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EVALUATION OF EFFICACY OF TENELIGLIPTIN BY QUANTIFICATION WITH ACTIVE GLUCAGON LIKE PEPTIDE-1 (GLP-1) FROM INDIAN HUMAN PLASMA USING NOVEL BIO-ANALYTICAL METHOD IN LC-ESI-MS/MS

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ABSTRACT

Teneligliptin is an inhibitor of DPP-4 enzyme by which treated hyperglycemic patient. The main target of this proteonomic study was to evaluation of the efficacy of teneligliptin on inhibition of DPP-4 enzyme. In previous literature several studies depended on quantifying the concentration of DPP-4 enzyme by ELISA method. But in this study inhibition of DPP-4 enzyme by teneligliptin was evaluated by quantification of the active concentration of Glucagon like peptide-1(GLP-1) by a novel validated LC-MS/MS QTRAP (API-4000) method. Active GLP-1 is an endogenous compound, so before administration of reference preparation, endogenous compound active GLP-1 concentration in human plasma was 31±11 fg/ml and in the case of test preparation 42±11 fg/ml. After treating reference preparation the maximum plasma concentration of active GLP-1 was 330 ± 28.0 fg/ml (Cmax) at 1.05 ±0.18 hr (tmax) and after administration of test product in the fasting state teneligliptin tablet IP 10mg the maximum plasma concentration of active GLP-1 was 141.00 ± 11.00 fg/ml (Cmax) at 1.01 ± 0.00 hr (tmax) then decrease its concentration and again increase it to 71.00 ± 5.10 fg/ml at (Cmax)14.01 ± 0.00hr (tmax) after administration of a second dose of teneligliptin tablet IP 10mg after 12hrs of first dosing time of

administration. This method is highly selective, sensitive, specific, low ion suppressive, highly recovered, validated according to US-FDA and EMA guidelines, and successfully used for quantifying pharmacokinetic parameters of active concentration of peptide and teneligliptin from Indian healthy human plasma.

KEYWORDS: Active-GLP-1 assay, Teneligliptin, Efficacy study, Bioequivalence study, LC-MS/MS QTRAP.

Abbreviations

DPP-4: Dipeptidyl peptidase-4; GLP-1: Glucagon like peptide-1; USFDA: United States Food and Drug Administration; CDSCO: Central Drugs Standard Control Organization; LLOQ: Lower limit of quantification; LQC: Low quality control; MQC: Middle quality control; HQC: High quality control; EMA: European Medicine Agency; API: Active Pharmaceutical Ingredient; LOD: Limit of Detection; ESI: Electro spray ionization; ME: Matrix Effect; SPE: Solid phase extraction, ACN : Acetonitrile, MeOH: Methanol.

1. INTRODUCTION

Gliptin class of anti-diabetic drugs like teneligliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor.[1,11,12,13] An incretin hormone called glucagon-like peptide-1 (GLP-1) is a 37-amino-acid peptide that is released by the Langerhans cells in the intestine in response to meals.[2,16,17] This GLP-1 has insulinotropic action and helps to uptake of glucose by cells and reduces the glucose concentration in the plasma by stimulating insulin release and inhibiting glucagon release from pancreatic cells.[3, 18, 19, 37, and 38] **[Figure: 1].** Glucagon like peptide- 1 is a very poorly active drug containing 1-37 amino acids, however after cleavage division endogenously between the 6th and 7th position, it produces a more biologically active peptide containing 7-37 hydroxyl group peptide.[4,20,38] But 7-36 amide containing GLP-1 is a natural compound in which glycine at the C-terminal has been replaced with an amide group and analogue to GLP-1.[5] GLP-1 has two biological forms: active and inactive.[6, 21, 22, 23, 39, 40] The active form contains 7-36 amino acids and dissolves in an aqueous solution at P^H7.4, but inactive states contain 9-36 amino acids and are insoluble in an aqueous solution at PH7.4and have no insulinotropic activity.[7, 24, 25, 27] After eating, the dipeptidyl peptidase-4 (DPP- 4) enzyme quickly cleaves the two amide groups of the terminal amino acids of active glucagon- like peptide-1, turning it from active to inactive GLP-1.[8,28,29,30,40] Glucagon like peptide-1is present in deficient concentrations in human plasma.[9, 31-33] Teneligliptin inhibits the enzyme DPP-4 and increases the concentration of active GLP-1 and decreases the

concentration of 9-36 containing amino acid, as a result, increases secretion of insulin and reduce the plasma glucose concentration.[10,34-36]**[Figure: 2]**

This study's primary goal was to assess teneligliptin's effectiveness by measuring active GLP-1(7-35) from human plasma after the anti-diabetic medication had been administered. The literature review revealed that different articles were published based on the pharmacokinetic characteristics of teneligliptin, one of which was Mandal.P. et al. However, this is a novel GLP-1technique to assess the effectiveness of teneligliptin in Indian human plasma.

2. Experimental

2.1.Chemicals and reagents

ACN, MeOHwere used for HPLC and LC-MS/MS grade respectively and formic acid and ammonium acetate were used as an AR grade. A protease inhibitor cocktail (100X) was used during blood collection time. HPLC Grade Milli-Q water was applied for analysis.

2.2. Preparation of calibration and quality control sample concentrations

2.2.1. Standard concentration and quality control sample preparation of active GLP-1.

The prepared stock solution concentration of GLP-1 was 200µg/ml in DMSO solution. The calibration concentrations were 15.63, 31.25, 62.5, 125, 250, and 500, 1000 fg/ml were prepared by dilution with a mixture of acetonitrile and water at 1:1 and by spiking into human plasma. Thequality control samples concentration were 15.63, 46.88, 375 and 750fg/ml for LLOQ, LQC, MQC and HQC respectively, prepared from the intermediate concentration of GLP-1 and by spiking into human plasma. The plasma was extracted by the solid phase extraction method.

2.2.2. Standard concentration and quality control sample preparation of teneligliptin

The intermediate concentrations of teneligliptin were prepared from a 1mg/ml stock solution. From the intermediate concentrations working concentrations of teneligliptin were prepared and 500µl of higher concentration was spiked into 500µl of blood plasma. After that all standard concentrations were prepared by serial dilution. Plasma extracted by protein precipitation technique. The usable standard concentrations were 15.63, 31.25, 62.5, 125, 250, 500 and 1000ng /ml and LLOQ, LQC, MQC and HQC concentrations were 15.63, 46.88, 375 and 750 ng/ml.

2.3. Liquid chromatography quadruple tandem mass spectrometry (LC-MS/MS API-4000 QTRAP) analysis

2.3.1. LC-MS/MS of active GLP-1analysis

In order to quantify the 36 amino acids that make up active glucagon like peptide-1, which were ionised in LC-MS/MS API-4000 QTRAP, Phenomenex Kinetex 5 C18 100A 50*3mm column was employed. 0.1% formic acid in Milli Q water with 10mM ammonium acetate as an aqueous solvent and 0.1% formic acid in ACN as an organic solvent were the mobile phases utilised for this ionisation. For enhancing the ionisation of active glucagon amino acids like peptide-1, the concentrations of mobile phases were utilised in a gradient fashion.In the gradient curve organic solvent of 15% was used for 0.01min to 0.50min and gradually increased to 45% from 0.50minto 3.00min after that increased this concentration to 60% and ran from 3.00min to 5.00min then again increased this concentration was to 95% from 5.00min to 7.50min. From this timing gradually decrease the concentration of organic solvent (increasing concentration of aqueous solvent) to 60% up to 8.5min and again decrease to 45% up to 9.5 min then reduce to 15% upto 10min run time for washing purposes.**[Figure: 3].** Mass instrumental optimisation parameters forGLP-1 are illustrated in **[Table-1].**

2.3.2. LC-MS/MS of Teneligliptin

Teneligliptin was quantified by LC-MS/MS API-4000 QTRAP for the evaluation of efficacy, and a PhenomenexKinetex 5 C18 100A 50*3mm column was employed to elute teneligliptin from the analytical material. The aqueous solvent used was 0.1% formic acid in milli Q water with 10mM ammonium acetate, and the organic solvent utilised was 0.1% formic acid in methanol. Teneligliptin was ionised and then graded for analysis. In this case, 10% organic solvent was ran from 0.01 to 1.50 minutes, increased to 90% from 1.50 to 4.00 minutes, and then lowered to 10% from 4.00 minutes. 7.00 minutes after the runtime has ended for washing reasons. **[Figure: 4].** Mass instrumental optimisation parameters for teneligliptin are illustrated in **[Table-2].**

2.4. Plasma sample preparation

2.4.1. Blood collection procedure

The protease inhibitor powder was dissolved in 1ml deionised water into the bottle to make the 100 times concentrated stock solution and diluted with buffer (0.2M Phosphate buffer solution p^H 7.5) to make 100ml of 1X (1:100) protease inhibitor solution. Then 10µl of protease inhibitor solution was used in 2ml of freshly collected plasma sample and

immediately mixed with handshaking and stored this blood sample in -20ºc.

2.4.2. Active GLP-1 plasma sample preparation by solid phase extraction

Solid phase extraction cartridges were used for extraction of analyte from plasma by solid phase extraction technique. 500µl human plasma was collected in a blood collection tube containing protease inhibitor cocktail (100X) with the internal standard, was acidified with 10 µl of 50% acetic acid then rotated or vortexed for 10min. After vortexingalkalise this solution by using 500 µl of 10% ammonium hydroxide solution and vortexed again for 10min. Then this 1000 µl solution was applied to solid phase extraction.

After pretreatment using dilute sample (1:3 v/v) containing 2% formic acid of cartridge for eliminating protein binding, then 1000 µl of prepared plasma sample was loaded in it as hydrophobic and ionic interactions retained a resulting analyte. Then it was washed firstly with 10% ammonium hydrate for elution interference, then again washed in a second time with 15% acetonitrile to overcome the interference of elution. Finally, glucagon like peptide-1 was eluted with 400 µl of elution buffer (acetonitrile: water: acetic acid 55:15:30), which was diluted with 100 µl of 70% acetic acid. A 10µl aliquot of the 300µl of elute was then injected into the chromatographic system using an autosampler vial.

2.4.2. Teneligliptin plasma sample preparation by protein precipitation technique

Protein precipitation technique was used to extract the plasma; 100 µl of plasma was obtained, precipitated with 400 µl of acetonitrile containing 1 g/ml of metoprolol (IS), and vortexed for 10 min. 300 µl of the plasma was then centrifuged for 10 min. at 10,000 rpm at -20^{0}C. The supernatant was taken, diluted ten times, and then put into vials for autosampler injection.

2.5. Method validation[14,15]

The bio-analytical method of active GLP-1 and teneligliptin was validated as per US-FDA and EMA guidelines.

2.5.1. Method validation of active GLP-1

Active glucagon-like peptide-1 was analysed by using standard calibration concentration 15.63to 1000fg/ml in which LLOQ, LQC, MQC and HQC values were present. Active GLP-1 was quantified in this assay by using the linear regression formula Y= m X+ c where Y was the ratio of analyte peak area and internal standard peak area and X was the percentage of

analyte concentration and standard internal concentration, m was the slope and c was the intersect and also used weighing factor 1/X*X. According to US-FDA guidelines, three-day linearity freshly prepared samples were quantified for accuracy, precision and linear calibration curve. The stability of the active GLP-1 plasma sample was validated by analysis of freeze-thaw, bench top, auto-sampler, and short term and long-term stability samples. Suppression of ionisation of active GLP-1 in mass spectrometry was validated by matrix effect, and recovery samples validated extraction of active GLP-1 from plasma and selection of method.

2.5.2. Method validation of teneligliptin

Teneligliptin was analysed by using standard calibration concentrations of 15.63, to 1000 ng/ml in which LLOQ, LQC, MQC and HQC values were present. Teneligliptin was quantified in this assay by using the linear regression formula Y= m X+ c where Y was the ratio of analyte peak area and internal standard peak area and X was the ratio of analyte concentration and internal standard concentration, m was the slope and c was the intersect and also used weighing factor 1/X*X. According to US-FDA guidelines, three-day linearity freshly prepared samples were quantified for accuracy, precision and linear calibration curve. The stability of the teneligliptin plasma sample was validated by analysis of freeze thaw, bench top, auto-sampler, short term and long term stability samples. Suppression of ionisation of teneligliptin in mass spectrometry was validated by matrix effect, and recovery samples validated extraction of teneligliptin from plasma and selection of method.

2.6. Ethical guideline

This study was carried out according to clinical research guidelines for medical research involving human subjects [59th WMA General Assembly, Seoul, October 2008] and as per ICMR and Indian GCP guidelines.

This quantification of active GLP-1 for evaluation of the efficacy of teneligliptin study protocol and informed consent form, case record form and subject information sheet was submitted to the HURIP Independent Bio-ethics committee, Kolkata, India. CDSCO registration: ECR/103/Indt/WB/2013/RR-19 valid up to 21-Nov.-2024.

2.7. Efficacy study by comparative pharmacokinetic and pharmacodynamic parameters of Teneligliptin and assay of active GLP-1

This study, which involved 24 healthy male volunteers, compared teneligliptin hydrobromide

hydrate IP equivalent to teneligliptin 10mg (twice daily) film-coated tablets with once-daily Eternex-T tablets, each of which contains teneligliptin IP 20mg. It was a randomised open-label, oral, multiple-dose steady state research with two treatments and two time points.

For DPP-4 analysis, pre-dose blood samples were collected an hour prior to the dose, and post- dose blood samples were collected at 0, 0.5, 1, 5, 6, 8, 10, 12, 14, 16, 18, and 24 hours. Days three through seven saw the collection of pre-dose blood samples, which included 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 20.0, 24.0, and 48.0, and 72.0. hours for post-dosing samples.

3. RESULT AND DISCUSSION

3.1. Bio-analytical method development of active GLP-1 and Teneligliptin

3.1.1. Method development of active GLP-1

GLP-1 is a peptide consisting of 36 or 37 amino acids derived from proglucagon secreted from intestinal Langerhans cells. This peptide is truncated terminally at N-position, resulting in 7-36 or 7-37 amino acids containing GLP-1 and can be amidated. Active glucagon like peptide-1 was ionised in positive mode in Triple Quadrupole LC/MS/MS Mass Spectrometer API 4000 (Q- Trap). The parent ion $[M+H]^+$of active GLP-1 was m/z 825.6 **[His-Ala- Glu- Gly- Thr- Phe- Thr-]** and the fragmented ion or product ion was m/z 643.4 **[His-Ala- Glu- Gly- Thr-]** at 200msec by using declustering potential 40V and collision energy 50V, collision cell exit potential 15V. This method was developed in gradient technique with flow 0.5ml/min. **[Figure-5(a,b)]**

3.1.2. Method development of teneligliptin[27]

Teneligliptin was ionised in positive mode in Triple Quadrupole LC/MS/MS Mass Spectrometer API 4000 (Q-Trap). The parent ion $[M+H]^+$ of active teneligliptin was m/z 427.3 and the fragmented ion or product ion was m/z 243.2 at 200msec using declustering potential 90V and collision energy 39V, collision cell exit potential 15V. This method was developed in gradient technique with flow 0.5ml/min. **[Figure: 6]**

3.2. Method validation of active GLP-1

The percentage of nominal concentrations of calibration standard was 92.88% to 105.46% where the LLOQ and LOD were 15.63 and 1.10ng/ml respectively and the regression value was 0.9740. Some stability parameters result like freeze thaw stability, short term, long term, bench top and autosampler stability were 92.11 to 107.82%, 92.34 to 101.56%, 92.03 to

104.35%, 91.43 to 104.12% and 90.88 to 107.50% respectively and matrix factor of analyte and IS were 0.86 to 0.89 and 0.90 to 0.92 respectively. The recovery result of analyte and IS were 94.12 to 97.14% and 90.32 to 96.00%, respectively.

3.3 Method validation of Teneligliptin

The percentage of nominal concentrations of calibration standard was 90.65% to 101.35% where the LLOQ and LOD were 15.63 and 0.73ng/ml respectively and the regression value was 0.9980. Some stability parameters result like freeze thaw stability, short term, long term, bench top and autosampler stability were 91.01 to 97.12%, 90.14 to 91.26%, 93.23 to 94.35%, 93.43 to 94.22% and 92.68 to 97.35% respectively and matrix factor of analyte and IS were 0.89 to 0.90 and 0.92 to 0.95 respectively. The recovery result of the analyte and IS were 92.02 to 95.44% and 92.02 to95.03% respectively.

3.4. Pharmacokinetic study of Teneligliptin

The maximum plasma concentration of 288.88±22.09 ng/ml (Cmax) was achieved at the time of 1.05±0.16 hr following administration of the reference preparation using the ETERNEX®-T tablet (each tablet contains teneligliptin IP 20mg) as a multiple-dose (once daily) in the fasting state (tmax). While administering the test preparation, teneligliptin tablet IP 10mg (each film- coated tablet contains teneligliptin hydrobromide hydrate IP Eq. to teneligliptin 10mg), as a multiple-dose (twice daily) in the fasted state, produced the maximum plasma concentration 198.18±15.08 ng /ml (Cmax) at the time 1.01±0.00 hr (Tmax). The area under the plasma concentration time curve (AUC_{0-t}) after administering the reference preparation was 2773.89±188.55 ng.hr/ml. The area under the plasma concentration curve (AUC_{0-t}) for the test preparation administration was 2672.63±213.61ng.hr/ml. The reference preparation produced an area under the plasma concentration time curve up to infinity ($AUC_{0-\alpha}$) of 2725.77 ±210.38 ng.hr./ml when given in several doses while the subject was fasting. In comparison, administering the test preparation resulted through an $AUC_{0-\alpha}$ of 2716.13 ±225.16 ng.hr/ml (area under the plasma concentration time curve up to infinity). Plasma elimination half-life ($T_{1/2}$) afteradministration of the reference preparation was 11.53 ±0.51 hours, whereas $T_{1/2}$ after administration of the test preparation was 10.76 ±0.75 hours. Plasma elimination constant (Kel) after administration of the reference preparation was 0.077 ±0.002 hr^{-1}, but after administration of the test preparation, it was 0.058 ±0.005 hr^{-1}. **[Figure: 7, 8],[Table: 3]**

3.5. Assay of active GLP-1

From the assay values of active GLP-1 in human plasma, it was observed that after administration of the reference product ETERNEX®-T tablet (each tablet contains teneligliptin IP 20mg) in thehigher concentration in this plasma of active GLP-1 was 0.000331 ± 0.000028 ng/ml or 331 ± 28.0fg/ml (Cmax) at 1.05±0.18hr (tmax). After administration of the test product in the fasting state, teneligliptin tablet IP 10mg (each film-coatedtabletcontainsteneligliptin hydrobromide hydrate IP Eq. to teneligliptin 10mg) the maximum plasma concentration of activeGLP-1 was 0.000141 ± 0.000011 ng/ml or 141.00 ± 11.00fg/ml (Cmax) at 1.01 ± 0.00hr (tmax) then decrease its concentration and again increase it to 0.000071 ± 0.0000051or 71.00 ± 5.10 fg/ml at (Cmax)14.00 ± 0.00 hr (tmax) after administration of a second dose of teneligliptin tablet IP 10mg (each film-coatedtabletcontainsteneligliptin hydrobromide hydrate IP Eq. to teneligliptin 10mg) after 12hrs of first dosing time of administration. Where, before administration of reference preparation, endogenous compound active GLP-1 concentration in human plasma was 0.000031±0.000011ng/ml and CV% 37.64 and in the case of test preparation 0.000042±0.000011 ng/ml and CV % 18.62. **[Figure: 9, 10], [Table: 4]**

3.6. Efficacy study of Teneligliptin

Glucagon was found to be derived from proglucagon, a precursor to glucagon, and eliminated from the endocrine cells of the mucous cell of intestine when food was present in the intestine. This active form of glucagon like peptide-1, known as active GLP-1(7-36 amide), acts on the GLP-1 receptor (G-protein coupled receptor), causing endocrine cells to release insulin. After entering the bloodstream, the enzyme DPP-4 acts on the active GLP-1 and two of its amino- terminal amino acids, cleaving and converting them into the inactive GLP-1 (9-36 amide), which inhibits the release of insulin from the endocrine cells in the pancreas, intestine, and other endocrine organs. Teneligliptin is one of the DPP-4 enzyme inhibitors which after administration into the body, inhibits the DPP-4 enzyme from converting inactive GLP-1 from active GLP-1. So, active GLP-1 concentration increases in the blood after administration of the DPP-4 inhibitordrug teneligliptin. From the study it was observed that after administration of reference preparation of teneligliptin 20mg percentage of DPP-4 inhibition was nearby 100% but after administration of teneligliptin 10mg (twice on 24hr.) at first nearby 70% inhibition then greater than 100% inhibition observed on DPP-4 enzyme after administration of second dose (10mg) of 12hrs. **[Figure: 11]**

The formula for calculation of DPP-4 inhibition

% DPP-IV Inhibition= 100 x (1-D0/Dt)

Where D0 = was the DPP-4 activity measured predose (i,e the concentration. of active GLP-1pre-dose)

Dt = was the DPP-4 activity measured at time‘t’ (i,e the concentration of active GLP-1 post-dose)

3.7. Steady state concentrations of Teneligliptin

Pre dose samples (24hrs. – Pre-Dose Day 2, 48hrs. – Pre-Dose Day 3, 72hrs. – Pre-Dose Day 4, 96hrs. – Pre-Dose Day 5, 120hrs – Pre-Dose Day 6, 144hrs – Pre-Dose Day 7) were used for quantification of steady state concentration of teneligliptin by validated LC-MS/MS method. From the quantification report of pre dose samples it was observed that after administration of reference preparation of teneligliptin 20mg at 24hr, 48hr, 72hr, 96hr, 120hr, 144hr the concentrations of teneligliptin were 0.44,17.65,22.99, 17.60, 21.64, 38.25ng/ml respectively that is 19.76 ± 2.27 ng/ml in an average steady state concentration after 144hrs. But after administration of test preparation of teneligliptin 10mg on twice a day at 24hr, 48hr, 72hr, 96hr, 120hr, 144hr the concentrations of teneligliptin were 5.96, 35.56, 32.46, 37.02, 35.89, 39.91 ng/ml respectively that is 31.13 ± 3.75 ng/ml in an average steady state concentration after 144hrs**. [Figure: 12]**

Figures

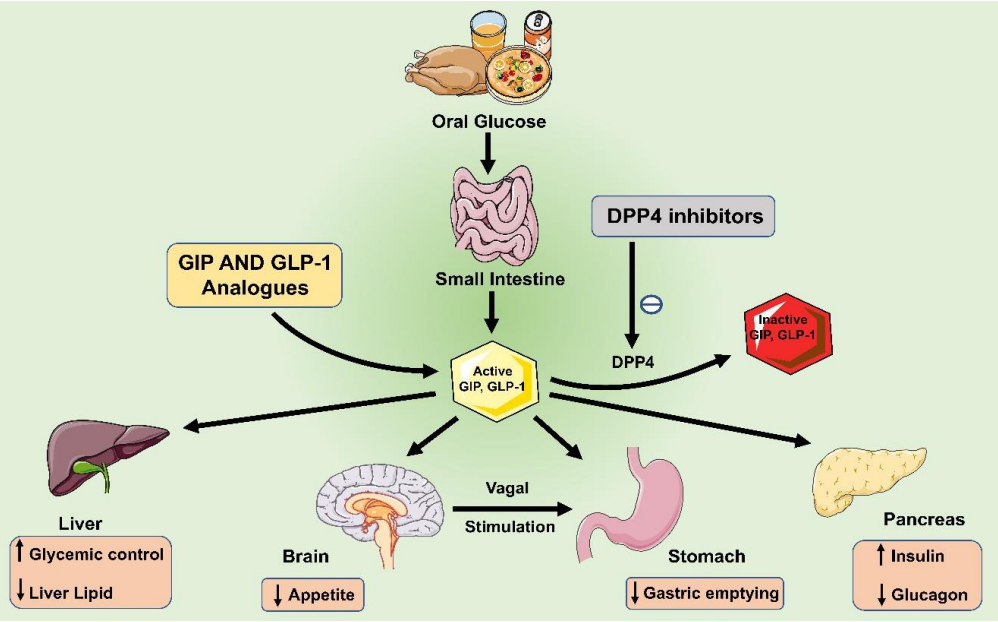

Figure 1: Mechanism of action of Active GLP-1.

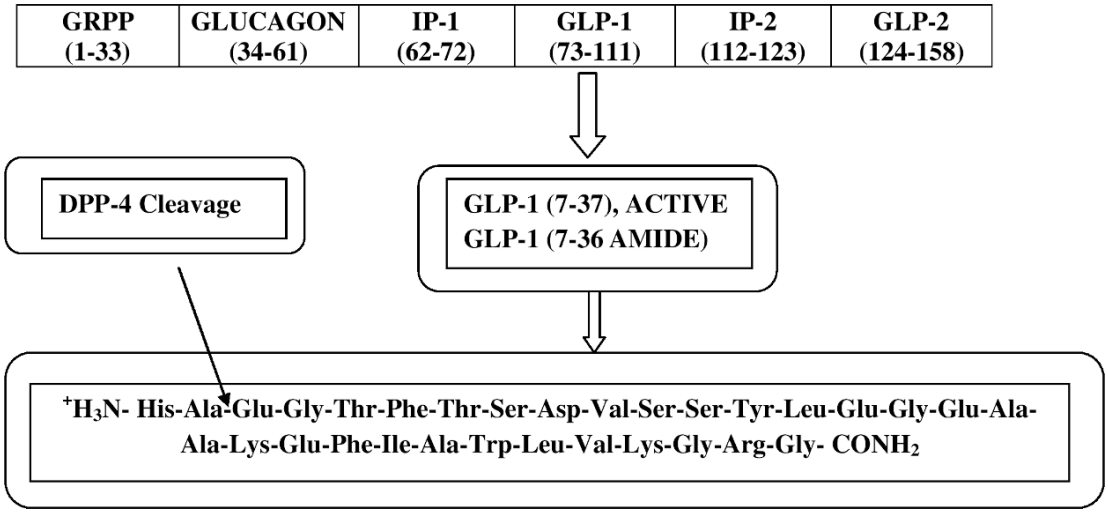

Figure 2: Amino acid sequance of GLP-1.

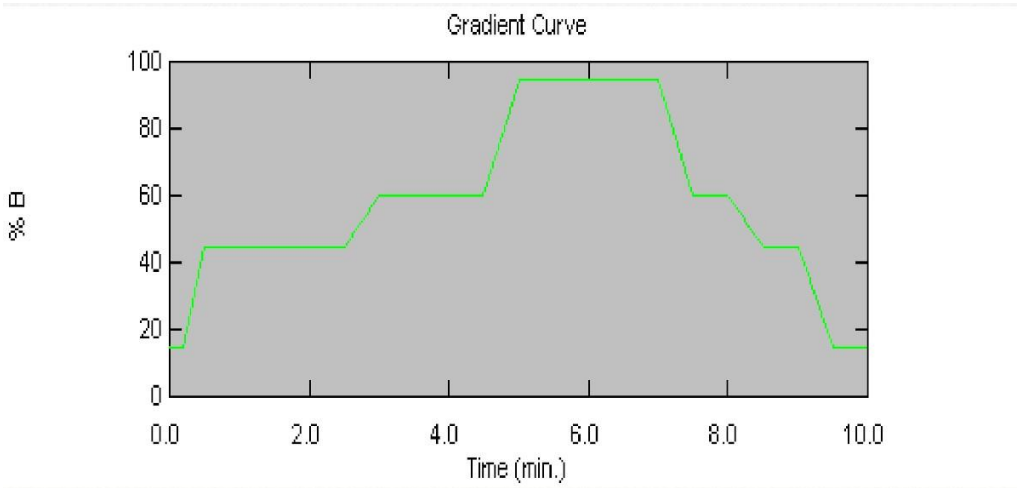

Figure-3 Gradient curve of Active GLP-1 method.

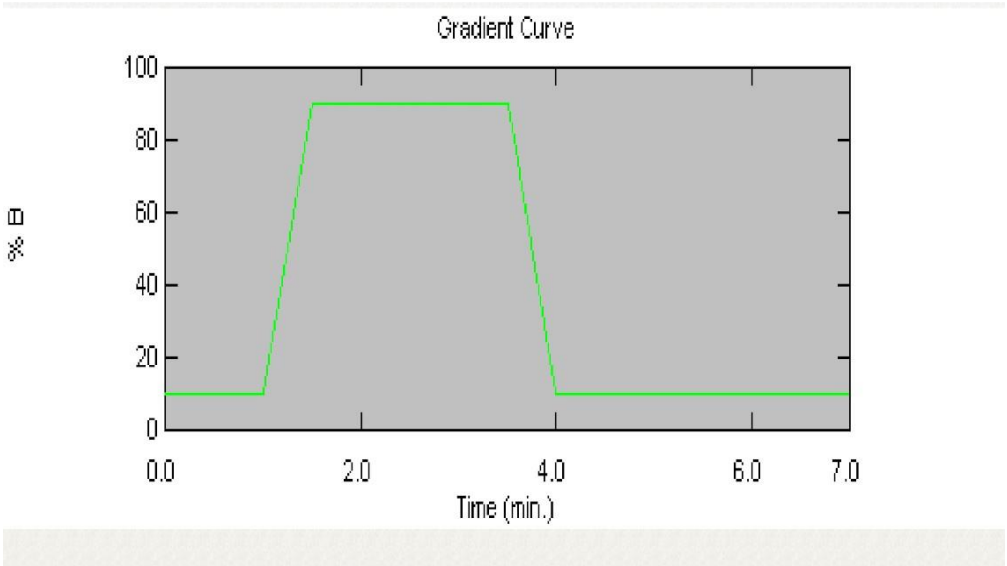

Figure 4: Gradient curve of method development of Teneligliptin.

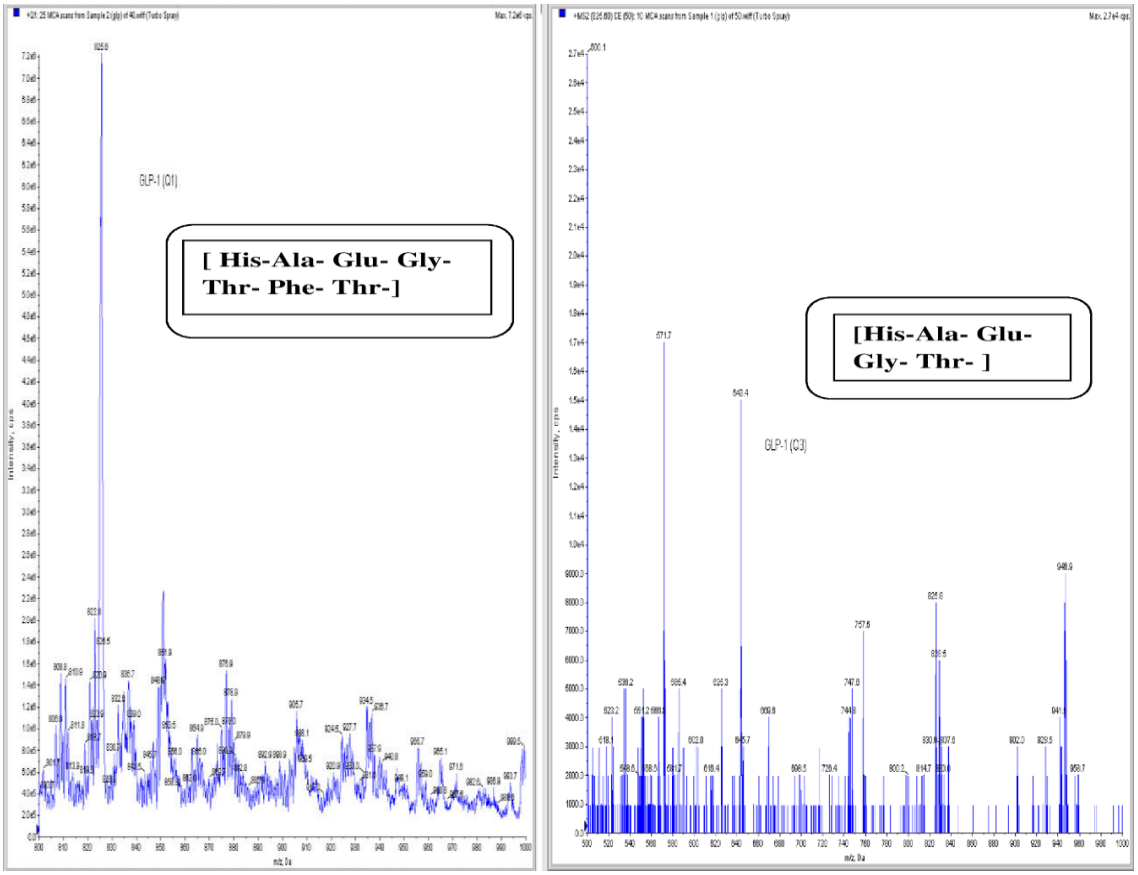

Figure 5a Parent ion and product ion of Active GLP-1.

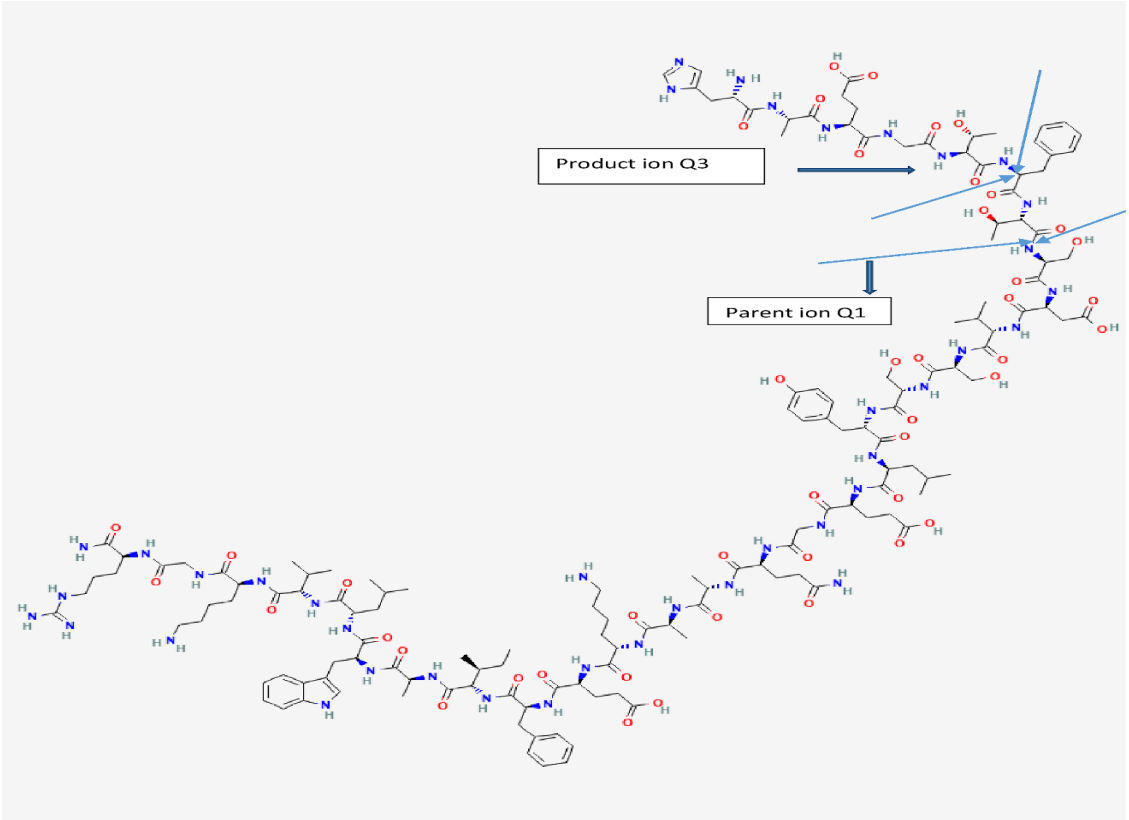

Figure: 5b Parent ion (Q1) and Product ion (Q3) of active GLP-1.

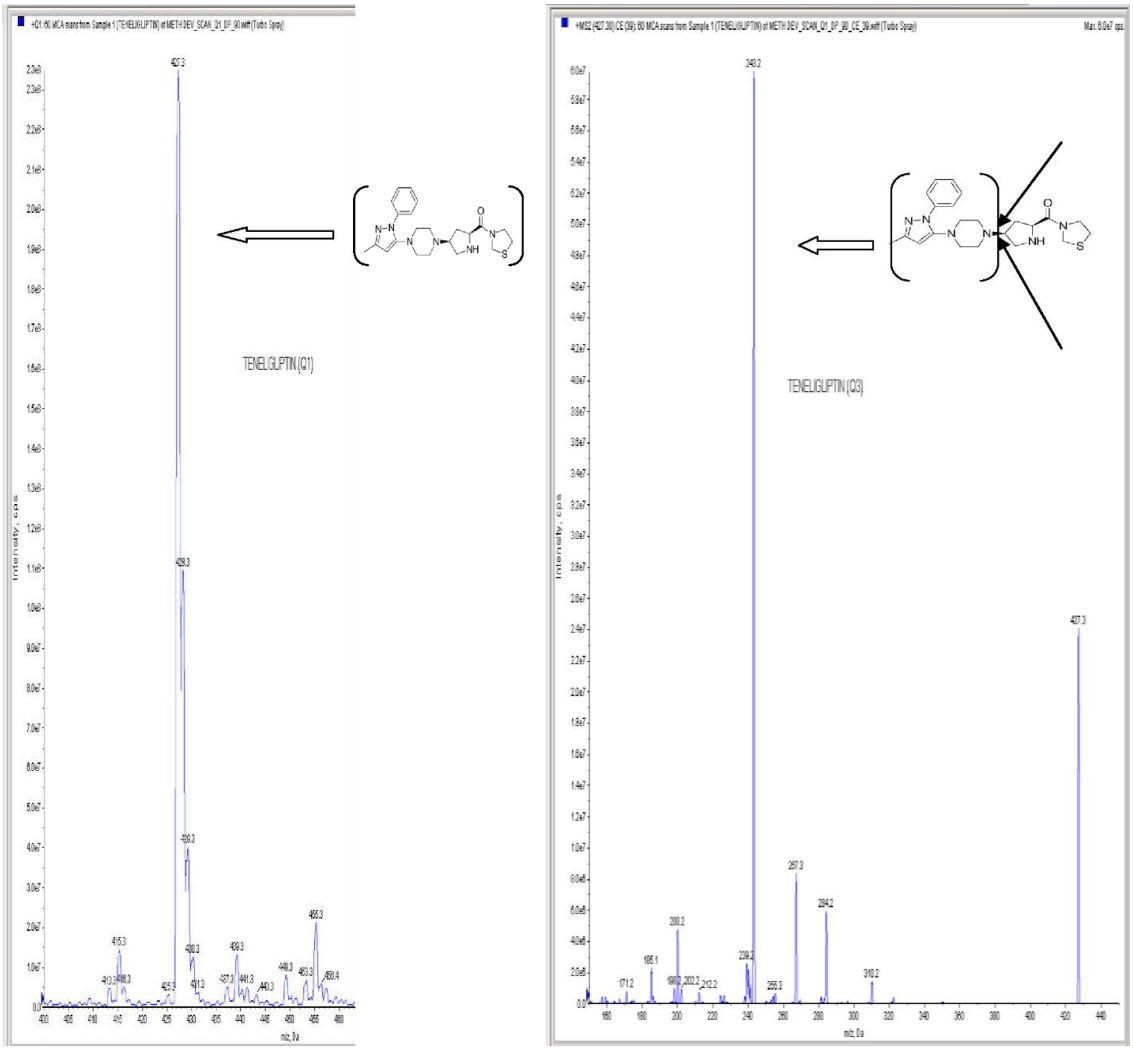

Figure 6 Parent ion and product ion of Teneligliptin.

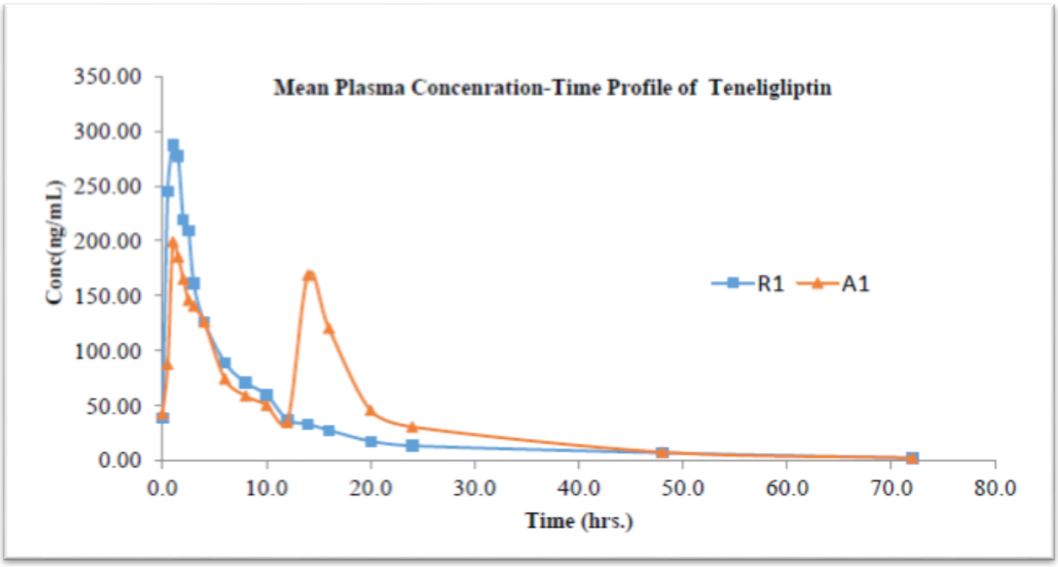

Figure 7: Mean plasma concetration- time profile of Teneligliptin where R1: Reference preparation, A1: Test preparation.

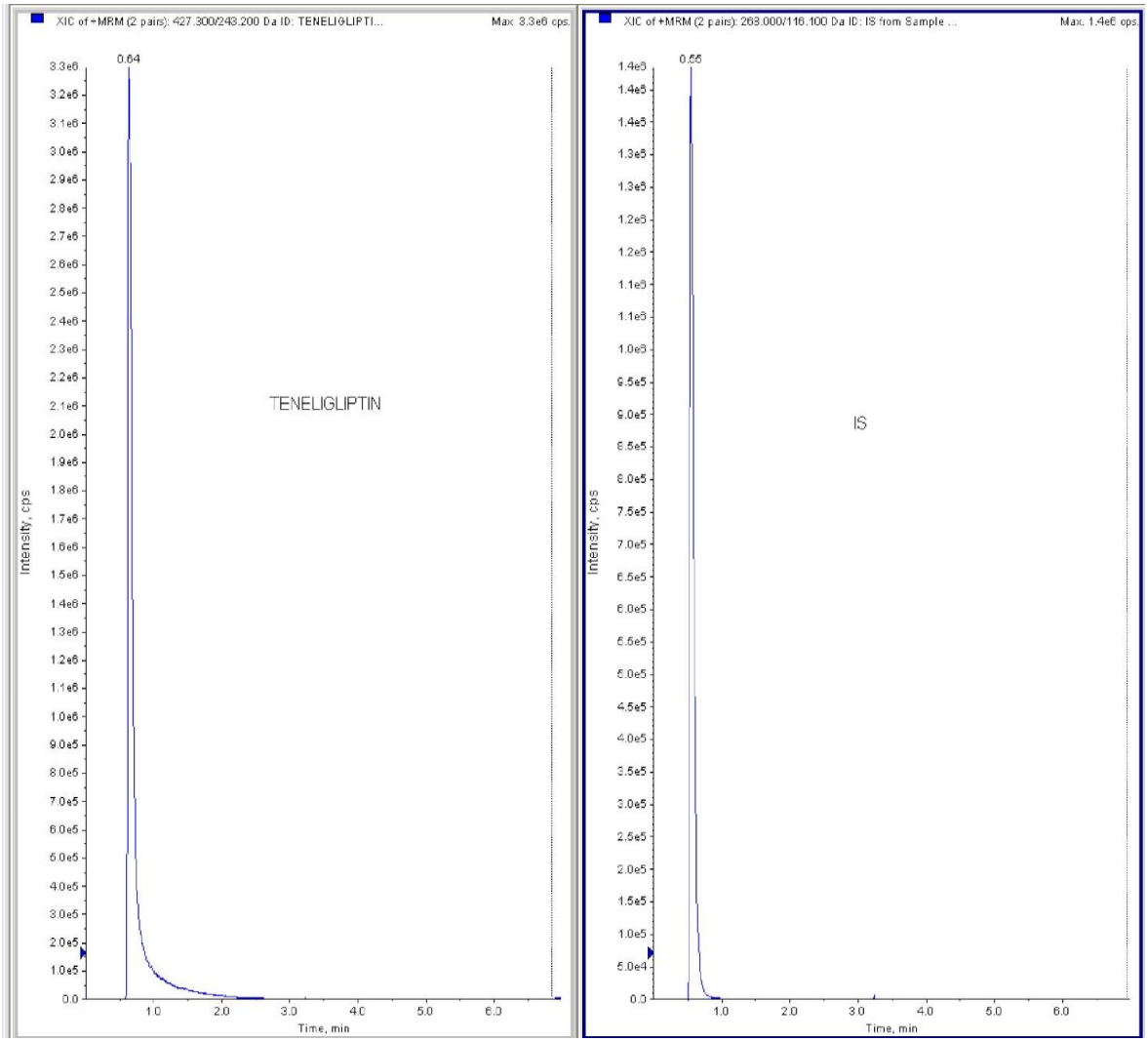

Figure 8 MRM Chromatogram of Teneligliptin.

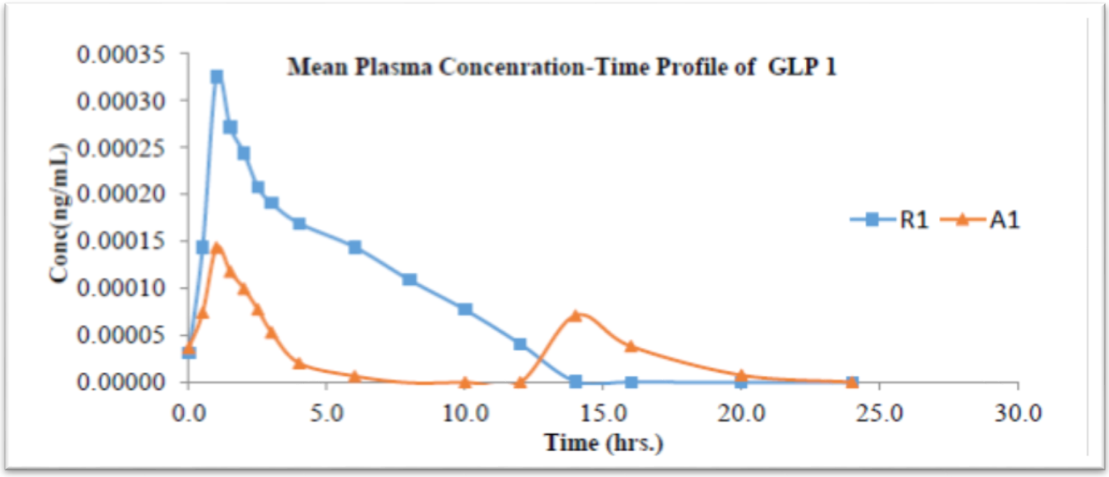

Figure 9: Mean plasma concetration time profile of active GLP-1 where R1: Reference preparation, A1: Test preparation.

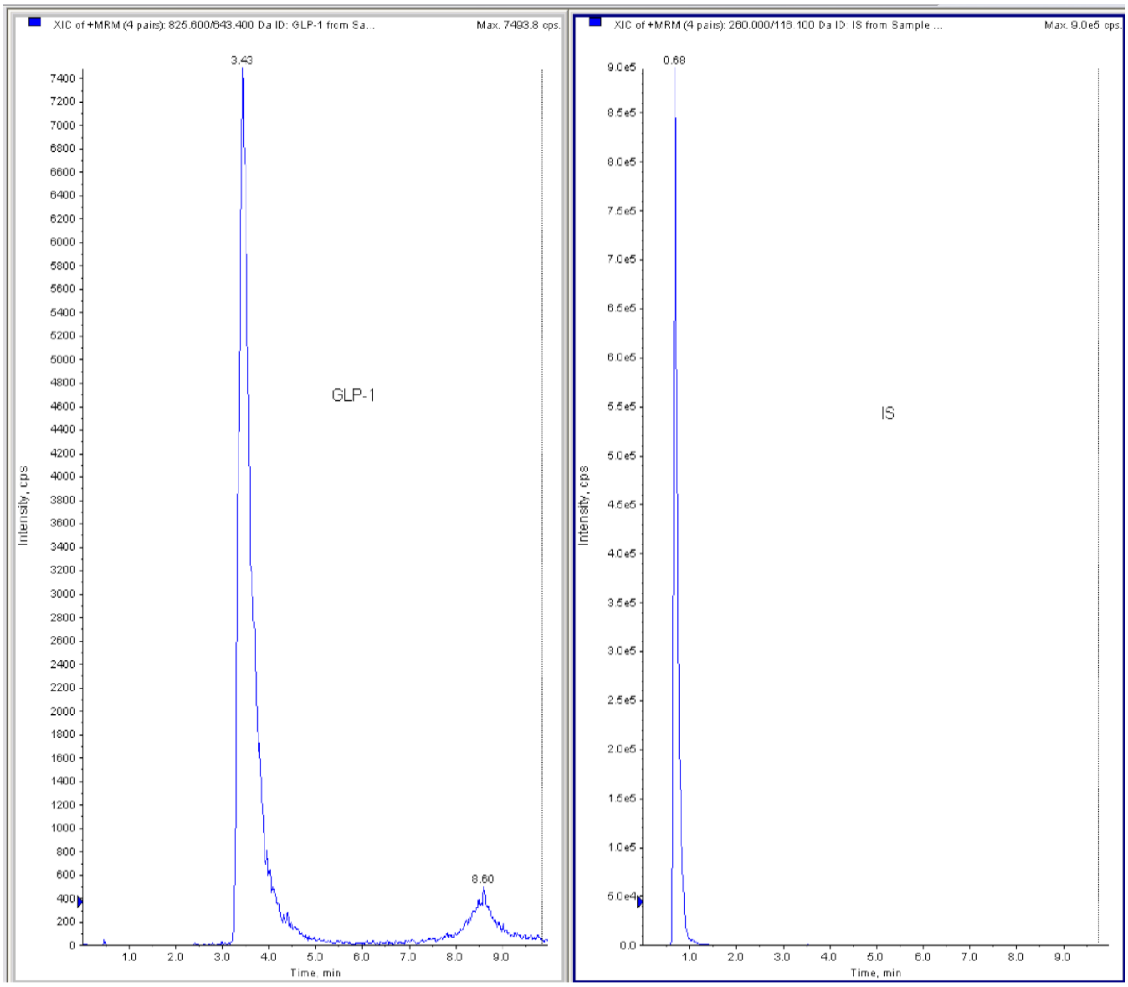

Figure 10: MRM Chromatogram of Active GLP-1.

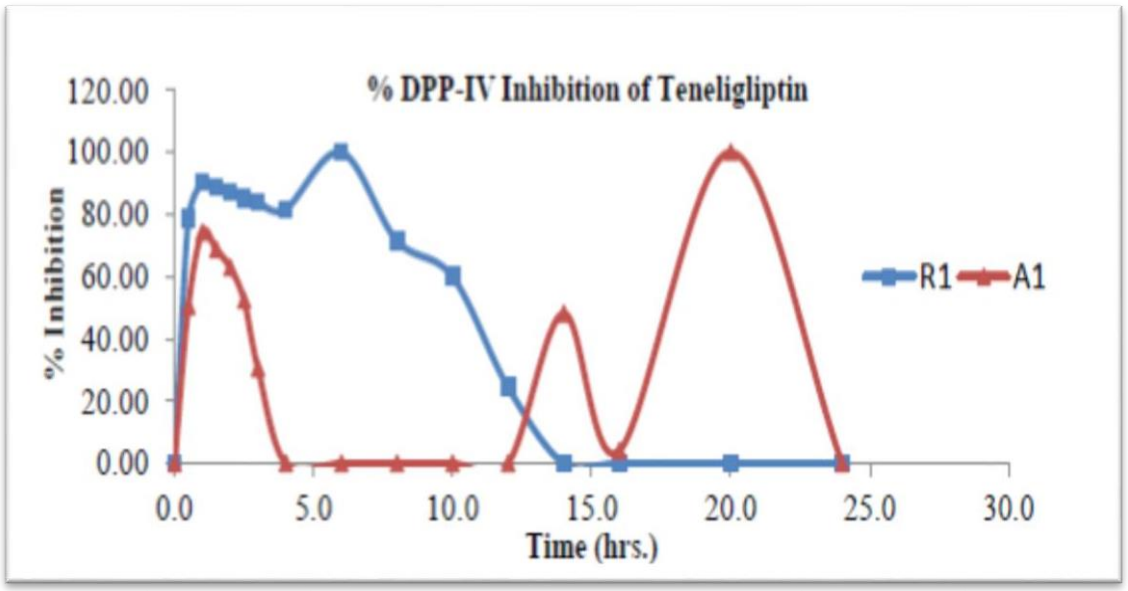

Fig 11: Percentage inhibition of DPP-4 where R1: Reference preparation, A1: Test preparation.

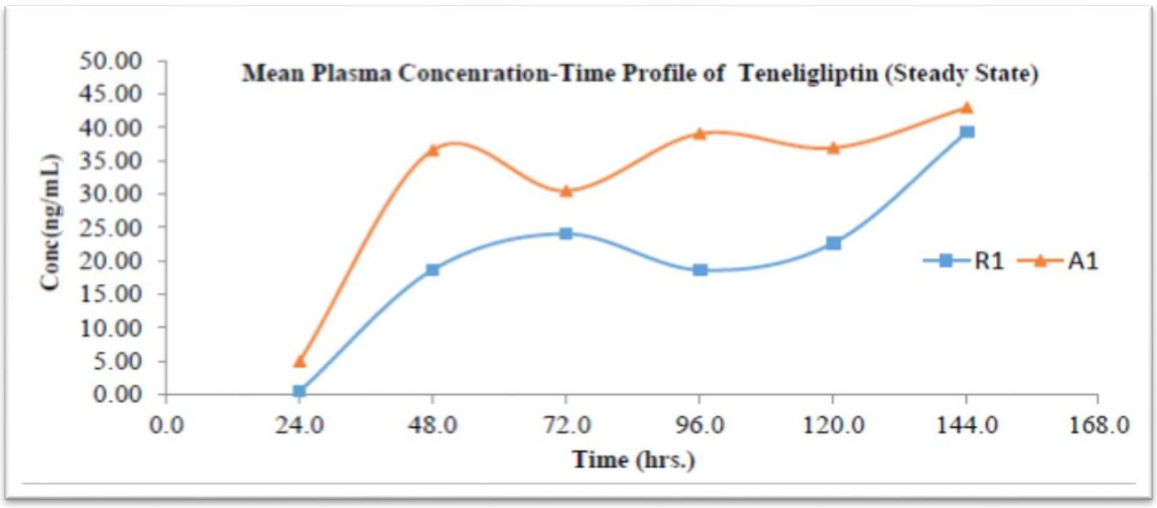

Figure 12: Mean plasma concentration time profile of Teneligliptin in steady state whereR1: Reference preparation, A1: Test preparation.

Table-1: Optimized instrumental (mass) parameters for analyte and IS.

Parameter(s)	Value
Ionization mode	MRM (+ve)
Source temperature (ºC)	400
Dwell time per transition (msec)	100
Curtain gas (psi)	20
CAD gas (psi)	3
Ion spray voltage (V)	5500.00
Ion source gas 1 (psi)	55
Ion source gas 2 (psi)	45
Focussing potential (V)	400
Declustering potential (V)	40 (GLP) and 20 (IS)
Entrance potential (V)	10
Collision energy (V)	50 (GLP) and 26 (IS)
Collision cell exit potential (V)	15 (analytes and IS)
Transition pair of GLP (analyte)	825.6/643.4
Transition pair of Propranolol (IS)	260.0/116.1

Table-2: Optimized instrumental (mass) parameters for analyte and IS.

Parameter(s)	Value
Ionization mode	MRM (+ve)
Source temperature (ºC)	400
Dwell time per transition (msec)	100
Curtain gas (psi)	20
CAD gas (psi)	3
Ion spray voltage (V)	5500.00
Ion source gas 1 (psi)	55
Ion source gas 2 (psi)	45
Focussing potential (V)	400
Declustering potential (V)	90 (Teneligliptin) and 20 (IS)

Entrance potential (V)	10
Collision energy (V)	39 (Teneligliptin) and 31(IS)
Collision cell exit potential (V)	15 (analytes and IS)
pair of Teneligliptin(analyte)	427.3/243.2
Transition pair of Metoprolol (IS)	268.0/116.1

Table- 3: pharmacokinetic parameters in 24 volunteers with the reference and test preparation of Teneligliptin.

Pharmacokinetic parameters	**Reference Preparation (R1)**		**Test Preparation (A1)**	
Cmax (ng./ml.)	**Mean**	288.88	**Mean**	198.18
	± S.D.	22.09	**± S.D.**	15.08
Tmax (hr.)	**Mean**	1.05	**Mean**	1.01
	± S.D.	0.16	**± S.D.**	0.00
AUC 0-t (ng. hr./ml.)	**Mean**	2773.89	**Mean**	2672.63
	± S.D.	188.55	**± S.D.**	213.61
AUC 0-□ (ng. hr. /ml.)	**Mean**	2725.77	**Mean**	2716.13
	± S.D.	210.38	**± S.D.**	225.16
kel (hr.$^{-1}$)	**Mean**	0.077	**Mean**	0.058
	± S.D.	0.002	**± S.D.**	0.005
T1/2 (hr.)	**Mean**	11.53	**Mean**	10.76
	± S.D.	0.51	**± S.D.**	0.75
Relative Bioavailability (%)	100 %		**103.78%**	
Log Transformed Relative Bioavailability	100%		**93.31%**	

Table- 4: pharmacokinetic parameters in 24 volunteers with the reference and test preparation of Active GLP-1.

Pharmacokinetic parameters	**Reference Preparation (R1)**		**Test Preparation (A1)**	
Cmax (fg./ml.)	**Mean**	331.00	**Mean**	141.00 & 71.00
	± S.D.	28.00	**± S.D.**	11.00 &5.10
Tmax (hr.)	**Mean**	1.05	**Mean**	1.01 & 14.00
	± S.D.	0.18	**± S.D.**	0.00

4.0. DISCUSSION

From my knowledge and my point of viewed, after previous literature studing it was observed that all study related to GLP were on its mechanism and on its clinical study and also related to genomic study. But this study totally difference from the previous study because in this study depends on efficacy of teneligliptin which is a DPP-4 inhibitor. In previous study on efficacy of teneligliptin was quantified DPP-4 enzyme concentration in plasma or serum by ELISA method.But in this efficacy study of teneligliptin, active GLP-1

concentrations was quantified in human plasma before and after administration of teneligliptin of multiple dose by using validated LC- MS/MS method and then from that inhibition of DPP-4 enzyme by teneligliptin was calculated and also quantified steady state concentrations of teneligliptin after administration of multiple dose of teneligliptin by using USFDA validated LC-MS/MS method. The multiple dose bioequivalence study of teneligliptin tablet IP 10mg (each film coated tablet contains teneligliptin hydrobromide hydrate IP Eq. to teneligliptin 10mg) was conducted in 24 healthy adult, human, male volunteers with two preparations of teneligliptin. Teneligliptin was detected in plasma from 0.0 to 72.0 hrs. for both the preparations (Day 7 pre dose & post dose). Peak plasma levels of teneligliptin were achieved between 1.0 to 1.5 hrs. for both preparations (Day 7 post-dose). On the study day, the mean peak plasma levels of teneligliptin with reference preparation, ETERNEX®-T tablet ranged between 243.64 – 344.54 ng/ml (Day 7 post-dose). While the test preparation, teneligliptin tablet IP 10mg, ranged between 168.28 – 242.39 ng/ml (Day 7 post-dose). Based on comparison of the AUC0-t for teneligliptin after multiple dose administration, the relative bioavailability & log-transformed relative bioavailability of the test preparation, teneligliptin tablet IP 10mg, were 103.78% & 93.31%, respectively with that of the reference preparation, ETERNEX®-T tablet. The study observed that the active GLP-1 maximum concentration was 331 ± 28.0 fg/ml at 1.05 ±0.18 hr in the case of reference preparation and at this time, DPP-4 enzyme inhibition was nearly 100%. In the case of test preparation active GLP-1 maximum concentration was 141.00 ± 11.00 fg/ml (Cmax) at 1.01 ± 0.00 hr then decreased its concentration and again increased it to 71.00 ± 5.10 fg/ml at (Cmax) 14.01 ± 0.00 hr (tmax) and at this time DPP-4 enzyme inhibition was nearly 71% inhibition then greater than 100% inhibition of DPP-4 enzyme after administration of second dose (10mg) of 12hrs.

5.0. CONCLUSION

Typically Glucagon like peptide-1 are present in the human body but it has two parts: one is active and another is inactive. When food in the stomach or in the intestine, a powerful regulator of glucose homeostasis, glucagon-like peptide-1 is produced by endocrine cells and increases the glucose-dependent insulin secretion. But the DPP-4 enzyme cleaved this active Glucagon like peptide-1 to inactive Glucagon like peptide-1. This study concluded that after administration of the teneligliptin, active GLP-1 concentration increases with the concentration of teneligliptin in human plasma at the same Tmax. Again it was observed that in the case of the test sample active GLP-1 concentration increases with the repeated dose

interval. So. It was concluded that teneligliptin inhibits the DPP-4 enzyme and the percent of inhibition is nearly 100% (shown in the result). As a result, the plasma concentration of active glucagon like peptide -1 was at the same Tmax and maintained steady-state concentrations of teneligliptin in human plasma up to 144hr after treating test and reference preparation. So it was proved that teneligliptin is a DPP-4 inhibitor and inhibits the DPP-4 enzyme nearby 100%. Based on the pharmacokinetic parameters studied, it can be concluded that the test preparation, teneligliptin tablet IP 10mg (each film-coated tablet contains teneligliptin hydrobromide hydrate IP Eq. to teneligliptin 10mg), was bioequivalent with the reference preparation, ETERNEX®-T pill (each tablet contains teneligliptin IP 20mg), and test preparation was highly efficacious than reference preparation in case of inhibition of DPP-4 enzyme.

6.0. Safety Assessment

At each check-in and check-out, a clinical check up and physical parameters measures were taken. Additionally, before administering investigational products, vital signs were taken. Data on QT prolongation were obtained by ECG (during the study period). Studies on liver damage were conducted before and after teneligliptin treatment. Each individual underwent a thorough physical examination as well as hematopoietic, hepatic, and renal function testing in the lab. No negative effects were observed during the entire investigation.

Conflict of interest

The authors declare that there is no conflict of interest.

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Author contribution

S.K. and P.M. conceived the idea; P.M. operated LC-MS/MS and collected relevant data; M.A.S.; R.B; S.C; and A.S. prepared the manuscript; P.M. completed the format and

proofreading of the manuscript.

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