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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULANEOUS ESTIMATION OF DAPAGLIFLOZIN PROPANEDIOL MONOHYDRATE AND VILDAGLIPTIN 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 Vildagliptin 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 Vildagliptin in Tablet Dosage FORM has been developed. The separation was achieved Column Agilent Eclipse XDB (150 mm x 4.6 mm x 5 µ), Gradient program20 mM potassium dihydrogen phosphate buffer: Acetonitrile as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 210nm, temperature 35◦C retention time of Dapagliflozin and Vildagliptin was found to be 9.11 and 2.58 min. The method has been validated for linearity, accuracy and precision. Linearity observed for Dapagliflozin Propanediol Monohydrate and Vildagliptin 25.15-746.25µg/ml. Developed method was found to be accurate, precise and rapid for estimation of Dapagliflozin Propanediol Monohydrate and Vildagliptin 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, Vildagliptin, 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 Dapagliflozin and Vildagliptin is shown in Figure. [4-5] Vildagliptin inhibit the action of DPP-4 enzymes and slow down the rapid degradation of incretins. Dapagliflozin 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 Vildagliptin 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 Dapagliflozin and Vildagliptin. Also, it is validated for market product named Dapagligen-V SR containing Dapagliflozin and Vildagliptin in tablet dosage form.

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 Dapagligen-V SR was purchased from local market.

IR identification and wavelength selection

The individual standard drugs, Dapagliflozin and Vildagliptin 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.

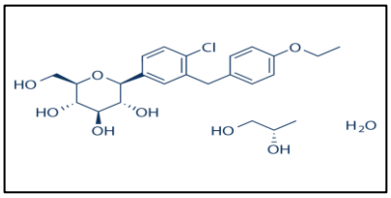

Figure 1. Structure of Dapagliflozin

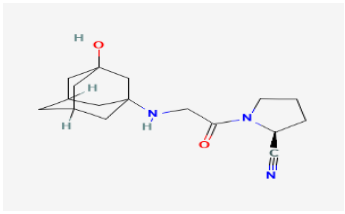

Figure 2. Structure of Vildagliptin

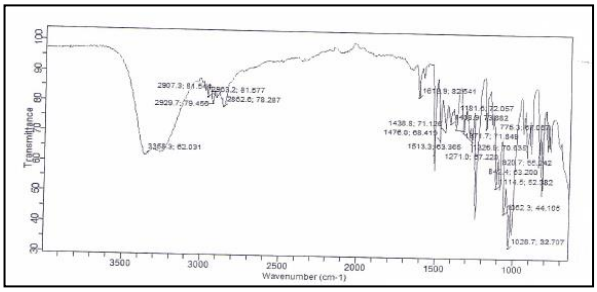

Figure 3: IR spectrum of Dapagliflozin (API)

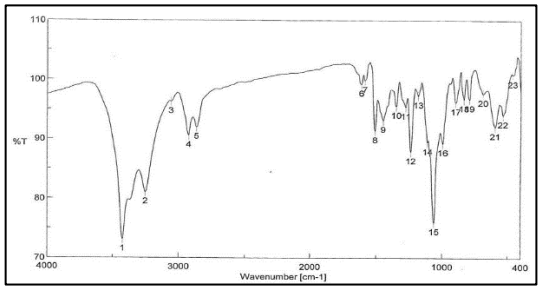

Figure 4: IR Spectrum of Dapagliflozin (Std.)

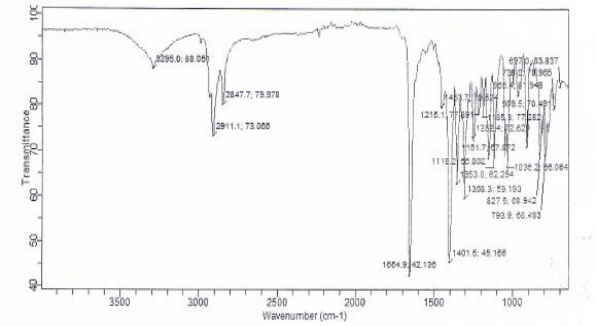

Figure 5: IR spectrum of Vildagliptin (API)

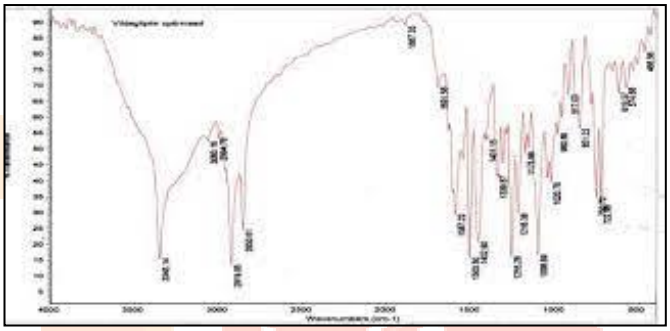

Figure 6: IR Spectrum of Vildagliptin (Std.)

Table 1: IR spectrum of Dapagliflozin

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 Vildagliptin

sr. No.	Functional group	Observed value	Standard value
1	O-H stretching	3295.0	3300-2500
2	N-H stretching	2911.1	3000-2800
3	C-H stretching	2847.7	2830-2695
4	C=O	1654.9	1800-1770

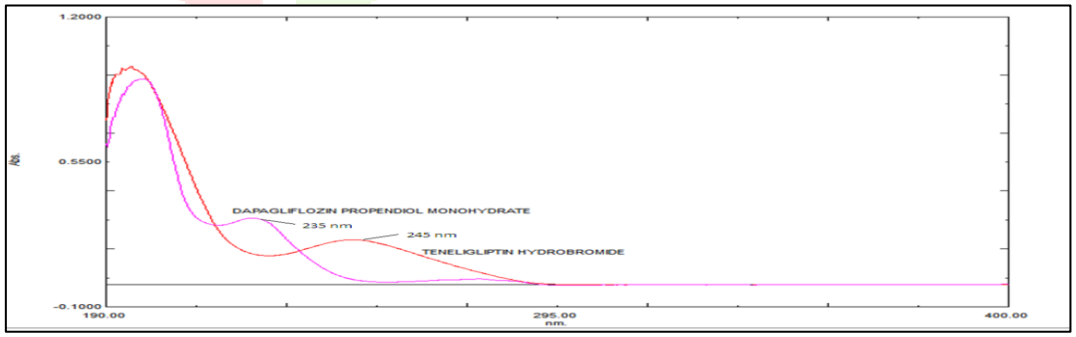

Figure 7: Determination of wavelength maximum (235 nm)

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 Dapagliflozin and Vildagliptin were further diluted with methanol into 10 ml volumetric flask which contain 20,40,60,80 and 200,400,600,800 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 0-800 µg/ml for both drugs.

Preparation of sample solution

Ten tablets were weighed accurately. Powder equivalent to 10 mg of Dapagliflozin and 100 mg of Vildagliptin 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

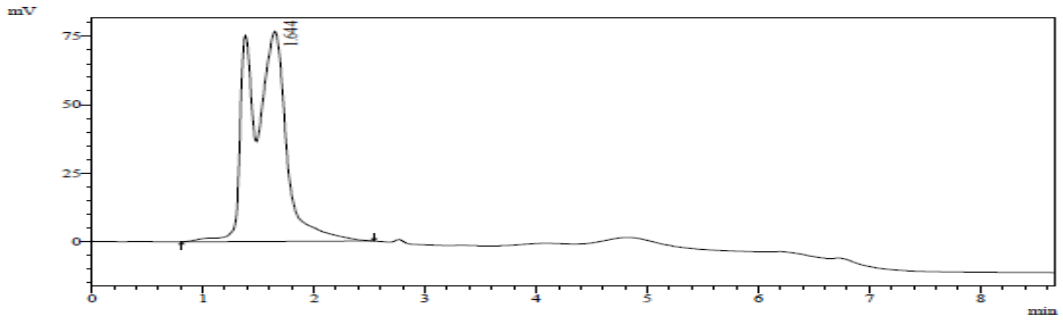

Figure 8: Chromatogram for Vildagliptin 500 ppm Buffer: ACN (50:50)

Trial-2

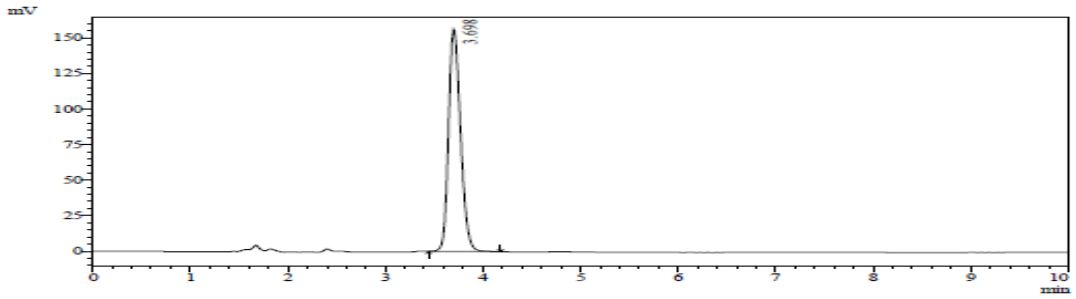

Figure 9: Identification peak of Vildagliptin 500 ppm Buffer: ACN (90:10)

Trial-3

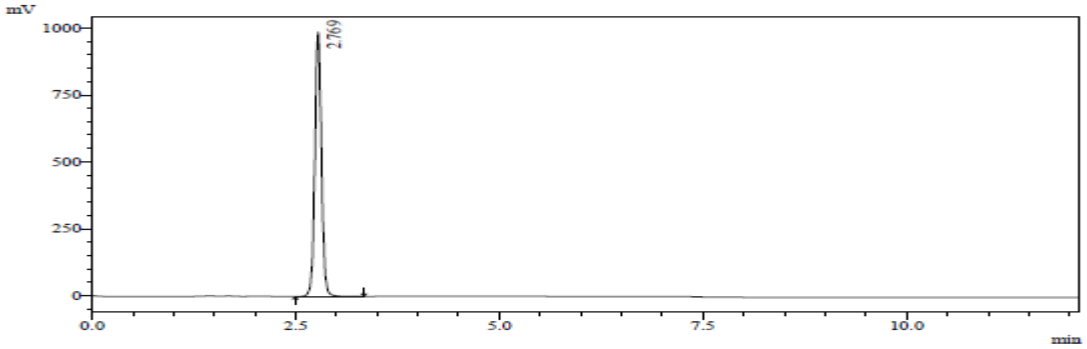

Figure 10: Identification peak of Dapagliflozin 50 ppm Buffer: ACN (50:50)

Trial-4

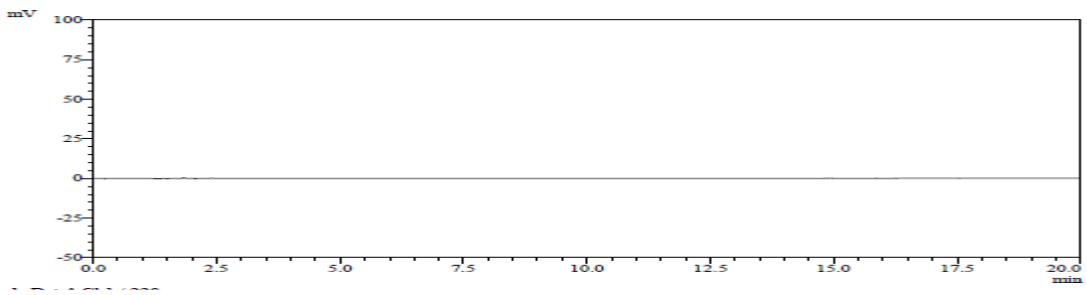

Figure 11: Identification peak of Dapagliflozin 50 ppm Buffer: ACN (90:10)

Trial-5

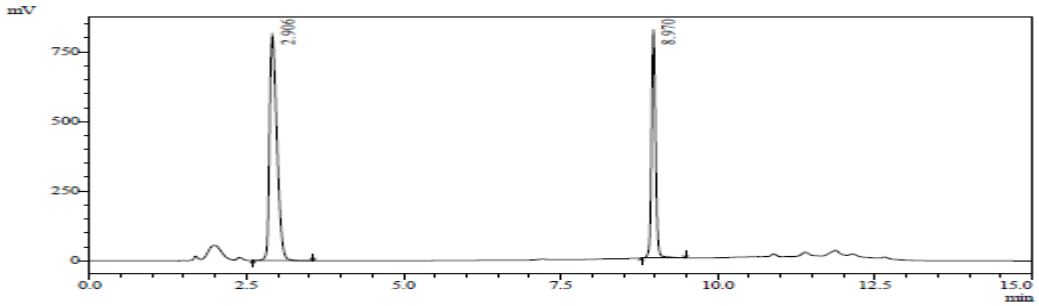

Figure 12: Identification peak of Vildagliptin 500 ppm + Dapagliflozin 50 ppm ACN (Gradient)

Trial-6 (Final)

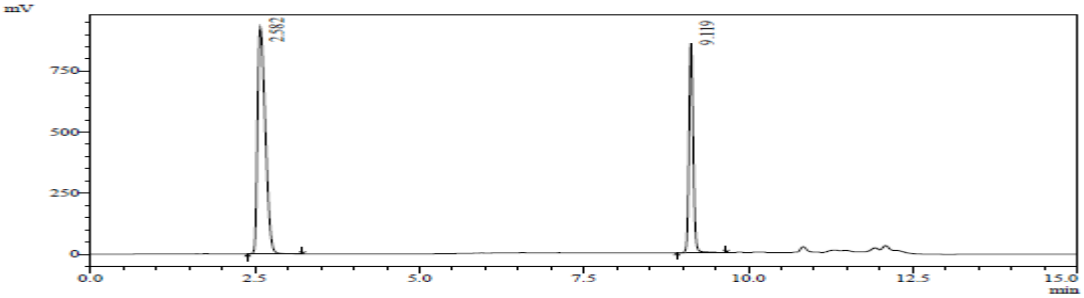

Figure 13: Identification peak of Vildagliptin 500 ppm + Dapagliflozin 50 ppm ACN (Gradient)

Table: 3 Mobile phase selection

Sr. no	Mobile phase composition	Inference
1	Buffer: ACN (50:50)	Peak splitting observed
2	Buffer, :ACN (90:10)	Vildagliptin peak elutes properly
3	Buffer: ACN (50:50)	Dapagliflozin peak elutes properly
4	Buffer, :ACN (90:10)	dapagliflozin peak did not elute
5	Buffer, :ACN (Gradient)	peaks eluted (Further trials taken to shorten and optimize method.)
6	Buffer, :ACN (Gradient)	Peak observed both drugs observed with good shape and tailing factor.

IV. METHOD VALIDATION

Specificity

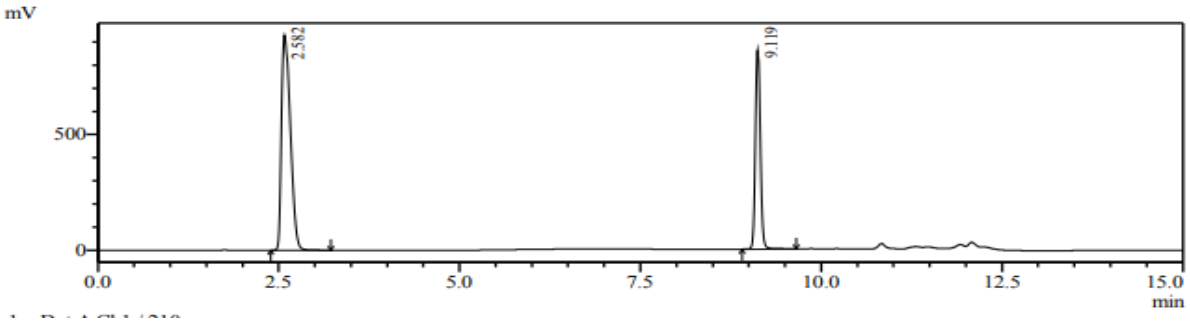

Figure 14: Chromatogram of Standard Dapagliflozin and Vildagliptin

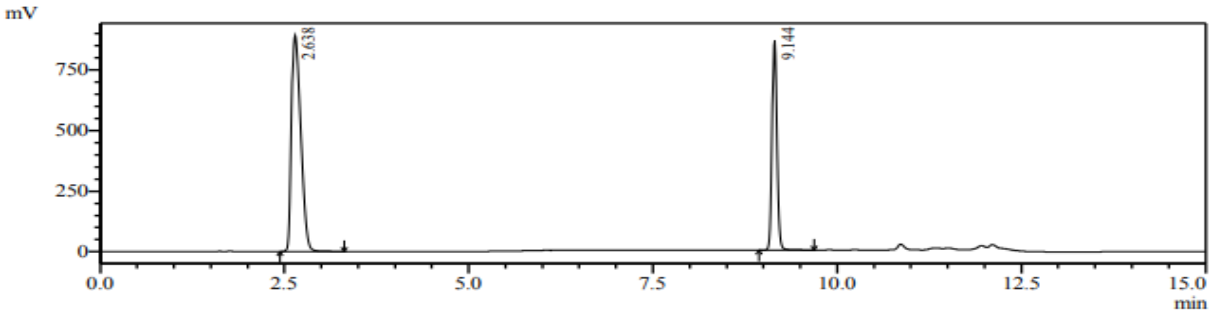

Figure 15: Chromatogram of Sample Dapagliflozin and Vildagliptin

Linearity

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

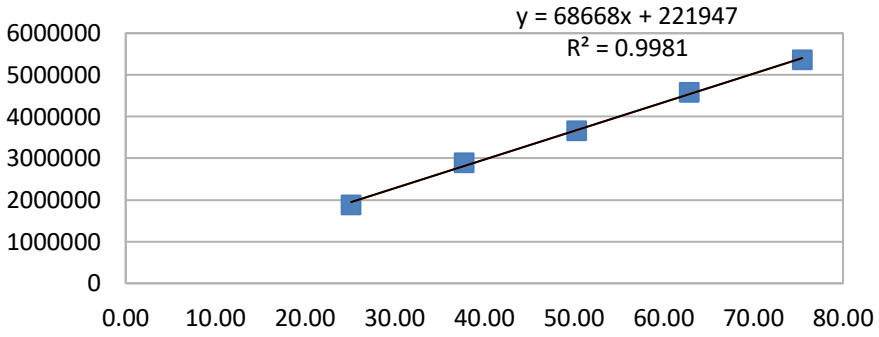

Figure 16: Calibration Curve of Dapagliflozin

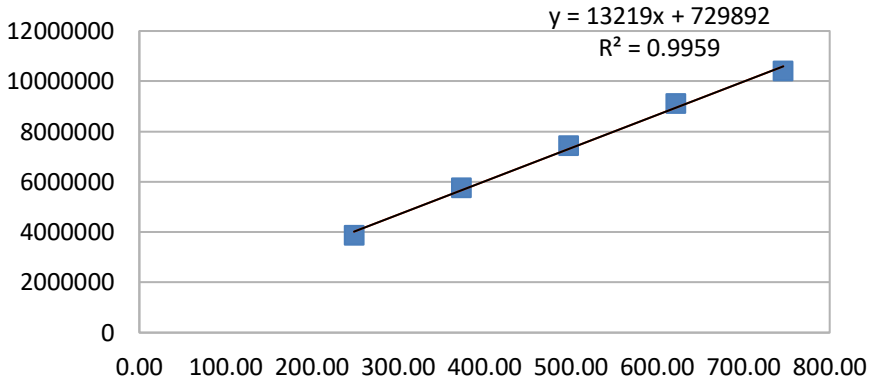

Figure 17: Calibration curve of Vildagliptin

Table 5: Linearity study of DAPA and VILDAGLIPTIN

Dapagliflozin		Vildagliptin	
Concentration (µg/ml)	**Peak Area**	**Concentration (µg/ml)**	**Peak Area**
25.15	1884072	248.75	3851809
37.72	2895511	373.13	5752906
50.30	3661881	497.50	7427858
62.87	4577351	621.88	9100109
75.45	5360562	746.25	10398684

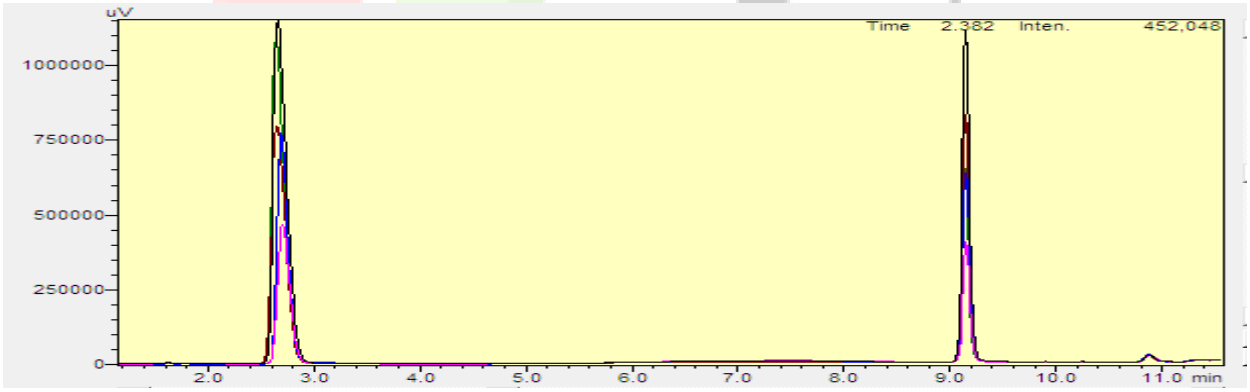

Figure 18: Overlain linearity chromatogram of Dapagliflozin and Vildagliptin

Repeatability

The data for repeatability of peak area measurement for Dapagliflozin and Vildagliptin based on six measurements of same solution. The % RSD for Dapagliflozin and Vildagliptin 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 6: Repeatability study

Concentrationof Dapagliflozin (µg/ml)	Dapagliflozin		Concentration of Vildagliptin(µg/ml)	Vildagliptin	
	Mean ± SD (n=6)	% RSD		Mean ± SD (n=6)	% RSD
50	3800953.833 ± 2708.34	0.1	500	7636789± 9206.83	0.1

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

Drug	Conc. (µg/ml)	Intra-day precision		Inter-day precision	
		Mean ± SD(n=3)	% RSD	Mean ± SD (n=3)	% RSD
Dapagliflozin	50	3798728.000±7863.04	0.1	3816044± 5418	0.2

Table 8: Intraday & Inter-day precision study of Vildagliptin

Drug	Conc. (µg/ml)	Intra-day precision		Inter-day precision	
		Mean ± SD (n=3)	% RSD	Mean ± SD (n=3)	% RSD
Vildagliptin	500	7652010±8814.7481	0.1	7666116±5734	0.1

Accuracy:

Table 9: Recovery study for Dapagliflozin and Vildagliptin

Drug	% Of Level	Amount (mg/ml)	AmountAdded (mg/ml)	Total Amount Found (mg/ml)	% Recovery ± SD (n=3)
Dapagliflozin	50 %	1	1.56	1.58	101.1± 0.2
	100 %	1	3.12	3.10	99.1 ± 0.15
	150 %	1	4.52	4.56	100.9 ± 0.15
Vildagliptin	50 %	1	12.44	12.86	103.5 ± 0.11
	100 %	1	24.88	24.76	99.5 ±0.16
	150 %	1	35.07	34.80	99.30 ±0.1

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: 10 Robustness data for Dapagliflozin and Vildagliptin

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%)
Dapagliflozin	3837264	3868683	4257302	3518176	3845045	3849100
	3845464	3874598	4255999	3518273	3842172	3858784
	3877412	3872955	4259210	3522133	3847850	3861837
% R.S.D	0.6	0.1	0.0	0.1	0.1	0.2
Vildagliptin	7735079	7692985	8530597	6988803	7837556	7486199
	7733372	7684370	8520731	6992510	7825938	7498001
	7735324	7690543	8522151	6992533	7834120	7507746
% R.S.D	0.0	0.1	0.1	0.0	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 12: Limit of Detection and Limit of Quantitation Data of Dapagliflozin and Vildagliptin

Dapagliflozin	Vildagliptin
LOD = 3.3 x (SD / Slope) = 3.3 x (1366615.442 / 68667.83154) = 65.67 mg/ml = 0.06567 µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (2604894.761 / 13218.85668) = 650.297 mg/ml = 0.650 µg/ml
LOQ = 10 x (SD / Slope) = 10 x (1366615.442 / 68667.83154) =199.1173 mg/ml = 0.199 µg/ml	LOQ = 10 x (SD / Slope) = 10 x (2604894.761 / 13218.85668) = 197.0630 mg/ml = 0.1970 µg/ml

V. Forced Degradation Condition

1. Acid Degradation

Accurately measured 2 ml of Vildagliptin standard stock and 2 ml dapagliflozin standard stock solutions were taken into 20 mL volumetric flask. 2 ml 0.5 N HCl was added into the flask. The flask was kept on table top at temperature 60ºC for 6 hours. Solution was then neutralized with 2 ml 0.5 N NaOH Volume was made up to the mark with water and injected in to HPLC system.

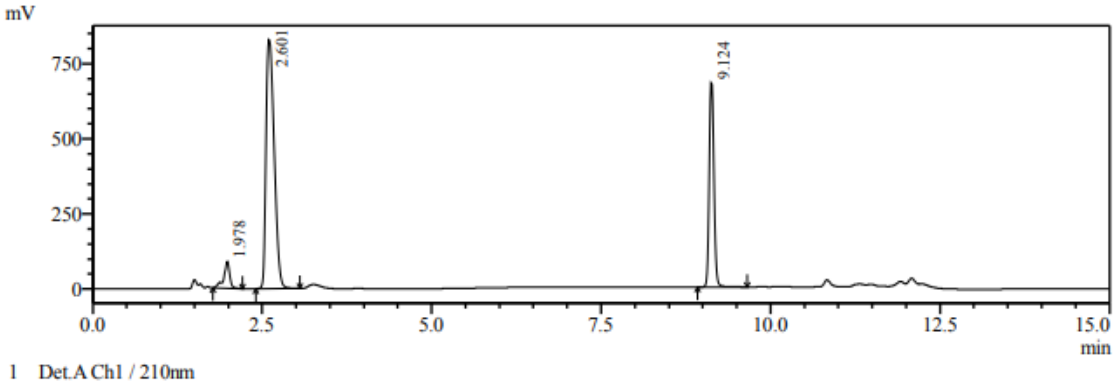

Figure 19: Chromatogram of Dapagliflozin and Vildagliptin under Acid Degradation Standard

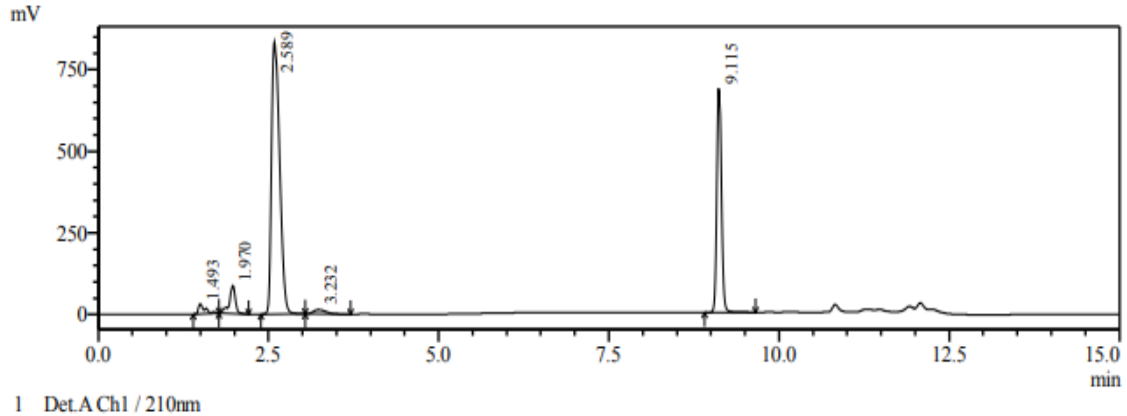

Figure 20: Chromatogram of Dapagliflozin and Vildagliptin under acid degradation Sample

2. Base Degradation

2 ml of API stock solution (2 ml of sample stock solution for sample) was taken into 20 mL volumetric flask. 2 ml 0.5 N NaOH was added into the flask. The solution was kept at 60 °C water bath for 6 hours. Solution was then allowed to cool down and then neutralized with 2 ml 0.5 N HCl Volume was made up to the mark with water and injected in to HPLC system.

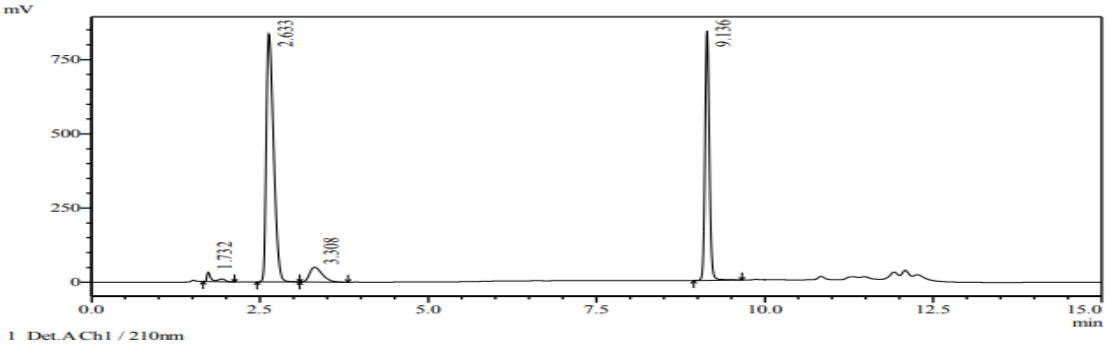

Figure 21: Chromatogram of Dapagliflozin and Vildagliptin under base degradation Standard

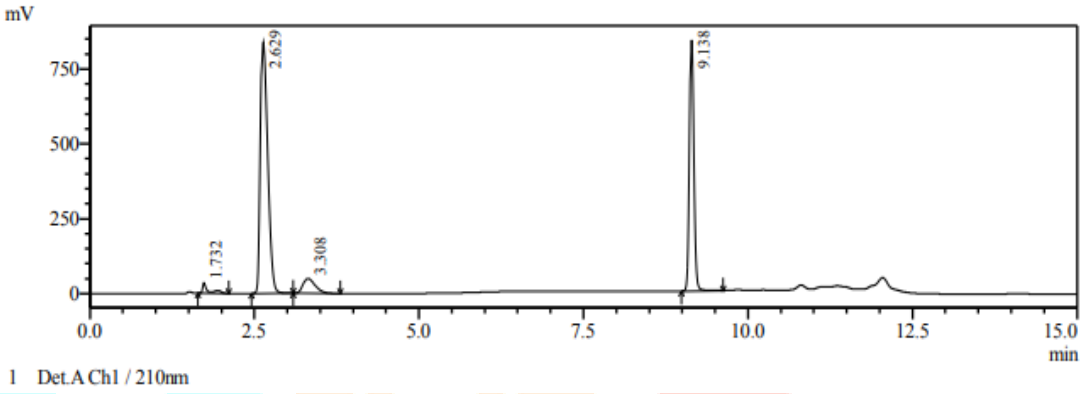

Figure 22: Chromatogram of Dapagliflozin and Vildagliptin under base degradation Sample

3. Oxidative Degradation

2 ml of API stock solution (2 ml of sample stock solution for sample) was taken into 20 mL volumetric flask. To this 2 ml 0.3% Hydrogen Peroxide was added. Solution was kept at room temperature for 6 hours and made volume up to mark with water filtered and injected in to HPLC system.

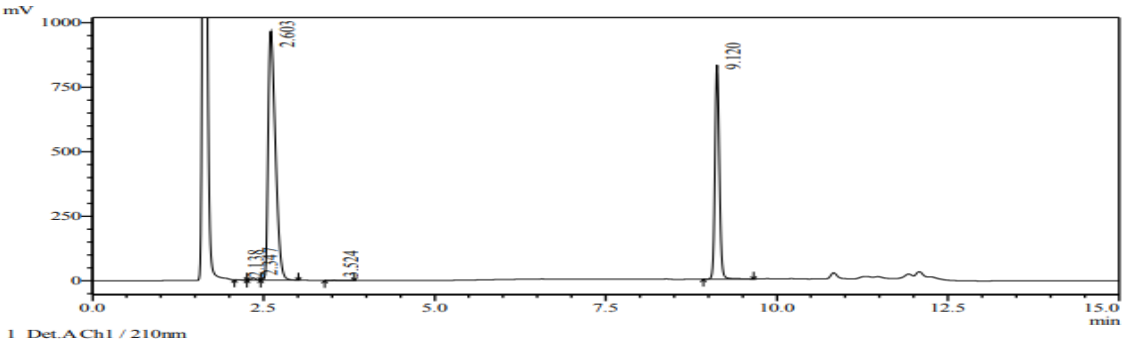

Figure 23: Chromatogram of Dapagliflozin and Vildagliptin under oxidation degradation Standard

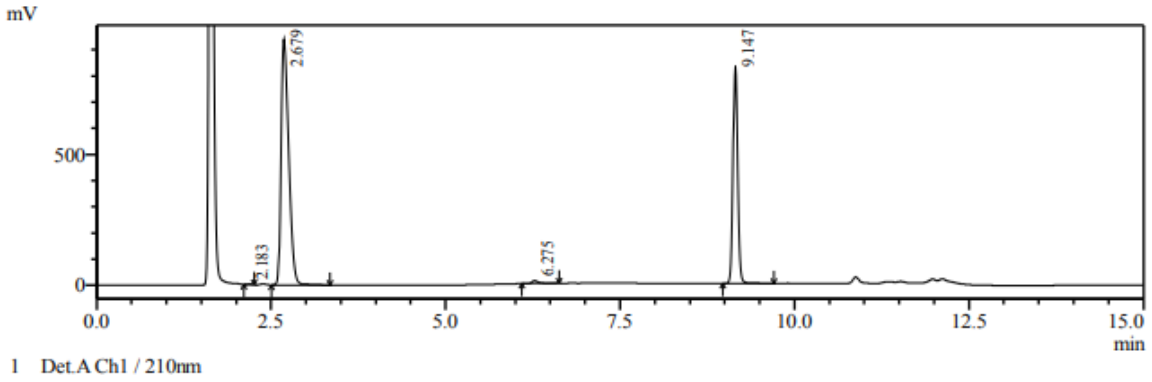

Figure 24: Chromatogram of Dapagliflozin and Vildagliptin under oxidation degradation Sample

4. Photo Degradation

Tablet powder and APIs were kept into sunlight for 72 hours. Solutions were made as per test method Solutions were made as per method preparation and injected into HPLC.

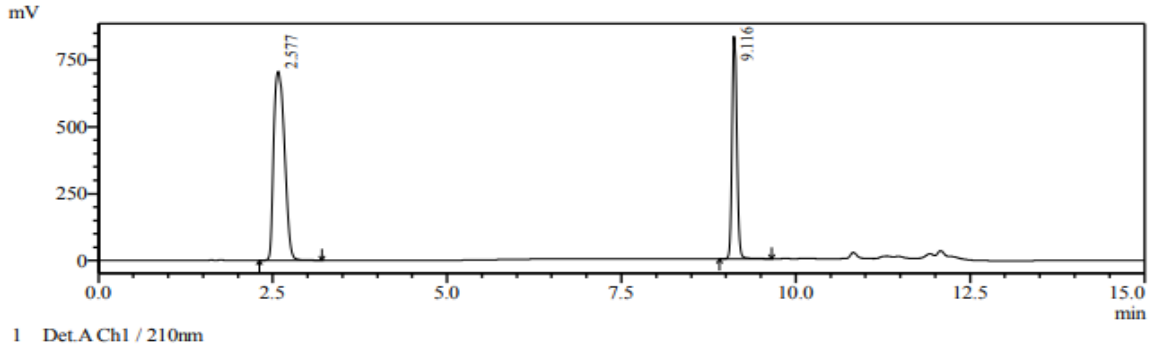

Figure 25: Chromatogram of Dapagliflozin and Vildagliptin under Photo degradation Standard

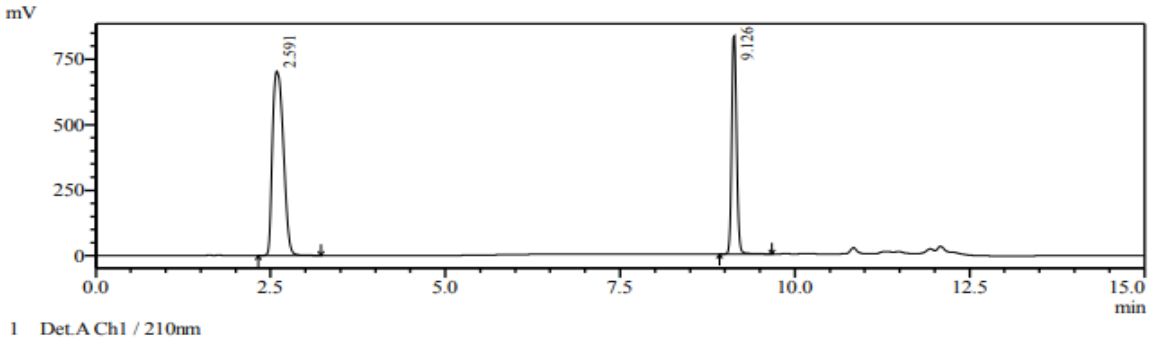

Figure 26: Chromatogram of Dapagliflozin and Vildagliptin under Photo degradation Sample

5. Thermal Degradation

Tablet powder and APIs were kept into hot air oven at 80 °C for 24 hours and then solutions were made as per test sample preparation and chromatographed.

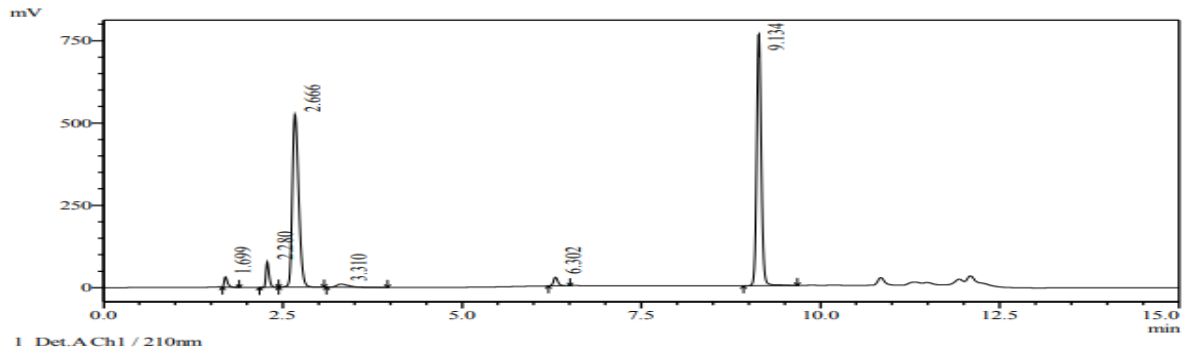

Figure 27: Chromatogram of Dapagliflozin and Vildagliptin under thermal degradation Standard

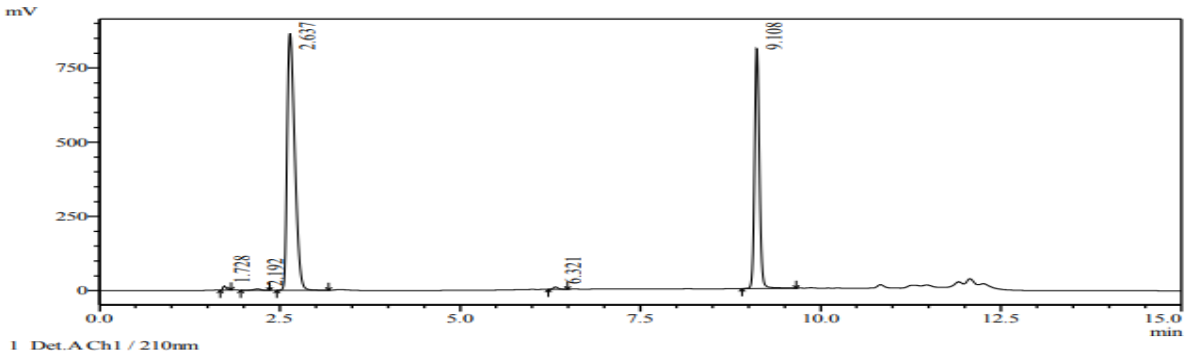

Figure 28: Chromatogram of Dapagliflozin and Vildagliptin under thermal degradation Sample

Table 15: Result of stability study of Dapagliflozin and Vildagliptin

Condition	% Degradation Dapagliflozin		% Degradation Vildagliptin	
	Sample	Standard	Sample	Standard
Acid	9.053142	20.45777	13.15718	13.15718
Base	15.6735	85.34509	47.79671	47.79671
Oxidation	-85.5425	-85.8191	7.168879	7.168879
Thermal	10.26131	10.65582	18.35495	18.35495
Photo	2.941928	2.935015	3.21844	3.21844

RESULT AND DISCUSSION

The present work aimed development and validation of stability indicating RP-HPLC method for simultaneous estimation of Dapagliflozin and Vildagliptin. The melting point of Dapagliflozin (74-78 °C) and Vildagliptin (149-155 °C) was found in the range. Method was developed in mobile phase containing Gradient program potassium dihydrogen phosphate buffer: ACN. Detection was carried out at 210 nm. Method was validated as per ICH guidelines. Linearity and regression data were shown in table and Figure. % Recovery was within the range (99% - 106%). 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 -86% to 11% 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

Vildagliptin 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, Dapagliflozin 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 Dapagliflozin and Vildagliptin. In RP-HPLC method, good resolution and separation of two drugs was achieved. Gradient program potassium dihydrogen phosphate buffer: ACN, mobile phase. Retention time of Dapagliflozin and Vildagliptin were found to be 9.11and 2.58 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 Dapagliflozin and Vildagliptin in tablets.
Forced degradation study of Dapagliflozin and Vildagliptin 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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