

# RP- HPLC Method for Simultaneous Estimation of Dapagliflozin and Saxagliptin in Bulk Samples

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## Abstract

A simple, rapid reversed-phase high performance liquid chromatographic method had been developed and validated for estimation of dapagliflozin and saxagliptin in bulk samples. The estimation was carried out on Phenomenex Luna C18 (25 cm x 4.60 mm, particle size 5 µm) column with a mixture of 10mM phosphate buffer (pH 6.8): Acetonitrile; 40: 60 (v/v) as mobile phase. UV detection was performed at 260 nm. The method was validated for linearity, accuracy, precision, specificity and sensitivity as per ICH norms. The developed and validated method was successfully used for the quantitative analysis of commercially available dosage form. The retention time was 2.95 and 3.53 min for meropenem and vaborbactam respectively and total run time was 10 min. at a flow rate of 1.0 mL/min. The calibration curve was linear over the concentration range of 40.00 - 240.00 µg/ mL for meropenem and 60.0 - 360.00 µg/mL for vaborbactam. The LOD and LOQ values were found to be 1.54 and 4.54 µg/ mL for meropenem and 4.60 and 13.65 µg/ mL for vaborbactam respectively. The low percentage coefficient of variance confirm the suitability of the method for the simultaneous estimation of Dapagliflozin and Saxagliptin in bulk samples.

**Keywords:** Dapagliflozin, Saxagliptin, RP-HPLC, Validation

## INTRODUCTION

Chemically Dapagliflozin is (2S,3R,4R,5S,6R)-2-[4-chloro-3-(4-ethoxybenzyl)phenyl]-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol as shown in figure 1. Dapagliflozin inhibits subtype 2 of the sodium-glucose transport proteins (SGLT2), which is responsible for at least 90% of the glucose reabsorption in the kidney. Use of Dapagliflozin leads to blood glucose to be eliminated through the urine, which can lead to weight loss and tiredness.<sup>1,2</sup> Dapagliflozin was approved by FDA on 8<sup>th</sup> January 2014.

While Saxagliptin HCl is (1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl)acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile as shown in figure 2. Saxagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor antidiabetic for the treatment of type 2 diabetes. DPP-4 inhibitors are a class of compounds that work by affecting the action of natural hormones DPP-4 inhibitors are a class of compounds that work by affecting the action of natural hormones.<sup>3,4</sup> Saxagliptin HCl was approved by FDA on 31<sup>st</sup> July 2009.

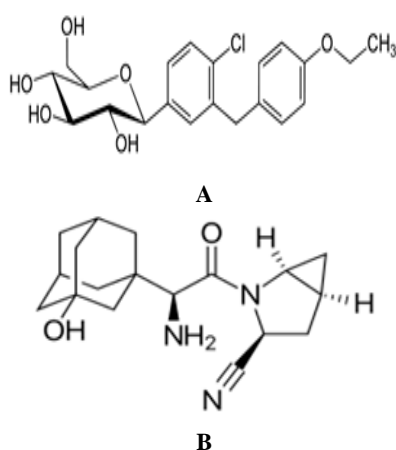


Fig.1.0: Chemical structures of A) Dapagliflozin and B) Saxagliptin HCl

Both the drugs either alone or in combination therapy are used to treat type 2 Diabetes mellitus. Literature survey revealed estimation of Dapagliflozin and Saxagliptin HCl by HPLC method either in alone or in combination with other drugs.<sup>5-10</sup> Literature survey revealed that there were no any official or

reported methods available for the estimation of both the drugs in combination.

In this present study, an attempt was made to develop simple, rapid, reliable, accurate and economical RP-HPLC method for estimation of Dapagliflozin and Saxagliptin HCl in bulk samples combined tablet dosage formulation with better sensitivity, precision, and accuracy by using BDS column.

## EXPERIMENTAL

### Apparatus

RP-HPLC was performed with an Agilent chromatographic system equipped with 1200 series isocratic pump UV-visible and a Rheodyne universal loop injector of injection capacity 50 µL. The monitoring software was Ezichrome Elite. The equipment was controlled by a PC workstation. Compounds were separated on a 25 cm x 4.6 mm i.d, 5-µm particle, Phenomenex-Luna C18 column under reversed-phase partition chromatographic conditions. The flow rate was 1.0 mL/min and injection volume was 20 µL, analyte were monitored at 260 nm and run time was 7 min.

### Chemicals and reagents

Working Standards of pharmaceutical grade Dapagliflozin (DAP) and Saxagliptin HCl (SAX) were obtained as gift samples from Micro labs, Bangalore. All the chemicals and reagents used were of HPLC grade and purchased from Merck, Mumbai, India.

### Preparation of standard stock solution

Standard stock solution of Dapagliflozin (DAP) and Saxagliptin HCl (SAX) pure drugs prepared by accurately weighing about 100 mg drugs and transferring in to 100 mL volumetric flask and dissolved in acetonitrile.

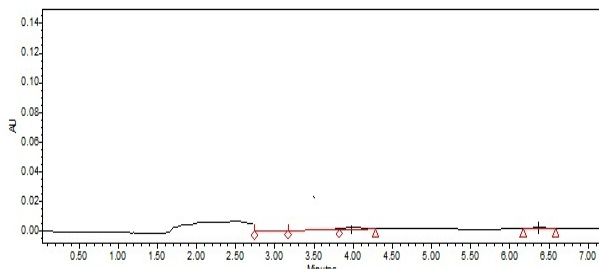
## METHOD VALIDATION

The proposed method was validated as per ICH guidelines. The parameters studied for validation were system suitability, specificity, linearity, precision, ruggedness, robustness, limit of detection and limit of quantification, filter validation and solution stability<sup>[10]</sup>.

### Selectivity and Specificity

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix. If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective. It has been observed

that there were no peaks of diluents and placebo at main peaks. Hence, the chromatographic system used for the estimation of Dapagliflozin (DAP) and Saxagliptin HCl (SAX) was very selective and specific. Specificity studies indicating that the excipients did not interfere with the analysis. The standard solution shown symmetric peak with retention times of 2.86 min for Dapagliflozin (DAP) and Saxagliptin HCl (SAX). The results were depicted in Fig.2.0.

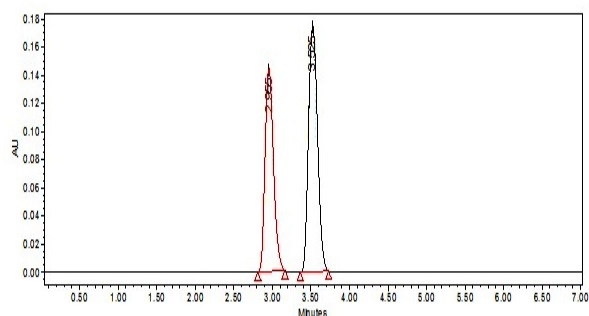


**Fig.2.0. Chromatogram of blank sample**  
System suitability

Standard solution was prepared as per the proposed method and injected into the HPLC system in six replicates and the results were depicted in Table. 1.0 and Fig.3.0.

**Table 1.0: System suitability results of DAP and SAX**

<b>Retention Time</b>	Dapagliflozin	2.95 min
	Saxagliptin HCl	3.53 min
<b>Peak Area</b>	Dapagliflozin	111223
	Saxagliptin HCl	83213
<b>Theoretical plates</b>	Dapagliflozin	8569
	Saxagliptin HCl	9577
<b>Tailing Factor</b>	Dapagliflozin	0.07
	Saxagliptin HCl	0.32
<b>Resolution</b>	Dapagliflozin	-
	Saxagliptin HCl	5.61



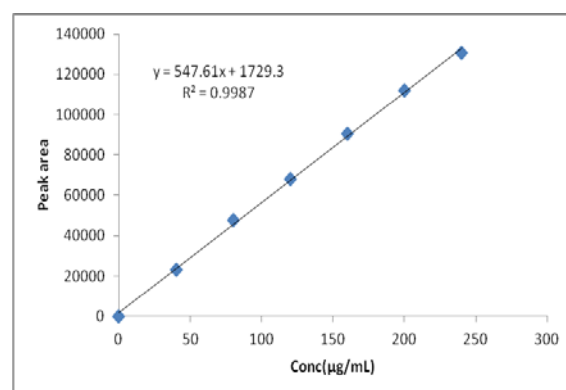
**Fig.3.0. Chromatogram of Standard sample**

**Linearity & Range**

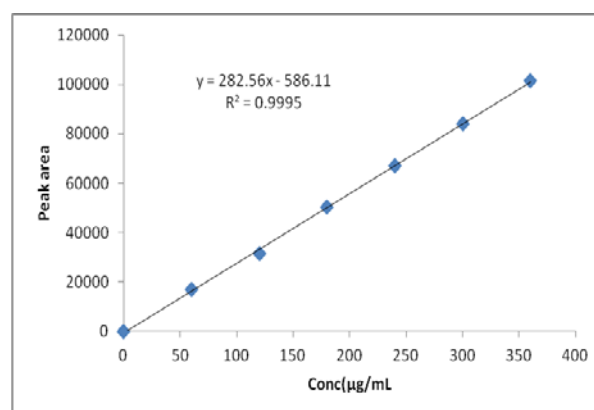
A series of standard concentrations were prepared from 50 % to 150 % of the target concentration of Dapagliflozin (DAP) and Saxagliptin HCl (SAX). Linearity was assessed by performing single measurement at several analyte concentration varying quantities of stock standard solution diluted with the mobile phase to get final concentrations of 40, 80, 120, 160, 200, 240 µg/mL of DAP and 60, 120, 180, 240, 300, 360 µg/mL of SAX. Injection was made at intervals of 10.0 min. Linearity of DAP was found to be exist between 40-240 µg/mL and for SAX was 60 - 360 µg/mL. The chromatograms were recorded and linearity graph was plotted by using peak area of drug against respective concentrations to obtain the linearity range. The results were depicted in Table.2.0 and Fig.4.0 to 5.0.

**Table.2.0: Linearity and range of DAP and SAX**

S.No.	Concentration	Area of	Concentration	Area of
1	40	22988	60	16998
2	80	47605	120	31602
3	120	68028	180	50459
4	160	90704	240	67137
5	200	112120	300	84014
6	240	130654	360	101710
Concentration	40-240 µg/mL		60-360 µg/mL	
Slope (m)	547		282	
Correlation	0.9987		0.9995	



**Fig.4.0: Linearity of Dapagliflozin (DAP)**



**Fig.5.0: Linearity of Saxagliptin HCl (SAX)**

**Precision**

The intra-day and inter-day precision studies were carried out using a test sample assay method with six replicates on the same day and different days. The results were depicted in Table. No- 3.0 and 4.0.

**Table. 3.0: Intraday precision data for DAP and SAX**

Sample. No	Area of	Area of
1	110164	84015
2	110145	84037
3	111162	84052
4	111133	84062
5	110152	84034
6	111160	84014
Mean	110653	84036
SD	546.76	19.29
%RSD	0.49	0.02

**Table.4.0: Interday precision data for DAP and SAX**

Sample. No	Area of	Area of
1	110165	85031
2	110138	85137
3	110222	85012
4	110142	85034
5	110163	84511
6	110122	85038
Mean	110159	84961
SD	34.98	224.60
% RSD	0.03	0.26

**Ruggedness**

This is to prove the lack of influence of operational and environmental variables of the test results by using the method. Ruggedness is a measure of reproducibility of test results under the variation in conditions normally expected from system to system and from analyst to analyst. It was carried out by using a test sample assay method with six replicates using different analyst.

**Robustness**

Robustness was performed by change in mobile phase ratio, mobile phase flow rate and wavelength of the detector. The test was carried out by small variation in the chromatographic conditions at a concentration equal to standard concentrations 200 µg/mL for DAP and 300 µg/mL for SAX and % change was calculated. % Change in the results was calculated.

**Limit of detection and Limit of quantification**

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using the following equation as per ICH guidelines.

$$\text{LOD} = 3.3 \times \sigma / S; \text{LOQ} = 10 \times \sigma / S;$$

Where  $\sigma$  is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

**Solution Stability**

Solution stability was assed using standard and test stock solutions. These stocks were prepared and stored at room temperature and refrigerated conditions (2-8°C) for 36 h and % differences were calculated.

**Filter validation**

A study was conducted to determine the effect of filter on the assay, dissolution and impurities. Test solution was prepared as per the test method. Some portion of the above solution was filtered through three different filters namely 0.45µ PVDF filter, 0.45µ PTFE and 0.45µ Nylon filter and some portion was centrifuged and injected into the HPLC system. The % difference values between centrifuged and filtered sample were calculated.

**RESULT AND DISCUSSION**

In this RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate analytes. The mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time and resolution. The system with 10 mM phosphate buffer (pH 6.8): acetonitrile (40: 60) (v/v) at flow rate of 1.0 mL/min was found to be robust method. The developed method was validated as per the ICH guidelines for the quantification of Dapagliflozin (DAP) and Saxagliptin HCl (SAX) in bulk samples.

A suitability test was applied to various system suitability parameters and the results obtained were within acceptable limits of tailing factor  $\leq 2.0$  and theoretical plates  $>2000$ . The

calibration curve was constructed with series of concentration in the range of 40-240 µg/mL and 60-360 µg/mL for Dapagliflozin (DAP) and Saxagliptin HCl (SAX).

The correlation co-efficient of meropenem (MEP) and vaborbactam (VAB) was found to be  $>0.998$ . This concluded that the method was linear throughout the range selected. Specificity was studied for the quantification of impurities in Dapagliflozin (DAP) and Saxagliptin HCl (SAX). From the results it was indicated that none of impurities were interfere at analytes retention time. Hence the developed method was specific.

The precision of the method was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample with in the day (intraday) and next consequent three days for inter day precision. For each cases % RSD was calculated and results were the acceptable limits. The low values of RSD indicate that the method is precise.

Robustness test was carried out by small variation in the chromatographic conditions and % change was calculated. The % change in the results was calculated and it was found robust as % change was below 2.0 %.

A signal-to-noise ratio 2:1 is generally considered acceptable for estimating the detection limit. LOD is found to be 5.888 µg/mL for Dapagliflozin (DAP) and 0.225 µg/mL for Saxagliptin HCl (SAX) and LOQ is found to be 17.841 µg/mL for Dapagliflozin (DAP) and 0.683 µg/mL for Saxagliptin HCl (SAX).

Sample and standard solution are stable at 5°C for 36 h as the % difference in the area was found to be less than 2.0 %. Filter interference was done on three types of 0.45 µ filters (Nylon, PVDF, PTFE) and the % difference was found to be below 2.0 % for sample solutions and standard solutions calculated against centrifuged samples and standard.

**CONCLUSION**

A new, reversed-phase HPLC method has been developed for simultaneous analysis of Dapagliflozin (DAP) and Saxagliptin HCl (SAX) in bulk samples. It was shown that, the method was linear, accurate, reproducible, repeatable, precise, selective and specific proving the reliability of the method. The run time is relatively short (7 min), which enables rapid determination of many samples in routine and quality control analysis.

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