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METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF METFORMIN HYDROCHLORIDE AND SAXAGLIPTIN IN BULK AND MARKETING PREPARATION

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ABSTRACT

A simple, accurate and precise spectrophotometric method has been developed for simultaneous determination of Saxagliptin (SAXA) and Metformin hydrochloride (MET) in a laboratory mixture. In absorbance ratio method, isobestic point is observed at 227 nm. Isobestic point (227nm) is considered as λ_2 and absorbance maxima of Saxagliptin (210nm) is considered as λ_1 . Saxagliptin and Metformin hydrochloride were quantified using principle that absorbance difference between two points on mixture spectra is directly proportional to concentration of components of interest and independent of interfering component. All dilutions were prepared in distilled water. Linearity range was observed in the concentration range of solution 5-50 $\mu\text{g/ml}$ for Saxagliptin and 2-16 $\mu\text{g/ml}$ for Metformin hydrochloride. The methods were validated statistically and recovery study was performed to confirm the accuracy of both drugs.

Keywords – Saxagliptin, Metformin Hydrochloride, Ultraviolet spectroscopy, Absorbance ratio method.

1. INTRODUCTION

Metformin Hydrochloride 1, 1-dimethylbiguanide hydrochloride is taken as first line drug of choice for the treatment of type-2 diabetes and also used in treatment of polycystic ovary disorder. It can also be used for other diseases where insulin resistance is an important factor. Metformin improves hepatic and peripheral tissue sensitivity to insulin without the problem of serious lactic acidosis¹. Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state² MET acts as anti-hyperglycemic and lowers the blood glucose level by inhibiting hepatic glucose production, gluconeogenesis and increasing peripheral utilization of glucose. Metformin hydrochloride is official in I.P., U.S.P., and B.P³. It is a biguanide drug well known as antidiabetic drug, the mechanism of action of metformin is simulates glycolysis in peripheral tissue⁴. Saxagliptin (SAXG) is chemically (1S, 3S, 5S)-2-[(2S)-2-Amino-2-(3 hydroxytricyclo [3.3.1.1^{3,7}] dec-1-yl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile previously identified as BMS-477118. This is new oral hypoglycemic agent of the new dipeptidyl peptidase- 4 (DPP-4) inhibitor class of drugs. The empirical formula is $\text{C}_{18}\text{H}_{25}\text{N}_3\text{O}_2$, H_2O and the molecular weight is 333.43⁵⁻⁸. Saxagliptin recently approved for the treatment of type-2 diabetes mellitus⁹. It has been used in conjunction with exercise and diet to improve glycaemic control in patients with type 2 diabetes and is to be used with metformin, a sulphonylurea or pioglitazone

when blood sugar levels are not adequately controlled by one of these agents alone^{10, 11}. Literature survey reveals that the drug can be estimated only by LC-MS/MS¹⁰, Spectrophotometric method¹³ have been reported.

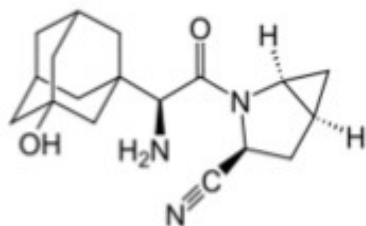


Fig. 1: structure of Saxagliptin

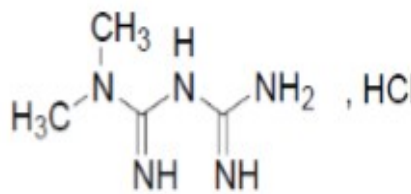


Fig. 2: structure of metformin hydrochloride

2. MATERIALS AND METHODS

2.1 Instrumentation: UV-visible double beam spectrophotometer, Jasco model 630 with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 mm matched quartz cells was used.

2.2 Reagents and chemicals used

- ✓ Saxagliptin
- ✓ Metformin Hydrochloride
- ✓ Distilled water

2.3 Preparation of stock solution

2.3.1 Saxagliptin standard solution:

100mg of Saxagliptin was accurately weighed and it was transferred into clean, dry 100ml volumetric flask and dissolved with sufficient volume of distilled water to get concentration of 1000 μ g/ml. 10ml of the stock solution was further diluted in a 100ml volumetric flask with distilled water to get a concentration 100 μ g/ml.

2.3.2 Metformin Hydrochloride standard solution:

Standard stock solution: 100mg of Metformin Hydrochloride was accurately weighed and it was transferred into clean, dry 100ml volumetric flask and dissolved with sufficient volume of distilled water. The volume was made up to 100ml with distilled water to get concentration of 1000 μ g/ml. 10ml of the stock solution was further diluted in a 100ml volumetric flask with distilled water to get a concentration 100 μ g/ml.

2.3.3 Determination

Working standard solutions of both the drugs were scanned in the UV range of 200nm to 400nm, using distilled water as Blank. The peaks obtained were noted and the peak having highest absorbance was taken as wavelength maximum.

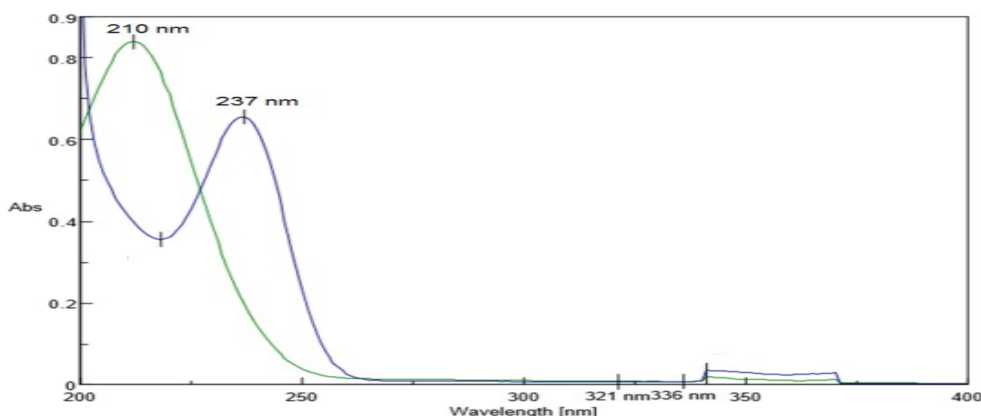


Fig. 3: wavelength maximum of Saxagliptin (210nm) and Metformin Hydrochloride (237nm)

2.4 Method A: Absorbance ratio method

The absorbance ratio method is the simultaneous equation procedure. It depends on the property that for a substance, which obeys Beer's law at all wavelengths, the ratio of absorbance at any two wavelengths is constant value independent of concentration or wavelength. This ratio is also referred to as a Q-value. In the quantitative assay of two components in mixture by the absorbance ratio method; absorbance is measured at two wavelengths, one being the λ_{max} of one component λ_1 and other being the wavelength of equal absorptivity of the two components λ_2 . i.e. isobestic point. A series of standard solutions of Saxagliptin and Metformin hydrochloride in the concentration range of 5-50 $\mu\text{g/ml}$ and 2-16 $\mu\text{g/ml}$ respectively were prepared and were measured at 210nm and 227nm (isobestic point). Calibration curves and absorptivity values were calculated.

The concentration of two drugs was calculated by using the following equations

$$C_x = \frac{Q_m - Q_x}{Q_y - Q_x} \times \frac{A_1}{a_{y1}}$$
$$C_y = \frac{Q_m - Q_y}{Q_x - Q_y} \times \frac{A_1}{a_{x1}}$$
$$Q_m = \frac{A_2}{A_1}; \quad Q_x = \frac{a_{x2}}{a_{x1}}; \quad Q_y = \frac{a_{y2}}{a_{y1}}$$

A_2 and A_1 = Absorbance of the sample solution at 210nm and 227nm

a_{x1} and a_{x2} = Absorptivity of Saxagliptin at 210nm and 227nm

a_{y1} and a_{y2} = Absorptivity of Metformin hydrochloride at 210nm and 227nm

2.5 Validation of UV method for the Assay of Metformin Hydrochloride and Saxagliptin

Validation of an analytical method is a process to establish that the performance characteristics of the developed method to meet the requirements of the intended analytical application. Typical analytical parameters used in assay validation according to ICH guidelines are: sensitivity, linearity, range, accuracy, precision, system precision and method precision, intraday precision and interday precision.

2.5.1 Sensitivity

Metformin Hydrochloride standard stock solution

10mg of Metformin Hydrochloride was accurately weighed and it was transferred into a clean, dry 100ml volumetric flask and dissolved with sufficient volume of distilled water. The volume was made up to 100ml with distilled water to get concentration of 100 $\mu\text{g/ml}$.

Working standard stock solution

Aliquots from stock solution was transferred into 8 separate 10ml volumetric flasks and volume made up to 10 ml with the distilled water to obtain the concentrations 2, 4, 6, 8, 10, 12, 14 and 16 $\mu\text{g/ml}$.

Saxagliptin standard stock solution

10mg of Saxagliptin was accurately weighed and it was transferred into a clean, dry 100ml volumetric flask and dissolved with sufficient volume of distilled water. The volume made up to 100 ml with distilled water to get concentration of 100 $\mu\text{g/ml}$.

Working standard stock solution

Aliquots from stock solution was transferred into 9 different 10ml volumetric flasks and volume made up to 10ml with distilled water to obtain the concentrations 10, 15, 20, 25, 30, 35, 45 and 50 µg/ml.

Determination

Absorbance of working standard solutions of Metformin hydrochloride and Saxagliptin was taken at 210nm and at 227nm.

2.5.2 Linearity and Range

Metformin hydrochloride standard stock solution

10mg of Metformin Hydrochloride was accurately weighed and it was transferred into a clean and dry 100 ml volumetric flask and dissolved with sufficient volume of distilled water. The volume was made up to 100ml with distilled water to get concentration of 100 µg/ml.

Working standard solution

Aliquots from standard solution were withdrawn in the volumes of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6ml and transferred into different 10ml volumetric flasks. The volumes were made up with the distilled water to get concentrations ranging from 2, 4, 6, 8, 10, 12, 14 and 16 µg/ml.

Standard stock solution of Saxagliptin

Accurately weighed 10mg of Saxagliptin was transferred into a clean and dry 100ml volumetric flask and dissolved with sufficient volume of distilled water. The volume was made up to 100ml with distilled water to get concentration of 100 µg/ml.

Working standard solution

Aliquots from standard solution were transferred in volumes of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0ml into different 10ml volumetric flasks. The volumes were made up with the distilled water to get concentration ranging from 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 µg/ml.

Determination

Six replicates per concentration were studied. Absorbance of working standard solutions of Metformin Hydrochloride and Saxagliptin were taken at the 210nm and 227nm. Graph of concentration (on X- axis) Vs mean response (on Y- axis) was plotted for both the drugs separately. The regression equation, Y- intercept and correlation coefficient, were calculated.

2.5.3 Accuracy

Preparation of standard stock solution

50mg of Metformin Hydrochloride and 5mg of Saxagliptin were accurately weighed and transferred to a 100ml volumetric flask and dissolved in sufficient volume of distilled water. The volume made up to 100ml with distilled water to get the concentration of 500 µg/ml of Metformin hydrochloride and 50 µg/ml of Saxagliptin.

Preparation of sample stock solution

Twenty tablets containing 5mg of Saxagliptin and 50mg of Metformin Hydrochloride were weighed and finely powdered. Accurately weighed amount of powder which is equivalent to 5mg of Saxagliptin and 50mg of Metformin Hydrochloride was transferred to 100ml volumetric flask. The volume was made up to 100ml with distilled water to get the concentration 50µg/ml of Saxagliptin and 500µg/ml of Metformin hydrochloride.

Preparation of standard and sample mixture

Level I (80%): Volume of 1ml sample stock solution and 0.8ml standard stock solution was transferred into 10ml volumetric flask and volume made up with distilled water.

Level II (100%): Volume of 1ml sample stock solution and 1ml standard stock solution was transferred into 10ml volumetric flask and volume made up with distilled water.

Level III (120%): Volume of 1ml sample stock solution and 1.2ml standard stock solution was transferred into 10ml volumetric flask and volume made up with distilled water.

Determination

The absorbances of resulting solutions were recorded at wavelengths 210nm and 227nm. Concentrations of Saxagliptin and Metformin hydrochloride in each solution were calculated.

2.5.4 Precision

Preparation of standard mixture solution

5mg of Saxagliptin and 50mg of Metformin Hydrochloride was accurately weighed and transferred to 100ml volumetric flask. The volume was made up to 100ml with distilled water to get the concentration 50µg/ml of Saxagliptin and 500µg/ml of Metformin Hydrochloride.

Working solution

Standard mixture solution of volume 1.0ml was transferred to 10ml volumetric flask and volume adjusted with distilled water to get the concentration 5µg/ml of Saxagliptin and 50µg/ml of Metformin Hydrochloride.

System Precision

The absorbance of six determinations of working solution was recorded at wavelengths 210nm and 227nm. The % RSD was calculated for the absorbance of replicates.

Method Precision

The absorbance of six determinations of working solution was taken at wavelengths 210nm and 227nm. Concentration of Metformin Hydrochloride and Saxagliptin in each replicate was calculated from simultaneous equation. The % RSD was calculated from the concentrations of Metformin Hydrochloride and Saxagliptin.

Inter-day Precision

The absorbance of six determinations of working sample solution was taken at wavelengths 210nm and 227nm on different days. Concentration of Metformin Hydrochloride and Saxagliptin in each replicate was calculated from simultaneous equation. The %

RSD was calculated from the concentrations of Metformin Hydrochloride and Saxagliptin. The standard deviation and relative standard deviation were calculated.

Intra-day Precision

The absorbance of six determinations of working sample solution was taken at wavelengths 210nm and 227nm on different intervals in the same day. Concentration of Metformin Hydrochloride and Saxagliptin in each replicate was calculated from the equation. The % RSD was calculated from the concentrations of Metformin Hydrochloride and Saxagliptin six determinations of working solution. The standard deviation and relative standard deviation were calculated.

3. RESULTS AND DISCUSSION

3.1 Sensitivity

Absorbance of standard solutions of Metformin Hydrochloride and Saxagliptin was measured at 227nm and 210nm. Sandell's sensitivity (Π) for both the drugs was calculated from formula, at both the wavelengths.

$$\text{Sandell's sensitivity} = \frac{\text{concentration of drug in } \mu\text{g}/100\text{ml}}{\text{absorbance}} \times 0.001$$

Table 01: Sensitivity Data of Metformin Hydrochloride

Conc. $\mu\text{g}/\text{ml}$	Metformin Hydrochloride	
	Absorbance at 227 nm	Sensitivity ($\mu\text{g}/\text{cm}^3/\text{Au}$)
2	0.1721	1.16
4	0.3345	1.19
6	0.5178	1.15
8	0.7332	1.09
10	0.9210	1.08
12	1.0459	1.14
14	1.2549	1.11
16	1.3934	1.14
Mean		1.132

Table 2: Sensitivity Data of Saxagliptin

Conc. $\mu\text{g}/\text{ml}$	Saxagliptin	
	Absorbance at 227 nm	Sensitivity ($\mu\text{g}/\text{cm}^3/\text{Au}$)
5	0.1489	3.35
10	0.2592	3.85
15	0.3409	4.40
20	0.3802	5.26
25	0.4628	5.40
30	0.5186	5.78
35	0.5833	6.00
40	0.6622	6.04
45	0.7186	6.26
50	0.7976	6.26
Mean		5.26

3.2 Linearity and Range

The linearity in response for Metformin Hydrochloride and Saxagliptin was observed in the concentration range of 2-16 μ g/ml and 5-50 μ g/ml respectively for both the drugs, with percentage curve fittings found to be well within the limits of acceptance criteria.

Table 3: Linearity Range Data of Metformin Hydrochloride

Volume of stock solution(ml)	Volume adjusted(ml)	Concentration μ g/ml	Absorbance at 210nm	Absorbance at 227nm
0.2	10	2	0.1407	0.1721
0.4	10	4	0.2831	0.3345
0.6	10	6	0.4215	0.5178
0.8	10	8	0.5978	0.7332
1	10	10	0.7217	0.921
1.2	10	12	0.8499	1.059
1.4	10	14	1.0245	1.254
1.6	10	16	1.132	1.3934

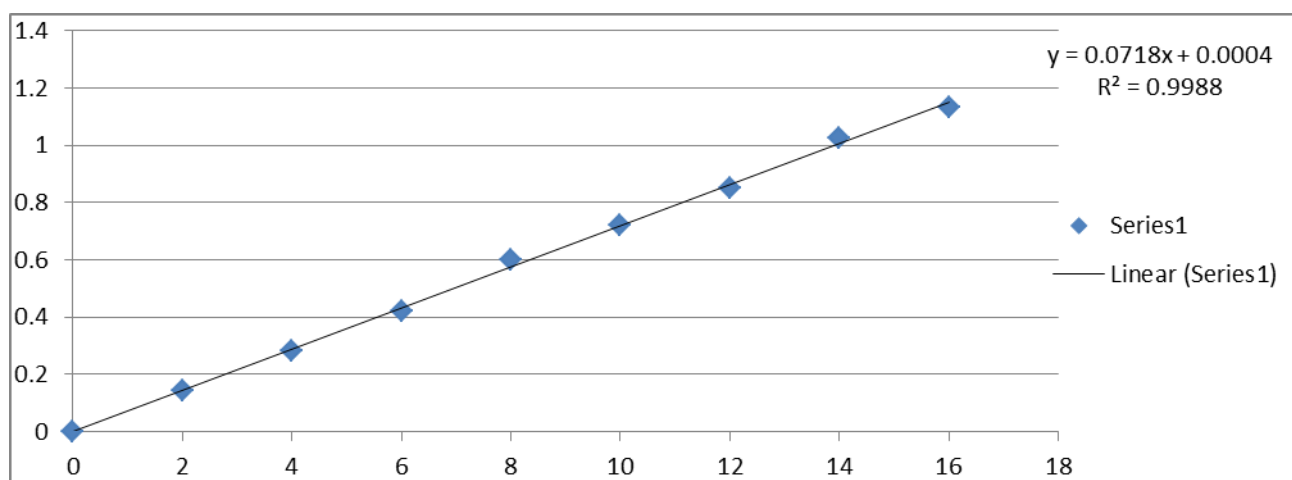


Fig 4: Linearity range graph of Metformin Hydrochloride at 210nm

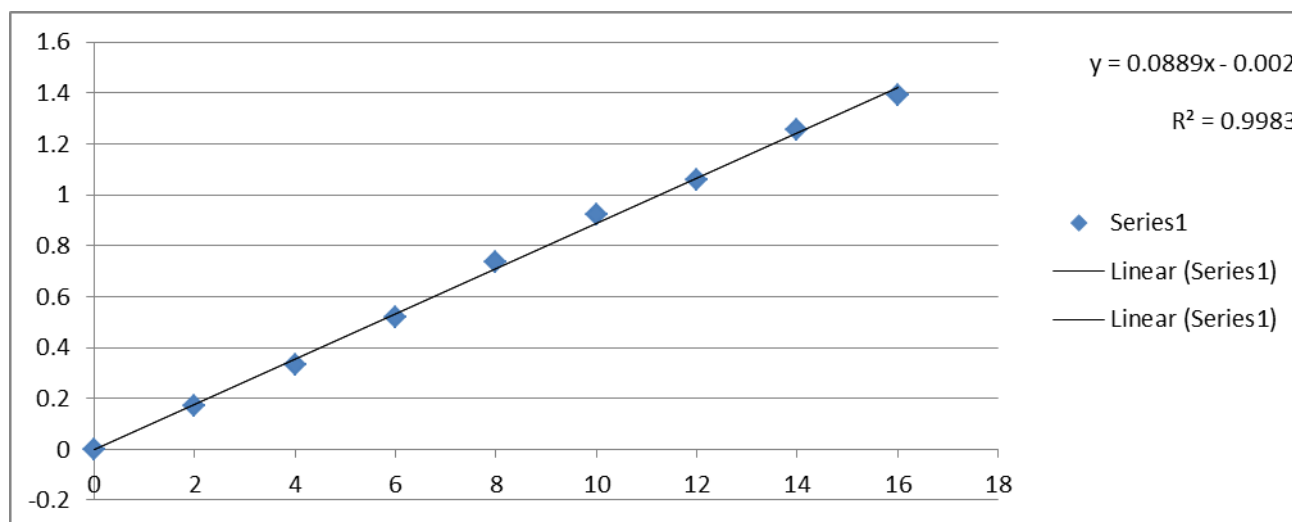


Fig 5: Linearity range graph of Metformin Hydrochloride at 227nm

Table 4: Linearity Range Data of Saxagliptin

Volume of stock solution(ml)	Volume adjusted(ml)	Concentration $\mu\text{g/ml}$	Absorbance at 210nm	Absorbance at 227nm
0.5	10	5	0.2041	0.1001
1	10	10	0.3262	0.1991
1.5	10	15	0.4304	0.3004
2	10	20	0.5274	0.3511
2.5	10	25	0.6283	0.4129
3	10	30	0.7231	0.5087
3.5	10	35	0.8239	0.5863
4	10	40	0.9464	0.6622
4.5	10	45	1.0317	0.7459
5	10	50	1.1576	0.8259

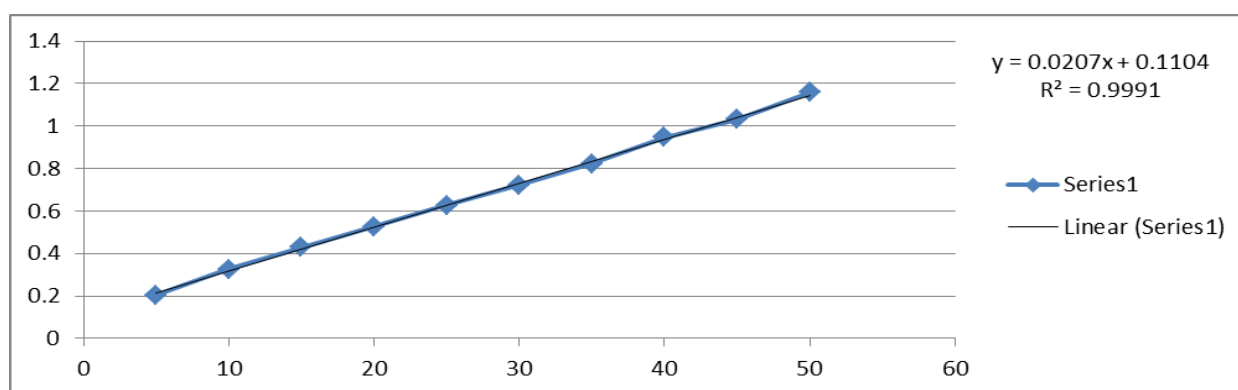


Fig 6: Linearity range graph of Saxagliptin at 210nm

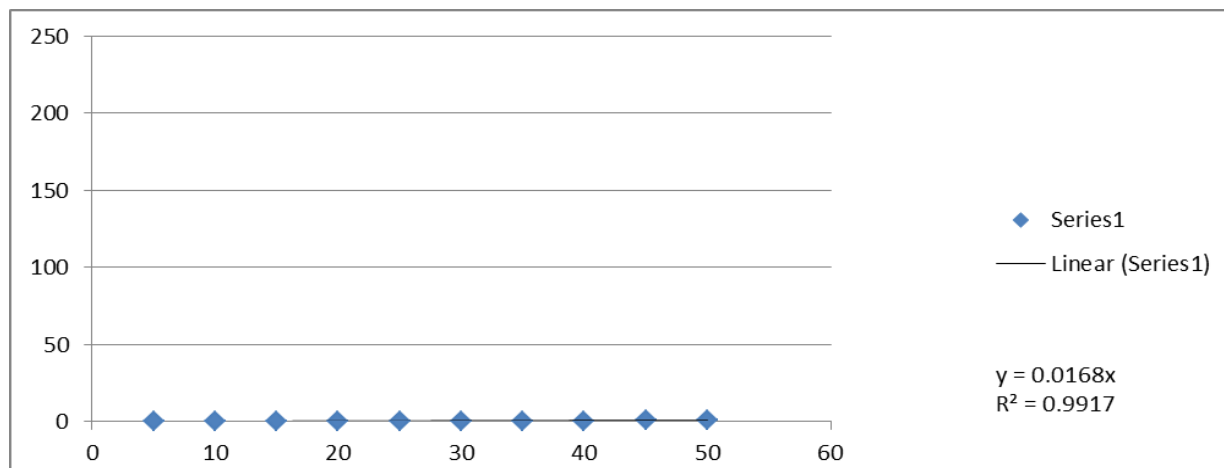


Fig 7: Linearity range graph of Saxagliptin at 227nm

Table 5: Linearity report of Metformin Hydrochloride and Saxagliptin

Parameters	Results observed				Acceptance Criteria
	Metformin Hydrochloride		Saxagliptin		
	288nm	271.5nm	288nm	271.5nm	
Linearity range (µg/ml)	2-16 µg/ml	2-16 µg/ml	5-50 µg/ml	5-50 µg/ml	-
Regression equation	0.0718x + 0.0004	0.0889x - 0.002	0.0207x + 0.1104	0.0157x+0.0369	-
Correlation coefficient	0.9988	0.9983	0.9991	0.99974	0.99
Intercept	0.0004	0.002	0.11	0.03	-
Slope	0.071	0.089	0.020	0.015	-

Assay of marketed formulation

Table 6: content of Metformin Hydrochloride and Saxagliptin found in tablets

Stock. Vol	Concentration obtained($\mu\text{g/ml}$)		Amount of drug in tablet (mg)		Amount obtained in %	
	Met	Saxa	Met	Saxa	Met	Saxa
0.5	9.95	2.47	49.9	4.94	99.5	98.8
1	19.97	4.96	49.97	4.96	99.85	99.2
1.5	29.96	7.45	49.97	4.96	99.85	99.2
2	39.96	9.96	49.98	4.98	99.9	99.6
2.5	49.98	12.48	49.99	4.99	99.95	99.8
Average			49.962	4.966	99.81	99.32

Accuracy Determination

The absorbances of resulting solutions were recorded at wavelengths of 227nm and 210nm. Concentrations of Metformin Hydrochloride and Saxagliptin in each solution were calculated from absorbance ratio equation.

Table 7: Recovery Data of Standard Mixture

Level (%)	Sample conc. ($\mu\text{g/ml}$)		Total Conc. ($\mu\text{g/ml}$)		Amount of std recovered($\mu\text{g/ml}$)		% Recovery of standard	
	Met	Saxa	Met	Saxa	Met	Saxa	Met	Saxa
80%	49.8	4.72	35.71	8.81	45.91	4.09	99.43	102.25
100%	49.8	4.72	39.74	9.45	49.94	5.03	99.7	100.6
120%	49.8	4.72	43.71	10.69	43.91	5.97	99.62	99.5

3.3 Precision

3.3.1 System Precision

The absorbance of six determinations of working solution was recorded wavelengths 210nm and 227nm. The %RSD was calculated for the absorbance of replicates.

Table 8: System Precision Data of Metformin Hydrochloride and Saxagliptin

Replicates	Absorbance A ₁ (210nm)	Absorbance A ₂ (227nm)
1	0.2831	0.3345
2	0.2822	0.3312
3	0.2845	0.3465
4	0.2901	0.3315
5	0.2801	0.3323
6	0.2811	0.3302
Mean	0.2835	0.334
Standard deviation	0.01235	0.0145
% RSD	0.4356	0.4341

3.3.2 Method Precision

The absorbance of six determinations of working solution was taken at wavelengths 210nm and 227nm. Concentration of Metformin Hydrochloride and Saxagliptin in each replicate was calculated from absorbance ratio equation. The % RSD was calculated from the concentrations of Metformin Hydrochloride and Saxagliptin.

Table 9: Method Precision Data of Metformin Hydrochloride and Saxagliptin

Replicates	Concentration in (µg/ml)	
	Metformin Hydrochloride	Saxagliptin
1	49.94	9.87
2	49.96	9.88
3	49.95	9.85
4	49.94	9.86
5	49.95	9.84
6	49.94	9.88
Mean	49.946	9.863
Standard deviation	0.01453	0.01231
%RSD	0.0290	0.1248

Inter-day Precision

Table 10: Inter-day Precision Data of Metformin Hydrochloride and Saxagliptin

Replicates	Date interval	Concentration in µg/ml	
		Metformin Hydrochloride	Saxagliptin
1	4/10/2017	49.94	9.87
2	4/10/2017	49.95	9.88
3	5/10/2017	49.97	9.87
4	5/10/2017	49.94	9.86
5	6/10/2017	49.95	9.84
6	6/10/2017	49.94	9.88
Mean		49.976	9.842
Standard deviation		0.02451	0.02134
%RSD		0.1214	0.1451

Intra-day Precision

Table 11: Intra-day Precision Data of Metformin Hydrochloride and Saxagliptin

Replicates	Time interval	Concentration in µg/ml	
		Metformin Hydrochloride	Saxagliptin
1	10:00AM	49.94	9.88
2	11:00AM	49.95	9.87
3	12:00 PM	49.95	9.85
4	1:00 PM	49.96	9.86
5	2:00 PM	49.98	9.85
6	3:00 PM	4.94	9.87
Mean		49.95	9.863
Standard deviation		0.01364	0.01252
%RSD		0.0273	0.1269

Report

As the values of % RSD were within the acceptable limits, both the method as well as the system provides good precision.

4. CONCLUSION

Saxagliptin and Metformin Hydrochloride were estimated at 210nm and 227nm in distilled water, Saxagliptin and Metformin Hydrochloride obey Beer-Lamberts law in concentration range of 5-50µg/ml and 2-16µg/ml respectively. The method was validated as per ICH and USP guidelines. The result of recovery study was found to be within the prescribed limit of 98 – 101 %.

These methods were accurate, simple, rapid, precise, reliable, sensitive, and reproducible and can be used for further quantitative analysis of Saxagliptin and Metformin Hydrochloride.

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