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Development and Validation of Saxagliptin and Dapagliflozin in Tablet Dosage Form by Uv-Simultaneous Equation Method

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ABSTRACT

Saxagliptin and Dapagliflozin is an anti-diabetic drug used mainly in the treatment of type 2 diabetes mellitus. Simple, accurate, precise, reproducible, requiring no prior separation and economical procedures for simultaneous estimationin tablet dosage form has been developed. Method employs formation and solving of simultaneous equation using 220nmand 204 nm as two analytical wavelengths for both drugs in alcohol. The method was validated as per ICH guidelines. Saxagliptin and Dapagliflozin at their respective λmax 220nm and 204 nm shows linearity in a concentration range 5-25µg/ml with correlation coefficient in the range of 0.00493-0.00767 for simultaneous equation method.The proposed methods are recommended for routine analysis since it is rapid, simple, accurate and also sensitive and specific. The main objective of the work undertaken was to develop UV spectrophotometric method for the simultaneous use of Saxagliptin and Dapagliflozin in the combined tablet dosage by simultaneous equation method and to validate the proposed methods as per ICH guidelines.

Key words: Saxagliptin,Dapagliflozin, UV-Simultaneous Equation

I. INTRODUCTION

Saxagliptin is a DPP-4 inhibitor used for the management of type 2 diabetes mellitus, treatment of type 2 diabetes to improve the glycaemic control in combination of other drugs or monotherapy.DDP-4 inhibitors are a class of compounds that act by affecting the action of natural hormones in the body called incretins[1-2]. Incretins decreases blood sugar by the body. Dapagliflozin is a sodium -glucose co-transporter 2 used in the management of type 2 diabetes mellitus.Dapagliflozin inhibits the sodium-glucose cotransporter 2 (SGLT2) which is primarily located in the proximal tubule of the nephron[3-4]. SGLT2 facilitates 90% of glucose resorption in the kidneys and so its inhibition allows for the glucose to be excreted in the urine[5-6]. This excretion allows for the better glycaemic control and potentially weight loss in patients with type 2 diabetes mellitus.However, there is no UV spectrophotometric method for study of Saxagliptin and Dapagliflozin in tablet dosage form[7]. This communication forms the first report of simple, sensitive, and reproducible methods for the simultaneous estimation of Saxagliptin and Dapagliflozin by UV spectroscopy[8-9].

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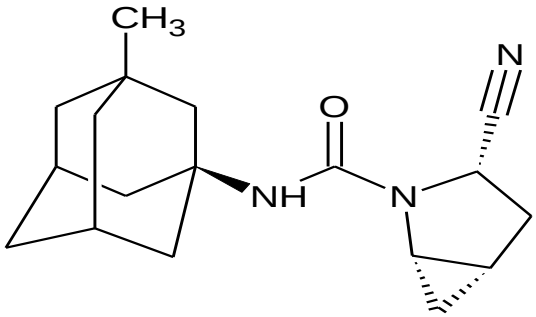

Figure 1: Structure of Saxagliptin

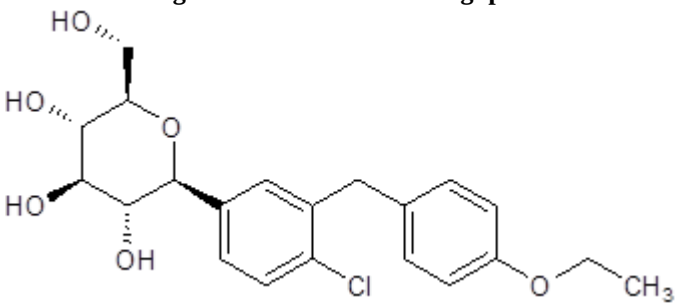

Figure 2: Structure of Dapagliflozin

II. MATERIALS AND METHODS

Instruments

Spectral runs were made on Jasco V560 double beam spectrophotometer and ME29 UV-Visible spectrophotometer with a pair of 10mm quartz cells.

Reagents and Chemicals

Saxagliptin and Dapagliflozin reference standard provided by Zenith Pharma, Tirur,Kerala. Alcohol by Bajaj Hindustan Ltd, Mumbai. The commercially available QTERN (containing Saxagliptin 5mg and Dapagliflozin 10mg), marketed by AstraZeneca Pharma India Limited.

Preparation of Standard Drug Solution

Standard stock solution containing Saxagliptin (SAXA) and Dapagliflozin (DAPA) were prepared individually by dissolving 100 mg of Saxagliptin and 100 mg Dapagliflozin RS separately in 25 ml alcohol. It was then sonicated for 10 minutes and the final volume of both solutions was made up to 50 ml in a 50 ml standard flask to obtain a concentration of 1 mg/ml. (solution A). From the above solution, accurately pipetted out 5.0ml into a 50 ml standard flask and the volume were made up to the mark using alcohol. The resulting solution had a concentration of 100 µg/ml.

Determination of Absorption Maxima

Solution containing 10µg/ml of Saxagliptin and Dapagliflozin were scanned separately in the range of 200-400 nm to determine the wavelength of maximum absorption for both drugs. SAXA and DAPA showed absorbance maxima at 220 nm (λ_1) and 204nm (λ_2) respectively.

Simultaneous Equation Method

Two wavelengths selected for the method are 220 nm and 204 nm that are absorption maxima's of SAXA and DAPA in respectively alcohol. The stock solutions of both drugs were further diluted separately with alcohol to get a series of standard solution of 5-50µg/ml of SAXA and DAPA. The absorbance was measured at the selected wavelengths and absorptivity's ($A_{1\%}^{1cm}$) 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[10-11].

$$C_x = \frac{A_2 a_{y1} - A1 a y2}{a_{x2} a_{y1} - a x 1 a y 2} \ldots \text{Eq. (i)}$$

$$C_y = \frac{A_1 a_{x2} - A2 a x 1}{a_{x2} a_{y1} - a x 1 a y 2} \ldots \text{Eq. (ii)}$$

Where A_1 and A_2 are absorbance's of sample at 220 nm and 204 nm respectively, a_{x1} and a_{x2} are absorptivity's of SAXA at 220 and 204 nm respectively, a_{Y1} and a_{Y2} are absorptivity's of DAPA at 220 nm and 204 nm respectively. C_x and C_y are the concentrations of SAXA and DAPA respectively in the diluted sample.

Determination of SAXA and DAPA in Tablets

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Twenty tablets of QTERN were weighed; average weight of one tablet was calculated and finely powdered with the help of a mortar and pestle. A quantity of powder equivalent to 50 mg of Saxagliptin (containing 25mg of Dapagliflozin) was weighed accurately and transferred to a glass stoppered flask. The powder was extracted initially with 15 ml ofalcohol by sonication for 10 mints and filtered through whatmann no:1 filter paper to a 50 ml standard flask. The residue was further extracted twice with 10 ml of alcohol and transferred to the same standard flask through the same filter paper. The volume was made up to the mark using alcohol. The resulting solution had a concentration of 400 µg/ml of Dapagliflozin and 2000 µg/ml Saxagliptin. From the above solution, accurately pipetted out 1ml and transferred to a 50 ml standard flask. Then the volume was made up to the mark using alcohol to obtain a concentration of 10µg/ml of Dapagliflozin and 5µg/ml of Saxagliptin. The absorbances of resulting solution were measured at 220 nm and 204 nm. Values are substituted in the respective formula to obtain concentrations.

III. RESULTS

The absorption spectra were observed with maximum absorption at 220 nm and 204 nm for Saxagliptin and Dapagliflozin respectively. The spectra obtained are shown in figure.

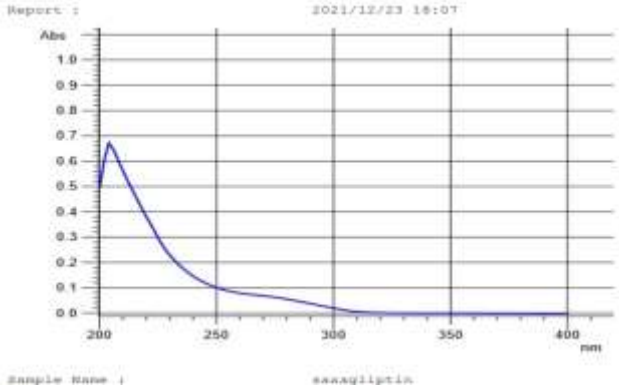

Figure 3: UV Absorption spectra of Saxagliptin in alcohol with absorption maximum at 220 nm

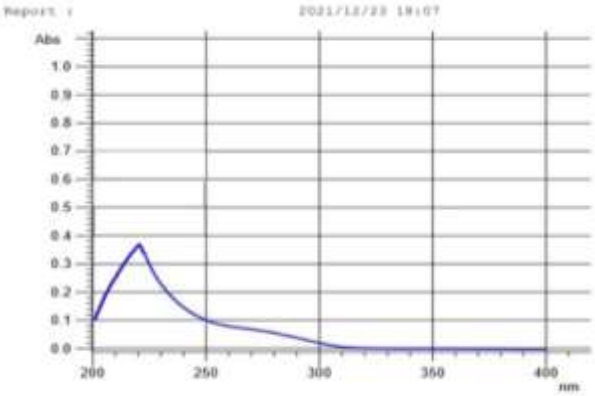

Figure 4: UV Absorption spectra of Dapagliflozin RS in alcohol with absorption maximum at 204 nm

IV. METHOD VALIDATION

The developed method was validated as per ICH guidelines for Linearity, precision, specificity, LOD and LOQ.

1. LINEARITY

The linear response of Saxagliptin and Dapagliflozin was determined by analysing five different concentrations of standard solution ranging from 5-50 µg/ml. The absorbance of each solution was measured at 220 nm and 204 nm with alcohol as blank. The calibration curve of absorbance v/s concentration was plotted and correlation co-efficient and regression line equation was determined.

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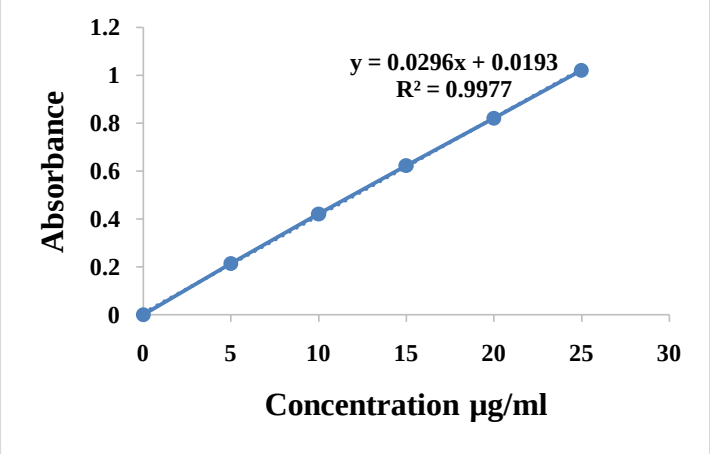

Figure 5: Linearity plot of Saxagliptin RS in alcohol at 220 nm

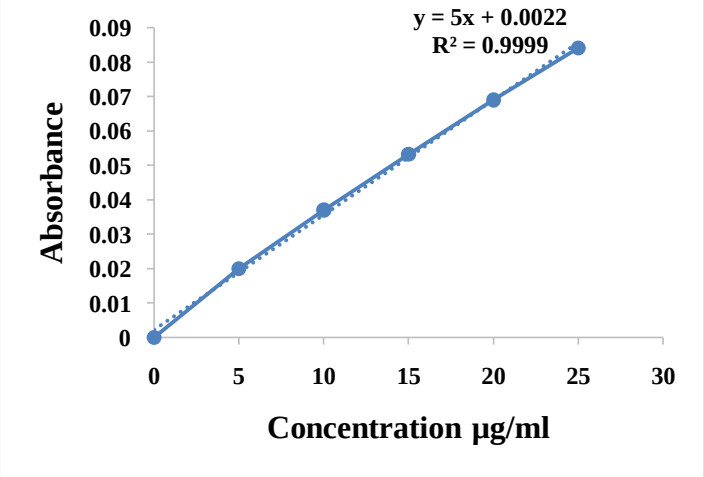

Figure 6: Linearity plot of Saxagliptin RS in alcohol at 204 nm

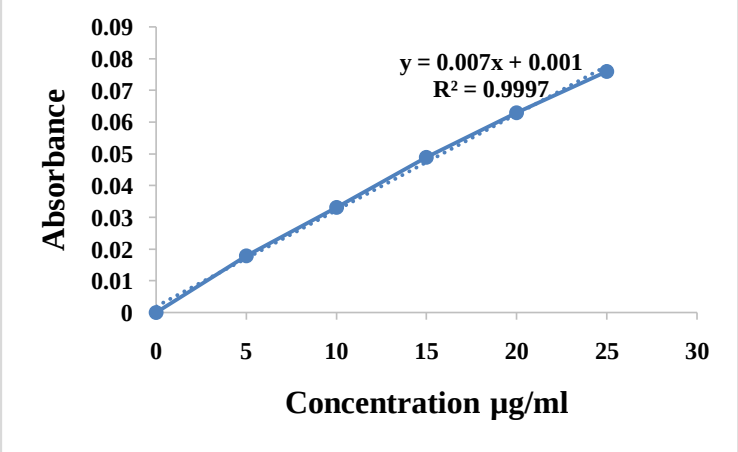

Figure 7: Linearity plot of Dapagliflozin RS in alcohol at 220 nm

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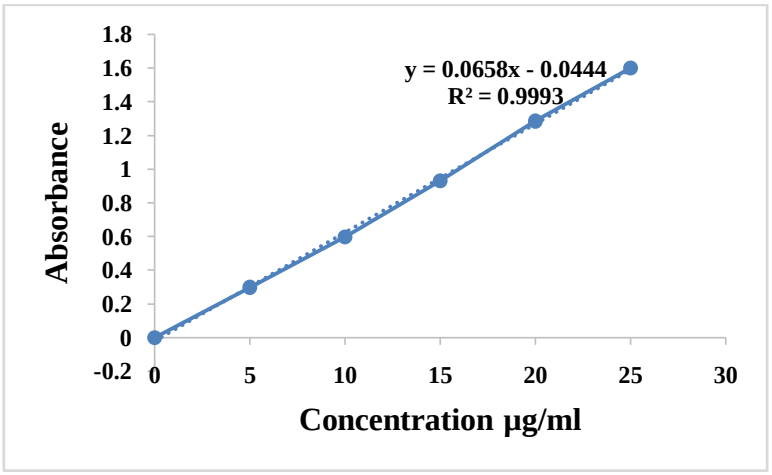

Figure 8: Linearity plot of Dapagliflozin RS in alcohol at 204 nm

2. PRECISION

Precision was determined in two levels – Repeatability and Intermediate Precision

➢ Repeatability

The repeatability of the method was studied by using five determinations at 100 % test concentration i.e., mixture of 10µg/ml of DAPA and 5µg/ ml of SAXA. The results are tabulated in **Table 1** and the statistical validation is given in table 2.

Table 1: Results of Repeatability Study

Sl.no.	Amount present (label claim) mg/ tablet		Amount obtained mg/tablet		Percentage label claim	
	SAXA	DAPA	SAXA	DAPA	SAXA	DAPA
1	5	10	4.9557	9.987	94.55	99.87
2	5	10	4.9799	9.238	99.78	92.38
3	5	10	4.9123	9.426	97.42	94.53
4	5	10	4.931	9.538	99.85	95.38
5	5	10	4.931	9.644	94.32	96.78

Table 2: Repeatability Study- Statistical Validation

Components	Mean of label claim (%)	Standard deviation (SD)	Relative standard deviation (% RSD)	Coefficient of variation

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SAXA	97.184	0.48201	0.49596	0.0049
DAPA	95.78	0.49807	0.52001	0.00520

➢ Intermediate Precision

The intermediate precision was studied by using five determinations of the mixture of 10µg/ ml of DAPA and 5µ/ml of SAXA. The stock solution was prepared and analysed at the same time on three consecutive days. The absorbance of the resulting solution was measured at 220 nm and 204 nm. The variations of the results on three days were analysed and the statistical validation was done.The results are given in **Table 3**.

Table 3: Results of Intermediate Precision

Components	**Mean of label claim (%) (n= 5)**	**Standard deviation (SD)**	**Relative standard deviation (%RSD)**	**Coefficient of variation (CV)**
SAXA	97.69	0.4823	0.4937	0.00493
DAPA	95.30	0.7313	0.7674	0.00767

Limit of Detection and Quantitation (LOD and LOQ)

LOD and LOQ were determined by linearity curve method and by using the equations.

LOD = 3.3 (σ/S)

LOQ =10 (σ/S)

Where σ is the standard deviation of the response and 'S' is the slope of the linearity curve.

Table 7: LOD and LOD data

Method parameters	**SAXA**		**DAPA**	
	220 nm	204 nm	220 nm	204nm
LOD (µg/ml)	0.3525	0.3287	0.3287	0.6015
LOQ (µg/ml)	1.0682	0.9961	1.1883	1.8229

SUMMARY OF RESULTS

PARAMETERS	**RESULTS**	
UV Detection Wavelength (nm)	Saxagliptin	Dapagliflozin
	220 nm	204 nm

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Linearity range	5- 25µg/ml	5- 25µg/ml
Regression equation at 220 nm at 204 nm	 Y=0.296x+0.0193 Y=5x+0.0022	 Y=0.007x+0.001 Y=0.0658x-0.0444
Precision % RSD Inter day Repeatability	 0.4823 0.49596	 0.7674 0.52001
LOD (µg/mL) at 220 nm at 204 nm	 0.3525 0.3287	 0.3287 0.6015
LOQ (µg/mL) at 220 nm at 204 nm	 1.0682 0.9961	 1.1883 1.8229

V. DISCUSSION

Standard calibration curves for Saxagliptin and Dapagliflozin were linear with correlation coefficient (R^2) values in the range of 0.9909-0.9999 at the selected wavelengths and the values were average of five reading with standard deviation in the range of 0.4823-0.7313. The calibration curves were repeated three times in a day and the average % RSD was found to be 0.4823 for SAXA and 0.7674 for DAPA.

VI. CONCLUSION

The most striking feature of this method is its simplicity, economy, and rapidity. The method gives accurate and precise results for the analysis of Saxagliptin and Dapagliflozin in dosage forms.

VII. ACKNOWLEDGEMENT

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