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Research Article

## Development and Validation of UV Spectroscopic Method for Simultaneous Estimation of Dapagliflozin and Saxagliptin in marketed formulation

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### ABSTRACT

Simple, precise and accurate UV-Spectrophotometric simultaneous equation method for estimation of dapagliflozin (DAPA) and saxagliptin (SAXA) were developed and validated as per ICH guidelines. This Method involves solving of simultaneous equations based on measurement of absorbance at two wavelengths 222 nm and 276 nm ( $\lambda_{\max}$  of SAXA and DAPA) in phosphate buffer pH 6.8. Both the drugs obey the Beer's law in the concentration ranges 5-25 $\mu$ g/ml. % Recovery for both the drugs were in the range of 98.44-99.05% indicating excellent accuracy. The methods were precise, with a relative standard deviation of less than 2% for both drugs. The developed methods were validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values. Thus, method can be used for routine monitoring of drugs in industry for the assay of bulk drugs and commercial formulation (QTERN tablets, Astra Zeneca).

**Keywords:** Dapagliflozin, Saxagliptin, Spectrophotometric analysis, Simultaneous equation method.

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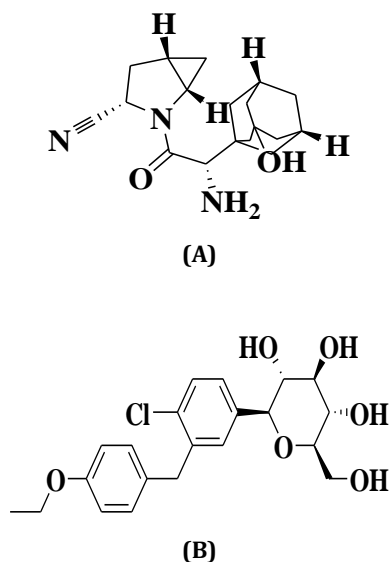
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### INTRODUCTION

Saxagliptin is chemically called as (1S, 3S, 5S)-2-[(2S)-2-Amino-2-(3 hydroxytricyclo [3.3.1.1<sup>3,7</sup>] dec-1-yl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile (Figure 1A). It is the oral hypoglycemic (antidiabetic) agent, class of dipeptidyl peptidase (DPP-4) inhibitor [1]. Saxagliptin was inhibiting the activity of dipeptidyl peptidase-4(DPP-4) enzyme by increasing the insulin production in response to meal and decreasing the gluconeogenesis rate in the liver, in blood glucose regulation is thought to be through degradation of GIP [2] and the degradation of GLP-1 [3]. Saxagliptin was used for the treatment of type-2 diabetics in the form of mono or combination of with other drugs. Dapagliflozin is chemically called as (1S)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-D-glucitol (Figure 1B). It is a highly selective, sodium-Glucose Co-Transporter 2 (SGLT2). Dapagliflozin blocking the activity of the sodium-glucose transport proteins, which is regulates for at least 90% of the glucose reabsorption in the kidney and obstructs the

transporter mechanism causes blood glucose to be removed through the urine. Dapagliflozin is improved the glycaemic control in patients with type 2 Diabetes Mellitus [4]. A survey of literature revealed the availability of number of analytical methods for the quantitative determination of DAPA and SAXA alone or combination with other drugs. The reported methods were mainly based on liquid chromatographic estimation using UV-VIS, fluorescence, electrochemical or mass spectrometry detectors. The available methods are based on spectrophotometry, thin layer chromatography or high performance liquid chromatography [5-23]. However, no UV-Spectrophotometric simultaneous equation method is available for simultaneous determination of the DAPA and SAXA in combined pharmaceutical dosage form. In the present study, an attempt was made to develop a simple, precise and accurate method for the simultaneous estimation of these drugs in combined pharmaceutical dosage form and validate as per International Conference on Harmonization (ICH) guidelines.



**Figure 1** Chemical structure of (A) Saxagliptin and (B) Dapagliflozin

## MATERIALS AND METHODS

### Reagents and chemicals

DAPA and SAXA standard were obtained from scan research laboratories, Bhopal as gift sample. QTERN tablets Purchased from Local market. Methanol, acetonitrile were procured from Rankem, RFCL Limited, New Delhi, India. Potassium dihydrogen phosphate AR and NaOH AR grade were procured from Merck. Milli Q water was used throughout the study. Other chemicals used were of analytical grade.

### Instrument

In UV-spectrophotometric method, Labindia model-3000+ series was used, which is a wavelength accuracy  $\pm 1$  nm, with 1cm quartz cells.

### Method development

#### Standard stock solution (Stock-A)

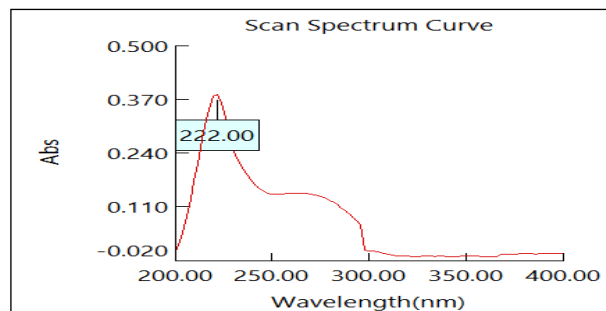
Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 80ml phosphate buffer pH 6.8 in 100 ml volumetric flask. The flask was sonicated for about 10 min to solubilizing the drug and the volume was made up to the mark 100ml with phosphate buffer pH 6.8 to get a concentration of 1000  $\mu\text{g/ml}$  (Stock-A) for both drugs.

#### Sub Stock Solution (Stock-B)

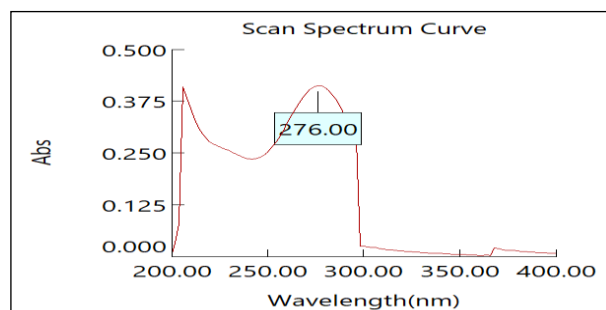
Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of SAXA and DAPA and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with phosphate buffer pH 6.8 that gave concentration of 100  $\mu\text{g/ml}$  (Stock-B).

#### Determination of $\lambda_{\text{max}}$

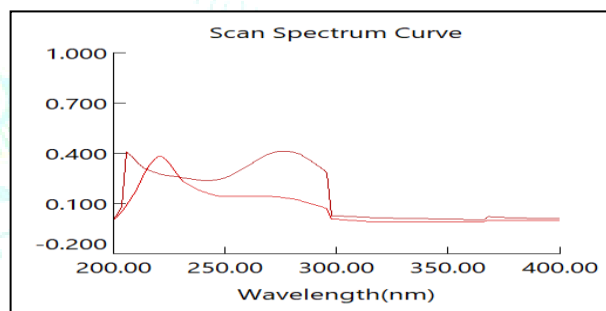
10  $\mu\text{g/ml}$  standard solutions of both SAXA and DAPA were prepared from respective sub-stock solutions. Both the solutions were scanned in the wavelength region of 200-400 nm and the  $\lambda_{\text{max}}$  was found to be 222 nm and 276 nm for SAXA and DAPA respectively. They were scanned in the wavelength range of 200-400 nm and the overlain spectrum was obtained (Figure 2-4).



**Figure 2** Determination of  $\lambda_{\text{max}}$  of SAXA



**Figure 3** Determination of  $\lambda_{\text{max}}$  of DAPA



**Figure 4** overlay spectra of SAXA and DAPA

#### Preparation of calibration curve

From the standard stock solution of each drug, appropriate aliquots were pipette out into a series of 10 ml volumetric flasks. The volume was made up to the mark with phosphate buffer pH 6.8 to get a set of solutions having a concentration range of 5-25 $\mu\text{g/ml}$  for both drugs. Triplicate dilutions of each drug solutions were prepared separately. The prepared working solutions of SAXA and DAPA were scanned 222 nm and 276 nm, respectively. The absorbance's were recorded and were plotted against the concentrations to obtain their respective calibration curves.

#### Simultaneous equation method (Vierordt's)

Working standard solution from the standard stock solution prepared in concentration 40 $\mu\text{g/ml}$  of SAXA and 2 $\mu\text{g/ml}$  of DAPA were scanned in the spectrum mode over the range of 200-400 nm against phosphate buffer pH 6.8 as blank and the overlain spectra of the two were recorded. SAXA showed an absorbance peak at 222.0 nm, whereas DAPA at 276.0 nm. The overlain spectra also showed isoabsorptive points at 235.0 nm. Due to difference in absorbance maxima and having no interference with each other so both drug can be simultaneously estimated by simultaneous equation method. Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two wavelengths selected for the method are 222.0 nm and 276.0 nm that are  $\lambda_{\text{max}}$  of SAXA and DAPA respectively. The absorbances were measured at the selected wavelengths and

absorptivities ( $A_{1\%}^{1\text{cm}}$ ) for both the drugs at both wavelengths were determined as mean of five independent determinations. Concentrations in the sample were obtained by using following equations

$$C_{\text{saxa}} = \frac{A_{1\text{ay}_2} - A_{2\text{ay}_2}}{ax_{1\text{ay}_2} - ax_{2\text{ay}_1}} \dots\dots\dots \text{Eq (1)}$$

$$C_{\text{dapa}} = \frac{A_{1\text{ax}_2} - A_{2\text{ax}_1}}{ax_{1\text{ay}_2} - ax_{2\text{ay}_1}} \dots\dots\dots \text{Eq (2)}$$

Where,  $A_1$  and  $A_2$  are absorbances of mixture at 222.0 nm and 276.0 nm respectively,  $ax_1$  and  $ax_2$  are absorptivities of SAXA at  $\lambda_1$  (222.0 i.e.  $\lambda_{\text{max}}$  of SAXA) and  $\lambda_2$  (276.0 i.e.  $\lambda_{\text{max}}$  of DAPA) respectively and  $ay_1$  and  $ay_2$  are absorptivities of DAPA at  $\lambda_1$  and  $\lambda_2$  respectively.  $C_{\text{DAPA}}$  and  $C_{\text{SAXA}}$  are concentrations of SAXA and DAPA respectively. Figure 4 represent the overlain spectra of both the drugs and the criteria for obtaining maximum precision [i.e. absorbance ratio ( $A_2/A_1$ )/ $ax_2/ax_1$  and  $ay_2/ay_1$ ] by this method were calculated and found to be outside the range of 0.1-2.0 which is satisfied for both the SAXA and DAPA [24].

#### Methods validation

Validation of the method was carried out in accordance with the International Conference on Harmonization Q2B guidelines 2005 [25].

#### Linearity

The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and estimate into the UV and the results was recorded. The results of linearity are reported in Table 1 and figure 5 & 6.

#### Accuracy

The validity and reliability of proposed methods were assessed by recovery studies by using standard addition method. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. Result of recovery study shown in Table 2.

#### Precision

Precision was determined by repeatability and Intermediate precision (Day to day precision, Analyst-to-Analyst) of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less than 2 indicate the precision of method. Result of precision shown in Table 3.

#### Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve Table 4.

#### Assay of Tablet Formulation

Mixed Blends of SAXA and DAPA were weighed and ground to a fine powder; amount equal to 10mg of DAPA was taken in 10 ml volumetric flask. The present in this amount of marketed tablets (QTERN tablet, Astra Zeneca) was 5mg

SAXA. Then 5ml of phosphate buffer pH 6.8 was added and the flask was sonicated for about 10 min to solubilizing the drug present in tablet formulation and the volume was made up to the mark with Buffer. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with buffer to get the final concentrations of both drugs in the working range. The absorbance of final dilutions was observed at selected wavelengths and the concentrations were obtained from simultaneous equation method. The procedure was repeated for five times Table 5.

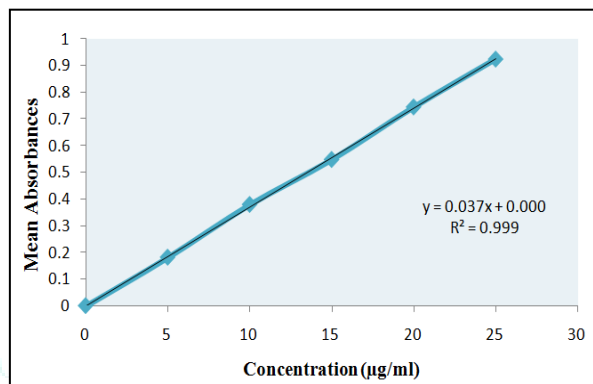


Figure 5: Calibration Curve of SAXA

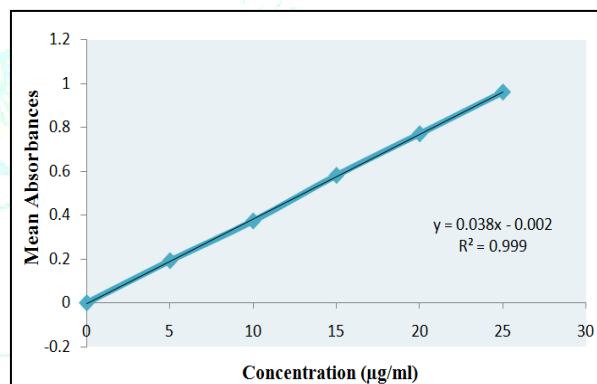


Figure 6: Calibration Curve of DAPA

## RESULTS AND DISCUSSION

Method development by UV-Spectrophotometer is cost effective and time saving as compared to HPLC method of analysis [26]. Thus, for estimation of routine sample of drugs simple, rapid, sensitive and accurate analytical UV methods were utilized which reduces unnecessary tedious sample preparations and use of costly materials. To develop suitable methods of analysis, various solvents were studied. Based on sensitivity of the method and non-toxic behaviour phosphate buffer pH 6.8 was selected as a solvent for the methods. Overlain spectra (Figure 4) shows that at  $\lambda_{\text{max}}$  of SAXA (222 nm) interference of DAPA and at  $\lambda_{\text{max}}$  of DAPA (276nm) interference of SAXA occurs which suggested development of simultaneous equation method. The optimized methods showed good reproducibility and mean recovery with  $98.956 \pm 0.114$  (SAXA),  $99.090 \pm 0.141$  (DAPA) and  $98.870 \pm 0.951$  (SAXA),  $99.059 \pm 0.358$  (DAPA) respectively. The standard deviation, coefficient of variance and standard error were obtained for SAXA and DAPA were satisfactorily low. Result of precision at different levels was found to be within acceptable limits (RSD < 2). Thus, the method provides a simple, convenient, rapid and accurate way to determine SAXA and DAPA simultaneously.

## CONCLUSION

A new, simple, sensitive and economical UV spectrophotometric method was developed for the simultaneous estimation of SAXA and DAPA in their tablet formulation. Validation of developed methods was performed according to ICH guidelines. The standard deviation, % RSD for the methods are low, reflecting a high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. Vierordt's method has the advantage of being simple, economic, rapid and subsequently not required sophisticated technique, instrument and costly solvents. Thus, the proposed methods can be successfully applied for determination and dissolution testing of SAXA and DAPA in commercial formulation (Qtern).

**Table 1 Results of Linearity of Saxagliptin and Dapagliflozin**

PARAMETER	Saxagliptin	Dapagliflozin
Concentration ( $\mu\text{g/ml}$ )	5-25	5-25
Correlation Coefficient ( $r^2$ )*	0.999	0.999
Slope (m)*	0.037	0.038
Intercept (c)*	0.000	-0.002

\*value of three replicate

**Table 2 Results of Recovery Study**

% LEVEL	% MEAN $\pm$ SD*	
	Saxagliptin	Dapagliflozin
80%	98.736 $\pm$ 1.076	98.549 $\pm$ 1.875
100%	98.870 $\pm$ 0.951	99.059 $\pm$ 0.358
120%	98.449 $\pm$ 0.211	99.000 $\pm$ 0.465

\* Value of three replicate and five concentrations.

**Table 3 Results of Precision**

PARAMETER	% MEAN $\pm$ SD*	
	Saxagliptin	Dapagliflozin
Repeatability	99.086 $\pm$ 0.084	98.860 $\pm$ 0.064
Intermediate precision		
Day to day precision	99.071 $\pm$ 0.070	99.070 $\pm$ 0.107
Analyst-to-Analyst	98.816 $\pm$ 0.107	99.345 $\pm$ 0.087
Reproducibility	98.956 $\pm$ 0.114	99.090 $\pm$ 0.141

\* Value of five replicate and five concentrations

**Table 4 LOD and LOQ OF SAXA and DAPA**

Name	LOD ( $\mu\text{g/ml}$ )	LOQ ( $\mu\text{g/ml}$ )
Saxagliptin	1.23	3.25
Dapagliflozin	0.95	2.72

**Table 5 Assay of Tablet Formulation**

	% Conc. Found	
	SAXA	DAPA
Mean	98.092	93.070
S.D.	1.708	10.573
% RSD	1.742	11.360

\*Average of three replicate

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