wjpmr, 2020,6(7), 243-248

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN & SAXAGLIPTIN BY RP-HPLC METHOD

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Article Received on 19/05/2020 **Article Revised on 09/06/2020** **Article Accepted on 29/06/2020**

ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Dapagliflozin and Saxagliptin in Tablet dosage form. Chromatogram was run through Std BDS 150 x 4.6 mm, 5µ. Mobile phase containing Buffer Perchloric acid: Acetonitrile taken in the ratio 50:50 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.1% OPA. Temperature was maintained at 30°C. Optimized wavelength selected was 220 nm. Retention time of Dapagliflozin and Saxagliptin were found to be 2.266min and 2.805min. %RSD of the Dapagliflozin and Saxagliptin were and found to be 0.9 and 0.6 respectively. %Recovery was obtained as 99.72% and 99.60% for Dapagliflozin and Saxagliptin respectively. LOD, LOQ values obtained from regression equations of Dapagliflozin and Saxagliptin were 0.12, 0.36 and 0.02, 0.06 respectively. Regression equation of Dapagliflozin is y = 20173x + 18271, and y = 4124x + 2572 of Saxagliptin. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

KEYWORDS: Dapagliflozin, Saxagliptin, RP-HPLC.

NTRODUCTION

Chemically Dapagliflozin (DPG) inhibits subtype-2 of the sodium-glucose transport proteins (SGLT2), which is responsible for at least 90% of the glucose reabsorption in the kidney. Blocking this transporter causes blood glucose to be eliminated through the urine.[6] The efficacy of this medication class has yet to be determined, but in initial clinical trials, dapagliflozin lowers HbA_{1c} by 0.90 percentage points when added to metformin. Structure of the DPG was shown in figure 1 (A).[1]

Chemically Saxagliptin (SXG) is a dipeptidyl peptidase-4 (DPP-4) inhibitor antidiabetic for the treatment of type 2 diabetes. DPP-4 inhibitors are a class of compounds that work by affecting the action of natural hormones in the body called incretins. Incretins decrease blood sugar by increasing consumption of sugar by the body, mainly through increasing insulin production in the pancreas, and by reducing production of sugar by the liver. [Bristol-Myers Squibb Press Release] DPP-4 is a membrane associated peptidase which is found in many tissues, lymphocytes and plasma. DPP-4 has two main mechanisms of action, an enzymatic function and another mechanism where DPP-4 binds adenosine deaminase, which conveys intracellular signals via dimerization when activated. Saxagliptin forms a reversible, histidine-assisted covalent bond between its nitrile group and the S630 hydroxyl oxygen on DPP-4. The inhibition of DPP-4 increases levels active of glucagon like peptide 1 (GLP-1), which inhibits glucagon production from pancreatic alpha cells and increases production of insulin from pancreatic beta cells. Structure of the SXG was shown in figure 1 (B).[2]

Literature survey reveals there are several methods to estimated these drugs in single or in combination of two or three drugs.[5-9] But there is only very few HPLC methods are available for simultaneous estimation of DPG and SXG, so the scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation processes.

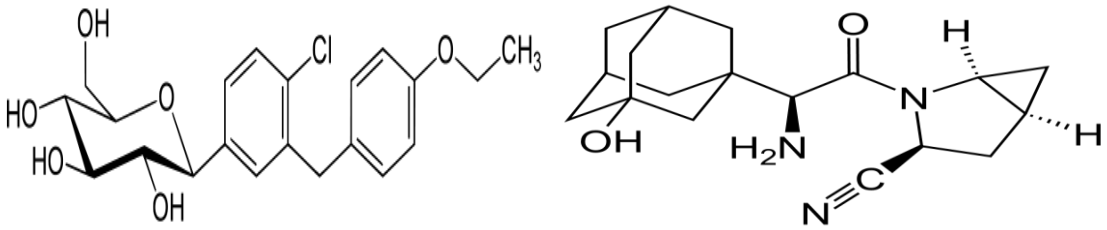

Figure 1: Structure of (A) Dapagliflozin (B) Saxagliptin.

MATERIALS AND METHODS

Reagents and Chemicals: Dapagliflozin and Saxagliptin pure drugs (API), Combination Dapagliflozin and Saxagliptin tablets (QTERN), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dehydrogenate ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Instrumentation: HPLC (waters 2695) system with Empower-2 software and 2996 module photo diode array detector equipped with a quaternary solvent delivery pump, automatic sampler unit, BDS C18 (4.6 x 150mm, 5µm). As part of experimentation, additional equipment such as sonicator (ultrasonic cleaner power sonic 420), pH meter, vacuum oven (wadegati), water bath and other glassware were used for the present investigation.

Chromatographic conditions: The BDS C18 (4.6 x 150mm, 5µm) column was used for analytical separation. Potassium dihydrogen ortho phosphate and one drop of triethyl amine in every 100ml of OPA (0.1%) and Acetonitrile was taken in the ratio of (50:50%v/v) mobile phase for the investigation with a flow rate of a 1 ml/min. The temperature was maintained at 30^0C. The injection volume was 10µl and the UV detection was achieved at 220nm.

Preparation of potassium dihydrogen ortho phosphate buffer (pH:3.0): Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 3.45 with dil. Orthophosphoric acid solution.

Preparation of mobile phase

Buffer: Water - in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water.

Preparation of mixture Standard stock solution: Accurately weighed 10 mg of Dapagliflozin, 5 mg of Saxagliptin and transferred to individual 10 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2. (1000µg/ml of Dapagliflozin and 500µg/ml of Saxagliptin).

Preparation of Sample (Tablet) stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 10 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (1000µg/ml of Dapagliflozin and 500µg/ml of Saxagliptin).

Optimized chromatographic conditions

Column Used : BDS C18 (4.6 x 150mm, 5µm)
Mobile phase : 50% OPA (0.1%): 50% Acetonitrile
Flow rate : 1ml/min
Wavelength : 220.0 nm
Temperature : 30°C
Injection Volume: 10.0µl

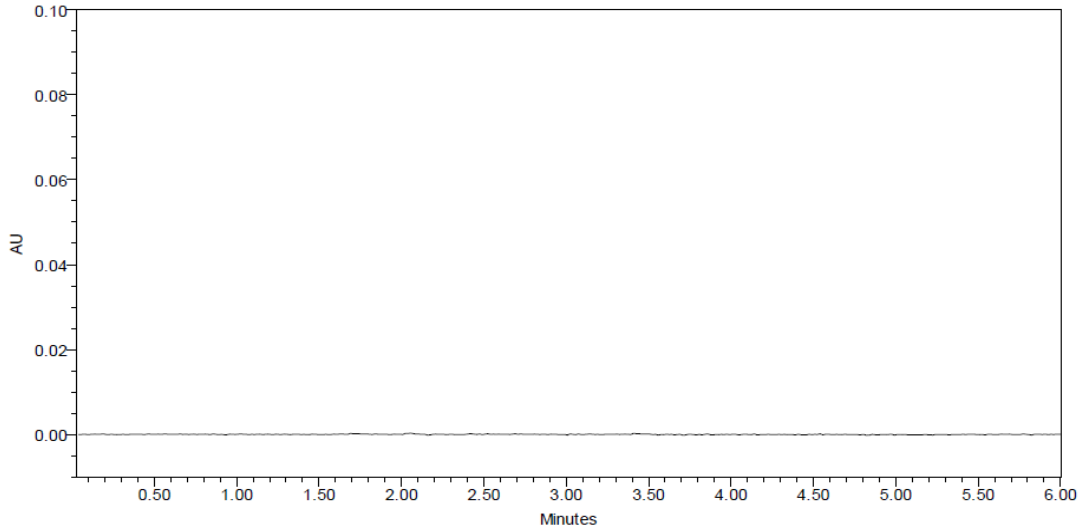

Figure 2: Blank chromatogram.

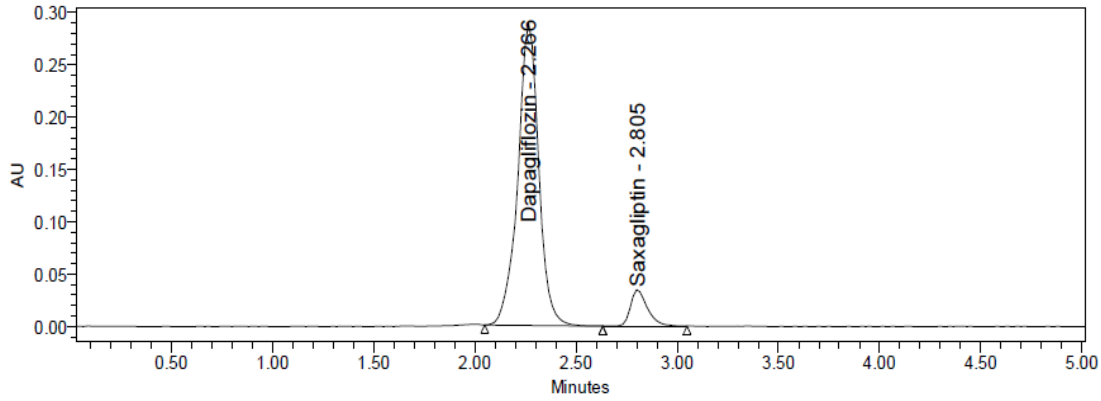

Figure 3: Chromatogram of standard mixture of DPG & SXG.

	Peak Name	RT	Area	USP Tailing	USP Resolution	USP Plate Count
1	Dapagliflozin	2.266	2032360	1.33	3.2	5623
2	Sxagliptin	2.805	202920	0.98	4	2612

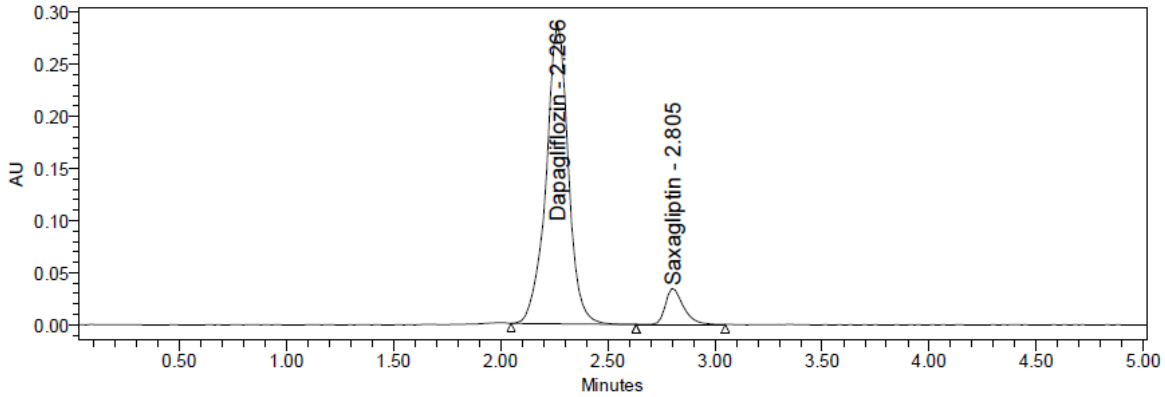

Figure 4: Chromatogram of sample mixture of DPG & SXG.

Validation

The above optimized chromatographic method has been validated for the assay of DPG & SXG using the following parameters [International Conference on Harmonization (ICH) 1995]. Linearity was studied to find out the relationship of concentration with Peak area. Six different concentrations of Dapagliflozin and Saxagliptin (DPG & SXG)drug mixtures respectively.

Each concentration of solution was injected into the HPLC and chromatogram was recorded. The calibration graph was constructed by plotting the peak versus the final concentration of the each drug (µg/ml) and the corresponding regression equation derived. Precision was studied to find out variations in the test methods of mixtures of Dapagliflozin (10mg)+ Saxagliptin (5mg) respectively. The precision of each method was ascertained separately from the peak area by actual determination of five replicates of a fixed amount of Dapagliflozin (10mg)+ Saxagliptin (5mg) respectively. The %RSD (percentage relative standard deviation) was calculated for precision and ruggedness. The accuracy of the method was shown by analyzing the model mixtures containing 80,100 and 120% of Dapagliflozin and Saxagliptin. After the measurement, the Amount found and individual recoveries were calculated. Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on the linearity data using the formulae LOD = 3.3×standard deviation /slope; LOQ = 10×standard deviation /slope. Robustness was performed by following the same method with different flow rate.

RESULTS AND DISCUSSION

The regression equation for DPG was found to be y = 20173x + 18271 (slope, intercept and correlation coefficient were found to be 20173, 18271 and 0.999 respectively) and linear over beer's range of 25-150µg/ml. The regression equation for SXG was found to be y = 4124x + 2572 (slope, intercept and correlation coefficient were found to be 4124, 2572 and 0.999 respectively) and linear over beer's range of 12.5-75 µg/ml. Linearity graph of DPG & SXG were shown in Figure 5 & 6 respectively. Linearity data was shown in table 1. The precision and ruggedness were determined using the % RSD of the peak area for six replicate preparations of the drug. %RSD of system precision for Dapagliflozin and Saxagliptin were and found to be 0.9 and 0.7 respectively. %RSD of method precision for Dapagliflozin and Saxagliptin were and found to be 0.9 and 0.6 respectively. % recovery was obtained as 99.72% and 99.60% for Dapagliflozin and Saxagliptin respectively. The calculated RSD values were less than 2. Precision and ruggedness data are presented in Table 2. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures contained 50%, 100% and 150% of standard solution of drug DPG & SXG and along with 5 µg/mL of placebo solution within the linearity ranges. The mean percentage recoveries were found to be 99.40% and 99.36% w/w for 50%, 100% and 150% respectively. The results of accuracy were shown that the developed method have a good percentage recovery at different concentrations of drugs. LOD for DPG & SXG was found to be 0.12µg/ml and 0.02µg/ml respectively. LOQ for DPG & SXG was found to be 0.36µg/ml and 0.06µg/ml respectively. Summary of all the validation parameter shown in table 3.

Table 1: Linearity table for DPG & SXG.

Dapagliflozin		Saxagliptin	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
25	543171	25	543171
50	1029638	50	1029638
75	1534748	75	1534748
100	2015960	100	2015960
125	2564897	125	2564897
150	3030548	150	3030548

Table 2: System precision table of DPG & SXG.

S. No	Area of Dapagliflozin	Area of Saxagliptin
1.	2004545	202059
2.	2026029	203810
3.	2045009	201711
4.	2037805	204921
5.	2058268	201655
6.	2022506	203366
Mean	2032360	202920
S.D	18824.1	1326.5
%RSD	0.9	0.7

Table 3: Summary of validation data of DPG & SXG.

Parameters		Dapagliflozin	Saxagliptin	LIMIT
Linearity Range (µg/ml)		25-150µg/ml	12.5-75 µg/ml	R< 1
Regressioncoefficient		0.999	0.999	
Slope(m)		20173	4124	
Intercept(c)		18271	2572	
Regression equation (Y=mx+c)		y = 20173x + 18271	y = 4124x + 2572	
Assay (% mean assay)		99.40%	99.36%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.9	0.7	NMT 2.0%
Method precision %RSD		0.9	0.6	NMT 2.0%
Accuracy %recovery		99.72%	99.60%	98-102%
LOD		0.12	0.02	NMT 3
LOQ		0.36	0.06	NMT 10
Robustness	**FM**	1.2	1.4	%RSD NMT 2.0
	FP	1.3	1.1	
	MM	1.0	1.0	
	MP	1.1	0.4	
	TM	0.5	0.7	
	TP	0.8	0.8	

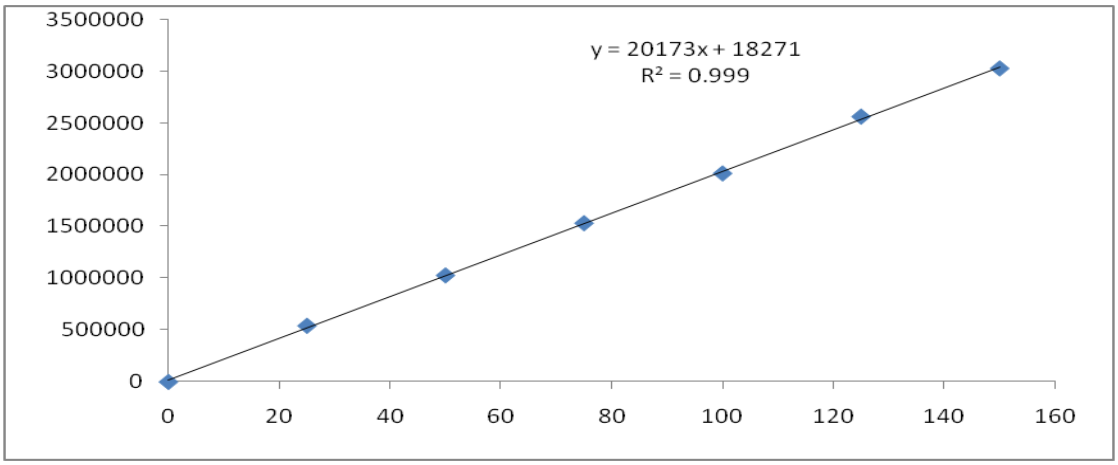

Fig. 7: Linearity curve of Dapagliflozin.

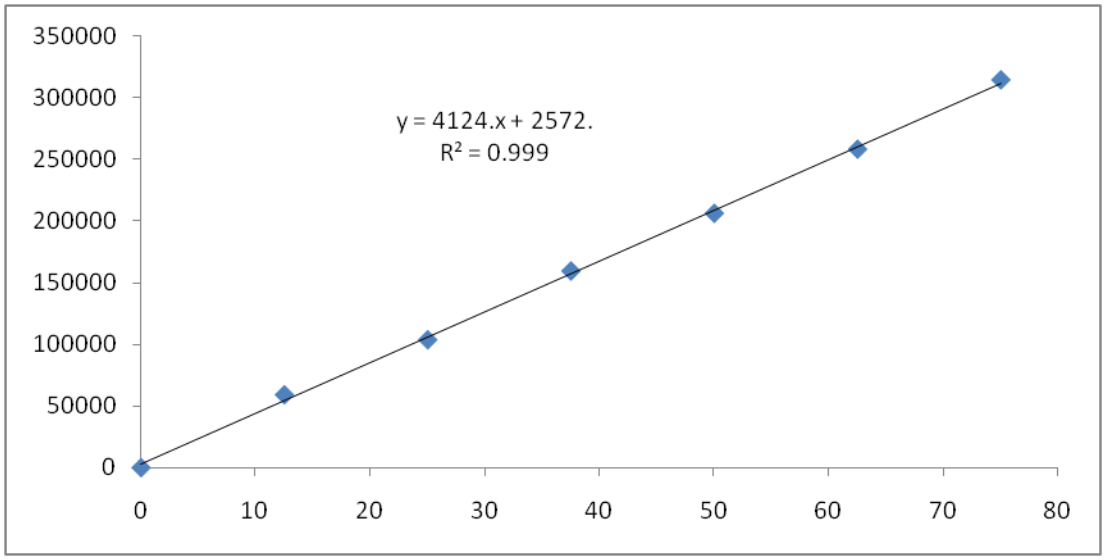

Fig. 8: Linearity curve of Saxagliptin.

Table 4: Degradation data of DPG & SXG.

Type of degradation	Dapagliflozin			Saxagliptin		
	AREA	%Recovered	%Degraded	AREA	%Recovered	%Degraded
Acid	1942082	95.46	4.54	193192	95.11	4.89
Base	1983063	97.48	2.52	197428	97.20	2.80
Peroxide	2001677	98.39	1.61	199428	98.18	1.82
Thermal	2015131	99.05	0.95	201535	99.22	0.78
Uv	2017472	99.17	0.83	201587	99.24	0.76
Water	2014647	99.03	0.97	201535	99.22	0.78

Degradation

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

Conclusion

A simple, accurate, precise method was developed for the simultaneous estimation of the Dapagliflozin and Saxagliptin in Tablet dosage form was developed and the proposed method as suitable for routine analysis of MXF & BRF.

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