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Development and Validation of RP-HPLC Method for Simultaneous Estimation of Dapagliflozin and Saxagliptin in Tablet Dosage Form

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ABSTRACT

The simple, specific, precise and accurate method has been developed for RP-HPLC for simultaneous estimation of Dapagliflozin and Saxaglptin in tablet dosage form. The detection wavelength of Dapagliflozin and Saxagliptin by taking overly was found to be 215nm. In RP-HPLC method separation was achieved by Cosmosil C18 (250×4.6ID) column with the mobile phase Methanol:10m Potassium phosphate buffer (80:20v/v). The pH 6.8 of was adjusted by orthophosphoric acid. The flow rate is 0.8ml/min. The retention time dapagliflozin and saxagliptin was found to be 6.15min and 4.96min for Dapagliflozin and Saxagliptin respectively. A good linear response was obtained in the range of 5-10µg/ml of each respectively. The recoveries of dapagliflozin and saxagliptin were found in range of 99.51-99.82% and 99.26-99.48% respectively. The LOD (Limit of detection) of Dapagliflozin and Saxagliptin was found to be 0.04262µg/ml and 0.004065µg/ml respectively and LOQ (Limit of Quantitation) of Dapagliflozin and Saxagliptin was found to be 0.0371µg/ml and 0.0246µg/ml .The % recovery was found to be 99.51%-99.54% for DAPA and 99.43%-99.26% for SAXA respectively. The proposed method were validated as per ICH guidelines by means of different parameters like Linearity, Precision, Accuracy, LOD and LOQ, range of selectivity, Roubstness and Rouggedness as per ICH guidelines and successfully applied to the estimation of Dapagliflozin and Saxagliptin in tablet dosage form.

INTRODUCTION

A nature of this drug is competitive inhibitor of the sodium glucose transport subtype 2 protein, dapagliflozin blocks glucose reabsorption which one carried out into the kidney which help into the elimination of blood glucose from the urine. The common side effect includes urinary tract and vaginal infection. The 90% glucose reabsorption carried out into the kidney. They block the transporter which responsible for blood glucose eliminated through urine Saxagliptin its new oral hypoglycemic agent. It is a dipeptidyl peptidase-4 (DPP-4) inhibitor. They affect the action of hormone known as incretins. Incretins lowers the blood sugar level by increasing insulin production in pancreas.

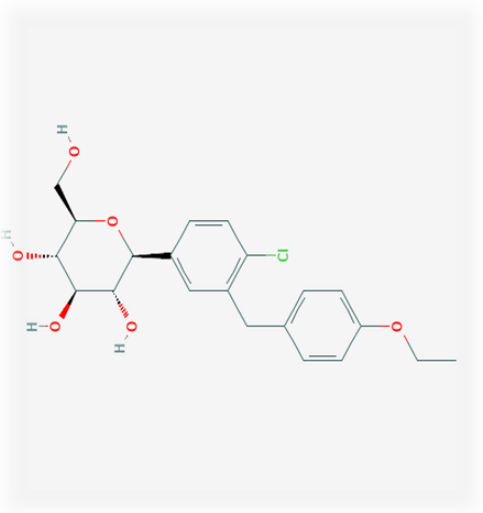


Fig. No. 1: Structure of Dapagliflozin

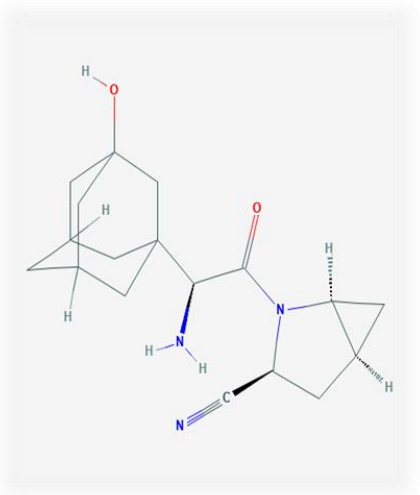


Fig. No. 2: Structure of Saxagliptin

The literature review says that various analytical method reported for Dapagliflozin and Saxagliptin in UV-spectrometry, HPLC, RP-HPLC individually and common. This work shows that the Development and validation of RP-HPLC method for simultaneous estimation of Dapagliflozin and Saxagliptin in tablet dosage form.

MATERIALS AND METHODS

Materials Dapagliflozin and Saxagliptin pure API, Combination tablet of both drugs (QTERN), Distilled water, Phosphate buffer, Methanol, orthophosphoric acid, Acetonitrile.

Instruments Electronic balance, pH meter, Ultrasonicator, HPLC system, Uv-VIS spectrophotometer.

Methods

Diluent: Based upon the solubility the diluent was selected. Methanol: 10m potassium phosphate buffer (80:20).

Preparation of Std. stock solution Weigh accurately 10mg of Dapagliflozin and Saxagliptin respectively in 100ml of volumetric flask in 10ml of mobile phase and make the final volume upto mark and labeled them as standard stock solutions of 1000µg/ml of both drugs.

Preparation of Sample stock solutions:- 20 tablets were weigh accurately and calculate the average weight of tablets, then weight equivalent of one tablet were taken and add 10ml of mobile phase and sonicated for 20min make the final volume upto the mark and filter by HPLC filter paper it makes 1000µg/ml of sample stock solution.

Preparation of Buffer: 10M Potassium Phosphate buffer: Weigh accurately 0.136gm of potassium dihydrogen phosphate in 1000ml of volumetric flask then degassed to sonicate upto 20min and finally make up the volume with water and finally pH 3.8 was adjusted with orthophosphoric acid.

Determination of Analytical wavelength: The both drug is scanned in range 200-400nm. The analytical wavelength of Dapagliflozin and Saxagliptin was found to be 215nm.

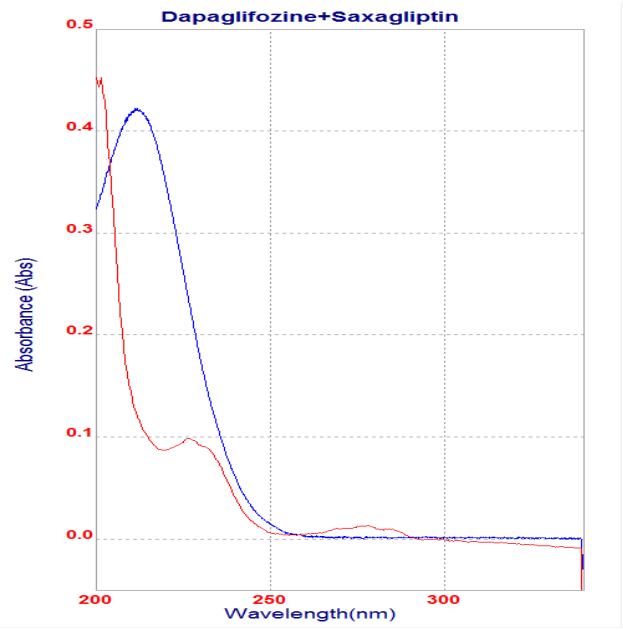


Fig. No. 3 Overlain spectra of DAP and SAX

RESULTS AND DISCUSSION

Dapagliflozin and Saxagliptin having good resolution, plate count and tailing factor, asymmetry, so that's why this method was optimized and validated. All the system suitability parameters was within the range as per ICH guidelines.

Linearity The various concentrations of Dapagliflozin (5µg/ml-25µg/ml) and Saxagliptin (5µg/ml-25µg/ml) were made. Linearity equations of Dapagliflozin was y=14085x + 28543 and Saxagliptin was y= 88082x +28955 correlation coefficient of both drugs was 0.999 for both drugs.

Precision Precision is called as the closeness of agreement between a series of measurements obtained from multiple samples of the same sample. The known concentrations of Dapagliflozin and Saxagliptin has been analysed into HPLC column n the same day. The precision determined by injecting the samples of same concentrations on 2 different days. The peak area all conc. were taken and standard deviations, % Relative standard deviation was calculated.

Accuracy Accuracy tested by standard addition method at diff levels 100,120,150%. A known amount of drugs was added on sample of every level. The recovery of Dapagliflozin and Saxagliptin were calculated it found to be 99 ±82%.

LOD (Limit of detection) LOD (Limit of detection) and LOQ (Limit of Quantitation) of Dapagliflozin and Saxagliptin determined by calibration curve method. Dilutions were prepared in linearity range. The average area was plotted against concentrations and they are calculated by following formula.

Robustness HPLC conditions were modified to evaluate the analytical robustness. The changes in flow rate and wavelength**.**

Linearity

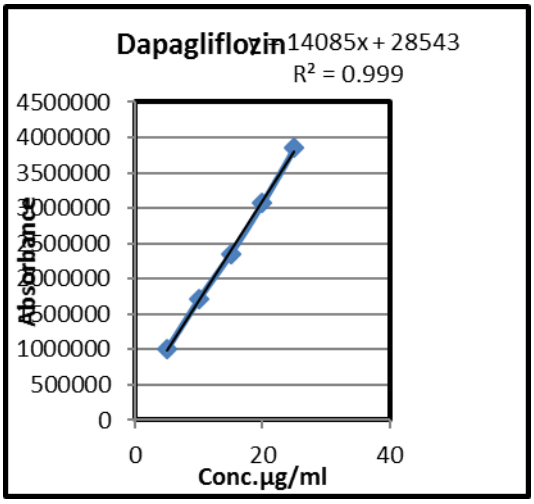
Linearit of the Dapagliflozin and Saxagliptin in which relationship was established at these ranges between area under peak and concentrations. Good linearity was proved the high coefficient value that all shows in following tables.

Table No. 1: Linearity data of Dapagliflozin

Standard conc.	5ug/ml	10ug/ml	15ug/ml	20ug/ml	25ug/ml
Replicates	Peak Area (µV.sec)				
1	100557	171221	235520	307004	384791
2	101180	171323	235468	307110	384572
3	100677	171389	235356	307019	384672
4	100610	171580	235534	307213	384542
5	100709	171478	235409	307003	384789
Mean	100746	171398	235457	307069	384673
SD	77.15136	84.64041	63.058174	107.38715	123.57588
% RSD	0.076671	0.049407	0.026780	0.0349687	0.0321253

Table No. 2: Linearity data for Saxaglitpin

Standard conc.	5ug/ml	10ug/ml	15ug/ml	20ug/ml	25ug/ml
Replicates	Peak Area(µV.sec)				
1	141066	586806	105405	147582	189860
2	141123	586704	105361	147489	189769
3	141219	586891	105365	147545	189721
4	141356	586834	105442	147768	189679
5	141043	586332	105128	147589	189880
Mean	141161	586713	105340	147594	189781
SD	117.09967	307.60743	24.331052	46.822359	70.59981
% RSD	0.082912	0.0524313	0.0230895	0.0317357	0.037200



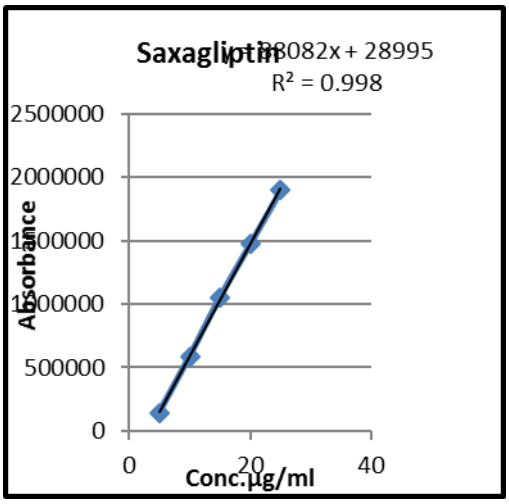


Fig. No. 4: Calibration curve for Dapagliflozin Fig. No. 5: Calibration curve for Saxagliptin

Precisions:

The precision of the Dapagliflozin and Saxagliptin was found to be within the limits (RSD<2).

Table No. 3: Inter-day precision for Dapagliflozin

Conc. (µg/ml)	Peak area (µV.sec)			Mean area (µV/sec)	±SD	%RSD
	Day 1	Day 2	Day 3			
5	235520	235634	235611	235561.7	674.3493	0.028627
15	235525	235490	235545	235542.3	794.8693	0.033746
25	235639	235503	235697	235663.2	52.11525	0.022110

Table No. 4: Inter-day precision for Saxagliptin

Conc. (µg/ml)	Peak area (µV.sec)			Mean area (µV/sec)	±SD	%RSD
	Day 1	Day 2	Day 3			
5	1054331	1053982	1054173	105416.2	174.7598	0.01657
15	1055198	1054290	1055303	105493.1	557.0245	0.05280
25	1056198	10566234	1055456	105530.3	439.1552	0.04158

Table No. 5: Intra-day precision for Dapagliflozin

Conc. (µg/ml)	Peak area (µV.sec)			Mean area (µV/sec)	±SD	%RSD
	Day 1	Day 2	Day 3			
5	235419	235423	235535	235498.65	65.848310	0.027965
15	235522	235438	235521	235459.00	48.211340	0.020472
25	235551	235445	235467	235487.67	55.940441	0.041588

Table No. 6: Intra-day precision for Saxagliptin

Conc. (µg/ml)	Peak area (µV.sec)			Mean area (µV/sec)	±SD	%RSD
	Day 1	Day 2	Day 3			
5	1054154	1053634	1053612	10553800	306.77027	0.029110
15	1054416	1054123	104312	1054283.6	148.54667	0.014089
25	1055213	1056931	1055814	1055986	871.81936	0.082559

Accuracy:

Three levels 80%, 100%, 120% for accuracy shown in below table and it done by standard addition method in which known amount of standard drug was added into blank and standard solutions of Dapagliflozin and Saxagliptin respectively. The average accuracy and % RSD was within the limit.

Table No. 7: Recovery data for Dapagliflozin

Recovery Level	Dapagliflozin					
	Area (µV.sec)	Amt. added (mg)	Amt. recovered (mg)	% Recovery	Average recovery	%RSD
80%	1005571	8	7.50	98.92	99.51.	0.56
	1005727	8	7.92	99.60		
	1006082	8	8.07	100.01		
100%	2355200	10	10.03	100.02	99.82	0.49
	2351887	10	10.01	100.1		
	2356369	10	9.92	99.27		
120%	3843712	12	11.99	99.99	99.54	0.86
	3844912	12	11.82	98.55		
	3851912	12	12.00	100.08		

Table No. 8: Recovery data of Saxagliptin

Recovery Level	Saxagliptin					
	Area (µV.sec)	Amt. added (mg)	Amt. recovered (mg)	% Recovery	Average recovery	%RSD
80%	10510	8	7.80	98.56	99.43	0.77
	10515	8	7.99	100		
	10613	8	7.97	99.72		
100%	14548	10	10.01	100.01	99.48	0.63
	14106	10	9.96	99.66		
	14184	10	9.87	98.79		
120%	187794	12	11.82	98.50	99.26	0.87
	184673	12	12.02	100.2		
	188460	12	11.77	99.09		

LOD, LOQ The limit of detection (LOD) for dapagliflozin and saxagliptin was found to be 0.042625µg/ml and 0.004065µg/ml respectively. The limit of quantitation (LOQ) for dapagliflozin and saxagliptin was found to be 0.0371µg/ml and 0.02464µg/ml respectively. The all value was found to be within the limit.

Robustness:- The method parameters like change in flow rate, change in wavelength was maintained. System suitability parameters were not much affected and all parameters passed. % RSD were within the acceptable limit.

Table No. 9: Robustness data for change in flow rate.

System suitability parameter	Drug	Change in flow rate (ml/min)		RSD	
		0.7ml/min	0.9min/ml	0.7min/ml	0.9min/ml
Peak area	DAPA	17172	17095	0.69	0.06
	SAXA	58651	58667	0.17	0.15
Therotical plates	DAPA	8198	9926	0.35	0.37
	SAXA	9697	9953	0.62	0.25
Tailing Factor	DAPA	1.08	1.08	0.53	0.57
	SAXA	1.12	1.13	0.51	0.89
Retention Time (Min)	DAPA	5.37	6.01	0.45	0.25
	SAXA	4.41	4.53	0.56	0.55

Table No. 10: Robustness data for change in wavelength

System suitability parameter	Drug	Change in Wavelength (nm)		RSD	
		213	218	213	218
Peak area	DAPA	17272	17122	0.43	0.22
	SAXA	58756	58845	0.16	0.13
Therotical plates	DAPA	8698	5489	0.89	0.83
	SAXA	8764	8137	0.85	0.21
Tailing Factor	DAPA	1.07	1.09	0.89	0.53
	SAXA	1.13	1.11	0.51	0.86
Retention Time (Min)	DAPA	5.76	5.68	0.70	0.63
	SAXA	4.89	4.94	0.75	0.47

Assay AstraZenica pharmaceuticals (QTERN) the label claim Dapagliflozin 10mg and Saxagliptin 5mg. Average % assay for Dapagliflozin and Saxagliptin found to be 100% and 99.88% respectively.

Table 11: Analysis of marketed formulation

Sr. No	Wt. of stand. (mg)		Peak area of (µV.sec)		Label claim (%)		Mean	
	DAP	SAXA	DAPA	SAXA	DAPA	SAXA	DAPA	SAXA
1	10mg	5mg	105485	235520	100.02	100	100	99.88
2			105153	235188	99.96	99.99		
3			105492	235757	100.04	99.97		
						S.D.	0.04163	0.04264
						%RSD	0.01527	0.01532

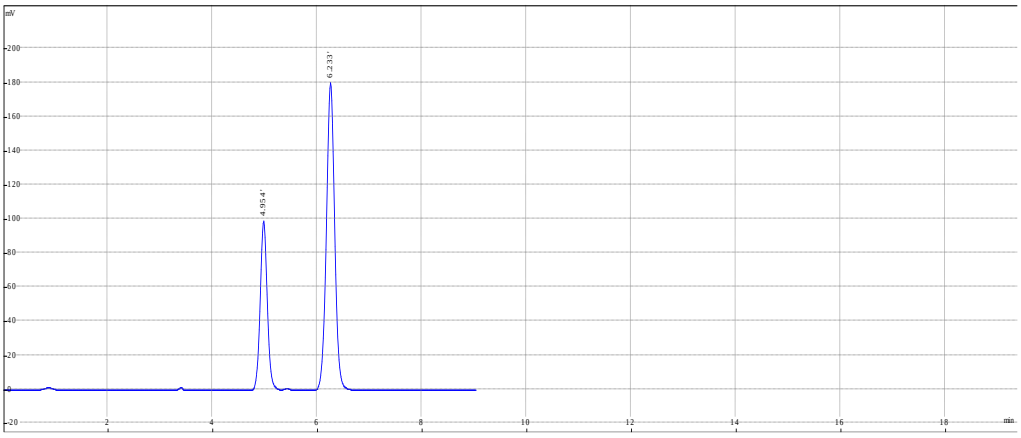


Fig. No. 6 Chromatogram of Tablet formulation of Dapagliflozin and Saxagliptin

CONCLUSION

The simple, precise and accurate, robust method for estimation of the Dapagliflozin and Saxagliptin in tablet dosage form by RP-HPLC method was developed and validated. The good resolution with short analysis time below 10min. All the validation parameters were found to be within the limits as per ICH guidelines. No interference of additives is encountered in both of the developed method.

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