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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULANEOUS ESTIMATION OF DAPAGLIFLOZIN PROPANEDIOL MONOHYDRATE AND TENELIGLIPTIN IN TABLET DOSAGE FORM

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ABSTRACT: Simple, rapid, economical, precise and accurate Stability indicating RP- HPLC method for the estimation of Dapagliflozin Propanediol Monohydrate and Teneligliptin in Tablet Dosage Form has been developed. A reverse phase high performance liquid chromatographic method was developed for the estimation of Dapagliflozin Propanediol Monohydrate and Teneligliptin in Tablet Dosage FORM has been developed. The separation was achieved Column Kromasil C18 (150 x 4.6) 5 µm ID, Gradient program0.1%TFA: Methanol, as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 235 nm retention time of DAPA and TENE was found to be 9.47and 3.61 min. The method has been validated for linearity, accuracy and precision. Linearity observed for Dapagliflozin Propanediol Monohydrate and Teneligliptin 49.94-298.24 µg/ml. Developed method was found to be accurate, precise and rapid for estimation of Dapagliflozin Propanediol Monohydrate and Teneligliptin in Tablet Dosage Form. The drug was subjected to stress condition of hydrolysis, oxidation, photolysis and Thermal degradation, under same chromatographic condition. The stress samples were assayed on RP-HPLC system.

KEYWORDS:
Dapagliflozin Propanediol Monohydrate, Teneligliptin, Stability indicating RP- HPLC Method, Validation.

I . INTRODUCTION:

Diabetes is chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Hyperglycemia, also known as raised blood glucose or raised blood sugar, is a Common impact of uncontrolled diabetes and over time leads to genuine harm of body, particularly the nerves and blood vessels. [1]. Type 2 diabetes also called as non-insulin-dependent diabetes it means that your body doesn't use insulin properly. Mostly the people control their blood sugar levels by healthy eating and exercise, and some are using medication. [2] Although type 2 diabetes is more prevalent in elderly adults, instances in younger people have increased because to the rise in the number of obese children. [3]. Structure of DAPA and TENE is shown in Figure. [4-5] TENE inhibit the action of DPP-4 enzymes and slow down the rapid degradation of incretins. DAPA inhibiting SGLT2, DAPA blocks reabsorption of filtered glucose in the kidney, increasing urinary glucose excretion and reducing blood glucose levels. [6-8] By the literature survey it was found that analytical methods are available for estimation of DAPA and TENE alone and with other combination. [9-15]. So, there is thought to perform Stability indicating RP-HPLC method development and validation for simultaneous estimation of tablet dosage form. With the advent of International Conference on Harmonization (ICH) guidelines, the requirement of establishment of stability -indicating assay method (SIAM) has become more clearly mandated. The guidelines explicitly require conduct of forced decomposition studies under a variety of conditions, like pH, light, oxidation, etc. and separation of drug from degradation products. [16-17] Thus, the objectives of this work are to develop a new sensitive stability indicating RP-HPLC method for simultaneous determination of DAPA and TENE. Also, it is validated for market product named Zita-D containing DAPA and TENE in tablet dosage form. [18]

II .MATERIALS AND METHODS

Shimadzu HPLC, LC 2010 CHT model and LC Solution software was used. Acetonitrile, methanol, Diammonium hydrogen phosphate, Mili-Q water and ortho phosphoric acid of AR grade from Merck Life Science Pvt. Ltd, was used. A commercial dosage form Zita-D was purchased from local market.

IR identification and wavelength selection

The individual standard drugs, DAPA and TENE were mixed with KBr and KBr pallets were prepared. These KBr pallets of drugs were used for FTIR analysis. And then FTIR spectra were interpreted and results were co-related with M.P., UV spectra and solubility to confirm identity of individual drugs. Wavelength was selected from the overlay spectra of above solutions.

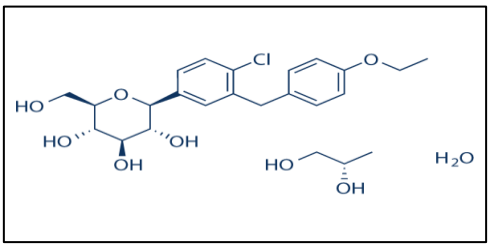

Figure 1. Structure of DAPA

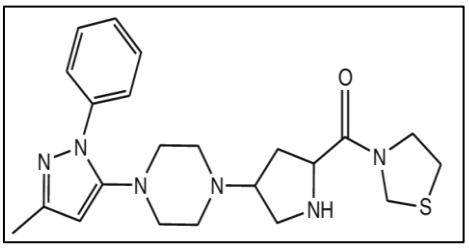

Figure 2. Structure of TENE

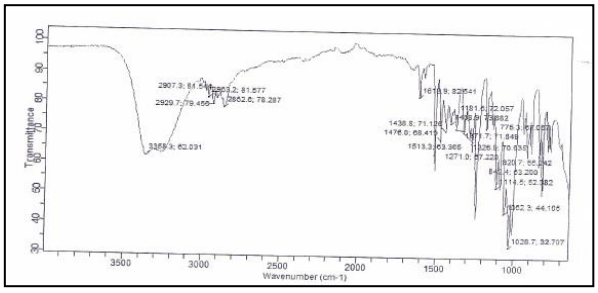

Figure 3: IR spectrum of DAPA (API)

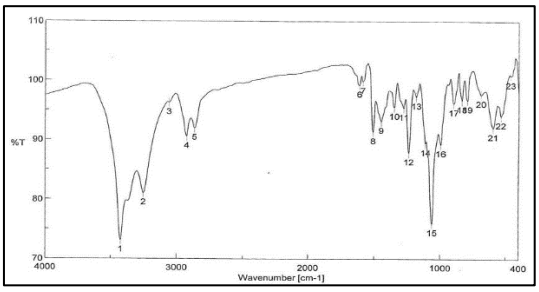

Figure 4: IR Spectrum of DAPA (Std.)

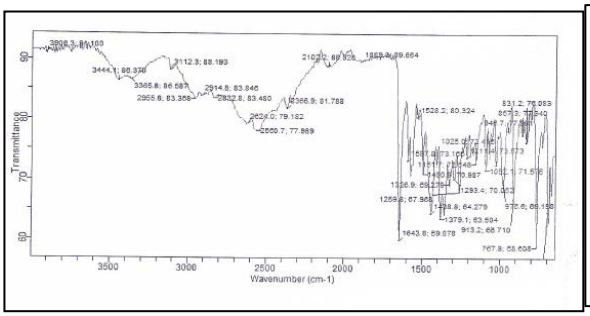

Figure 5: IR spectrum of TENE (API)

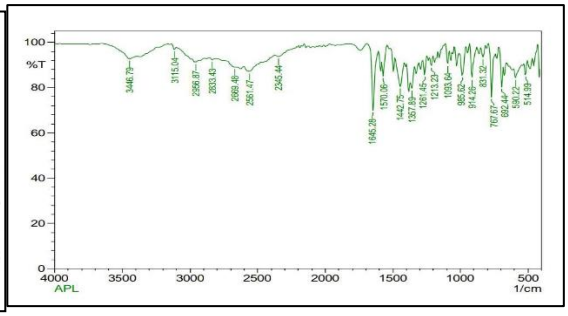

Figure 6: IR Spectrum of DAPA (Std.)

Table 1: IR spectrum of DAPA

Sr. No.	Functional group	Observed value	Standard value
1	O-H stretching	3369.3	3300-3400
2	C-H stretching	28802.2	2850-3000
3	C-O stretching	1271.0	1300-1000
4	C-CL stretching	820.7	750-850

Table 2: IR spectrum of TENE

sr. No.	Functional group	Observed value	Standard value
1	N-H stretching	3365.8	3500-3100
2	C-H stretching	2955.8	2850-3000
3	N-H bending	1643.8	1650-1620
4	C-N stretching	1269.8	1350-1000

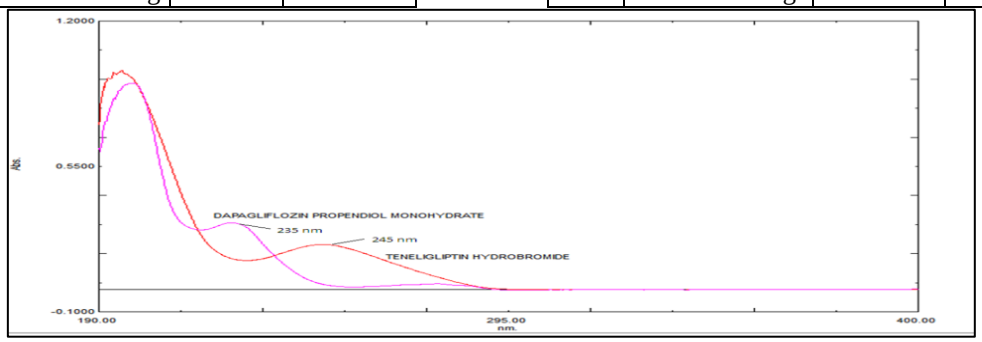

Figure 7: Determination of wavelength maximum (235 nm)

Selection of Mobile phase

Various mobile phases were tried. Trial contains various mobile phases which consisted of Acetonitrile, methanol, Sodium dihydrogen phosphate, sodium hydroxide and hydrochloric acid, ortho phosphoric acid in different proportions with various pH and different volumes at flow rate 1 ml/min were tried. Chromatogram in optimized mobile phase is shown in Figure.

Standard stock solution of DAPA and TENE were further diluted with methanol into 10 ml volumetric flask which contain 0,50,100,150,200,250,300 µg/ml for both drugs and volume was made up mark with mobile phase. Scan in HPLC and plot the graph of peak area vs concentration. Calibration curve were plotted over a concentration range of 0-300 µg/ml for both drugs.

Preparation of sample solution

Ten tablets were weighed accurately. Powder equivalent to 10 mg of TENE and 20 mg of TENE was weighed and transferred in a 50ml volumetric flask containing 25 ml of methanol. The mixture was sonicated for 10 minutes to dissolve the content. Then volume was made up to the mark with methanol with intermittent shaking. The resultant solution was filtered through 0.45 µm membrane filter. Further **10 ml of the clear filtrate was** taken into 20 ml volumetric flask and diluted up to the mark.

METHOD DEVELOPMENT

Trial-1

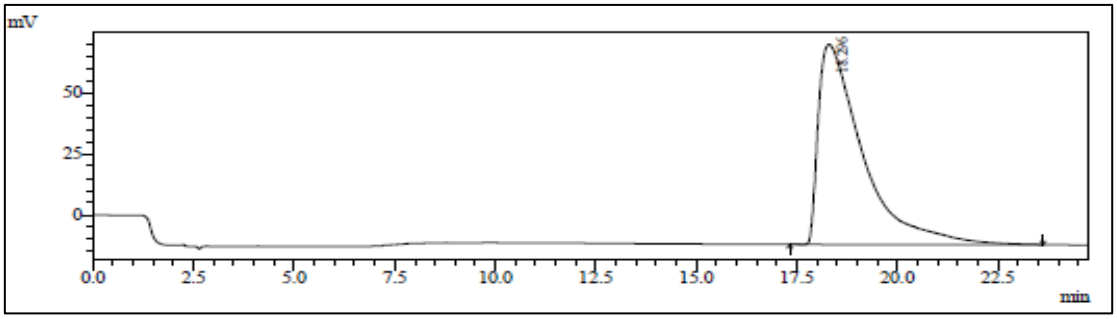

Figure 8: Chromatogram for Dapagliflozin 100 ppm Water: Methanol (50:50)

Trial-2

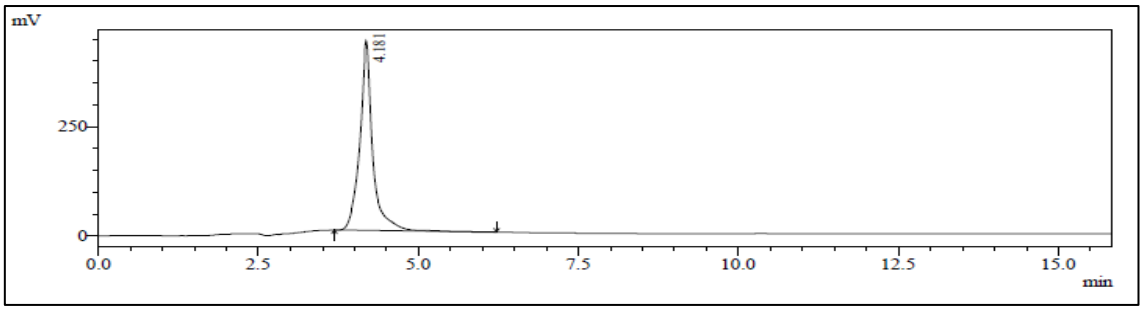

Figure 9: Identification peak of Teneligliptin 200 ppm Water: Methanol (20:80)

Trial-3

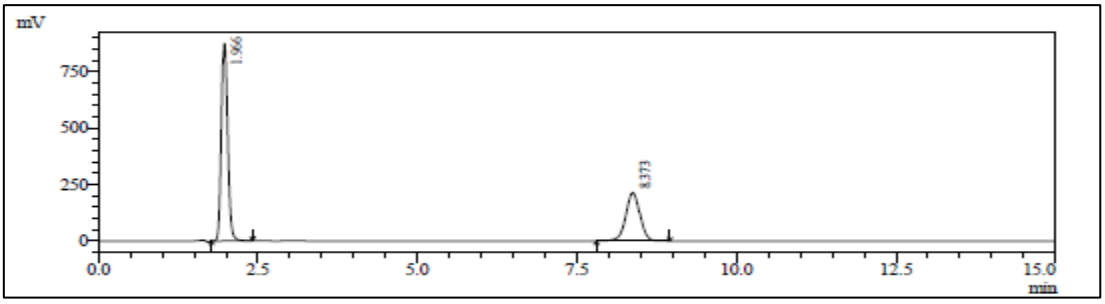

Figure 10: Identification peak of Teneligliptin 200 ppm + Dapagliflozin 100 ppm0.1%TFA: Methanol (40:60)

Trial-4

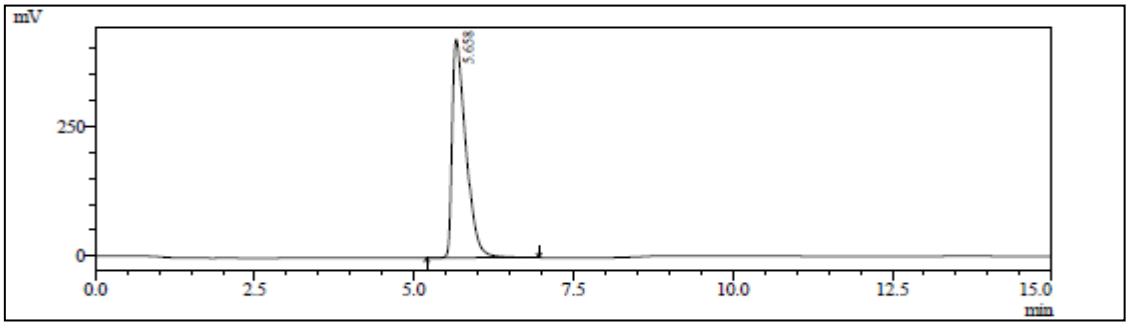

Figure 11: Identification peak of Teneligliptin 200 ppm + Dapagliflozin 100 ppm 0.1%TFA: Methanol (50:50)

Trial-5

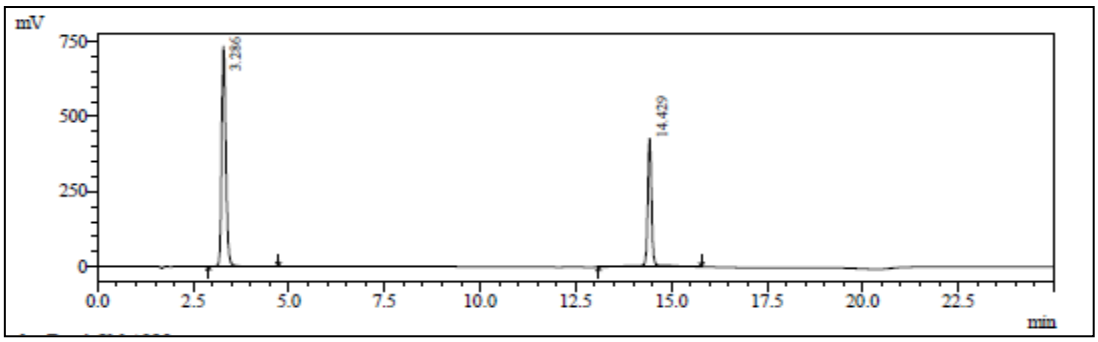

Figure 12: Identification peak of Teneligliptin 200 ppm + Dapagliflozin 100 ppm 0.1%TFA: Methanol

Trial-6 (Final)

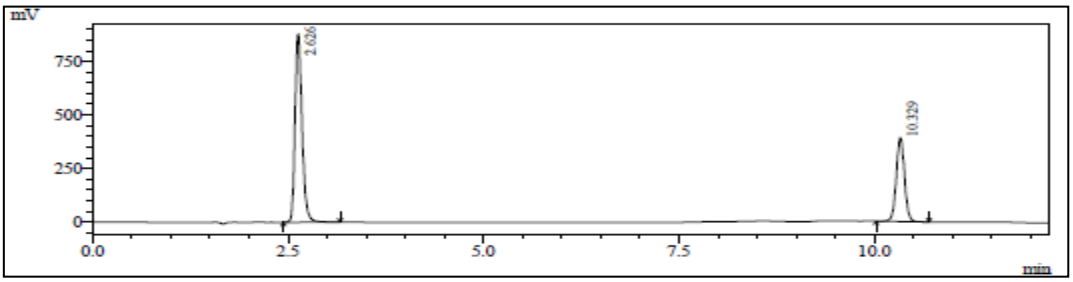

Figure 13: Identification peak of Teneligliptin 200 ppm + Dapagliflozin 100 ppm 0.1%TFA: Methanol

Table: 3 Mobile phase selection

Sr. no	Mobile phase composition	Inference
1	Water: Methanol (50:50)	Peak elutes very late
2	Water: Methanol (20:80)	Irregular peak shape
3	0.1%TFA: Methanol (40:60)	peak elutes in void volume (Teneligliptin theoretical plates very less)
4	0.1%TFA: Methanol (50:50)	dapagliflozin peak did not elute
5	0.1%TFA: Methanol (Gradient)	peaks eluted (Further trials taken to shorten and optimize method.)
6	0.1%TFA: Methanol (Gradient)	Peak observed both drugs observed with good shape and tailing factor.

IV. METHOD VALIDATION

Specificity

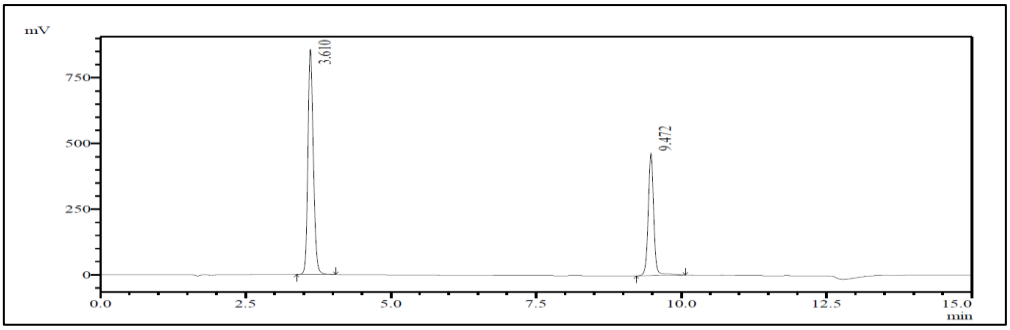

Figure 14: Chromatogram of Standard DAPA and TENE

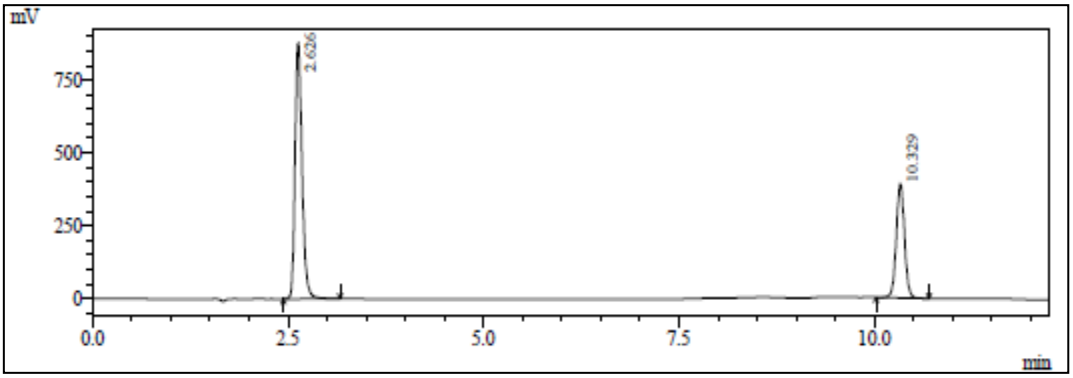

Figure 15: Chromatogram of Sample Hydrochlorothiazide and Clonidine HCL

Linearity

For the linearity study 5,10,15,20,25,30 ml of DAPA, 5,10,15,20,25,30 ml of TENE was mixed in six 10ml volumetric flask and volume was made up to mark by Methanol. Calibration curve DAPA and TENE are shown in figure.

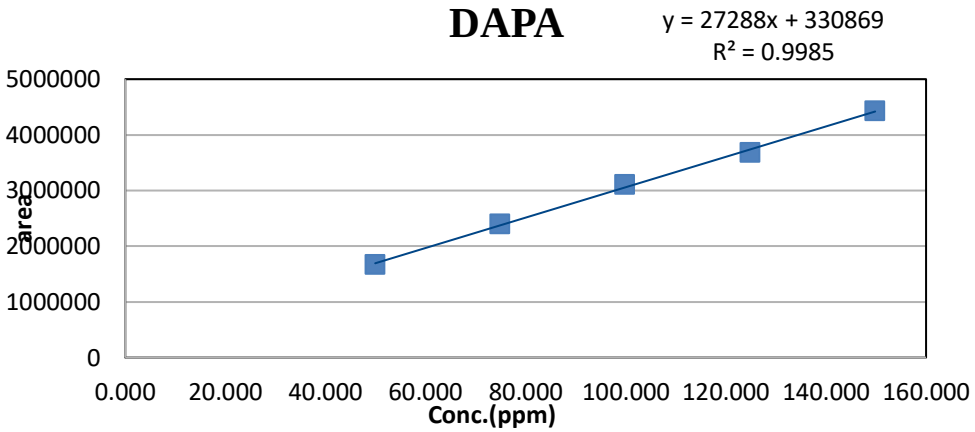

Figure 16: Calibration Curve of DAPA

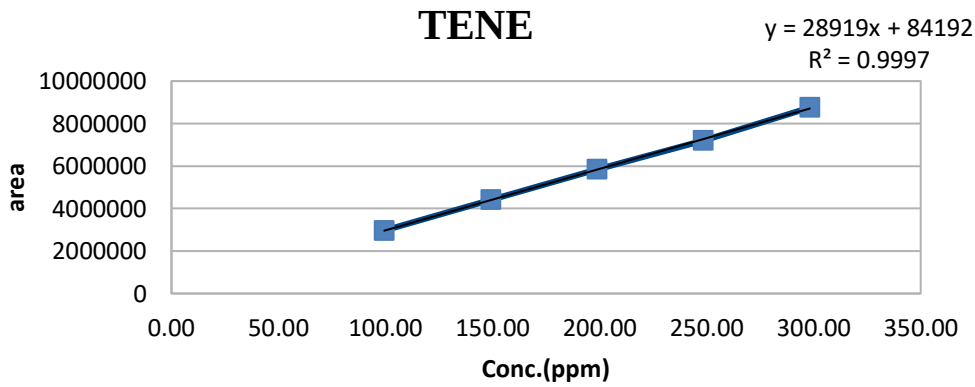

Figure 17: Calibration curve of TENE

Table 4: Linearity study of DAPA and TENE

DAPA		TENE	
Concentration (µg/ml)	**Peak Area**	**Concentration (µg/ml)**	**Peak Area**
49.941	1666376	99.41	2966047
74.912	2398093	149.12	4405454
99.883	3104782	198.83	5840588
124.854	3681411	248.53	7203834
149.824	4431728	298.24	8754131

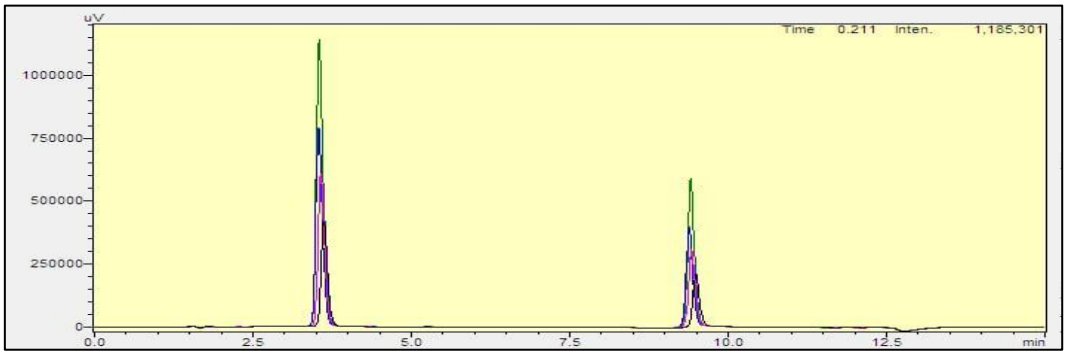

Figure 18: Overlain linearity chromatogram of DAPA and TENE

Repeatability

The data for repeatability of peak area measurement for DAPA and TENE based on six measurements of same solution. The % RSD for DAPA and TENE are shown in table.

Intra-day and inter-day Precision

In the intra-day studies, three repeated injections of standard solution were made and response factor of drug peaks and % RSD were calculated. In inter-day variation studies, three injections of standard solution were made for three consecutive days and response of drug peaks and % RSD were calculated.

Table 5: Repeatability study

Concentrationof DAPA(µg/ml)	DAPA		Concentration of TENE(µg/ml)	TENE	
	Mean ± SD (n=6)	% RSD		Mean ± SD (n=6)	% RSD
100	3055892.4±43362.9	0.8	200	5573370.6±7724.68	0.1

Table 6: Intraday & Inter-day precision study of DAPA

Drug	Conc. (µg/ml)	Intra-day precision		Inter-day precision	
		Mean ± SD(n=3)	% RSD	Mean ± SD (n=3)	% RSD
DAPA	100	2874584 ±10095.44893	1.0	3044795± 46814.22969	0.9

Table 7: Intraday & Inter-day precision study of TENE

Drug	Conc. (µg/ml)	Intra-day precision		Inter-day precision	
		Mean ± SD (n=3)	% RSD	Mean ± SD (n=3)	% RSD
TENE	200	5791485±9387.053265	0.2	5569949±7549	0.6

Accuracy:

Table 8: Recovery study for DAPA and TENE

Drug	% Of Level	Amount (µg/ml)	AmountAdded (µg/ml)	Total Amount Found (µg/ml)	% Recovery ± SD (n=3)
DAPA	50 %	1	3.17	3.19	101.0± 0.32
	100 %	1	6.34	6.40	100.95 ± 0.04
	150 %	1	9.51	9.52	101.55 ± 0.27
TENE	50 %	1	3.03	3.07	101.3 ± 0.2
	100 %	1	6.07	6.04	100.11 ±0.06
	150 %	1	9.10	9.12	101.00 ±0.02

Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 10 %).
2. Temp of Mobile phase was changed (± 5°C).
3. Ratio of Mobile phase was changed (± 2 %). The results were shown in table.

Table:9 Robustness data for DAPA and TENE

Drug	Area at Temp. (-5°C)	Area at Temp. (+5°C)	Area at Flow (-10% ml/min)	Area at Flow (+10% ml/min)	Area at Mobile Phase (-2%)	Area at Mobile Phase (+2%)
DAPA	3114388	3050905	3226746	2732741	3062417	3049193
	3101013	3034457	3236867	2743528	3092137	3002911
	3180887	2993141	3237226	2761936	3077136	3063233
% R.S.D	1.4	1.0	0.2	0.5	0.5	1.0
TENE	5619095	5648000	6230914	5111269	5626950	5615996
	5618981	5642223	6237301	5108958	5625648	5617423
	5626722	5639910	6228827	5099599	5631084	5629262
% R.S.D	0.1	0.1	0.1	0.1	0.1	0.1

LOD and LOQ

Calibration curve was repeated for Three times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where, SD = Standard deviation of intercepts. The results were shown in table.

Table 10: Limit of Detection and Limit of Quantitation Data of DAPA and TENE

DAPA	TENE
LOD = 3.3 x (SD / Slope) = 3.3 x (1078198/ 27288.04832) = 130.3887 mg/ml = 0.130 µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (2273189 / 28918.65858) = 259.40081 mg/ml = 0.259 µg/ml
LOQ = 10 x (SD / Slope) = 10 x (1078198/ 27288.04832) =395.1173 mg/ml = 0.395 µg/ml	LOQ = 10 x (SD / Slope) = 10 x (2273189 / 28918.65858) = 786.0630 mg/ml = 0.786 µg/ml

V. Forced Degradation Condition

1. Acid Degradation

- **Acid degradation Standard:** Accurately measured 2 ml of Teneligliptin standard stock and 2 ml dapagliflozin standard stock solutions were taken into 20 mL volumetric flask. 2 ml 1 N HCl was added into the flask. The flask was kept on table top at room temperature for 8 hours. Solution was then neutralized with 2 ml 1 N NaOH Volume was made up to the mark with water and injected in to HPLC system.
- **Acid degradation Sample:** 10 ml of filtered sample stock solution was taken into 20 ml volumetric flask. To this, 2 ml 1 N HCl was added. The flask was kept on table top at room temperature for 8 hours. Solution was then neutralized with 2 ml 1 N NaOH. Volume was made up to the mark with water and injected in to HPLC system.

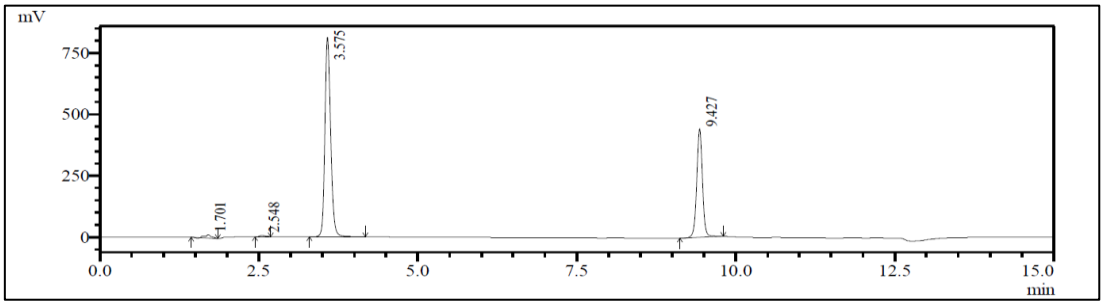

Figure 19: Chromatogram of DAPA and TENE under Acid Degradation Standard

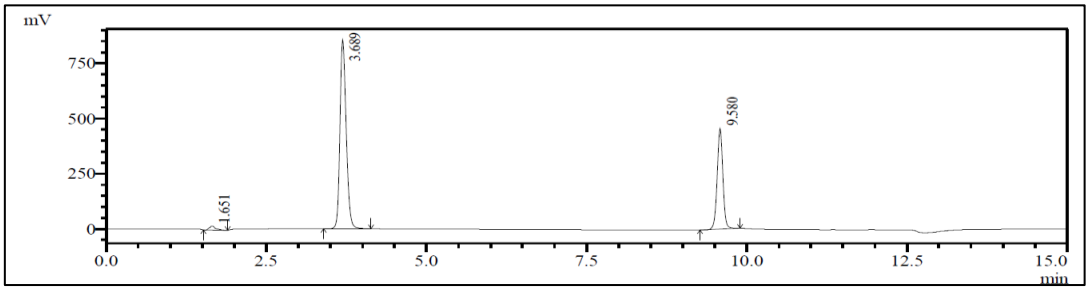

Figure 20: Chromatogram of DAPA and TENE under acid degradation Sample

2. Base Degradation

- **Base degradation Standard:** Accurately measured 2 ml of Teneligliptin standard stock and 2 ml dapagliflozin standard stock solutions were taken into 20 mL volumetric flask. 2 ml 1 N NaOH was added into the flask. The flask was kept on table top at room temperature for 6 hours. Solution was then neutralized with 2 ml 1 N HCL. Volume was made up to the mark with water and injected in to HPLC system
- . **Base degradation Sample**10 ml of filtered sample stock solution was taken into 20 ml volumetric flask. To this, 2 ml 1 N NaOH was added. The flask was kept on table top at room temperature for 6 hours. Solution was then neutralized with 2 ml 1 N HCL. Volume was made up to the mark with water and injected in to HPLC system.

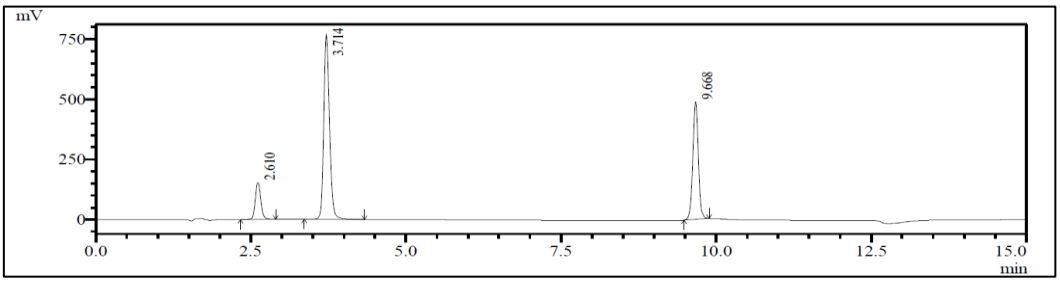

Figure 21: Chromatogram of DAPA and TENE under base degradation Standard

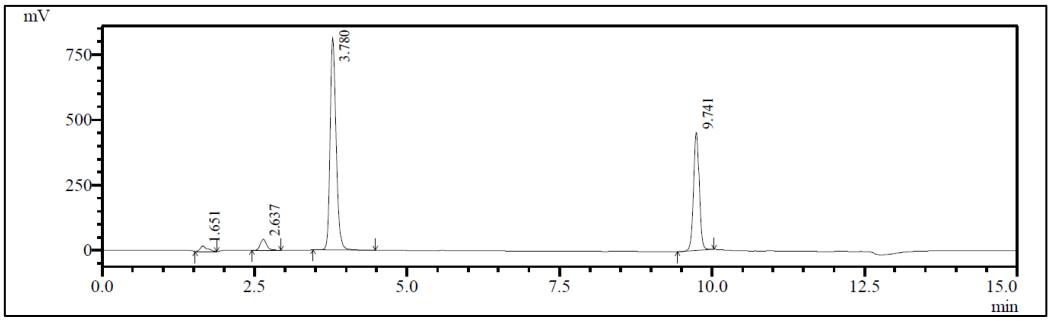

Figure 22: Chromatogram of DAPA and TENE under base degradation Sample

3. Oxidative Degradation

- **Peroxide degradation Standard:** Accurately measured 2 ml of Teneligliptin standard stock and 2 ml dapagliflozin standard stock solutions were taken into 20 mL volumetric flask and 1 ml 3 % H2O2 was added and solution was kept at room temperature for 60 min for an Oxidative hydrolysis and made volume up to mark with water filtered and injected in to HPLC system.

- **Peroxide degradation Sample:** 5 ml stock solution into 20 ml volumetric flask and 0.2 ml 3% H2O2. Set for 1 hours at room temperature. Volume made with diluents and injected into HPLC.

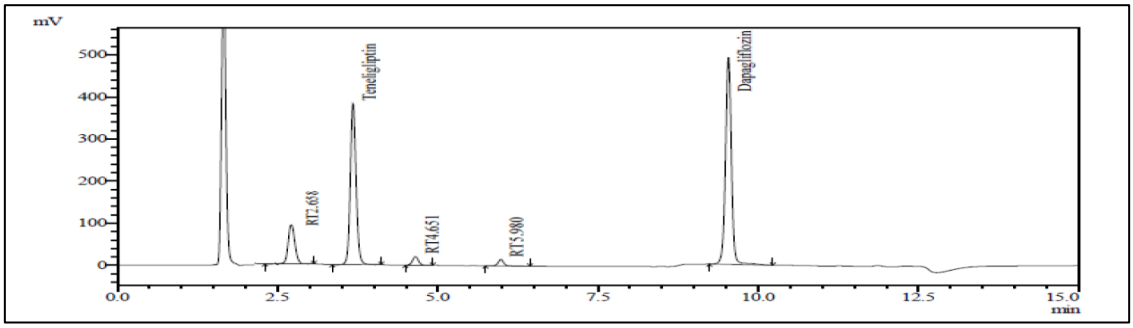

Figure 23: Chromatogram of DAPA and TENE under oxidation degradation Standard

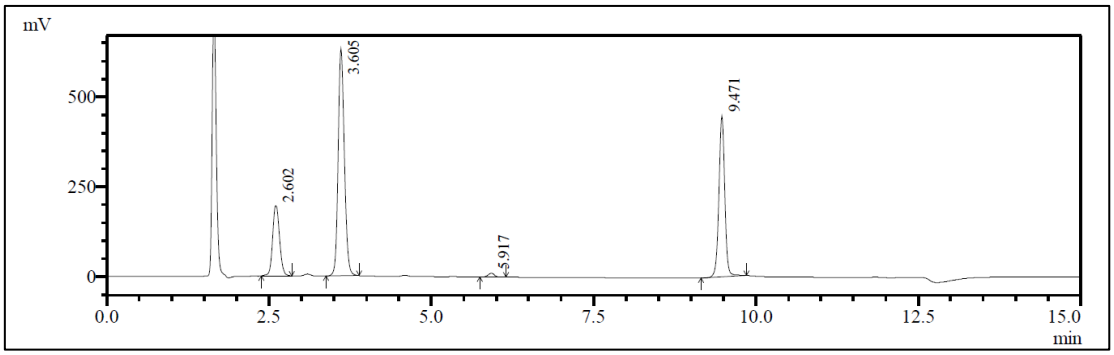

Figure 24: Chromatogram of DAPA and TENE under oxidation degradation Sample

4. Photo Degradation

- Tablet powder and APIs were kept into photo chamber and exposed to 200 watt-hours per square meter, 200-800 nm (UV+ visible) to achieve 1.2 million lux hours. Solutions were made as per method preparation and injected into HPLC.

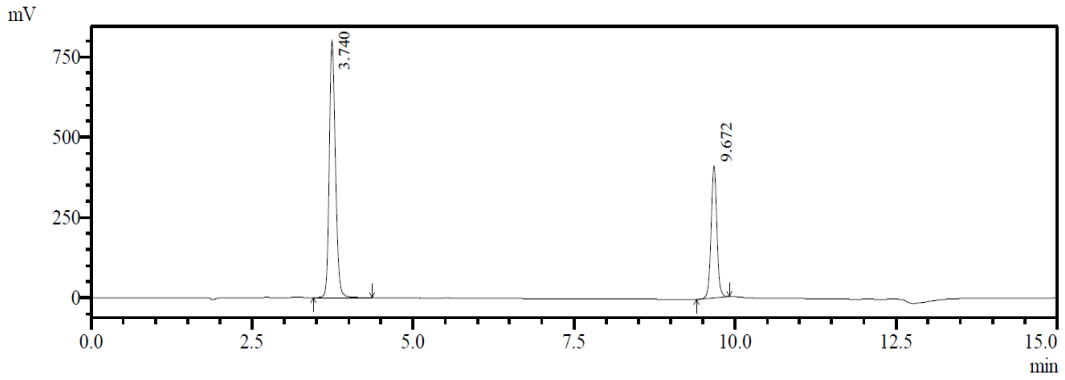

Figure 25: Chromatogram of DAPA and TENE under Photo degradation Standard

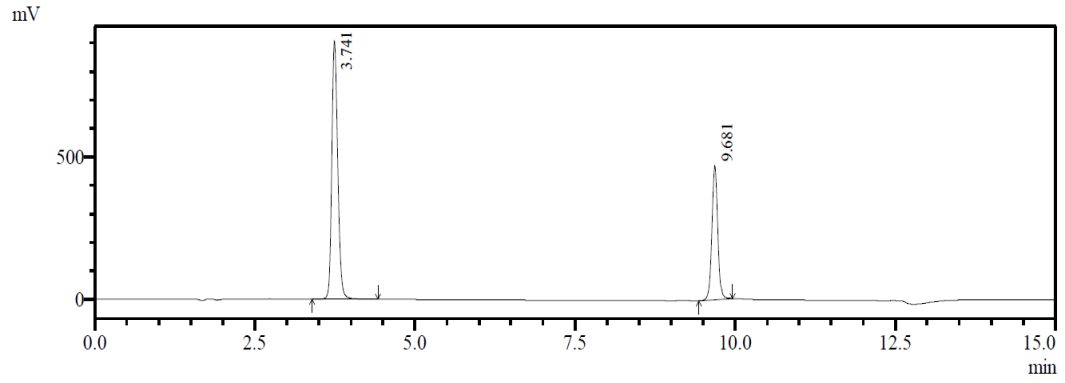

Figure 26: Chromatogram of DAPA and TENE under Photo degradation Sample

5. Thermal Degradation

- Tablet powder and APIs were kept into hot air oven at 70°C for 48 hours and then solutions were made as per test sample preparation and chromatographed.

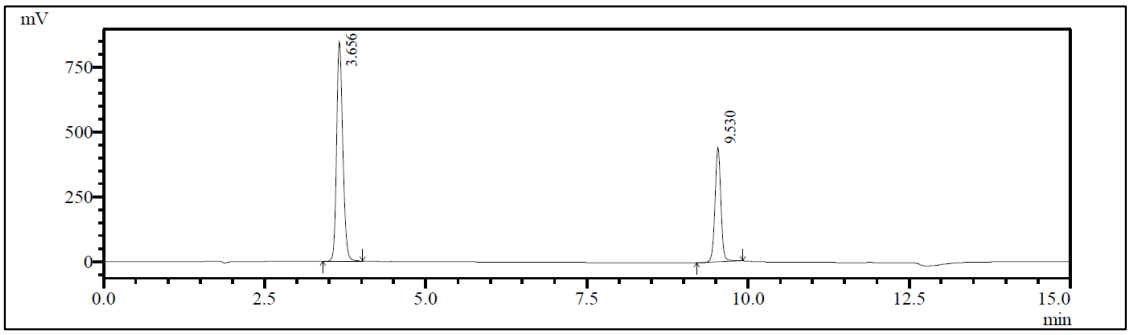

Figure 27: Chromatogram of DAPA and TENE under thermal degradation Standard

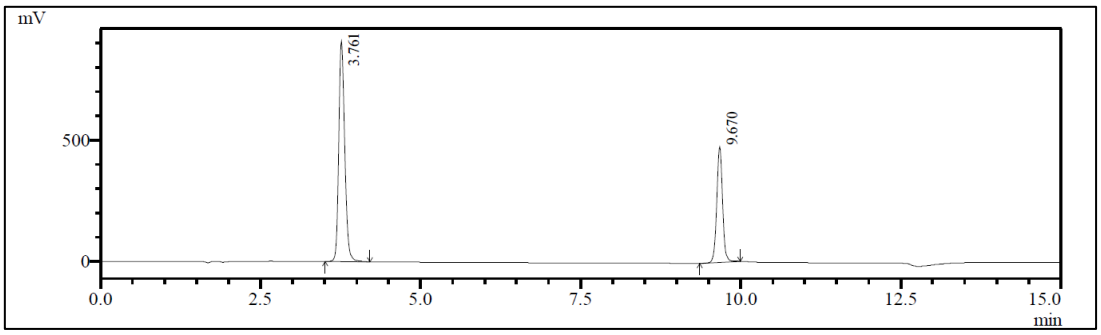

Figure 28: Chromatogram of DAPA and TENE under thermal degradation Sample

Table 11: Result of stability study of DAPA and TENE

Condition	% Degradation DAPA		% Degradation TENE	
	Sample	Standard	Sample	Standard
Acid	9.6	9.4	4.7	7.1
Base	9.6	0.8	9.0	10.2
Oxidation	9.8	6.9	30.8	44.9
Thermal	1.0	1.9	1.4	0.7
Photo	5.0	3.5	3.5	3.6

RESULT AND DISCUSSION

The present work aimed development and validation of stability indicating RP-HPLC method for simultaneous estimation of DAPA and TENE. The melting point of DAPA (75-79 °C) and TENE (208-213 °C) was found in the range. Method was developed in mobile phase containing Gradient program0.1%TFA: Methanol. Detection was carried out at 235 nm. Method was validated as per ICH guidelines. Linearity and regression data were shown in table and Figure. % Recovery was within the range (99% - 102%). Results were shown in table. Hence it is found that the developed method is accurate. %RSD values were <2 for repeatability, intra-day and inter-day precision. Results were shown in table. So, the developed method was found to be precise. LOD and LOQ values were shown in table. LOD & LOQ confirms the method to be sensitive. Small changes were carried out in mobile phase and flow rate for robustness study, in that % RSD of area was found to be <2. So, the developed method was found to be robust. Various forced degradation conditions were performed in proposed method and it can efficiently separate all the degradation products from the drugs. % degradation values are 1% to 31% degradation of the drug substance, have been considered as reasonable and acceptable for validation of chromatographic assays. So, the developed method is stability indicating.

CONCLUSION

TENE inhibit the action of DPP-4 enzymes and slow down the rapid degradation of incretins. and increase incretin levels (GLP-1 and GIP), which inhibit glucagon release, which in turn increases insulin secretion, decreases gastric emptying, and decreases blood glucose levels. DAPA inhibiting SGLT2, DAPA blocks reabsorption of filtered glucose in the kidney, increasing urinary glucose excretion and reducing blood glucose levels.

RP-HPLC method was developed for simultaneous estimation DAPA and TENE. In RP-HPLC method, good resolution and separation of two drugs was achieved. Gradient program0.1%TFA: Methanol, mobile phase. Retention time of DAPA and TENE were found to be 9.47and 3.61 min respectively with a flow rate of 1 ml/min. The proposed method was accurate and precise. Therefore, proposed method can be used for routine analysis of DAPA and TENE in tablets.

Forced degradation study of DAPA and TENE was performed by RP-HPLC method which includes Acid, Base, Oxidative and Thermal degradation. Results of degradation were found within limit.

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