IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULANEOUS ESTIMATION OF DAPAGLIFLOZIN PROPANEDIOL MONOHYDRATE AND SITAGLIPTIN PHOSPHATE MONOHYDRATE IN TABLET

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ABSTRACT:simple, rapid, economical, precise and accurate stability indicating rp- hplc method for the estimation of dapagliflozin propanediol monohydrate and sitagliptin phosphate monohydrate 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 sitagliptin phosphate monohydrate in tablet dosage form has been developed. the separation was achieved column Kromasil C18 (150 x 4.6) 5 µm ID, gradient program 20 mM potassium dihydrogen phosphate buffer : Aceonitrile, as mobile phase, at a flow rate of 1 ml/min. detection was carried out at 220 nm retention time of dapa and Sita was found to be 8.71 and 2.94 min. the method has been validated for linearity, accuracy and precision. linearity observed for dapagliflozin propanediol monohydrate and sitagliptin phosphate monohydrate 25.68-755.83 µg/ml. developed method was found to be accurate, precise and rapid for estimation of dapagliflozin propanediol monohydrate and sitagliptin phosphate monohydrate 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, sitagliptin phosphate monohydrate, 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. diabetes is group of conditions where the body cannot produce enough or any insulin, cannot properly use the insulin that is produced, or cannot do a combination of either.When any of these things happen, the body is unable to get sugar from the blood into your cells. This can lead to high blood sugar levels.Glucose, the from of sugar found in your blood, is one of your main energy sources. A lack of insulin or a build up in your blood. This can lead to health problems. [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 SITA is shown in Figure. [4-5] SITA Sitagliptin increases insulin production and decreases hepatic glucose overproduction. Sitagliptin prolongs the action of GLP-1 and GIP. By enhancing active incretin levels, sitagliptin increases insulin production and lowers glucagon secretion from alpha cells, which decreases hepatic glucose overproduction.. 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 SITA 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] Thus, the objectives of this work are to develop a new sensitive stability indicating RP-HPLC method for simultaneous determination of DAPA and SITA. Also, it is validated for market product named UDAPA-S 10/100containing DAPA and SITA in tablet dosage form. [17]

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 UDAPA-S 10/100 was purchased from local market.

IR identification and wavelength selection

The individual standard drugs, dapagliflozin propanediol monohydrate and sitagliptin phosphate monohydrate 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.

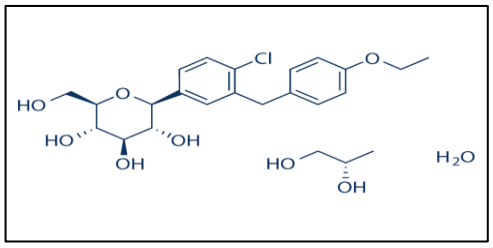

Figure 1. Structure of dapagliflozin

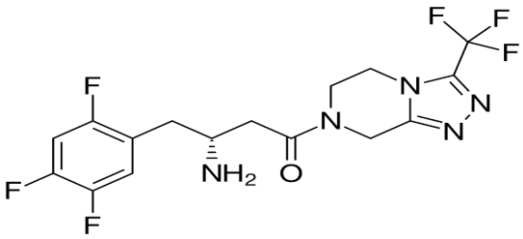

Figure 2. Structure of sitagliptin

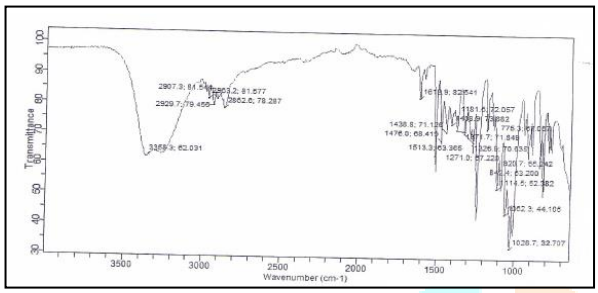

Figure 3: IR spectrum of dapagliflozin (API)

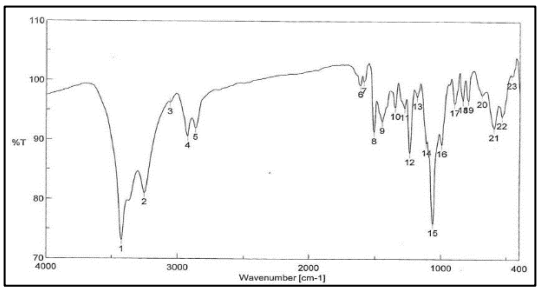

Figure 4: IR Spectrum of dapagliflozin (Std.)

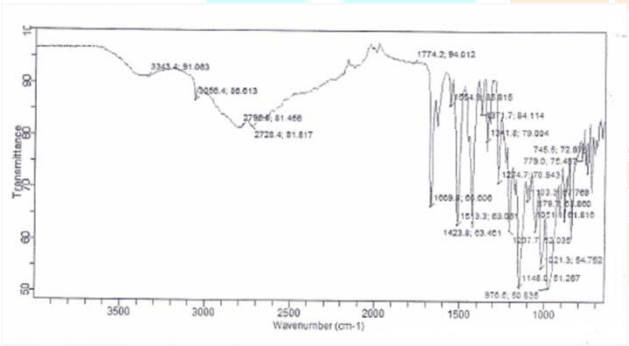

Figure 5: IR spectrum of sitagliptin (API)

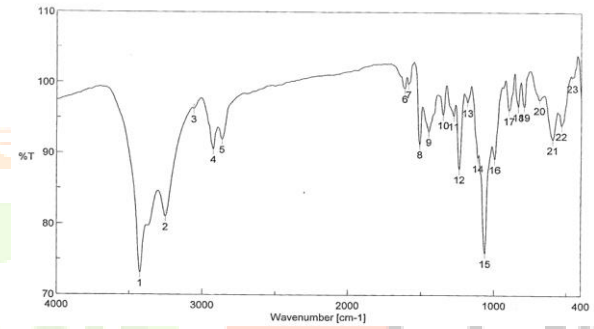

Figure 6: IR Spectrum of sitagliptin (Std.)

Table 1: IR spectrum of DAPA

Sr. No.	Functionalgroup	Observed value	Standardvalue
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 sitagliptin

sr. No.	Functionalgroup	Observed value	Standard value
1	O=H Stretching	3343.4	3500-3100
2	C=O Stretching	1669.8	2850-3000
3	C-H Bending	1423.8	1650-1620
4	C-F	835.2	1350-1000

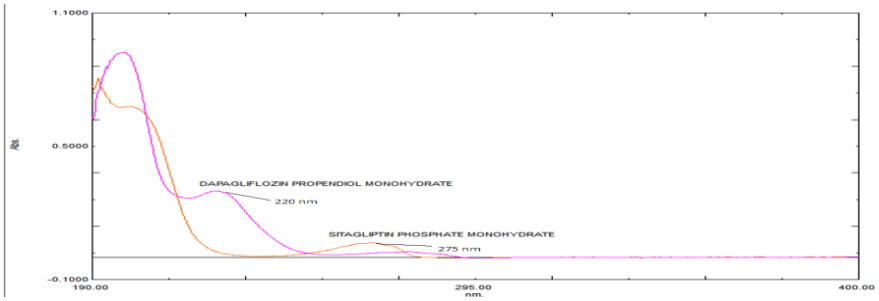

Figure 7: Determination of wavelength maximum (220nm)

Selection of Mobile phase

Various mobile phases were tried. Trial contains various mobile phases which consisted of Acetonitrile, methanol, Sodium dihydrogen phosphate, 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 sitagliptin were further diluted with Acetonitrial into 10 ml volumetric flask which contain 0,100,200,300,400,500,600,700,800 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 0-300 µg/ml for both drugs.

Preparation of sample solution

Stock sample solution: To determine the content of sitagliptin and dapagliflozin simultaneously in a pharmaceutical dosage form, 20 tablets were accurately weighed and triturated to make a smooth powder. An accurately weighed portion of the powder equivalent to 100 mg of sitagliptin and 10 mg of dapagliflozin was transferred into 50 ml volumetric flask containing 20 ml of acetonitrile. The mixture was sonicated for 10 minute to dissolve the content. Then volume was made upto the mark with diluent with intermittent shaking. The resultant solution was filtered through 0.45 µm membrane filter. Sample solution: Further 5 ml of the clear filtrate was taken into 20 ml volumetric flask and diluted upto the mark with diluent to get a final concentration of 500 ppm sitagliptin and 50 ppm dapagliflozin.

METHOD DEVELOPMENT

Trial-1

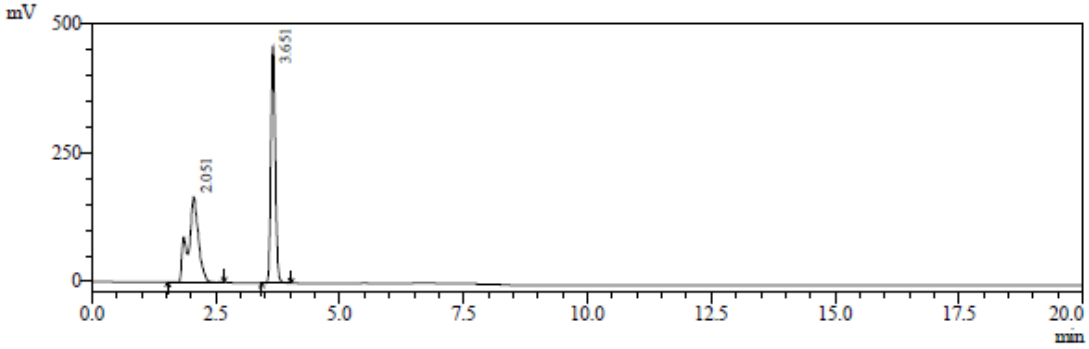

Figure 8: 500 ppm sitagliptin and 50 ppm dapagliflozin.: buffer:ACN (40:60)

Trial-2

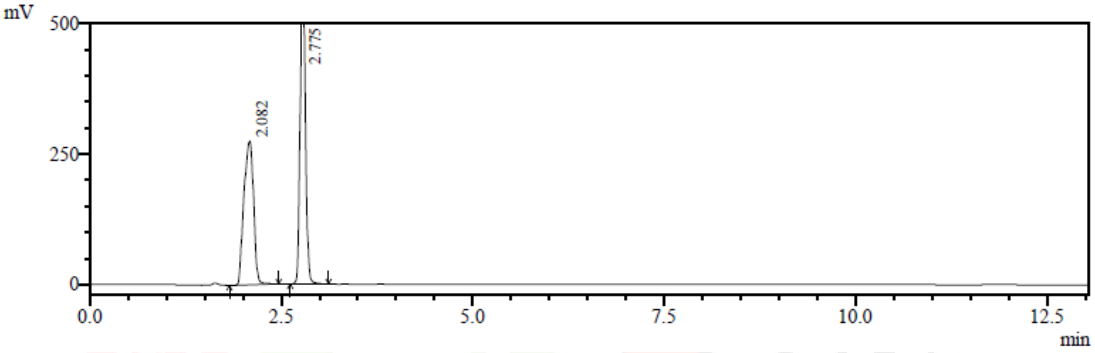

Figure 9: 500 ppm sitagliptin and 50 ppm dapagliflozin.: buffer:ACN (40:60)

Trial-3

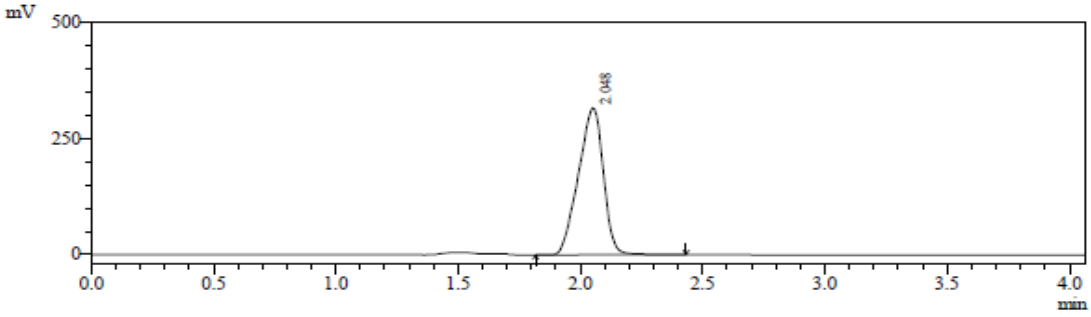

Figure 10: 500 ppm sitagliptin and 50 ppm dapagliflozin.: buffer:ACN (65:35)

Trial-4

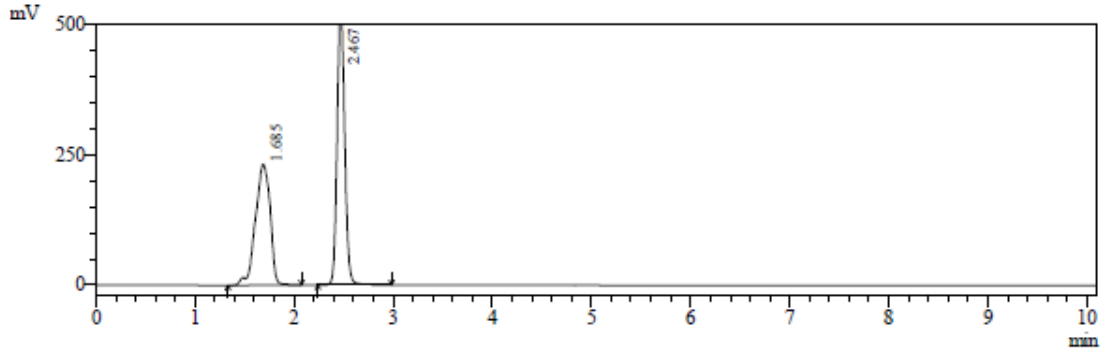

Figure 11: 500 ppm sitagliptin and 50 ppm dapagliflozin buffer:ACN (50:50)

Trial-5

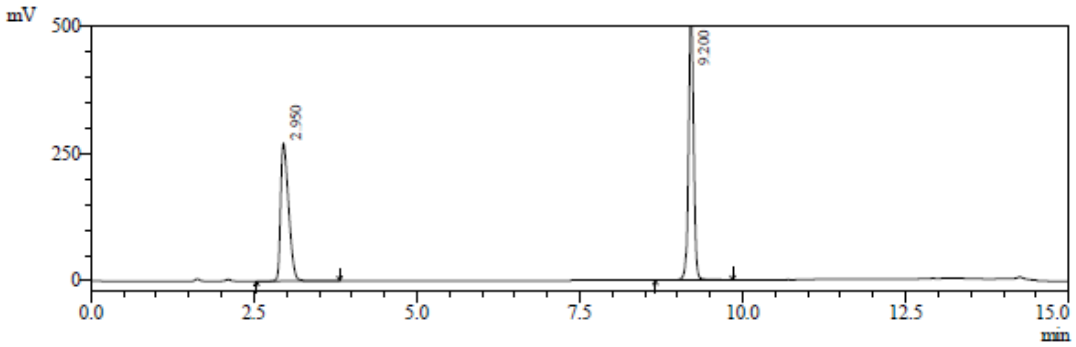

Figure 12: 500 ppm sitagliptin and 50 ppm dapagliflozin.: Buffer: ACN

Trial-6 (Final)

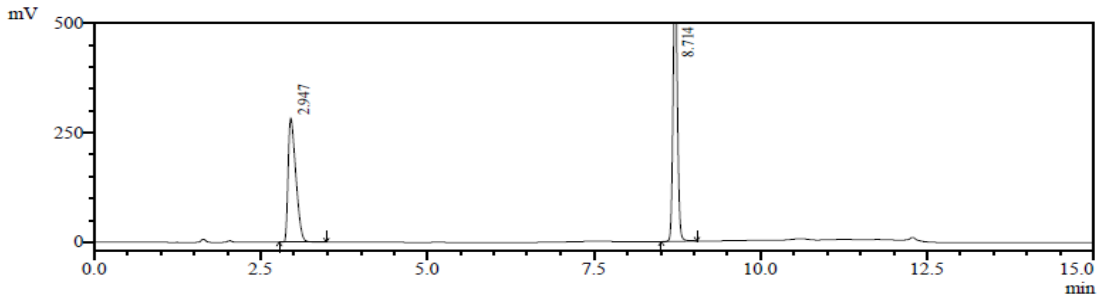

Figure 13: 500 ppm sitagliptin and 50 ppm dapagliflozin.: Buffer: ACN

Table: 3 Mobile phase selection

Sr. no	Mobilephasecomposition	Inference
1	buffer: ACN (40:60)	Sitagliptin peak split
2	Buffer :ACN (40:60)	Irregular peak shape
3	buffer: ACN (65:35)	dapagliflozin peak did not elute
4	buffer: ACN (50:50)	Both peak eluted but very less number of theoretical plates of sitagliptin peak
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

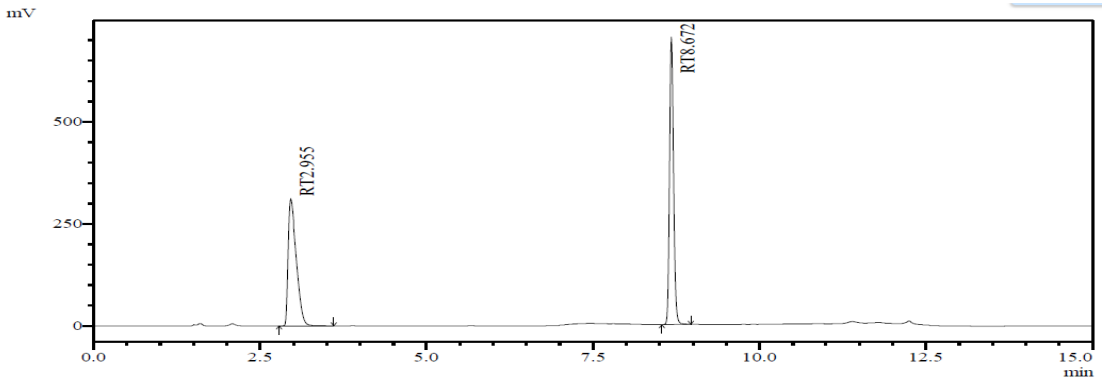

Figure 14: Chromatogram of Standard dapagliflozin and sitagliptin

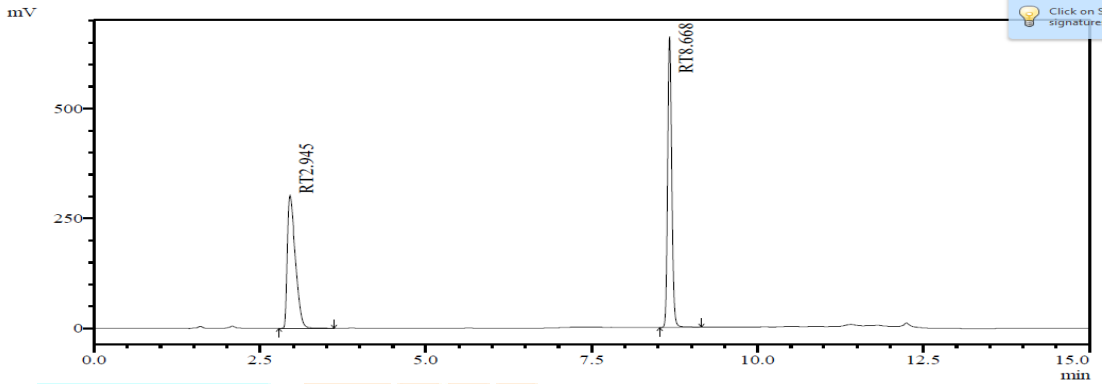

Figure 15: Chromatogram of Sample dapagliflozin and sitagliptin

Linearity

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

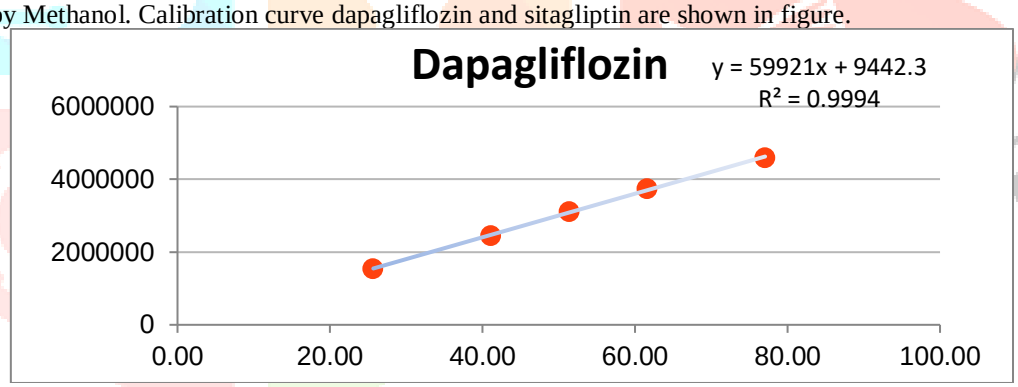

Figure 16: Calibration Curve of dapagliflozin

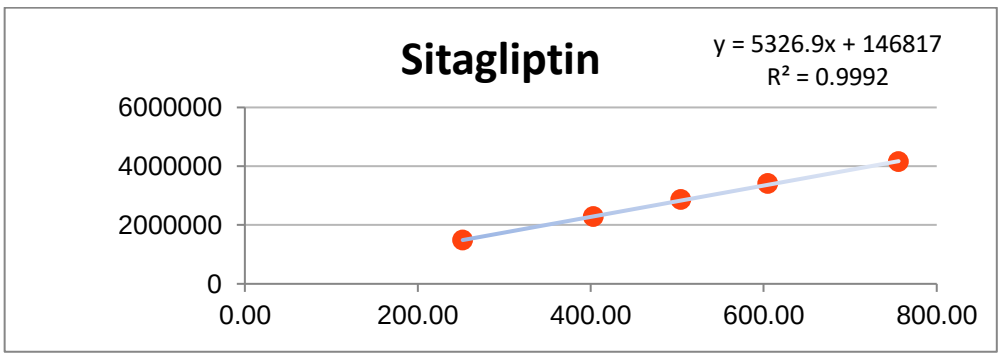

Figure 17: Calibration curve of sitagliptin

Table 5: Linearity study of dapagliflozin and sitagliptin

dapagliflozin		sitagliptin	
Concentration (µg/ml)	**PeakArea**	**Concentration (µg/ml)**	**PeakArea**
25.68	1546711	251.94	1480318
41.09	2443683	403.11	2271227
51.37	3106951	503.89	2860169
61.64	3741746	604.67	3401185
77.05	4597919	755.83	4141952

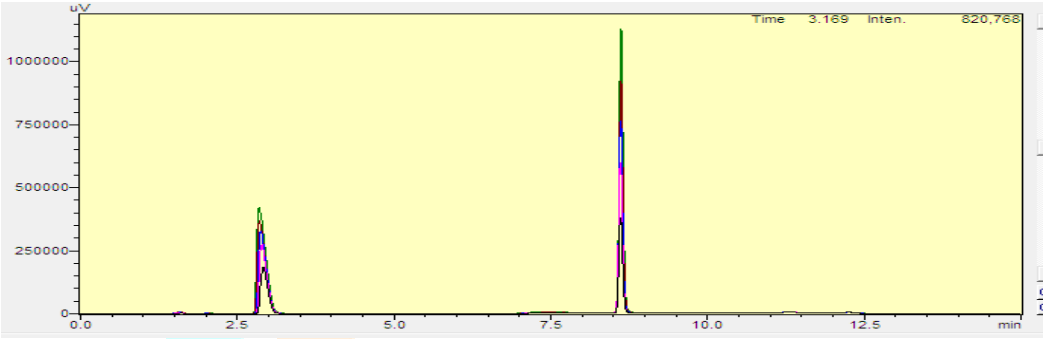

Figure 18: Overlain linearity chromatogram of dapagliflozin and sitagliptin

Repeatability

The data for repeatability of peak area measurement for dapagliflozin and sitagliptin based on six measurements of same solution. The % RSD for dapagliflozin and sitagliptin 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)	DAPA		Concentration of sitagliptin (µg/ml)	sitagliptin	
	Mean ± SD(n=6)	%RSD		Mean ± SD(n=6)	%RSD
50	2854479 ± 796177.4	0.3	500	2640793 ± 3558.59	0.2

Table 7: Intraday & Interday precision study of dapagliflozin

Drug	Conc.(µg/ml)	Intra-dayprecision		Inter-dayprecision	
		Mean ± SD(n=3)	%RSD	Mean ± SD(n=3)	%RSD
dapagliflozin	50	2842005 ± 9649.82	0.1	2849553 ± 2948.523727	0.3

Table 8: Intraday & Interday precision study of sitagliptin

Drug	Conc.(µg/ml)	Intra-dayprecision		Inter-dayprecision	
		Mean ± SD (n=3)	% RSD	Mean ± SD (n=3)	% RSD
sitagliptin	500	2626546 ± 5547.404141	0.7	2637071 ± 19293	0.1

Accuracy:

Table 9: Recovery study for dapagliflozin and sitagliptin

Drug	% OfLevel	Amount (µg/ml)	AmountAdded(µg/ml)	TotalAmountFound(µg/ml)	% Recovery ± SD (n=3)
dapagliflozin	50 %	1	0.66	0.66	100.9± 0.
	100 %	1	1.32	1.34	101.6 ± 0.1
	150 %	1	1.98	1.98	100.2 ± 0.1
sitagliptin	50 %	1	6.87	7.00	101.8 ± 0.05
	100 %	1	13.74	13.61	99.1 ±0.05
	150 %	1	20.62	20.28	99.5 ±1.0

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 sitagliptin

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	2955791	2954087	3260841	2685363	2958041	2951452
	2953574	2943280	3266988	2689316	2951394	2945766
	2951421	2952451	3258768	2670614	2941979	2938794
% R.S.D	**0.1**	**0.2**	**0.1**	**0.4**	**0.3**	**0.2**
sitagliptin	2735699	2720830	3026063	2476430	2767905	2702697
	2736933	2717186	3017776	2475630	2759274	2700073
	2740358	2722828	3023936	2467342	2766345	2698625
% R.S.D	**0.1**	**0.1**	**0.1**	**0.2**	**0.2**	**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 sitagliptin

dapagliflozin	sitagliptin
LOD = 3.3 x (SD / Slope) = 3.3 x (1172424.065/ 59921.27311) = 64.56804426 mg/ml = 0.0645604426 µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x(1022526.844/ 5326.87528) = 633.4556232mg/ml = 0.633 µg/ml
LOQ = 10 x (SD / Slope) = 10 x (1172424.065/ 59921.27311) = 195.660740 mg/ml = 0.195660740 µg/ml	LOQ = 10 x (SD / Slope) = 10 x(1022526.844/ 5326.87528) = 1919.562220 mg/ml = 1.919562220 µg/ml

V. Forced Degradation Condition

1. Acid Degradation

➢ **Acid degradationStandard:** 5 ml of sample stock solution (for standard, 5 ml of sitagliptin and 5 ml dapagliflozin standard stock solution) was taken into 20 mL volumetric flask. 1 ml 1 N HCl was added into the flask. The flask was refluxed at 60 °C 12 hours. Solution was then allowed to cooled down and then neutralized with ml 1 N NaOH. Volume was made upto the mark with diluent and chromatographed

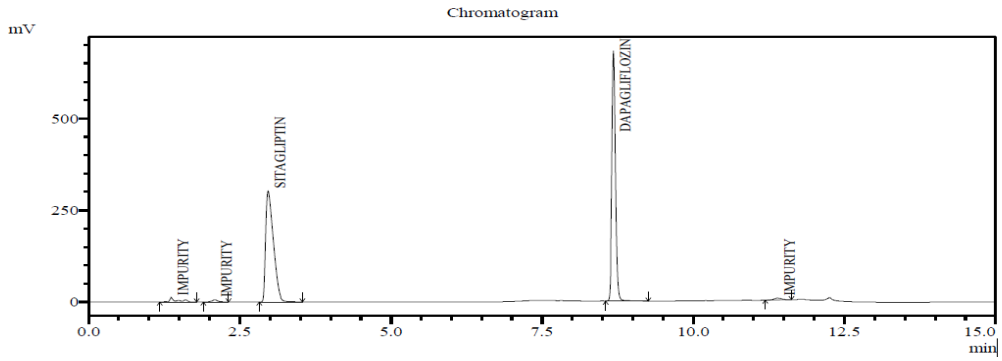

Figure 19: Chromatogram of dapagliflozin and sitagliptin Acid Degradation Standard

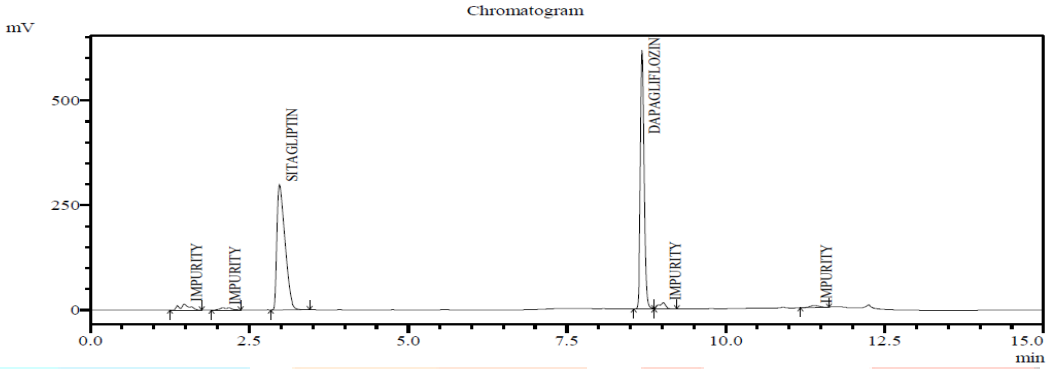

Figure 20: Chromatogram of dapagliflozin and sitagliptin acid degradation Sample

2. Base Degradation: 5 ml of sample (5 ml of mixed standard for standard degradation) solution was taken into 20 mL volumetric flask. 1 ml N HCl was added into the flask. The flask was kept at room temperature for 12 hours. Solution was then neutralized with 1 ml N NaOH. Volume was made upto the mark with diluent and chromatographed.

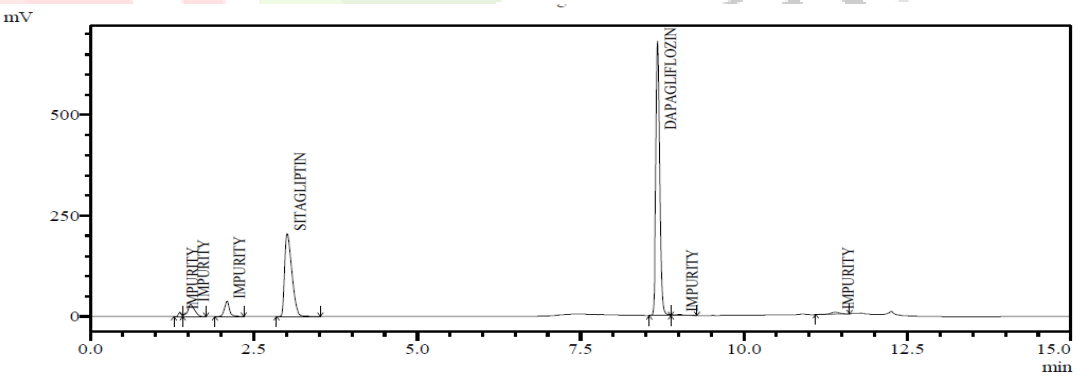

Figure 21: Chromatogram of dapagliflozin and sitagliptin base degradation Standard

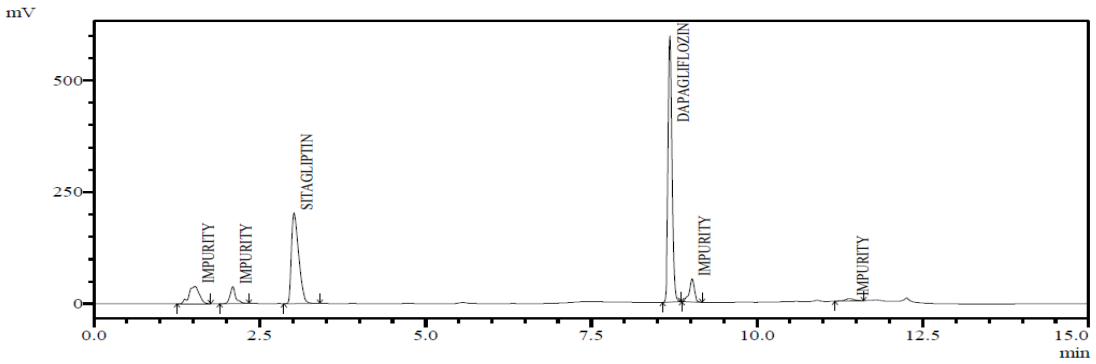

Figure 22: Chromatogram of dapagliflozin and sitagliptin base degradation Sample

3. Oxidative Degradation: 5 ml of sample (5 ml of mixed standard for standard degradation) solution was taken into 20 mL volumetric flask. 1 ml 3% H2O2 was added into the flask. The flask was kept at room temperature for 12 hours. Volume was made upto the mark with diluent and chromatographed.

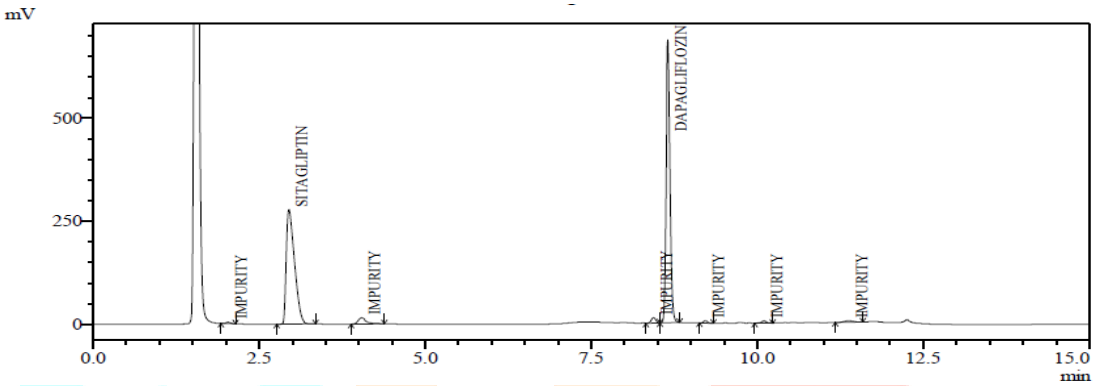

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Figure 23: Chromatogram of dapagliflozin and sitagliptin under oxidation degradation Standard

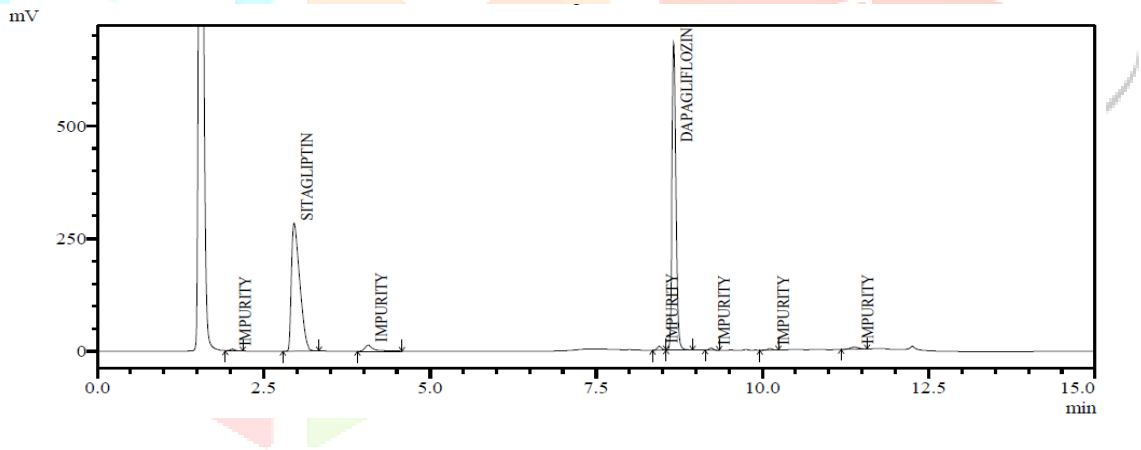

Figure 24: Chromatogram of dapagliflozin and sitagliptin under oxidation degradation Sample

4. Photo Degradation: Tablet powder and APIs were kept into sunlight for 72 hours. Solutions were made as per test method and then chromatographed.

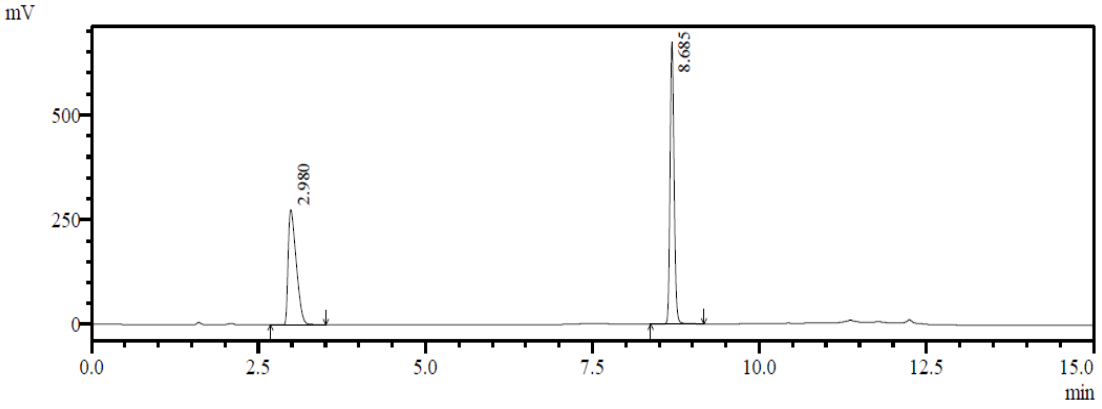

Figure 25: Chromatogram of dapagliflozin and sitagliptin under Photo degradation Standard

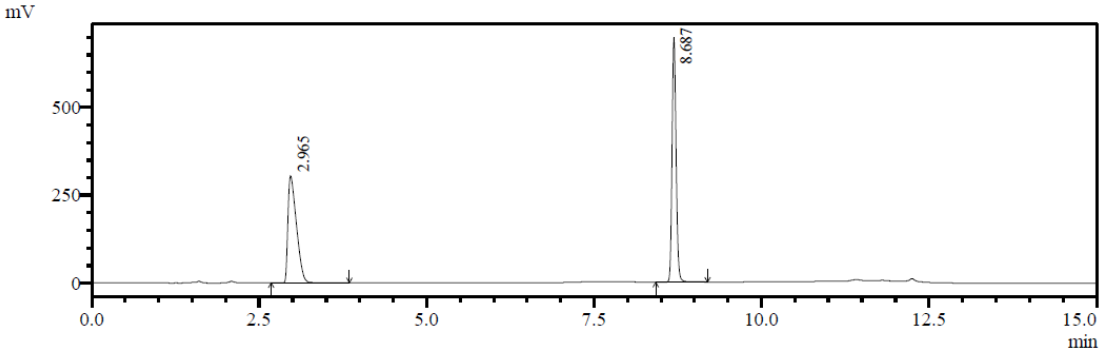

Figure 26: Chromatogram of dapagliflozin and sitagliptin under Photo degradation Sample

5. Thermal Degradation: Tablet powder and APIs were kept into hot air oven at 60 °C for 48 hours and then solutions were made as per test sample preparation and chromatographed.

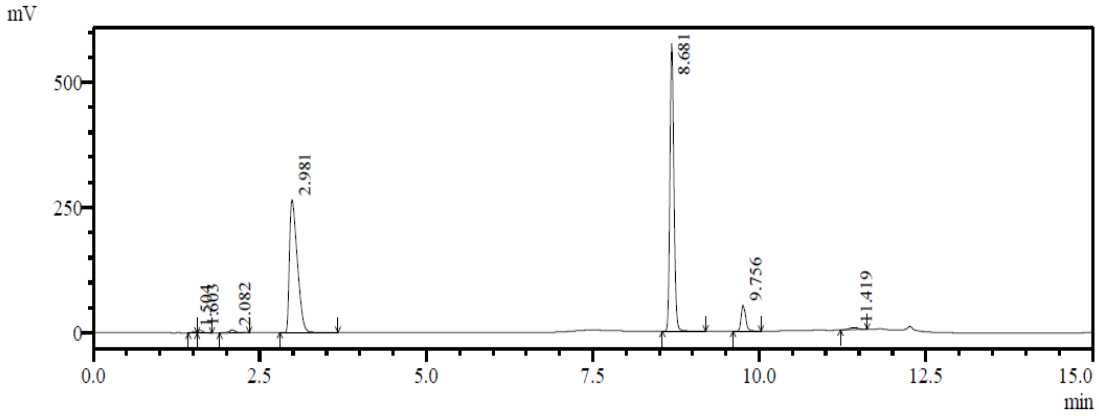

Figure 27: Chromatogram of dapagliflozin and sitagliptin under thermal degradation Standard

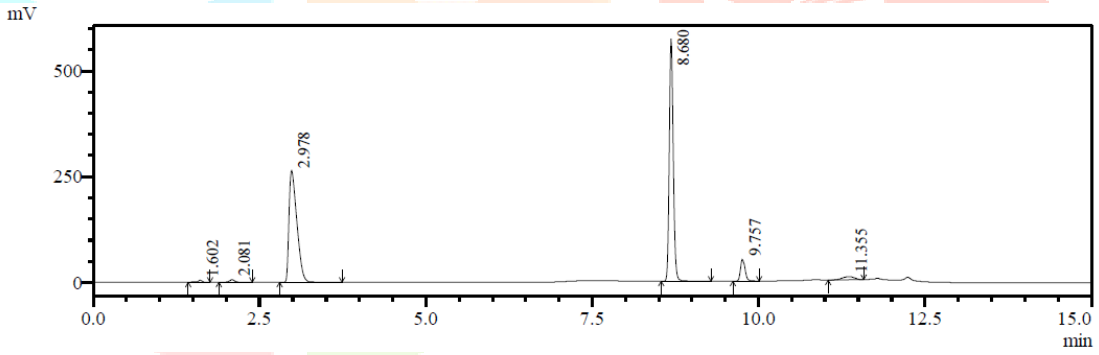

Figure 28: Chromatogram of dapagliflozin and sitagliptin under thermal degradation Sample

Table 15: Result of stability study of dapagliflozin and sitagliptin

Condition	% Degradation dapagliflozin		% Degradation sitagliptin	
	Sample	Standard	Sample	Standard
Acid	11.5	2.5	3.8	2.8
Base	3.1	3.3	3.6	3.3
Oxidation	1.4	0.5	10.3	13.4
Thermal	4.1	1.0	4.2	0.5
Photo	-0.2	3.3	0.0	0.6

RESULT AND DISCUSSION

The present work aimed development and validation of stability indicating RP-HPLC method for simultaneous estimation of dapagliflozin and sitagliptin The melting point of dapagliflozin (75-79 °C) and sitagliptin (206-210 °C) was found in the range. Method was developed in mobile phase containing Gradient program20 mM potassium dihydrogen phosphate buffer: Aceonitrile. Detection was carried out at 220 nm. Method was validated as per ICH guidelines. Linearity and regression data were shown in table and Figure. % Recovery was within the range (99.1% - 101.8%). 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 12% 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

Sitagliptin increases insulin production and decreases hepatic glucose overproduction. Sitagliptin prolongs the action of GLP-1 and GIP. By enhancing active incretin levels, sitagliptin increases insulin production and lowers glucagon secretion from alpha cells, which decreases hepatic glucose overproduction dapagliflozin 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 sitagliptin. In RP-HPLC method, good resolution and separation of two drugs was achieved. Gradient program 20 mM potassium dihydrogen phosphate buffer: Aceonitrile, mobile phase. Retention time dapagliflozin and sitagliptin were found to be 8.71and 2.94 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 sitagliptin in tablets.

Forced degradation study of dapagliflozin and sitagliptin 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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