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BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF GEMIGLIPTIN TARTRATE SESQUIHYDRATE.

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ABSTRACT
A simple, selective, specific, accurate Liquid Chromatography -Mass Spectrometry (LC-MS) method was developed and validated for the determination of Gemigliptin in Human Plasma. The accuracy and precision data must fulfill the requirements for the quantification of analytes in biological matrices to produce data for bioavailability, bioequivalence, etc. The separation of the analyte was carried out on Phenomenex Gemini C_{18} , (3 µm, 30.0 × 2.0 mm) column and the mobile phase containing 0.1% Formic acid in 10 mM Ammonium acetate in water : Acetonitrile (10:90% v/v) at a flow rate of 0.5 mL/min. The retention times of Gemigliptin and Sitagliptin (Internal Standard) were 3.29 min and 4.22 min simultaneously and the total run time was 5.5 min. Monitoring of the fragmentation of m / z 490.160 → 338.100 and 408.142 → 235.000 performed during MS/MS detection of Gemigliptin and Internal Standard (I.S.) on the mass spectrometer. The overall recovery of Gemigliptin and IS was 89.81% and 89.52% respectively. The method was validated over the concentration range of 1 ng/mL to 2000 ng/mL. The method was validated for linearity, accuracy, precision, specificity, selectivity, inter and intraday precision, LQC, HQC.

KEYWORDS: Gemigliptin, LC-MS, Bioanalytical Method, validation.

1. INTRODUCTION[1-4]
Type 2 diabetes mellitus is the 7th leading cause of death in world wide. Approximately 1.5 million deaths occurred in 2014 are directly due to type 2 diabetes mellitus. More than 80% deaths are occurring due to Diabetes in developing countries. Unhealthy diet, lack of physical exercise, stress and more usage of tobacco increasing the risk of developing type 2 diabetes mellitus.

Type 2 diabetes mellitus is a chronic metabolic disease characterized by impaired insulin secretion and insulin resistance in insulin-targeting tissues (liver, skeletal muscle and adipocytes). Insulin resistance in these tissues is accompanied by decreased glucose utilization by muscle and fat cells and increased hepatic glucose output, leads to hyperglycemia. Insulin resistance is compensated by excessive insulin secretion. The over secretion of insulin gradually decreases the islet beta-cell reserve and its function. The complexity in treatment of type 2 diabetes mellitus is welcoming new drugs for specific cure.

Gemigliptin is chemically 3(s)-3-amino-4- (5,5-difluro oxopiperidino [2,4 (trifluromethyl) -5,6,7,8 -tetra hydro [3,4-d] pyrimidine-7-yl] butan-1-one exhibits a unique structure. Gemigliptin a dipeptidyl peptidase inhibitor(DPP-4 inhibitor), has recently been approved in the Korea for the therapy of type 2 diabetes. DPP-4 inhibitors represent a new therapeutic approach to the treatment of type 2 diabetes that functions to stimulate glucose dependent insulin release and reduce glucagons levels, by inhibiting the inactivation of incretins, particularly glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP), thereby improving glycemic control. DPP-4 is a membrane associated peptidase which is found in many tissues, lymphocytes and plasma. DPP-4 has two main mechanisms of action, an enzymatic function and another mechanism where DPP-4 binds adenosine deaminase, which conveys intracellular signals via dimerization when activated.

Literature survey reveals that, till date there is no LC-MS method has been reported in literature for the determination of Gemigliptin in rat plasma. Hence, authors attempted to develop a simple and reproducible LC-MS method for quantification of Gemigliptin which helps the researchers for therapeutic drug monitoring and

pharmacokinetics.

The aim of this work is, to develop a simple, selective and sensitive method, which employs liquid-liquid extraction technique for sample preparation and liquid chromatography with electrospray ionisation-tandem mass spectrometry for quantification of Gemigliptin in rat plasma.

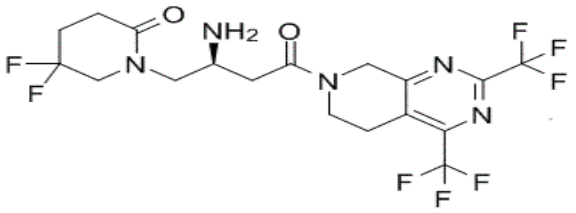

Figure 1: Structure of Gemigliptin

2. EXPERIMENTAL[5-14]

2.1 Chemicals and reagents

The pure drug form of Gemigliptin is collected from Precise Chemipharma Pvt. Ltd Talegaon Dindori, Maharashtra. Methanol (HPLC grade), Acetonitrile (HPLC grade), Milli-Q water, Formic acid, DMSO were purchased from Merck, Rankem. Buffered blank plasma was procured from blood bank for the preparation of plasma calibration standards and quality control samples. For specificity check experiment different samples of plasma were procured from Lions Blood bank and Research Centre, Vapi.

2.2 Instrumentation

Liquid Chromatographic Mass Spectroscopy for quantitative estimation of Gemigliptin LC-MS instrument having Waters Acquity UPLC system. Mass spectrometric detection was performed on Mass spectrometric detection was performed on triple Quadrupole mass spectrometer, equipped with turbo ion spray inter-face. The analytes and IS were detected using a Waters XEVO TQ mass spectrometer (Waters corporation, Milford, USA) equipped with Z spray source.

The precursor to product ion transitions for both analytes and IS were as follows: Gemigliptin m/z 490.160→338.100, Repaglinide m/z 408.142→ 235.000 with dwell time 100 ms. Data acquisition and calculations were performed using Analyst software, version 4.1.2 was used for controlling the hardware and data handling.

2.3 Chromatographic conditions

Chromatographic separation was performed on Phenomenex Gemini C_{18} column (3 µm, 30.0 × 2.0 mm) was used. The analyte and IS were separated by using the mobile phase 0.1% Formic acid in 10 mM Ammonium acetate in water: Acetonitrile (10:90% v/v) by gradient delivered with a flow rate of 0.5 mL/min. The injection volume was 2µl and the total chromatographic run time was 5.5 min. The column oven temperature was set at 40.0 ± 2.0°C.

2.4 Mass spectrometric conditions

Ionization mode: Positive ionization. Resolution: Q1 Unit; Q3 Unit.

MRM conditions

Parameters	Q1 (amu)	Q3 (amu)	Dwell Time (msec)	DP (volts)	CE (volts)	CXP (volts)
Gemigliptin	490.160	338.100	100	36.13	10.00	13.00
Sitagliptin	408.142	235.000	100	45.00	18.00	16.00

2.4 Preparation of calibration standards and quality control samples

Standard stock solutions of Gemigliptin and internal standard (Sitagliptin) were prepared by dissolving their accurately weighed amounts in methanol to give a final concentration of 1mg/mL. Individual working solutions of analyte were prepared by appropriate dilution of their stock solutions in methanol. All the solutions were stored in refrigerator at below 10◦ C and were brought to room temperature before use. Working solution of internal standard (Sitagliptin, 50 µg/mL) was prepared daily in methanol and was stored at room temperature.

Calibration standards and quality control (QC) samples were prepared by spiking blank plasma with the working solutions (5%) prepared from independent stock weightings. K3 EDTA anticoagulant blank plasma was collected from human. Calibration standards were prepared in plasma at concentrations of 1, 2, 100, 200, 400, 800, 1200, 1600 and 2000 ng/mL. Quality control samples were prepared at 1 ng/mL (LLOQ QC), 3 ng/mL (LQC), 800 ng/mL (MQC) ,1600 ng/mL (HQC) and 2000 ng/mL (ULOQ).

2.5 Sample Preparation

A plasma sample (0.5 mL) was pipetted into a 15 mL glass tube, then 50µL of IS working solution (0.5 µg/mL) and 1mL of organic solvent, Perchloric acid (Precipitating agent) were added. And vortex mix it for 10 min then centrifuge it at 10000 rpm for 15 min at 8000 rpm. After vortex mixing for 10 s, 4 mL aliquot of the extraction solvent, was added and the sample was vortex-mixed for 5 min. The organic layer (3 mL) was transferred to a glass tube and evaporated to dryness

using an evaporator at 40°C under a stream of nitrogen. Then the dried extract was reconstituted in 250 µL of mobile phase and a 10 µL aliquot was injected into the chromatographic system.

2.6 METHOD VALIDATION: SYSTEM SUITABILITY

System suitability experiment is performed to check whether system is suitable or not to carry out experiments. System suitability was performed by injecting six consecutive injections using aqueous MQC at least once in a day.

Acceptance criteria: % CV of retention time (t_R) and area ratio should be ≤ 4.00%.

SYSTEM PERFORMANCE

System performance experiment is carried out to check that system is capable to perform as per its predetermined specifications or not. For system performance extracted STD BL, ULOQ and LLOQ samples are processed and injected for determination of % interference in blank and % carryover effect of system.

System performance is carried out before each batch experiment.

Acceptance criteria

Peak area of analyte should be more than or equal to 5.0 times of LLOQ sample when compared to 1st acquired STD BL(ULOQ). Carryover observed in both STD BL injected after ULOQ should be ≤ 20.0% for analyte and ≤5.0% for ISTD response compared to analyte and ISTD response respectively of LLOQ sample. % Interference observed in first acquired STD BL (before ULOQ) is > 20.0% for analyte and > 5.0% for ISTD in this case repeat same experiment by using separately processed samples.

SPECIFICITY

Specificity is performed by using 10 different plasma lots among which 7 lots of normal plasma having K3EDTA as anticoagulant, 1 lot of haemolysed plasma, 1 lot of lipidemic plasma and 1 lot of plasma having sodium heparin as anticoagulant.

Sample processed: STD BL and LLOQ.

Compare response of interfering peak at t_R of analyte and ISTD in STD BL against response of extracted LLOQ sample.

Acceptance criteria

Response of interfering peak at t_R of analyte should be ≤20.0% and at t_R of ISTD should be ≤5.0% response of ISTD of respective LLOQ samples. At least 80% of matrix lots (excluding haemolysed, heparinised, and lypemic matrix lots) with intended anticoagulant should be within aforementioned acceptance criteria.

Linearity Procedure

LLOQ should cover at least 4-5 half-life of the reported Cmax. ULOQ should cover the expected Cmax value. When no reference is available, it should be decided on basis of development studies results. Calibration curve must contain minimum six calibration standards. Selection of calibration standard expressed as multiple of LLOQ and percent of ULOQ are as follows:

Code	9 std calibration curve
STD-1/ CS-1	LLOQ
STD-2/ CS-2	2 LLOQ
STD-3/ CS-3	5% ULOQ
STD-4/ CS-4	10% ULOQ
STD-5/ CS-5	20% ULOQ
STD-6/ CS-6	40% ULOQ
STD-7/ CS-7	60% ULOQ
STD-8/ CS-8	80% ULOQ
STD-9/ CS-9	100% ULOQ

Protocol for calibration level selection

PRECISION

For intra and inter-run precision LLOQ, HQC, MQC,LQC and ULOQ samples should be within 15.00% CV and for LLOQ it should be 20.00% CV.

ACCURACY

Acceptance criteria

For intra and inter-run accuracy LLOQ, HQC, MQC,LQC and ULOQ samples should be within ±15% mean accuracy and for LLOQ it should be ±20 % mean accuracy.

DILUTION INTEGRITY OF STOCK SOLUTIONS (DISS)

It is performed to demonstrate that dilution of samples should not affect accuracy and precision. If applicable dilution integrity should be demonstrated by spiking matrix with an analyte concentration above ULOQ and diluting this sample with blank matrix (at least 5-10 times per dilution factor). Dilution integrity experiment is performed by using n ≥ 5 samples of dilution integrity by spiking matrix with an analyte concentration 4-5 times above ULOQ and diluting this sample with blank matrix (at least 5-10 times per dilution factor).

Preparation of Dilution Integrity Spiked Solution

Prepare drug intermediate of 2 mg/mL from drug stock solution This prepared solution called as DISS (1/10). 5% spiking of DISS (1/10) solution spiked into blank plasma and final concentration of spiked solution became 100µg/mL (AUL QC). AUL QC further 10 times diluted with plasma which gives 1000 ng/mL which is 5 times than ULOQ.

Acceptance Criteria: % CV of dilution integrity sample should be ≤ 15.00 % and accuracy should be within 15.00 %.

STABILITY

Short Term Stock Solution Stability (STSS)

Stability of drug and ISTD stock solution should be evaluated for at least 06 hours. For STSS stock solutions are stored in refrigerator for minimum of 06 hours and after stability period retrieve it and make ULOQ vial from drug stock and ISTD dilution vial from ISTD stock solution. Inject aqueous ULOQ and ISTD dilution vial compare it with freshly prepared aqueous ULOQ and ISTD dilution.

Long Term Stock Solution Stability (LTSS)

Stability of drug and ISTD stock solution should be evaluated for relevant time period. For LTSS stock solutions are stored in refrigerator for 20 days and after stability period retrieve it and make ULOQ vial from drug stock and ISTD dilution vial from ISTD stock solution. Inject aqueous ULOQ and ISTD dilution vial compare it with freshly prepared aqueous ULOQ and ISTD dilution.

Short Term Working Solution Stability (STWSS)

Stability of drug and ISTD working solution should be evaluated for at least 06 hours. For STWSS working solution are stored in refrigerator for minimum of 06 hours and after stability period retrieve it and make ULOQ, LLOQ vial from drug stock and ISTD dilution vial from ISTD stock solution. Inject aqueous ULOQ, LLOQ and ISTD dilution vial compare it with freshly prepared aqueous ULOQ, LLOQ and ISTD dilution.

Long Term Working Solution Stability (LTWSS)

Stability of drug and ISTD working solution should be evaluated for relevant time period. For LTWSS working solution are stored in refrigerator for 20 days and after stability period retrieve it and make aqueous ULOQ, LLOQ vial from drug stock and ISTD dilution vial from ISTD stock solution. Inject ULOQ and ISTD dilution vial compare it with freshly prepared aqueous ULOQ, LLOQ and ISTD dilution.

Bench Top Stability (BT) (Short Term Stability of Analyte in Matrix).

It should be performed at higher quality control and lower quality control level for six replicates. Prepare spiked sample of HQC and LQC and stored at room temperature for a specific time period. Generally, time period is about time required from spiking of sample to transfer in to vials. Use freshly spiked calibration curve and quality control standard for determination of stability samples. For BT kept spiked HQC and LQC for 06 hours at room temperature. BT stability samples were analyzed along with freshly spiked CCs and freshly prepared QCs 6 replicates of each LQC and HQC samples as per procedure.

Stability of Dry Extract (DE)

DE experiment was carried out whenever sample processing involves evaporation step. DE stability was conducted by using previously processed and dried stability samples. Freshly spiked replicates of each LQC and HQC samples were prepared and processed as per sample preparation procedure. After drying, dry extract stability samples were stored at -20 ± 5ºC for a period of at least 24 hours or as per requirement. DE stability samples were analyzed along with freshly spiked CCs and freshly prepared QCs 6 replicates of each LQC and HQC samples as per procedure.

Freeze and Thaw Stability

QC samples (at high and low level) are stored and frozen in freezer at intended temperature and thereafter thawed at room or processing temperature. After complete thawing, samples are refrozen again applying same conditions. At each cycle, samples should be frozen for at least 12 hours before they are thawed. Number of cycles in freeze- thaw stability should equal or exceed that of freeze/thaw cycles of study samples. It is perform to demonstrate that accuracy and precision is not change upon freezing and thawing cycle. Freeze and thaw stability experiment is performing by processing $n \geq 5$ sample (at high and low level) of freeze thaw stability along with freshly spiked calibration curve and quality control sample. Storage temperature: -20 ± 5°C and -78 ± 8°C.

3 RESULTS AND DISCUSSION LC-MS Method development

For consistent and reliable estimation of analytes it was necessary to give equal importance for optimization of extraction procedure along with chromatographic and mass spectrometric conditions. Analyte and ISTD were tuned in positive polarity mode using electro spray ionization technique. The Q1 and the MS scans were made in infusion mode and further compound and gas parameters were optimized in flow injection analysis. The [M+H] peaks were observed at m/z of 490.160 and 408.142 for Gemigliptin and Sitagliptin respectively. Most abundant product ions were found at m/z of 338.100 and 235.000 for both Gemigliptin and Sitagliptin by applying sufficient collision activated dissociation gas and collision energy. A 5% change in ion spray voltage and gas parameters did not affect the signal intensity.

Protein precipitation extraction was initiated with individual tubes. Impact of different solutions and their concentration on recovery of analytes was monitored. During the optimization of chromatographic conditions and extraction procedure, more emphasis was given to improve the sensitivity and recovery. No significant matrix effects were observed with the proposed chromatographic and extraction conditions.

3.1 SYSTEM SUITABILITY

% CV of Analyte t_R, ISTD t_R and Area ratio for drug was observed 0.078, 0.069 and 0.533425 respectively for Gemigliptin which is not more than 4.00% as per acceptance criteria.

3.2 SYSTEM PERFORMANCE

% Carryover in STD BL2 and STD BL3 was found to be 0.32% and 0.24% respectively at t_R of Gemigliptin which is not more than 20% and % Carry over in STD BL2 and STD BL3 were .0044% and 0.0021% respectively at t_R Sitagliptin which is not more than 5%. So results are accepted as per acceptance criteria.

% Interference in STD BL was found to be 0.85% at t_R of Gemigliptin which is not more than 20% and % carry over STD BL1 was found to be 0.011% at t_R of Sitagliptin which is not more than 5%. So results are accepted as per acceptance criteria.

% Interference at retention time of Drug & ISTD was found to be 0.90% to 0.06% and 0.02% to 0.0005% at LLOQ was found to be respectively Gemigliptin and Sitagliptin which is within acceptance criteria .All plasma lots are not interfering at t_R of analyte and ISTD, so these plasma lots can be used for further validation experiments.

3.3 SPECIFICITY OR SELECTIVITY

3.4 RECOVERY

% Overall recovery for Drug and ISTD was found to be 89.81% and 89.52% respectively for Gemigliptin and Sitagliptin.

Table: 1 Drug Recovery

Sr.no	HQC		MQC		LQC	
	Extracted Peak Area	Un- Extracted Peak Area	Extracted Peak Area	Un- Extracted Peak Area	Extracted Peak Area	Un- Extracted Peak Area
Mean	5107034.33	5528534.5	2609697	2892884	18832	21678.1667
SD	89438.1636	49447.329	76874.7869	60034.2166	575.4629441	231.509323
% CV	1.75127398	0.89440211	2.94573611	2.0752376	3.055771793	1.06793774
% Mean recovery	92.37591505		90.21091063		86.87081472	
% Overall recovery	89.8192135					

Table: 2 ISTD recovery

Sr.no	ISTD	
	Extracted Peak Area	Un- Extracted Peak Area
Mean	1501259	1676874
SD	59498.51	67695.26
% CV	3.963241	4.036992
% Mean Accuracy	89.52726711	

3.5 MATRIX FACTOR

Variability in ISTD normalized Matrix factor, as measured by coefficient of variation was found to be 2.28% for HQC level and 1.38% for LQC level for Gemigliptin which was ≤ 15% according to acceptance criteria.

Table: 3 Matrix Factor

Matrix lot no.	HQC			LQC		
	MF of Analy te	MF of ISTD	ISTD Normali zed Factor	MF of Analyte	MF of ISTD	ISTD Normaliz ed Factor
1	0.99	0.96	1.03	1.03	0.88	1.17
2	0.98	0.97	1.01	1.04	0.89	1.16
3	0.98	0.96	1.02	1.03	0.87	1.18
4	1	0.95	1.05	1.02	0.88	1.15
5	0.99	1	0.99	1.05	0.88	1.19
6	0.97	0.95	1.02	1.02	0.89	1.14

Haemolytic	0.98	0.98	1	1.03	0.88	1.17
Haemolytic	0.97	1	0.97	1.04	0.89	1.16
Lipidemic	0.99	0.96	1.03	1.04	0.87	1.19
Lipidemic	1	0.97	1.03	1.03	0.88	1.17
Mean			1.015			1.172
SD			0.023214			0.016193
% CV			2.28709			1.381679

3.5 LINEARITY

% Mean accuracy of concentration of calibration curve standards of 3 P & A run was found within ± 15% and % CV of 3 P & A run was found to be < 15%. R^2 value of 3 calibration curve was found to be > 0.998.

Table: 4 Linearity

STD ID	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6	STD 7	STD 8	STD 9
Nominal Conc. (ng/mL)	**1 ng/mL**	**2 ng/mL**	**100 ng/mL**	**200 ng/mL**	**400 ng/mL**	**800 ng/mL**	**1200 ng/mL**	**1600 ng/mL**	**2000 ng/mL**
Mean	0.935	1.888	89.954	193.742	386.625	782.541	1109.503	1560.382	1871.735
SD	0.066	0.102	3.533	3.277	3.140	5.875	15.767	38.987	42.292
%CV	7.100	5.419	3.927	1.691	0.812	0.750	1.421	2.494	2.259
Mean Accuracy	93.543	94.4	89.954	96.871	96.656	97.817	92.458	97.523	93.586

3.5 PRECISION AND ACCURACY

Intra-run Precision for all HQC, MQC and LQC samples was found to be ≤ 3.54% for Gemigliptin which is within acceptance limit of 15.00%. Intra-run Precision for all LLOQ QC sample was found to be ≤ 2.26% for Gemigliptin which is within acceptance limit of 20.00%. Intra-run Accuracy for all HQC, MQC and LQC samples was found to be ≤ 9.78% for Gemigliptin which is within acceptance limit of ± 15.00%. Intra-run Accuracy for all LLOQ QC quality control sample was found to be ≤ 16.67% for Gemigliptin which is within acceptance limit of ± 20.00%.

Inter-run Precision for all HQC, MQC and LQC samples was found to be ≤ 7.06% for Gemigliptin which is within acceptance limit of 15.00%. Inter-run Precision for all LLOQ quality control sample was found to be ≤ 4.86% for Gemigliptin which is within acceptance limit of 20.00%. Inter-run Accuracy for all HQC, MQC and LQC samples was found to be ≤ 9.13 for Gemigliptin which is within acceptance limit of ± 15.00%. Inter-run Accuracy for all LLOQ quality control sample was found to be ≤ 9.79% for Gemigliptin which is within acceptance limit of ± 20.00%.

Table: 5 Intra-run Precision and Accuracy

	QC Sample	Mean	SD	% CV	% Mean Accuracy
P & A I	**LLOQ QC**	0.985667	0.008383	0.850442	95.5333
	LQC	2.923333	0.040623	1.389629	93.2222
	MQC	791.4717	8.415551	1.063279	95.7047
	HQC	1559.918	55.22345	3.54015	93.4323
	ULOQ	1910.692	63.24548	3.310083	93.8679
P & A II	**LLOQ QC**	0.933333	0.021134	2.26441	83.3333
	LQC	2.932	0.038063	1.298196	91.6222
	MQC	804.505	6.241195	0.775781	95.9797
	HQC	1653.122	10.13791	0.613258	96.3056
	ULOQ	1986.445	5.39546	0.271614	92.6558
P & A III	**LLOQ QC**	0.975833	0.013963	1.430884	89.91667
	LQC	2.938833	0.021255	0.723239	91.47778
	MQC	805.14	6.21081	0.771395	90.22583
	HQC	1659.438	3.649983	0.219953	93.98729
	ULOQ	1996.227	1.787072	0.402785	95.21367

Table: 6 Inter-run Precision and Accuracy

P & A	LLOQ QC	LQC	MQC	HQC	ULOQ
	1 ng/mL	3 ng/mL	800 ng/mL	1600ng/mL	2000 ng/mL
Mean	0.902167	2.786833	726.96383	1514.538	1830.962
SD	0.043861	0.070845	51.360842	25.47699	49.29439
% CV	4.861714	2.542121	7.0651166	1.682162	2.692268
% Mean Accuracy	90.21667	92.89444	90.870479	94.65865	91.54808

3.5 DILUTION INTEGRITY

% Mean accuracy of dilution quality control was found to be 95.93%, 92.07% and 94.48% for Gemigliptin P & A I, P & A II, P & A III respectively which is within acceptance criteria. % Mean accuracy of dilution quality control is within ± 15% and % CV of dilution quality control is within 15%, so dilution of sample does not affect precision and accuracy of method.

Table: 7 Dilution Integrity

P & A ID	P & A I	P & A II	P & A III
QC	DQC 10000ng/mL	DQC 10000 ng/mL	DQC 10000 ng/mL
Mean	9593.166667	9207.83333	9448.666667
SD	1136.016623	1132.95427	1247.806983
% CV	11.84193564	12.3042438	13.20617
%Mean Accuracy	95.931	92.078	94.486

3.8 STABILITY

Stability tests were conducted to evaluate the analyte stability in stock solutions and in plasma samples under different conditions. The short term and long term stock solution and working solution stability was determined by comparing the area response of the analytes (stability samples) with the response of the sample prepared from fresh stock solution. Bench-top stability, dry extract stability, freeze–thaw stability were tested at LQC and HQC levels using six replicates at each level. Samples were considered to be stable if assay values were within the acceptable limits of accuracy (±15%) and precision (±15% CV).

Table: 8 Summary of stability data of Gemigliptin in human plasma:

Stability	QC Level	A	% CV	B	%CV	% Change
STSS	ULOQ	1837.06	0.330	1929.65	1.416	4.79
LTSS	ULOQ	1821.39	0.108	1922.39	1.148	5.25
STWSS	LLOQ	0.918	0.480	0.965	3.975	4.86
	ULOQ	1794.53	0.663	1830.96	2.692	4.44
LTWSS	LLOQ	0.805	0.441	0.842	0.824	4.39
	ULOQ	1841.65	0.282	1904.27	2.146	3.28
BT	LQC	2.850	2.541	2.897	0.846	1.62
	HQC	1478.68	2.557	1525.81	0.528	3.08
DE	LQC	2.869	2.983	2.936	1.463	2.28
	HQC	1493.34	0.518	1571.68	0.766	4.98
SE (5 ± 3°C)	LQC	2.787	4.773	2.865	0.508	2.72
	HQC	1544.08	2.774	1592.41	5.591	3.03
SE (Ambient temperature)	LQC	2.854	0.006	2.954	0.006	3.38
	HQC	1527.22	3.280	1540.56	2.803	0.86
FT (-20 ± 5°C)	LQC	2.767	2.225	2.816	4.913	1.74
	HQC	1547.27	0.426	1554.63	0.230	0.47

FT (-78 ± 8°C)	**LQC**	2.871	5.601	2.964	0.827	3.13
	HQC	1542.11	0.213	1588.77	0.140	2.93

A: Mean concentration of stability samples B: Mean concentration of comparison samples.

CALIBRATION CURVE

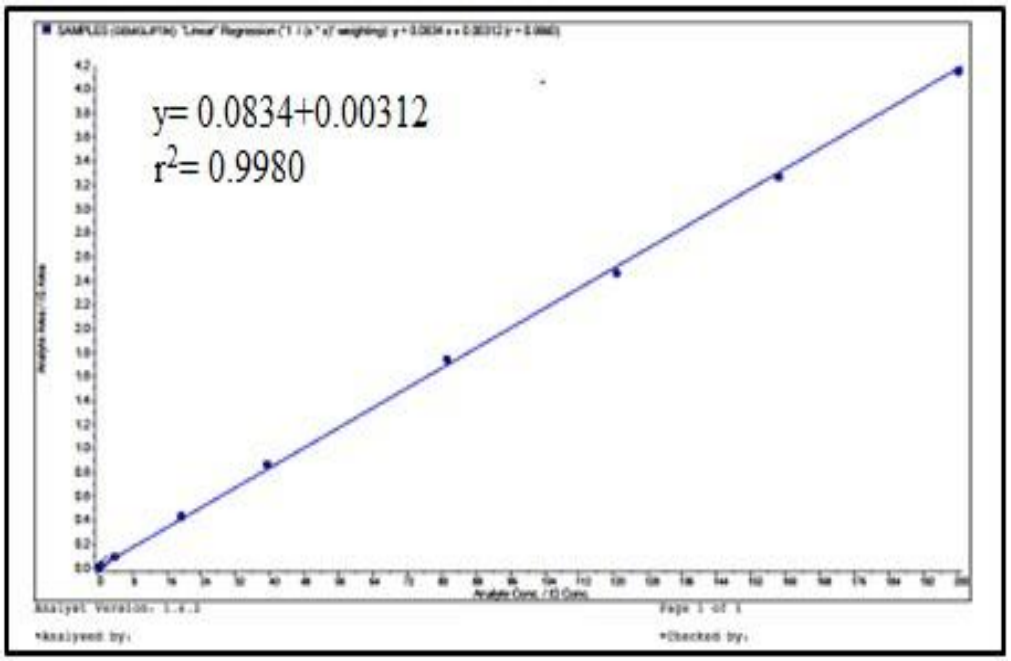

Figure 2: Calibration curve of Gemigliptin

CHROMATOGRAMS

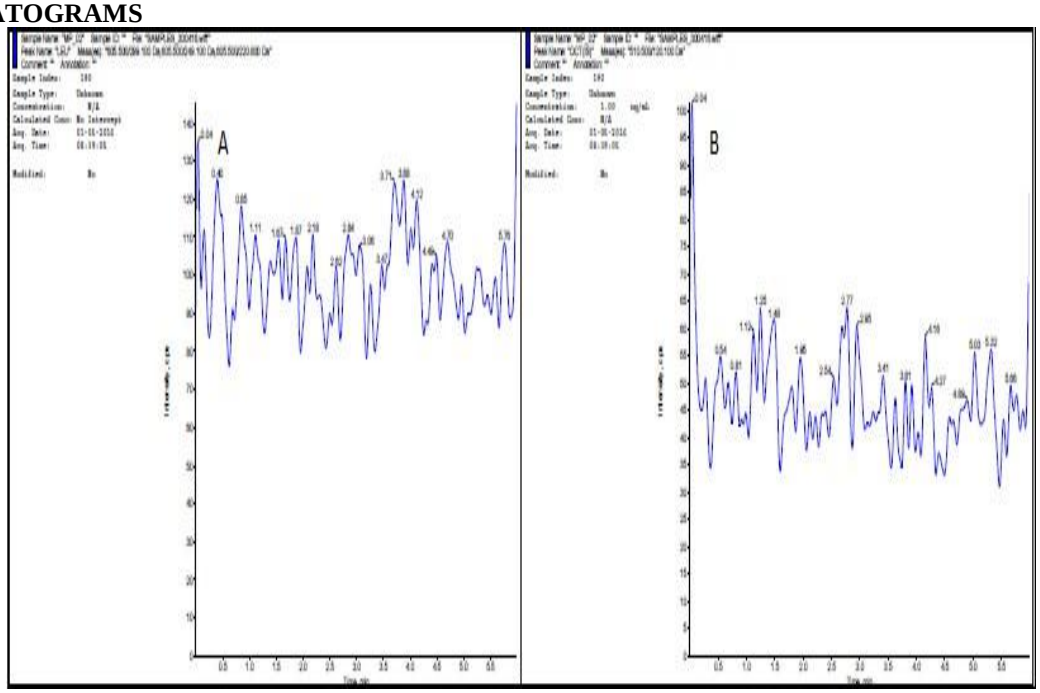

Figure 3: BLANK

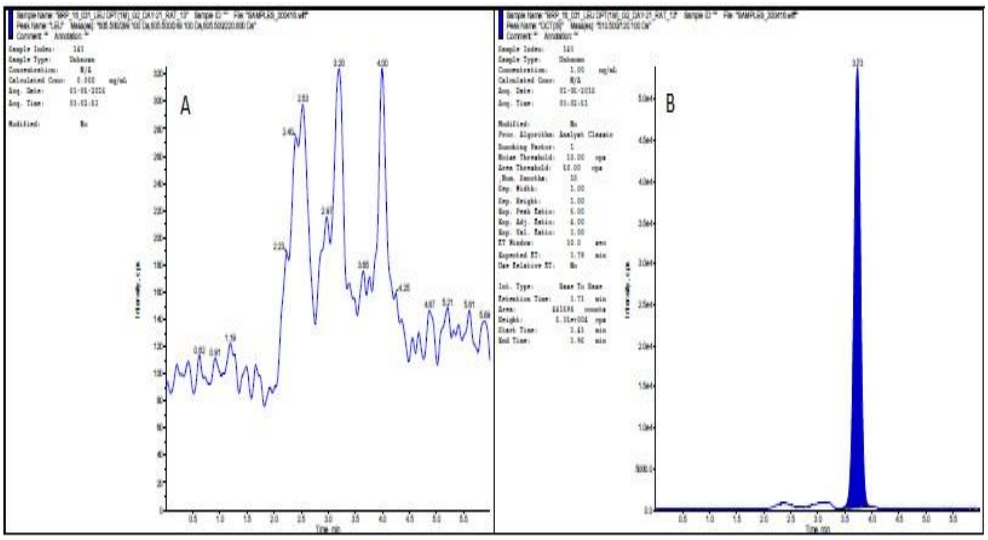

Figure 4: BLANK-IS

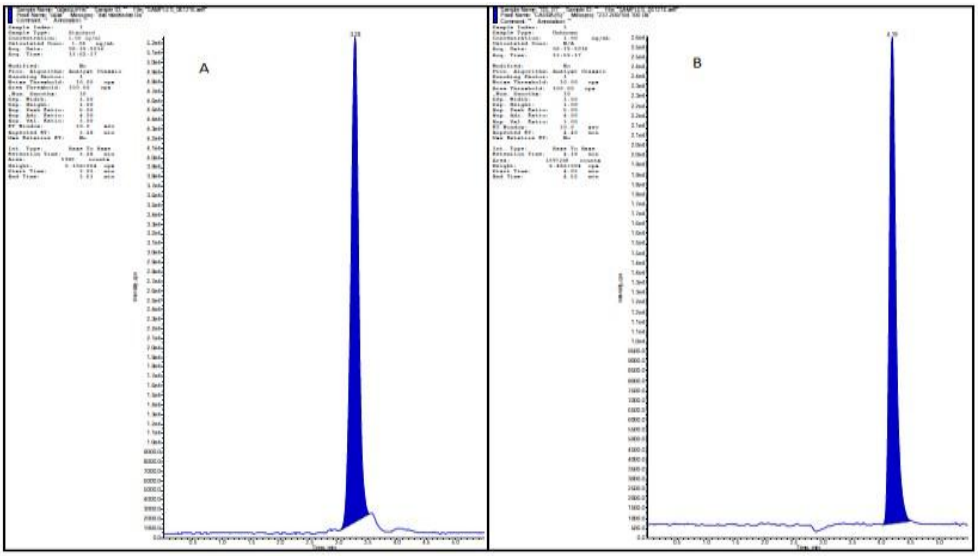

Figure 5: Representative of chromatograms of STD 1

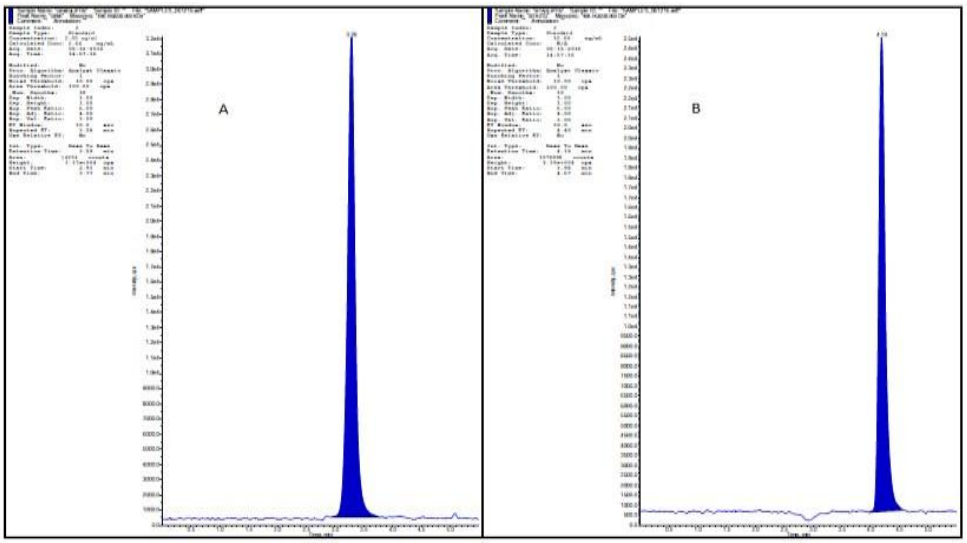

Figure 6: Representative of chromatograms of STD 2

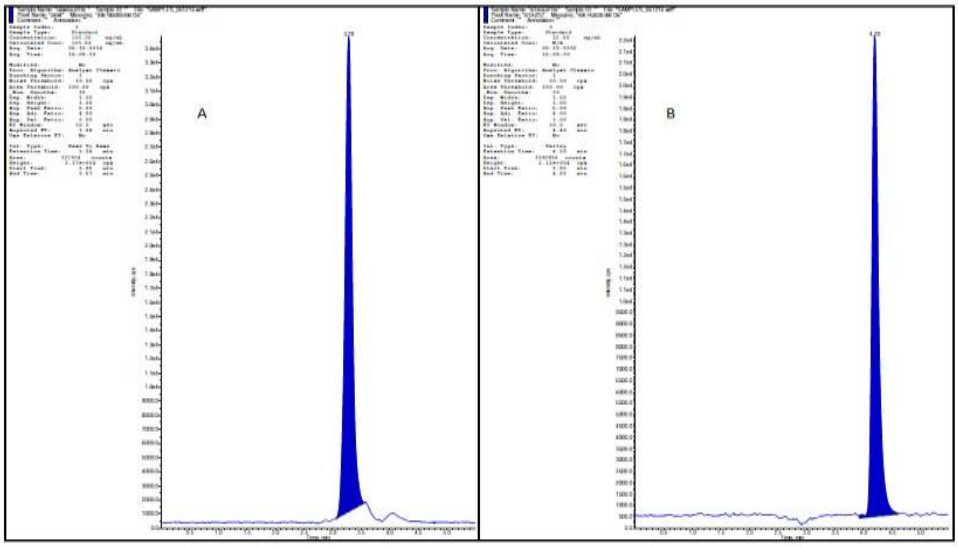

Figure 7: Representative of chromatograms of STD 3

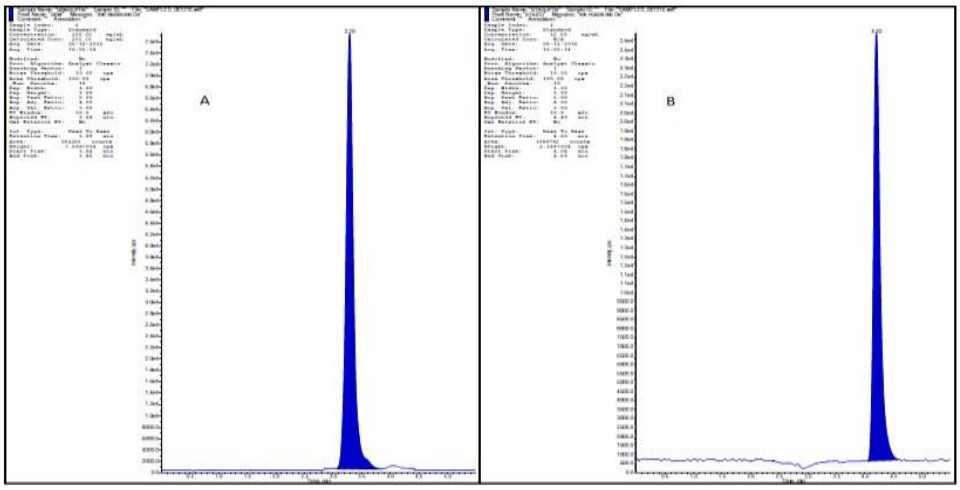

Figure 8: Representative of chromatograms of STD 4

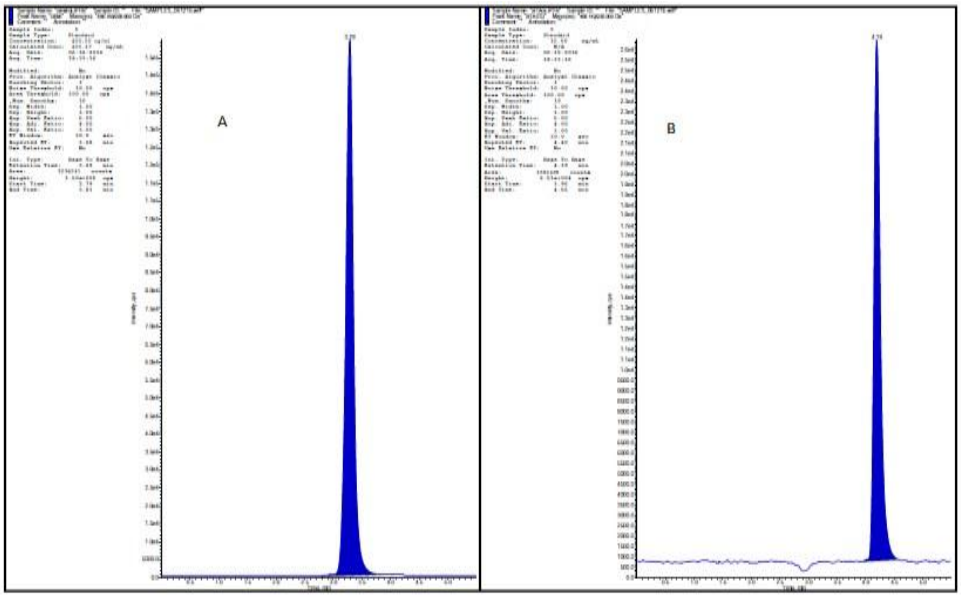

Figure 9: Representative of chromatograms of STD 5

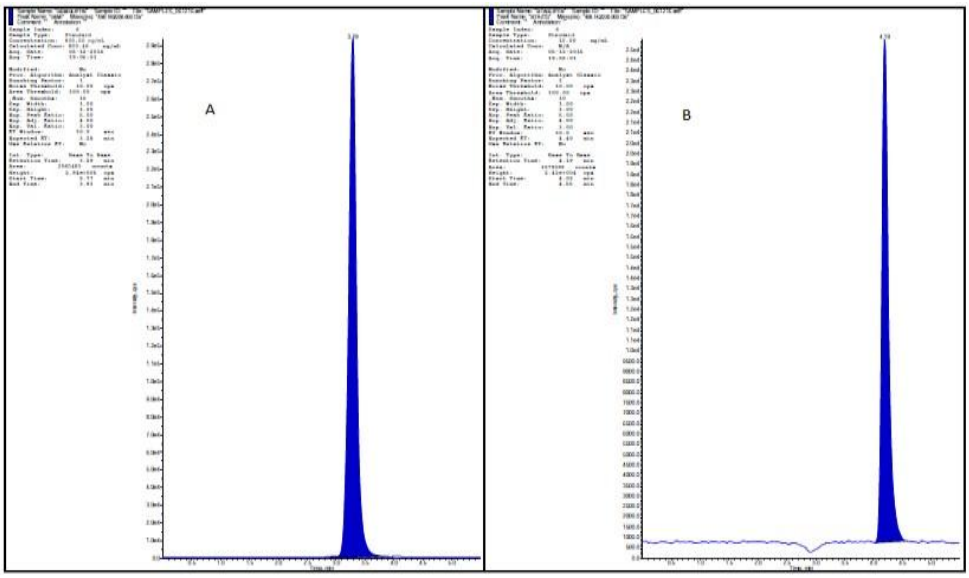

Figure 10: Representative of chromatograms of STD 6

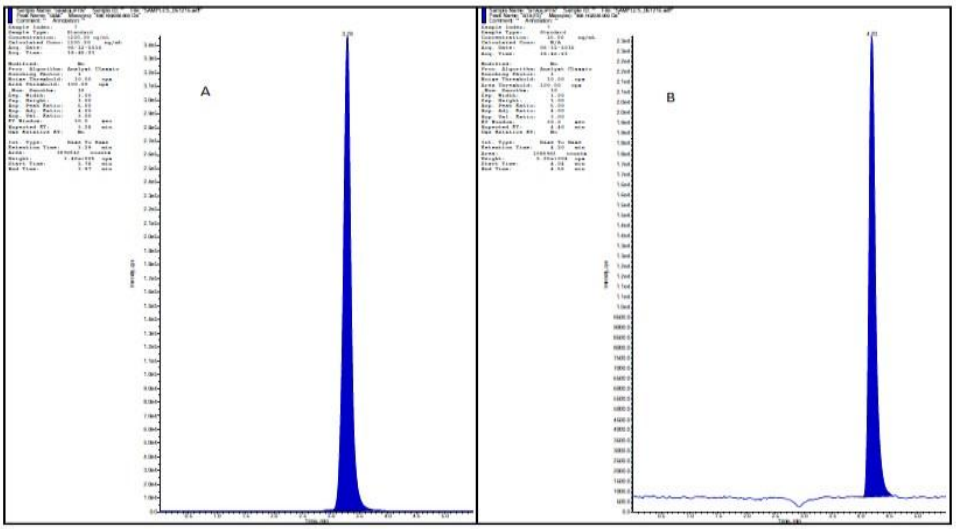

Figure 11: Representative of chromatograms of STD 7

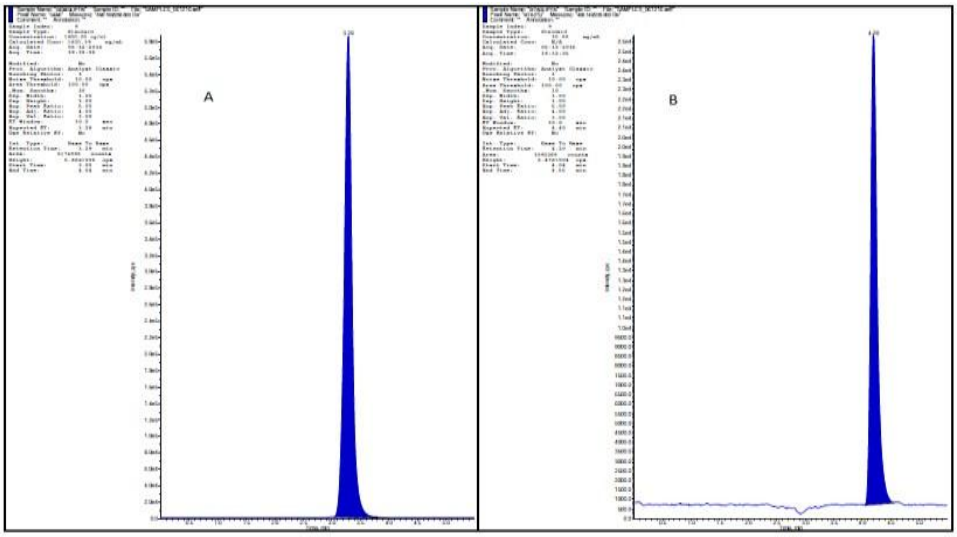

Figure 12: Representative of chromatograms of STD 8

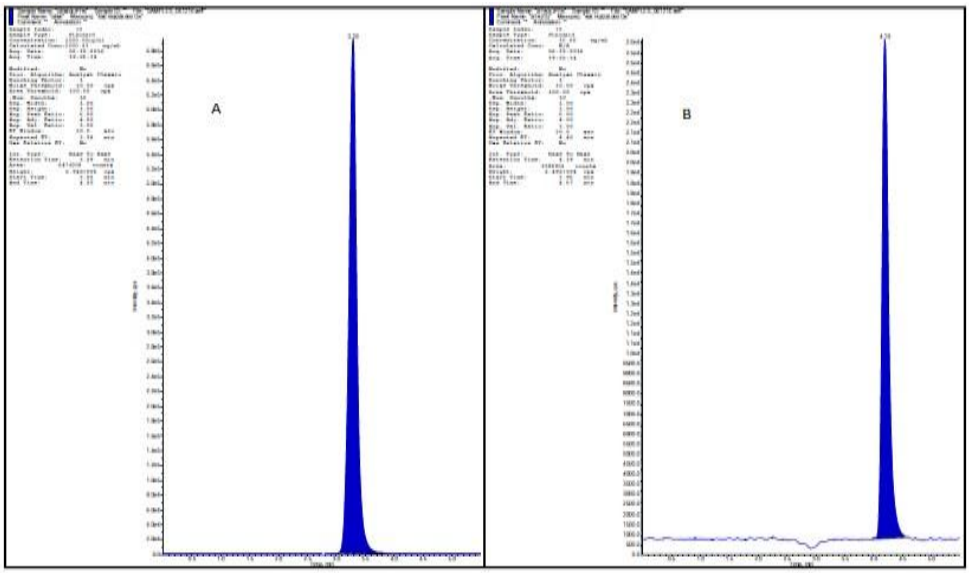

Figure 13: Representative of chromatograms of STD 9

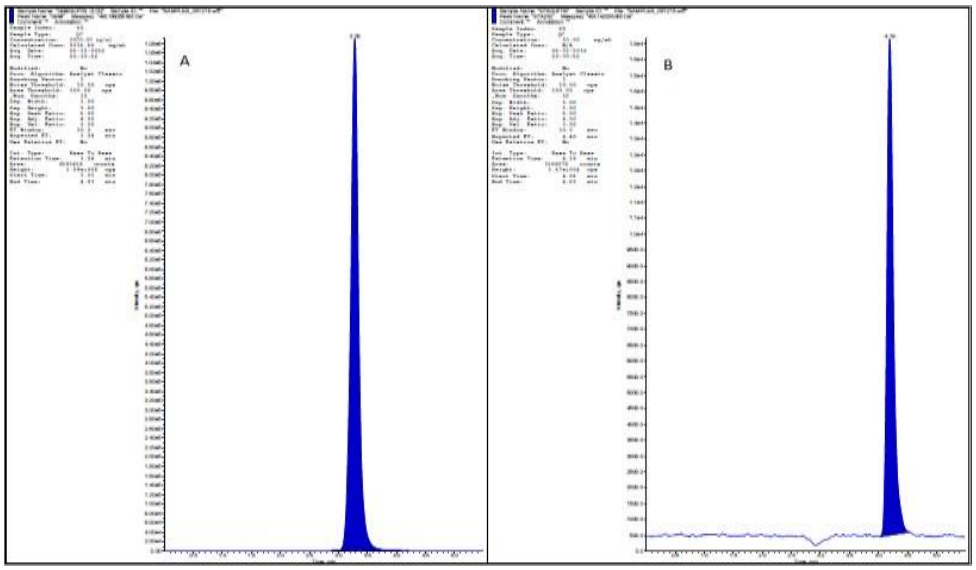

Figure 14: Representative of chromatograms of LLOQ

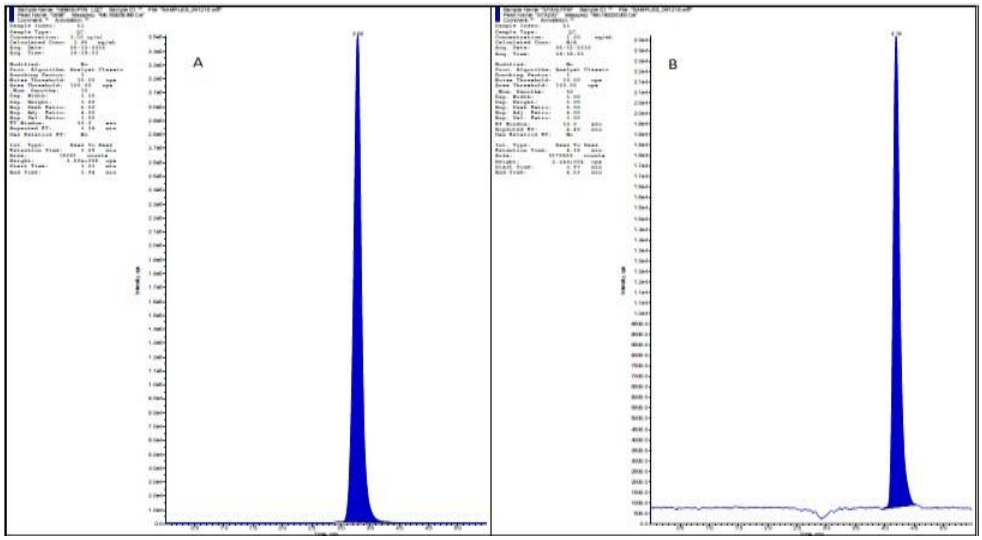

Figure 15: Representative of chromatograms of LQC

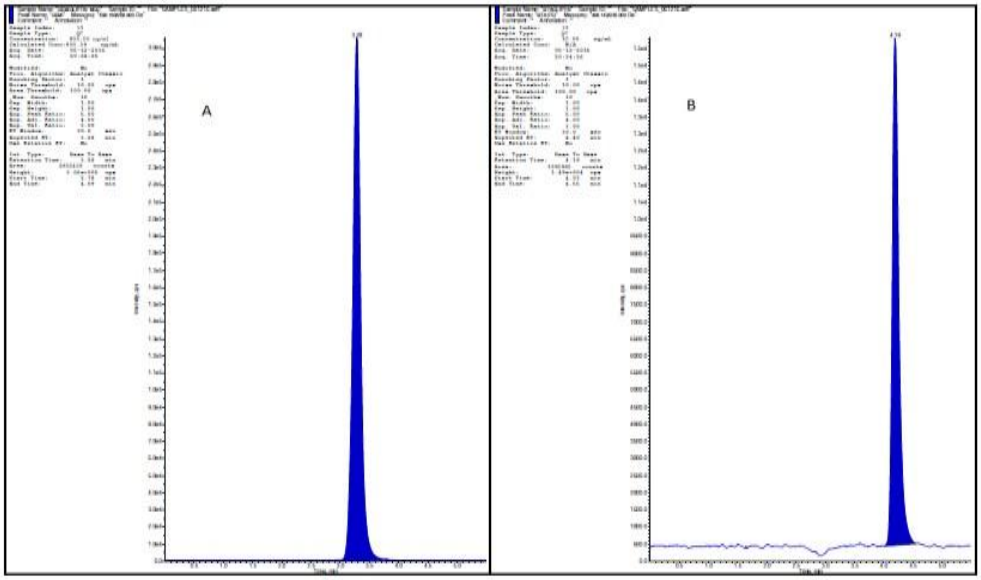

Figure 16: Representative of chromatograms of MQC

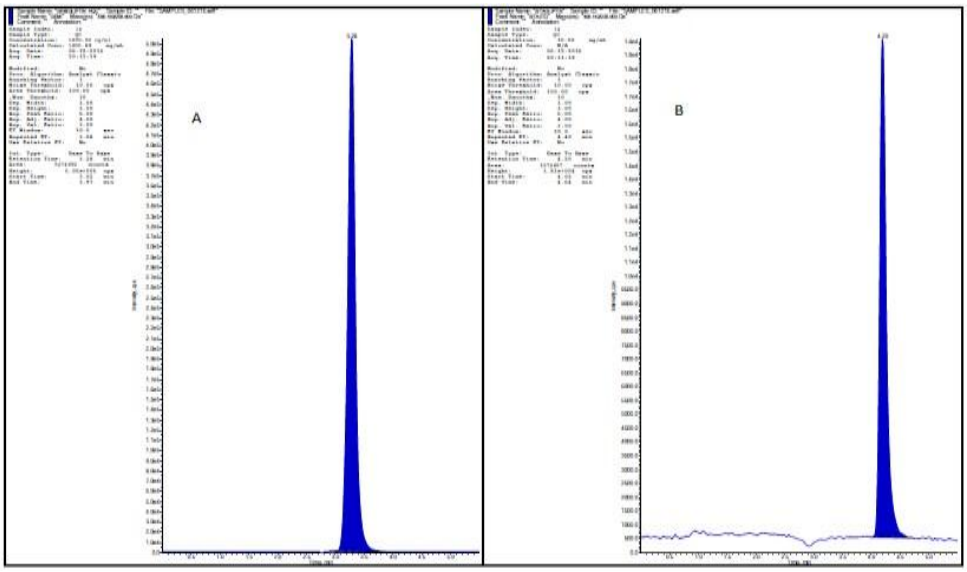

Figure 17: Representative of chromatograms of HQC

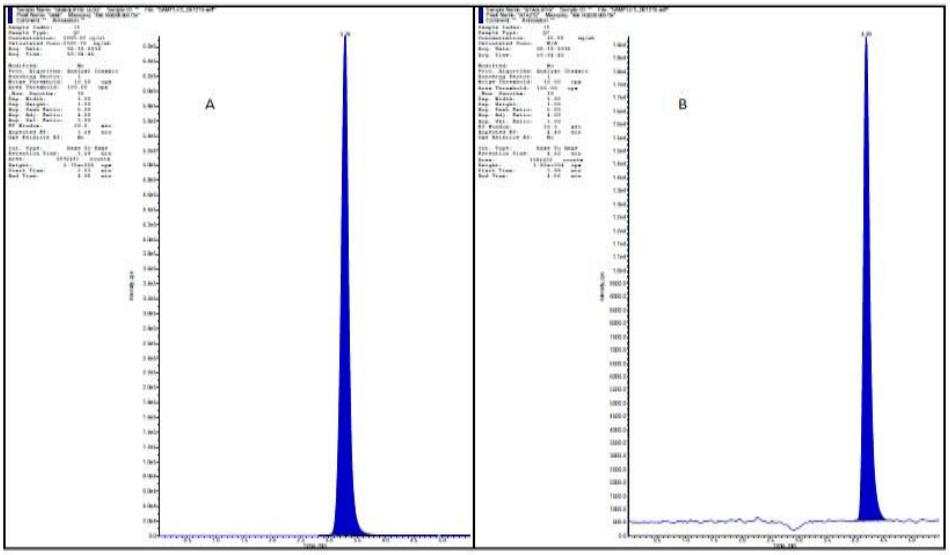

Figure 18: Representative of chromatograms of ULOQ

4 CONCLUSION

The proposed method of LC-MS has proved to be simple, sensitive, accurate, precise and reliable. The method is specific due to the selectivity of the mass spectrometry. The method offers high selectivity with a LOQ of 1 ng/mL. The validated method can be successfully used to a clinical and toxicity studies. Use of Sitagliptin as an ISTD will not compromise the accuracy of analytical results. The high throughput method can reduces overall processing time. So the proposed study is proved to apply this method for the estimation of Gemigliptin in human plasma.

5 ACKNOWLEDGEMENTS

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