



## DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF SAXAGLIPTIN AND DAPAGLIFLOZIN.

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### ABSTRACT

The present work describes a validated reverse phase high performance liquid chromatographic method for simultaneous estimation of saxagliptin and dapagliflozin in synthetic mixture. Chromatography was performed on a ODS inertsil C18 (250 mm x 4.6 mm i.d., 5 µm particle size) column with mobile phase containing 0.05M Potassium dihydrogen phosphate (pH 4.5±0.1 using orthophosphoric acid) : Methanol (35: 65). The flow rate was 1.0 ml/min and the eluent was monitored at 227 nm. The selected chromatographic conditions were found to effectively separate Saxagliptin (RT- 2.68min) and dapagliflozin (RT- 5.8 min). (Figure-3) Linearity for Saxagliptin and

Dapagliflozin were found in the range of 2.5-7.5 µg/ml and 5-15µg/ml respectively. The proposed method was found to be fast, accurate, precise, and reproducible and can be used for simultaneous estimation of these drugs.

**KEYWORDS:** Saxagliptin, Dapagliflozin, Reversed-phase HPLC.

### INTRODUCTION

Saxagliptin of 1S,3S,5S)-2-[(2S)-2-AMINO-2-(3-HYDROXYADAMANTAN-1-YL)ACETYL]-2-AZABICYCLO[3.1.0]HEXANE-3-CARBONITRILE, represents the class of Dipeptidase -4 (DPP-4) inhibitor in the management of oral hypoglycemic.<sup>[1]</sup> Saxagliptin exerts its action by hypoglycemic condition in diabetes type 2. saxagliptin inhibits DPP -4 enzyme activity for 24 hour period. The determination of Saxagliptin has been carried out in tablets by RP-HPLC<sup>[3-10]</sup> in bulk and solid dosage forms. Dapagliflozin of 2S,3R,4R,5S,6R)-2-(4-Chloro-3-(4-ethoxybenzyl)phenyl)-6- (hydroxyethyl)tetrahydro-2H-pyran-3,4,5-triol belongs to SGLT2

inhibitors.<sup>[2]</sup> Literature survey revealed that few analytical methods have been reported for the estimation of Dapagliflozin included RP-HPLC.<sup>[11-16]</sup> The analytical methods for simultaneous determination of Saxagliptin with other combination has been reported by RP-HPLC<sup>[5-9]</sup>, UV Spectrophotometry.<sup>[9]</sup> The analytical methods for simultaneous determination of Dapagliflozin with other combination has been reported by RP-HPLC.<sup>[15]</sup>

The present work describes a validated reverse phase HPLC method for simultaneous determination of these drugs in tablets.

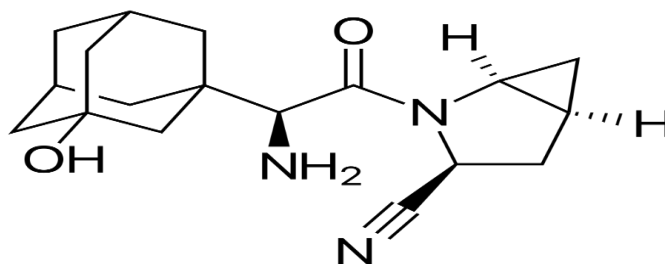


Figure 1. Structure of Saxagliptin.

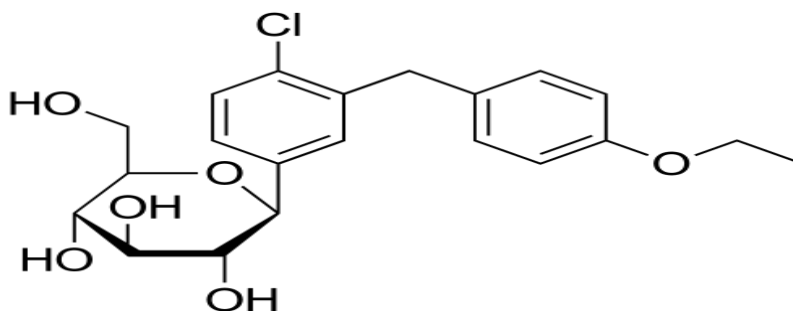


Figure 2. Structure of Dapagliflozin.

## EXPERIMENTAL WORK

### Apparatus

A RP-HPLC instrument (YL-Instrument) equipped with UV-Visible detector (Shimadzu, model 1800), manual injector with 20  $\mu$ l loop, and C18 column (250 mm  $\times$  4.6 mm id, 5  $\mu$ m particle size) and YL-Clarity software were used. Analytical weight balance (Mettler Toledo, Schwerzenbach, Switzerland), and ultra sonic cleaner (Branson ultrasonic corporation), pH meter (Sytonic) used during the study.

### Reagents and Materials

Saxagliptin and Dapagliflozin were received as a gift sample from Vaibhav Analytical Services. Acetonitrile of HPLC grade, Methanol HPLC grade, Water of HPLC grade. The

water for RP-HPLC was prepared by triple glass distillation and filtered through a nylon 0.45  $\mu\text{m}$  – 47 mm membrane filter (Gelman Laboratory, Mumbai, India).

### **Chromatographic Conditions**

Phenomenex C18 column (250 mm x 4.6 mm i.d., 5  $\mu\text{m}$  particle size) was used at ambient temperature. The mobile phase was consist of 0.05 M Potassium dihydrogen phosphate (pH adjusted to 4.5  $\pm$ 0.1 using orthophosphoric acid): Methanol (35: 65) at a flow rate of 1.0 ml/min. The mobile phase was filtered through a nylon 0.45  $\mu\text{m}$ -47 mm membrane filter and degassed before use. The elution was monitored at 227 nm, and the injection volume was 20  $\mu\text{l}$ .

### **Preparation of stock solution (Saxagliptin 50 $\mu\text{g/ml}$ and Dapagliflozin 100 $\mu\text{g/ml}$ )**

An accurately weighed quantity of standard Saxagliptin (5 mg) and Dapagliflozin (10 mg) Were transferred to 100 ml volumetric flasks and volumes were made up to mark with mobile phase to mark with mobile phase to get 50  $\mu\text{g/ml}$  of Saxagliptin and 100  $\mu\text{g/ml}$  of Dapagliflozin.

### **Mobile phase**

0.05 M Potassium dihydrogen phosphate (pH adjusted to 4.5 $\pm$  0.1 using diluted orthophosphoric acid), Methanol (45 : 65) was used, sonicated and filtered (Milipore) through 0.45  $\mu\text{m}$  filter.

### **SAXA and DAPA Standard Stock Solutions**

For HPLC analysis 5 mg of SAXA and 10 mg DAPA powder was weighed accurately using sartorius precision balance (readability 0.01 mg) and transferred in to 100 ml volumetric flask, dissolved and diluted to 100 ml with mobile phase to produce stock solution containing 50  $\mu\text{g/ml}$  of SAXA and 100  $\mu\text{g/ml}$  DAPA respectively.

### **Sample Solution**

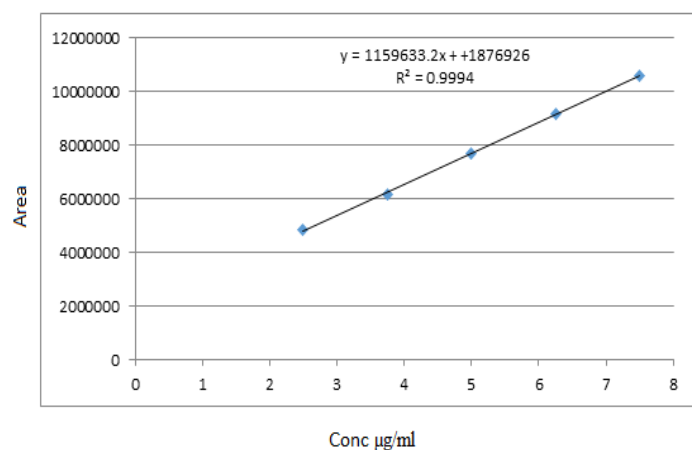
An accurately weighed powder of synthetic mixture equivalent to 5 mg Saxagliptin and 10 mg Dapagliflozin was dissolved in 50 ml diluent (mobile phase). Sonicate solution for 15 min. Filter with whatman filter paper and transferred in 100 ml volumetric flask and volume was made up to mark with a mobile phase.

### Determination of wavelength of maximum absorbance

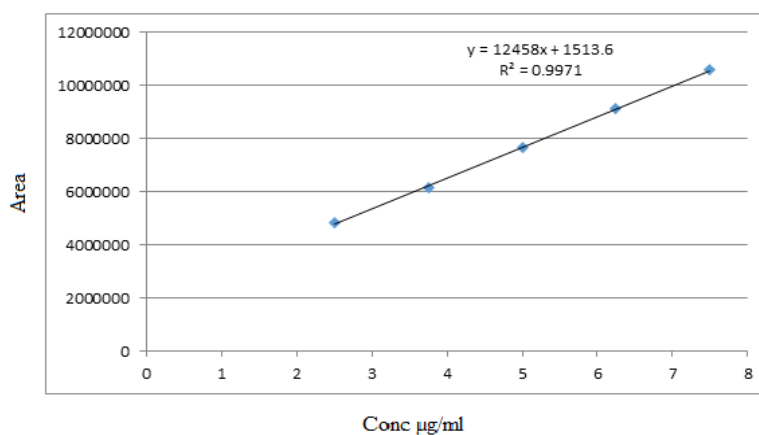
5 µg/ml solutions of Saxagliptin and Dapagliflozin were separately prepared in mobile phase. Each solution was scanned between 200-400 nm in double beam UV-visible spectrophotometer (Shimadzu, model 1800). Wavelength was selected from the overlay spectra of SAXA and DAPA. Both the components show reasonably good response at 227nm.

### Calibration curve (Linearity)

0.5, 0.75, 1, 1.25, 1.5, ml of Saxagliptin (SAXA) and Dapagliflozin (DAPA) was transferred to 10 ml volumetric flasks from both 50 µg/ml of Saxagliptin and 100µg/ml of Dapagliflozin stock solution and volume was made up to mark with mobile phase to get final concentration of Saxagliptin (2.5,3.75,5,6.25,7.5 µg/ml) and Dapagliflozin (5,7.5,10,12.5,15µg/ml). Plot the graph for area Vs time to get calibration curve.



**Linearity graph of saxagliptin.**



**Dapagliflozin linearity Graph.**

**Accuracy (% Recovery)**

Accuracy of the methods was assured by use of the standard addition technique, involving analysis of formulation samples to which certain amounts of authentic drugs were added. The resulting mixtures were assayed, and the results obtained for both drugs were compared to those expected. The recovery experiments were carried out in triplicate by spiking previously analyzed samples (SAXA 2.5 µg/ml and DAPA 5 µg/ml) with three different concentrations of standards (SAXA 1.25, 5, 3.75 µg/ml and DAPA 2.5, 5, 7.5 µg/ml). The good recoveries with the standard addition method prove the good accuracy of the proposed methods. (Table-3 & Table 4).

**Method Precision**

The precision of the method was demonstrated by inter - day and intra- day variation studies. In the intra-day studies, 3 repeated injections of standard solution were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies, 3 injection of standard solution were made for 3 consecutive days and response of drugs peaks and percentage RSD were calculated. From the data obtained, the developed RP-HPLC method was found to be precise. (Table 5 & 6).

**Limit of Detection and Limit of Quantification**

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations as per International Conference on Harmonization (ICH) guidelines.

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where  $\sigma$  = the standard deviation of the response and S = Slope of calibration curve.

**Robustness**

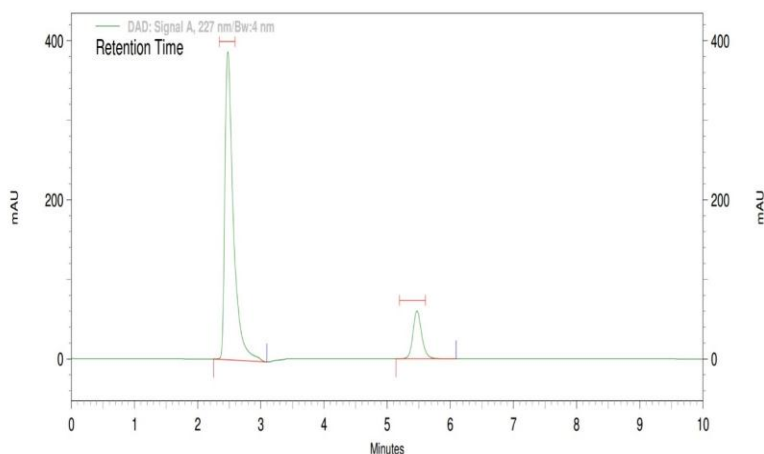
Robustness was carried by varying three parameters from the optimized chromatographic conditions. No significant change was observed as per table 6.

**Analysis SAXA and DAPA in combined Dosage forms**

Pharmaceutical formulation of SAXA and DAPA. The responses of formulations were measured at 227 nm for quantification of SAXA and DAPA by using RP-HPLC present in sample solution were determined by the response in to the regression equation for SAXA and DAPA both in the method and INDA by using RP-HPLC. Results are given in Table 2.

## RESULT AND DISCUSSION

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of 0.05M Potassium dihydrogen phosphate (pH 4.5  $\pm$  0.1 using Orthophosphoric acid):Methanol (35:65), and 1.0 ml/min flow rate proved to be better than the other mixtures in terms of resolution and peak shape. The optimum wavelength for detection was set at 227 nm at which much better detector responses for both drugs were obtained. As it was shown in Fig. 3 the retention times were 2.68 min for SAXA and 5.8 min for DAPA. The calibration graphs for DAPA and SAXA were constructed by plotting the peak area versus their corresponding concentrations, good linearity for both was found over the range Saxagliptin range 2.5-7.5  $\mu$ g/ml and Dapagliflozin range 5-15  $\mu$ g/ml. Results obtained by applying the RP-HPLC method showed that the concentrations of DAPA and SAXA can be simultaneously determined in prepared mixtures. The proposed method has been applied to the assay of SAXA and DAPA in Physical mixture. The validity of the method was further assessed by applying the standard addition technique. The results obtained indicate the additives. Present do not interfere with analysis of the studied mixtures. System suitability test parameters for SAXA and DAPA for the RP-HPLC method are reported in Table 1. The optical and regression characteristics and validation parameters are reported in Table 2.



**Figure 3.** A typical RP-HPLC chromatogram of SAXA (5  $\mu$ g/ml) and DAPA (10  $\mu$ g/ml) with corresponding retention time.

**Table 1: Statistical analysis of parameters required for system suitability testing of the HPLC method.**

System suitability parameter	SAXA	DAPA
Retention Time	2.68	5.8
Tailing Factor	1.1	1.10
Resolution	12.38	
Theoretical plate	1660	1562

**Table 2: Optical and Regression characteristics and validation Parameters of HPLC Method for SAXA and DAPA both Drug.**

Parameter	Saxagliptin	Dapagliflozin
Calibration Range( µg/ml)	2.5 -7.5	10-15
Regression Equation	$y = 1159633.2x + 1876926$	$y = 12593.1x + 1513.6$
Slope (m)	1159633.2	12593.1
Intercept (c)	1876926	1513.6
Correlation co-efficient (r)	0.999	0.997
Inter Day (%RSD, n=3)	0.11- 0.47	0.14 -0.29
Intra Day (%RSD, n=3)	0.48 -0.70	0.69 -0.96
Detection Limit(µg/ml)	0.10	0.33
Quantitation limit(µg/ml)	0.30	10.21

**Table 3: Data of recovery study for SAXA by HPLC method.**

Conc level (%)	Sample amount (µg/ml)	Amount added (µg/ml)	Amount Recoverd (µg/ml)	% Recovery	% Mean recovery ± SD (n=3)
50	2.5	1.25	1.230	98.47	99.24 ± 0.741
	2.5	1.25	1.241	99.30	
	2.5	1.25	1.249	99.95	
100	2.5	2.5	2.56	102.49	102.56 ± 0.234
	2.5	2.5	2.57	102.83	
	2.5	2.5	2.55	102.38	
150	2.5	3.75	3.71	99.09	98.72 ± 0.466
	2.5	3.75	3.70	98.89	
	2.5	3.75	3.68	98.20	

**Table 4: Data of recovery study for DAPA by HPLC method.**

Conc level (%)	Sample amount (µg/ml)	Amount added (µg/ml)	Amount Recoverd (µg/ml)	% Recovery	% Mean recovery ± SD (n=3)
50	5	2.5	2.49	99.99	99.82 ± 0.181
	5	2.5	2.49	99.85	
	5	2.5	2.49	99.63	
100	5	5	4.95	99.15	99.15 ± 0.055
	5	5	4.96	99.10	
	5	5	4.90	99.21	
150	5	7.5	7.78	103.78	103.75 ± 0.043
	5	7.5	7.78	103.77	
	.5	7.5	7.77	103.70	

**Table 4: Precision Intra day study of SAXA and DAPA.**

Saxagliptin			Dapagliflozin		
Conc. $\mu\text{g/mL}$	Area Mean $\pm$ SD (n =3)	%RSD	Conc. $\mu\text{g/mL}$	Area Mean $\pm$ SD (n =3)	%RSD
3.75	6129672 $\pm$ 31609.231	0.52	7.5	977416 $\pm$ 7214.652	0.74
5	7647909 $\pm$ 53419.624	0.70	10	1227439 $\pm$ 8515.1526	0.69
6.25	9166305 $\pm$ 44231.520	0.48	12.5	1583521 $\pm$ 15156.448	0.96

**Table 5: Precision study of Interday OF SAXA and DAPA.**

Saxagliptin			Dapagliflozin		
Conc. $\mu\text{g/mL}$	Area Mean $\pm$ SD (n =3)	%RSD	Conc. $\mu\text{g/mL}$	Area Mean $\pm$ SD (n =3)	%RSD
3.75	6145164 $\pm$ 28691.437	0.47	7.5	974299 $\pm$ 2534.077	0.26
5	7648494 $\pm$ 32770.99	0.43	10	1222684 $\pm$ 3598.39	0.29
6.25	9187634 $\pm$ 10211.6165	0.11	12.5	1588253 $\pm$ 2166.8563	0.14

**Table 6: Robustness.**

Parameters		Area (n = 3)	
		Saxagliptin	Dapagliflozin
pH ( $\pm 0.1$ )	3.3	7870090	1214520
	3.5	7655611	1227851
	3.7	7871252	1214782
	Mean $\pm$ SD	<b>7798597 <math>\pm</math> 123829.50</b>	<b>1219051 <math>\pm</math> 7622.14</b>
	RSD	<b>1.59</b>	<b>0.63</b>
Flow rate ( $\pm 0.02\text{ml/min}$ )	0.98	7670210	1224312
	1	7655611	1227851
	1.02	7676161	1219245
	Mean $\pm$ SD	<b>7667327 <math>\pm</math> 10573.92</b>	<b>1223803 <math>\pm</math> 4325.54</b>
	RSD	<b>0.14</b>	<b>0.35</b>
Mobile phase Methanol: Buffer $\text{KH}_2\text{PO}_4$ ( $\pm 2\text{ mL}$ )	63:37	7671312	1225632
	65:35	7655611	1227851
	67:33	7670110	1214214
	Mean $\pm$ SD	<b>7665678 <math>\pm</math> 8738.68</b>	<b>1222566 <math>\pm</math> 7317.35</b>
	RSD	<b>0.11</b>	<b>0.60</b>



**Table 7: Application of the proposed method to the Physical mixture.**

Saxagliptin			Dapagliflozin		
Labelled Amount (mg)	Amount Found (mg)	% Assay	Amount Found (mg)	Amount Found (mg)	% Assay
	5.02	100.4		10.01	100.1
5	5.05	101	10	10.03	100.3
	5.03	100.6		10.04	100.4
Mean $\pm$ SD	5 $\pm$ 0.015	101 $\pm$ 0.3055	Mean $\pm$ SD	10 $\pm$ 0.015	100 $\pm$ 0.1528
% RSD	0.30	0.30	% RSD	0.15	0.15

**CONCLUSION**

The RP-HPLC method was found simply, Sensitive, accurate and precise. This method can be applied to synthetic mixture for simultaneous estimation of Saxagliptin and dapagliflozin.

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