UHPLC Analytical Method Development of Teneligliptin in Oral Solid Dosage Form by Reverse Phase Chromatographic Technique

P. Lavanya Reddy[1], Dr. J. N. Suresh Kumar[2],Mr.B.Satya Prasad[3]*
1. Research Scholar, 2. Professor & Principal, 3. Assistant Professor
1,2,3. Narasaraopet Institute of Pharmaceutical Sciences, Narasaraopet, Guntur-A.P

ABSTRACT :

A separation step is usually a necessary part of both a qualitative and quantitative analysis. The result of typical quantitative analysis can be computed from two measurements. One is the mass or volume of sample to be analyzed and the second one is the measurement of some quantity that is proportional to the amount of analyte in that sample and normally completes the analysis. A simple, reproducible and efficient reverse phase Ultra high-performance liquid chromatography (RP-UHPLC) method has been developed for estimation of Teneligliptin in its tablet dosage form. Separation was done by using mobile phase consists of Mixture of 1-OctaneSulphonic acid sodium salt (pH adjusted to 3.5 ±0.05 with Orthophosphoric acid): Acetonitrile (50:50,v/v). Chromatography separations were carried out on Phenomenex Luna C18 column (50 X 3.0mn; 3µm) at a flow rate of 0.5ml/min and UV detection at 245nm and the retention time for Teneligliptin is 1.02 minutes. The linear dynamic response was found to be in the concentration of 50µg-150µg/ml. The slope, intercept and Correlation coefficient was found to be 0.9996, respectively. The percentage recovery of Teneligliptin was found to be 99.50 - 100.06%. Proposed methods were found to be simple, accurate, precise and rapid and could be used for routine analysis.

***Keywords*:-** Teneligliptin, Dosage Form, Phenomenex Luna.

INTRODUCTION :-

The pharmaceutical analysis is a branch of chemistry, which involves the series of process for the identification, determination, quantitation, and purification. This is mainly used for the separation of the components from the mixture and for the determination of the structure of the compounds. The different pharmaceutical agents are Plants, Minerals Microorganisms and Synthetic compounds[1]

Pharmaceutical analysis plays a vital role in the Quality Assurance and Quality control of bulk drugs. Analytical chemistry involves separation, identification, and determining the relative amounts of components in a sample matrix; Pharmaceutical analysis is a specialized branch of analytical chemistry that derives its principles from various branches of sciences like physics, microbiology, nuclear science, and electronics etc. Qualitative analysis is required before a quantitative analysis can be undertaken.

A separation step is usually a necessary part of both a qualitative and quantitative analysis. The result of typical quantitative analysis can be computed from two measurements. One is the mass or volume of sample to be analyzed and the second one is the measurement of some quantity that is proportional to the amount of analyte in that sample and normally completes the analysis.

The main steps that are performed during a chemical analysis are the following:

(1) Sampling,
(2) Field Sample Pretreatment,
(3) Laboratory Treatment,
(4) Laboratory Assay,
(5) Calculations, And
(6) Results Presentation.

Each must be executed correctly in order for the analytical result to be accurate. Some analytical chemists distinguish between an analysis, which involves all the steps, and an assay, which is the laboratory portion of the analysis[2]

The complete analysis of a substance consists of 5 main steps.

1. Sample preparation / Sampling
2. Dissolution of the sample,
3. Conversion of the analyte into a form suitable for measurement.
4. Measurement
5. Calculation and interpretation of the measurement

Need for pharmaceutical Analysis

1. New Drug Development.
2. Method Validation as for ICH Guidelines
3. Research in Pharmaceutical Sciences
4. Clinical Pharmacokinetic Studies

When promising results are obtained from explorative validation performed during the method development phase, then only full validation should be started. The process of validating a method cannot be separated from the actual development of method condition[3]

Chromatographic Techniques

In 1903 a Russian botanist Mikhail Tswett produced a colorful separation of plant pigments through calcium carbonate column. Chromatography word came from Greek language chroma = color and graphein = to write i.e. color writing or chromatography [4, 5] During 1970's, most chemical separations were carried out using a variety of techniques including open-columnchromatography,paper chromatography, and thin-layer chromatography. However, these chromatographic techniques were inadequate for quantification of compounds and resolution between similar compounds. During this time, pressure liquid chromatography began to be used to decrease flow through time, thus reducing purification times of compounds being isolated by column chromatography. However, flow rates were inconsistent, and the question of whether it was better to have constant flow rate or constant pressure was debated[6]. High pressure liquid chromatography was developed in the mid-1970's and quickly improved with the development of column packing materials and the additional convenience of online detectors. In the late 1970's, new methods including reverse phase liquid chromatography allowed for improved separation between very similar compounds. By the 1980's HPLC was commonly used for the separation of chemical compounds. New techniques improved separation, identification, purification and quantification far above the previous techniques. Computers and automation added to the convenience of HPLC. Improvements in type of columns and thus reproducibility were made as such terms as micro-column, affinity columns, and Fast HPLC began to immerge. By the 2000 very fast development was undertaken in the area of column material with small particle size technology and other specialized columns. The dimensions of the General Introduction typical HPLC column are 100-300 mm in length with an internal diameter between 3-5 mm. The usual diameter of micro-columns, or capillary columns, ranges from 3 μm to 200 μm [7]. In this decade sub 2 micron particle size technology (column material packed with silica particles of < 2μm size) with modified or improved HPLC instrumentation becomes a popular with different instrument brand name like UPLC (Ultra Performance Liquid Chromatography) of Waters and RRLC (Rapid Resolution Liquid Chromatography) of Agilent.

Classification of Chromatographic Techniques

I] According to the nature of stationary and mobile phase

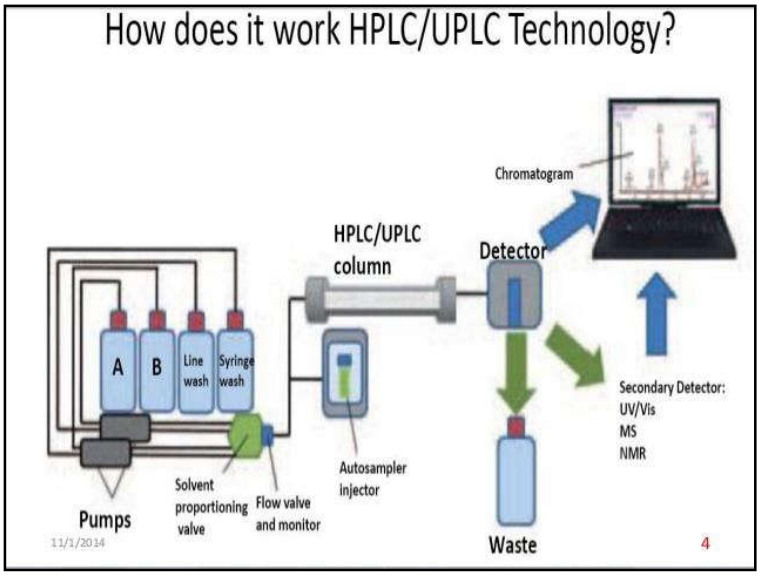
- Gas Solid Chromatography
- Gas Liquid Chromatography
- Solid Liquid Chromatography
- Liquid Liquid Chromatography

II] According to mechanisms of separation, chromatographic methods are divided into

1. Adsorption chromatography
2. Partition chromatography
3. Ion exchange chromatography
4. Ion pair chromatography
5. Size exclusion or gel permeation chromatography
6. Affinity chromatography
7. Chiral phase chromatography.

RP-UPLC :-

UPLC is a chromatographic technique that can separate a mixture of compounds, and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture. Utilizing a small packaging particle sizes columns and ultra-high pack pressure UPLC can be regarded as a new invention for liquid chromatography. The major differences between HPLC and UPLC are the use of smaller particles in the column of the UPLC and the greater pressure at which the UPLC operates. Advantages of this UHPLC is decreases the length of column, reduces solvent consumption& saves time. Therefore by using smaller particles, speed and peak capacity (number of peaks resolved per unit time in gradient separations) can be extended to new limits. The technology takes full advantage of chromatographic principles to run separations Using columns packed with smaller particles (less than2.5µm) and/or higher flow rates.



Principle:

The underlying principle of UPLC is based on the van Deemter relationship which explains the correlation between flow rate and plate height[8]. The van Deemter equation (i) shows that the flow range with the smaller particles is much greater in comparison with larger particles for good results [9,10,11,12]Van Demter equation, which is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency).

$$H=A+B/v+Cv$$

Where;

A, B and C are constants

v is the linear velocity, the carrier gas flow rate.

- The A term is independent of velocity and represents "eddy" mixing. It is smallest when the packed column particles are small and uniform.
- The B term represents axial diffusion or the natural diffusion tendency of molecules. This

effect is diminished at high flow rates and so this term is divided by v.

- The C term is due to kinetic resistance to equilibrium in the separation process.
- The kinetic resistance is the time lag involved in moving from the gas phase to the packing stationary phase and back again.

Efficiency is proportional to column length and inversely proportional to the particle size. Smaller particles provide increased efficiency as well as the ability to work at increased linear velocity without a loss of efficiency, providing both resolution and speed. Efficiency is the primary separation parameter behind UPLC since it relies on the same selectivity and retentivity as HPLC. In the fundamental resolution (Rs).

MATERIALS & METHODS :-

- **1-Octane sulfonic acid Sodium salt -** HPLC Grade Maker: RANKEM
- **Acetonitrile** HPLC Grade Maker: RANKEM
- **Ortho-Phosphoric Acid - AR Grade Maker: RANKEM**
- **Methanol: HPLC Grade**
- **HPLC Grade Water**

METHOD USED FOR ASSAY OF TENELIGLIPTIN TABLETS STRENGTH: 20 mg

Chromatographic parameters:

Column : Phenomenex Luna C18, 50×3.0mm, 3µm particle size
Flow rate : 0.5 mL/min
Run Time : 3 minutes
Wavelength : 245nm
Temperature : Ambient
Injection Volume : 2µL
Mobile phase composition: pH 3.5
Buffer: Acetonitrile (50:50v/v)

Preparation of pH 3.5 Buffer solution: Weigh and dissolve 1.00 g of 1-Octane sulfonic acid Sodium salt to 1000 ml of water. Adjust pH of solution to 3.5with dilute orthophosphoric acid.

Preparation of mobile phase:

1. Mix pH 3.5 Buffer solution and acetonitrile in the ratio 50:50(v/v)
2. Filter through 0.45µm membrane filter and degas for about 10min.

Note: Do not use the mobile phase preparation beyond 2days of bench top or 5days in refrigerator.

Diluent: Use mobile phase as diluent.

Preparation of standard stock solution:

Weigh accurately and transfer about 75 mg Teneligliptin Hydrobromide working standard which is equivalent to about 50 mg of Teneligliptin into a 100ml volumetric flask, add about 35ml of diluent, sonicate to dissolve the material completely, dilute to volume with diluent and mix.

Preparation of standard solution:

1. Pipette 5ml of the above solution into a 25ml volumetric flask, dilute to volume with diluent and mix.
2. Filter through 0.45µm filter.

Note: Do not use the standard preparation beyond 2days in refrigerator.

Test preparation:

1. Weigh and crush not less than 20 tablets using mortar and pestle.

2. Weigh and transfer the tablet powder equivalent to about 10 mg of Teneligliptin into a 100ml volumetric flask, add about 75ml of diluent, sonicate for 30minutes with intermediate shaking and dilute to volume with diluent.
3. Centrifuge a portion of above solution at 2500rpm for about 10minutes by using centrifuge tubes with caps.
4. From the above supernatant solution is used.
5. Filter through 0.45µm filter.
6. **Note:** Do not use the test preparation beyond 2days on bench top or 5days in refrigerator.

System suitability:

1. Inject about 10 µL portion of standard solution into the chromatographic system and measure the response of major peak.
2. The tailing factor for Teneligliptin peak should be NMT 2.0
3. The RSD for the area of Teneligliptin peak obtained from the 5 replicates injections of standard preparation should be NMT 2.0%.

Procedure:

Inject about 10 µL portion of diluent and test preparation into the chromatograph, record the chromatogram and measure the response of major peak.

Calculations:

Sample area x Standard dilution x purity x average weight x 100 X 0.6829

Standard area x Sample dilution x 100 x label claim Where: 0.6829 is conversion factor

ASSAY METHOD DEVELOPMENT AND OPTIMISATION :-

1. Detection method & Selection of wavelength:

Known concentration of Teneligliptin working standard was taken and dissolved in solvent such that the standard solution contains about 10ppm. Placebo and blank solutions were also prepared. All these solutions were scanned between 200 to 400nm using UV-Visible spectrophotometer. From the UV spectrum at the drug shows good absorbance 245 nm.

After reviewing the chromatograms and peak purity chromatograms a wavelength of 245 is selected as the optimum wavelength for this drug.

2. Selection of column:

As the Method is UHPLC various columns cannot be selected as the method is highly accurate, hence a particular UHPLC column is selected i.e., Phenomenex Luna C18(2) ; 3µm ; (3*50mm) which gave good peak shape, retention time, tailing factor, column efficiency. Hence Phenomenex column is selected

3. Optimization of mobile phase:

On the basis of retention property study results of the drug and reviewing the results“1-Octane sulfonic acid Sodium salt” is decided as the buffer preparation to be used.

Selection of pH of the buffer:

‘pH’ plays very an important role in achieving the chromatographic separation as it controls the elution properties by controlling ionization characteristics.

By altering the pH of the mobile phase different trials were carried out and finally optimized pH was found to be 3.5

At pH 3.5 peak shape, peak tailing and theoretical plate count was found to be satisfactory and hence 3.5 is decided as the pH of the Buffer.

Mobile phase composition:

Many trials on composition of Buffer and Acetonitrile were made to decide the ultimate composition of the mobile phase.

After reviewing many trials good peak shape, retention time, tailing factor, theoretical plates are obtained with the mobile phase composition Buffer: ACN (50:50). Hence it was finalized.

4. Selection of flow rate:

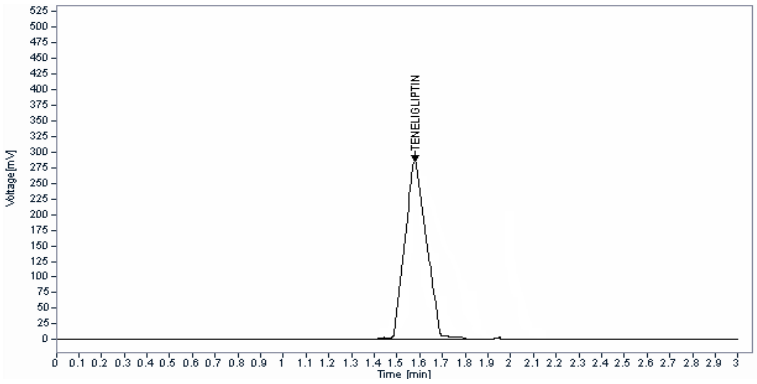
Flow rate selection is done depending on the retention time, peak symmetry, hence many trials were made to decide the flow rate and 0.5 ml /min was found to be acceptable as the peak is sharp. Hence 0.5 ml/ min flow rate was selected for this project

METHOD DEVELOPMENT PHASE CHROMATOGRAMS: TRAIL-I

Chromatographic Conditions:

Mobile phase	:	Water : Methanol (80 :20% v/v)
Column	:	Phenomenex Luna C18 (50 mm x 3.0 mm, 3.0µm)
Flow rate	:	0.5 ml
Wavelength	:	245 nm
Column Temperature	:	Ambient
Injection Volume	:	2µl

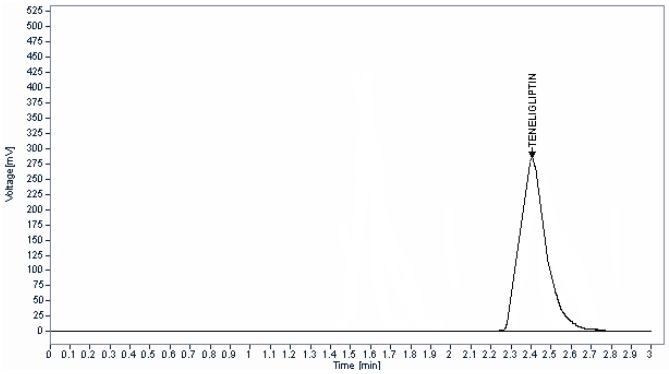
Observation: From the above chromatogram it was observed that the peak is broad and shows tailing.



TRAIL II :

Chromatographic Conditions:

Mobile phase	:	Water : ACN (70:30 % v/v)
Column	:	Phenomenex Luna C18 (50 mm x 3.0 mm, 3.0µm)
Flow rate	:	0.5 ml
Wavelength	:	245 nm
Column Temperature	:	Ambient
Injection Volume	:	2µl



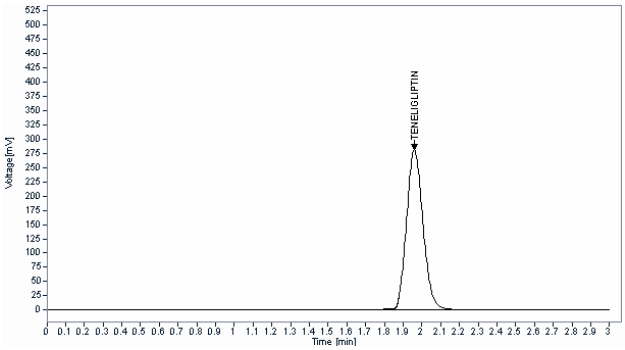
Observation: From the above chromatogram it was observed that the peak shape and tailing factor is not upto the aim of the project.

TRAIL III :-

Chromatographic Conditions:

Mobile phase	:	ACN: Phosphate buffer pH 6.0 (30:70% v/v)
Column	:	Phenomenex Luna C18 (50 mm x 3.0 mm, 3.0µm)
Flow rate	:	0.5 ml
Wavelength	:	245 nm
Column Temperature	:	Ambient
Injection Volume	:	2µl

Observation: From the above chromatogram it was observed that the peak shape is not sharp and retention time is not upto the aim of the project.

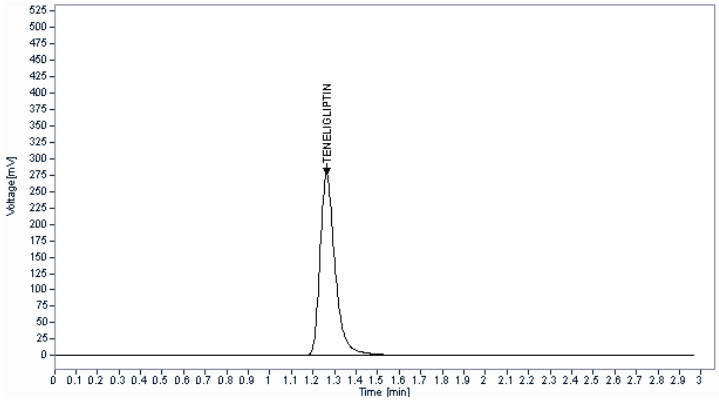


TRAIL IV:

Chromatographic Conditions:

Mobile phase	:	ACN: Phosphate buffer pH 4.5 (40 :60 % v/v)
Column	:	Phenomenex Luna C18 (50 mm x 3.0 mm, 3.0µm)
Flow rate	:	0.5 ml
Wavelength	:	245 nm
Column Temperature	:	Ambient
Injection Volume	:	2µl

Observation: From the above chromatogram it was observed that the peak shape is not sharp and retention time is not upto the aim of the project.

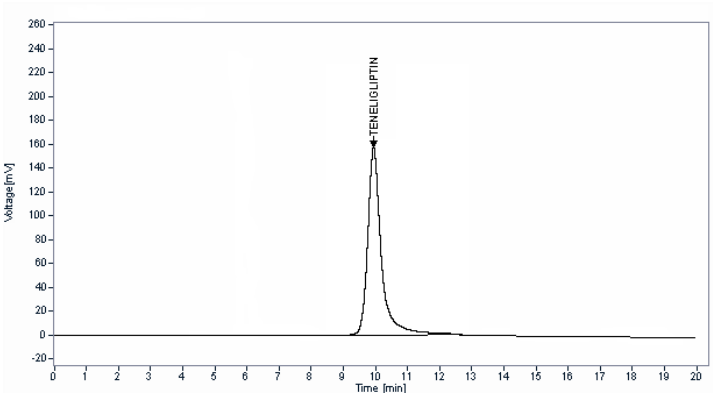


TRAIL V:

Chromatographic Conditions:

Mobile phase	:	Methanol: Phosphate buffer pH 4.0 (50:50 % v/v)
Column	:	Phenomenex Luna C18 (50 mm x 3.0 mm, 3.0µm)
Flow rate	:	0.5 ml
Wavelength	:	245 nm
Column Temperature	:	Ambient
Injection Volume	:	2µl

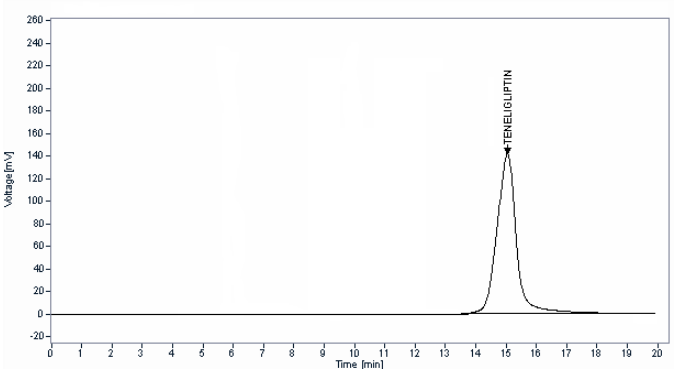
Observation: From the above chromatogram it was observed that theobtained Retention time, peak shape, tailing factor, theoretical plates is not upto the aim of the project



TRAIL VI : Chromatographic Conditions:

Mobile phase	:	ACN: 1-Hexane sulfonic acid Sodium salt pH 3.5(50:50 % v/v)
Column	:	Phenomenex Luna C18 (50 mm x 3.0 mm, 3.0µm)
Flow rate	:	0.5 ml
Wavelength	:	245 nm
Column Temperature	:	Ambient
Injection Volume	:	2µl

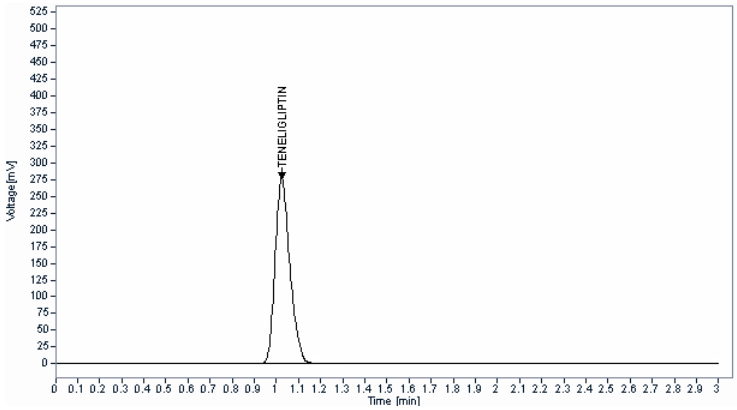
Observation: From the above chromatogram it was observed that the obtained Retention time, peak shape, tailing factor, theoretical plates is not upto the aim of the project.



TRAIL VI :

Chromatographic Conditions:

Mobile phase : Methanol: 1-Octane sulfonic acid Sodium salt pH 3.5(60:40%V/V)
Column: Phenomenex Luna C18 (50 mm x 3.0 mm, 3.0µm)
Flow rate : 0.5 ml
Wavelength : 245 nm Column
Temperature : Ambient Injection
Volume : 2µl



Observation: From the above chromatogram it was observed that the obtained Retention time, peak shape, tailing factor, theoretical plates is not up to the aim of the project.

TRIAL VIII:

Chromatographic Conditions:

Mobile phase	:	ACN: 1-Octane sulfonic acid Sodium salt pH 3.5 (50:50 % v/v)
Column	:	Phenomenex Luna C18 (50 mm x 3.0 mm, 3.0µm)
Flow rate	:	0.5 ml
Wavelength	:	245 nm
Column Temperature	:	Ambient
Injection Volume	:	2µl

Observation: From the above chromatogram it was observed that the obtained Retention time, peak shape, tailing factor, theoretical plates, satisfies the aim of the project.

Hence this is optimized chromatographic conditions.

Optimized Chromatographic Conditions for Teneligliptin was		
Mobile phase	:	Buffer :ACN(50:50 v/v)
Buffer	:	1.0 g of 1-Octane sulfonic acid Sodium salt to 1000 ml of water. Adjust pH of solution to 3.5with dilute orthophosphoric acid
pH	:	3.5
Column	:	Phenomenex Luna C18 (50 mm x 3.0mm, 3.0µm)
Flow rate	:	0.5 ml/min
Wavelength	:	245 nm
Column Temperature	:	Ambient
Injection Volume	:	2µl
Run time	:	3 minutes

SYSTEM SUITABILITY:

1. Inject about 2 µL portion of standard solution into the chromatographic system five injections and measure the response of major peak.
2. The tailing factor for Teneligliptin peak should be NMT2.0
3. The RSD for the area of Teneligliptin peak obtained from the 5replicates injections of standard preparation should be NMT 2.0%.

Injection	**Retention time**	**Area**	**Theoretical plates**	**Tailing factor**
BLANK	0.0	0	0	0.00
STANDARD-1	1.02	1361.354	2125	1.05
STANDARD-2	1.02	1365.524	2123	1.05
STANDARD-3	1.02	1364.443	2124	1.05
STANDARD-4	1.02	1363.557	2126	1.06
STANDARD-5	1.02	1364.756	2125	1.06
AVERAGE	-	1363.927	-	-
STDEV	-	1.602	-	-
%RSD	-	0.1	-	-

Fixed chromatographic conditions are as follows:

Instrument : UHPLC_AGILENT_1220 INFINITY LCwith UV detector and auto sampler.
Column : Phenomenex Luna C18, 50×3.0mm, 3µm particle size.
Flow rate : 0.5 mL/min
Wavelength : 245nm
Temperature : Ambient
Injection Volume : 2µL
Mobile phase composition: pH 3.5
Buffer: Acetonitrile (50:50V/v)

ASSAY METHOD VALIDATION

1. PRECISION

Precision covers

- System precision
- Method precision

System Precision

Six replicate injection of homogeneous standard solution indicate performance of UHPLC instrument under chromatographic conditions. Six sample preparations were prepared and injected at the nominal concentration level i.e., 100 ppm. The results obtained are as shown in Table-2

SYSTEM PRECISION

Sample ID	Rt (min)	Area	Theoretical Plates (N)	Tailing Factor
SYSTEM PRECISION-1	1.02	1361.254	2125	1.05
SYSTEM PRECISION-2	1.02	1365.746	2153	1.03
SYSTEM PRECISION-3	1.03	1363.547	2145	1.06
SYSTEM PRECISION-4	1.02	1364.253	2136	1.05
SYSTEM PRECISION-5	1.02	1366.215	2162	1.05
SYSTEM PRECISION-6	1.02	1365.158	2135	1.06
AVERAGE	3.964	1364.362	2143	1.05
STDEV	-	1.807	-	-
%RSD	-	0.132	-	-
LIMITS	NMT 1%	NMT 2%	NLT 2000	NMT 2

Since the %RSD was found to be below 2%, the system precision parameter passed.

Method precision/Repeatability:

This is determined by preparing 9 samples of homogeneous sample mixture at 3 Concentrations and 3 replicates each and the results obtained are as shown and chromatogram .

METHOD PRECISION

Test Preparation	Injection	Area	%Assay
Low Level	1	1090.24	99.35
	2	1090.05	99.05
	3	1090.34	99.82
Middle Level	1	1364.28	99.76
	2	1364.01	99.78
	3	1364.20	99.28
	1	1635.00	99.43
	2	1634.56	99.64

High Level	3	1634.19	99.17
Mean for Assay			99.48
Standard Deviation			0.285
%RSD			0.286

ACCURACY:

Dosage blends of Teneligliptin tablets was spiked with 10%,20%,and 30 % of known amount of Standard and to obtain110%, 120% and 130% with respect to labeled amount of drug in the formulation. It has been prepared in such a way that the average weight of tablet is kept constant and the weight of API was varied. Test solution was injected and the assay was performed as per the test method. From this "%Recovery" and mg recovered were calculated. The results are as shown in the following Table No: 4 and follows chromatograms.

$$\% \text{ Recovery} = \frac{\text{Sample peak area}}{\text{Standard peak area}} \times \frac{\text{Dilution factor of standard}}{\text{Dilution factor of sample}} \times \text{Potency} \times 100$$

ACCURACY

Spiked Level	API Added (mg)	API Recovered (mg)	%Recovery
110 %	2.0001	1.9917	99.58
110 %		1.9919	99.59
110 %		1.9943	99.71
120%	4.0002	3.9804	99.50
120%		3.9925	99.81
120 %		3.9943	99.85
130 %	6.0004	6.0040	100.06
130%		5.9902	99.83
130 %		5.9819	99.69

Acceptance criteria here are that the % Recovery calculated should be between 98% to 102%. Here percentage Recovery is calculated using the above formula.

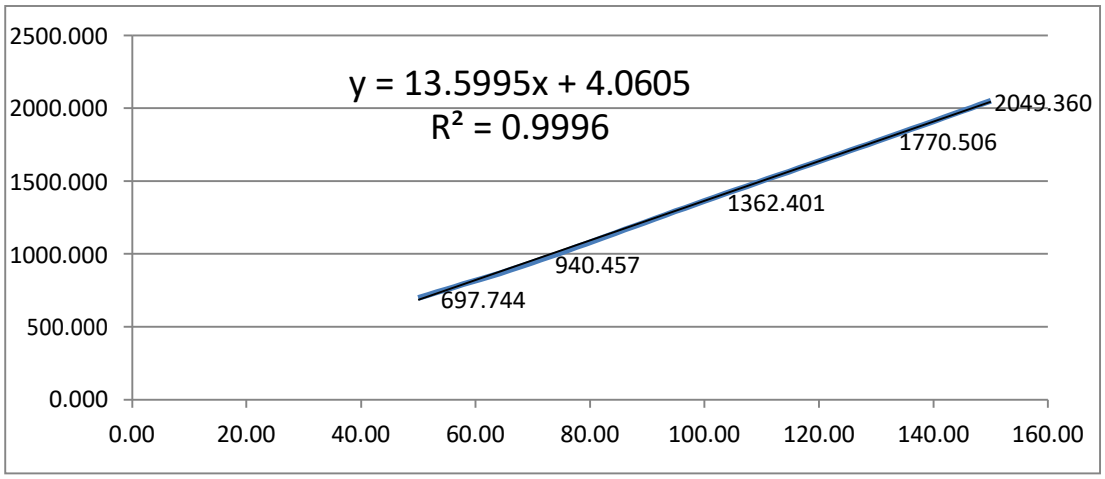
LINEARITY

Linearity has been demonstrated in the range of 50-150% of target concentration of the assay. Five series of standard solutions in the above concentration range were injected in duplicate. Standard solution in concentrations of 50, 70, 100, 130, 150 ppm were prepared and injected and the peaks areas and other statistical data are given in Table No: 5. A calibration curve was determined for the drug independently by plotting the peak areas obtained against concentrations (in percentage). There exists a linear relationship in the two graphs for the two concentration ranges which are prepared. From the data obtained correlation coefficient, Y-intercept and slope were calculated to provide mathematical estimates of the degree of linearity.

Chromatograms are as shown below, From the chromatograms, average area of Teneligliptin is calculated and is tabulated as follows. From this response values a linear curve is obtained as shown in below.

LINEARITY CURVE FOR TENELIGLIPTIN

	API Percentage				
	50%	**70%**	**100%**	**130%**	**150%**
Trial-1	697.839	940.541	1362.465	1770.863	2049.276
Trial-2	697.648	940.374	1362.337	1770.148	2049.443
Average	697.744	940.458	1362.401	1770.506	2049.360



Linearity Curve for Teneligliptin

RESULTS AND DISCUSSION :-

The new analytical method for the UHPLC method was established for Teneligliptin then optimized and then applied on pharmaceutical dosage forms.

Various mobile phase systems were prepared and used to provide an appropriate chromatographic separation, but the proposed mobile phase comprising of Buffer and Acetonitrile in the ratio 50: 50 gave a better resolution and sensitivity.

The detection was carried out by using UV detector at 245nm using Phenomenex Luna C18; 50 mm x 3.0 mm,3µm .Among the several flow rates tested, the flow rate of 0.5 ml was found to be the best for Teneligliptin with respect to retention times and theoretical plates.

The retention time is 1.02 for Teneligliptin. The asymmetry factor or the tailing factor was found to be 1.0 for Teneligliptin, which indicates symmetrical nature of the peak

System suitability parameters such as retention time, tailing factor, capacity factor and number of theoretical plates were calculated. The number of theoretical plates was found to be around 2100 for Teneligliptin, which indicates efficient performance of the column. These parameters represent the specificity of the method.

Linearity range was evaluated by the visual inspection of plot of peak area as a function of analyte concentration and the corresponding calibration graphs were shown. From the linearity

studies, the specified concentration range was determined. It was observed that Teneligliptin was linear in the range of 50% to 150% for the target concentrations.

The validation of the proposed method was verified by system precision and method precision. The %RSD for system precision and method precision of Teneligliptin was tabulated.

The validation of proposed method was verified by recovery studies. The percentage recovery range was found to be within the limits for Teneligliptin. This is a good index of accuracy, specificity and repeatability of the method. The obtained results were tabulated.

Placebo interference studies were made by injecting placebo alone, then the standard and the placebo along with the standard. They did not show any interference of placebo at the RT of the analyte peak

Study of ruggedness was made by conducting the study on different system and by two analysts. The results were found to be in limits and were tabulated and hence the developed method is found to be rugged.

Summary & Conclusion

A simple, reproducible and efficient reverse phase Ultra high-performance liquid chromatography (RP-UHPLC) method has been developed for estimation of Teneligliptin in its tablet dosage form. Separation was done by using mobile phase consists of Mixture of 1-OctaneSulphonic acid sodium salt (pH adjusted to 3.5 ±0.05 with Orthophosphoric acid): Acetonitrile (50:50,v/v). Chromatography separations were carried out on Phenomenex Luna C18 column (50 X 3.0mn; 3µm) at a flow rate of 0.5ml/min and UV detection at 245nm and the retention time for Teneligliptin is 1.02 minutes. The linear dynamic response was found to be in the concentration of 50µg-150µg/ml. The slope, intercept and Correlation coefficient was found to be 0.9996 respectively. The percentage recovery of Teneligliptin was found to be 99.50 - 100.06%. Proposed methods were found to be simple, accurate, precise and rapid and could be used for routine analysis.

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