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A Novel Stability-Indicating Method for the Simultaneous Estimation of Saxagliptin and Dapagliflozin in Rat Serum by Using UV Spectroscopy

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Abstract

A new approach developed for stability-indicating, simultaneous estimation of Saxagliptin and Dapagliflozin in rat serum by using UV spectroscopy. Saxagliptin detection wave length was at 222 nm with water is solvent and Dapagliflozin detection wave length was at 274 nm with phosphate buffer pH 6.8 is solvent. Both drugs are obeyed the beers-lamberts concentration range was founds to be 1-10 µg/mL. The present method was optimized and validated in spiked rat serum according to ICH guidelines. All validation parameters were found to be within the acceptable limits and stability-indicating studies were conducted under different conditions founds in negligible. The present method was simple and sensitive; it was successfully adopted for the simultaneous estimation of Saxagliptin and Dapagliflozin in rat serum samples by using UV spectroscopy.

Keywords: Saxagliptin; Dapagliflozin; Serum; Spectroscopic method; Rat

Introduction

Saxagliptin is chemically called as (1S, 3S, 5S)-2-[(2S)-2-Amino-2-(3 hydroxytricyclo [3.3.1.13, 7] dec-1-yl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile (Figure 1). It is the oral hypoglycemic (anti-diabetic) 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 gluconeogensis 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 2). 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 glyceamic control in patients with type 2 Diabetes Mellitus [4].

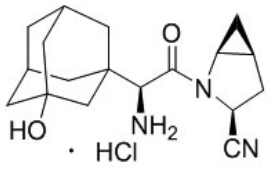

Figure 1: Chemical structure of saxagliptin.

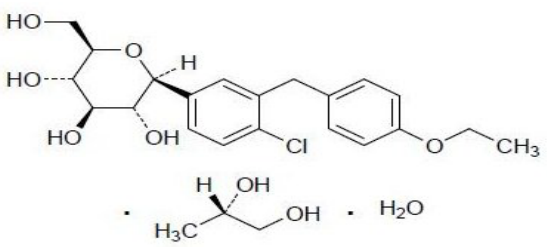

Figure 2: Chemical structure of dapagliflozin.

Few analytical methods based on UV [5-8], RP-HPLC [9,10] and LC-MS/MS [11] for the estimation of saxagliptin and dapagliflozin. Although literature survey reveals that various methods were reported for the saxagliptin and dapagliflozin both for single determination and in combination with other drugs, but no method determination has been reported for the analysis of this combination of these drugs in rat serum. Stability studies were performed under different conditions like acid, base, and oxide, thermal and neutral hydrolysis as per the guidelines of ICH [11]. In this method solvents are aqueous solvents, λ max range is lesser and fully validated when compared with previous literature which uses organic solvents, gives higher λ max value and incomplete validated data, because the present method was simple, sensitive and more accurate. Serum is extracted by using liquid liquid extraction to inhibit the protein binding to drug molecules before the UV detection. Once extraction is carried out serum proteins are get filtered which do not interfere in UV detection. The present method was simple and sensitive; it was successfully adopted for the simultaneous estimation of saxagliptin and dapagliflozin in rat serum bulk and pharmaceutical dosage form.

Experimental

Instrument

ElicoSL164 UV-Visible spectrophotometer with double beam detector configuration. The above instruments had automatic wavelength accuracy 0.1 nm and matched quartz cells and weighing balance (Elico, India).

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Materials

Blank rat serum, saxagliptin and dapagliflozin were gift samples from Spectrum Pharma Research Solutions, Hyderabad, India. Formulations were procured from local Market, Tanuku, India. All reagents and chemicals used were purchased form Nico chemicals Pvt. Ltd.

Solvent section

Saxagliptin soluble in water and dapagliflozin soluble in phosphate buffer pH 6.8 were selected for the solvents through the experiment.

Wavelength selection

Saxagliptin and dapagliflozin working concentration is 10 µg/mL was prepared and scanned UV range 200-400 nm using a water and phosphate buffer pH 6.8 as a blank. The maximum absorbance shown at 222 nm for saxagliptin and maximum absorbance shown at 274 nm for dapagliflozin, respectively. The λ maximum graphs of saxagliptin and dapagliflozin were shown in Figures 3 and 4.

Method Development

Saxagliptin standard preparation

100 mg of saxagliptin pure drug was weighed and taken in dry and clean conical flask, and then it will dissolve in 100 ml of water. 10 ml of solution took from stock solution and then made up to 100 ml with water and further dilutions were made to produce the concentration is 10 µg/mL.

Dapagliflozin standard preparation

100 mg of dapagliflozin pure drug was weighed and taken in dry and clean conical flask then it dissolved in 100 ml of phosphate buffer pH 6.8. 10 ml of solution took from stock solution and then made up to 100 ml with phosphate buffer pH 6.8 and further dilutions were made to produce the concentration is 10 µg/mL.

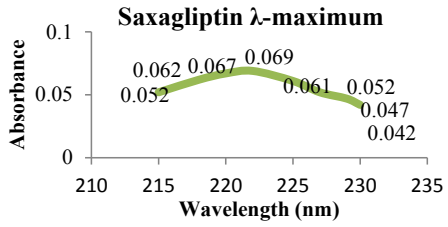

Figure 3: Saxagliptin λ-maxima.

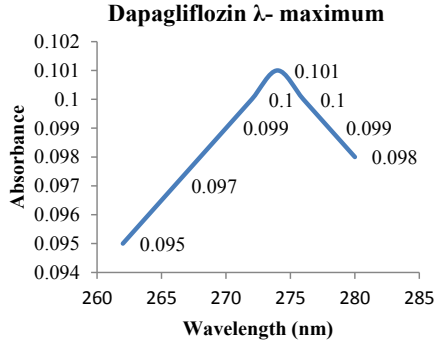

Figure 4: Dapagliflozin λ-maximum.

Preparation of calibration curve of saxagliptin

A calibration curve was plotted over a concentration range of 1-10 µg/mL for saxagliptin. Precisely measured standard stock solution of saxagliptin (1, 3, 5, 7, 8, 9 and 10 ml) was transferred to a series of 10 ml volumetric flasks and the volume to each flask was adjusted to 10 ml with water. Calibration curve was prepared by plotting concentration of saxagliptin on X-axis and their respective absorbance's on Y-axis. Calibration data are presented in Table 1.

Preparation of calibration curve of dapagliflozin

A calibration curve was plotted over a concentration range of 1-10 µg/mL for dapagliflozin. Precisely measured standard stock solution of dapagliflozin (1, 3, 5, 7, 8, 9 and 10 ml) was transferred to a series of 10 ml volumetric flasks and the volume to each flask was adjusted to 10 ml with phosphate buffer pH 6.8. Calibration curve was prepared by plotting concentration of dapagliflozin on X-axis and their respective absorbance's on Y-axis. Calibration data are presented in Table 2.

Method Validation

The developed method was validated for various parameters like linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and ruggedness according to ICH guidelines.

Linearity

This manifests linear relationship in the range of 1-10 µg/mL of saxagliptin and dapagliflozin.

Precision

The intra-day precision analysed in the same day and inter-day precision analysed for three consecutive days. The results were indicated by calculated per cent relative standard deviation.

Accuracy

Accuracy was carried out at three different levels 40%, 80% and 120%. The percentage of accuracy was calculated as mean ± standard deviation.

S. No	Concentration (µg/mL)	Absorbance
0	0	0
1	1	0.032
2	3	0.080
3	5	0.192
4	7	0.257
5	8	0.293
6	9	0.338
7	10	0.380

Table 1: Calibration data of saxagliptin.

S. No	Concentration (µg/ml)	Absorbance
1	1	0.038
2	3	0.080
3	5	0.119
4	7	0.163
5	8	0.188
6	9	0.210
7	10	0.230

Table 2: Calibration data of dapagliflozin.

Limit of detection (LOD)

Formula for measuring of limit of detection:

LOD=3.3 σ/S

Where,

Σ: Standard deviation

S: Slope

Limit of quantification (LOQ)

Formula for measuring of limit of quantification:

LOD=10 σ/S

Where,

Σ: Standard deviation

S: Slope

Stability-indicating studies

A stability-indicating study was conducted as per ICH Q2 (R1) regulations of both drugs like saxagliptin and dapagliflozin and founds its negligible.

Serum studies

This manifests linear relationship in the range of 40-84 µg/mL of saxagliptin and dapagliflozin, in spiked rat serum, respectively.

Results and Discussion

Selection of wavelength

The spectra of saxagliptin in water showed absorption at 222 nm shown in Figure 3 and the spectra of dapagliflozin showed in phosphate buffer pH 6.8 absorption at 274 nm shown in Figure 4, respectively.

Linearity

The linearity for the proposed method was investigated at seven concentration levels (1-10 µg/mL) of reference standard saxagliptin and dapagliflozin, respectively. The linearity of saxagliptin and dapagliflozin were shown in Tables 1 and 2 and Figures 3 and 4.

Precision

The percent RSD value of intra-day and inter-day precision were found to be 0.021 and 0.014 for saxagliptin and 0.02 and 0.0128 for dapagliflozin, respectively, as shown in Table 3. The percentage of RSD values of Saxagliptin and Dapagliflozin was not more than 2%. The value of percentage of RSD is within the limits.

Accuracy

The percentage of recovery of saxagliptin was found to be 99.01% and percentage recovery of dapagliflozin was found to be 99.08% shown in Table 4, respectively. The range of recovery of saxagliptin and dapagliflozin is within the limits.

Limit of detection

The limit of detection of saxagliptin and dapagliflozin were obtained the values 0.088 µg/mL and 0.197 µg/mL it indicates the high sensitivity of the proposed method, data shown in Table 4.

Limit of quantitation

The limit of quantitation of saxagliptin and dapagliflozin were obtained the value 0.267 and 0.598 µg/mL, it indicates the high sensitivity of the proposed method, data shown in Tables 4 to 6 (Figures 5 and 6).

Stability-indicating studies

Stability-indicating studies performing like acid, base, oxide and neutral hydrolysis on saxagliptin and dapagliflozin respectively. The percentage of degradation of saxagliptin and dapagliflozin found within the limits.

Sample number	Assay of saxagliptin	Assay of saxagliptin	Assay of dapagliflozin	Assay of dapagliflozin
	Intra-day precision	Inter-day precision	Intra-day precision	Inter-day precision
1	97.50	97.51	98.22	98.23
2	97.53	97.52	98.23	98.24
3	97.55	97.54	98.24	98.23
4	97.54	97.53	98.23	98.22
5	97.50	97.50	98.22	98.21
6	97.51	97.52	98.20	98.21
Mean	97.52	97.53	98.22	98.22
S.D	0.0210	0.0140	0.020	0.0126
%RSD	0.0210	0.0140	0.020	0.0128

Table 3: Precision data of saxagliptin and dapagliflozin.

Drug name	Level of addition (%)	Amount added (mg)	Drug found (mg/mL)	% Recovery	Average % recovery
saxagliptin	40	4	3.99	99.75	
	80	8	7.89	98.62	99.01 ± 0.0143
	120	12	11.84	98.66	
dapagliflozin	40	4	3.97	99.25	99.08 ± 0.025
	80	8	7.82	97.75	
	120	12	11.97	100.25	

Table 4: Accuracy data of saxagliptin and dapagliflozin.

S. No	Parameter	Saxagliptin	Dapagliflozin
1	Absorption maxima (nm)	222	274
2	Linearity (mcg/ml)	1-10	1-10
3	Standard regression equation	Y=0.0374x	Y=0.0234x
4	Correlation coefficient (r^2)	0.9989	0.9932
5	Molar extinction coefficient	0.0123	0.0116
6	Accuracy (% recovery ± SD)	99.01 ± 0.0143	99.08 ± 0.025
7	Precision	97.52% (Intra-day precision) and 97.53% (Inter-day precision)	98.22% (Intra-day precision) and 98.28% (Inter-day precision)
8	Sandell's sensitivity (mg/cm 2/0.001 absorbance unit)	0.0813	0.0862
9	LOD (µg/mL)	0.088	0.197
10	LOQ (µg/mL)	0.267	0.598

Table 5: Validation parameters of saxagliptin and dapagliflozin.

Drug name	Type of stress conditions	Amount of drug to be taken (mg)	Amount of solution taken (µg/mL)	Test absorbance	Standard absorbance	% Degradable
Saxagliptin	Acidic conditions	10	1	0.422	0.123	97.09
	Basic conditions	10	1	0.117	0.123	89.05
	Oxidation condition	10	1	0.921	0.123	98.67
	Neutral condition	10	1	0.130	0.123	90.54
Dapagliflozin	Acidic conditions	10	1	0.048	0.116	75.90
	Basic conditions	10	1	0.064	0.116	81.90
	Oxidation condition	10	1	0.095	0.116	87.80
	Neutral condition	10	1	0.033	0.116	64.90

Table 6: Stability-indicating data of saxagliptin and dapagliflozin.

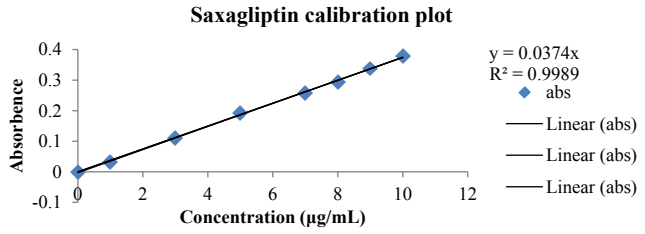

Figure 5: Saxagliptin calibration plot.

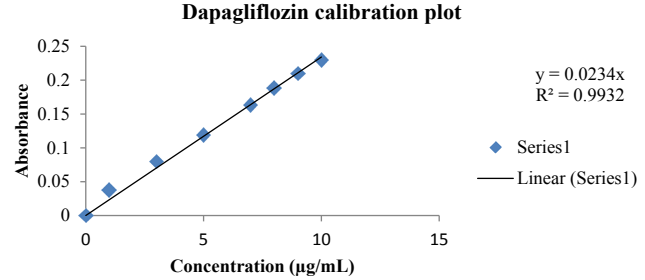

Figure 6: Dapagliflozin calibration plot.

Serum Studies

Estimation of saxagliptin in spiked rat serum

Calibration plot: The manifest of calibration plot in the range of 40-84 µg/ml of saxagliptin shown in Figure 7 and Table 7.

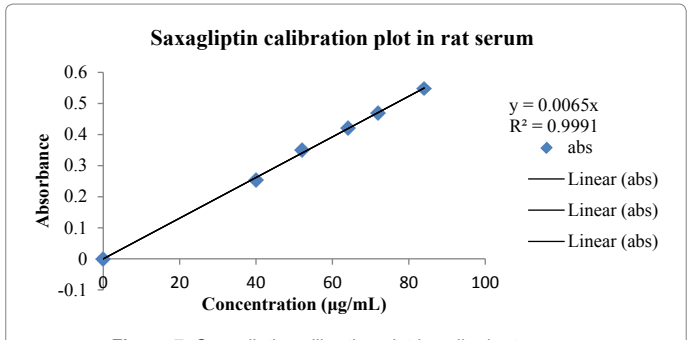

Figure 7: Saxagliptin calibration plot in spiked rat serum.

S. No	Concentration (µg/mL)	Absorbance
1	40	0.254
2	52	0.350
3	64	0.420
4	72	0.469
5	84	0.548

Table 7: Saxagliptin calibration data in spiked rat serum.

Estimation of dapagliflozin in spiked rat serum

Calibration plot: The manifest of calibration plot in the range of 40-84 µg/ml of dapagliflozin shown in Figure 8 and Table 8.

Conclusion

A simple, sensitive and appreciable stability-indicating simultaneous

S. No	Concentration (µg/mL)	Absorbance
1	40	0.140
2	52	0.185
3	64	0.232
4	72	0.271
5	84	0.318

Table 8: Dapagliflozin calibration data in spiked rat serum.

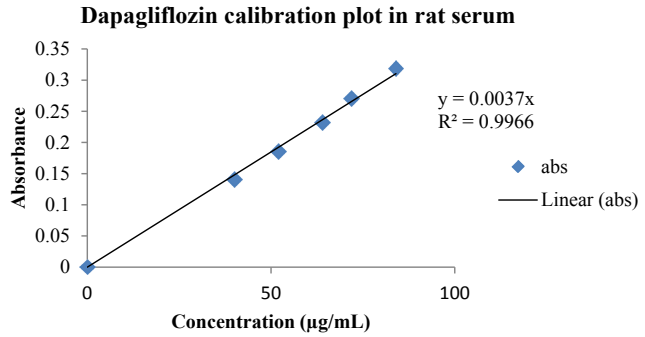

Figure 8: Dapagliflozin calibration plot in spiked rat serum.

estimation of saxagliptin and dapagliflozin in spiked rat serum is by using UV spectroscopy. The method was fast and economical and it was also selective and sensitive to the desirable range. The results of the analysis were validated as per ICH guidelines and by recovery studies. The stability-indicating nature of the proposed method was established by performing forced degradation, which provided degradation behaviour of saxagliptin and dapagliflozin under various conditions. *In vitro* rat studies were conducted in spiked rat serum with saxagliptin and dapagliflozin by using UV spectroscopy. It will help to improve the sensitivity of the method in future works on saxagliptin and dapagliflozin.

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