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Development and validation of UV spectroscopic method for simultaneou estimation of dapagliflozin and saxagliptin in synthetic mixture

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ABSTRACT

Aim

Simple, precise and accurate UV-Spectrophotometric Simultaneous Equation method for estimation of Dapagliflozin and Saxagliptin were developed and validated as per ICH guidelines.

Experimental and Results

The objective of the work is to develop UV spectroscopic method for simultaneous estimation of Dapagliflozin (DAPA) and Saxagliptin (SAXA). This Method involve solving of simultaneous equations based on measurement of absorbance at two wavelengths 223 nm and 212 nm. Both the drugs obey the Beer's law in the concentration ranges 4-24 µg/mL and 5-50 µg/mL respectively. Results of the methods were validated statistically. Novel, simple, sensitive, rapid, accurate and economical Spectrophotometric methods have been developed for simultaneous estimation of Dapagliflozin and Saxagliptin .The method can be used to estimate the amount of Dapagliflozin and Saxagliptin in mixture containing Dapagliflozin and Saxagliptin.

Conclusion

The results of estimation and validation parameters like accuracy, precision, ruggedness, linearity and range were studied for all the developed methods and were found to be within limits. The proposed method can be adopted for routine quality control for estimation of drug in formulation.

Keywords: Dapagliflozin, Saxagliptin, Antidiabetic, Spectrophotometric analysis, Simultaneous equation method

INTRODUCTION

Dapagliflozin is a highly selective, orally active and reversible inhibitor of the human Sodium-Glucose Co-Transporter 2 (SGLT2), the major transporter responsible for the renal glucose reabsorption. It improves glyceamic control in patients with Type 2 Diabetes Mellitus by inhibiting the Sodium-Glucose Co-Transporter 2, intern by reducing glucose reabsorption. [1]

Dapagliflozin is supplied as a crystalline solid. Dapagliflozin is inhibiting renal glucose reabsorption through the solid glucose cotranspoter (SGLT) offers an insulin-independent alternative to controlling blood glucose concentrations in patients with type 2 diabetes. Dapagliflozin is a first generation, selective SGLT inhibitor that blocks glucose transport with about 100-fold selective for SGLT2 over SGLT1. [2]

Chemically, it is as shown in Fig. 1, (2*S*,3*R*,4*R*,5*S*,6*R*)-2-[4-chloro-3-(4ethoxybenzyl) phenyl]-6-(hydroxymethyl) tetrahydro-2*H*-pyran-3,4,5triol.It has a molecular formula of $\mathbf{C_{21}H_{25}ClO_6}$ and molecular weight of 408.873 g/mol. [3]

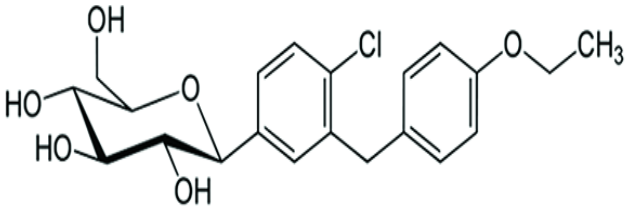

Fig. 1: Chemical structure of Dapagliflozin

Saxagliptin hydrochloride is a new oral hypoglycemic (antidiabetic drug) of the dipeptidyl peptidase-4 inhibitor class of drugs. Saxagliptin is part of a class of diabetes medications called dipeptidyl peptidase-4 (DPP-4) inhibitors. DPP-4 is an enzyme that breaks down incretin hormones. As a DPP-4 inhibitor, Saxagliptin slows down the breakdown of incretin hormones, increasing the level of these hormones in the body. It is this increase in incretin hormones that is responsible for the beneficial actions of Saxagliptin, including increasing insulin production in response to meals and decreasing the amount of glucose that the liver produces. Because in cretin hormones are more active in response to higher blood sugar levels, the risk of dangerously low blood sugar (hypoglycemia) is low with Saxagliptin. Saxagliptin is chemically (1S, 3S, 5S)-2-[(2S)-Amino (3-hydroxytricyclo [3.3.1.13,7] dec-1-yl)acetyl]-2-azabicyclo[3.1.0] hexane-3carbonitrilemonohydrochloride with empirical formula is $\mathbf{C_{18}H_{25}N_3O_2.HCl}$ and molecular weight 351.87 [4]

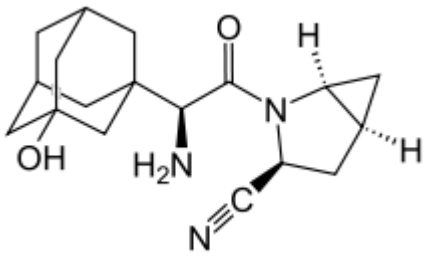

Figure 2: Chemical structures of Saxagliptin.

Saxagliptin monohydrate is a white to light yellow or light brown, non-hygroscopic, crystalline powder. It is sparingly soluble in water at 24°C ± 3°C, slightly soluble in ethyl acetate, and soluble in methanol, ethanol, isopropyl alcohol,acetonitrile, acetone, and polyethylene glycol 400 (PEG 400) [5]

MATERIALS AND METHODS

Materials

Dapagliflozin (purity 99.99%) and Saxagliptin (purity 99.99%) samples were provided by Cipla Pharmaceuticals, Mumbai. respectively. Methanol were procured from Merck, Germany. All chemicals were of analytical grade and were used without further purification. Double distilled water was used in the present study. UV-Vis spectrophotometer (1800, Shimadzu, Japan) with spectral bandwith of 0.1 nm and wavelength accuracy of ± 0.5 nm. [6]

Preparation of Calibration Curve

Preparation of Standard Calibration Curve of Dapagliflozin in Water: Methanol (80:20) Solution

Accurately weighed 100 mg of Dapagliflozin was dissolved in 100 ml of Water: Methanol (80:20) solution (stock solution).Then 4, 8, 12, 16, 20, 24, µg/ml of above solution was transferred in a 100 ml volumetric flask and volume was made up to the mark with Water:Methanol(80:20) solution to make concentrations 4, 8, 12, 16, 20, 24, µg/ml concentration. The absorbance of each of these solutions were measured at the selected wavelengths (i.e. 223 nm and 212 nm) using UV spectrophotometer and plotted against concentration. The concentration range over which the drugs obeyed beer's law was chosen. The range was found to be 4-24 µg/ml at both the wavelength.

Preparation of Standard Calibration Curve of Saxagliptin in Water: Methanol (80:20) Solution

Accurately weighed 100 mg of Saxagliptin was dissolved in 100 ml of Water: Methanol (80:20) Solution (Stock solution).Then 5,10, 20, 30, 40, 50, µg/ ml of above solution was transferred in a 100 ml volumetric flask and volume was made up to the mark with Water: Methanol (80:20) solution to make 5,10, 20, 30, 40, 50, µg/ ml concentration. The absorbance of each of these solutions were measured at the selected wavelengths (i.e., 223 nm and 212 nm) using UV spectrophotometer and plotted against concentration. The concentration range over which the drugs obeyed beer's law was chosen. The range was found to be 5-50 µg/ml at both the wavelength.

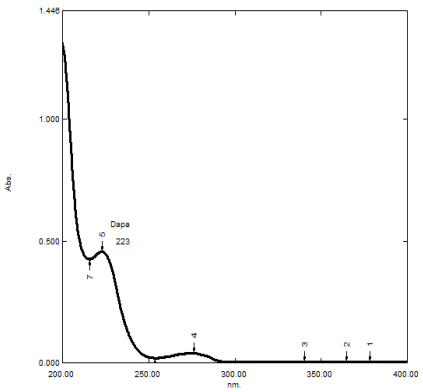

Figure 3: UV spectrum of DAPA

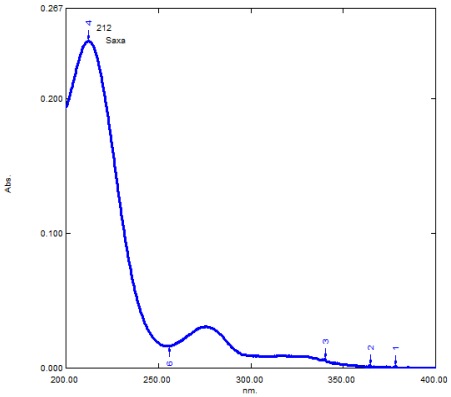

Figure 4: UV spectrum of SAXA

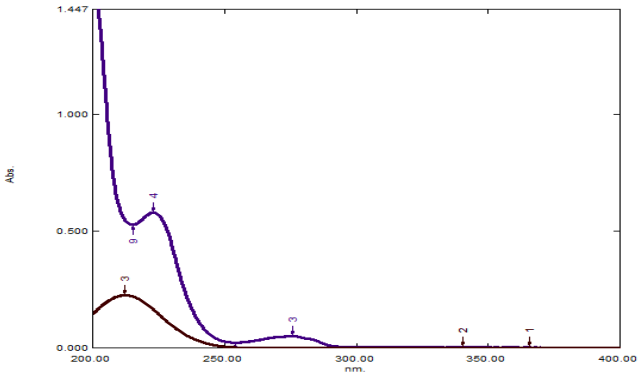

Figure 5: Overlay Spectrum of DAPA and SAXA

Table 1: Standard calibration Table for DAPA and SAXA

Sr. No.	DAPA		SAXA	
	Concentration in µg/ml	Absorbance	Concentration in µg/ml	Absorbance
1	`4	0.165	5	0.099
2	8	0.336	10	0.231
3	12	0.501	20	0.463
4	16	0.668	30	0.633
5	20	0.831	40	0.879
6	24	0.969	50	1.09

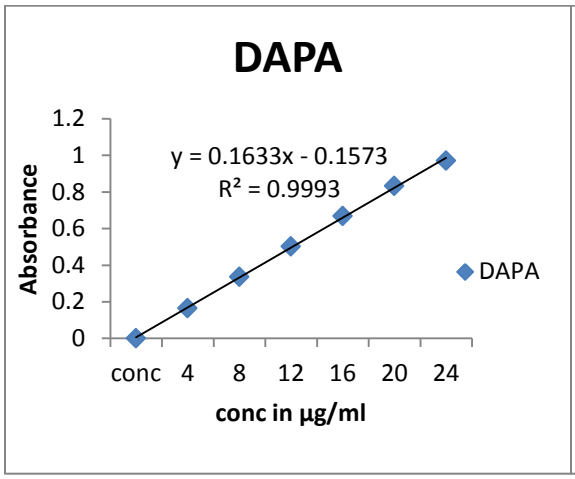

Figure 6: Calibration curve of DAPA

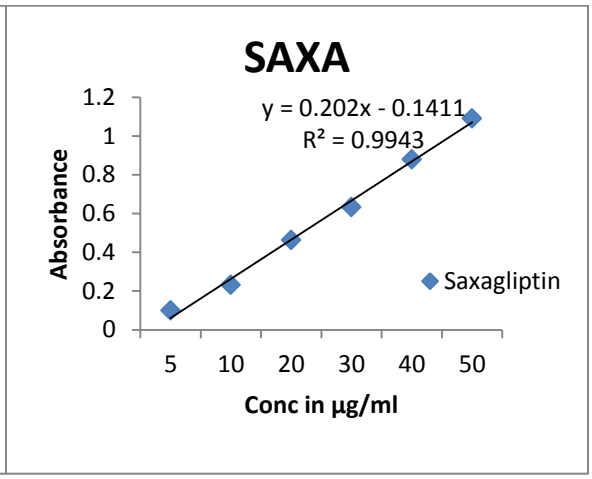

Figure 7: Calibration curve of SAXA

Table 2: Optical characteristics and other Parameters

Parameters	DAPA	SAXA
Detection wavelength	223 nm	212 nm
Linearity range	4-24 µg/ml	5-50 µg/ml
Slope	0.163	0.202
Intercept	0.157	0.141
Correlation coefficient	0.999	0.994
Regression equation (y = mx + c)	Y = 0.163x-0.157	Y = 0.202x - 0.141

Table 3: Absorptivities Values of DAPA at 223 nm and 212 nm

Sr.No.	Absorbance at	
	223 nm	**212 nm**
1	0.432	0.424
2	0.433	0.425
3	0.432	0.425
Mean	**0.432**	**0.425**
SD	**0.0006**	**0.0005**
%RSD	**0.133**	**0.135**

Development of Simultaneous Equation for Dapagliflozin and Saxagliptin

Absorptivity from all the concentration was calculated for both the drugs in methanol Solution and used for the development of simultaneous equation [7, 8].

$$Cx = \frac{A_2 a_{Y1} - A_1 a_{Y2}}{a_{X2} a_{Y1} - a_{X1} a_{Y2}} \dots\dots\dots\dots I$$

$$Cy = \frac{A_1 a_{X2} - A_2 a_{X1}}{a_{X2} a_{Y1} - a_{X1} a_{Y2}} \dots\dots\dots\dots II$$

Where,

Cx and Cy =Concentration of DAPA and SAXA respectively

A1 and A2 = Absorbance at 223 nm and 212 nm

ax1= 432 ax2= 424 ay1= 152 ay2= 182

Thus,

$$C_X = \frac{A_2 X152 - A_1 X182}{-14140}$$

$$C_Y = \frac{A_1 X43 - A_2 X533}{-14140}$$

Table 4: Analysis of DAPA and SAXA in Synthetic Mixture

Sr.No.	Absorbance at		%Estimation	
	223 nm	**212nm**	**DAPA**	**SAXA**
1	0.508	0.515	99.92	99.96
2	0.506	0.513	99.43	99.71
3	0.505	0.513	99.36	99.67
		Mean	**99.57**	**99.78**
		SD	**0.305**	**0.157**
		%RSD	**0.306**	**0.157**

Validation of Proposed method [9, 10]

The Proposed method was validated as per the ICH guidelines. To the pre-analysedsample solutions (10 µg/ml of DAPA and 5 µg/ml of SAXA), a known amount of standard solutions of the pure drugs (DAPA and SAXA) were added i.e. 8, 10, and 12 µg/m1 of DAPA and 4, 5 and6 µg/ml of SAXA (standard stock solution) was added, and total conc. of above dilution is measured by using equation I and II

Validation Parameters

Accuracy

Accuracy of an analytical method is the closeness of the test results obtained by that of the true value. It was ascertained on the basis of recovery studies performed at different levels of concentrations

Table 5: Results of Recovery Studies

Sr.NO.	Amount Added (µg/ml)		Absorbance at		Amount Recovered		% Recovery	
	DAPA	SAXA	223 nm	212 nm	DAPA	SAXA	DAPA	SAXA
1	18	9	0.916	0.929	18.04	8.95	100.5	99.00
2	20	10	1.017	1.032	19.96	10.10	99.70	102.2
3	22	11	1.122	1.138	22.07	10.98	100.66	99.83
						Mean	**100.2**	**100.3**
						SD	**0.514**	**1.66**
						%RSD	**0.512**	**1.65**

Table 6: Results of Accuracy Studies

Sr.No.	Amount Added (µg/ml)		% Recovery	
	DAPA	SAXA	DAPA	SAXA
1	18	09	100.50	99.00
2	20	10	99.70	102.2
3	22	11	100.60	99.83
		Mean	**100.2**	**100.3**
		SD	**0.514**	**1.66**
		% RSD	**0.512**	**1.65**

Precision

Precision of an analytical method is the degree of agreement among individual test results. It is expressed as ± S.D. or % RSD of series of measurements. Precision of the method was verified by using stock solutions in the ratio of 10:5 containing 10 µg/ml DAPA and 5 µg/ml of SAXA. System repeatability was done by repeating the assay three times of three replicate dilutions of the same concentration after every two hours on the same day for intraday precision. Interday precision was carried out by performing the assay of tree sample sets after 24 hours and 48 hours, results are reported in Table 7 & 8

Table 7: Results of Precision Studies (Intra-day)

Sr.No.	Absorbance at		%Estimation*	
	223 nm	212 nm	DAPA	SAXA
Obser.1	0.5076	0.5150	99.78	99.89
Obser.2	0.5080	0.5153	99.85	99.92
Obser.3	0.5080	0.5156	99.49	101.96
		Mean	**99.70**	**100.59**
		SD	**0.190**	**1.186**
		%RSD	**0.190**	**1.179**

* Results are mean of three replicates

Table 8: Results of Precision Studies (Inter-day)

Sr.No.	Absorbance at		%Estimation*	
	223 nm	212 nm	DAPA	SAXA
Day 1	0.5080	0.5153	99.85	99.92
Day 2	0.5076	0.5153	99.42	101.92
Day 3	0.5056	0.5130	99.36	99.67
		Mean	**99.54**	**100.50**
		SD	**0.267**	**1.233**
		%RSD	**0.268**	**1.226**

* Results are mean of three replicates

Ruggedness

Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by two analyst using same operational and environmental conditions, the results are reported in Table 9.

Table 9: Results of Different analyst studies

Sr.No.	Absorbance at		%Estimation*	
	223 nm	212 nm	DAPA	SAXA
Analyst 1	0.5056	0.5133	99.00	101.71
Analyst 2	0.5060	0.5130	99.79	99.67
		Mean	**99.39**	**100.69**
		SD	**0.558**	**1.442**
		%RSD	**0.561**	**1.432**

* Results are mean of three replicates

Robustness

It expresses the precision within laboratories, Variation like different solvent. Robustness of the methods was assessed by carrying out assay 3 times with different solvent by using same equipment. The results of the same are presented in Table 10.

Table10: Results of Different solvent studies

Solvent	Absorbance at		%Estimation*	
	223 nm	212 nm	DAPA	SAXA
Water:Methanol(85:15)	0.5076	0.5150	99.78	99.89
Water:Methanol(80:20)	0.5063	0.5136	99.50	99.74
		Mean	**99.60**	**99.81**
		SD	**0.197**	**0.106**
		%RSD	**0.197**	**0.106**

* Results are mean of three replicates

DISCUSSION

Proposed method for simultaneous estimation of Dapagliflozin and Saxagliptin in combined sample solutions was found to be simple, accurate and reproducible. Table.2 shows data for optical characteristics. Data for validation and precision studies are given in Table. 3, 4, 5, 6,7,8,9 and 10. Once the equations are determined, analysis required only the measuring of the absorbances of the sample solution at the two wavelengths selected, followed by a few simple calculations. The standard deviation (S.D.), relative standard deviation (%R.S.D.) calculated are low, indicating high degree of precision of the method. The %R.S.D. is less than 2% as required by USP and ICH guidelines complies in our method.

CONCLUSION

The method was successfully used to estimate the amount of Dapagliflozin and Saxagliptin in synthetic mixture containing 10 mg of Dapagliflozin and 5 mg of Saxagliptin. By observing validation parameters, method was found to be specific, accurate, precise, repeatable and reproducible. This method is simple in calculation, hence can be employed for routine analysis of synthetic mixture as well as dissolution testing.

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