JOTCSA Journal of the Turkish Chemical Society Section: A Chemistry

TURKISH CHEMICAL SOCIETY

Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Dapagliflozin Propanediol Monohydrate and Teneligliptin Hydrobromide Hydrate in Synthetic Mixture

Hiteksha Dobariya[1*], Urvi Chotaliya[1]

[1] B. K. Mody Government Pharmacy College, Rajkot, Gujarat, India, Postal code: 360003

Abstract: For the quantitative measurement of Dapagliflozin propanediol monohydrate and Teneligliptin hydrobromide hydrate in synthetic mixture form in the presence of its degradants, precise, accurate, robust, cost-effective, and isocratic stability indicating RP-HPLC method was developed and validated. The mobile phase comprises [Methanol: 20 mM Ammonium formate (70:30 v/v)] at a flow rate of 1.0 ml/min, injection volume of 20 µl, and UV detection at 225 nm. Separation was accomplished using Gemini, C18 column. Teneligliptin hydrobromide hydrate and Dapagliflozin propanediol monohydrate were eluted with retention times of 6.65 minutes and 4.20 minutes, respectively. This procedure was approved following ICH guideline Q2 (R1). The calibration plots for Dapagliflozin propanediol monohydrate and Teneligliptin hydrobromide hydrate have correlation coefficients of 0.9995 and 0.9996 over the concentration ranges of 5-100 µg/ml and 10-200 µg/ml respectively. For Dapagliflozin propanediol monohydrate and Teneligliptin hydrobromide hydrate, accuracy ranged from 99.81-100.78% and 99.13-100.69%, respectively. For Dapagliflozin propanediol monohydrate and Teneligliptin hydrobromide hydrate, the LOD was found to be 0.947 µg/ml and 1.355 µg/ml. In contrast, the LOQ was 2.869 µg/ml and 4.107 µg/ml, respectively. The findings demonstrated the applicability of the devised approach for routine analysis of Teneligliptin hydrobromide hydrate and Dapagliflozin propanediol monohydrate in a synthetic mixture form with its degradants.

Keywords: Dapagliflozin propanediol monohydrate, Teneligliptin hydrobromide hydrate, RP- HPLC, Stability indicating method, Validation

Submitted: April 26, 2023. **Accepted**: August 17, 2023.

Cite this: Dobariya H, Chotaliya U. Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Dapagliflozin Propanediol Monohydrate and Teneligliptin Hydrobromide Hydrate in Synthetic Mixture. JOTCSA. 2023;10(4):1025-1034.

DOI: https://doi.org/10.18596/jotcsa.1288000.

***Corresponding author.** E-mail: hiteksha134@gmail.com

1. INTRODUCTION

The chemical name of Dapagliflozin propanediol monohydrate (DAPA) is (2S)-propane-1,2-diol (2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4-ethoxyphenyl)methyl]phenyl}-6-(hydroxymethyl)oxane-3,4,5-triol hydrate, Structure shown in Figure 1. Dapagliflozin propanediol monohydrate is an SGLT-2 inhibitor and treats type-2 diabetes mellitus (1).

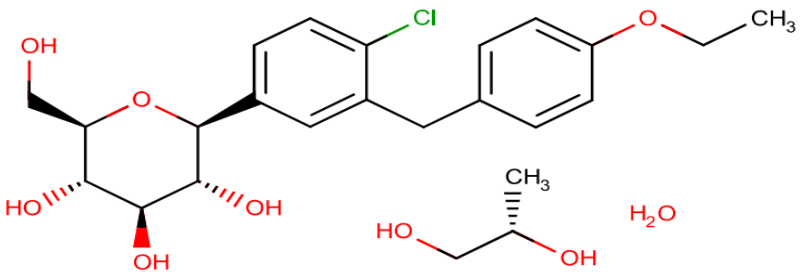

Figure 1: Structure of Dapagliflozin propanediol monohydrate

Teneligliptin hydrobromide hydrate (TENE) is {(2S,4S)-4-[4-(5-Methyl-2-phenylpyrazol-3-yl) piperazin-1-yl] pyrrolidin-2-yl} (1,3 thiazolidin-3-yl) methanone; hydrate; pentahydrobromide, Structure shown in Figure 2. It is indicated for treating type 2 diabetes mellitus (2).

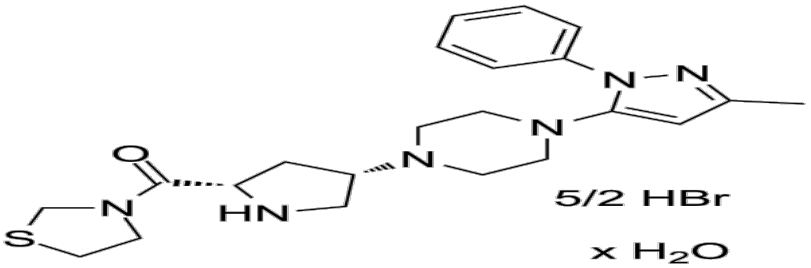

Figure 2: Structure of Teneligliptin hydrobromide hydrate

Analytical quality by design (AQbD) and CCD help in regulatory compliance for RP-HPLC method development, stress testing, or stability-indicating methods (3-6). UV spectrophotometric methods (simultaneous equation method/ Vierodt') and LC-MS are widely acceptable for the simultaneous estimation of pharmaceutical combinations (7-9). The presence of impurities or degradants critically affects the stability and pharmacological action of pharmaceutical API and drug products (10-14). The purpose of stability and related substance study is to provide evidence on how the quality of a drug substance or product varies with time under the influence of various environmental factors (15-16).

Developing this approach has been clearly mandated since the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) was created. The information on how the quality of the drug changes with changes in environmental elements, such as pH, humidity, temperature, light, etc., can be provided by stability-indicating methods. Moreover, SIM aids a formulator in selecting appropriate vehicles when creating novel formulations (17-18).

A literature review showed a UV (19) method for combining Dapagliflozin propanediol monohydrate and Teneligliptin hydrobromide hydrate. Still, no single approach is available for stability, indicating simultaneous estimation of Dapagliflozin propanediol monohydrate and Teneligliptin hydrobromide hydrate (20-33). There were many analytical methods reported for Dapagliflozin propanediol monohydrates, such as RP-HPLC (34-36), RP-HPLC (stability indicating method) (37), and UV spectroscopy (38-39) in combination with saxagliptin, metformin, and sitagliptin. There were few analytical methods reported for Teneligliptin hydrobromide hydrates, such as RP-HPLC (40-43), RP-HPLC (stability indicating method) (44-45), and UV spectroscopy (46-47) reported in combination with metformin, rosuvastatin, remogliflozin, and pioglitazone.

2. MATERIALS AND METHODS

2.1. Materials and Chemicals

Dapagliflozin propanediol monohydrate and Teneligliptin hydrobromide hydrate were supplied by Merril Pharma Pvt. Ltd. as gift samples and manufactured by Morphan Laboratories and Aalidhra Pharma Chem. respectively. This fixed dose combination is under clinical trial phase 3. So, the synthetic mixture was used for the estimation. Dapagliflozin propanediol monohydrate is White to off-white powder, and Teneligliptin hydrobromide hydrate is White/off-white crystalline powder.

2.2. HPLC Instrumentation and Chromatographic Conditions

The method was developed using a Shimadzu HPLC instrument with a Photodiode Array Detector. At 40℃, [MeOH:20mM Ammonium formate (70:30 v/v)] was used as mobile phase, which was pumped at a flow rate of 1 ml per minute, and Gemini, C18, (250×4.6 mm, 5µm) column as a stationary phase. The successful detection had a wavelength of 225 nm.

2.3. Preparation of Solutions

2.3.1. Preparation of standard stock solution of 400 µg/ml of Dapagliflozin propanediol monohydrate

A 100 ml volumetric flask combined 40 mg of Dapagliflozin propanediol monohydrate with 70% of the diluent. The resulting mixture was subjected to sonication to facilitate dissolution. Subsequently, the remaining diluent was added to reach the desired volume mark on the flask, followed by additional sonication to ensure complete dissolution. The concentration of Dapagliflozin propanediol monohydrate in the resulting solution will be 400 µg/ml.

2.3.2. Preparation of Standard stock solution of 800 µg/ml of Teneligliptin hydrobromide hydrate

An 80 mg of atorvastatin calcium was introduced into a volumetric flask with a capacity of 100 ml. Subsequently, 70% of the diluent was added, followed by sonication to facilitate dissolution. The remaining diluent was added, and the resulting mixture was stirred well. The resulting solution will contain a Teneligliptin hydrobromide hydrate concentration at 800 µg/ml.

3. FORCED DEGRADATION STUDIES

Acid hydrolysis was carried out by using 1 N HCl for 48 hours, and base hydrolysis was carried out by using 1 N NaOH for 6 hours and then neutralized the mixture solution and made up to the final volume. Oxidative hydrolysis was carried out using 3% H_2O_2 for 30 minutes in a thermal degradation sample placed in a hot air oven at 70℃ for 60 hours. The photodegradation sample was carried out using a UV chamber for 60 hours.

4. METHOD VALIDATION

Analytical validation parameters for this proposed method were determined according to the ICH Q2(R1) guideline (48).

4.1. Linearity

Linearity has been established over six different concentrations by plotting the calibration curve of peak area v/s concentration.

4.2. Accuracy

Drug to drug spiking accuracy was done at three levels: 80%, 100%, and 120%. Three sets were prepared for each level, and the percentage recovery was calculated.

4.3. Precision

Interday and intraday precision were performed by taking 80, 100, and 120 percent of the target concentration. Repeatability was achieved by taking six replicate injections of the standard preparation. The outcome was noted as Relative Standard Deviation (RSD).

4.4. Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were found by the equation as per ICH guidelines.

LOD= 3.3 x σ / S and LOD= 10 x σ / S

Where, σ was the SD of the response, and S was the mean slope of the calibration curves.

4.5. Specificity

In specificity, % interference was measured after injecting a blank (mobile phase), placebo; standard DAPA and TENE solution spiked with excipients, and a test solution.

4.6. Robustness

Robustness was performed by deliberated change in flow rate (± 0.1 ml/min), column oven temperature (40 ± 1°C), and mobile phase composition. Robustness was calculated in terms of RSD.

4.7. Assay of synthetic mixture

10 mg of dapagliflozin propanediol monohydrate and 20 mg of teneligliptin hydrobromide hydrate were added into a 100 ml volumetric flask, followed by excipients used in single oral unit dosage form, to determine the concentration of dapagliflozin propanediol monohydrate and teneligliptin hydrobromide hydrate in a synthetic mixture. Both substances were evaluated for assay at 40 µg/ml final concentrations for dapagliflozin propanediol monohydrate and 80 µg/ml for teneligliptin hydrobromide hydrate.

5. RESULTS AND DISCUSSION

5.1. Optimized chromatographic conditions

In Figure 3, Dapagliflozin propanediol monohydrate had a retention time of 6.65 min, while Teneligliptin hydrobromide hydrate had a retention time of 4.20 min under optimized chromatographic conditions.

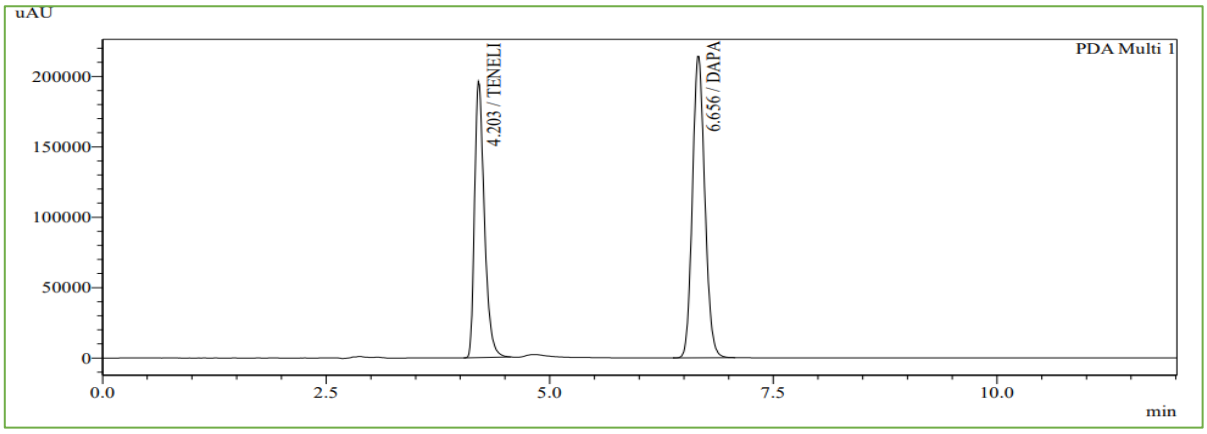

Figure 3: Optimized Chromatogram of Standard DAPA TENE

5.2. System suitability parameter

The system suitability parameters were calculated, and it was found that every parameter was within the acceptable range. It is mentioned in Table 1.

Table 1: System suitability parameter of optimized condition

Peak	Ret. Time	Area	Theoretical Plate	Resolution	Tailing Factor
DAPA	6.65 ± 0.1	2063684	10017 ± 100	10.211	1.14 ± 0.1
TENE	4.20 ± 0.1	1498051	6141 ± 100	0.00	1.36 ± 0.1

5.3. Forced degradation study

Acid hydrolysis was carried out as specified, and as shown in Figure 4, the percent degradation for DAPA and TENE was 4.84 and 5.77, respectively. According to Figure 5, the degradation amount was 5.30 for DAPA and 24.13 for TENE during base hydrolysis. In oxidative hydrolysis, the percent degradation for the DAPA and TENE shown in Figure 6 was 5.81 for DAPA and 23.93 for TENE. Thermal degradation was carried out as instructed, and as shown in Figure 7, the percent degradation was 0.29 for DAPA and 0.57 for TENE. According to Figure 8 and Table 2, the results for photo deterioration were 0.24 for DAPA and 0.35 for TENE in terms of percent degradation.

Table 2: Forced degradation summary

Degradation Condition	R.T.		%Degradation		Peak purity index	
Name	DAPA	TENE	DAPA	TENE	DAPA	TENE
Acid hydrolysis	6.67	4.27	4.84	5.77	1	1
Base hydrolysis	6.66	4.19	5.30	24.13	1	1
Oxidative hydrolysis	6.65	4.13	5.81	23.93	1	1
Thermal degradation	6.66	4.14	0.29	0.57	1	1
Photolytic degradation	6.67	4.13	0.24	0.35	1	1

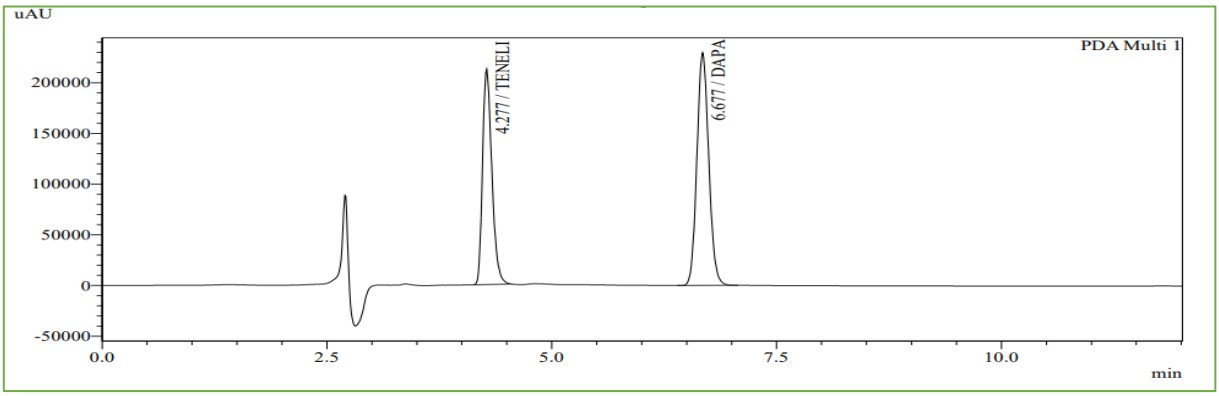

Figure 4: Forced degradation chromatogram of acid degradation

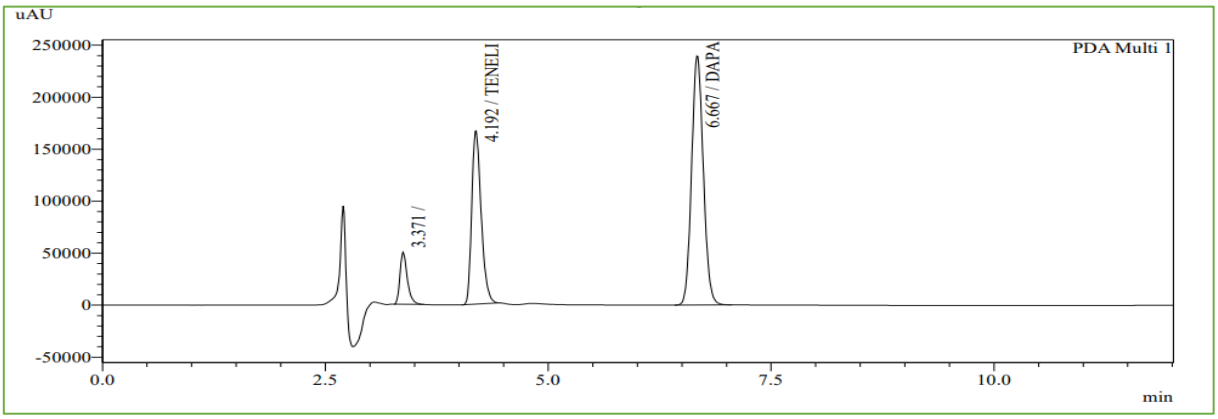

Figure 5: Forced degradation chromatogram of base degradation

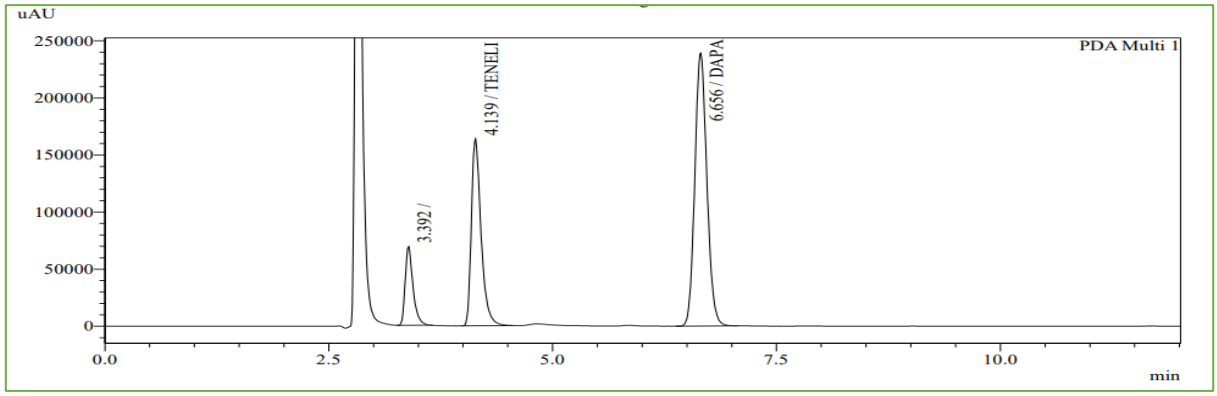

Figure 6: Forced degradation chromatogram of oxidative degradation

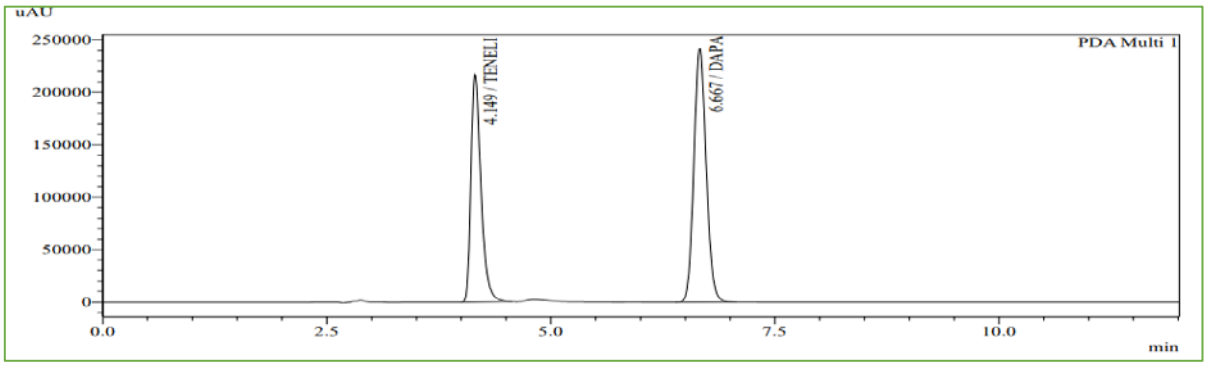

Figure 7: Forced degradation chromatogram of thermal degradation

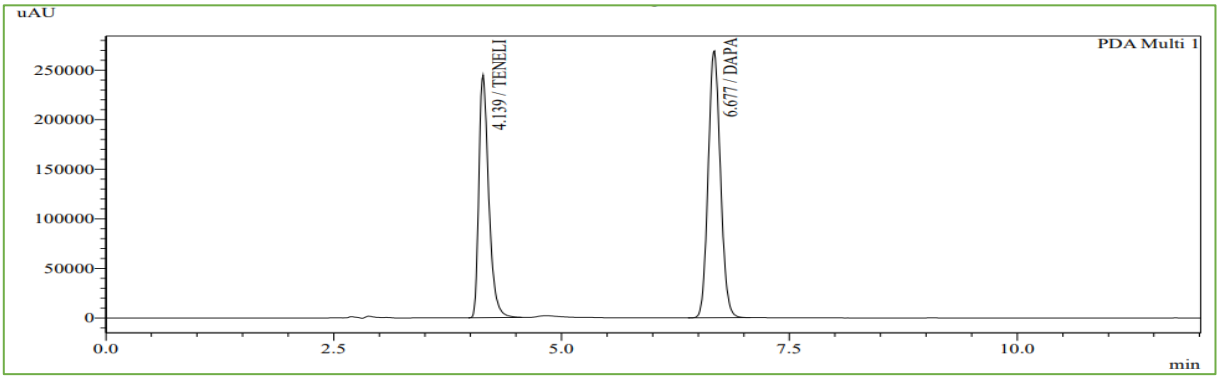

Figure 8: Forced degradation chromatogram of photodegradation

5.4. METHOD VALIDATION

5.4.1. Linearity

DAPA was found to have linear responses in the concentration range of 5-100 µg/ml, and TENE was found to have linear responses in the concentration range of 10-200 µg/ml. The calibration curves for Dapagliflozin propanediol monohydrate and Teneligliptin hydrobromide hydrate, respectively, are shown in Figures 9 and 10.

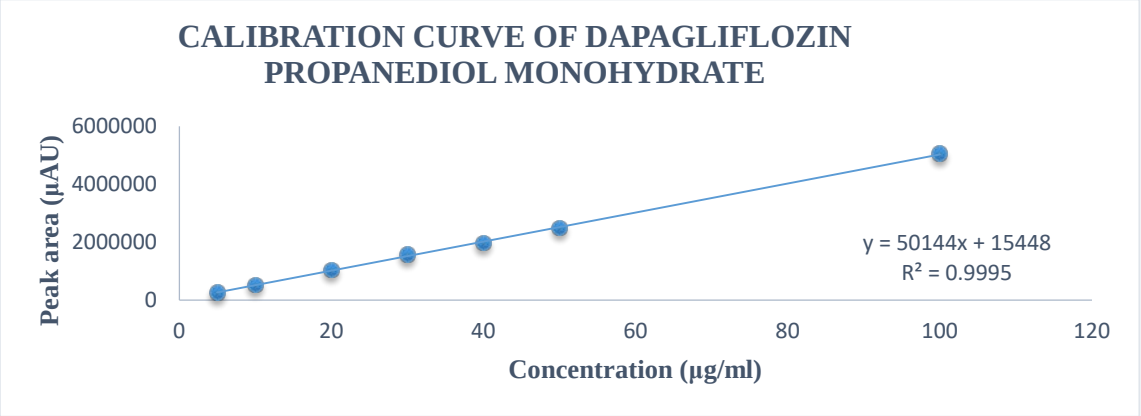

Figure 9: Calibration curve of Dapagliflozin propanediol monohydrate

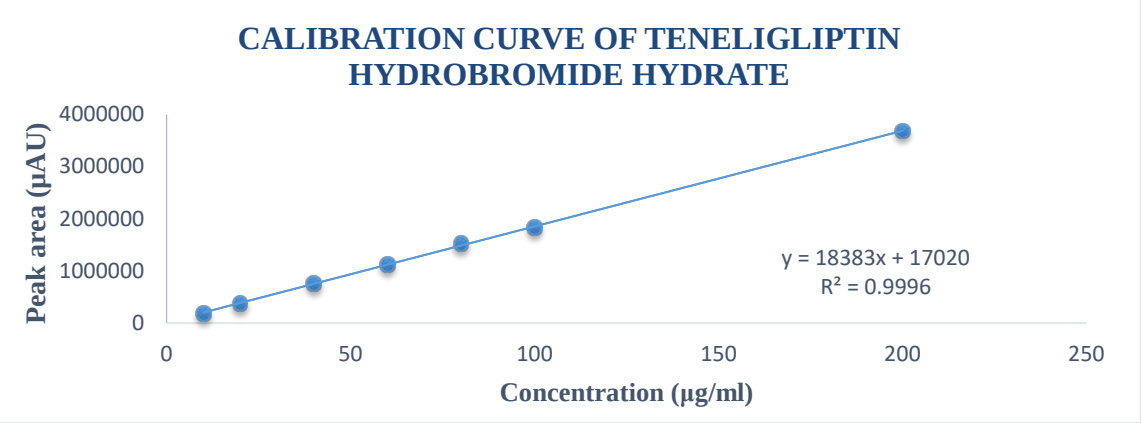

Figure 10: Calibration curve of Teneligliptin hydrobromide hydrate

5.4.2. Accuracy

As indicated in Table 3, the percentage recovery for Dapagliflozin propanediol monohydrate ranged from 99.81-100.78%, whereas the percentage recovery for Teneligliptin hydrobromide hydrate ranged from 99.13-100.69%.

5.4.3. Precision

The repeatability, intraday, and interday precision of the RP-HPLC method were tested using RSD, which was found to be greater than 2. Results are shown in Table 3, demonstrating the precision of the analyzed method.

Table 3: Accuracy and Precision data of DAPA & TENE

LEVEL	ACCURACY		PRECISION					
	% Drug recovery		% RSD					
			Repeatability		Intra-day		Inter-day	
	DAPA	TENE	DAPA	TENE	DAPA	TENE	DAPA	TENE
80	100.66	100.67	0.0018	0.014	0.014	0.011	0.017	0.009
100	99.90	99.31			0.032	0.023	0.004	0.003
120	100.77	99.12			0.025	0.029	0.009	0.010

5.4.4. LOD and LOQ

Using the average slope and standard deviation of intercepts, the LOD and LOQ were calculated. For DAPA and TENE, the LOD was discovered to be 0.94 g/ml and 1.35 µg/ml, respectively. For DAPA and TENE, the LOQ was found to be 2.86 µg/ml and 4.10 µg/ml, respectively.

5.4.5. Specificity

By comparing the chromatograms of the blank, mobile phase, and standard solution, it is demonstrated that no excipient interference with the peak of dapagliflozin propanediol monohydrate

and teneligliptin hydrobromide hydrate has ever been seen.

5.4.6. Robustness
When various parameters, including flow rate, wavelength, and mobile phase ratio, were purposefully altered, it was discovered that the method was reliable and robust. When the parameters were intentionally altered, the relative standard deviation of the peak area was less than 2%.

5.4.7. Assay of synthetic mixture
The concentrations of Dapagliflozin propanediol monohydrate and Teneligliptin hydrobromide hydrate in a synthetic mixture containing 10 mg of Dapagliflozin propanediol monohydrate and 20 mg of Teneligliptin hydrobromide hydrate were calculated using the developed method, and the results showed that they were 100.3% and 101.7%, respectively.

6. CONCLUSION

This study presents the development and validation of a stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method for the identification of Dapagliflozin propanediol monohydrate and Teneligliptin hydrobromide hydrate in a synthetic combination. Validation was conducted for Dapagliflozin propanediol monohydrate and Teneligliptin hydrobromide hydrate in accordance with the ICH Q2(R1) requirements. Through the utilization of RP-HPLC, the obtained results indicate that the accuracy falls within the range of 99.81-100.78 percent for DAPA and 99.13-100.69 percent for TENE. The precision of Dapagliflozin propanediol monohydrate and Teneligliptin hydrobromide hydrate was found to be less than 2% in terms of relative standard deviation (RSD). Additionally, all other parameters met the guidelines set by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). These results indicate that the method employed in this study was effective and suitable for accurately quantifying Dapagliflozin propanediol monohydrate and Teneligliptin hydrobromide hydrate in a synthetic mixture. The approach that has been devised effectively achieves the total separation of alkali and oxidative degradants from the primary peak. The pharmacological combination under investigation is currently in phase 3 of clinical trials, indicating that there are currently no commercially available formulations. Consequently, a synthetic combination is employed for the purpose of technique development.

7. CONFLICT OF INTEREST

The authors report no conflicts of interest.

8. ACKNOWLEDGMENTS

The authors are grateful to MERRIL PHARMA PVT. LTD. for providing standard samples of Dapagliflozin propanediol monohydrate and teneligliptin hydrobromide hydrate and B.K. MODY GOVERNMENT PHARMACY COLLEGE, RAJKOT, and SAURASHTRA UNIVERSITY to offer facilities to work.

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