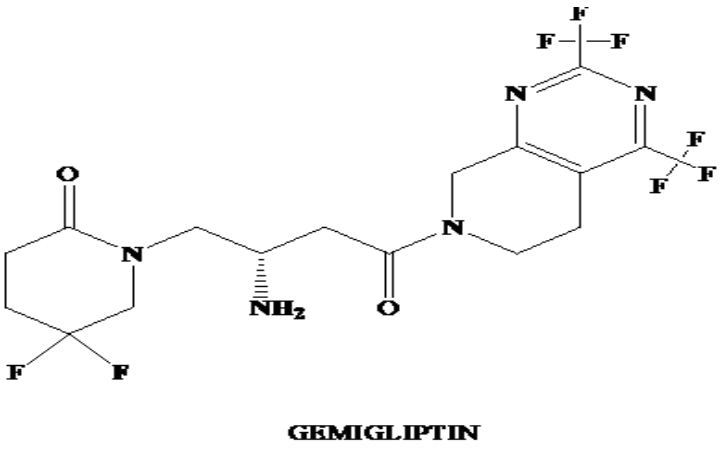

Figure 1. Gemigliptin

MATERIALS AND METHODS

Material

Gemigliptin was received as a gift Sample from Manus Akkteva Biopharma LLP, Ellisbridge, Ahmedabad. Methanol (HPLC grade) and Water were procured from Merk India.

INSTRUMENT[6]

The instrument used was Agilent 1220 LC series HPLC instrument. The instrument is consist of Agilent 1220 LC pump and variable wavelength programmable UV detector and 20 µl inject port.

Chromatographic conditions

C_{18} Column Zorbax Eclipse XDB- C_{18} (4.6×250mm×5µ) was used for separation. The mobile phase containing Methanol: Water in the ratio 20:80 v/v. was delivered at flow rate 1 ml/ min and elution was monitored at 233 nm. Injection volume was 20 µl and analysis was performed at ambient temperature.

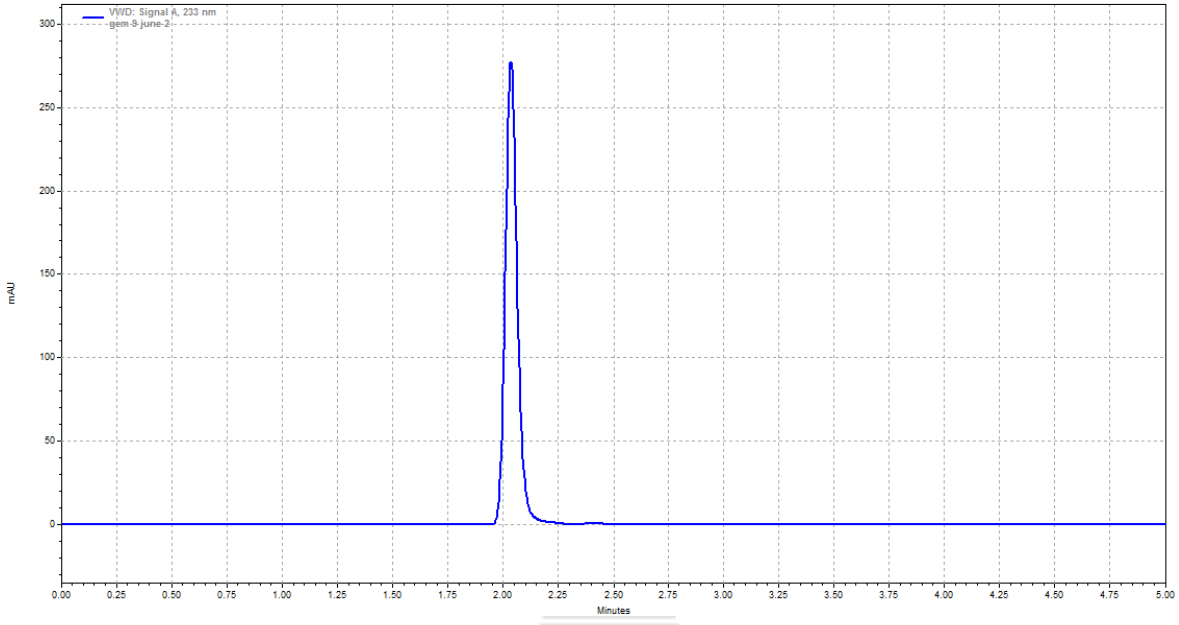

Figure 2. Gemigliptin Chromatogram

Preparation of mobile phase:

A mobile phase consisting of Methanol (HPLC grade), water in the ratio of 20:80 v/v was prepared and then filtered through a 0.45 µ membrane filter.

Preparation of standard stock solution:

Accurately, about 10 mg of standard Gemigliptin was weighed and transferred to separate 10 ml volumetric flasks. The drugs was dissolved in methanol then volume made up to the mark with the same solvent to obtain a standard stock solution of drug of concentration 1000 µg/ml.

Preparation of working solution:

Appropriate volume 0.01 ml of a standard stock solution of Gemigliptin Hydrochloride was transferred into 10 ml volumetric flask, diluted to mark with Distilled Water to give a desired concentration of drug. The resulting solution was scanned at 233 nm.

Construction of calibration curve

Appropriate aliquots of the standard stock solutions of Gemigliptin were pipetted out and transferred to a series of 10 ml volumetric flasks respectively. The volume was made up to the mark with water to obtain working standard solutions of Gemigliptin. The concentrations 1-35 µg/ml of Gemigliptin and. From these solutions, 20 µl injections of each concentration of the drug were injected into the HPLC system three times separately. Evaluation of the drug was performed with the UV detector set at 233 nm and the peak areas were recorded. The standard calibration curve for Gemigliptin was plotted as peak area Vs the respective concentration of Gemigliptin. Good linearity was obtained in the concentration range of 1- 35 µg/ml for Gemigliptin.

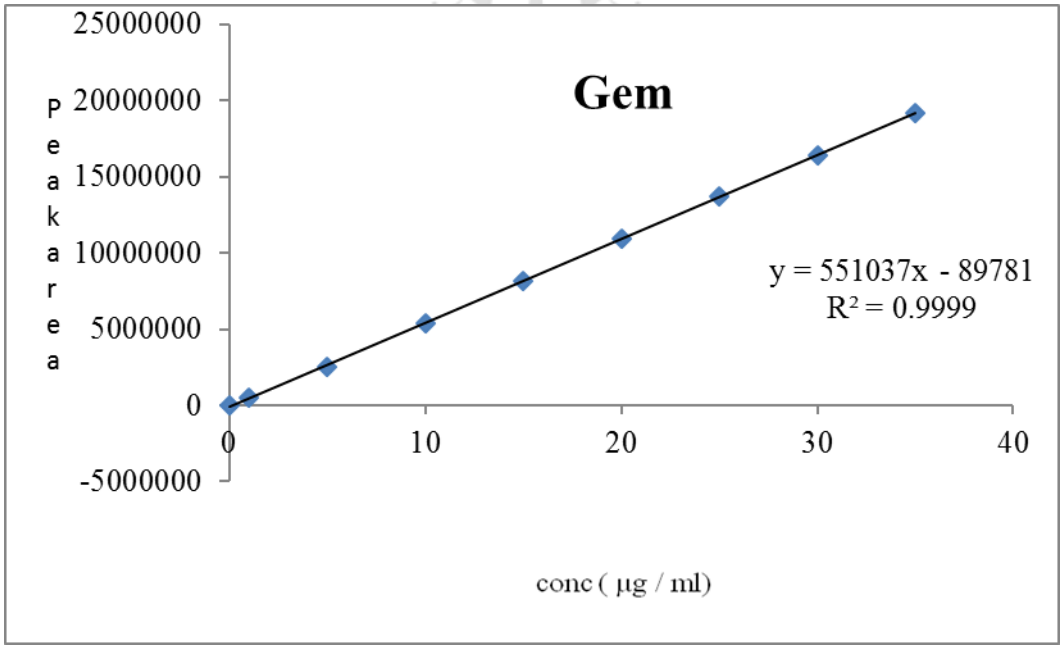

Figure No. 3: Calibration Curve of Gemigliptin

VALIDATION OF PROPOSED METHOD

The proposed method was validated as per ICH guidelines[7,8] The solutions of the drugs were prepared as per the earlier adopted procedure is given in the experiment.

a) Recovery study

The accuracy of an analytical method is the closeness of the test results obtained by that of the true value. The accuracy of the proposed method has been carried out by recovery studies. It was performed by recovery study using standard addition method at 80, 100, and 120 % level; the known amount of standard Gemigliptin was added to the pre-analyzed sample (8, 10, 12 µg/ml) and subjected them to the proposed HPLC method. Results are shown in Table No. 2.

b) Precision

The precision of an analytical method is the degree of agreement among individual test results. The precision of the method was verified by using stock solutions containing 1 µg/ml Gemigliptin. System repeatability was done by repeating the assay three times of the same concentration after every two hours on the same day for intraday precision. Inter-day precision was carried out by performing the assay sample sets after 24 hours and 48 hours, results are reported in Table No. 3.

c) Sensitivity

The sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 3.3 SD/S and LOQ = 10 SD/S, where SD is the residual standard deviation and S is the slope of the line. LOD was found to be 0.38 ng/ml & LOQ were found to be 1 ng/ml.

d) Ruggedness

From the stock solution, a sample solution of Gemigliptin (1 µg/ml) was prepared and analyzed by two different analysts using similar operational and environmental conditions. Peak area was measured for the same concentration solutions. The results are shown in Table No. 4.

e) Linearity and range

It was performed using different test concentrations. The response was Linear in the range of 1 to 35 µg/ml for Gemigliptin (Figure no. 1).

RESULT

Developed HPLC for determination of Gemigliptin was sensitive, specific, precise, rugged and robust.

For this method, the retention time was found to be 2.397 min and mobile phase was Methanol: Water 20:80 v/v and the flow rate was 1.0 ml/ min. % label claim for Gemigliptin found to be 99.85 indicative of the accuracy of the method.

Statistical analysis proves developed method is rapid and economical & can be used for routine analysis of said drug.

CONCLUSION

The proposed method for the determination of Gemigliptin is accurate, precise, linear, robust, simple and rapid. Hence the present RP-HPLC method is suitable for ascertaining the quality control of Gemigliptin bulk drug.

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Table No. 1: Calibration Table for Gemigliptin

Concentration in µg/ml	Peak Area of GEM
00	000000
01	538640
05	2503750
10	5385400
15	8130240
20	10964000
25	13717650
30	16452400
35	19196050

Table No. 2: Result of Recovery Study

Level of % recovery	Amount present (µg)	Amount of standard added (µg)	The total amount recovered (µg)	% Recovery
80	1	430912	9694160	99.98
100	1	538540	1078432	100.13
120	1	646368	1182045	99.74
			Mean	99.95
			SD	0.19230
			% RSD	0.19239
			SE	0.11102

(n=3) *Average of three replicate at each level of recovery S.D –Standard deviation, % RSD-Relative standard deviation.

Table No. 3: Result of Intraday Precision

Sr. No.	Conc. In µg/ml GEM	Peak Area	% Estimation
1	1	538540	99.98
2	1	539254	100.11
3	1	538500	99.97
		Mean	100.020
		SD	0.07784
		% RSD	0.07783
		SE	0.04494

(n=3) *Average of three replicate at each level of recovery S.D –Standard deviation , % RSD-Relative standard deviation.

Table No. 4: Result of Ruggedness

Sr. No.	Conc. In µg/ml	Peak Area GEM	% Estimation GEM
Analyst 1	1	530720	98.52
Analyst 2	1	538747	100.01
			99.26
			1.047225
			1.054931
			0.604616

(n=3) *Average of three replicate at each level of recovery S.D –Standard deviation, % RSD-Relative standard deviation.

Table 5: Summary of validation parameter

Parameters	**GEM**
Linearity range [µg/ml]	5-35
Regression equation [Y = mX +C]	y = 55103x - 89781
Recovery [% RSD]	99.95
Precision [% RSD]	
Intra-day	99.97
Inter-day	99.98
Ruggedness [% RSD]	99.26
Specificity	Specific
LOD	0.38
LOQ	1