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Simultaneous Estimation of Dapagliflozin and Teneligliptin by Analytical Method Development Using HPLC

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KEYWORDS

Dapagliflozin and Teneligliptin method development, HPLC method for dapagliflozin and Teneligliptin. Dapagliflozin and Teneligliptin combination method development, Method Development.

ABSTRACT:

Analytical methods need to be validated or revalidated prior to their introduction into routine analyses. Chromatography is an analytical techniques based on the separation of molecules due to differences in their structure and/or composition. In general, Chromatography involves moving a sample through the system over a stationary phase. The molecules in the samples will have different affinities and interaction with the stationary support, leading to separation of Molecules. Samples components that display stronger interaction with the stationary phase will move more slowly through the column than components with weaker interaction. Different compounds can be separated from each other as they move through the column. Chromatographic separation can be carried out using a variety of stationary phases. High-Performance liquid chromatography (HPLC) is types of liquid Chromatography used to separate and quantify compounds that have been dissolved in Solution.

INTRODUCTION:

HPLC can be used to determine the amount of a specific compound in a solution.

These different dosages analyzed by different method including crystal structure elution, polarimetry, UV, IR, HPLC, LCMS, and such more technique are useful in different types of analysis in different dosage form. But now a day most important and better techniques in HPLC and GC by using HPLC simple reverse phase Chromatographic method develop for the determination of active content and relative impurities, while it is better to find stability indicating method for analysis. Because after some time impurities present in the product increase more than its limits and if impurities elute very close to the main drug the possibility of merging impurities in to main drug in cresses and it result in to the failure of method.

MATERIAL AND METHODS:

MATERIALS AND INSTRUMENTS

Materials:

Drugs

Table No. 1 Procurement of Drug Samples

Sr. No.	Name of the Drug	Taken from
1	Dapagliflozin and Teneligliptin	Vidisha Aalytical

Reagents

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Table No. 2 List of Reagents Used

Sr. No.	Chemicals/ Reagents/ Solvents	Supplier	Grade
1	Methanol	Merck	HPLC grade
2	Acetonitrile	Merck	HPLC grade
3	Water	Siddhi Lab	HPLC grade

Instruments:

Table No. 3 List of Instruments used

UV – Visible Spectrophotometer Double beam UV- Visible spectrophotometer	
Model	UV 550
Make	Jasco

Analytical Balance Azcet High Precision Balance	
Model	CY 224C
Maximum	220 gm
Minimum	0.001gm
pH Meter Digital pH meter	
Make	LabMan
Sonicator Bio-technic Ultra Sonicator	
Capacity	13.5 Litre
Filter	
Membrane	Nylon 0.45 µm
Membrane	PVDF 0.45 µm

EXPERIMENTAL WORK :

PRELIMINARY CHARACTERIZATION OF DRUG :

Color, odour and appearance :-Dapagliflozin Propanediol Monohydrateand Teneligliptin Hydrobromide hydrate was evaluated for parameters like color; odour & appearance are shown in result**.**

Teneligliptin hydrobromide hydrate factor calculations

Moleculaer weight of Teneligliptin hydrobromide hydrate: 628.9

Moleculaer weight of Teneligliptin: 426.6

$$Factor = \frac{Molecular\ weight\ of\ \text{Teneligliptin}}{Molecular\ weight\ of\ \text{Teneligliptin hydrobromic}}$$

$$Factor = \frac{426.6}{628.9}$$

Factor = 0.678

Example:

When we want to weigh 10 mg of Teneligliptin, in that case we need to calculate the weight of Teneligliptin hydrobromide hydrate as follows:

Weight of Teneligliptin hydrobromide hydrate = weight of Teneligliptin / factor

So, Weight of Teneligliptin hydrobromide hydrate = 10 / 678

Weight of Teneligliptin hydrobromide hydrate = 14.75 mg

When we will weigh 14.75 mg of Teneligliptin hydrobromide hydrate, it will contains only 10 mg of Teneligliptin.

Dapagliflozin Propanediol Monohydrateand factor calculations

Moleculaer weight of Dapagliflozin Propanediol Monohydrate: 502.98

Moleculaer weight of Dapagliflozin: 408.873

$$Factor = \frac{Molecular\ weight\ of\ \text{Dapaglifloz}}{Molecular\ weight\ of\ \text{Dapagliflozin Propanediol}}$$

$$Factor = \frac{408.873}{502.98}$$

Factor = 0.813

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Example:

When we want to weigh 10 mg of Dapagliflozin, in that case we need to calculate the weight of Dapagliflozin Propanediol Monohydrateand hydrate as follows:
Weight of Dapagliflozin Propanediol Monohydrateand = weight of Dapagliflozin / factor
So, Weight of Dapagliflozin Propanediol Monohydrateand = 10 / 678
Weight of Dapagliflozin Propanediol Monohydrateand = 12.30 mg
When we will weigh 12.30 mg of Dapagliflozin Propanediol Monohydrateand, it will contains only 10 mg of Dapagliflozin.

Determination of solubility
The solubility was determined in Water and Methanol at a concentration of 3mg/mL as follows and are given in results**.**

Water:
Teneligliptin: Weighed approx 44.25 mg of Teneligliptin hydrobromide hydrate (Equivalent to 30 mg of Teneligliptin) and sonicated for 5-10 minutes to dissolve in 10 ml of Water.
Dapagliflozin: Weighed approx 36.90 mg of Dapagliflozin Propanediol Monohydrate (Equivalent to 30 mg of Dapagliflozin) sonicated for 5-10 minutes to dissolve in 10 ml of Water.
Methanol:
Teneligliptin: Weighed approx 44.25 mg of Teneligliptin hydrobromide hydrate (Equivalent to 30 mg of Teneligliptin) and sonicated for 5-10 minutes to dissolve in 10 ml of Methanol.
dapagliflozin: Weighed approx 36.90 mg of Dapagliflozin Propanediol Monohydrate (Equivalent to 30 mg of Dapagliflozin) sonicated for 5-10 minutes to dissolve in 10 ml of Methanol.
Selection of analytical wavelength-

Selection of solvent -
Methanol was selected as the solvent for dissolving Teneligliptin and Dapagliflozin.
Preparation of standard stock solutions-
Teneligliptin: In order to prepare stock solution, weighed accurately 14.75 mg Teneligliptin hydrobromide hydrate (Equivalent to 10 mg of Teneligliptin) and transferred into 20 ml volumetric flask, added 15 ml of methanol and sonicated to dissolve the standard completely and diluted up to the mark with methanol (500 PPM).
Further diluted 0.8 mL to 20 mL with methanol. (20 PPM)
Dapagliflozin: In order to prepare stock solution, Weighed accurately 12.30 mg of Dapagliflozin Propanediol Monohydrate (Equivalent to 10 mg of Dapagliflozin) and transferred into 20 ml volumetric flask, added 15 ml of methanol and sonicated to dissolve the standard completely and diluted up to the mark with methanol (500 PPM).
Further diluted 0.8 mL to 20 mL with methanol. (20 PPM)

Selection of analytical wavelength
Methanol as a blank and Teneligliptin and Dapagliflozin standard solution (20 PPM each) was scanned from 400 nm to 200 nm. Absorption maxima was determined for both drug. Teneligliptin and Dapagliflozin showed Q-point at 238 nm shown in results.

Method Development by RP – HPLC

Teneligliptin: In order to prepare stock solution, weighed accurately 29.50 mg Teneligliptin hydrobromide hydrate (Equivalent to 20 mg of Teneligliptin) and transferred into 20 ml volumetric flask, added 15 ml of methanol and sonicated to dissolve the standard completely and diluted up to the mark with methanol (1000 PPM).
Further diluted 1 mL to 10 mL with Mobile phase (100 PPM). It was prepared in mobile phase of each trial and injected in development trials.
Dapagliflozin: In order to prepare stock solution, Weighed accurately 24.60 mg of Dapagliflozin Propanediol Monohydrate (Equivalent to 20 mg of Dapagliflozin) transferred into 20 ml volumetric flask, added 15 ml of methanol and sonicated to dissolve the standard completely and diluted up to the mark with methanol (1000 PPM).
Further diluted 1 mL to 10 mL with Mobile phase (100 PPM). It was prepared in mobile phase of each trial and injected in development trials.

Selection of analytical wavelength for HPLC method development: Analytical wavelength for the

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examination was selected from the Q-point from the spectrophotometric analysis and it was 238 nm**.**

Optimization of HPLC method

Following trials are taken for estimation of Teneligliptin and Dapagliflozin.

Principle: Reversed Phase Liquid Chromatography with Isocratic elution and UV detection.

Trial 1:
Chromatographic Conditions:
Standard solution: Teneligliptin 100 PPM and Dapagliflozin100 PPM
Detector: U.V. Detector
Column: Inertsil ODS-3V
Column Dimension: (150 mm X 4.6 mm i.d.) 5µm
Column Oven temperature: 40°C
Injection Volume: 20 µl
Wavelength: 238 nm
Mobile phase: Methanol : water (70:30)
Flow Rate: 1.0 ml/min
Observation: The trial 1 results are shown in results.

Trial 2:
Chromatographic Conditions:
Standard solution: Teneligliptin 100 PPM and Dapagliflozin100 PPM
Detector: U.V. Detector
Column: Inertsil ODS-3V
Column Dimension: (150 mm X 4.6 mm i.d.) 5µm
Column Oven temperature: 40°C
Injection Volume: 20 µl
Wavelength: 238 nm
Mobile phase: Acetonitrile : water (70:30)
Flow Rate: 1.0 ml/min
Observation: The trial 1 results are shown in results.

Trial 3:
Chromatographic Conditions:
Standard solution: Teneligliptin 100 PPM and Dapagliflozin100 PPM
Detector: U.V. Detector
Column: Inertsil ODS-3V
Column Dimension: (150 mm X 4.6 mm i.d.) 5µm
Column Oven temperature: 40°C
Injection Volume: 20 µl
Wavelength: 238 nm
Mobile phase: Methanol : 0.05% OPA in water (70:30)
Flow Rate: 1.0 ml/min
Observation: The trial 3 results are shown in results.

Trial 4:
Chromatographic Conditions:
Standard solution: Teneligliptin 100 PPM and Dapagliflozin100 PPM
Detector: U.V. Detector
Column: Inertsil ODS-3V
Column Dimension: (150 mm X 4.6 mm i.d.) 5µm
Column Oven temperature: 40°C
Injection Volume: 20 µl
Wavelength: 238 nm
Mobile phase: Methanol : 0.05% OPA in water (60:40)
Flow Rate: 1.0 ml/min
Observation: The trial 4 results are shown in results.

Optimized Chromatographic condition: Trial no. 4 considered as optimized chromatography which is as follows:
Chromatographic Conditions:
Detector: U.V. Detector
Column: Inertsil ODS-3V
Column Dimension: (150 mm X 4.6 mm i.d.) 5µm
Column Oven temperature: 40°C
Injection Volume: 20 µl
Wavelength: 238 nm
Mobile phase: Methanol : 0.05% OPA in water (60:40)
Flow Rate: 1.0 ml/min
Run time: 14 Minutes

Preparation of System suitability stock solutions:
Teneligliptin: Weighed 29.50 mg Teneligliptin hydrobromide hydrate (Equivalent to 20 mg of Teneligliptin) and transferred in 50 mL volumetric flask, added 35 mL of methanol, sonicated to dissolve it, made volume up to the mark with methanol. (400 PPM)
Dapagliflozin: Weighed accurately 20 mg Dapagliflozin Propanediol Monohydrate (Equivalent to 20 mg of Dapagliflozin) transferred into 50 ml volumetric flask, added 35 ml of methanol and sonicated to dissolve the standard completely and diluted up to the mark with methanol (400 PPM).

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System suitability standard mixture solution:

Pipette out 1.0 mL of Teneligliptin standard stock solution and 0.5 mL of Dapagliflozin standard stock solution and transferred in 20 mL volumetric flask, made volume up to the mark with mobile phase as a diluent.

(Teneligliptin = 20 ppm)

(Dapagliflozin= 10 ppm)

20 PPM of Teneligliptin and 10 PPM of Dapagliflozinare the working concentration.

Marketed formulation contains Teneligliptin (10 mg) and Dapagliflozin (20 mg) in the ratio of 1:2 hence concentration is selected in this ratio.

System suitability is a Pharmacopoeial requirement and is used to verify, whether the chromatographic system is adequate for analysis to be done. The tests were performed by collecting data from Five replicate injection of standard drug solution and the results are recorded.

Acceptance criteria

1. RSD should not be more than 2.0 % for five replicate injections of standard.
2. USP Tailing Factor/ Asymmetry Factor is not more than 2.0.
3. The column efficiency as determined for Plate Count should be more than 2000.

Analysis of marketed Test sample:

Marketed test sample Having Name Zita-D tablets are selected for analysis and for doing validation. It contains Teneligliptin 10 mg and Dapagliflozin 20 mg in the ratio of 1:2.

Average weight of test sample (Zita-D**):**

Weighed the 20 tablets at a time and calculated average weight of tablet by following formula:

Average weight (mg) = Weight of 20 tablets (mg) / 20

Sample preparation of Marketed test sample:

Weighed 20 tablets transferred in mortar pestle and crushed to fine powder. Mixed the contents with butter paper uniformly. Weighed the powder material equivalent to 50 mg of Teneligliptin and 25 mg of Dapagliflozin (639.5 mg of powder material). Transfer it in a clean and dried 100 mL of volumetric flask, added 70 ml of methanol sonicated it for 15 minutes with intermittent shaking. Made the volume up to the mark with methanol. Filter the solution through suitable 0.45 µ syring filter discarding 3-5 mL of filtrate. Further diluted 1 ml of filtrate to 25 ml with diluent. (20 PPM of Teneligliptin and 10 PPM of Dapagliflozin)

Sample Prepared in duplicate. Summary of sample preparation as follows:

Sample	Sample (mg)	Diluted to (mL)	Volume taken (mL)	Diluted to (mL)
Sample 1	639.5	100	1.0	25
Sample 2	639.1	100	1.0	25

Formula for % Assay calculation:

$$\%\ Assay\ of\ Teneligliptin = \frac{Teneligliptin\ Spl\ area}{Teneligliptin\ Std\ avg\ area}\ X\ \frac{Teneligliptin\ STD\ wt\ (mg)}{50}\ X\ \frac{1.0}{20}\ X\ \frac{100}{Tablet\ sample\ weight\ (mg)}\ X\ \frac{25}{1.0}\ X\ \frac{Avg\ wt\ of\ tablet\ (mg)}{Label\ claim\ of\ Teneligliptin\ (mg)}\ X\ F\ X\ 100$$

$$\%\ Assay\ of\ \text{Dapagliflozin} = \frac{\text{Dapagliflozin}Spl\ area}{\text{Dapagliflozin}Std\ avg\ area}\ X\ \frac{\text{Dapagliflozin}STD\ wt\ (mg)}{50}\ X\ \frac{0.5}{20}\ X\ \frac{100}{Tablet\ sample\ weight\ (mg)}\ X\ \frac{25}{1.0}\ X\ \frac{Avg\ wt\ of\ tablet\ (mg)}{Label\ claim\ of\ \text{Dapagliflozin}(mg)}\ X\ F\ X\ 100$$

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VALIDATION OF RP-HPLC METHOD

The developed method for estimation of Dapagliflozin and Teneligliptin was validated as per ICH guidelines for following parameters.

1) FILTRATION STUDY:

Filtration study of an analytical procedure checks the interference of extraneous components from filter, deposition on filter bed and compatibility of filter with sample.

This study was conducted with Test sample solution. (Zita-D Tablet solution)

Filtration study carried out with unfiltered (Centrifuged at 3000 RPM for 5 minutes) and filtered test solution. During filtration activity 0.45 µm PVDF and 0.45 µm Nylon syringe filters used by discarding 5 mL of aliquot sample.

2) STABILITY OF ANALYTICAL SOLUTION

Stability study was conducted for standard and test sample solution. Stability study was performed at normal laboratory conditions.

The solution was stored at normal illuminated laboratory conditions and analyzed after 12 hours and 24 hours.

Standard and Test solution stability study was performed by calculating the difference between results of test solution at each stability time point to that of initial.

3) SPECIFICITY:

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

Following solution shall be prepared and injected to prove the specificity nature of the method. (Checked peak purity for standard and test sample solution)

I. Blank (Diluent)
II. Placebo
III. Teneligliptin and Dapagliflozin Standard solution mixture
IV. Tablet test sample solution

Analyzing marketed test sample contains excipients (additives) which are totally unknown. So Placebo prepared at lab level by using formula as follows:

Sr. No.	Ingredients	Role	Qty (mg)
1	Lactose	Filler	80
2	Starch	Binder	5
3	Magnesium stearate	Lubricant	5
4	Talc	Glidant	5
5	Crospovidone	Disintegrants	5
Total			100 mg

Total 10 gm of placebo prepared:

Placebo Sample solution preparation:

Weighed 535.0 mg of placebo material (Which is equivalent to 50 mg of Teneligliptin and 25 mg of Dapagliflozin) and transferred to clean and dried 100 mL of volumetric flask. Added 70 mL of methanol, sonicated for 15 minutes with intermittent shaking. After 15 minutes allow to cool the solution to room temperature and made volume up to the mark with methanol. Filtered the solution through suitable 0.45 µ syringe filter discarding 3-5 mL of initial filtrate. Further dilute 1.0 ml of filtered stock solution to 25 ml with diluent, injected the resultant solution and chromatograms were recorded.

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4) LINEARITY AND RANGE

Preparation of linearity solution

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

5 levels of Linearity was performed from 50% to 150% of working concentration

Linearity stock solutions:

Teneligliptin: Weighed 29.50 mg Teneligliptin hydrobromide hydrate (Equivalent to 20 mg of Teneligliptin) and transferred in 50 mL volumetric flask, added 30 mL of methanol, sonicated to dissolve it completely, made volume up to the mark with methanol. (400 PPM)

Dapagliflozin: Weighed 26.40 mg of Dapagliflozin Propanediol Monohydrate (Equivalent to 20 mg of Dapagliflozin) transferred in 100 mL volumetric flask, added 70 mL of methanol, sonicated to dissolve it completely, made volume up to the mark with methanol. (200 PPM)

Linearity levels prepared as follows:

Level	Teneligliptin Stock solution (mL)	DapagliflozinStock solution (mL)	Diluted to with diluent	Teneligliptin Conc (µg/mL)	DapagliflozinConc (µg/mL)
50%	0.50	0.50	20	10.00	5.00
75%	0.75	0.75	20	15.00	7.50
100%	1.00	1.00	20	20.00	10.00
125%	1.25	1.25	20	25.00	12.50
150%	1.50	1.50	20	30.00	15.00

Determination

Each level injected in triplicate and mean area calculated. Calibration curve was plotted graphically as a function of analyte concentration in µg/mL on X-axis Vs mean area on y-Axis as given in results.

Acceptance criteria

Correlation Coefficient: NLT 0.98

Intercept: To be report

Slope: To be report

5) Limit of Detection (LOD) and Limit of Quantitation (LOQ):

Detection limit:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Quantitation limit:

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

As per ICH Q2R1 guidelines LOD and LOQ was determined by using the approach Based on the Calibration Curve in which residual standard deviation of a regression line was calculated and determined the LOD and LOQ by using following formula:

$LOD = 3.3\ \sigma / S$

$LOQ = 10\ \sigma / S$

Where,

σ = residual standard deviation of a regression line

S = Slope of regression line

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6) ACCURACY (% RECOVERY)

The accuracy of the analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value of the value found,

Accuracy will be conducted in the range from 50 % to 150 % of working concentration. Solution of each accuracy level was prepared in triplicate. Calculated % Recovery for each sample, Mean % recovery for each level and overall recovery and also calculated % RSD for each level and % RSD for overall recovery.

Accuracy levels details:

Refer Following table for each sample:

Level (%)	Teneligliptin HBr Hydrate API (mg)	Dapagliflozin Propanediol Monohydrate API (mg)	Wt of Placebo (mg)	Diluted to (mL)	Volume taken (mL)	Diluted to (mL)	Teneligliptin Added Conc (µg/mL)	Dapagliflozin Added Conc (µg/mL)
50	36.8	15.60	535.1	100	1	25	9.98	5.07
	37.0	15.40	535.2	100	1	25	10.03	5.01
	37.2	15.70	535.0	100	1	25	10.09	5.11
100	73.8	30.8	535.6	100	1	25	20.01	10.02
	73.4	30.3	535.2	100	1	25	19.91	9.85
	73.6	30.9	535.7	100	1	25	19.96	10.05
150	110.6	46.50	534.8	100	1	25	29.99	15.12
	110.8	46.30	535.3	100	1	25	30.05	15.06
	110.5	46.70	535.1	100	1	25	29.97	15.19

Procedure for preparation of Accuracy sample solution:

Take clean and dried 9 volumetric flask of 100 mL. Weighed aprrox 535.0 mg of placebo and transferred in each 100 mL volumetric flask. Weighed Teneligliptin hydrobromide hydrate and Dapagliflozin Propanediol Monohydrate API as per accuracy level and transferred in same 100 ml voluetric flask. Added 70 mL of methanol and sonicated it for 15 minutes with intermittent shaking. Made the volume up to the mark with methanol. Filter the solution through 0.45 µ Nylon syring filter discarding 3-5 mL of filtrate. Further dilute 1.0 ml of filtrate to 25 ml with diluent.

Acceptance criteria

1. % Recovery for each sample and Mean recovery and overall recovery should be in the range of 98-102%.
2. The Relative Standard Deviation should not be more than 2.0%.

7) PRECISION

Precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous test under the prescribed conditions. Precision is of two types, Repeatability and Intermediate precision. It is performed on tablet test sample.

I.Repeatibility:

Preparation of sample solution (6 Samples prepared):

Weighed 20 tablets transferred in mortar pestle and crushed to fine powder. Mixed the contents with butter paper uniformly. Weighed the powder material equivalent to 50 mg of Teneligliptin and 25 mg of Dapagliflozin (639.5 mg of powder material). Transfer it in a clean and dried 100 mL of volumetric flask, added 70 ml of methanol sonicated it for 15 minutes with intermittent shaking. Made the volume up to the mark with methanol. Filter the solution through Nylon 0.45 µ syring filter discarding 3-5 mL of filtrate. Further diluted 1 ml of filtrate to 25 ml with diluent.

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(20 PPM of Teneligliptin and 10 PPM of Dapagliflozin) Six samples prepared.

Precision (Repeatability) Sample details are as follows:

Sample No.	Test powder material (mg)	Diluted to (mL)	Volume taken (mL)	Diluted to (mL)
1	639.5	100	1	25
2	639.1	100	1	25
3	639.7	100	1	25
4	639.8	100	1	25
5	639.2	100	1	25
6	639.7	100	1	25

Acceptance criteria:
% Assay: 90-110% for each sample and mean assay value
% RSD for % assay value of 6 samples: NMT 2%

II.Intermediate precision

It is performed by doing analysis on another day to check reproducibility of results. Samples prepared in same manner as that of Repeatability parameter (6 Samples prepared).

Intermediate Precision Sample details are as follows:

Sample No.	Test powder material (mg)	Diluted to (mL)	Volume taken (mL)	Diluted to (mL)
1	639.4	100	1	25
2	639.5	100	1	25
3	638.9	100	1	25
4	638.7	100	1	25
5	639.2	100	1	25
6	639.6	100	1	25

Acceptance criteria:
% Assay: 90-110% for each sample and mean assay value
% RSD for % assay of 6 samples of Intermediate precision**:** NMT 2
% RSD for Total 12 samples: NMT 2% for test results (6 of Repeatability and 6 of Intermediate precision)

8) ROBUSTNESS

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Determination: Standard solution were injected under different chromatographic conditions as shown below.

a). Changes in flow rate by ±10%. (± 0.1ml/min)
b). Change in column oven temperature. (± 2ºC)

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c) Change in wavelength (± 3 nm)

RESULTS AND CONCLUSION :

Selection of analytical wavelength

1) Blank Methanol:

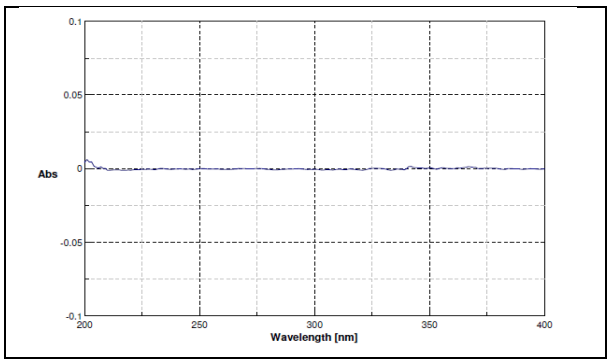

Fig. No. 1 UV spectrum of methanol as a blank

2) Teneligliptin STD solution: (20 PPM)

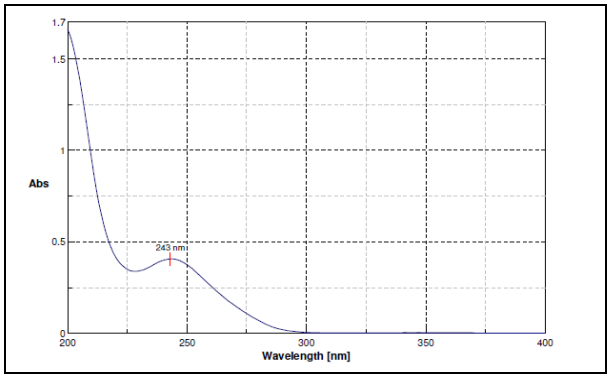

Fig. No. 2 UV spectrum of Teneligliptin

3) Dapagliflozin STD solution: (20 PPM)

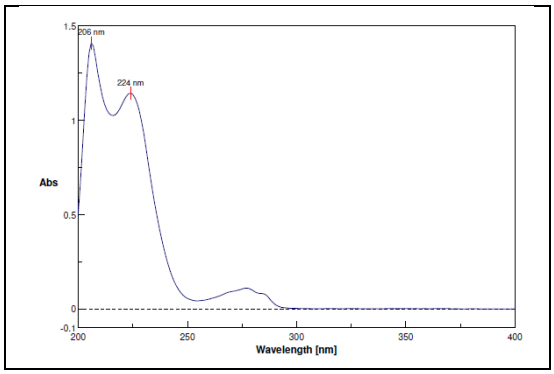

Fig. No. 3 UV spectrum of Dapagliflozin

4) Overlay plot: (Each 20 PPM)

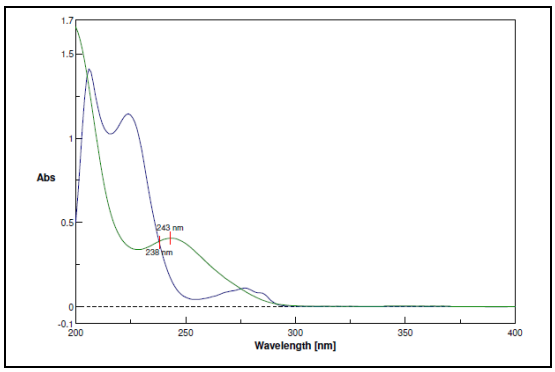

Fig. No. 4 Overlay UV spectrum of Teneligliptin & Dapagliflozin

Observation: Both standard solutions were scanned between 200 nm to 400nm. Q- absorption point was determined for both drugs. It is shown in **Figure No.4**. 238 nm considered as an analytical wavelength for further determination.

8.3 Method Development by RP – HPLC

8.3.1 Optimization of HPLC method

Trial 1:

Chromatogram:

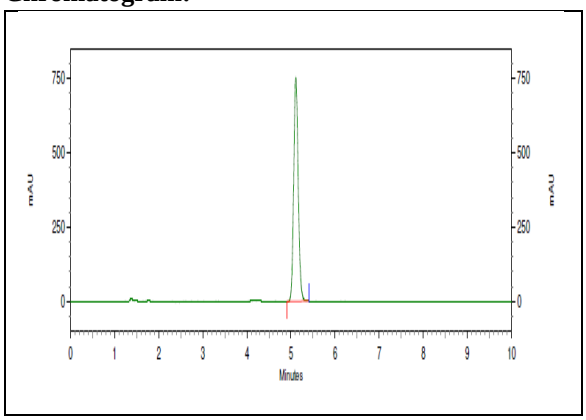

Fig. No. 5 Typical chromatogram of Dapagliflozin Trial 1

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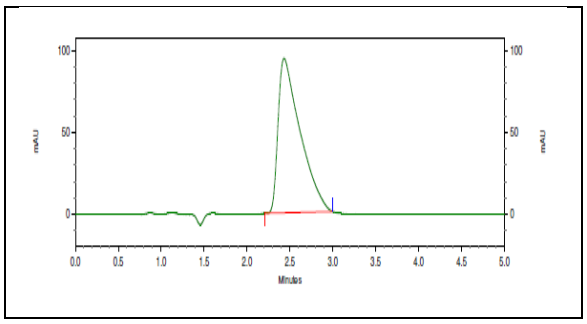

Fig. No. 6 Typical chromatogram of Teneligliptin Trial 1

Observation: Dapagliflozin eluted at 5.11 minutes with acceptable chromatography and Teneligliptin eluted at 2.43 minutes with unacceptable chromatography (TP = 391, asymmetry = 2.21)

Conclusion: Method rejected.

Trial 2:

Chromatogram:

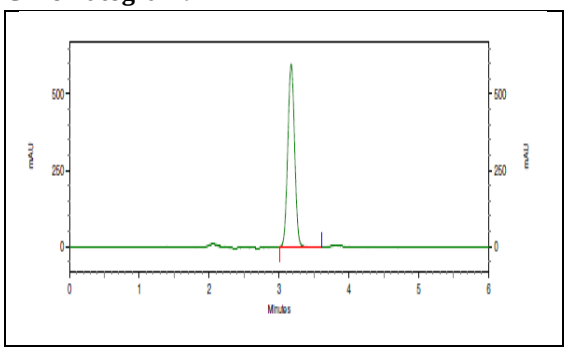

Fig. No. 7 Typical chromatogram of Dapagliflozin Trial 2

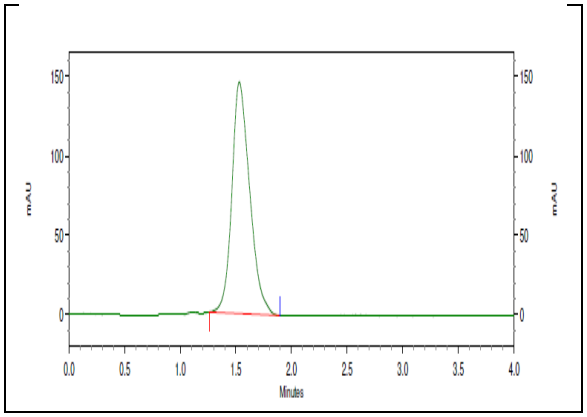

Fig. No. 8 Typical chromatogram of Teneligliptin Trial 2

Observation: Dapagliflozin eluted at 3.17 minutes with acceptable chromatography and Teneligliptin eluted at 1.53 minutes with unacceptable chromatography (Peak shape is not sharp, TP = 419)

Conclusion: Method rejected.

Trial 3:

Chromatogram:

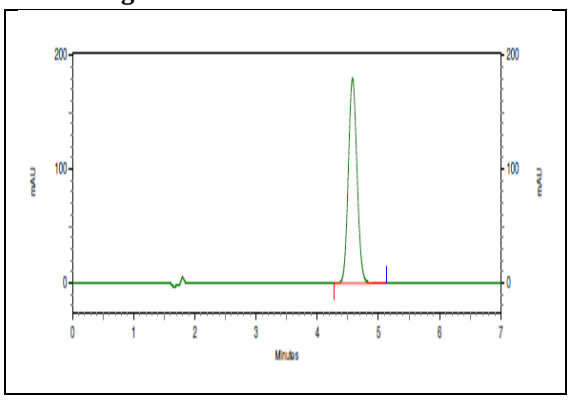

Fig. No. 9 Typical chromatogram of Dapagliflozin Trial 3

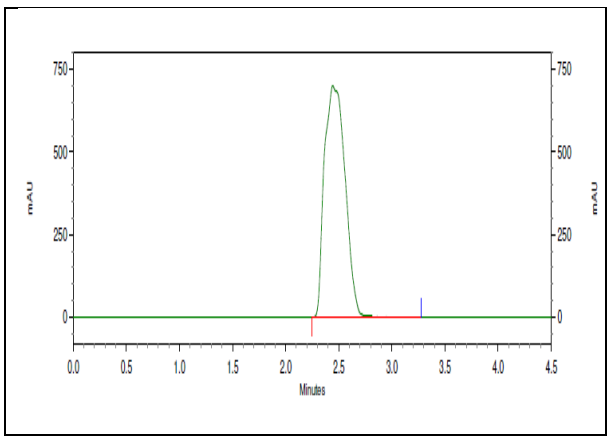

Fig. No. 10 Typical chromatogram of Teneligliptin Trial 3

Observation: Dapagliflozin eluted at 4.58 minutes with acceptable chromatography and Teneligliptin eluted at 2.44 minutes with unacceptable chromatography (Peak shape is not sharp, TP = 241)

Conclusion: Method rejected.

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Trial 4:

Chromatogram:

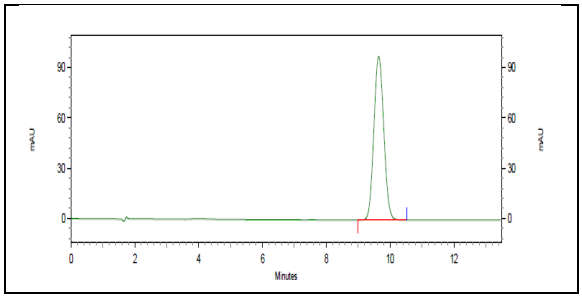

Fig. No. 11 Typical chromatogram of Dapagliflozin Trial 4

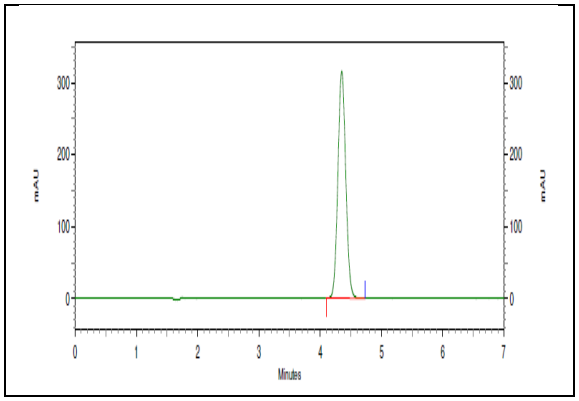

Fig. No. 12 Typical chromatogram of Teneligliptin Trial 4

Observation: Dapagliflozin and Teneligliptin eluted with acceptable chromatography.

Conclusion: Need to inject on mixture to check chromatography.

Mixture chromatogram (each 100 ppm)

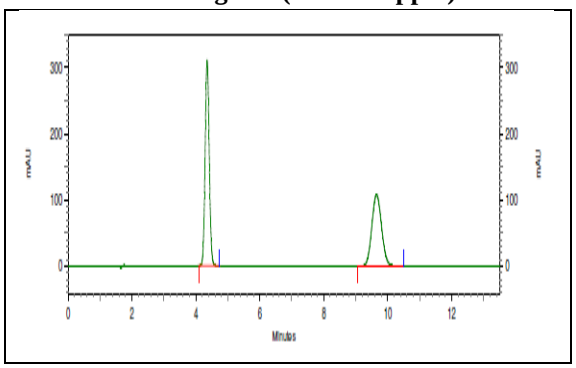

Fig. No. 13 Typical chromatogram of Mixture Trial 4

Observation: Both drugs eluted with very good chromatography and with good resolution

Teneliptin: R.T.= 4.35, TP = 5387 Asymmetry = 1.09

Dapagliflozin: R.T.= 9.65, TP = 4621 Asymmetry = 1.08

Resolution = 13.17

Conclusion: From the observations of trials first to four, it was concluded that chromatographic conditions in trial four gives better peak, good retention time, good tailing factor, Theoretical plates and good resolution (Resolution 13.17) therefore chromatographic conditions in trial four was subjected for method validation

Conclusion:

The study concludes that a reliable and efficient RP-HPLC method has been developed and validated for the simultaneous estimation of Dapagliflozin and Teneligliptin in pharmaceutical dosage forms. The optimized chromatographic conditions include an Inertsil ODS-3V column, a mobile phase of methanol and 0.05% OPA in water (60:40), and UV detection at 238 nm. This method demonstrates good resolution between the two drugs, with retention times of approximately 3.5 minutes for Teneligliptin and 5.5 minutes for Dapagliflozin. The validation process, conducted according to ICH guidelines, confirms the method's specificity, linearity, accuracy, precision, and robustness. Linearity was established over a concentration range of 50-150% of the working concentration, with correlation coefficients exceeding 0.98 for both drugs, indicating a strong linear relationship between concentration and response. Furthermore, the study concludes that the developed method is highly accurate and precise, with recovery rates between 98-102% and relative standard deviations (RSD) below 2% for both repeatability and intermediate precision studies. The method's robustness was confirmed through deliberate variations in flow rate, column oven temperature, and detection wavelength. Limits of detection (LOD) and quantification (LOQ) were successfully determined using the calibration curve approach, and the stability of analytical solutions was established for up to 24 hours under normal laboratory conditions. These findings collectively demonstrate that

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the developed RP-HPLC method is suitable for routine quality control analysis of Dapagliflozin and Teneligliptin in pharmaceutical formulations, offering a simple, accurate, and reliable approach for their simultaneous quantification..

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