IJPSR (2021), Volume 12, Issue 1 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 31 December 2019; received in revised form, 18 April 2020; accepted, 20 April 2020; published 01 January 2021

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN AND SAXAGLIPTIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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Keywords:

Dapagliflozin, Saxagliptin, Validation, Linearity, Recovery, ICH guide

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ABSTRACT: An analytical method consists of a detailed, stepwise list of instructions to be followed in the qualitative, quantitative, or structural analysis of a sample for one or more analytes and using a specified technique. A novel, simple, precise, sensitive, and reproducible RP-HPLC method for the simultaneous estimation of Dapagliflozin and Saxagliptin in bulk and pharmaceutical formulation was developed and validated. The separation was carried out on Symmetry C_8 (4.6 × 150 mm, 3.5 µm, Make: XTerra) column with buffer: acetonitrile in the ratio of 70:30 %v/v (pH 3) as the mobile phase at the flow rate of 1 ml/min. The eluent detection was carried out using a UV-Visible detector at 221 nm. The retention time of Dapagliflozin and Saxagliptin was 2.83 min and 4.35 min, respectively. Linearity was observed Dapagliflozin and Saxagliptin in the concentration range of 25-125 µg/ml and 12.5-62.5 µg/ml, respectively. The % mean recovery of Dapagliflozin and Saxagliptin was found to be 99.90 and 99.99, respectively. The present study demonstrates the applicability of chromatographic method to develop a new, sensitive, single RP-HPLC method for the simultaneous quantitative determination of Dapagliflozin and Saxagliptin in a fixed pharmaceutical dosage form. Hence, this method can be conveniently adopted for routine analysis in quality control laboratories.

INTRODUCTION: Analytical chemistry is a branch of chemistry that deals with the identification of compounds and mixtures (qualitative analysis) or the determination of the proportions of the constituents (quantitative analysis). In the modern pharmaceutical industry, high-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development, and production. The development of new chemical entities (NCEs) is comprised of two major activities: drug discovery and drug development [1, 2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(1).314-20

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(1).314-20

It is one of the significant roles for the physician in the treatment of patients with type-2 diabetes. Type-2 diabetes is known as diabetes mellitus. It is a long term metabolic disorder that is characterized by high blood sugar and insulin resistance [3, 4].

To overcome these problems, an oral combination therapy of Dapagliflozin and Saxagliptin was introduced for the treatment of Type-2 diabetes. Dapagliflozin is a drug of the gliflozin class which specifically inhibits subtype 2 of the sodium-glucose transport proteins (SGLT2) which are responsible for at least 90% of the glucose re-absorption in the kidney. Saxagliptin is an oral hypoglycemic drug which inhibits the dipeptidyl peptidase-4 (DPP-4) enzyme and reduces the blood glucose level [4, 5]. Clinical trials for Dapagliflozin and Saxagliptin oral combination therapy indicate that it is safety, efficacy, rapid and intensive control over high blood glucose level.

So, the combination of Dapagliflozin and Saxagliptin is clinically approved for the treatment of Type-2 diabetes **Fig. 1** and **Fig. 2** [5, 6]. The techniques commonly used are titration, precipitation, spectroscopy, chromatography, *etc*. High-performance liquid chromatography (HPLC) is the fastest-growing analytical technique for the analysis of drugs [1]. Its simplicity, high specificity, and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids [7]. For this instance, an attempt has been made to develop and validate a novel RP-HPLC method for the simultaneous estimation of Dapagliflozin and Saxagliptin in the combined tablet dosage form.

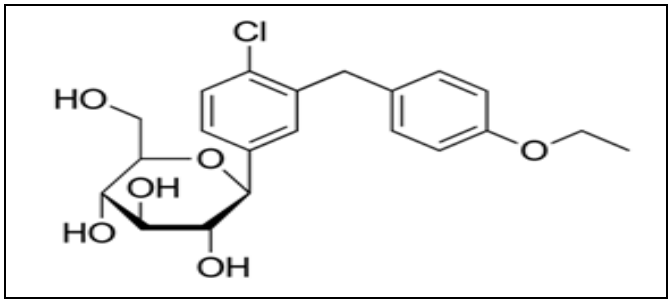

FIG. 1: CHEMICAL STRUCTURE OF DAPAGLIFLOZIN

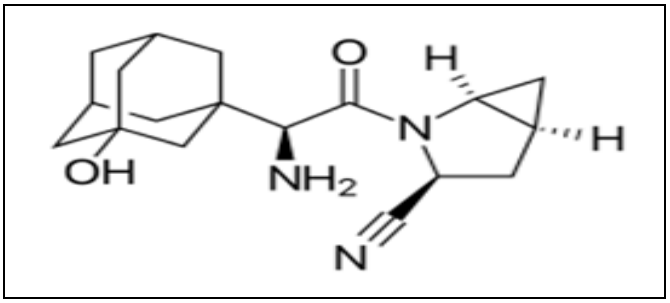

FIG. 2: CHEMICAL STRUCTURE OF SAXAGLIPTIN

MATERIALS AND METHODS: An isocratic RP-HPLC method was performed on a Waters 515 PDA 2998 Detector HPLC system equipped with empowering software for processing and data collecting. Symmetry C_8 (4.6 × 150 mm, 3.5 µm, Make: XTerra) column is used as a stationary phase. An ultrasonic bath sonicator (Frontline FS 4, Mumbai, India), semi-micro analytical balance (India), and Whatman filter paper no. 41 is used in the study.

Dapagliflozin and Saxagliptin were procured from Manus Aktteva Biopharma Ltd., India. Acetonitrile of HPLC grade was procured from Merck Specialities Private Limited, Mumbai, India. Water and orthophosphoric acid of HPLC grade was obtained from Rankem Ltd., India. HPLC grade of potassium dihydrogen orthophosphate was procured from Rankem Ltd., India. Qtern® tablets were procured from AstraZeneca Pharmaceuticals.

The separation was carried out on Symmetry C8 (4.6 × 150 mm, 3.5 µm, Make: XTerra) column with buffer: acetonitrile in the ratio of 70:30 %v/v (pH 3) as the mobile phase at the flow rate of 1ml/min. The eluent detection was carried out using a UV-Visible detector at 221 nm. The injection volume was 20 µl, and the analysis was performed at ambient temperature.

Preparation of Dapagliflozin and Saxagliptin Mixed Standard Drug Stock Solutions: [8] The mixed standard drug stock solutions of Dapagliflozin and Saxagliptin were prepared by dissolving 10 mg of Dapagliflozin and 5 mg of Saxagliptin in 10 mL of the mobile phase into a 10 mL of volumetric flask and then sonicated to dissolve it completely to get a concentration of 1000 µg/mL of Dapagliflozin and 500 µg/mL of Saxagliptin.

Preparation of Sample Solution: [9] Sample solution was prepared from Qtern® tablets. Twenty tablets of Qtern® were taken and weighed individually, and the average weight of twenty tablets was calculated. From this calculation, the weight of each tablet is determined. Each tablet of Qtern® contains 10 mg of Dapagliflozin and 5 mg of Saxagliptin. After weighing, twenty tablets of Qtern® were crushed and mixed in a mortar and pestle to produce a powder. An accurately weighed quantity of powder equivalent to 10 mg of Dapagliflozin and 5 mg of Saxagliptin was transferred into a clean, and dry 10 mL volumetric flask and then mobile phase was added and sonicated to dissolve it completely and filtered through 0.45 µm nylon membrane filter and volume was made up to the mark with the same mobile phase to get the concentration of 1000 µg/mL of Dapagliflozin and 500 µg/mL of Saxagliptin.

An aliquot of 0.5 mL was pipette out from the above solution and then transferred into a 10 ml of volumetric flask and diluted up to mark with the mobile phase to get the concentration of 50 µg/mL

of Dapagliflozin and 25 µg/mL of Saxagliptin solution.

Validation: [10] The method was validated for linearity, precision, specificity, accuracy, and robustness as per the ICH guidelines. Linearity was observed Dapagliflozin and Saxagliptin in the concentration range of 25-125 µg/ml and 12.5-62.5 µg/ml, respectively. The % mean recovery of Dapagliflozin and Saxagliptin was found to be 99.90 and 99.99, respectively.

Accuracy was determined at three different levels of 50%, 100%, and 150% of the target concentration of the active ingredient by adding a known amount of each standard to previously analyzed tablet samples. Precision was studied to determine intraday variation by performing six replicate assays of the tablet sample. The % RSD was calculated for intraday precision. Robustness of the method was carried out by deliberately changing the mobile phase composition by altering the proportion of the organic phase by ±10% and flow rate by ±0.1 mL. Specificity was by using a placebo of commonly used tablet excipients [11-13].

RESULTS AND DISCUSSION: The present RP-HPLC method for the simultaneous estimation of Dapagliflozin and Saxagliptin in bulk and pharmaceutical dosage forms was established and validated as per ICH guidelines. This method was intended for rapid and accurate estimation of Dapagliflozin and Saxagliptin in bulk and pharmaceutical dosage forms. Good separation of the chromatographic peaks was observed, and no interfering peaks are found. A number of commercially available HPLC columns and various mobile phases were used for the development of the RP-HPLC method for the simultaneous estimation of Dapagliflozin and Saxagliptin in bulk and pharmaceutical dosage forms **Fig. 5, 6** and **7**. The best response was obtained with Symmetry C_8 (4.6 × 150 mm, 3.5 µm particle size); Waters 515 PDA 2998 Detector HPLC system and mobile phase contained a mixture of Potassium dihydrogen orthophosphate (pH adjusted to 3 with orthophosphoric acid) and Acetonitrile (30:70, v/v) was delivered at a flow rate of 1 mL/min. Quantitation was attained with a PDA detector at 221 nm depends on peak area. The retention time of Dapagliflozin and Saxagliptin was 2.831 min and 4.357 min with a resolution of 4.718.

Linearity was established for Dapagliflozin and Saxagliptin in the range of 25-125 µg/mL for Dapagliflozin, and 12.5-62.5 µg/mL for Saxagliptin with correlation coefficients (r=0.999) and the percentage recoveries were between 99.82%-100.01% for Dapagliflozin and 99.95%-100.04% for Saxagliptin, respectively **Table 2**, **Fig. 3** and **4**.

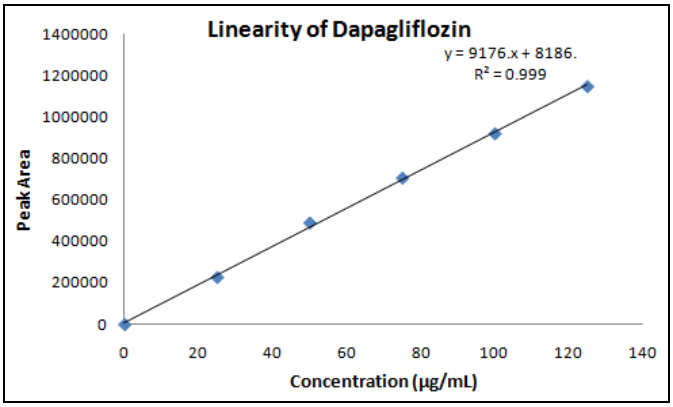

FIG. 3: LINEARITY GRAPH OF DAPAGLIFLOZIN

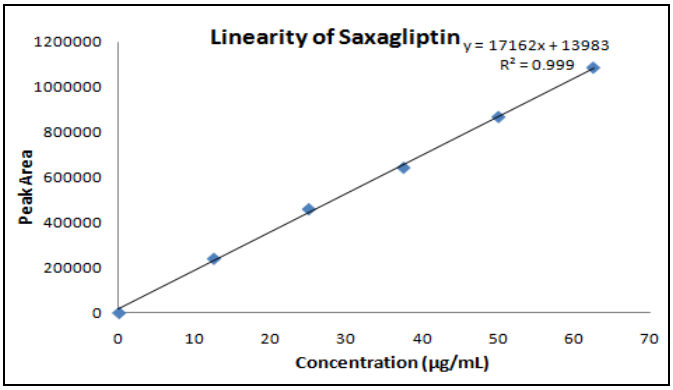

FIG. 4: LINEARITY GRAPH OF SAXAGLIPTIN

TABLE 1: SYSTEM SUITABILITY PARAMETERS OF DAPAGLIFLOZIN AND SAXAGLIPTIN

Parameters	Dapagliflozin	Saxagliptin	Acceptance limits
Retention time (min)	2.831	4.357	-----
Theoretical plates (N)	2332	3218	Not less than 2000
Asymmetry factor	1.02	1.04	Not more than 2
Resolution		4.718	More than 2
Linearity range (µg/mL)	25-125	12.5-62.5	-----
Limit of detection (LOD) (µg/mL)	0.23	0.08	-----
Limit of quantification (LOQ) (µg/mL)	0.68	0.24	-----

TABLE 2: LINEARITY OF DAPAGLIFLOZIN AND SAXAGLIPTIN

Concentration of Dapagliflozin (µg/mL)	Peak Area	Concentration of Saxagliptin (µg/mL)	Peak Area
25	227288	12.5	240522
50	490023	25	460387
75	706102	37.5	643827
100	919842	50	869362
125	1147218	62.5	1087692

TABLE 3: OPTICAL AND REGRESSION PARAMETERS OF DAPAGLIFLOZIN AND SAXAGLIPTIN

Optical and regression parameters	Dapagliflozin	Saxagliptin
Detection wavelength (nm)	221	
Linearity range (µg/mL)	25-125	12.5-62.5
Regression Equation (y=mx+C)	9176x+8186	17162x+13983
Slope (m)	9176	17162
Intercept (C)	8186	13983
Correlation coefficient (r)	0.999	0.999
Limit of detection (µg/mL)	0.23	0.08
Limit of quantification (µg/mL)	0.68	0.24

The RSD % values of accuracy for Dapagliflozin and Saxagliptin were found to be < 2 %, which indicate the accuracy of the proposed method **Table 4** and **5**. The RSD % values of method precision were found to be 0.11% for Dapagliflozin and 0.08 % for Saxagliptin, respectively, and for the system, precision was found to be 0.19 % for Dapagliflozin and 0.45 % for Saxagliptin, respectively **Table 6, 7, 8,** and **9**.

The RSD % values of reproducibility were found to be 0.024% for Dapagliflozin and 0.02% for Saxagliptin, respectively **Table 10** and **11** reveal that the proposed method is precise. LOD values were found to be 0.23 µg/mL for Dapagliflozin and 0.08 µg/mL for Saxagliptin and LOQ values were found to be 0.68 µg/mL for Dapagliflozin and 0.24 µg/mL for Saxagliptin **Table 2**. The RSD % values of robustness studies were found to be < 2%, which indicate the robustness of the proposed method **Table 12** and **13**. These reports show that the proposed method was accurate and precise for the simultaneous determination of Dapagliflozin and Saxagliptin in bulk and pharmaceutical combined dosage forms.

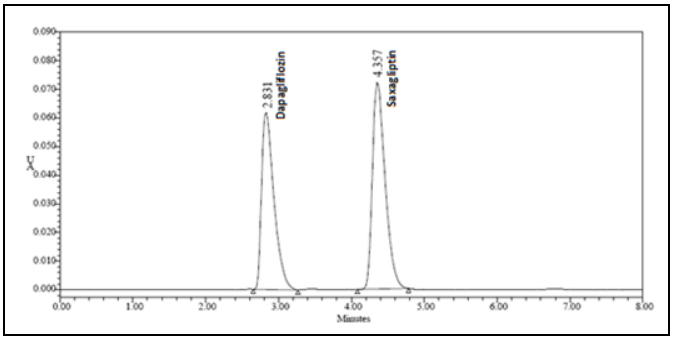

FIG. 5: REPRESENTATIVE CHROMATOGRAM OF STANDARD SOLUTION OF DAPAGLIFLOZIN AND SAXAGLIPTIN

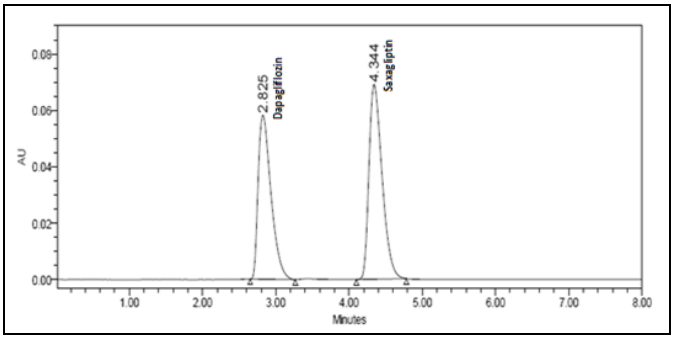

FIG. 6: REPRESENTATIVE CHROMATOGRAM OF TEST SOLUTION OF DAPAGLIFLOZIN AND SAXAGLIPTIN

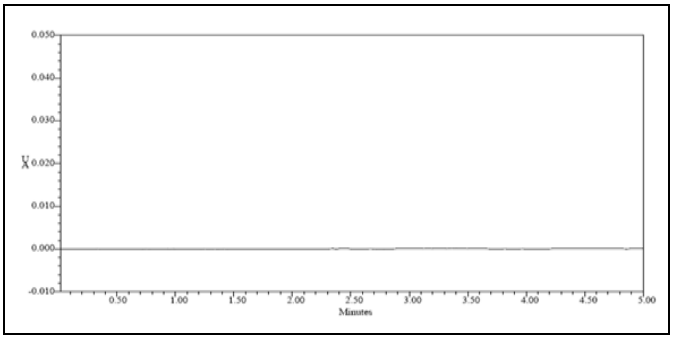

FIG. 7: REPRESENTATIVE CHROMATOGRAM OF BLANK

TABLE 4: RESULTS OF ACCURACY STUDIES OF DAPAGLIFLOZIN

Concentration Level in %	Amount added (µg/mL)	Amount recovered (µg/mL)	% Recovery	% Mean Recovery	RSD %
S_1:50%	25	24.96	99.84	99.89	0.24
S_2:50%	25	24.92	99.68		
S_3:50%	25	25.04	100.16		
S_4:100%	50	49.94	99.88	99.82	0.18
S_5:100%	50	49.81	99.62		
S_6:100%	50	49.98	99.96		
S_7:150%	75	75.01	100.01	100.01	0.03
S_8:150%	75	75.03	100.04		
S_9:150%	75	74.98	99.97		

TABLE 5: RESULTS OF ACCURACY STUDIES OF SAXAGLIPTIN

Concentration Level in %	Amount added (µg/mL)	Amount recovered (µg/mL)	% Recovery	% Mean Recovery	RSD %
S_1:50%	12.5	12.48	99.84	99.95	0.12
S_2:50%	12.5	12.49	99.92		
S_3:50%	12.5	12.51	100.08		
S_4:100%	25	25.02	100.08	100.04	0.14
S_5:100%	25	24.97	99.88		
S_6:100%	25	25.04	100.16		
S_7:150%	37.5	37.48	99.95	9.98	0.04
S_8:150%	37.5	37.51	100.03		
S_9:150%	37.5	37.49	99.97		

TABLE 6: METHOD PRECISION OF DAPAGLIFLOZIN

Injection no.	99.95	0.12	Assay %
1			99.88
2			99.83
3	100.04	0.14	99.78
4			100.02
5			99.94
6	99.98	0.04	100.07
	Average		
	SD		
	RSD %		0.11

TABLE 7: METHOD PRECISION OF SAXAGLIPTIN

Injection no.	Name of the drug	Concentration (µg/mL)	Assay %
1	Saxagliptin	25	99.89
2	Saxagliptin	25	100.03
3	Saxagliptin	25	99.86
4	Saxagliptin	25	99.97
5	Saxagliptin	25	99.89
6	Saxagliptin	25	100.03
	Average		99.95
	SD		0.075299
	RSD %		0.08

TABLE 8: SYSTEM PRECISION OF DAPAGLIFLOZIN

Injection no.	Name of the drug	Concentration (µg/mL)	Assay %
1	Dapagliflozin	50	491920
2	Dapagliflozin	50	492188
3	Dapagliflozin	50	490332
4	Dapagliflozin	50	490228
5	Dapagliflozin	50	491988
6	Dapagliflozin	50	490389
	Average		491174
	SD		945.2421
	RSD %		0.19

TABLE 9: SYSTEM PRECISION OF SAXAGLIPTIN

Injection no.	Name of the drug	Concentration (µg/mL)	Assay %
1	Saxagliptin	25	491920
2	Saxagliptin	25	492188
3	Saxagliptin	25	490332
4	Saxagliptin	25	490228
5	Saxagliptin	25	491988
6	Saxagliptin	25	490389
	Average		463183
	SD		2095.138
	RSD %		0.45

TABLE 10: RUGGEDNESS AND REPRODUCIBILITY OF DAPAGLIFLOZIN

	Laboratory-1 (Assay %)-HPLC-1				Laboratory-2 (Assay %)-HPLC-2			
Concentration (µg/mL)	Analyst-1		Analyst-2		Analyst-1		Analyst-2	
	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2
50	100.02	99.89	99.87	99.92	100.03	99.81	99.87	100.08
50	99.99	99.97	99.99	99.97	99.94	99.93	99.98	99.97
50	99.83	99.93	99.96	100.03	99.77	99.96	100.02	99.62
50	100.01	99.96	99.92	99.98	99.98	99.89	99.94	99.69
50	99.97	99.99	100.01	100.02	99.95	100.02	99.68	99.63
50	100.04	100.01	99.94	100.01	100.14	99.99	99.94	100.09
Average	99.98	99.96	99.95	99.99	99.97	99.93	99.91	99.85
SD	0.08	0.04	0.05	0.04	0.12	0.08	0.12	0.22
RSD %	0.08	0.04	0.05	0.04	0.12	0.08	0.12	0.22
Intermediate precision within-laboratories variations (n=24)								
	Laboratory-1 (Assay %)-HPLC-1				**Laboratory-2 (Assay %)-HPLC-2**			
Average	99.97				99.91			
SD	0.02				0.05			
RSD %	0.02				0.05			
Reproducibility between laboratories (n=48) (Assay %)								
Average	99.94							
SD	0.024							
RSD %	0.024							

TABLE 11: RUGGEDNESS AND REPRODUCIBILITY OF SAXAGLIPTIN

	Laboratory-1 (Assay %)-HPLC-1				Laboratory-2 (Assay %)-HPLC-2			
Concentration	**Analyst-1**		**Analyst-2**		**Analyst-1**		**Analyst-2**	
(µg/mL)	**Day-1**	**Day-2**	**Day-1**	**Day-2**	**Day-1**	**Day-2**	**Day-1**	**Day-2**
25	99.44	99.89	99.98	100.03	100.03	99.67	99.34	100.03
25	99.89	99.97	99.97	100.04	99.89	99.92	99.89	99.81
25	99.56	99.88	99.93	99.98	100.02	99.69	99.92	99.79
25	100.07	99.79	99.94	99.56	99.89	99.93	100.05	100.07
25	99.95	100.11	99.78	100.02	99.74	100.01	100.09	99.87
25	99.93	100.04	99.98	99.87	99.81	99.71	99.83	99.67
Average	99.81	99.95	99.93	99.92	99.90	99.82	99.85	99.87
SD	0.25	0.12	0.08	0.19	0.11	0.15	0.27	0.15
RSD %	0.25	0.12	0.08	0.19	0.11	0.15	0.27	0.15
Intermediate precision within-laboratories variations (n=24)								
	Laboratory-1 (Assay %)-HPLC-1				**Laboratory-2 (Assay %)-HPLC-2**			
Average	99.90				99.86			
SD	0.06				0.03			
RSD %	0.06				0.03			
Reproducibility between laboratories (n=48) (Assay %)								
Average	99.88							
SD	0.02							
RSD %	0.02							

TABLE 12: ROBUSTNESS DATA OF DAPAGLIFLOZIN

Variations in method parameters	Retention Time (min)	Average peak area*	RSD %	System suitability parameters	
				Theoretical plates	Asymmetry
Buffer : Acetonitrile (37:63,v/v)	3.446	875485	0.05	2341	1.02
Buffer : Acetonitrile (23:77,v/v)	2.841	724885	0.11	2206	1.02
0.9 mL/min Flow rate	3.446	875715	0.05	2341	1.01
1.1 mL/min Flow rate	2.405	602098	0.08	2288	1.02

* mean of six determinations

TABLE 13: ROBUSTNESS DATA OF SAXAGLIPTIN

Variations in method parameters	Retention Time (min)	Average peak area*	RSD %	System suitability parameters	
				Theoretical plates	Asymmetry
Buffer : Acetonitrile (37:63,v/v)	5.329	1066390	0.46	2890	1.01
Buffer : Acetonitrile (23:77,v/v)	4.180	870664	0.6	2724	1.03
0.9 mL/min Flow rate	5.329	1063581	0.024	2990	1.04
1.1 mL/min Flow rate	3.688	732120	0.221	2806	1.01

CONCLUSION: The developed method is simple, precise, and accurate. Hence, the RP-HPLC method can be applied for the routine analysis of Dapagliflozin and Saxagliptin in bulk and pharmaceutical dosage forms.

ACKNOWLEDGEMENT: The authors wish to thank the management of Mewar University, Chittorgarh, Rajasthan, India, Pellets Pharma Limited, Sangareddy, Telangana, India and School of Pharmacy, Anurag Group of Institutions, Hyderabad, Telangana, India for providing the necessary equipment for research, constant encouragement, facilities, and support.

CONFLICTS OF INTEREST: The author declares that there is no conflict of interest to disclose.

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How to cite this article:
Mirzaee V, Jafarzadeh A, Tahmasebi A and Jalalpour S: Zin*giber officinale* effect on *Helicobacter pylori* infection in human; a systematic review. Int J Pharm Sci & Res 2021; 12(1): 314-20. doi: 10.13040/IJPSR.0975-8232.12(1).314-20.