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ORIGINAL ARTICLE

Method Development and Validation for Simultaneous Estimation of Dapagliflozin & Saxagliptin by using Reverse phase -High performance Liquid Chromatography

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ABSTRACT
A simple, economical and highly selective RP-HPLC method for the estimation of Dapagliflozin and Saxagliptin in API and tablet dosage form has been developed. Separation was done on Cosmosil C18 (250mm x 4.6ID;5µ) Methanol:10mM KH_2PO_4 Buffer (90:10) at a flow rate of 0.8 ml/min. Detection was carried out at 220nm. RT of Dapagliflozin and Saxagliptin were found to be 5.8 and 6.1 respectively. The method was linear at the concentration range 10-50 mg/ml for dapagliflozin and 5-25 mg/ml for saxagliptin with correlation coefficient of (R^2) 0.9999 & 0.9993 respectively. LOD values of Dapagliflozin & Saxagliptin were 0.2254 & 0.1481 and LOQ of Dapagliflozin & Saxagliptin were 0.6832 & 0.448. Developed methods follow ICH Q2 (R1) criteria and economical; hence, applied for routine quality analysis in laboratories.
Keywords: *Dapagliflozin, HPLC, Saxagliptin, Simultaneous Estimation, Validation.*

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INTRODUCTION

When taken together, dapagliflozin and saxagliptin may significantly improve glycemic control without increasing the risk of weight gain and hypoglycemia, which can also occur when taking other type 2 diabetes drugs. (1) Dapagliflozin, a sodium glucose co-transporter-2 inhibitor, has the chemical name 2S,3R,4R,5S,6R)-2-[4-Chloro-3-(4-ethoxybenzyl) phenyl]-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol. The chemical formula is $C_{24}H_{33}ClO_8$, and its molecular weight is 408.987. The kidney's ability to reabsorb glucose depends on these sodium-glucose co-transporters. (2-4)

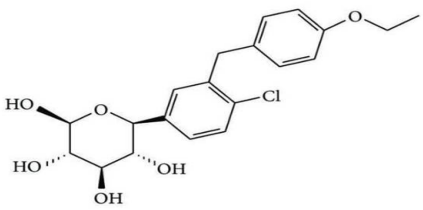

Fig.1. Structure of Dapagliflozin

Saxagliptin is an oral dipeptidyl peptidase-4 (DPP-4) inhibitor that lowers blood sugar levels and prevents diabetes with the IUPAC nomenclature (1S, 3S, 5S). - 2[(2S)-2-(3-hydroxy-1-adamantyl)-acetyl].-2-azabicyclo [3.1.2] Hexane-3-Carbonitrile. (5) C18H25N3O2 has a chemical formula with a molecular weight of 315.41 g/mole. (6) Gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are two active forms of augmentin that are produced when the medication inhibits the protein/enzyme dipeptidyl peptidase 4 (DPP-4). (7)

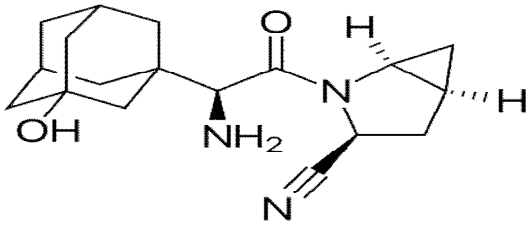

Fig.2. Structure of Saxagliptin

MATERIAL AND METHODS

Equipment

The chromatographic separation was performed on Cosmosil C18 column (255 mm x 4.6ID,5µ) equipped with P-3000-M Reciprocating pump(40MPa), UV detector with HPLC workstation software. Double beam UV-Visible spectrophotometer for spectroscopic determinations and wenser high precision electronic balance was used for weighing purpose in the study.

Chemicals and reagents

Dapagliflozin and Saxagliptin pure drug (API), Combination of Dapagliflozin and Saxagliptin tablet (Qtern 5/10mg), Water were purchased from Qualigens, Methanol was procured from Merck Specialties Private Limited and Potassium dihydrogen phosphate buffer from Hexon laboratories, Ortho-phosphoric acid.

Chromatographic conditions

An HPLC binary gradient system was utilized for the analysis of drugs. An instrument called Cosmosil C18 (250mm x 4.6ID, particle size: 5 micron) was used to carry out the chromatographic separation. For the analysis, a UV-3000-M detector was employed. Software called HPLC Workstation was used to record the data. Using a Wenser ultrasonicator, the mobile phase was degassed. The mobile phase used in this RP-HPLC technique was made up of Methanol:10 mM KH_2PO_4 Buffer (90:10) pH:3, which was adjusted with o-phosphoric acid. The analysis was done at room temperature with a flow rate of 0.8 ml per minute.

Preparations of solutions

Preparation of standard stock solution

precisely weighed 10 milligrams of saxagliptin and dapagliflozin before adding 10 milliliters of solvent (mobile phase) to each volumetric flask and 10 minutes of sonication. Diluents were added to flasks, which were designated as standard stock solutions 1 and 2. Thus, a 1000 ppm solution is generated.

Preparation of sample stock solution

20 tablets were weighed and the average weight of each tablet was calculated, all tablets were grounded into fine powder 228.2 mg was weighed and dissolved it into 10ml to get 1000 ppm of solution.

RESULT AND DISCUSSION

Selection of Wavelength

UV-VIS scan applied to the solution of Dapagliflozin, and Saxagliptin was within the range of 200-400 nm. A wavelength of 220 nm was selected for analysis.

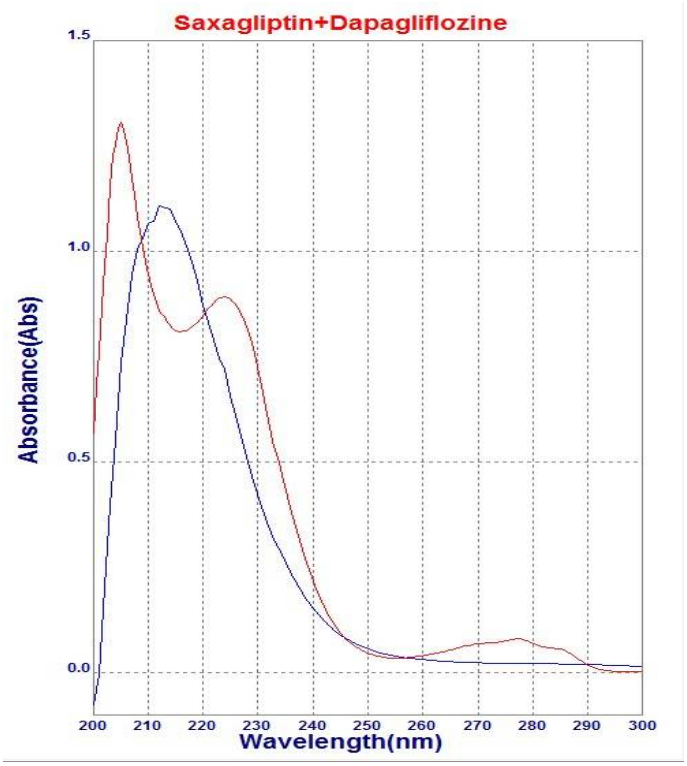

Figure 3: UV Spectra of dapagliflozin & saxagliptin

METHOD VALIDATION

Following ICH guidelines, analytical method is validated for linearity, accuracy, precision, limit of detection, limit of quantitation, robustness, ruggedness and system suitability parameters as per (Q2 R1).

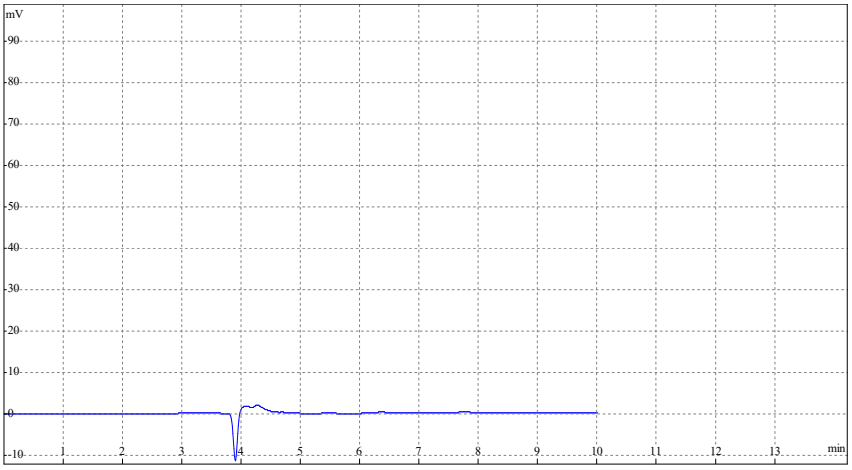

Figure 4: Typical chromatogram of Blank

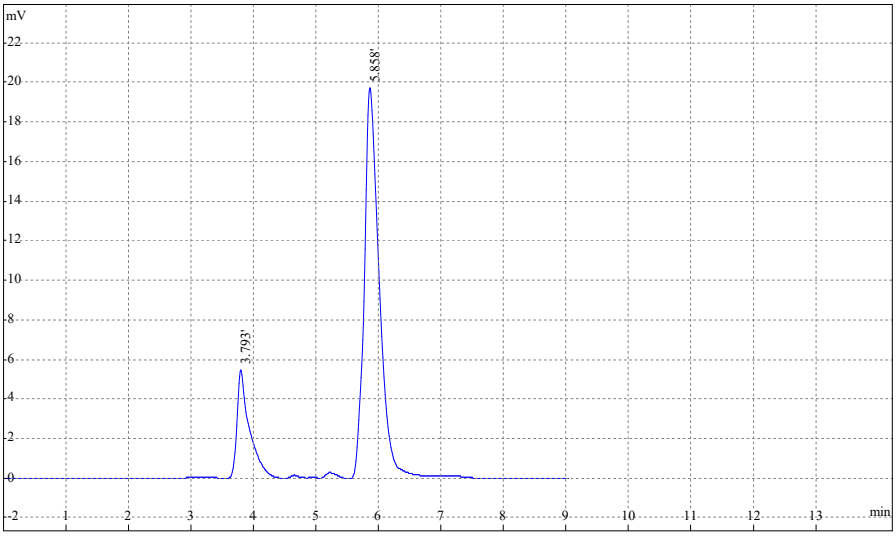

Figure 5: Standard chromatogram of dapagliflozin and saxagliptin.

LINEARITY

The correlation coefficient will be ≥0.999 for the range of 80% to 120% of the target concentration. Linearity was studied with the help of calibration curve using different concentration range of 10-50 mg/ml for dapagliflozin and 5-25 mg/ml for saxagliptin respectively. The correlation coefficient (R^2) for given drug is found to be 0.9999 & 0.9993 respectively. The calibration curve for given drug were shown in figure 6 and 7.

Table no.1: Linearity data of dapagliflozin

Sr. No.	Concentration	Area
1	10	330610
2	20	683091
3	30	1028052
4	40	1397002
5	50	1744493

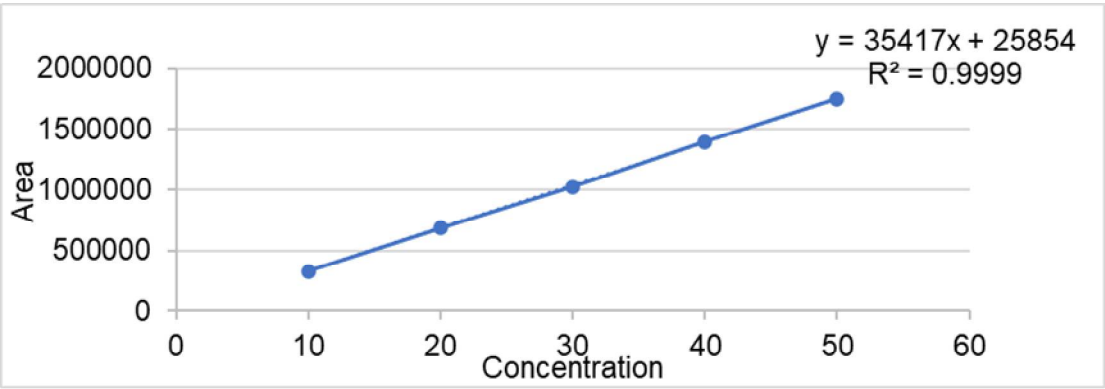

Figure 6: Calibration curve for dapagliflozin

Table no.2: Linearity data of saxagliptin

Sr.No.	Concentration	Area
1	5	106508
2	10	215111
3	15	339362
4	20	448827
5	25	554567

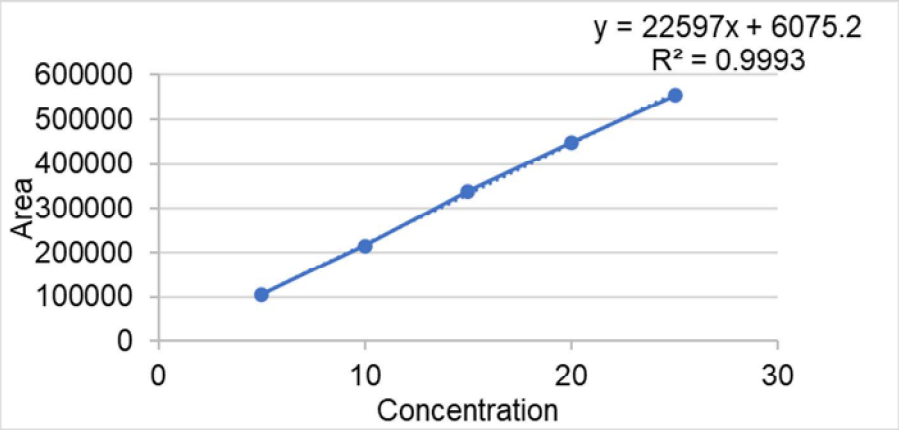

Figure 7: Calibration curve for saxagliptin

PRECISION

For drug substances and drug products, respectively, the RSD should be 1% and 2%. For small components, it should be within ±5%, but above the quantitation limit, it could be as high as 10%. Precision were studied by measuring inter-day (by injecting of samples over two consecutive days) and intra-day (repeatability which was carried out by analyzing the drug solutions within same day).

Precision studies of dapagliflozin

Table no.3: Inter-day study of dapagliflozin

			Standard Deviation		Accuracy	Precision
Sr. No.	**Conc.**	**Area**	**Mean**	**SD**	**%SD**	**%RSD**
	30	1028052				0.156010908
1	30	1029566	1030861.333	3634.455301	0.35256491	
	30	1034966				
	30	1051251				
2	30	1049687	1049809	1385.03574	0.13193216	
	30	1048489				

Table no.4: Intraday study of dapagliflozin

			Standard Deviation		Accuracy	Precision
Sr. No.	**Conc.**	**Area**	**Mean**	**SD**	**%SD**	**%RSD**
1	30 30 30	1028052 1029566 1034966	1030861.333	3634.455301	0.35256491	0.140565383
2	30 30 30	1047491 1059105 1053453	1053349.667	5807.6895	0.55135438	

Precision studies of saxagliptin

Table no.5: Inter-day study of saxagliptin

			Standard Deviation		Accuracy	Precision
Sr. No.	**Conc.**	**Area**	**Mean**	**SD**	**%SD**	**%RSD**
1	15 15 15	339362 343407 342161	341643.3333	2071.591256	0.6063608	0.221987174
2	15 15 15	301018 302052 306283	303117.6667	2789.586051	0.92029807	

Table no.6: Intraday Study of Saxagliptin

			Standard Deviation		Accuracy	Precision
Sr. No.	**Conc.**	**Area**	**Mean**	**SD**	**%SD**	**%RSD**
1	15 15 15	339362 343407 342161	341643.3333	2071.591256	0.6063608	0.143936479
2	15 15 15	312394 314915 313511	313606.6667	1263.219828	0.40280388	

RECOVERY

The mean recovery will be within 90 to 110% of the theoretical value for non-regulated products. Accuracy of an analytical procedure is closeness of test results to the true value. Accuracy was determined by standard addition method. The study was determined by spiking known amount of standard stock to the test solution prepared from formulation. The solutions were analyzed for mean recovery and %RSD. The studies were performed for both drugs at three different levels.

Table no.7: Recovery study of dapagliflozin

Sr. No	% Composition	Area of standard (Area Units)	Area of sample (Area Units)	% Recovery (%)	Conc. Taken (ppm)	Conc. Found (ppm)
1	50% Recovery	1028052	1025188	99.72141487	30	29.91642446
2	100% Recovery	1397002	1381760	98.90894931	40	39.56357972
3	150% Recovery	1744493	1737662	99.60842491	50	49.80421246

Table no.8: Recovery study of saxagliptin

Sr. No	% Composition	Area of standard (Area Units)	Area of sample (Area Units)	% Recovery (%)	Conc. Taken (ppm)	Conc. Found (ppm)
1	50% Recovery	339362	338053	99.6142762	15	14.94214143
2	100% Recovery	448827	446353	99.44878539	20	19.88975708
3	150% Recovery	554567	551395	99.42802222	25	24.85700556

Limit of detection and Limit of Quantification

The limit of quantitation for chromatographic methods has been described as the concentration that gives a signal-to-noise ratio (3:1). In this method, σ represents the standard deviation of the responses, while S is the mean of the calibration curve slopes.

Table no.9: Limit of detection and Limit of quantification study of saxagliptin and dapagliflozin

Sr. No.	Drug	SD	Slope	LOD	LOQ
1	Saxagliptin	507.178	11298	0.148140149	0.44890954
2	Dapagliflozin	2419.934	35417	0.225478787	0.68326905

ROBUSTNESS

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameter and provides an indication of its reliability during normal usage. The robustness was performed by change in wavelength, change in pH.

Table no.10: Robustness study of dapagliflozin

Sr. No.	Change in parameter	Concentration	Area	Mean	SD	% RSD
1	Change in wavelength	20 20 20	683091 678006 678200	679765.7	2881.46	0.4238896
2	Change in pH	20 20 20	683091 681132 671545	678589.3	6178.7	0.910522

Table no.11: Robustness study of saxagliptin

Sr. No.	Change in parameter	Concentration	Area	Mean	SD	% RSD
1	Change in wavelength	10 10 10	215111 219206 216524	216947	2080.01	0.9587654
2	Change in pH	10 10 10	215111 219029 215947	216695.7	2063.51	0.95226

Assay procedure

The assay performed on marketed formulation. Prepared sample and standard solution were injected into HPLC and peak areas were recorded. Finally, percentage assay of drug was calculated.

Sr. No	Drug	Concentration	Area of standard	Area of sample	% Assay
1	Saxagliptin	15 ppm	339362	336426	99.1348472
2	Dapagliflozin	30 ppm	1028052	1025651	99.7664515

Table no.12: Assay study of Saxagliptin & Dapagliflozin

An efficient and simple HPLC method was developed and validated for the simultaneous determination of Saxagliptin and Dapagliflozin in their combined dosage form. The chromatogram was run through Cosmosil C18 column (250mm x 4.6ID, Particle size: 5 micron) using a mobile phase consisting of Methanol:10mM KH2PO4 Buffer (90:10) at a flow rate of 0.8 ml/min. Drug peaks were well separated and detected by a UV detector at 220nm. The retention times of Dapagliflozin and Saxagliptin were found to be 5.8 and 6.1 minutes, respectively. The method linear in the range of 10-50 mg/ml for Dapagliflozin and 5-25 mg/ml for Saxagliptin, with correlation coefficients (R^2) of 0.9999 and 0.9993 respectively. The limits of detection (LOD) were determined to be 0.2254 and 0.1481 for Dapagliflozin and Saxagliptin, respectively, while the limits of quantification (LOQ) were found to be 0.6832 and 0.448 for Dapagliflozin and Saxagliptin, respectively.

CONCLUSION

The developed HPLC method provides a robust and reliable means for the simultaneous determination of Dapagliflozin and Saxagliptin in API and combined tablet dosage form. The method offers several advantages, including simplicity, efficiency, and cost-effectiveness. Notably, the optimization of retention times has led to a decreased run time, further enhancing the method's practicality and economy. With its high sensitivity, precision, and accuracy, this method can be readily recommended in pharmaceutical laboratories for routine analysis.

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