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STABILITY INDICATING OF GEMIGLIPTIN SIMULTANEOUSLY ESTIMATION IN ITS DEGRADATION PRODUCT IN MARKET BY RP-HPLC METHOD

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Gemigliptin, RP-HPLC method, forced degradation, validation

ABSTRACT

To develop simple, accurate, precise, rapid and economic Stability Indicating RP-HPLC method for the Gemigliptin. In RP- HPLC, estimation of Gemigliptin was carried out by using Shimadzu LC-2010, using Sheisdo C18 (250 * 4.6 mm, 5µm) column and with mobile phase composition of Acetonitrile : Methanol : water (40:40:20 % v/v/v), at a flow rate of 1.0 ml/min was used. Detection was carried out at 280 nm. Retention time of Gemigliptin was found to be 2.735 min. The force degradation of Gemigliptin was carried out by using acid hydrolysis, alkaline hydrolysis, oxidative degradation and thermal degradation. The force degradation study for Gemigliptin indicates that the drug significantly degrades under Alkaline and oxidative conditions. All the method was found to be simple, accurate, economical, robust and reproducible. The proposed method was successfully applied for the simultaneous estimation of Gemigliptin and its degradation product. RP-HPLC method was found to be linear over the range of 50-300 µg/ml for Gemigliptin. The method has been validated for linearity, accuracy and precision, LOD, LOQ and system suitability according to ICH guideline.

INTRODUCTION:

Gemigliptin is an anti-diabetic drug in the class of DPP-4 inhibitors. It's a prescription medicine used along with diet and exercise to improve blood sugar (glucose) control in adults with type 2 diabetes[1]. IUPAC name of the drug is (3S) 3-amino-4-(5,5-difluoro-2-oxopiperidino)[2,4-di(trifluoromethyl)-5,6,7,8-tetrahydro[3,4-d]pyrimidin-7-yl] butan-1-one. The molecular formula of the drug is C18H19F8N5O2 [2](figure 1). The dug is having a molecular weight of489.36g/mol [3]. Gemigliptin is an oral anti hyperglycemic agent (anti diabetic drug) of the new dipeptidyl peptidase4 (DPP4) inhibitor class of drugs. It is well known that glucose lowering effects of DPP4 inhibitors are mainly mediated by GLP1 and gastric inhibitory polypeptide (GIP) incretin hormones which are inactivated by DPP4[4]. DPP4 is a serine protease located on the cell surfaces

throughout the body. In plasma, DPP4 enzyme rapidly inactivates incretins including GLP1 and GIP which are produced in the intestine depending on the blood glucose level and contribute to the physiological regulation of glucose homeostatis. Active GLP1 and GIP increase the production and release of insulin by pancreatic beta cells[5].GLP1 also reduces the secretion of glucagon by pancreatic alpha cells, thereby resulting in a decreased hepatic glucose production. However these incretins are rapidly cleaved by DPP4 and their effects last only for a few minutes[7]. DPP4 inhibitors block the cleavage of the gliptins and thus lead to an increase insulin level and a reduced glucagon level in a glucose dependent way. These results in a decrease of fasting and postprandial glycemia, as well as HbA1c levels the drug is administered orally[8]. Clinical trials analysis stated that the drug shows its action, without depending on the dietary habits and body mass index (BMI). Stability indicating method gives the better idea about the drug and its degradation product it is affected to the potency of drug. There is a need of developing a new simple analytical method for the estimation of Gemigliptin [6]. Hence a simple stability indicating analytical method is developed for the estimation of Gemigliptin and its degradation product and the results are reported here.

MATERIALS AND METHOD:

1. RP-HPLC METHOD DEVELOPMENT

1.1 Instrumentation:

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of Gemigliptin anHPLC – [Shimadzu-LC-2010] instrument with shisedoC18(250mm x 4.6mm, 5 µm) column was used[9,10]. The instrument is equipped pump with autosampler and a detector running on Peak LC Solution Software[12].The mobile phase consists of Methanol: Acetonitrile: Water in 40:40:20 (v/v) with pH 3and the flow rate was maintained at 1.0 ml/min[11]. The mobile phase was freshly prepared and passed through nylon membrane filter of pore size of 0.45µm and it was degassed by sonicating for 5 min before it was used. The elution was monitored at wavelength of 280 nm with UV detector and the injection volume was 10µl.

1.2 Chemicals and Solvents: The pure drug form ofGemigliptin is collected from Manus Akkteva Biopharma LLP, Ellisbridge, Ahmedabad380006; Gujarat, India.Impurities would be synthesized in house by forced degradation at various conditions. Methanol, acetonitrile, orthophosphoric acid, water (HPLC grade) were purchased from S D fine-CHEMLimited, Mumbai and Sodium hydroxide, hydrochloric acid and hydrogen peroxide also purchased from S D fine-CHEM Limited, Mumbai, and Trifluoro acetic acid purchased from – SIGMA-ALDRICH CHEMIE GmbH, Germany.

1.3 UV detection: The maximum absorbance Standard solution of Gemigliptin (100 µg/ml) were scanned between 200-400 nm using UV-visible spectrophotometer. Wavelength was selected from the overlap spectra of standard solutions.

1.4 Preparation of stock and standard solution: 100mg of the standard drug was weighed accurately and was dissolved in 100ml of solvent. The stock solution of concentration 1000µg/ml was obtained. The solution was filtered through 0.45micron meter nylon membrane filter paper. Working standard solution of Gemigliptin was prepared by making various dilutions of the drug solution from the stock solution. six sets of the drug solution were prepared in the mobile phase containing Gemigliptin at a concentration of 50-300µg/ml. Each of this drug solution (10µl) was injected into the column and

the peak area and retention time was recorded.

1.5 Assay of Gemigliptin tablets (in house prepared tablets): Ten tablets of Gemiligliptin were weighed and average weight of a single tablet was calculated. Tablets were crushed and mixed using a mortar and pestle. Then drug sample equivalent to 25 mg of Gemigliptin is accurately weighed and transferred into a 25 ml volumetric flask and mixed with known amount of methanol and the active pharmaceutical ingredients were extracted into the methanol by vortex mixing followed by ultrasonication and then filtered through a nylon membrane of pore size 0.45µm. The drug sample was diluted by adding methanol to obtain a stock solution of 100µg/ml

METHOD VALIDATION: The method was validated according to International Conference on Harmonization guidelines for validation of analyticalprocedure.The Proposed method was validated according to ICH guidelines[6]. The parameters assessed were linearity, precision, accuracy, stability, LOD and LOQ figures 2, and 3.Table 1

Linearity: Linearity of the proposed method was evaluated according to the ICH guidelines by the analysis of working solutions of Gemiligliptin at different concentrations ranging from 50-300 µg/ml. The linearity of an analytical procedure is the ability to obtain test results that are directly proportional to the concentration (amount) of an analyte in the sample within a given range. Linearity was evaluated by linear-regression analysis. Corresponding peak area values of different concentrations were determined and graph was plotted between concentration on x-axis and peak area values on y-axis figure 4, table 2.

Precision: The precision of the analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision is usually expressed in variance, standard deviation or coefficient of variance of a series of measurements.

Recovery: The accuracy of method was determined by recovery, by spiking of standard drug solution to pre analyzed sample at three different levels i.e., at 80, 100, and 120%. The resultant solutions were then re-analyzed by the developed method. At each concentration, sample was injected thrice to check repeatability and from the data it was analyzed that the method was accurate.

Robustness: Robustness is the measure of analytical method to remain unaffected by small, deliberate variations in the method parameters. It provides its reliability during normal usage.

Limit of detection: Limit of detection of the individual analytical method is the lowest concentration of analyte in the sample that the method can detect but not necessarily quantify under stated experimental conditions. LOD not only depend on procedure of analysis but also on type of instrument.LOD is calculated using the formulae, LOD=S/N,Where Average Baseline Noise obtained from Blank was named as (S), Signal Obtained from LOD solution (0.25% of target assay concentration) was named (N).

Limit of Quantification: Limit of quantification of the individual analytical method is the lowest concentration of analyte in the sample, which can be quantitatively determined with suitable precision and accuracy under stated experimental conditions. The quantification limit is used particularly for the determination of impurities and degraded products. LOQ is calculated by the formula , LOQ= S/N,where S was Average Baseline Noise obtained from Blank, N was Signal Obtained from LOD

solution (0.75% of target assay concentration) figure [5]

Force Degradation Study:Stresstesting of the drug substance can help identify thelikely degradation products, which can in turn helpto establish the degradation pathways and theintrinsic stability of the molecule and to developand validate the stability indicating method[7]. Stress studies were performedon API solutions. Considering good solubility of Gemigliptin, all stress study solutions were preparedin methanol.Samples were withdrawn from stress study sample [8]

Preparation of standard stock solution of Gemigliptin: Accurately weighed 10 mg of standard Gemigliptin was transferred to 10 ml volumetric flask, dissolved in 5 ml methanol and diluted up to the mark with same solvent to get stock solution having strength 1000 µg/ml.

Preparation of working standard solution of Gemigliptin: Working standard solution (50 µg/ml) was prepared by diluting of 0.5 ml of stock solution of Gemigliptin to 10 ml with methanol.

Preparation of Sodium hydroxide (NaOH) solution: Preparation of 0.1m sodium hydroxide solution (0.1M NaOH) Sodium hydroxide (0.4 gm) was transferred to a 100 ml volumetric flask, dissolved in and diluted up to mark with water.

Preparation of hydrochloric acid (HCl) solution: Preparation of 0.1M hydrochloric acid solution (1M HCL) Hydrochloric acid (0.85 ml) was transferred to a 100 ml volumetric flask and diluted up to mark with water.

Forced degradation test: Preparation of solutions for forced degradation of Gemigliptin was carried out under acidic, alkaline, oxidative and Thermal conditions.

1. **Acid degradation:** Accurately weighed 10 mg of Gemigliptin was transferred to a 10 ml volumetric flask, dissolved in and diluted to mark with methanol. To 1 ml aliquot of the solution, 2 ml of 0.1M HCl was added. The solution was heated for 1 hr at 60ºC and transferred to a 10 ml volumetric flask, cooled, neutralized by 0.1M NaOH and diluted up to mark with methanol to get final concentration 100 µg/ml.

2.**Alkali degradation:** Accurately weighed 10 mg of Gemigliptin was transferred to a 10 ml volumetric flask, dissolved in and diluted to mark with methanol. To 1 ml aliquot of the solution, 2 ml of 0.1M NaOH was added. The solution was heated for 1 hr at 60ºC and transferred to a 10 ml volumetric flask, cooled, neutralized by 0.1M HCl and diluted up to mark with methanol to get final concentration 100 µg/ml.

3.**Oxidation:** Accurately weighed 10 mg of Gemigliptin was transferred to a 10 ml volumetric flask, dissolved in and diluted to mark with methanol. To 1 ml aliquot of the solution, 2 ml 3 % H2O2 was added. The solution was heated for 4 hours at room temperature and transferred to a 10 ml volumetric flask, cooled diluted up to mark with methanol to get final concentration 100 µg/ml.

4**. Thermal degradation:** Accurately weighed 10 mg of Gemigliptin was transferred to a 10 ml volumetric flask, dissolved in and diluted to mark with methanol. To 1 ml aliquot of the solution heated for 2 hours at 80ºC and transferred to a 10 ml volumetric flask, cooled diluted up to mark with methanol to get final concentration 100 µg/ml

Result and Discussion: Rp-Hplc Method Development And Validation Of Gemigliptin In Bulk Form. Table 12

Selection of Wavelength: The standard solution of Gemigliptin (50 µg/ml) was

scanned in the range of 200-400 nm against methanol as blank in UV-Visible Spectrophotometer. The UV spectrum of Gemigliptin was recorded at 280 nm

Linearity The calibration curve showed (Fig.3) good linearity in the range of 50-300µg/ml, for Gemigliptin with correlation coefficient (r2) of 0.997. A typical calibration curve has the regression equation of y = 9.074x + 39.50. Results are given in Table 4.

Precision Intraday precision was carried out using test samples prepared and analyzed on the same day. Interday precision was assessed by analysis of the same solutions on consecutive days. The low % RSD values below 2 indicate that the method is precise. Repeatabilty also performed. The results are given in table 6 & 7.

Recovery at each concentration, sample was injected thrice to check repeatability and from the RSD values it was analyzed that the method was accurate as % recovery values found to be in the range of 99.71% to 100.8 % at three different concentration, the result are given in table 5.

Robustness Small deliberate changes in chromatographic conditions such as change in mobile phase ratio (+ 2 %), change in pH (±2 units) and flow rate (± 2 units) were studied to determine the robustness of the method. The results were in favor of (% RSD< 2%) the developed RP-HPLC method for the analysis of Gemigliptin. The results are given in table 9.

Limit of Detection (LOD) and Limit of Quantification (LOQ) The LOD of was found to be 14.36 µg/ml and the LOQ 43.53 µg/ml estimated by using the standard formulas. The low values of LOD and LOQ illustrate that the developed method was sensitive, accurate and precise asit can detected and quantify with very low concentration. The results are given in table 8, 10.

Force degradation studies

RP-HPLC study of samples obtained on stress testing of Gemigliptin under different conditions using mixture of methanol, acetonitrile and water in the ratio 40:40:20 (v/v) with pH 3.0 as a mobile solvent system suggested the following degradation behavior. The chromatograms obtained on stress degradation, like Acid degradation and similarly other conditions were shown in figure.6-13.Tabel 13

DISCUSSION

A simple, accurate and precise RP-HPLC method for¬ the Gemigliptin in Bulk form has been developed and validated. Separation of drugs was carried out using methanol:¬ acetonitrile: water (pH-3.0) (%V/V) (40: 40: 20 :) mobile phases at 5 min. run time and 280 nm. The Rt value for Gemigliptin were found to be 2.735 min. The %RSD values for intra-day precision study were¬ ≤ 2.0% and inter-day study were ≥ 2.0%, confirming that the method was sufficiently precise The %RSD values of Robustness study were ≤ 2.0%, confirming that the proposed method was found to be robust enough to withstand such deliberate changes and allow routine analysis of the sample. The Forced degradation was carried out in various¬ stress condition like acid, alkali, oxidative and thermal. Maximum degradation in Gemigliptin was observed in a Acidic condition i.e. 12.45% in standard and 10.13% in Acid condition in Tablet. The peak of degraded component were in resolved¬ from the peak of main component and do not interfere with the API peak.

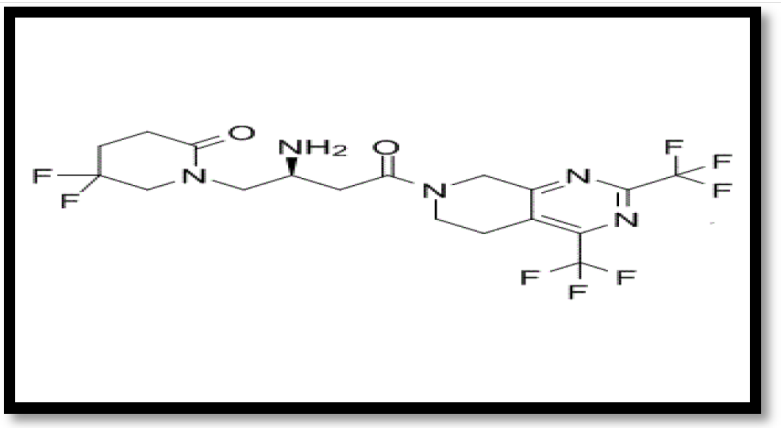

Figure 1: Structure of Gemigliptin

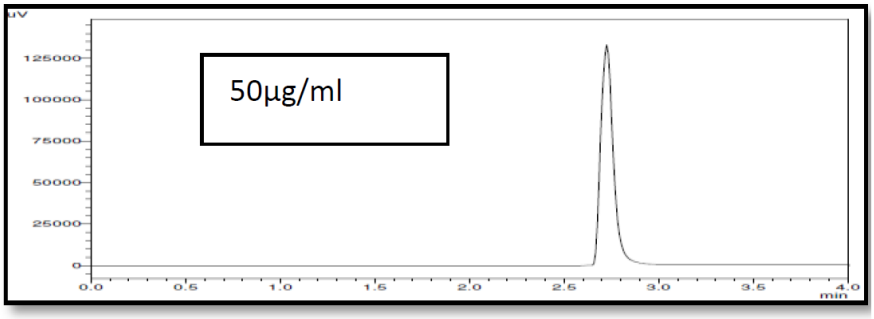

Figure: 2 Standard Chromatogram of Gemigliptin

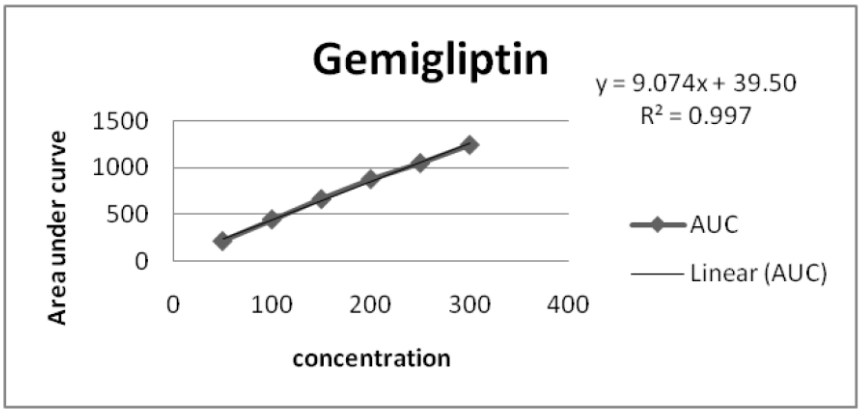

Figure:3 Calibration curve of Gemigliptin

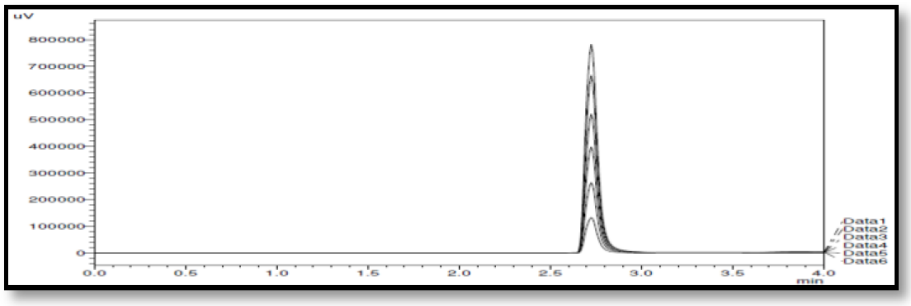

Figure : 4 Linearity Chromatogram of Gemigliptin

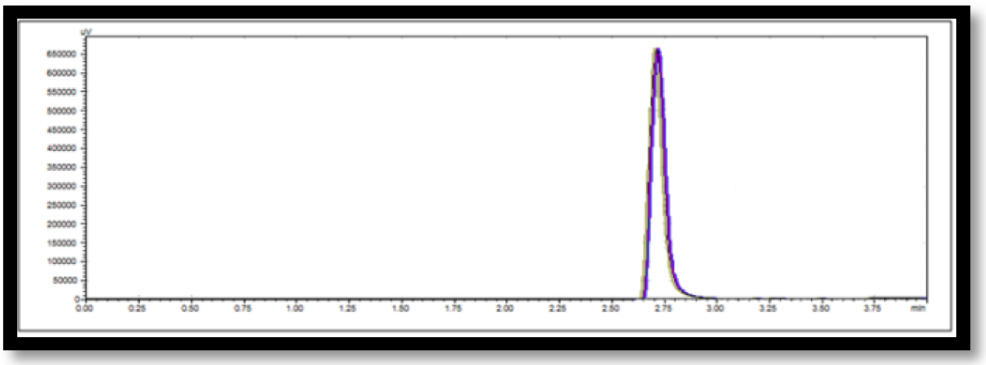

Figure: 5 System suitability for Gemigliptin

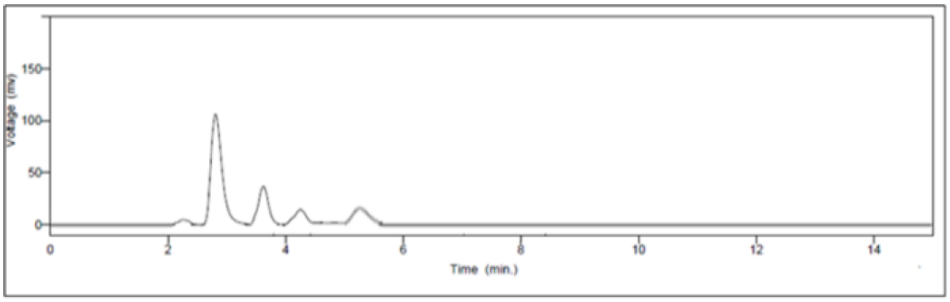

Figure: 6 Acid degradation of Gemigliptin in Bulk form

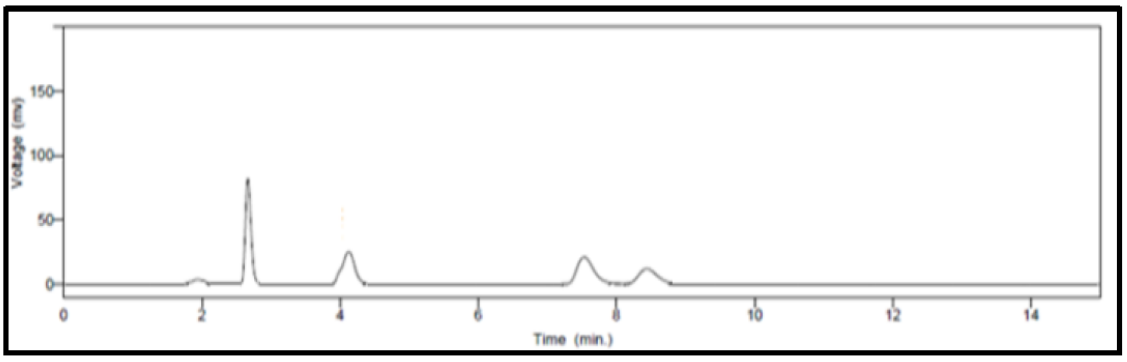

Figure: 7 Acid degradation of Gemigliptin Tablet (In House Prepare)

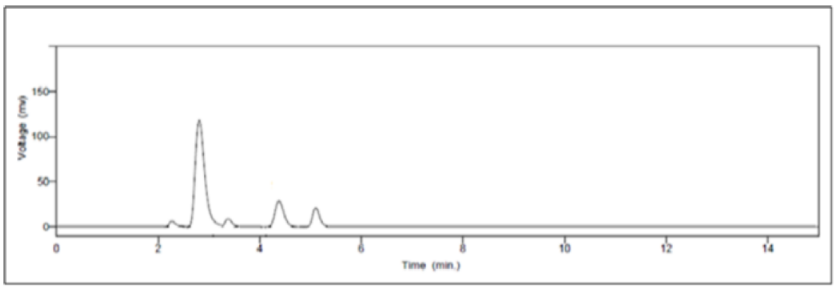

Figure: 8 Alkaline degradation of Gemiglptin in Bulk form (0.1 N NaOH, 1hr)

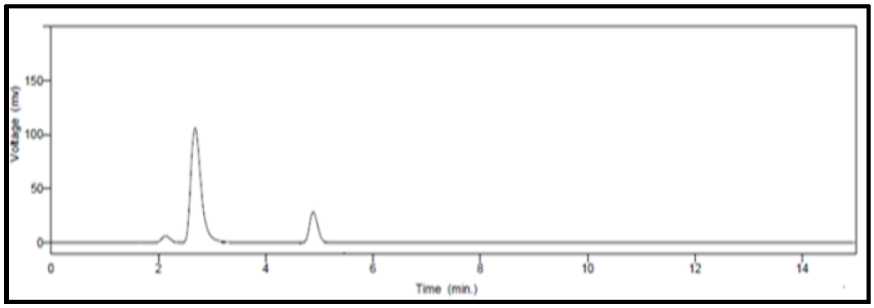

Figure: 9 Alkaline degradation of Gemigliptin Tablet (In House Prepare)

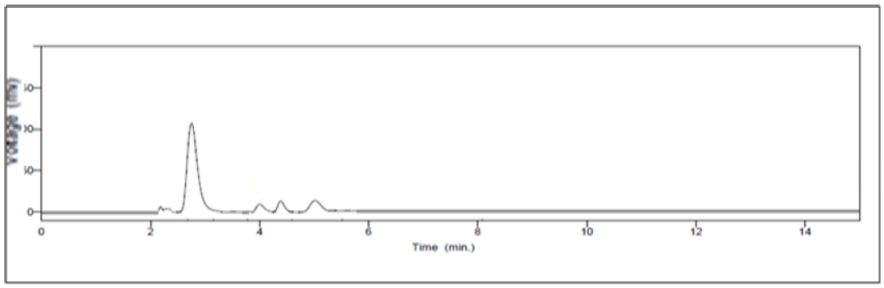

Figure: 10Oxidative Degradation of Gemigliptin in Bulk form Oxidative (3%H_2O_2, 4hr)

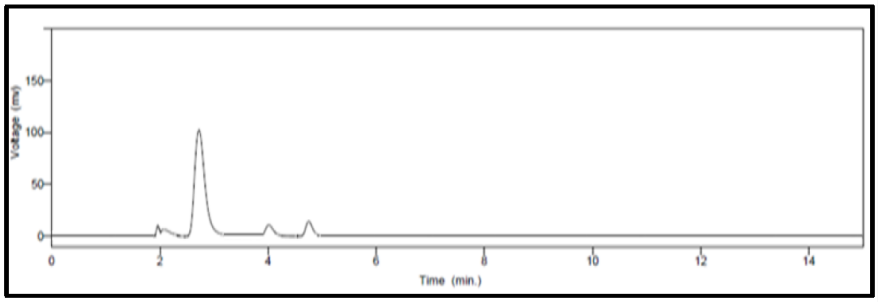

Figure: 11 Oxidative degradation of Gemigliptin Tablet (In House Prepare)

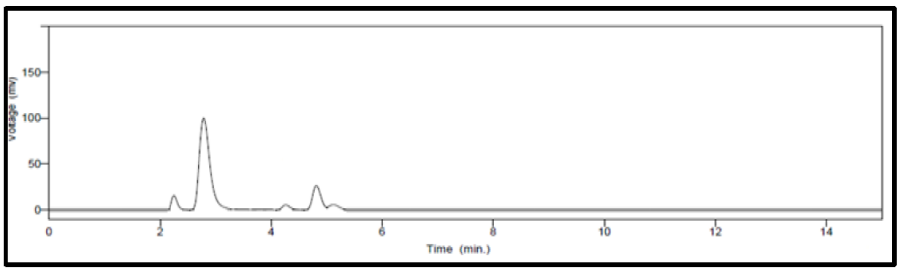

Figure: 12 Thermal degradation of Gemigliptin in Bulk

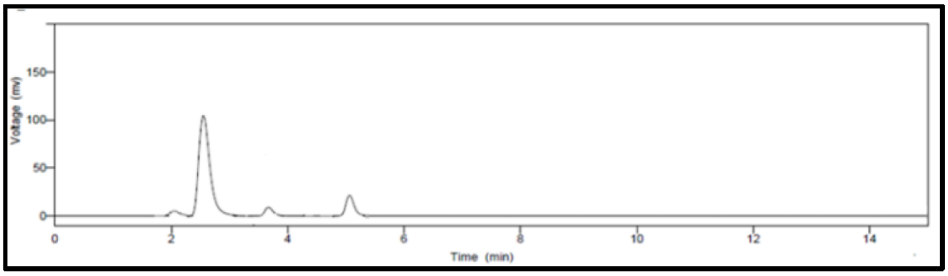

Figure: 13 Thermal degradation of Gemigliptin Tablet (In House Prepare)

Table: 1 Chromatograpic condition for Gemigliptin

Parameters	Specifications
Column	Shisedo C_{18} (250mm * 4.6mm, 5 µm)
Mobile phase	Acetonitrile : Methanol : Water (pH-3.0) (40:40:20 %V/V/V)
Flow rate	1 ml/min
Run time	5 min
Detection wavelength	280 nm
Retention time	2.735 min for Gemigliptin

Table: 2 Calibration Data of Gemigliptin

Conc. (µg/ml)	Area±SD
50	218.92±1.919
100	455.82±4.422
150	650.43±6.464
200	874.22±5.850
250	1048.78±6.870
300	1253.18±7.465

Table: 3 Assay Data of Gemigliptin

Brand name	Label Claim	% Mean Recovery ± SDn = 5
Gemigliptin Tablet (In house prepared)	**Gemigliptin (mg)** 50	**Gemigliptin** 100.43±0.9817

Table: 4 Linearity Data of Gemigliptin

Sr. No	Gemigliptin concentration (µg/ml) (n=3)	Peak Area (v) ± SD	%RSD
1	50	220.21 ±1.058	0.4807
2	100	453.62 ±4.097	0.9033
3	150	650.03 ±4.361	0.6709
4	200	875.89±3.986	0.4551
5	250	1046.32±6.429	0.6144
6	300	1252.12±7.216	0.5762

Table: 5 Accuracy Data of Gemigliptin

% Recovery	Target Conc.	Spiked Conc.	Final Conc.	Conc. obtained	% Assay	Mean±SD	% R.S.D
	100	80	180	180.62	100.44	99.93±0.372	0.3725
80 %	100	80	180	179.31	99.61		
	100	80	180	179.72	100.84		
	100	100	200	199.86	99.93	100.06±0.127	0.1270
100 %	100	100	200	200.19	100.09		
	100	100	200	200.36	100.18		
	100	120	220	220.13	100.05	99.96±0.081	0.0816
120%	100	120	220	219.86	99.93		
	100	120	220	219.79	99.90		

Table: 6 Repeatability Data for Gemigliptin

Sr. no	Peak Area (volt) (n= 3)		
	Gemigliptin (100µg/ml)	**Gemigliptin (150µg/ml)**	**Gemigliptin (200µg/ml)**
1	456.11	654.146	876.546
2	448.129	643.284	886.346
3	451.118	648.547	884.385
Mean	451.78	647.32	882.42
SD	4.032	5.4318	5.1854
% RSD	0.8924	0.8373	0.5876

Table: 7 Interday& intraday precision Data for Gemigliptin

Sr.no	Gemigliptin Concentration (µg/ml) (n=3)	Peak Area (V) ± SD Interday	Peak Area (V) ± SD Intraday	% RSD interday	% RSD intraday
1	100	453.97 ±12.992	457.64 ±3.285	2.8619	0.7179
2	150	657.69 ±11.064	651.36 ±6.656	1.6822	1.0219
3	200	872.57 ±7.4830	872.57 ±7.483	0.8575	0.8575

Table: 8 LOD and LOQ Data for Gemigliptin

Drugs	LOD (µg/ml)	LOQ (µg/ml)
Gemigliptin	14.36	43.53

Table: 9 Robustness Data for Gemigliptin

Sr. no.	Gemigliptin (100 µg/ml)					
	Ph		**Flow rate**		**Mobile phase**	
	+ 0.2 units	**-0.2 units**	**+0.2 units**	**-0.2 units**	**+ 2 %**	**-2%**
1	461.15	452.83	462.73	464.32	463.93	457.83
2	473.27	459.29	466.39	472.37	457.23	469.37
3	458.13	449.92	474.92	478.73	452.33	471.29
Mean	464.18	454.01	468.01	471.80	457.83	466.16
S.D	8.011	4.795	6.255	7.221	5.8232	7.280
% R.S.D	1.7258	1.0560	1.3365	1.5306	1.2719	1.5617

Table: 10 System Suitability of Gemigliptin

Gemigliptin (250µg/ml) (n=7)	Peak Area (volt)	Retention time (min)
	Gemigliptin	Gemigliptin
1	1050.11	2.746
2	1049.23	2.732
3	1057.41	2.761
4	1056.21	2.742
5	1063.46	2.758
6	1048.12	2.791
7	1051.13	2.772
Mean	1053.66	2.757
SD	5.5666	0.019
% RSD	0.5283	0.7204

Table: 11 System Suitability Parameter of Gemigliptin

Sr. No.	System suitability parameter	Gemigliptin	Specification as per IP 2010 and USP 34 NF 29
1	Retention time (min)	2.7574	-
2	Theoretical plate number (N)	4455	Not less than 2000
3	Tailing factor (T)	1.382	Not greater than 2.0

Table: 12 Summary of Validation Prameters of Gemigliptin

Sr No.	Parameters	Gmigliptin	REMARK
1	Linearity (µg/ml)	50-300 (µg/ml)	Linear
2	%Recovery	99.71-100.08	Accurate (98.0%-102%)
3	Precision((%RSD) Repeatability (n=3)	0.5876-0.8924	Precise (%RSD < 2)
	Inter-day (n=3)	0.8575-2.8619	
	Intra-day (n=3)	0.7179-1.0219	
4	LOD (µg/ml)	14.36	Sensitive
5	LOQ (µg/ml)	43.53	Sensitive
7	Robustness	Robust	Robust (No difference in result)

Table: 13 Result of force degradation (Bulk)

Sr. no	Stress Type	Gemi std R_t (min)	Gemi tab R_t (min)	No. of extra peak std	No. of extra peak tab	% Degradation std	% Degradation tab
1	Acid	2.811	2.796	4	4	12.45	10.13
2	Alkaline	2.792	2.771	4	2	7.56	6.2
3	Oxidation	2.751	2.762	5	4	9.8	9.1
4	Thermal	2.793	2.663	4	3	9.6	6.7

CONCLUSION:

A simple, rapid, accurate and precise RP-HPLC¬ method was developed and validated for estimation of Gemigliptin in Bulk form. For RP-HPLC method linearity range was found in¬ range of 50-300 µg/ml for Gemigliptin, Limit of detection and Limit of Quantification was found to be 14.36 µg/mL and 43.53 µg/mL respectively. % RSD for intraday ≤ 2 and interday precision was¬ found to be ≥ 2.Recovery greater than 98 but less than 102 for this method shows that the method is accurate and free from the interference of excipients used in formulation. So, the developed method can be used for routine¬ analysis and quality control test for Gemigliptin The stability-indicating method resolved the drug¬ peak and also the peaks of degradation products formed under variety of conditions. After exposure of Gemigliptin to stress conditions, it¬ was concluded that the drug is susceptible to Acid, Alkaline hydrolysis, oxidation degradation, and Thermal degradation. Therefore this method can be employed for¬ monitoring the stability of Gemigliptin and can be used for the routine analysis of the drug in pure and tablet dosage forms. The maximum degradation of Gemigliptin was¬ observed in Acidicdegradation i.e.12.45 % for standard and 10.13 % for Tablet formulation. RP-HPLC method was able to estimate Gemigliptin¬ accurately in presence of its degradation products. The method was validated as per ICH guidelines.

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