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"SIMULTANEOUS ESTIMATION AND VALIDATION OF DAPAGLIFLOZIN AND SAXAGLIPTIN IN BULK DRUG AND DOSAGE FORM BY RP-HPLC"

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ABSTRACT

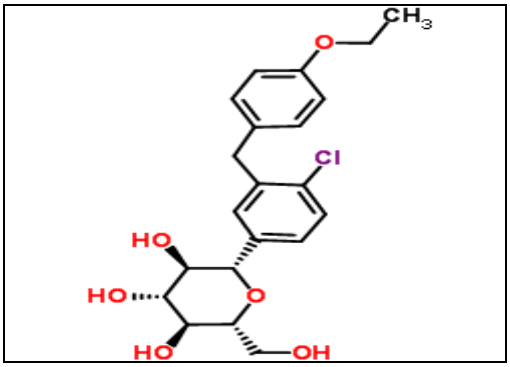
A simple, rapid, precise, accurate and RP-HPLC method has been developed and validated to estimate Saxagliptin and Dapagliflozin in bulk and in dosage form. The samples were eluated Grace C18 (250mm x 4.6ID, Particle size: 5 micron with mobile phase Methanol: water (80:20) at wavelength 225 nm. A good linear response was obtained in the range from 5-25µg/mL of Saxagliptin hydrochloride and 10-50 µg/mL of Dapagliflozin. The method was quantitatively evaluated in terms of linearity, precision, accuracy (recovery), selectivity and robustness as per ICH guideline.

KEYWORDS: RP-HPLC, Saxagliptin, Dapagliflozin.

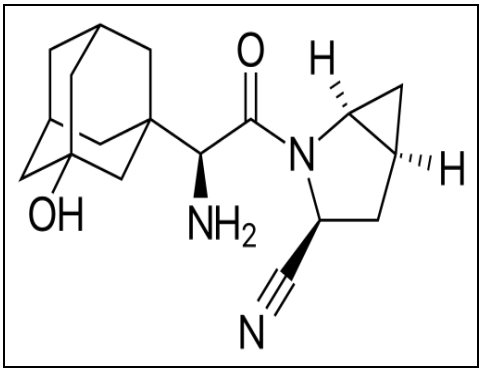
INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic progressive metabolic disorder characterized by absolute or relative insulin deficiency. Saxagliptin is chemically known as (1S, 3S, 5S)-2[(2S)-2-amino-2-(3-hydroxy-1- adamantyl) acetyl]2 azabicyclo hexane-3-carbonitrile) with molecular formula of C18H25N3O2 and molecular weight of 315.41g/mol. Saxagliptin is a selective and potent dipeptidyl peptidase (DPP)-4 inhibitor, approved as an adjunct to diet and exercise to improve glycemic control in type 2 diabetes mellitus (T2DM). In patients with T2DM, Dapagliflozin is chemically known as (1s)-1, 5- anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-D-glucitol. It has a molecular formula of C24H33ClO8 with molecular weight 408.98. Dapagliflozin is selective Sodium Glucose CoTransporter 2 inhibitor (SGLT 2). It acts by reducing the re absorption of glucose by the kidney, leading to excretion of excess glucose in the urine, thereby improving glycemic control in patients with type 2 diabetes mellitus. Though several methods are reported in literature for the estimation

of Saxagliptine and Dapagliflozin individually, no methods are reported for estimation of Saxagliptine and Dapagliflozin in combination. The objective of the present study is to develop a novel, simple, accurate, precise, economic method for the simultaneous estimation of Saxagliptine and Dapagliflozin and validate according to ICH guidelines.[3]



Ch. Str. of Dapagliflozin



Ch. Str. Saxagliptin

MATERIALS AND METHODS

All reagents and solvent were of analytical grade; they included water (HPLC grade), Methanol, ortho phosphoric acid, saxagliptin and dapagliflozin procured purchased from swapnrooop pharmaceuticals Aurangabad India. The tablets containing Saxagliptin hydrochloride 5 mg and Dapagliflozin 10 mg under brand name QTERN was procured from Astra Zaneca Pharma. Pvt. Ltd. India. The solution filtered through 0.45–l filters. HPLC Binary Gradient System, Model no.: HPLC 3000 Series, Company: Analytical Technologies Ltd, Detector: UV-3000-M, Column: Grace C18 (250mm x 4.6ID, Particle size: 5 micron.

Optimized Chromatographic Conditions

Mobile phase: Methanol: water (pH: 3)

System: Isocratic. Column

Stationary Phase column: Grace C18 (250mm x 4.6ID, Particle size: 5 micron

Flow rate: 0.9 mL/min

Detection wavelength: 225 nm

Injection volume: 20µL

Column oven temperature: 40°C

Diluent: Methanol: water (80:20)

Run Time: 8 Minutes

Retention Time: 3.866 min and 5.179 for Saxagliptin and Dapagliflozin

Standard Stock solution of dapagliflozin and saxagliptin

100 mg of each of Saxagliptin and Dapagliflozin were accurately weighed and transferred 100 mL Volumetric flask, added 50 ml diluent (Methanol: water 75:25 v/v) sonicated to dissolve. Make up to the volume with diluent and mixed to obtain 1000 µg/mL of Saxagliptin hydrochloride and Dapagliflozin respectively.

Preparation of the standard working solution

Take 100ug Dilute 100ml/ug mL of standard stock solution to volumetric flask, make up to the volume with diluent and mixed to obtain 1000 ug/mL of Saxagliptin hydrochloride and Dapagliflozin.

Preparation of working sample solution

Accurately weighed 10mg of saxagliptin and Dapagliflozin was added in volumetric flask containing some amount of mobile phase and volume was made up to the mark using mobile phase. The resulting solution was filtered through 0.45µ membrane filter and sonicated for three cycles each of 10 min. From the stock solution 1.0ml of stock was pipette out in triplicate and kept in three different volumetric flasks, cleaned previously and diluted up to 10ml by using mobile phase to obtain resultant solution of 10µg/ml.

Method validation

The chromatographic conditions were found to be appropriate for the quantitative determination. After the analytical conditions had been optimized, certain parameters such as the linearity, precision, accuracy (recovery), selectivity, and robustness were evaluated to validate the method.

Linearity

The linearity for Saxagliptin hydrochloride and Dapagliflozin were assessed by analysis of combined standard solution in range of 5-25 ug/mL and 10-50 ug/mL respectively by taking 1, 2, 3, 4, 5, ml solutions Linearity data for Saxagliptin Hydrochloride and Dapagliflozin are shown in Table No.1. Lineariry spectra of both drug and calibration curve of Saxagliptin Hydrochloride and Dapagliflozin are shown in Figure No 6, 7, 8.

Accuracy

Accuracy of the method was confirmed by recovery study at three level of standard addition drug solution was taken in three volumetric flasks. 50%, 100%, 150% of standard solution in

volumetric flask and diluted up to mark with Diluent. The area of each solution peak was measured at 225nm Results of recovery study are shown in Table No.2.

Precision

Precision for 3 intraday and interday was estimated using three concentrations in triplicate. Results for intraday and interday precision are shown in Table No.3 and 4 respectively.

LOD and LOQ (limit of Detection and Quantification)- LOD and LOQ are calculated as per ICH guideline. Results of LOD and LOQ are shown in Table No.5.

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 (± 0.2 ml/min) 0.8 ml/min and 0.9 ml/min,0.8ml/min.
2. Change in wavelength- Wavelength changed to 227nm,223nm,225nm

Assay

20ul of standard solution injected into chromatographic system, chromatograms were recorded and peak areas were measured .20ul of sample solution was injected into chromatographic system, chromatograms were recorded and peak areas were measured.

RESULTS AND DISCUSSION

Table 1: Linearity data of Saxagliptin and Dapagliflozin.

Sr. No.	Concentration µg/ml	Peak Area SAXA	Concentration ug/ml	Peak Area DAPA
1	5 ug/ml	210153	10ug/ml	445828
2	10ug/ml	438127	20ug/ml	879615
3	15ug/ml	618461	30ug/ml	1299622
4	20ug/ml	820473	40ug/ml	1734645
5	25ug/ml	1021474	50ug/ml	2252638

Table 2: Accuracy data of Saxagliptin and Dapagliflozin.

Sr. No.	% Concentration	Area	Amount added	Amount found	% recovery	Mean Recovery
1	50%	616178	15	14.94	99.63%	
2	50%	618461	15	14.94	99.63%	
3	50%	618461	15	14.94	99.63%	
4	100%	816932	20	19.91	99.56%	
5	100%	820473	20	19.91	99.56%	99.68%
6	100%	820473	20	19.91	99.56%	
7	150%	1019954	25	24.96	99.85%	
8	150%	1021474	25	24.96	99.85%	
9	150%	1021474	25	24.96	99.85%	

Sr. no.	% Concentration	Area	Amout added	Amout found	% recovery	Mean Recovey
1	50%	1295118	30	29.89	99.65%	
2	50%	129922	30	29.89	99.65%	
3	50%	1299622	30	29.89	99.65%	
4	100%	1734645	40	39.99	99.98%	
5	100%	1734645	40	39.99	99.98%	99.87%
6	100%	1734645	40	39.99	99.98%	
7	150%	2249312	50	49.93	99.87%	
8	150%	2252238	50	49.83	99.87%	
9	150%	2252238	50	49.83	99.87%	

Table 3: Robustness data.

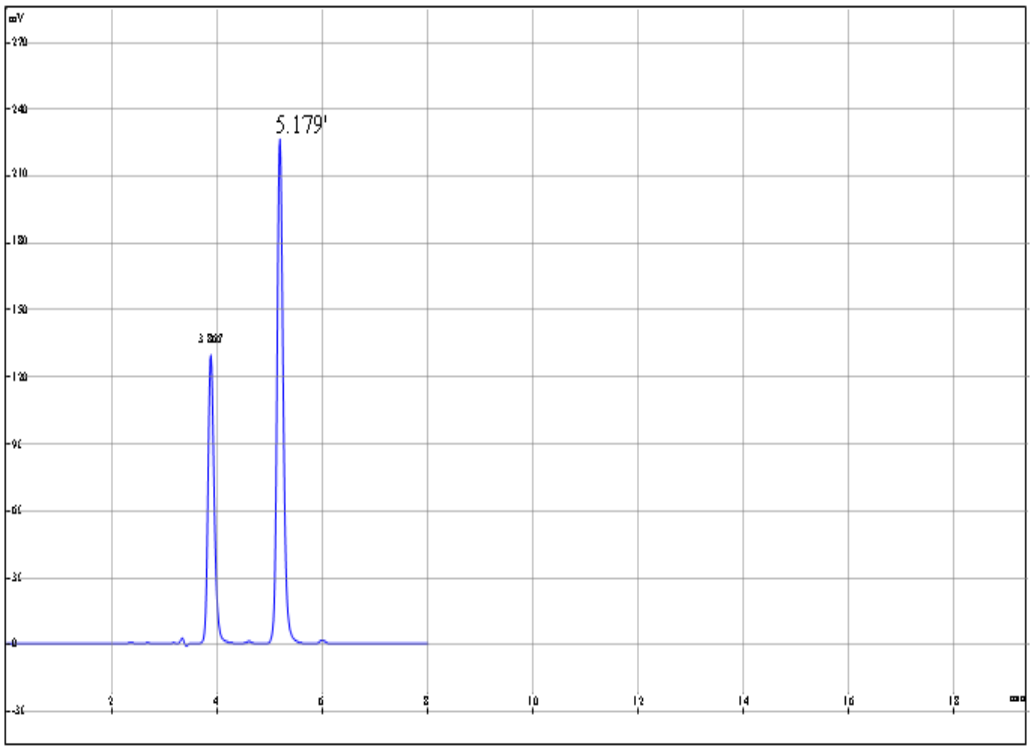
Parameter used for analysis	Saxagliptin	Dapagliflozin	Mean	SD	%RSD
Change in wavelength			Saxagliptin		
227nm	435430	876270	436891	0.30	0.3036
223nm	4372234	882938	Dapagliflozin		
225nm	438020	872887	877365	0.58	0.5829
Change in flow rate			Saxagliptin		
0.9ml/min	435980	882270	437080	1029.29	0.2354
0.5ml/min	438020	876284	Dapagliflozin		
0.7ml/min	437239	882938	880497	0.4161	0.4161

Table 4: Limit of Detection and limit of quantification of Saxagliptin and Dapagliflozin LOD and LOQ data.

LOD(ug/ml)	LOQ(ug/ml)
0.47106	1.4273
0.23757	0.71991

Table 5: Assay of Saxagliptin and Dapagliflozin.

Sr. No.	Drug Name	Standard Area	Sample Area	% assay
1	Saxagliptin	618461	612147	98.97
2	Dapagliflozin	1299622	1294073	99.57



Time	Area	Resolut.	T.Plate	Num Asy
3.866	1147472	5.79	4567	1.25
5.179	1894131	0.00	9067	1.18

Fig. 1: Typical chromatogram of Saxagliptin and Dapagliflozin.

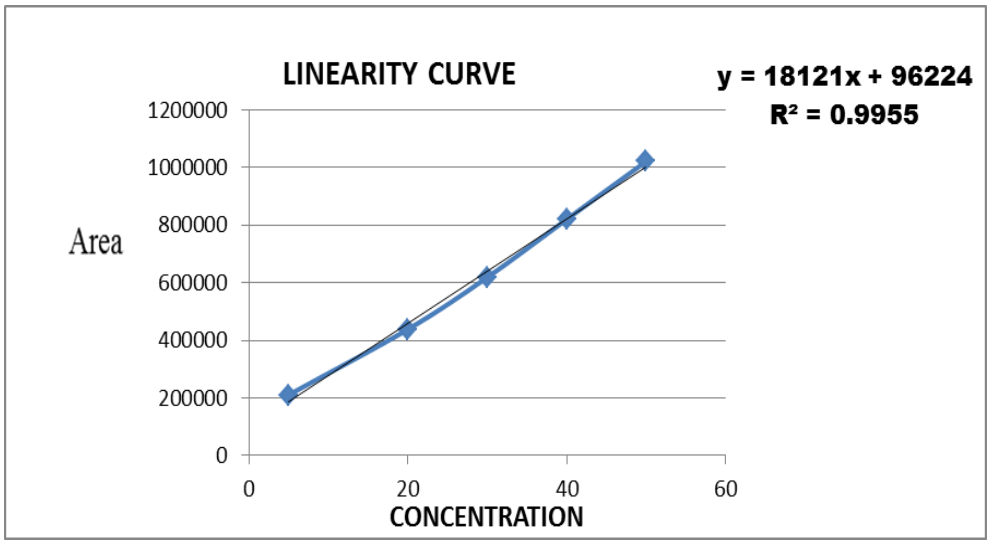


Fig. 2: Linearity Graph by HPLC of Saxagliptin.

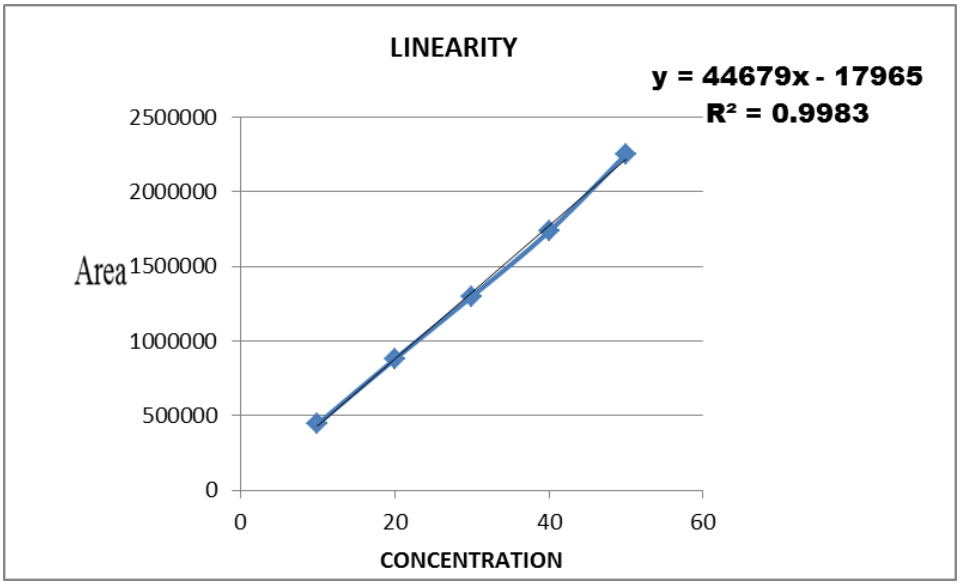


Fig. 3: Linearity graph of Dapagliflozin.

CONCLUSION

The developed RP-HPLC method is accurate, precise, robust, sensitive and selective. And the method is cost effective and less time consuming. It can successfully apply for estimation of Saxagliptin and Dapagliflozin in its pharmaceutical dosage form.

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