METHOD DEVELOPMENT AND VALIDATION OF AZELNIDIPINE AND TELMISARTAN IN HUMANPLASMA USING RP-HPLC

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Abstract

A simple, Accurate, precise method was developed for the simultaneous estimation ofAzelnidipine and Telmisartan in human plasma was developed and validated. By usingCentrifugation, the sample preparation was prepared. Chromatogram was run through StdInertsil C18 (150 x 4.8 mm, 5m) Mobile phase containing BufferDiSodiumHydrogenPhosphate: Acetonitrile taken in the ratio 65:35 was pumped throughcolumn at a flow rate of 1.0ml/min. Buffer used in this method wasDiSodiumHydrogenPhosphate buffer. For the separation of Azelnidipine and Telmisartan, Internal Standard [IS] used is Saxagliptin. The Temperature was maintained at 30°C.Optimized wavelength selected was 228nm. Retention time of Azelnidipine and Telmisartanand Internal Standard were found to be 2.139 min and 2.422 min and 3.025 The standardcurve was linear (R2 >0.995) over the concentration range of 6.0-240 ng/ml of telmisartan &0.45-18 ng/ml. All the analytical validation parameters were determined as per ICHguidelines The bioanalytical method developed approach was selective, robust, and reliable,as accuracy, precision, recovery, and other validation parameters were all within therecommendations' limitations. The peaks produced for the drug of interest and the internalstandard was well separated from one another without any plasma interferences, and thepeaks weresymmetrical with an adequate tailing factor. The method has the potential to bevery beneficial in therapeutic drug monitoring (TDM), bioequivalence research, pharmacokinetics studies, toxicology, and biomedical investigations.

Key words: - Azelnidipine and Telmisartan, Internal Standard, Rp HPLC, Bioanalysis, Human plasma.

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INTRODUCTION:

Bioanalytical techniques, employed for the quantitative determination of drugs and theirmetabolites in biological fluids and creates a specific procedure to enable a coalesce ofinterest to be identified and at the same time to be quantified in a matrix. A coalesce ismeasured by several procedures. The choice of analytical procedures involves manyconsiderations, such as: concentration levels, chemical properties of the analyte, specimenmatrix, cost of the analysis, experimental speed, quantitative or qualitative measurement,required precision and necessary equipment.Bioanalytical method validation comprises allcriteria determining data quality, such as selectivity, accuracy, precision, recovery, sensitivity, and stability.

DRUG ANALYSIS IN VARIOUS BIOLOGICAL MEDIA

Blood, urine, and faeces are the most acquired samples for biopharmaceuticalanalysis, especially if the drug or metabolite is poorly absorbed or substantially eliminated inthe bile.Saliva, breath, and tissue are examples of other media that can be used. The nature ofthe investigation heavily influences the selection of sampling media. In a clinicalpharmacokinetic investigation, for example, medication levels necessitate the use of blood,urine, and saliva. A bioavailability study may necessitate drug level data in blood and/orurine, but a drug identification or drug addiction concern may only necessitate one type ofbiological sample.The nature of the drug investigation heavily influences the selection of sample media. In aclinical pharmacokinetic study, for example, medication levels necessitate the use of blood,urine, and perhaps saliva. Bioavailability research may necessitate medication levelmeasurements in blood or urine. The steps involved in estimating medicines in biologicalfluid are sample collection, sample treatment, separation of the compound of interest from the matrix, and analysis.Bioanalysis can determine the therapeutic efficacy of a specific medicine. Bioanalysis isimportant in the pharmaceutical industry. The following steps are involved in bioanalysis.

- Biological fluid selection and collection
- Sample preparation -Analyte extraction from biological matrix.
- Analyte detection is accomplished through a variety of approaches.

The desired analyte should be extracted from the biological fluid after it has been selected.This phase in the bioanalytical approach is more crucial since sample preparation can be doneusing several extraction methods. The preparation of the sample takes time and should bedone carefully due to its importance. If the biological matrix is liquid, such as blood, plasma,or urine, liquid-liquid extraction is employed; if it is solid, liquid-solid extraction is utilized.

The following are the most well-known and widely utilized extraction methods

1. Protein precipitation method.
2. Liquid-liquid extraction method. (LLE)
3. Solid-phase extraction method. (SPE)

DRUGS ESTIMATION IN BIOLOGICAL SAMPLES USING HPLC

Due to the various advantages such as speed, specificity, consistency, accuracy, precision,and ease of automation in these methods, most of the drugs in multicomponent dosage formcan be analysed by the HPLC system. The HPLC approach avoids repetitive processes forextraction and isolation. In HPLC, there are distinct modes of differentiation. They areNormal Phase Mode, Inverted Phase Mode, Chromatography of Reversed Phase Ion Phase,Chromatography of Affinity and Chromatography of Size Exclusion.The quality of a drug plays an important role in ensuring the safety and efficacy of the drugs.Quality assurance and control of pharmaceutical and chemical formulations is essential forensuring the availability of safe and effective drug formulations to consumers. HenceAnalysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotalrole in assessing the suitability to use in patients. The quality of the analytical data dependson the quality of the methods employed in generation of the data (1) . Hence, development ofrugged and robust analytical methods is very important for statutory certification of drugs andtheir formulations with the regulatory authorities.The wide variety of challenges is encountered while developing the methods for differentdrugs depending on its nature and properties. This along with the importance of achieving theselectivity, speed, cost, simplicity, sensitivity, reproducibility, and accuracy of results gives anopportunity for researchers to come out with solution to address the challenges in getting thenew methods of analysis to be adopted by the pharmaceutical industry and chemicallaboratories. Different physio-chemical methods (1) are used to study the physicalphenomenon that occurs as a result of chemical reactions. Among the physio-chemicalmethods, the most important are optical

(refractometry, polarimetry, emission and fluorescence methods of analysis), photometry (photocolorimetry and spectrophotometry covering UV-Visible, IR Spectroscopy and nephelo turbidimetry) and chromatographic (column, paper, thin layer, gas liquid and high-performance liquid chromatography) methods. Methods such as nuclear magnetic resonance (NMR) and para magnetic resonance (PMR) arebecoming more and more popular. The combination of mass spectroscopy (MS) with gaschromatography is one of the most powerful tools available. The chemical methods includethe gravimetric and volumetric procedures which are based on complex formation; acid-base, precipitation, and redox reactions. Titrations in non-aqueous media and complexometry havealso been used in pharmaceutical analysis. The number of new drugs is constantly growing.

This requires new methods for controlling their quality. Modern pharmaceutical analysismust need the following requirements.

1. The analysis should take a minimal time.
2. The accuracy of the analysis should meet the demands of Pharmacopoeia.
3. The analysis should be economical.
4. The selected method should be precise and selective.

DRUG PROFILE:

Telmisartan:

Telmisartan is an angiotensin II receptor antagonist (ARB) used in themanagement of hypertension. Generally, angiotensin II receptor blockers (ARBs) such astelmisartan bind to the angiotensin II type 1 (AT1) receptors with high affinity, causinginhibition of the action of angiotensin II on vascular smooth muscle, ultimately leading to areduction in arterial blood pressure. Recent studies suggest that telmisartan may also havePPAR-gamma agonistic properties that could potentially confer beneficial metabolic effects.

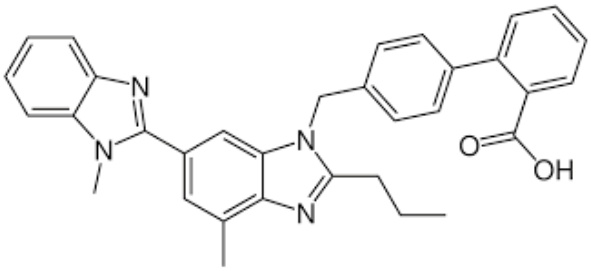

Fig no 1: structure of telmisartan

Azelnidipine:

Azelnidipine is a dihydropyridine calcium channel blocker. It is marketed byDaiichi-Sankyo pharmaceuticals, Inc. in Japan. It has a gradual onset of action and producesa long-lasting decrease in blood pressure, with only a small increase in heart rate, unlikesome other calcium channel blockers 3. It is currently being studied for post-ischemic strokemanagement.

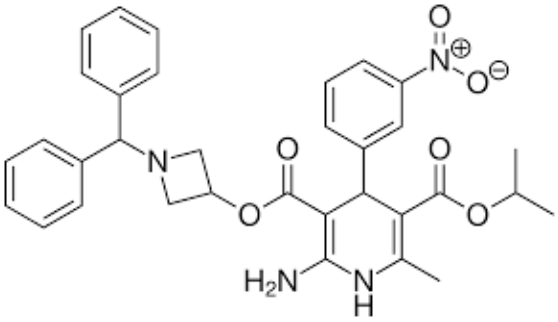

Fig no 2: structure of azelnidipine

MATERIALS AND METHODS

Materials

1. API:

Azelnidipine and Telmisartan API were obtained as a gift sample from Jai RamBiosciences, Kukatpally, Hyderabad, and Internal Standard from Akrivis Pharma pvtLtd.

2. Human plasma :

K 2 EDTA controlplasma	deccan pathological labs, Hyderabad

Table no 1: Human Plasma

3. Chemicals:

S.no	Chemical Name	Grade	Manufacturing company
1.	Distilled water		Rankem, Avantor performance material India limited
2.	Water	Analytical Reagent	Rankem, Avantor performance material India limited
3.	Acetonitrile	Analytical Reagent	Rankem, Avantor performance material India limited
4.	Phosphate buffer	Analytical Reagent	Rankem, Avantor performance material India limited
5.	Methanol	Analytical Reagent	Rankem, Avantor performance material India limited

6.	Sodium dihydrogen Phosphate	Analytical Reagent	Rankem, Avantor performance material India limited
7.	Ortho-phosphoric acid	Analytical Reagent	Rankem, Avantor performance material India limited

Table no 2: Chemicals and Solvents

4.Instruments:

S.no	Instrument	Company name	Brand name
1.	Electronic balance	Sartorious	Denver
2.	pH meter	Metsar	BVK enterprises
3.	Sonicator	Lab man	BVK enterprises
4.	Centrifuge	Thermo Fisher	-
5.	Vertex	Remi CM101	-
6.	Water	Acquity	HPLC Acquity

Table no 3: Instruments and Equipment

EXPERIMENTAL & ANALYTICAL METHODOLOGY:

Diluent: Based on the solubility of the drugs, diluent was selected are 0.01NSodiumdihydrogen phosphate and acetonitrile taken in the ratio of 65:35.

Extraction procedure: Take 950µl of plasma and 500µl of internal standard, 50µl of Azelnidipine and Telmisartanfrom the spikingsolutions of both into a centrifuging tube and add 2 ml of Acetonitrile go forcyclomixer for 15 sec. Then vertex for 2 min and finally centrifuge for 15 min at 3200 rpmspeed. After the centrifugation collect the sample and filter it directly inject 10 µL into.950µl of plasma +500 µl of internal standard +50 µl of Azelnidipine and Telmisartan
|
15 sec cyclomixer
|
1 ml of acetonitrile
|
Vertex for 2 min
|
Centrifuge for 5 min at 3200 rpm
|
Collection of supernatant samples
|
Filter the sample (polyvinylidene fluoride or polyvinylidene difluoride 0.45µ filter)
|
Inject 0.2 µL into HPLC System

PREPARATION OF AZELNIDIPINE AND TELMISARTAN STOCK:

Take 2.25 mg of Azelnidipine and30 mg Telmisartan in 500 ml volumetric flask and make the volume with diluent to produce10 µl. (4.5µg/ml of Azelnidipine and 60µg/ml of Telmisartan)

PREPARATION OF AZELNIDIPINE AND TELMISARTAN SPIKING SOLUTIONS:

From the above Azelnidipineand Telmisartan stock solution 0.05ml, 0.1ml, 0.15ml, 0.6ml, 1.0ml, 1.2ml, 1.6ml and 2.0 mlwas pipette and transferred to 8 individual 10 ml volumetric flask and make up the volume upto the mark with diluent to produce 0.0005 µg/ml, 0.0009µg/ml, 0.0014µg/ml, 0.0036 µg/ml,0.0090 µg/ml, 0.0108 µg/ml, 0.0144 µg/ml and 0.0180µg/ml of Azelnidipine and 0.0006µg/ml, 0.0012µg/ml, 0.00 18µg/ ml, 0.0048 µg/ml, 0.0120 µg/ml, 0.0144µg/ml, 0.0192 µg/mland 0.0240µg/ml of Azelnidipine

PREPARATION OF INTERNAL STANDARD SOLUTION (SAXAGLIPTIN):

Stock-1:

Take 10 mg of Saxagliptin in 100 ml volumetric flask and make up the volume withdiluent to produce 100µg/ml.

Stock-2:

From the above solution, take 1ml of solution into 10 ml volumetric flask and makeup the volume with diluent to produce 10µg/ml solutions.

Final concentration:

From the above solution, take 0.5ml of solution and spiking blankplasma with working stock dilutions of analyte to produce 0.2µg/ml ISD concentration

VALIDATION METHODOLOGY IN BIOANALYTICAL METHOD

System Suitability Parameter

System Suitability test are performed that the test mixture is essential to check thespecifications of a liquid chromatographic system. the System suitability testing limits areacceptance criteria that must be prior to sample analysis. The test is carried out by injectingsix samples of quality

control samples of MQC and check the criteria acceptance accordinglyas the % CV of the retention time (RT) should be ≤ 2.00 %.

Auto Sampler Carryover

Carry-over is an alteration of a measured concentration due to residual analyte from apreceding sample that remains in the analytical instrument, during validation carry-overshould be assessed by analysing blank samples after the calibration standard at the ULOQ.Carry-over in the blank samples following the highest calibration standard should not begreater than 20% of the analyte response at the LLOQ and 5% of the response for the IS.

Specificity and Screening of Biological matrix

Specificity is the ability of a bioanalytical method to detect and differentiate the analyte fromother substances, including its related substances (e.g., substances that are structurally like the analyte, metabolites, isomer, impurities, degradation products formed during samplepreparation or concomitant medications that are expected to be used in the treatment ofpatients with the intended indication). Specificity is determined by the injecting six samplesof standard solution and the LLOQC sample solution and check the % Interference Responseof interfering peaks in STD Bulk at the retention time of analyte should be ≤20.00 % of that inLLOQ and At least 80 % of the matrix lots (Biological Sample) with intended anticoagulantshould be within the acceptance criteria.

Sensitivity

Sensitivity is often interpreted as related to the detection/determination ability, LLOQ basedon precision and accuracy (bias) data, this is probably the most practical approach anddefines the LLOQ as the lowest concentration of a sample that can still be quantified withacceptable Limit. the sensitivity is performed by injecting six injections of lowerconcentration of sample (LLOQ) the acceptance criteria of sensitivity of LLOQ are At least67 % (4 out of 6) of samples should be within 80.00-120.00 %.

Matrix Factor evaluation

A matrix effect is defined as an alteration of the analyte response due to interfering and oftenunidentified component(s) in the sample matrix. During method validation it is necessary toevaluate the matrix effect between different independent sources/lots. The matrix effectshould be evaluated by analysing at least 3 replicates of low and high QCs (LQC andHQC), each prepared using matrix from at least 6 different sources/lots. The accuracy shouldbe within ±15% of the nominal concentration and the precision (per cent coefficient ofvariation (%CV)) should not be greater than 15% in all individual matrix sources/lots.

Linearity (Calibration Curve and Range)

The relationship between the nominal analyte concentration and the response of the analyticalplatform to the analyte, Calibration standards, prepared by spiking matrix with a knownquantity of analyte, span the calibration range and comprise the calibration curve. Calibrationstandards should be prepared in the same biological matrix as the study samples. Thecalibration range is obtained by injecting 6 concentrations of calibration standards notincluding blank and zero samples and establishing the concentration-response relation-ship by the sample regression model method and The % accuracy for all CC standards except ofLLOQ (STD 1) standard should be within 85.00-115.00 %. The % accuracy for LLOQstandard should be within 80.00-120.00 %.

Rugged Linearity

Linearity ruggedness is a measure for the susceptibility of a method to small changes thatmight occur during routine analysis, The calibration range is obtained by injecting 6concentrations of calibration standards not including blank and zero samples and establishing the concentration- response relationship by the sample regression model method and the %accuracy for all CC standards except of LLOQ (STD 1) standard should be within 85.00-115.00 %.The % accuracy for LLOQ standard should be within 80.00-120.00 %.

Precision and Accuracy (Intra-day)

Accuracy and precision should be determined by analysing the QCs within each run(within-run) and in different runs (between-run). Accuracy and precision should beevaluated using the same runs and data. The test is performed injecting the QCsamples were injected 6 replicates at each qc concentration level in each analyticalrun the overall accuracy at each concentration level should be within ±15% of thenominal concentration, except at the LLOQ, where it should be within ±20%. Theprecision (%CV) of the concentrations determined at each level should not exceed15%, except at the LLOQ, where it should not exceed 20%.

Rugged Precision and Accuracy (Inter-Day)
Accuracy and precision should be evaluated using the same runs and data. The test isperformed injecting the QC samples were injected 6 replicates at each qc concentration levelin each analytical run the overall accuracy at each concentration level should be within ±15%of the nominal concentration, except at the LLOQ, where it should be within ±20%. Theprecision (%CV) of the concentrations determined at each level should not exceed 15%,except at the LLOQ, where it should not exceed 20%.

Recovery
Recovery was determined by measuring the peak areas obtained from prepared plasmasamples with those extracted blank plasma spiked with standards containing the same areawith known amount of Drug The recoveries for Azelnidipine and Telmisartan at LQC, MQCand HQC levels the results demonstrated that the bioanalytical method had good extractionefficiency by injecting the six samples of LQC, MQC and HQC with the main drug andcheck the interference with unextracted and extracted, The % CV of recovery at each QClevel should be ≤ 15.00 %. The overall mean recovery % CV for all QC levelsshould be ≤20.00 %.

Recovery of Internal Standard
The measuring of peak areas obtained from prepared plasma samples with those extracted blank plasma spiked with Internal Standards containing the same area with known amount ofDrug, The recoveries for IS at 6 replicates the results demonstrated that the bioanalyticalmethod had good extraction efficiency by injecting the six samples and check the interferencewith unextracted and extracted, The % CV of recovery at each QC level should be ≤ 15.00 %.The overall mean recovery % CV for all QC levels should be ≤ 20.00 %.

Reinjection Reproducibility
Reproducibility of the method is assessed by replicate measurements of the QCs and is usuallyincluded in the assessment of precision and accuracy. However, if samples could be reinjected (e.g., in the case of instrument interruptions or other reasons such as equipment failure), reinjection reproducibility should be evaluated and included in the Validation Report orprovided in the Bioanalytical Report of the study where it was conducted. The reproducibilitywas performed by injecting the qc samples in 6 replicates and check the acceptance limits the %mean accuracy for LQC, MQC and HQC samples should be within 85.00-115.00 % and for theLLOQ QC sample it should be within 80.00-120.00 %.

Stabilities
Stability evaluations should be carried out to ensure that every step taken during sample preparation, processing, and analysis as well as the storage conditions used do not affect theconcentration of the analyte. The stability is assessed by long term stock solution stability andMatrix samples stability at -28±5 □C for 37 days & -80±5 0 C, stability testing is performed byinjecting the QCsamples of high and low concentrations (HQC and LQC) with taken biological matrix The mean concentration at each QC level should bewithin ±15% of thenominal.

RESULTS AND DISCUSSIONS
METHOD DEVELOPMENT:
Based on drug solubility and P ka Value following conditions has been used to develop themethod estimation of Azelnidipine and Telmisartan as per current ICH guidelines.Optimization of the chromatographic conditionsfordeveloping the method for the assay of Azelnidipine and Telmisartan, asystematic study of the effect of various factors was undertaken by varying oneparameter at a time and keeping all the other conditions constant. The following studieswere conducted for this purpose. A hypurity advance C18column was chosen as thestationary phase for this study. The mobile phase and the flow rate in order to getsharppeaks and base line separation of the components, the author has carriedout several experiments by varying the commonly used solvents, theircompositionsand flow rate. To effect ideal separation of the drug under isocratic conditions, mixturesof commonly used solvents like water, methanol, and acetonitrile with or withoutbuffers in different combinations were tested as mobile phases on a C18 stationaryphase. A binary mixture of acetonitrile and 0.01N Potassium dihydrogen ortho phosphate buffer in a ratio of 60:40 v/v was proved to be the most suitable of all the combinationssince the chromatographic peaks obtained were well defined and resolved and free fromtailing. A mobile phase flow rate of 0.2 mL/min was found to be suitable. The drugmolecule was tuned on the HPLC for the detection of Azelnidipine and Telmisartan andby injecting 0.15ng/mL and 6ng/ml concentration respectively. All the optimizedsystem suitability parameters within the limits results.

Optimized method:

Chromatographic conditions

Mobile phase :Acetonitrile: Na2HPo4 (35:65)
Flow rate :1.0ml/min
Column :Inertsil C18 (150mm x 4.8 mm, 5.0µ)
Detector wavelength : 228.nm
Column temperature : 30^0C
Injection volume : 10.0µL
Run time : 3.0min

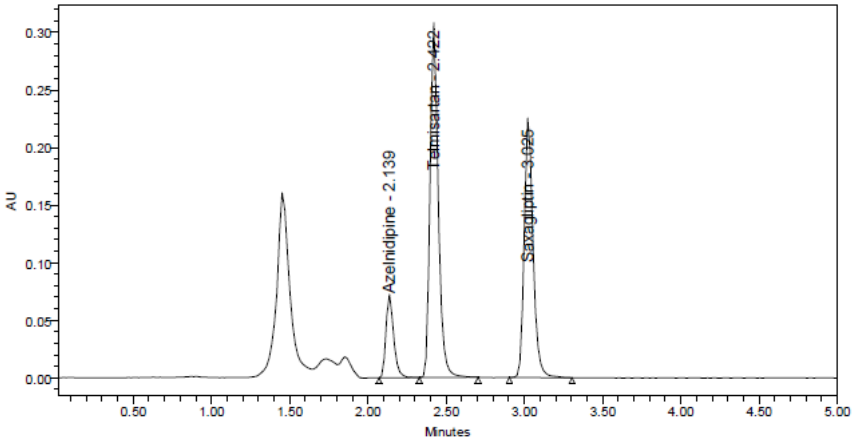

Fig no 3: Chromatogram of Optimized

	Peak Name	RT	Area	USP Plate Count	USP Resolution	USP Tailing
1	Azelnidipine	2.139	144211	8666.3		1.2
2	Telmisartan	2.422	775061	9533.2	3.0	1.2
3	Saxagliptin	3.025	2370299	13925.9	5.9	1.2

Table no 4: Observation of Optimized Chromatogram

Observation:

Azelnidipine and Telmisartan and Internal Standard were eluted at 2.139 min, 2.422 min, 3.025 respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated. Drugs were eluted with good retention time, resolution; all the system suitable parameters like Plate count and Tailing factor were within the limits.

METHOD VALIDATION

1) System suitability of Azelnidipine and Telmisartan

This system suitability method is intended to guarantee that the HPLC system is working in such a way that correct and reproducible data may be submitted to regulatory agencies with confidence. This procedure includes signal stability, carryover, and instrument response tests.

System Suitability						
Analyte	**Telmisartan**	**ISTD**	**Saxagliptin**			
Sample Name	**File Name**	**Analyte Area**	**Analyte RT (min)**	**ISTD Area**	**ISTD RT (min)**	**Area Ratio**
AQ MQC	**01**	**337595**	**2.41**	**2307729**	**2.97**	**0.1463**
AQ MQC	**02**	**335639**	**2.42**	**2309642**	**2.97**	**0.1453**
AQ MQC	**03**	**337096**	**2.42**	**2364362**	**2.97**	**0.1426**
AQ MQC	**04**	**336154**	**2.42**	**2345491**	**2.97**	**0.1433**
AQ MQC	**05**	**334181**	**2.43**	**2311353**	**2.98**	**0.1446**
AQ MQC	**06**	**337398**	**2.43**	**2391357**	**2.99**	**0.1411**
MEAN			**2.423**		**2.975**	**0.14386**
SD			**0.0083**		**0.0085**	**0.001906**

%CV			**0.34**		**0.29**	**1.33**
System Suitability Status	**Suitable**					
Acceptance Criteria:	**(+)**					
The % CV of the retention time (RT) should be ≤ 2.00 %.						
The % CV of the area ratio should be ≤ 5.00 %						
System Suitability						
Validation No.					**.**	
Analyte	**Azelnidipine**	**ISTD**	**Saxagliptin**			
Sample Name	**File Name**	**Analyte Area**	**Analyte RT (min)**	**ISTD Area**	**ISTD RT (min)**	**Area Ratio**
AQ MQC	**07**	**58823**	**2.12**	**2307729**	**2.97**	**0.0255**
AQ MQC	**08**	**58741**	**2.12**	**2309642**	**2.97**	**0.0254**
AQ MQC	**09**	**58649**	**2.13**	**2364362**	**2.97**	**0.0248**
AQ MQC	**10**	**58749**	**2.13**	**2345491**	**2.97**	**0.0250**
AQ MQC	**11**	**58331**	**2.14**	**2311353**	**2.98**	**0.0252**
AQ MQC	**12**	**58065**	**2.14**	**2391357**	**2.99**	**0.0243**
MEAN			**2.129**		**2.975**	**0.02505**
SD			**0.0084**		**0.0085**	**0.000453**
%CV			**0.40**		**0.29**	**1.81**
System Suitability Status	**Suitable**					
Acceptance Criteria:						
The % CV of the retention time (RT) should be ≤ 2.00 %.						
The % CV of the area ratio should be ≤ 5.00 %						

Table no 5: System Suitability of Azelnidipine and Telmisartan

Discussion:

plate count, tailing factor, resolution of Azelnidipine and Telmisartan was According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits. The % CV of the retention time (RT) should be ≤ 2.00 %.

2) Auto sampler carryover of Azelnidipine and Telmisartan

The carryover was tracked back to the injection valve and eradicated by converting from a partial loop injection to a full loop injection, which allowed more effective cleansing of the sample flow channel. The HPLC system's susceptibility to carryover was shown to be dependent on the detection method's absolute sensitivity and the mass of analyte injected at the assay's lower limit of quantitation (LLOQ).

Auto sampler Carryover				
Validation No.			**SOP No.**	
Analyte	Telmisartan		**ISTD**	Saxagliptin
Acquisition Batch ID			Date	
Sample ID	Peak Area		% Carryover	
	Drug	ISTD	Drug	ISTD
Unextracted samples				
RS	0	0	N/A	N/A
AQ ULOQ	7761757	2366845	**0.00**	**0.00**
RS	0	0		
AQ LLOQ	46499	2355803	N/A	N/A
Extracted samples				

STD Blk	0	0	N/A	N/A
ULOQ	7754232	2312563	**0.00**	**0.00**
LLOQ	45946	2256321	N/A	N/A
Acceptance Criteria:				
The carryover area response in subsequent injections of RS or STD Blk after aqueous or extracted ULOQ should be ≤ 20.00 % of the equivalent aqueous or extracted LLOQ standard area.				

Validation No.			**SOP No.**	
Analyte	Azelnidipine		**ISTD**	Saxagliptin
Sample ID	Peak Area		% Carryover	
	Drug	ISTD	Drug	ISTD
Unextracted samples				
RS	0	0	N/A	N/A
AQ ULOQ	1451641	2366845	**0.00**	**0.00**
RS	0	0		
AQ LLOQ	4530	2355803	N/A	N/A
Extracted samples				
STD Blk	0	0	N/A	N/A
ULOQ	1412361	2314528	**0.00**	**0.00**
STD Blk	0	0		
LLOQ	4365	2375621	N/A	N/A
Acceptance Criteria:				
The carryover area response in subsequent injections of RS or STD Blk after aqueous or extracted ULOQ should be ≤ 20.00 % of the equivalent aqueous or extracted LLOQ standard area.				

Table 6: Auto sampler carryover of Azelnidipine and Telmisartan

Discussion:

-The area obtained is less than 20 % of extracted LLOQ standard area to unextracted area by injected of replicate manner

3) Specificity and Screening of Biological Matrix

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present

Specificity and Screening of Biological Matrix						
Analyte	**Telmisartan**			**ISTD**	**Saxagliptin**	
Acquisition Batch ID					**Date**	
S.No.	**Sample ID**	**Response**		**% Interference**		**Pass/Fail**
		Drug	**ISTD**	**Drug**	**ISTD**	
1	**STD Blk1**	**0**	**0**	**0.00**	**0.00**	**Pass**
2	**LLOQ1**	**45985**	**234625**			
3	**STD Blk2**	**0**	**0**	**0.00**	**0.00**	**Pass**
4	**LLOQ2**	**46489**	**234562**			
5	**STD Blk3**	**0**	**0**	**0.00**	**0.00**	**Pass**
6	**LLOQ3**	**46752**	**237854**			
7	**STD Blk4**	**0**	**0**	**0.00**	**0.00**	**Pass**

8	**LLOQ4**	**46859**	**2326854**			
9	**STD Blk5**	**0**	**0**	**0.00**	**0.00**	**Pass**
10	**LLOQ5**	**46253**	**2375621**			
11	**STD Blk6**	**0**	**0**	**0.00**	**0.00**	**Pass**
12	**LLOQ6**	**46753**	**2324589**			
Acceptance Criteria:						
Response of interfering peaks in STD Blk at the retention time of analyte should be ≤20.00 % of that in LLOQ.						
Response of interfering peaks in STD Blk at the retention time of ISTD should be ≤ 5.00 % of that in LLOQ.						
At least 80 % of the matrix lots (excluding haemolysed, heparinised and lipemic matrix lots) with intended anticoagulant should be within the acceptance criteria.						

Validation No.				**SOP No.**		
Analyte	**Azelnidipine**			**ISTD**	**Saxagliptin**	
S.No.	**Sample ID**	**Response**		**% Interference**		**Pass/Fail**
		Drug	**ISTD**	**Drug**	**ISTD**	
1	**STD Blk1**	**0**	**0**	**0.00**	**0.00**	**Pass**
2	**LLOQ1**	**4529**	**234625**			
3	**STD Blk2**	**0**	**0**	**0.00**	**0.00**	**Pass**
4	**LLOQ2**	**4530**	**234562**			
5	**STD Blk3**	**0**	**0**	**0.00**	**0.00**	**Pass**
6	**LLOQ3**	**4598**	**237854**			
7	**STD Blk4**	**0**	**0**	**0.00**	**0.00**	**Pass**
8	**LLOQ4**	**4532**	**2326854**			
9	**STD Blk5**	**0**	**0**	**0.00**	**0.00**	**Pass**
10	**LLOQ5**	**4532**	**2375621**			
11	**STD Blk6**	**0**	**0**	**0.00**	**0.00**	**Pass**
12	**LLOQ6**	**4530**	**2324589**			
Acceptance Criteria:						
Response of interfering peaks in STD Blk at the retention time of analyte should be ≤20.00 % of that in LLOQ.						
Response of interfering peaks in STD Blk at the retention time of ISTD should be ≤ 5.00 % of that in LLOQ.						
At least 80 % of the matrix lots (excluding haemolysed, heparinised and lipemic matrix lots) with intended anticoagulant should be within the acceptance criteria.						

Table 7: Specificity and Screening of Biological Matrix of Azelnidipine and Telmisartan

Observation:

We did not find and interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.

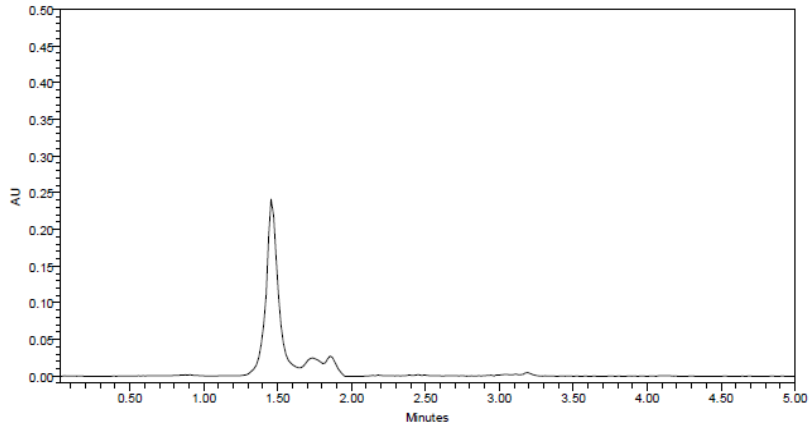

Fig no 4: Representative Chromatogram of a Blank Plasma Sample

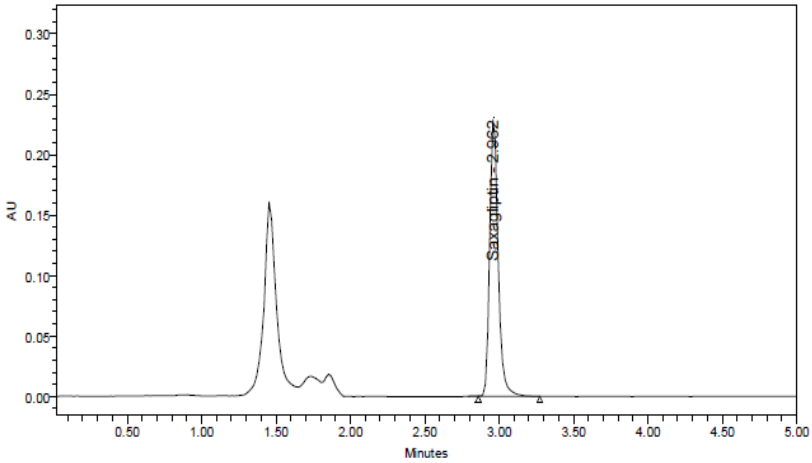

Fig no5: Representative Chromato gram of Blank Plasma with Internal Standard Sample

Discussion:

The response areas obtained of analyte and internal standard are less than 20% and 5 % of LLoq Area. We did not find and interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.

4) Sensitivity

A sensitivity is defined as "the lowest analyte concentration that can be measured with acceptable accuracy and precision i.e., LLoQ

Analyte	Azelnidipine	Saxagliptin
Replicate No.		**LLOQ**
		Nominal Concentration (ng/ml)
		0.450
		Nominal Concentration Range (ng/ml)
		(0.360-0.540)
		Calculated Concentration (ng/ml)
1		0.449
2		0.421
3		0.446
4		0.447
5		0.472
6		0.449
N		6
Mean		0.4473
SD		0.01618
% CV		**3.62**
% Mean Accuracy		**99.41**

Analyte	**Telmisartan**	**Saxagliptin**
Acquisition Batch ID		
Replicate No.		**LLOQ**
		Nominal Concentration (ng/ml)
		6.000
		Nominal Concentration Range (ng/ml)
		(4.800-7.200)
		Calculated Concentration (ng/ml)
1		**5.980**
2		**6.000**
3		**5.960**
4		**5.965**
5		**5.874**
6		**5.921**
N		**6**
Mean		**5.9500**
SD		**0.04548**
% CV		**0.76**
% Mean Accuracy		**99.17**

Table 8: Sensitivity of Azelnidipine and Telmisartan

Discussion:

The LLOQ concentration was found between 80 - 120 % and % Coefficient of variation found to be 0.87% of Azelnidipine and % of Telmisartan and mean of 6 injections was found to be 3.62 % and 0.76% of Azelnidipine and telmisartan within the acceptance limits. As the limit of Sensitivity % CV was less than "20%" the system Sensitivity was passed in this method.

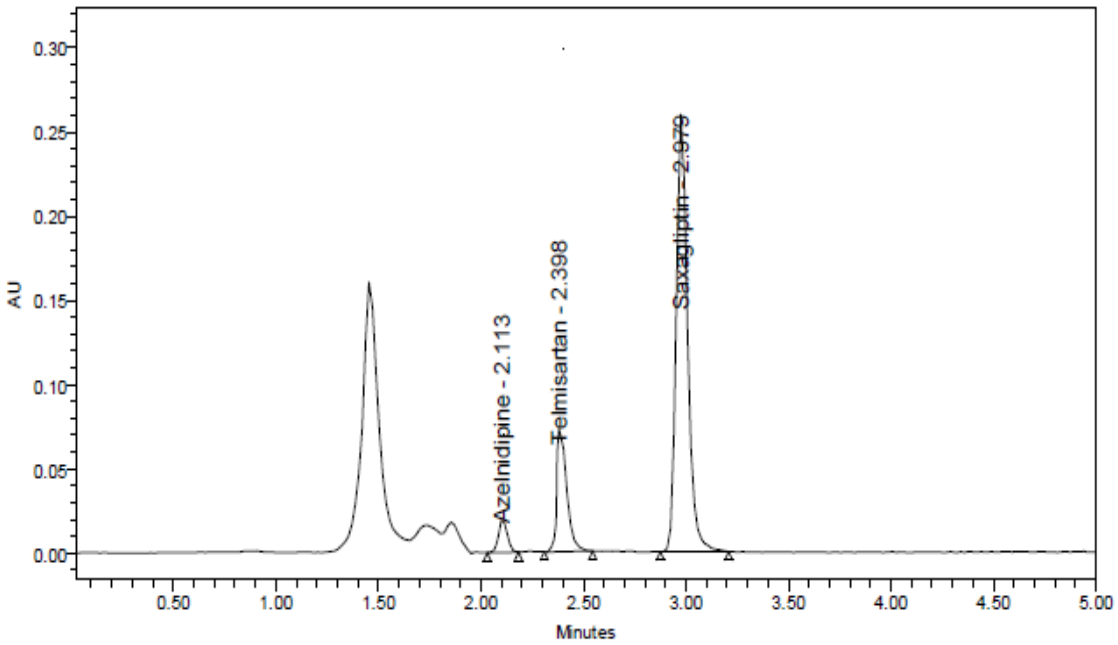

Fig no 6: LLOQ Chromatogram

Quality control samples

Standard zero sample:

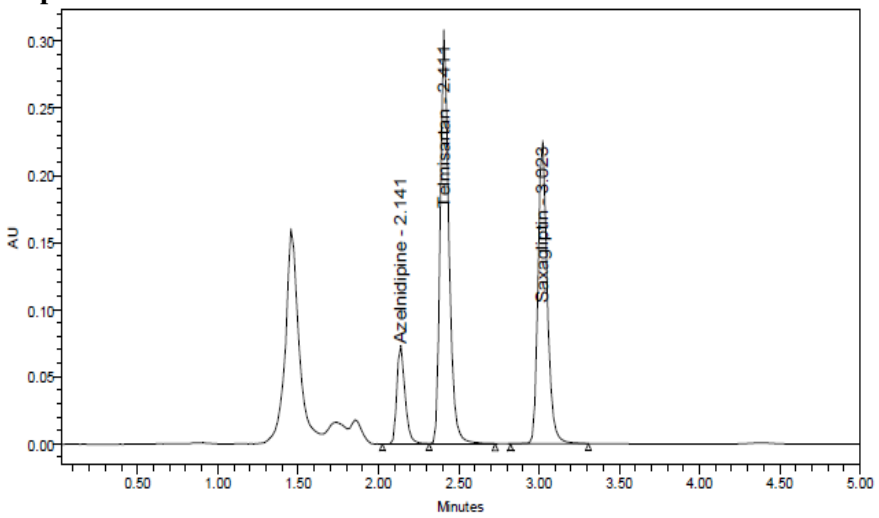

Fig no 7: chromatogram of standard Zero sample

QC-LLOQ

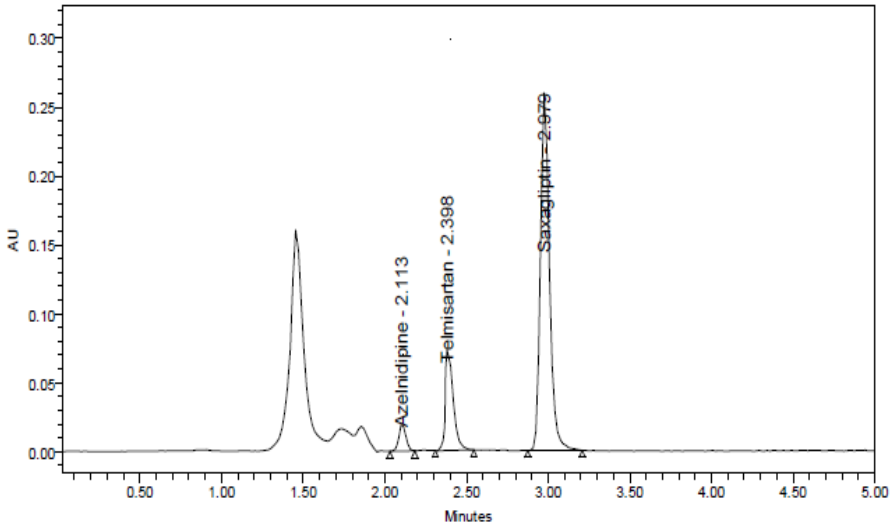

Fig no 8: chromatogram of QC-LLOQ sample

QC-LQC

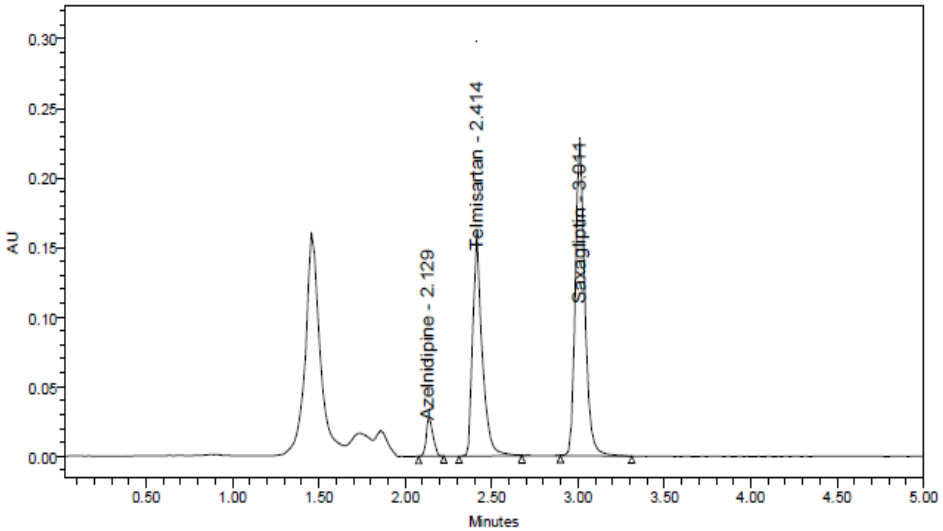

Fig no 9: chromatogram of QC-LQC sample

QC-MQC

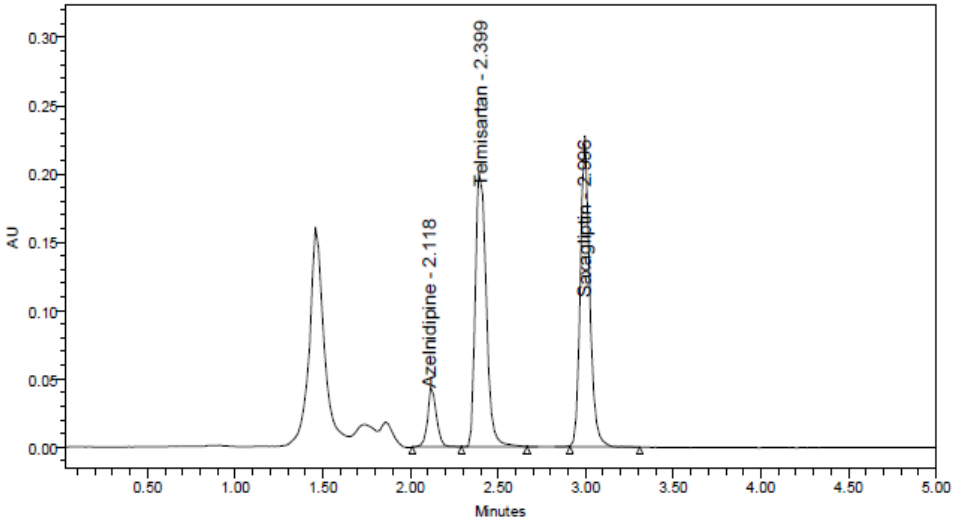

Fig no 10: chromatogram of QC-MQC sample

QC-HQC

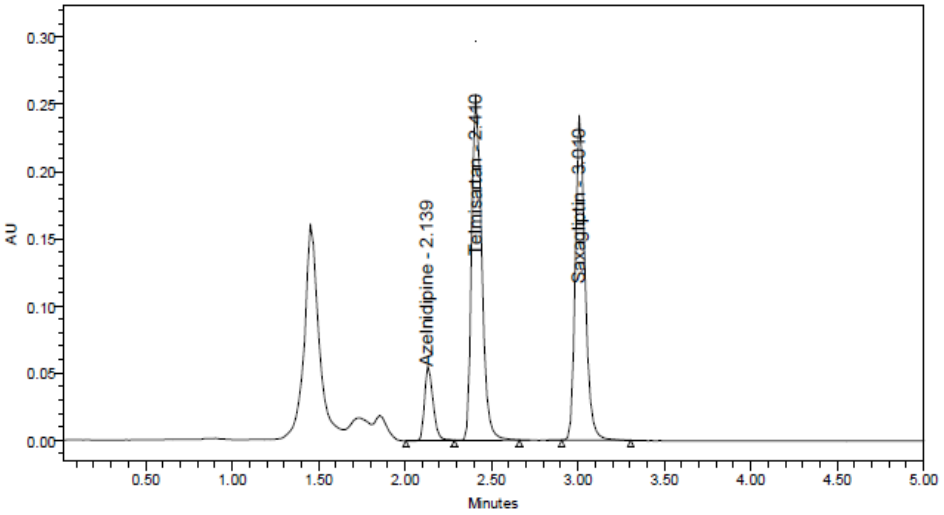

Fig no 11: chromatogram of QC-HQC sample l

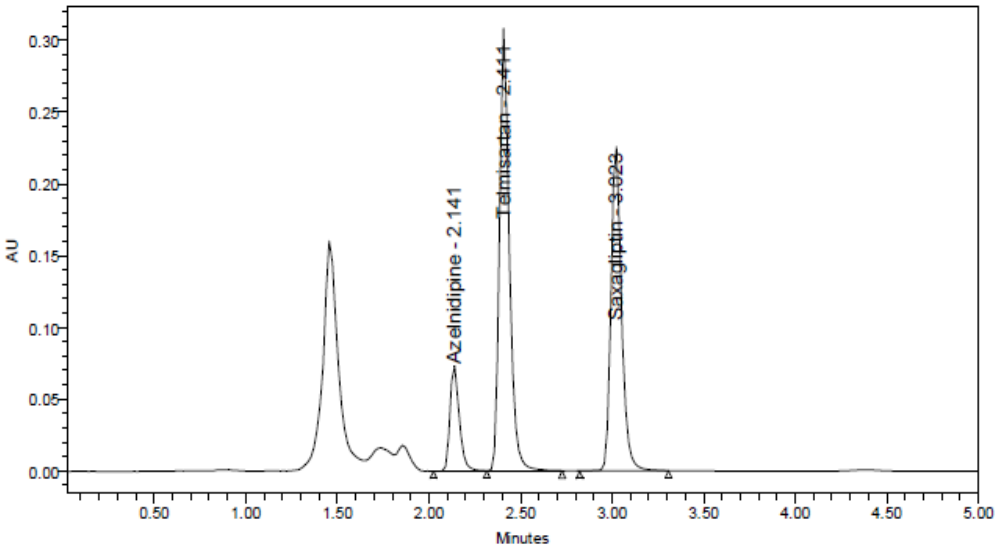

Fig no 12: chromatogram of ULOQ sample

5) Matrix factor evaluation

Analyte	Azelnidipine	ISTD	Saxagliptin
Acquisition Batch ID		Date	
S. No.	Plasma Lot No.	HQC	LQC
		Nominal Concentration (ng/ml)	
		14.400	1.350
		(12.240-16.560)	(1.148-1.553)
		Calculated Concentration (ng/ml)	
1	LOT1	14.325	1.285
		14.398	1.342
		14.365	1.345
2	LOT2	14.685	1.348
		14.000	1.350
		13.960	1.348
3	LOT3	14.398	1.350
		14.356	1.350
		14.398	1.354
4	LOT4	13.960	1.350
		13.980	1.345
		14.400	1.350
5	LOT5	14.265	1.346
		14.142	1.350
		14.400	1.348
6	LOT6	14.265	1.342
		14.000	1.342
		14.365	1.342
N		18	18
Mean		14.2590	1.3437
SD		0.20580	0.01508
% CV		1.44	1.12
% Mean Accuracy		99.02	99.53
No. of QC Failed		0	0

Analyte	Telmisartan	ISTD	Saxagliptin
Acquisition Batch ID		Date	
S. No.	Plasma Lot No.	HQC	LQC
		Nominal Concentration (ng/ml)	
		192.000	18.000
		Nominal Concentration Range (ng/ml)	
		(163.200-220.800)	(15.300-20.700)
		Calculated Concentration (ng/ml)	
1	LOT1	191.00	17.65
		190.00	17.98
		189.20	17.95
2	LOT2	189.90	17.86
		189.98	17.56
		191.32	17.98
3	LOT3	190.20	17.85
		192.30	17.98
		191.10	17.95
4	LOT4	191.20	17.96
		191.50	18.00
		192.30	17.87
5	LOT5	190.20	17.96
		191.40	17.85
		192.00	17.45
6	LOT6	192.00	17.65

		191.00	**17.85**
		191.25	**17.95**
N		**18**	**18**
Mean		**190.9917**	**17.8500**
SD		**0.90013**	**0.16306**
% CV		**0.47**	**0.91**
% Mean Accuracy		**99.47**	**99.17**
No. of QC Failed		**0**	**0**
Acceptance Criteria:			
At least 67 % (2 out of 3) of samples at each level should be within 85.00-115.00 %.At least 80 % (5 out of 6) of the matrix lot should be within the acceptance criteria.			
The % mean accuracy of back calculated concentration of LQC and HQC samples prepared from different biological matrix lots should be within 85.00-115.00 %.			

Table no 9: Matrix factor evaluations (absence of matrix factor)

Discussion:

The Evaluation of Matrix by injecting the QC samples of high and low concentrations in 6 lots the %Mean obtained was 99.47% and 99.17 of HQC and LOQ and % CV obtained are 0.47% and 0.91% of HQC and LOQ. As the limit of CV was less than "20%" the system Matrix was passed in this method of Telmisartan, the %Mean obtained was 99.02% and 99.53 of HQC and LOQ and % CV obtained are 1.44% and 1.12% of HQC and LOQ. As the limit of CV was less than "20%" the system Matrix was passed in this method of Azelnidipine.

6) Linearity:

Table 10: Linearity of Azelnidipine and Telmisartan

Analyte	**Azelnidipine**						**ISTD**	**Saxagliptin**
Acquisition Batch ID	**STD1**	**STD2**	**STD3**	**STD4**	**STD5**	**STD6**	**STD7**	**STD8**
	Nominal Concentration (ng/ml)							
	0.450	**0.900**	**1.350**	**3.600**	**9.000**	**10.800**	**14.400**	**18.000**
	Nominal Concentration Range (ng/ml)							
	(0.360-0.540)	**(0.765-1.035)**	**(1.148-1.553)**	**(3.060-4.140)**	**(7.650-10.350)**	**(9.180-12.420)**	**(12.240-16.560)**	**(15.300-20.700)**
	Back Calculated Concentration (ng/ml)							
P&A1	**0.448**	**0.889**	**1.348**	**3.58**	**8.90**	**10.80**	**14.36**	**17.89**
P&A2	**0.445**	**0.899**	**1.347**	**3.59**	**9.00**	**10.79**	**14.36**	**17.98**
P&A3	**0.449**	**0.897**	**1.346**	**3.59**	**8.90**	**10.79**	**14.40**	**17.96**
N	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Mean	**0.4473**	**0.8950**	**1.3470**	**3.5867**	**8.9333**	**10.7920**	**14.3690**	**17.9433**
SD	**0.00208**	**0.00529**	**0.00100**	**0.00577**	**0.05774**	**0.00520**	**0.02252**	**0.04726**
%CV	**0.47**	**0.59**	**0.07**	**0.16**	**0.65**	**0.05**	**0.16**	**0.26**
% Mean Accuracy	**99.41**	**99.44**	**99.78**	**99.63**	**99.26**	**99.93**	**99.78**	**99.69**

Analyte	**Telmisartan**						**ISTD**	**Saxagliptin**
Acquisition Batch ID	**STD1**	**STD2**	**STD3**	**STD4**	**STD5**	**STD6**	**STD7**	**STD8**
	Nominal Concentration (ng/ml)							
	6.000	**12.000**	**18.000**	**48.000**	**120.000**	**144.000**	**192.000**	**240.000**
	Nominal Concentration Range (ng/ml)							
	(4.800-7.200)	**(10.200-13.800)**	**(15.300-20.700)**	**(40.800-55.200)**	**(102.000-138.000)**	**(122.400-165.600)**	**(163.200-220.800)**	**(204.000-276.000)**
	Back Calculated Concentration (ng/ml)							
P&A1	**5.890**	**11.986**	**17.690**	**48.000**	**119.560**	**143.560**	**191.950**	**239.600**
P&A2	**5.986**	**11.876**	**17.980**	**47.650**	**120.000**	**143.950**	**192.000**	**239.562**
P&A3	**6.000**	**11.985**	**17.996**	**48.000**	**118.750**	**143.756**	**191.360**	**240.000**

N	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	
Mean	**5.9587**	**11.9490**	**17.8887**	**47.8833**	**119.4367**	**143.7553**	**191.7700**	**239.7207**	
SD	**0.05988**	**0.06322**	**0.17224**	**0.20207**	**0.63406**	**0.19500**	**0.35595**	**0.24265**	
%CV	**1.00**	**0.53**	**0.96**	**0.42**	**0.53**	**0.14**	**0.19**	**0.10**	
% Mean Accuracy	**99.31**	**99.58**	**99.38**	**99.76**	**99.53**	**99.83**	**99.88**	**99.88**	
Acceptance Criteria:									
The % accuracy for all CC standards except of LLOQ (STD 1) standard should be within 85.00-115.00 %.The % accuracy for LLOQ standard should be within 80.00-120.00 %.									
At least 75 % of CC standards should meet the acceptance criteria, including the LLOQ and highest CC standard (ULOQ). Any two consecutive points shall not be excluded.									
Response of interfering peaks in STD Blk and STD ZERO at the retention time of analyte should be ≤ 20.00 % of that in LLOQ.									
Response of interfering peaks in STD Blk at the retention time of ISTD should be ≤ 5.00 % of that in LLOQ.									

S.no	Final conc of Azelnidipine in ng/ml	Final conc of Telmisartan in ng/ml	ISD(area)	Drug(area) of Azelnidipine	Drug(area) of Telmisartan	Area ratio of Azelnidipine	Area ratio of telmisartan
1	0.45	**6.0**	2315824	**4553**	**46256**	**0.0020**	**0.02**
2	0.90	**12.0**	2354658	**9466**	**71251**	**0.0040**	**0.03**
3	1.35	**18.0**	2378287	**10978**	**89121**	**0.0046**	**0.04**
4	3.60	**48.0**	2340605	**34268**	**185682**	**0.0146**	**0.08**
5	9.00	**120.0**	2381532	**76442**	**391426**	**0.0321**	**0.16**
6	10.80	**144.0**	2319915	**88263**	**448562**	**0.0380**	**0.19**
7	14.40	**192.0**	2384084	**121682**	**586235**	**0.0510**	**0.25**
8	18.00	**240.0**	2383541	**151852**	**718523**	**0.0637**	**0.30**

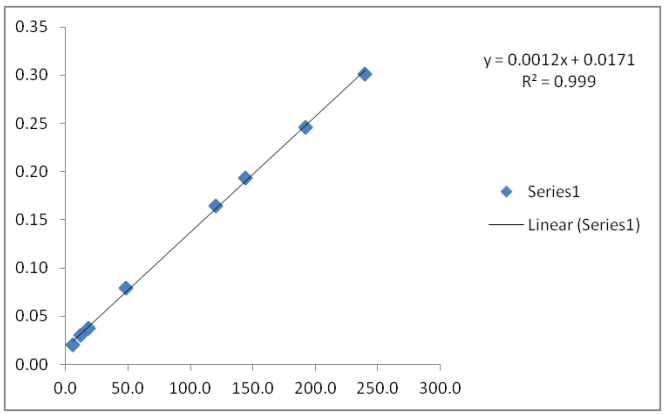

Fig. 13A RepresentativeCalibrationCurveforRegressionAnalysis of Telmisartan

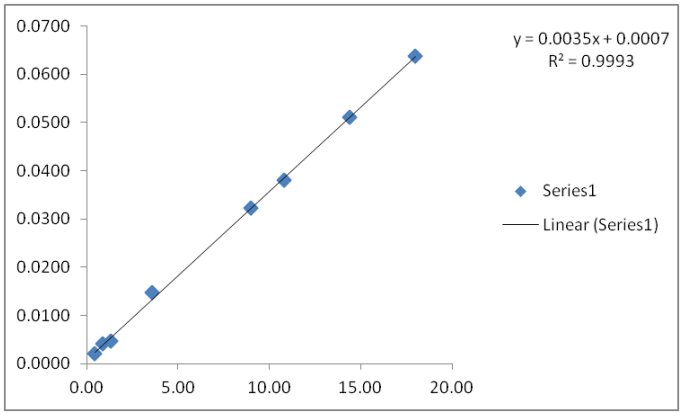

Fig. 13B Representative Calibration Curve for Regression Analysis of Azelnidipine

Discussion:

Calibration was found to be linear over the concentration range of 6.0 to 240µg /ml of Telmisartan and 0.45 to 18.0µg /ml of Azelnidipine. The coefficient correlation (r^2) value was found consistently greater than 0.999 in all the cases. This indicating linearity of results and an excellent correlation between peak area ratios for each concentration of analytes.

Acceptance criteria:

- Coefficient of correlation (r^2) should be≥0.98
- Deviation of LLOQ from nominal value can be±20%
- Deviation of standards other thanLLOQ from nominal value can be±15%
- No two consecutiveCCs must failto meet the above criteria.
- 75%oratleast 6 non zeroCCs including LLOQ& highest concentration must meet above criteria.

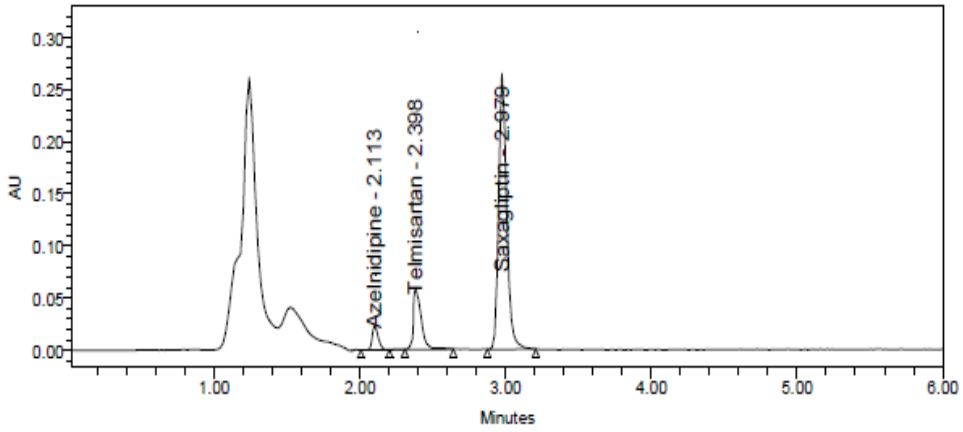

Fig no 14: Lineraity 1

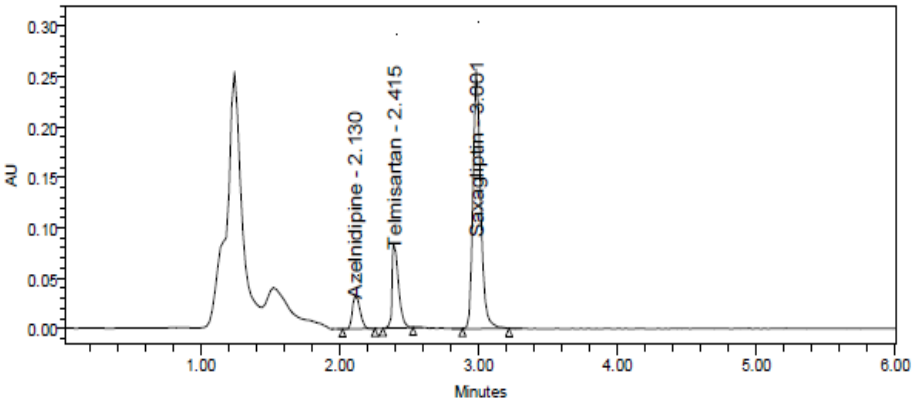

Fig no 15: Linearity 2

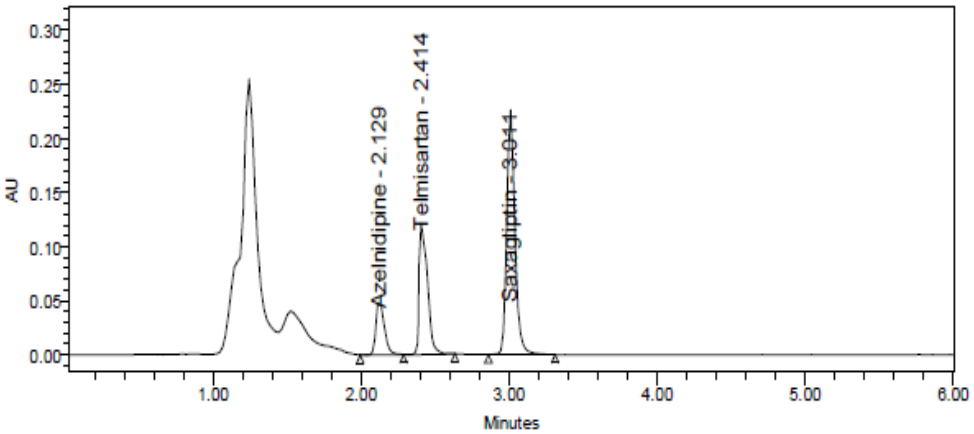

Fig no 16: Linearity 3

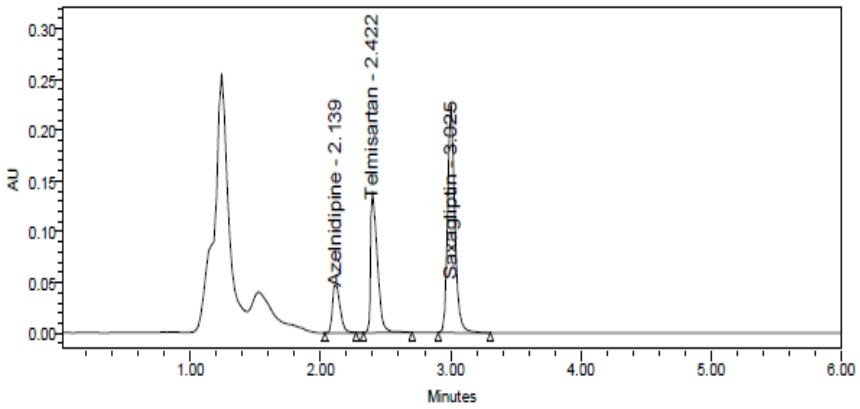

Fig no 17: Linearity 4

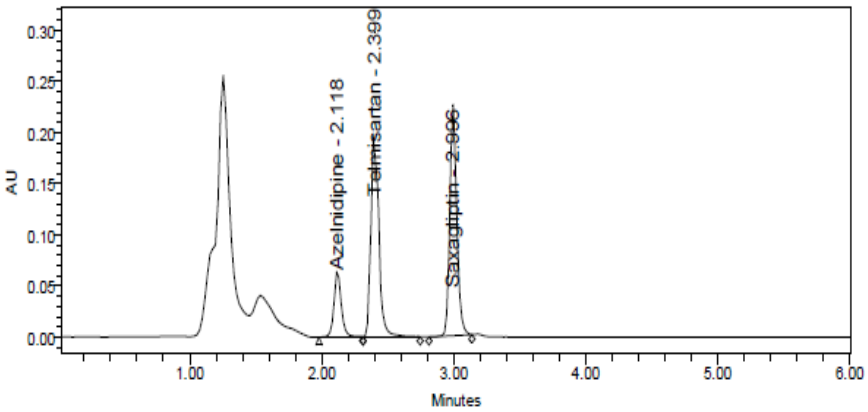

Fig no 18: Linearity 5

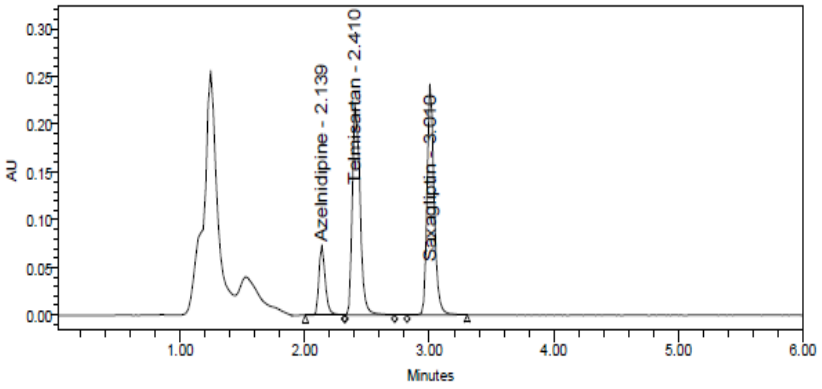

Fig no 19: Linearity 6

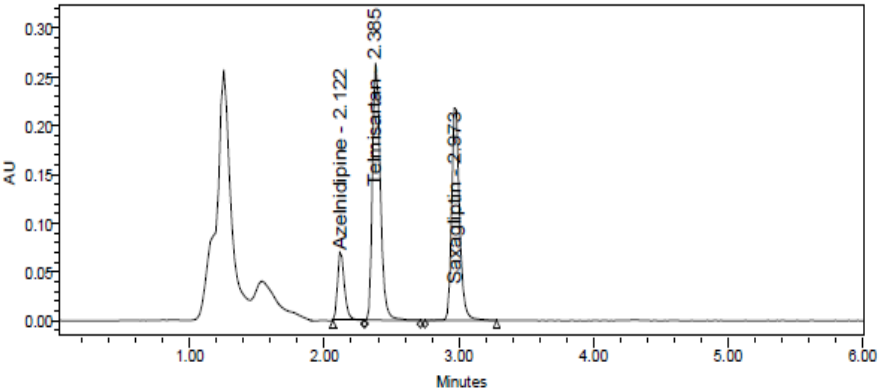

Fig no 20: Linearity 7

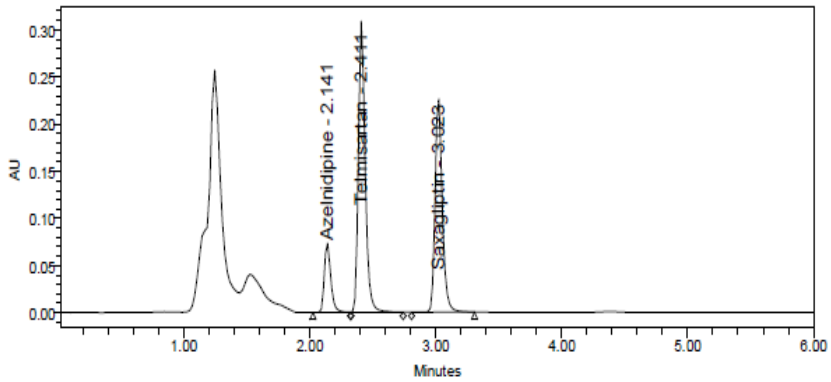

Fig no 21: Linearity 8

7) Precision and accuracy (intra-day runs of Azelnidipine and Telmisartan)

Analyte		Azelnidipine		Saxagliptin	
Acquisition Batch ID	Date	HQC	MQC1	LQC	LLOQ QC
		Nominal Concentration (ng/ml)			
		14.400	9.000	1.350	0.450
		Nominal Concentration Range (ng/ml)			
		(12.240-16.560)	(7.650-10.350)	(1.148-1.553)	(0.360-0.540)
		Back Calculated Concentration (ng/ml)			
		14.36	8.89	1.349	0.445
		14.39	8.98	1.348	0.442
		14.38	8.76	1.349	0.448
		14.39	8.96	1.350	0.447
		14.40	8.99	1.349	0.446
		14.38	8.96	1.348	0.449
N		6	6	6	6
Mean		14.3833	8.9233	1.3488	0.4462
SD		0.01366	0.08733	0.00075	0.00248
%CV		0.09	0.98	0.06	0.56
% Mean Accuracy		99.88	99.15	99.91	99.15
		14.38	8.99	1.335	0.447
		14.39	8.84	1.339	0.446
		14.39	8.76	1.348	0.442
		14.40	8.96	1.350	0.448
		14.38	8.99	1.348	0.443
		14.76	8.98	1.350	0.449
N		6	6	6	6
Mean		14.4500	8.9200	1.3450	0.4458
SD		0.15205	0.09695	0.00639	0.00279
%CV		1.05	1.09	0.47	0.63
% Mean Accuracy		100.35	99.11	99.63	99.07
		14.36	8.96	1.347	0.447
		14.39	8.99	1.338	0.449
		14.28	8.92	1.340	0.443
		14.36	8.96	1.342	0.448
		14.38	8.95	1.349	0.446
		14.41	8.99	1.338	0.447
N		6	6	6	6
Mean		14.3633	8.9617	1.3423	0.4467
SD		0.04502	0.02639	0.00468	0.00207
%CV		0.31	0.29	0.35	0.46
% Mean Accuracy		99.75	99.57	99.43	99.26
Between Batch Precision and Accuracy					
N		18	18	18	18
Mean		14.3989	8.9350	1.3454	0.4462
SD		0.09436	0.07477	0.00511	0.00234
%CV		0.66	0.84	0.38	0.52
% Mean Accuracy		99.99	99.28	99.66	99.16

Analyte		Telmisartan		Saxagliptin	
Acquisition Batch ID	Date	HQC	MQC1	LQC	LLOQ QC
		Nominal Concentration (ng/ml)			
		192.000	120.000	18.000	6.000
		Nominal Concentration Range (ng/ml)			
		(163.200-220.800)	(102.000-138.000)	(15.300-20.700)	(4.800-7.200)
		Back Calculated Concentration (ng/ml)			
		191.890	119.600	17.589	5.997
		191.875	118.700	17.600	5.898
		191.986	119.850	17.598	6.000
		191.200	120.000	18.603	5.998
		191.980	119.870	18.597	6.197
		191.845	119.700	17.599	6.198
N		6	6	6	6
Mean		191.7960	119.6200	17.9310	6.0480
SD		0.29754	0.47181	0.51822	0.12215
%CV		0.16	0.39	2.89	2.02
% Mean Accuracy		99.89	99.68	99.62	100.80
		191.256	118.600	18.600	6.003
		191.685	119.500	17.600	5.985
		191.745	120.200	17.621	6.000
		191.784	120.000	17.698	6.094
		191.652	119.000	17.596	5.890
		191.985	118.630	18.576	6.199
N		6	6	6	6
Mean		191.6845	119.3217	17.9486	6.0285
SD		0.24022	0.68791	0.49673	0.10573
%CV		0.13	0.58	2.77	1.75
% Mean Accuracy		99.84	99.43	99.71	100.48
		191.856	117.989	18.599	6.096
		191.784	118.999	18.596	6.198
		191.658	119.321	17.597	5.901
		191.784	118.121	17.593	5.900
		191.658	119.896	17.594	6.098
		191.987	120.879	17.596	6.099
N		6	6	6	6
Mean		191.7878	119.2008	17.9292	6.0487
SD		0.12495	1.09446	0.51769	0.12117
%CV		0.07	0.92	2.89	2.00
% Mean Accuracy		99.89	99.33	99.61	100.81
Between Batch Precision and Accuracy					
N		18	18	18	18
Mean		191.7561	119.3808	17.9362	6.0417
SD		0.22434	0.76800	0.48007	0.10994
%CV		0.12	0.64	2.68	1.82
% Mean Accuracy		99.87	99.48	99.65	100.70
Acceptance Criteria:					
The within and between batch precision for LQC, MQC and HQC samples should be ≤ 15.00 % and for the LLOQ QC, it should be ≤ 20.00 %.					
Intra batch					
At least 67 % (16 out of 24) of total QC samples and 50 % (3 out of 6) at each level should be within 85.00-115.00 % except LLOQ QC. LLOQ QC should be within 80.00-120.00 %.					
% Mean accuracy for LQC, MQC and HQC samples should be within 85.00-115.00 % and for the LLOQ QC sample it should be within 80.00-120.00 %.					
Intra batch					
% Mean accuracy between batch for LQC, MQC and HQC samples should be within 85.00-115.00 % and for the LLOQ QC sample it should be within 80.00-120.00 %.					

Table no11:precision data for intra-day runs of Azelnidipine and Telmisartan

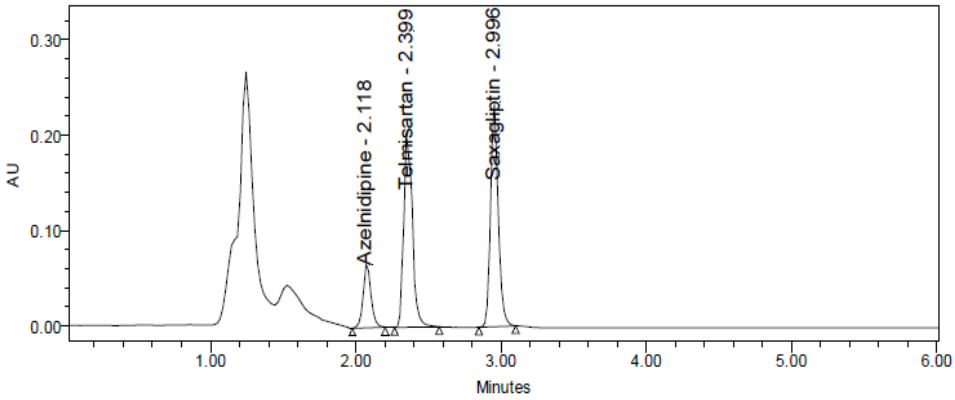

Fig no 22: Intraday precision 1

Discussion:

The intraday and inter day accuracy and precision was assessed by analysing six replicates at five different QC levels like **LLOQ, LQC, MQC and HQC**. Accuracy and precision method performance was evaluated by determined by six replicate analyses for Azelnidipine and Telmisartan at four concentration levels, i.e. ,0.45µg/ml(LLOQ), 1.35 µg/ml (LQC), 9.00 µg/ml (MQC) and 14.40 µg/ml HQC of Azelnidipine ,6.0µg/ml(LLOQ), 18.0 µg/ml (LQC), 120.0µg /ml (MQC) and 192.0 µg/ml HQC of telmisartan. The intra-day and inter day accuracy of plasma samples were assessed and excellent mean % accuracy was obtained with range varied from 99.96-100.35%, and 98.99%-99.93 % for intraday and 99.06%-100.02 and 98.91%-100.24 for inter day respectively. The precision (%CV) of the analytes and plasma samples were calculated and found to be <3% % for intraday and <12% for inter day respectively.

Acceptance criteria:

- **Precision:** Low, medium & high QCcon centrations should be within 15%&20% for LLOQ conc.
- **Accuracy:** Low, medium &high QC concentrations should bewithin ±15% &±20% for LLOQ concofnominal value

8) Rugged Precision and Accuracy (inter-day runs of Azelnidipine and Telmisartan)

Analyte		Azelnidipine		ISTD	Saxagliptin
P&A ID	Acquisition Batch ID	HQC	MQC1	LQC	LLOQ QC
		Nominal Concentration (ng/ml)			
		14.400	9.000	1.350	0.450
		Nominal Concentration Range (ng/ml)			
		(12.240-16.560)	(7.650-10.350)	(1.148-1.553)	(0.360-0.540)
		Calculated Concentration (ng/ml)			
Different Column		14.398	8.950	1.350	0.442
		14.396	8.976	1.348	0.443
		14.376	8.998	1.337	0.450
		14.386	8.997	1.336	0.446
		14.399	8.999	1.325	0.448
		14.498	8.998	1.335	0.446
N		6	6	6	6
Mean		14.4088	8.9863	1.3385	0.4458
SD		0.04456	0.01987	0.00922	0.00299
% CV		0.31	0.22	0.69	0.67
% Mean Accuracy		100.06	99.85	99.15	99.07
Different Analyst		14.496	8.986	1.341	0.448
		14.360	8.989	1.338	0.451
		14.389	8.999	1.347	0.438

		14.398	**9.000**	**1.332**	**0.442**
		14.387	**8.998**	**1.335**	**0.452**
		14.395	**8.986**	**1.349**	**0.447**
N		**6**	**6**	**6**	**6**
Mean		**14.4042**	**8.9930**	**1.3403**	**0.4463**
SD		**0.04697**	**0.00669**	**0.00668**	**0.00539**
% CV		**0.33**	**0.07**	**0.50**	**1.21**
% Mean Accuracy		**100.03**	**99.92**	**99.28**	**99.19**

Analyte		**Telmisartan**		**ISTD**	**Saxagliptin**
P&A ID	**Acquisition Batch ID**	**HQC**	**MQC1**	**LQC**	**LLOQ QC**
		Nominal Concentration (ng/ml)			
		192.000	**120.000**	**18.000**	**6.000**
		Nominal Concentration Range (ng/ml)			
		(163.200-220.800)	**(102.000-138.000)**	**(15.300-20.700)**	**(4.800-7.200)**
		Calculated Concentration (ng/ml)			
Different Column		**191.400**	**119.860**	**17.589**	**5.198**
		191.800	**118.980**	**18.590**	**6.200**
		191.400	**119.220**	**18.210**	**6.197**
		192.000	**119.989**	**17.600**	**5.900**
		191.400	**119.896**	**17.900**	**5.956**
		191.800	**120.200**	**18.597**	**6.196**
N		**6**	**6**	**6**	**6**
Mean		**191.6333**	**119.6908**	**18.0810**	**5.9412**
SD		**0.26583**	**0.47871**	**0.45786**	**0.38771**
% CV		**0.14**	**0.40**	**2.53**	**6.53**
% Mean Accuracy		**99.81**	**99.74**	**100.45**	**99.02**
Different Analyst		**192.200**	**119.980**	**18.100**	**5.985**
		191.200	**119.999**	**18.280**	**6.189**
		191.900	**119.796**	**17.590**	**6.184**
		191.100	**119.980**	**18.098**	**5.745**
		192.123	**119.000**	**17.599**	**5.965**
		191.500	**120.980**	**18.598**	**5.987**
N		**6**	**6**	**6**	**6**
Mean		**191.6705**	**119.9558**	**18.0442**	**6.0092**
SD		**0.47195**	**0.63105**	**0.39314**	**0.16475**
% CV		**0.25**	**0.53**	**2.18**	**2.74**
% Mean Accuracy		**99.83**	**99.96**	**100.25**	**100.15**
Acceptance Criteria:					
The within and between batch precision for LQC, MQC and HQC samples should be ≤ 15.00 % and for the LLOQ QC, it should be ≤ 20.00 %.					
At least 67 % (16 out of 24) of total QC samples and 50 % (3 out of 6) at each level should be within 85.00-115.00 % except LLOQ QC. LLOQ QC should be within 80.00-120.00 %.					
% Mean accuracy for LQC, MQC and HQC samples should be within 85.00-115.00 % and for the LLOQ QC sample it should be within 80.00-120.00 %.					

Table no 12: precision data for inter-day runs of Azelnidipine and Telmisartan.

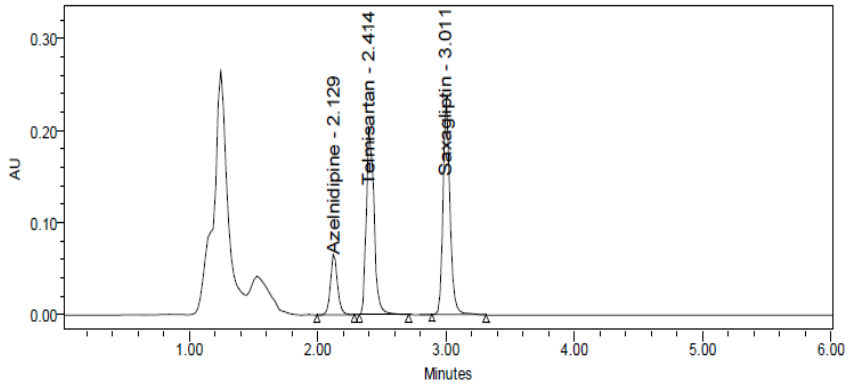

Fig no 23: Inter-day precision 2

Discussion:

The intraday and inter day accuracy and precision was assessed by analyzing six replicates at five different QC levels like **LLOQ, LQC, MQC and HQC**. Accuracy and precision method performance was evaluated by determined by six replicate analyses for Azelnidipine and Telmisartan at four concentration levels, i.e.,0.45µg/ml(LLOQ), 1.35 µg/ml (LQC), 9.00 µg/ml (MQC) and 14.40 µg/ml HQC of Azelnidipine , 6.0µg/ml(LLOQ), 18.0 µg/ml (LQC), 120.0µg/ml (MQC) and 192.0 µg/ml HQC of telmisartan. The intra-day and inter day accuracy of plasma samples were assessed and excellent mean % accuracy was obtained with range varied from 99.96-100.35%, and 98.99%-99.93 % for intraday and 99.06%-100.02 and 98.91%-100.24 for inter day respectively. The precision (%CV) of the analytes and plasma samples were calculated and found to be <3% % for intraday and <12% for inter day respectively.

Acceptance criteria:

§**Precision:** Low, medium & high Q Cconcentrations should be within 15%&20%fo r LLOQ conc.

§**Accuracy:** Low, medium &high QC concentrations should be within ±15% &±20% for LLOQ nominal value.

8) Recovery of Azelnidipine and Telmisartan-

Analyte	Azelnidipine					ISTD
Acquisition Batch ID						
Replicate No.	HQC		MQC1		LQC	
	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response
1	123658	121165	58665	58496	10250	10183
2	125985	124436	58665	58476	10314	10275
3	127986	125625	59163	58939	10564	10365
4	121865	121632	59165	58756	10363	10296
5	120845	123985	59657	58863	10389	10325
6	123635	124698	59576	58465	10252	10162
N	6	6	6	6	6	6
Mean	123996	123590	59149	58666	10355	10268
SD	2630.24	1786.34	426.46	213.00	116.80	79.94
% CV	2.12	1.45	0.72	0.36	1.13	0.78
% Mean Recovery	99.67		99.18		99.15	
Overall % Mean Recovery	99.337					
Overall SD	0.2915					
Overall % CV	0.29					

Analyte	Telmisartan		ISTD
Acquisition Batch ID			
Replicate No.	HQC	MQC1	LQC

	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response
1	**6398554**	**6357849**	**338652**	**335655**	**92425**	**91985**
2	**6347856**	**6389544**	**337456**	**336585**	**91945**	**91865**
3	**6349858**	**6346598**	**334785**	**334785**	**92056**	**91775**
4	**6385624**	**6326558**	**336587**	**339652**	**92056**	**91635**
5	**6378458**	**6345896**	**337455**	**336584**	**92045**	**91958**
6	**6359685**	**6345871**	**335698**	**335893**	**91986**	**91963**
N	**6**	**6**	**6**	**6**	**6**	**6**
Mean	**6370006**	**6352053**	**336772**	**336526**	**92086**	**91864**
SD	**20656.40**	**20952.55**	**1385.97**	**1671.47**	**172.21**	**136.85**
% CV	**0.32**	**0.33**	**0.41**	**0.50**	**0.19**	**0.15**
% Mean Recovery	**99.72**		**99.93**		**99.76**	
Overall % Mean Recovery	**99.801**					
Overall SD	**0.1106**					
Overall % CV	**0.11**					
#REF!						
Acceptance Criteria:						
The % CV of recovery at each QC level and for ISTD should be ≤ 15.00 %.						
The overall mean recovery % CV for all QC levels should be ≤ 20.00 %.						

Table no 13: Recovery of Azelnidipine and Telmisartan

Recovery - Internal standard

Acquisition Batch ID	**Date**	
S.No.	**Un extracted Area Ratio**	**Extracted Area Ratio**
1	**2345965**	**2307729**
2	**2345697**	**2309642**
3	**2365894**	**2364362**
4	**2345696**	**2345491**
5	**2365874**	**2311353**
6	**2339652**	**2391357**
N	**6**	**6**
Mean	**2351463.0**	**2338322.3**
SD	**11420.71**	**34721.72**
% CV	**0.49**	**1.48**
% Mean Recovery	**99.44**	
Acceptance Criteria:		
The % CV of recovery at each QC level and for ISTD should be ≤ 15.00 %.		
The overall mean recovery % CV for all QC levels should be ≤ 20.00 %.		

Table no 14: Recovery of Saxagliptin

Discussion:

Recovery was determined by measuring the peak areas obtained from prepared plasma samples with those extracted blank plasma spiked with standards containing the same area with known amount of Azelnidipine and Telmisartan and . The overall % mean recovery for was found to be 99.81% at LQC, MQC and HQC levels and % CV ranged from 0.11 % of telmisartan, The overall % mean recovery for was found to be 99.44% at LQC, MQC and HQC levels and % CV ranged from 0.29 % of Azelnidipine. The results demonstrated that the bioanalytical method had good extraction efficiency. The results demonstrated that the bioanalytical method had good extraction efficiency

Acceptance criteria:

- TheC.V%ofmeananalyte&ISTDrecoveriesmustbe≤15%foreachQCconclevel.
- The difference of% recovery between the lowest% recovery & highest% recovery should not be morethan 25%

Rugged Linearity:

Analyte	Telmisartan					ISTD	Saxagliptin	
P&A ID	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8
	Nominal Concentration (ng/ml)							
	6.000	12.000	18.000	48.000	120.000	144.000	192.000	240.000
	Nominal Concentration Range (ng/ml)							
	(4.800-7.200)	(10.200-13.800)	(15.300-20.700)	(40.800-55.200)	(102.000-138.000)	(122.400-165.600)	(163.200-220.800)	(204.000-276.000)
	Calculated Concentration (ng/ml)							
Different Column	Acquisition Batch ID						Date	
	5.925	11.865	17.650	48.100	119.865	143.560	191.560	239.600
Different Analyst	Acquisition Batch ID						Date	
	5.874	12.036	17.985	47.658	120.652	1144.000	191.260	238.650

Analyte	Azelnidipine					ISTD	Saxagliptin	
P&A ID	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8
	Nominal Concentration (ng/ml)							
	0.450	0.900	1.350	3.600	9.000	10.800	14.400	18.000
	Nominal Concentration Range (ng/ml)							
	(0.360-0.540)	(0.765-1.035)	(1.148-1.553)	(3.060-4.140)	(7.650-10.350)	(9.180-12.420)	(12.240-16.560)	(15.300-20.700)
	Calculated Concentration (ng/ml)							
Different Column	Acquisition Batch ID						Date	
	0.447	0.898	1.2560	3.587	8.956	9.156	13.980	17.950
Different Analyst	Acquisition Batch ID						Date	
	0.448	0.875	1.287	3.600	9.000	9.099	13.870	17.652
Acceptance Criteria:								
The % accuracy for all CC standards except of LLOQ (STD 1) standard should be within 85.00-115.00 %.The % accuracy for LLOQ standard should be within 80.00-120.00 %.								
At least 75 % of CC standards should meet the acceptance criteria, including the LLOQ and highest CC standard (ULOQ). Any two consecutive points shall not be excluded.								
Response of interfering peaks in STD Blk and STD ZERO at the retention time of analyte should be ≤ 20.00 % of that in LLOQ.								
Response of interfering peaks in STD Blk at the retention time of ISTD should be ≤ 5.00 % of that in LLOQ.								

Table no 14: Rugged Linearity of Azelnidipine and Telmisartan

Discussion: -

Linearity ruggedness is a measure for the susceptibility of a method to small changes that might occur during routine analysis, The calibration range is obtained by injecting 6 concentrations (6ng/ml-240ng/ml) of telmisartan, (0.45 ng/ml-18.0ng/ml) of Azelnidipine calibration standards not including blank and zero samples and establishing, The calibration curves were appeared linear and the coefficient of correlation was found to be 0.999 for Azelnidipine and Telmisartan.

Reinjection Reproducibility

Analyte	Azelnidipine		Temperature	
ISTD	Saxagliptin		2-8˚C	
Stability	Start	End	Duration	
Date	18 Mar 2023	20 Mar 2023	Hours (HH:MM)	
Time (HH:MM)	12:28	10:34	22:06	
P&A ID	HQC	MQC1	LQC	LLOQ QC
	Nominal Concentration (ng/ml)			

	14.400	**9.000**	**1.350**	**0.450**
	Nominal Concentration Range (ng/ml)			
	(12.240-16.560)	**(7.650-10.350)**	**(1.148-1.553)**	**(0.360-0.540)**
	Calculated Concentration (ng/ml)			
P&A01	**14.198**	**8.992**	**1.359**	**0.450**
	14.299	**8.999**	**1.320**	**0.459**
	14.398	**8.992**	**1.339**	**0.439**
	14.296	**8.993**	**1.320**	**0.440**
	14.398	**8.998**	**1.348**	**0.439**
	14.399	**9.089**	**1.350**	**0.447**
N	**6**	**6**	**6**	**6**
Mean	**14.3313**	**9.0105**	**1.3393**	**0.4457**
SD	**0.08190**	**0.03858**	**0.01627**	**0.00799**
% CV	**0.57**	**0.43**	**1.21**	**1.79**
% Mean Accuracy	**99.52**	**100.12**	**99.21**	**99.04**

Analyte	**Telmisartan**		**Temperature**	
ISTD	**Saxagliptin**		**2-8˚C**	
Stability	**Start**	**End**	**Duration**	
Date	**18 Mar 2023**	**20 Mar 2023**	**Hours (HH:MM)**	
Time (HH:MM)	**12:28**	**10:34**	**22:06**	
P&A ID	**HQC**	**MQC1**	**LQC**	**LLOQ QC**
	Nominal Concentration (ng/ml)			
	192.000	**120.000**	**18.000**	**6.000**
	Nominal Concentration Range (ng/ml)			
	(163.200-220.800)	**(102.000-138.000)**	**(15.300-20.700)**	**(4.800-7.200)**
	Calculated Concentration (ng/ml)			
P&A01	**191.65**	**119.86**	**17.61**	**5.898**
	191.96	**120.99**	**17.98**	**5.998**
	192.00	**120.00**	**18.02**	**5.987**
	191.98	**119.69**	**17.87**	**5.965**
	191.89	**119.89**	**17.96**	**5.898**
	191.96	**120.00**	**17.60**	**5.999**
N	**6**	**6**	**6**	**6**
Mean	**191.9067**	**120.0717**	**17.8400**	**5.9575**
SD	**0.13110**	**0.46413**	**0.18857**	**0.04769**
% CV	**0.07**	**0.39**	**1.06**	**0.80**
% Mean Accuracy	**99.95**	**100.06**	**99.11**	**99.29**
Note: Individual sample calculated concentration which appears in bold are out of acceptance criteria but included in statistical calculations.				
Reinjection Reproducibility has been proven at 2-8˚C for 46 Hr(s) 6 min(s).				
Acceptance Criteria:				
At least 67 % (16 out of 24) of total QC samples and 50 % (3 out of 6) at each level should be within 85.00-115.00 % except LLOQ QC. LLOQ QC should be within 80.00-120.00 %.				
The % mean accuracy for LQC, MQC and HQC samples should be within 85.00-115.00 % and for the LLOQ QC sample it should be within 80.00-120.00 %.				
The % CV for LQC, MQC and HQC samples should be ≤ 15.00 % and for the LLOQ QC it should be ≤ 20.00 %.				

Table no 15:Reinjection Reproducibility of Azelnidipine and Telmisartan

Discussion:

The % mean accuracy for LQC, MQC and HQC samples was found to be 99.95, 100.06, 99.11 and % Cv was found to be 0.07, 0.39, 1.06 and LLOQ was found 99.29% and % Cv was found to be 0.80% of telmisartan, The % mean accuracy for LQC, MQC and HQC samples was found to be 99.52, 100.12, 99.21 and % Cv was found to be 0.57,0.43, 1.21 and LLOQ was found 99.04% and % Cv was found to be 1.79% of Azelnidipine The results demonstrated that the bioanalytical method had good extraction efficiency.

Stabilities

Long term stock solution stability

Analyte	ISTD	Saxagliptin
Azelnidipine	Saxagliptin	
Acquisition Batch ID	Date	
Replicate No.	HQC	LQC
	Nominal Concentration (ng/ml)	
	14.400	1.350
	Nominal Concentration Range (ng/ml)	
	(12.240-16.560)	(1.148-1.553)
	Calculated Concentration (ng/ml)	
1	14.297	1.342
2	14.399	1.340
3	14.396	1.335
4	14.397	1.347
5	14.296	1.350
6	14.298	1.348
N	6	6
Mean	14.3472	1.3437
SD	0.05497	0.00568
% CV	0.38	0.42
% Mean Accuracy	99.63	99.53

Validation No.	SOP No.	
Analyte-Telmisartan	ISTD	Saxagliptin
Acquisition Batch ID		
Replicate No.	HQC	LQC
	Nominal Concentration (ng/ml)	
	192.000	18.000
	Nominal Concentration Range (ng/ml)	
	(163.200-220.800)	(15.300-20.700)
	Calculated Concentration (ng/ml)	
1	191.886	17.889
2	191.956	17.996
3	192.000	17.898
4	191.875	17.996
5	191.860	17.796
6	191.760	17.896
N	6	6
Mean	191.8895	17.9118
SD	0.08306	0.07556
% CV	0.04	0.42
% Mean Accuracy	99.94	99.51

Acceptance Criteria:	
At least 67 % (8 out of 12) of total QC samples and 50 % (3 out of 6) at each level should be within 85.00-115.00 %.	
The % mean accuracy of LQC and HQC should be within 85.00-115.00 %.	
The % CV of LQC and HQC samples should be ≤ 15.00 %.	

Table no 16: stability of Azelnidipine and Telmisartan (zero days)

Discussion-

In bench-top stability, six replicates of LQC & HQC samples (1.35 and 14.40 µg/ml) of Azelnidipine and(18.0 and 192.0 µg/ml) of telmisartan were analysed for 9 hours at room temperature on the laboratory bench. The % mean stability was calculated and found to 99.63% for LQC and 99.53% for HQC of Azelnidipine respectively. The % mean stability was calculated and found to 99.94% for LQC and 99.51% for HQC of Telmisartan respectively.

Matrix samples stability at -28±5 °C for 37 days

Analyte Name	**Azelnidipine**	**Temperature**	**-28**	**±5 ˚C**
Stability	**Start**	**End**	**Duration**	
Date	**10 Nov 2017**	**18 Dec 2017**	**Days**	**Hours (HH:MM)**
Time (HH:MM)	**15:23**	**12:58**	**37**	**21:35**
Acquisition Batch ID				
Replicate No.	**HQC**		**LQC**	
	Nominal Concentration (ng/ml)			
	1.600	**1.600**	**0.060**	**0.060**
	Nominal Concentration Range (ng/ml)			
	(1.360-1.840)	**(1.360-1.840)**	**(0.051-0.069)**	**(0.051-0.069)**
	Calculated Concentration (ng/ml)			
	Comparison Samples	**Stability Samples**	**Comparison Samples**	**Stability Samples**
1	**1.598**	**1.599**	**0.058**	**0.059**
2	**1.597**	**1.598**	**0.060**	**0.060**
3	**1.586**	**1.597**	**0.060**	**0.060**
4	**1.586**	**1.599**	**0.060**	**0.060**
5	**1.586**	**1.600**	**0.059**	**0.059**
6	**1.586**	**1.598**	**0.060**	**0.060**
N	**6**	**6**	**6**	**6**
Mean	**1.5898**	**1.5985**	**0.0595**	**0.0597**
SD	**0.00595**	**0.00105**	**0.00084**	**0.00052**
% CV	**0.37**	**0.07**	**1.41**	**0.87**
%Mean Accuracy	**99.36**	**99.91**	**99.17**	**99.44**
% Mean Stability	**100.55**		**100.28**	
Long Term Analyte Stability in Matrix of Norgestrel has been proven at Temperature -28 ± 5°C for 37 Days				
Acceptance Criteria:				
At least 67 % (8 out of 12) of total QC samples and 50 % (3 out of 6) at each level in stability and comparison samples should be within 85.00 -115.00 %.				
The % mean accuracy of back calculated concentration of LQC and HQC samples should be within 85.00-115.00 %.				
The % CV of LQC and HQC samples should be ≤ 15.00 %.The % Mean Stability of LQC and HQC samples should be within 85.00-115.00 %.				

Analyte Name	**Telmisartan**	**Temperature**	**-28**	**±5 ˚C**
Stability	**Start**	**End**	**Duration**	
Date	**10 Nov 2017**	**18 Dec 2017**	**Days**	**Hours (HH:MM)**
Time (HH:MM)	**15:23**	**12:58**	**37**	**21:35**
Acquisition Batch ID				
Replicate No.	**HQC**		**LQC**	
	Nominal Concentration (ng/ml)			
	192.000	**192.000**	**18.000**	**18.000**
	Nominal Concentration Range (ng/ml)			
	(163.200-220.800)	**(163.200-220.800)**	**(15.300-20.700)**	**(15.300-20.700)**
	Calculated Concentration (ng/ml)			
	Comparison Samples	**Stability Samples**	**Comparison Samples**	**Stability Samples**
1	**191.86**	**191.78**	**17.598**	**17.898**
2	**191.96**	**192.96**	**17.898**	**17.599**
3	**191.86**	**191.86**	**17.900**	**17.987**
4	**191.75**	**192.92**	**17.987**	**17.899**
5	**192.06**	**191.69**	**17.896**	**17.596**
6	**191.09**	**192.86**	**17.987**	**17.998**
N	**6**	**6**	**6**	**6**
Mean	**191.7633**	**192.3450**	**17.8777**	**17.8295**
SD	**0.34610**	**0.62571**	**0.14381**	**0.18459**
% CV	**0.18**	**0.33**	**0.80**	**1.04**
%Mean Accuracy	**99.88**	**100.18**	**99.32**	**99.05**
% Mean Stability	**100.30**		**99.73**	
Long Term Analyte Stability in Matrix of Norgestrel has been proven at Temperature -28 ± 5°C for 37 Days				
Acceptance Criteria:				
At least 67 % (8 out of 12) of total QC samples and 50 % (3 out of 6) at each level in stability and comparison samples should be within 85.00 -115.00 %.				
The % mean accuracy of back calculated concentration of LQC and HQC samples should be within 85.00-115.00 %.				
The % CV of LQC and HQC samples should be ≤ 15.00 %. The % Mean Stability of LQC and HQC samples should be within 85.00-115.00 %.				

Table no 17: Matrix samples stability at -28±5 °C for 37 days

Matrix samples stability at -80±5 °C for 37days

Method Validation No.		**SOP No.**		
Analyte Name	**Azelnidipine**	**Temperature**	**-80**	**±5 ˚C**
Acquisition Batch ID				
Replicate No.	**HQC**		**LQC**	
	Nominal Concentration (ng/ml)			
	14.400	**14.400**	**1.350**	**1.350**

	Nominal Concentration Range (ng/ml)			
	(12.240-16.560)	(12.240-16.560)	(1.148-1.553)	(1.148-1.553)
	Calculated Concentration (ng/ml)			
	Comparison Samples	Stability Samples	Comparison Samples	Stability Samples
1	14.389	14.299	1.348	1.342
2	14.250	14.397	1.350	1.325
3	14.592	14.486	1.300	1.340
4	14.498	14.393	1.349	1.347
5	14.399	14.299	1.356	1.342
6	14.397	14.400	1.342	1.338
N	6	6	6	6
Mean	14.4208	14.3790	1.3408	1.3390
SD	0.11532	0.07101	0.02050	0.00748
% CV	0.80	0.49	1.53	0.56
%Mean Accuracy	100.14	99.85	99.32	99.19
% Mean Stability	99.71		99.86	

Method Validation No.		SOP No.		
Analyte Name	Telmisartan	Temperature	-80	±5 ◦C
Replicate No.	HQC		LQC	
	Nominal Concentration (ng/ml)			
	192.000	192.000	18.000	18.000
	Nominal Concentration Range (ng/ml)			
	(163.200-220.800)	(163.200-220.800)	(15.300-20.700)	(15.300-20.700)
	Calculated Concentration (ng/ml)			
	Comparison Samples	Stability Samples	Comparison Samples	Stability Samples
1	191.860	192.680	17.596	17.998
2	191.680	191.968	17.997	17.589
3	192.760	191.860	17.912	17.810
4	191.960	191.690	17.578	17.914
5	192.960	191.040	17.598	18.000
6	192.960	191.960	18.600	17.614
N	6	6	6	6
Mean	192.3633	191.8663	17.8802	17.8208
SD	0.59200	0.52823	0.39611	0.18375
% CV	0.31	0.28	2.22	1.03
%Mean Accuracy	100.19	99.93	99.33	99.00
% Mean Stability	99.74		99.67	
Acceptance Criteria:				
At least 67 % (8 out of 12) of total QC samples and 50 % (3 out of 6) at each level in stability and comparison samples should be within 85.00 -115.00 %.				
The % mean accuracy of back calculated concentration of LQC and HQC samples should be within 85.00-115.00 %.				
The % CV of LQC and HQC samples should be ≤ 15.00 %.The % Mean Stability of LQC and HQC samples should be within 85.00-115.00 %.				

Table no 18: Matrix samples stability at -80±5 °C for 37 days

Discussion:

Long term stock solution stability for the Azelnidipine and Telmisartan was determined at a concentration of LQC-HQC level after a storage period of 37 days at -28°C& -80°C in refrigerator. The % mean stability of the Azelnidipine and Telmisartan was found to be 101.68%, 99.93% at 28 ± 5°C and 101.31%, 99.89% at 80 ± 5°C respectively. Long term stock solution stability for the was determined at a concentration of LQC-HQC level after a storage period of 37 days at -28°C& -80°C in refrigerator.

Summary of the result

Parameters	**Azelnidipine**	**Telmisartan**	**LIMIT**
Linearity Range(ng/ml)	0.45-18.0ng/ml	6.0-240.0ng/ml	R^2< 1
Regression coefficient	0.999	0.999	
Slope(m)	0.0035	0.0012	
Intercept(c)	0.0007	0.0171	
Regression equation (Y=mx+c)	y =0.0035x + 0.0007	Y = 0.0012x + 0.0171	
Specificity	Specific	Specific	No interference of any peak
Accuracy %recovery	98.81%	99.37%	80-120%
LLOQ	0.45ng/ml	6.0ng/ml	

Conclusion:

A simple, accurate, precise method was developed for the estimation of the Azelnidipine and Telmisartan in Human plasma using the Saxagliptin as internal standard. Retention time of Azelnidipine and Telmisartan was found to be 2.139min, 2.422min, which reach the level of both drugs possibly found in Human plasma. And Internal Standard retention time was found to be 3.025 Further, the reported method was validated as per the ICH guidelines and found to be well within the acceptable range.The proposed method is simple, rapid, accurate, precise, and appropriate for pharmacokinetic and therapeutic drug monitoring in the clinical laboratories.

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