Optimization of gradient RP-HPLC method for simultaneous quantification of dapagliflozin and teneligliptin in bulk and tablet formulations and their validation

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ABSTRACT

Background & Aim: The present RP-HPLC approach was created with the purpose of estimating Dapagliflozin and Teneligliptin in bulk and tablet formulations. It has been shown to be highly precise, straightforward, and stable. **Materials & Methods:** Eclipse XDB -C18, 150x4.6mm, 5micron column was pumped with a mobile phase of 0.025M potassium dihydrogen phosphate buffer (pH 2.50 adjusted by ortho-phosphoric acid) and acetonitrile in a gradient manner at a flow rate of 1.0 ml/min for chromatogram development. The optimal wavelength was 240 nm. The analysis was conducted at room temperature with a 20 µL injection volume. **Results & Discussion:** It was determined that the linearity ranges for Dapagliflozin and Teneligliptin are 2µg mL^{-1} to 12µg mL^{-1} and 4µg mL^{-1} to 24µg mL^{-1}, respectively. Dapagliflozin and Teneligliptin had retention durations of 3.8 and 7.7 min, respectively. The percentage recovery of Commercial Formulation DPG and TNG was found to be 99% and 99.2% respectively. RSD value not more than 2.0% for both active ingredients indicate stability of the solutions. **Conclusion:** The above-mentioned method was found to be highly precise, accurate, robust and specific enough to quantify the selected drugs with good resolution and high sensitivity, and its application for routine quality analysis is supported by validated reports.

KEYWORDS

Dapagliflozin, Teneligliptin, RP-HPLC, validation, gradient, stability

INTRODUCTION

As of the knowledge cutoff in September 2021, diabetes is indeed a significant global health issue.[1] According to the International Diabetes Federation (IDF), the estimated global prevalence of diabetes was 463 million people in 2019. Type 2 diabetes is the most common form of diabetes, accounting for the majority of diabetes cases worldwide. It is characterized by insulin resistance, where the body's cells do not respond effectively to insulin, and impaired insulin secretion by the pancreas. This results in elevated blood glucose levels, leading to hyperglycemia. Untreated or poorly controlled T2DM can lead to various complications, Renal failure, Cardiovascular complications, Diabetic retinopathy and maculopathy, Diabetic neuropathy [2]. The treatment of T2DM is typically aimed at reducing blood glucose levels and preventing complications. Lifestyle measures play a crucial role, including adopting a healthy diet, regular exercise, and weight management. Additionally, various antidiabetic medications [3]. are available, including oral medications and injectables, which work through different mechanisms to lower blood glucose levels. Dapagliflozin is indeed a sodium-glucose cotransporter 2 (SGLT2) inhibitor. SGLT2 is a protein responsible for reabsorbing glucose back into the bloodstream from the kidney's urine filtrate. By inhibiting SGLT2, dapagliflozin reduces the reabsorption of glucose, leading to increased glucose excretion in the urine [4,5]. As a result, there is a net reduction in blood glucose levels. This mechanism is independent of insulin and does not rely on increased insulin production by the pancreas. Teneligliptin, is a dipeptidyl peptidase-4 (DPP-4) inhibitor. DPP-4 is an enzyme responsible for breaking down incretin hormones (glucagon-like peptide-1 or GLP-1 and glucose-dependent insulinotropic peptide or GIP) in the body [6]. These hormones play a role in regulating blood glucose levels by stimulating insulin production and reducing glucagon release, which lowers blood glucose levels. By inhibiting DPP-4, teneligliptin increases the concentration and duration of the active incretin hormones, leading to enhanced insulin secretion in response to food

intake and reduced glucose production by the liver. This helps to lower blood glucose levels, especially after meals. The combination of teneligliptin and dapagliflozin in a fixed-dose formulation marketed under the brand name Zita D offers a synergistic approach to managing type 2 diabetes. By combining these two medications, their distinct mechanisms of action complement each other, leading to better glycemic control. Dapagliflozin works to reduce blood glucose levels by increasing glucose excretion in the urine, while teneligliptin enhances insulin secretion and reduces liver glucose production. As a result, this fixed-dose combination can be effective in lowering blood glucose levels and may be particularly beneficial for adult patients with uncontrolled type 2 diabetes, especially those with comorbidities. Despite previous analytical methods available for estimating DPG and TNG individually [7–14] or in combination with other drugs [15-29], there have been limited reports on simultaneous determination. The existing methods for simultaneous determination of DPG and TNG include a UV-spectroscopic method [30] and another RP-HPLC method [31]. However, the authors identified some limitations with the existing RP-HPLC method, such as being time-consuming and using methanol as an organic solvent, although is relatively cheap and easily available, it does have several drawbacks. One of the main concerns is its corrosiveness, which can lead to wear and tear of HPLC equipment and increase maintenance costs. Furthermore, its high affinity for water can cause issues with the stability of the mobile phase and make the analysis challenging. To address these limitations, the authors propose a new approach using acetonitrile as the organic solvent in RP-HPLC. Acetonitrile is chosen because it has higher elution strength than methanol for reversed-phase chromatography, leading to shorter analyte retention times. Additionally, acetonitrile-based solutions apply less pressure to the column when the flow rate is constant, making it more suitable for the analysis of a large number of biological samples. The developed RP-HPLC method is sensitive and capable of simultaneously determining the purity of DPG and TNG in bulk and tablet dosage forms. The method is validated following USFDA criteria, ensuring its accuracy and reliability for quantitative analysis. Overall, the new RP-HPLC method is considered successful in simultaneous

determination and is more efficient than existing methods, making it suitable for routine analysis of DPG and TNG in pharmaceutical formulations.

MATERIALS AND METHODS

The DPG and TNG, API samples necessary for this study are acquired from Sigma-Aldrich Chemicals, Mumbai, India. HPLC- Grade- Acetonitrile was obtained from Rankem Fine Chemicals and water contents of the mobile phase were of HPLC quality. The remainder of the required reagents, such as phosphoric acid, were obtained from Qualigen-Fine chemicals

Chromatographic conditions:

The current research is conducted on a waters 2996 high-performance liquid chromatograph with a PDA detector module, which included an automated injector with a 20 µL injection volume and a quadra- pump. Empower 2 software is loaded on the system for data processing and acquisition. 20 µL of the standard solution was injected into an Eclipse XDB -C18, 150 x 4.6mm, 5 µ column using a binary mobile phase consisting of Buffer: ACN (0.025M Potassium di-hydrogen orthophosphate in 1000 ml of water adjusted to pH 2.5 with ortho phosphoricacid) pumped in a gradient manner at a flow rate of 1.0 mL/min. This produced an effective separation, and 240nm was chosen as the detection wavelength. As a diluent, a mixture of acetonitrile and water at a ratio of 50:50 (v/v) was utilized. Before being injected into the HPLC system, both the mobile phase and standard solutions were filtered using 0.45 µm nylon membrane filter, and the entire experiment was conducted at room temperature. The 15 min gradient programme ran as follows (Table 1)

Preparation of Standard Solutions:

A standard solution of DPG (10 µg mL^{-1}) and TNG (20 µg mL^{-1}) was generated by dissolving DPG 10 mg and TNG 20 mg in 20 mL of diluent, and then diluting 5 mL of the resultant solution to 50 mL with the same solvent and further pipette out 1mL of the resultant solution into a 5mL volumetric flask and make up the volume with the diluent.

Preparation of drug stock Solution:

The sample solution was generated by weighing a tablet powder equivalent to 10 mg of DPG and 20mg

of TNG into a 50 mL Volumetric flask, dissolving it, and then diluting it to 50 mL with diluent. Take 5 mL of the resultant solution and dilute it with the same solvent to a volume of 50 mL which gives 100 µg $mL^{-1.}$

Preparation of Working Standard Drug Solution:

From the drug stock Solution pipette out 1mL and makeup to 5mL to give DPG (10 µg mL^{-1}) and TNG (20 µg mL^{-1}) solutions respectively.

METHOD VALIDATION

The method was validated in accordance with USFDA criteria for specificity, linearity and range, accuracy, precision, limit of quantification, limit of detection, and robustness.

Linearity:

For determination of linearity, six different concentration levels of sample solution (20%, 40%,60%, 80%,100%,120%), yielding in final strengths of 2-12µg mL^{-1} and 4-24 µg mL^{-1}, respectively for DPG and TNG were prepared by transferring aliquots of DPG and TNG stock drug solutions in various 5mL volumetric flasks and made the volume up to the 5mL with the mobile phase, (Table 4). Peak area against concentration of DPG and TNG is plotted to evaluate linearity, and regression equations, and further computed. The calibration curves of DPG and TNG were drawn over six distinct drug concentrations and three injections of each concentration were evaluated under the identical conditions.

Precision:

Establish the described Chromatographic conditions and allow the system to equilibrate so that the created method's precision may be evaluated. Standard and Drug solution 100 % (10 µgmL^{-1} of DPG and 20 µgmL^{-1} of TNG) were used to assess precision. On the same day, six replicates of the target concentrations were examined for intra-day variation, and six replicates were examined for inter-day variation on three different days.

Accuracy:

Nine determinations were performed on three spiked concentration levels (80%, 100%, and 120%) with 10% of ZITA-D® standard drug solution to determine the accuracy of the method recovery study (three replicates of each concentration). For each replicate sample, the percentage of recovery and RSD were determined.

LOD and LOQ:

Using this method, the LOD and LOQ of DPG and TNG were found by evaluating various dilute solutions beginning with concentrations of 20%, 10%, 5%, 2%, 1%, 0.5%, 0.2%, 0.1%, 0.05%, 0.02%, 0.01%, 0.005% and measuring signal-to-noise ratio. The limit of detection (LOD) is the concentration at which the signal-to-noise ratio is about 3:1, whereas the limit of quantification (LOQ) is the concentration at which the signal-to-noise ratio is approximately 10:1 with % RSD (n = 3) less than 10%.

Robustness:

The robustness was determined by making minor modifications to the following method conditions: (i) distinct columns (ii) Flow velocity: ± 0.1 mL/min. For each change, sample and reference solutions were evaluated. Modification was made in order to assess its impact on the methodology. The obtained data for each example was analyzed using the % RSD formula.

Specificity:

Checking if analyte reaction is altered by any possible interference, such as placebo or other endogenous substances, can be used to examine the specificity of a newly created method. For the aforesaid investigation, allow the system to equilibrate by optimising the chromatographic settings beforehand. After achieving a steady baseline, record the related responses initially with a blank and then with standard and sample solutions containing 10 μg/mL DPG and 20 μg/mL TNG.

RESULTS AND DISCUSSION

Optimization of experimental parameters:

In the present investigation, an attempt was made to change experimental circumstances in order to simultaneously estimate DPG and TNG in tablet dose forms. Stepwise study of various parameters which contribute to optimized method was done which includes the solvent, mobile phase constituents and its ratio, column, detection wavelength, pH etc.

Solvent and diluent selection

Both DPG and TNG were readily soluble in Acetonitrile and Water and a ratio of 50: 50 (v/v) of the above mentioned was selected as a diluent.

Wavelength selection

Using a UV-Visible spectrophotometer, standard DPG and TNG solutions were scanned from 200 to 400 nm. While the drugs demonstrated good absorbance at 240 nm in the UV spectrum, it was decided to move forward with further research.

Buffer selection

By reviewing and comparing multiple trials with different mobile phase compositions, the researchers were able to identify the gradient mobile phase comprising potassium dihydrogen phosphate buffer and acetonitrile as the best option. This optimized mobile phase provided good peak shapes, suitable retention times, low tailing factors, and high theoretical plates, all of which are crucial factors for a successful chromatographic analysis. The optimized method can now be used for the analysis of samples, providing accurate and precise quantification of the target analytes.

Selection of pH of the buffer:

By altering the pH of the mobile phase, so as to improve the elution properties by controlling ionization different trials were carried out and finally optimized pH was found to be 2.50 (adjusted by ortho-phosphoric acid) where peak shape, peak tailing and theoretical plate count was found to be satisfactory.

Mode of elution

Switching from isocratic elution to gradient elution can often lead to improved chromatographic separation and faster detection times. In the case of the method development attempted with isocratic

elution using the phosphate buffer (pH 2.5) and acetonitrile, the extended time for peaks to appear might be due to the limited ability of isocratic elution to efficiently resolve the components of the sample. In this case, the authors found that gradient elution was a more suitable approach to achieve their desired separation and detection goals.

METHOD VALIDATION

Linearity

According to the Linearity plots in Figures 3 and 4, the correlation coefficients for DPG and TNG were calculated to be 0.9996 and 0.9997, respectively, with a linearity range of 2 µg/mL to 12 µg/mL for DPG and 4 µg/mL to 24µg/mL for TNG.

Specificity

Comparing the chromatograms of blank, standard, and sample as depicted in Figure 5 revealed that analyte could be analyzed unambiguously, indicating that the established approach is specific.

Accuracy:

Based on recovery studies, three levels of accuracy for the new approach were determined. As seen in the (Table 2), the mean percent recovery for both drugs falls within the desired confidence interval demonstrating the method's accuracy.

Precision:

As shown in (Table 3), the % RSD values estimated from the peak area responses obtained after six injections of standard and sample solutions of DPG and TNG on same day for intraday precision and on three different days for interday precision which are less than 2 and sufficient to demonstrate the method's precision. Corresponding chromatograms are depicted in figure 6.

Robustness:

The approach is so robust that it was unaffected by a change in the flow rate of the mobile phase, as well as on various columns, as evidenced by the % RSD values (Table 4).

LOD and LOQ:

The method was found to be quite sensitive, where the values for LOD and LOQ for DPG and TNG, respectively, were determined to be 0.10% and 0.30% for both.

System suitability:

Fig. 5 depicted a typical chromatogram of regular DPG and TNG. TNG and DPG had retention durations of 3.8 and 7.7 min, respectively, and a resolution of 26.61. For DPG and TNG, respectively, the number of theoretical plates was calculated to be 18991.77 and 36057.29, and the tailing factors were 1.60 and 1.51, respectively, indicating effective column performance and are summarized (Table 5).

Solution Stability.

At periodic intervals of 6H,12H and 24H the stability of solutions was evaluated at ambient temperature. At each interval peak areas for the recorded chromatograms were calculated applying all the optimized conditions, and the observation from obtained data records RSD value not more than 2.0% for both active ingredients which reveals the stability of the solutions.

Assay of Commercial Formulation

Using the provided method, the assay of Commercial Formulation DPG and TNG in their combined dose form was successfully determined, and as shown in table 6, the percentage purity was found to be 99 and 99.2 for DPG and TNG, respectively.

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ABBREVIATIONS

DPG: Dapagliflozin; **TNG**: Teneligliptin; **API:** Active pharmaceutical ingredients; **RP-HPLC:** Reversed-phase high-performance liquid chromatography**; USFDA:** United States Food and drug administration; **KH_2PO_4**: Potassium Dihydrogen Phosphate; **OPA**: Ortho Phosphoric Acid; **LOD:** Limit of detection**; LOQ:** Limit of quantitation; **RT**: Retention Time; **RSD:** Relative standard deviation**; UV:** Ultraviolet **CV:** Co Variance**.**

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Table 1: Gradient programming of mobile phase

Time (Min)	Mobile phase-A(Buffer)	Mobile phase-B(ACN)
0-4	80	20
4-10	20	80
10-12	20	80
12-15	80	20
15	80	20

Table 2: Findings for DPG and TNG accuracy

DPG	**Concentration of unspiked standard(µg/ml)**	**Concentration of spiked standard(µg/ml)**	**Concentration of sample added(µg/ml)**	**Mean % recovery ±SD**	**%RSD**	**SEM**
80%	8	9.03	1	101.6±1.8	1.8	3.12
100%	10	11.01	1	101.1±0.2	0.2	0.35
120%	12	13.01	1	101.4±0.5	0.5	0.87
TNG						
80%	16	18.01	2	101.7±2.0	2.0	3.46
100%	20	22.01	2	100.6±0.9	0.9	1.56
120%	24	26.03	2	101.7±1.4	1.4	2.42

Table 3: Precision report of DPG and TNG

Intraday Precision	**Mean RT±SD**	**%RSD**	**Mean Area± SD**	**%RSD**
Standard				
DPG	3.87±0.01	0.25	3233611.5±25100	0.77
TNG	7.7±0.01	0.19	1611191±5272.1	0.32
Sample				
DPG	3.86±0.01	0.25	3247996.0±24975	0.77
TNG	7.6±0.01	0.15	1621287±7203.9	0.44
Interday Precision	Mean RT±SD	%RSD	Mean Area± SD	%RSD
Standard				
DPG	3.89±0.02	0.51	3233456.1±23500	0.72
TNG	7.6±0.02	0.26	1611235±5546	0.34
Sample				
DPG	3.88±0.03	0.77	3251998±24898	0.76
TNG	7.8±0.02	0.25	1622290±6578.2	0.40

*Mean n= 6 determinations

Table 4 : Method Robustness Analysis Outcomes

	DPG				**TNG**			
Parameter	**Mean area ±SD**	**%RSD**	**RT ±SD**	**%RSD**	**Mean area ±SD**	**%RSD**	**RT ±SD**	**%RSD**
Different column								
STD	3231473.9± 3064.1	0.1	3.9± 0.02	0.5	164526.9± 3536.4	0.2	7.8± 0.01	0.1
SAMPLE	3234443.3± 11930.2	0.4	3.9±0.02	0.5	1645263.9± 3536.4	0.4	7.8± 0.05	0.6
Flow decrease								
STD	3677945.1± 15786.1	0.4	4.2± 0.02	0.4	1852857.7± 5013.2	0.3	8.3± 0.01	0.1
SAMPLE	3664838.3± 32034.3	0.9	4.2± 0.03	0.7	1857908.6± 16569.3	0.9	8.3±0 .02	0.2
Flow Increase								
STD	2993380.0± 9377.0	0.3	3.6± 0.03	0.8	1518728.1± 4235.2	0.3	7.3±0 .01	0.1
SAMPLE	2968702.7± 33022.9	1.1	3.6± 0.02	0.5	1501348.6± 42243.8	0.9	7.3±0 .03	0.4

Mean*n=3 Determinations

Table 5: Summary of studies on system suitability

Parameter	**DPG**	**TNG**
RT (min)	3.8	7.7
Area(mV)	3198382	1611771
%Area	66.49	33.51
Tailing Factor	1.60	1.51
Theoretical plates	18991.77	36057.29
Resolution	-	26.61

Table 6: Results for assay of tablet formulation

		Peak Area	**RT**	**Label claim(mg)**	**%Assay**
DPG	Standard	3198382	3.872	10	99%
	Drug sample	3183119	3.870		
TNG	Standard	1611771	7.670	20	99.2%
	Drug sample	1596452	7.668		

FIGURE LEGENDS

Figure 1: (A) TNG's structure and (B) DPG's structure

Figure 2: Optimized Standard Chromatogram of DPG and TNG

Figure 3: Graph of linearity for DPG

Figure 4: Graph of linearity for TNG

Figure 5: Chromatograms Comparing Blank to sample and standard overlay

Figure 6: Overlay of standard and Sample Chromatograms Showing Precision

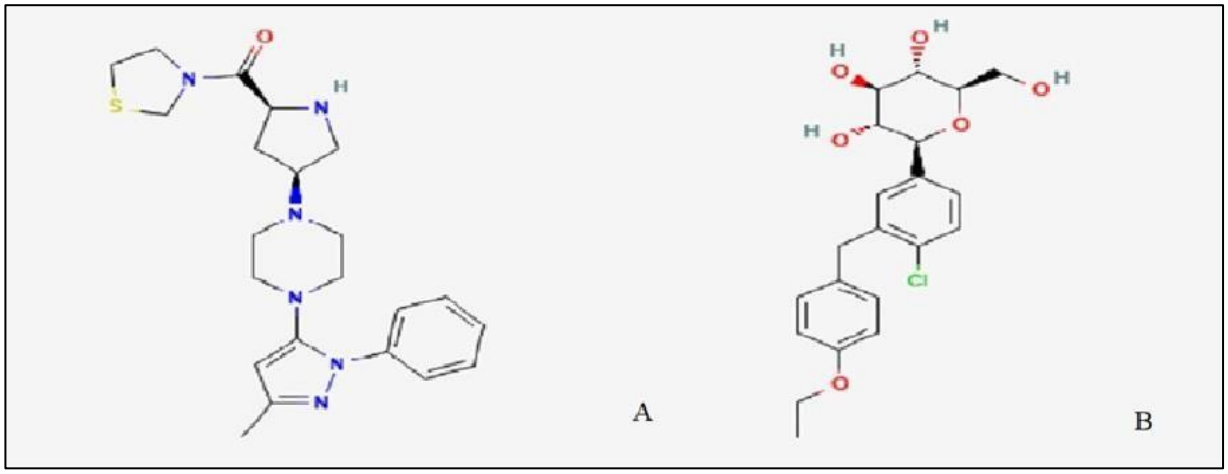


Figure 1

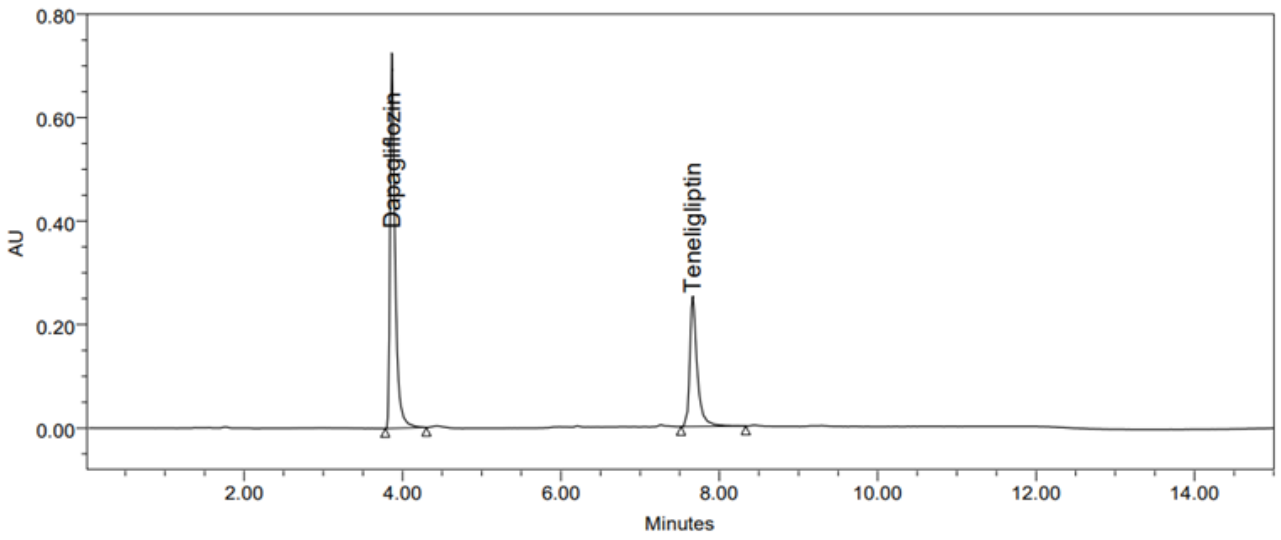


Figure 2

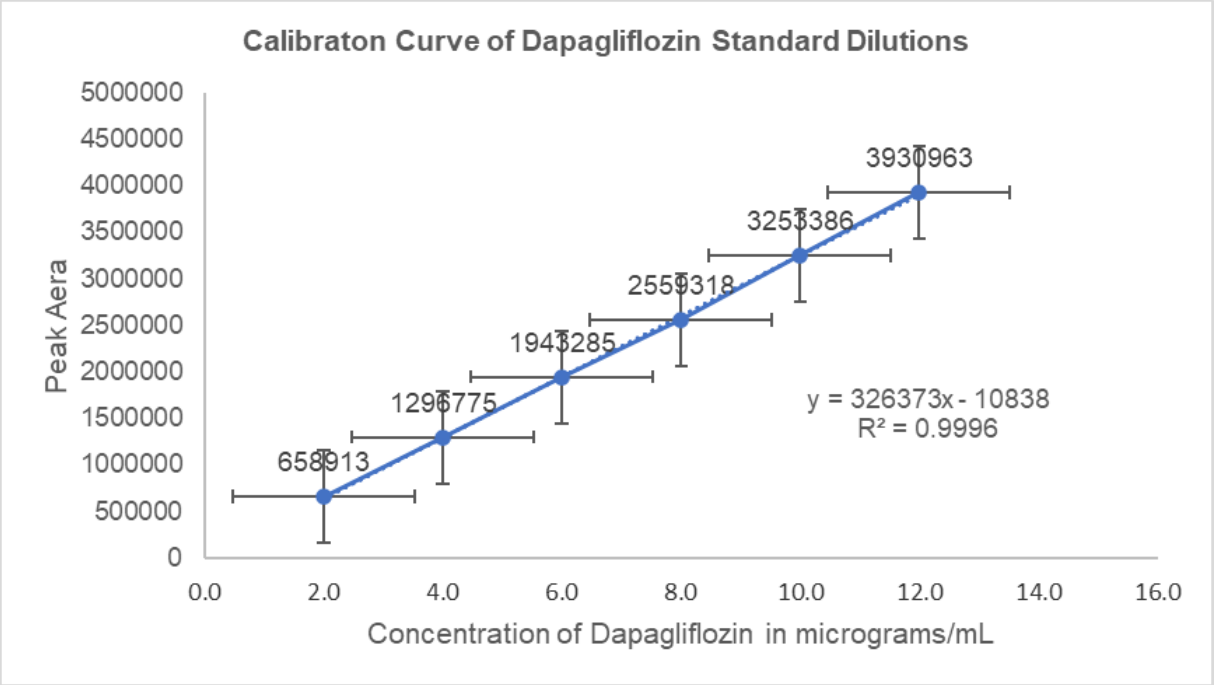


Figure 3

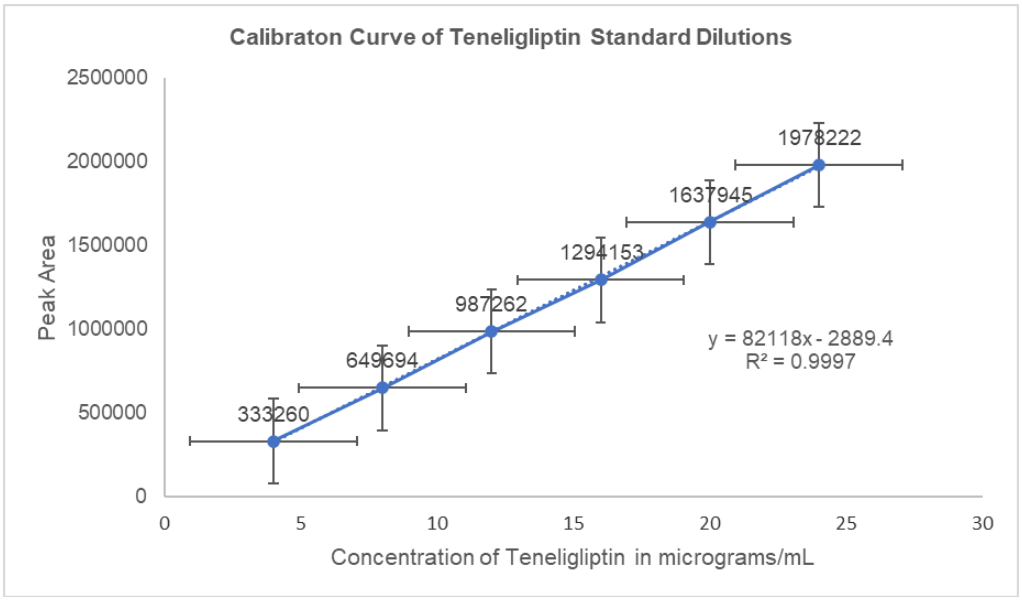
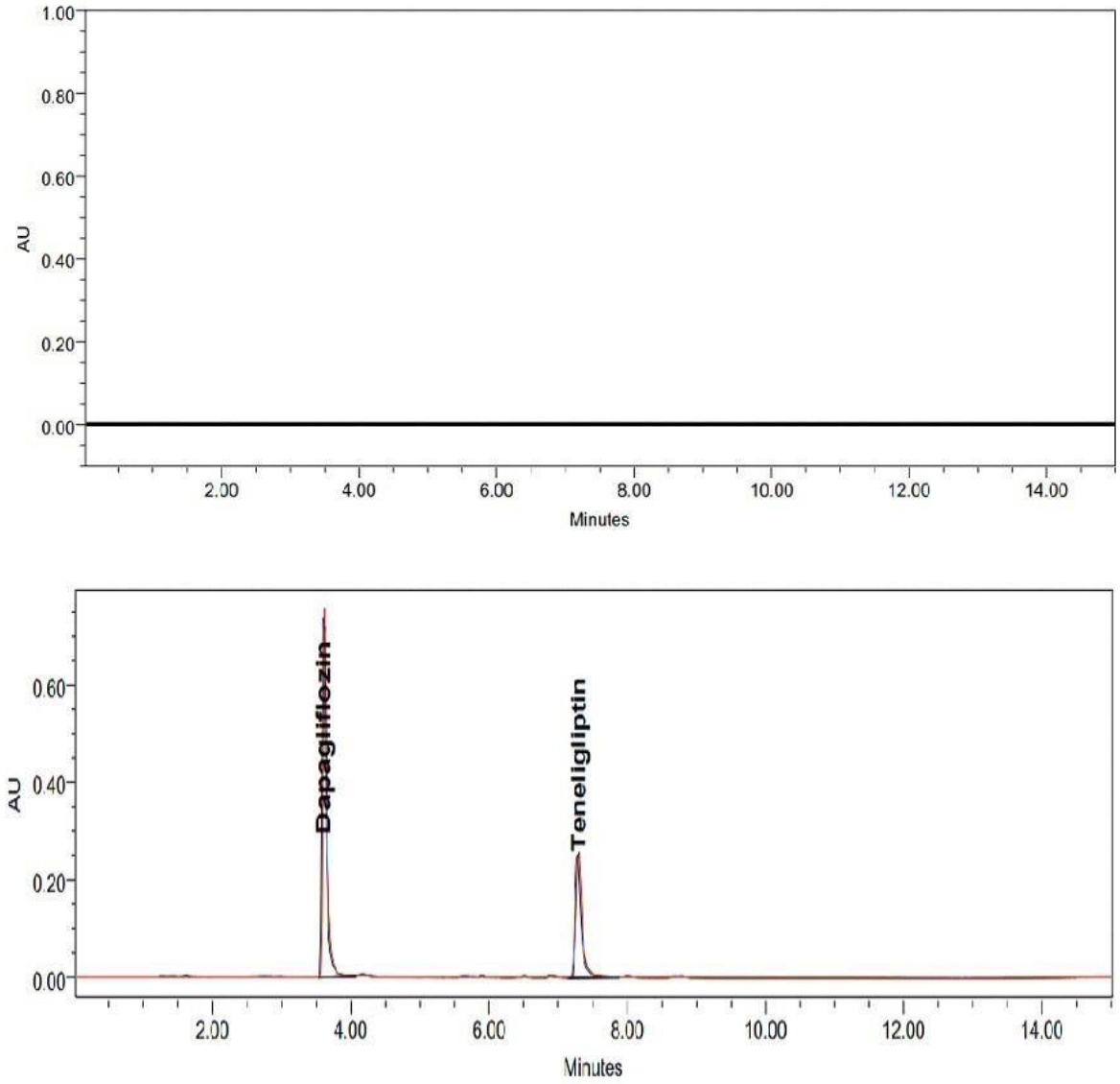


Figure 4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Minutes
Dapagliflozin
Teneligliptin
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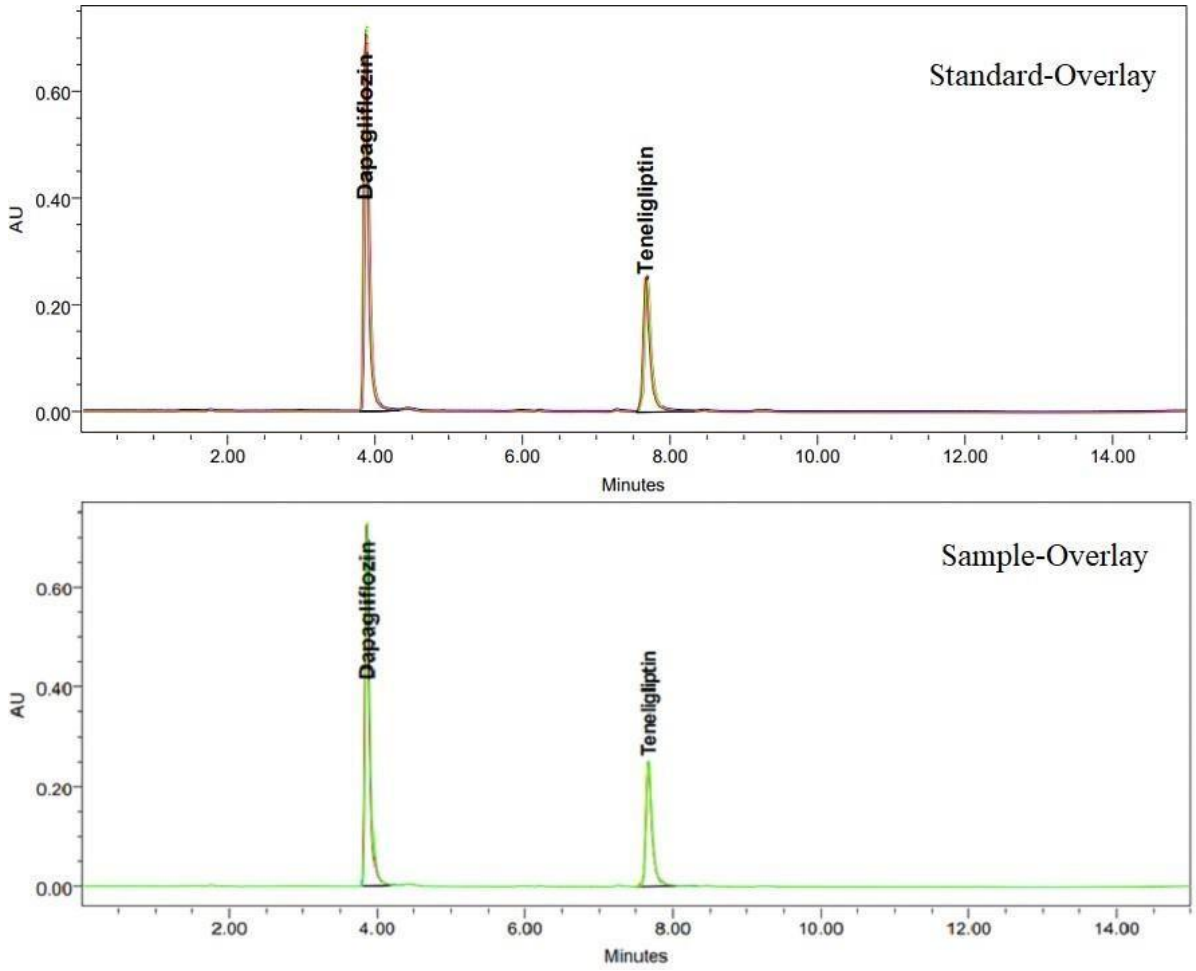
Figure 5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Minutes
Sample-Overlay
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Minutes

Figure 6