Research Article

Method Development and Validation for Determination of Metformin Hydrochloride and Saxagliptin in Bulk and Marketed 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 (227 nm) is considered as λ2 and absorbance maxima of Saxagliptin (210 nm) is considered as λ1. 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 µg/ml for Saxagliptin and 2-16 µg/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: 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 acidosis[1]. Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state[2] 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.P[3]. It is a biguanide drug well known as antidiabetic drug, the mechanism of action of metformin is simulates glycolysis in peripheral tissue[4].

Saxagliptin: Saxagliptin (SXG) is chemically (1S, 3S, 5S)-2[(2S)-2-Amino-2-(3 hydroxytricyclo [3.3.1.13, 7] dec1-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 $C_{18}H_{25}N_3O_2$, H_2O and the molecular weight is 333.43[5-8]. Saxagliptin recently approved for the treatment of type-2 diabetes mellitus[9]. 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/MS[10], Spectrophotometric method[13] have been reported.

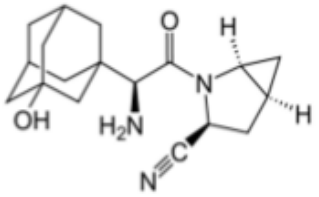

Fig. 1: structure of Saxagliptin

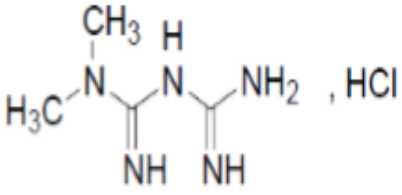

Fig. 2: structure of metformin hydrochloride

2. MATERIALS AND METHODS

Reagents and chemicals used

- ✓ Saxagliptin
- ✓ Metformin Hydrochloride
- ✓ Distilled water

Preparation of stock solution

Saxagliptin standard solution

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

Metformin Hydrochloride standard solution

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

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.

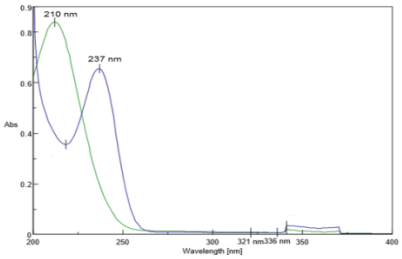

Fig. 3: wavelength maximum of Saxagliptin (210) and Metformin Hydrochloride (237)

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 λmax of one component λ1 and other being the wavelength of equal absorptivity of the two components λ2. i.e. isobestic point. A series of standard solutions of Saxagliptin and Metformin hydrochloride in the concentration range of 5-50 µg/ml and 2-16 µg/ml respectively were prepared and were measured at 210 and 227 (isobestic point). Calibration curves and absorptivity values were calculated.

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

$$C_x = \frac{Qm - Qx}{Qy - Qx} X \frac{A1}{ay1}$$

$$C_y = \frac{Qm - Qy}{Qx - Qy} X \frac{A1}{ax1}$$

$$Q_m = \frac{A_2}{A_1} ; \; Q_x = \frac{a_{x2}}{a_{x1}} ; \; Q_y = \frac{a_{y2}}{a_{y1}}$$

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

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.

1. Sensitivity

Metformin Hydrochloride standard stock solution

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

Working standard stock solution

Aliquots from stock solution was transferred into 8 separate 10 ml 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 16µg/ml.

Saxagliptin standard stock solution

10mg of Saxagliptin was accurately weighed and it was transferred into a clean, dry 100 ml 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 µg/ml.

Working standard stock solution

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

Determination

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

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 100 ml 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 1.6 ml and transferred into different 10 ml 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 10 mg of Saxagliptin 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 100 ml 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 5.0 ml into different 10 ml 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 where 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**.**

3. Accuracy

Preparation of standard stock solution

50 mg of Metformin Hydrochloride and 5 mg 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 100 ml 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 5 mg of Saxagliptin and 50 mg of Metformin Hydrochloride were weighed and finely powered. Accurately weighed amount of powder which is equivalent

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

Preparation of standard and sample mixture

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

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

Level III (120%): Volume of 1 ml sample stock solution and 1.2 ml standard stock solution was transferred into 10 ml 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.

4. Precision

Preparation of standard mixture solution

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

Working solution

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

A. 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.

B. 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.

RESULTS AND DISCUSSION

1. Sensitivity

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

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

Table 1: Sensitivity Data of Metformin Hydrochloride

Conc. µg/ml	Metformin Hydrochloride	
	Absorbance at 227	Sensitivity (µg/cm^3 /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. µg/ml	Saxagliptin	
	Absorbance at 227	Sensitivity (µg/cm^3 /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

2. Linearity and Range

The linearity in response for Metformin Hydrochloride and Saxagliptin was observed in the concentration range of 2-16 µg/ml and 5-50 µg/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 µg/ml	Absorbance at 210nm	Absorbance at 227 nm
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

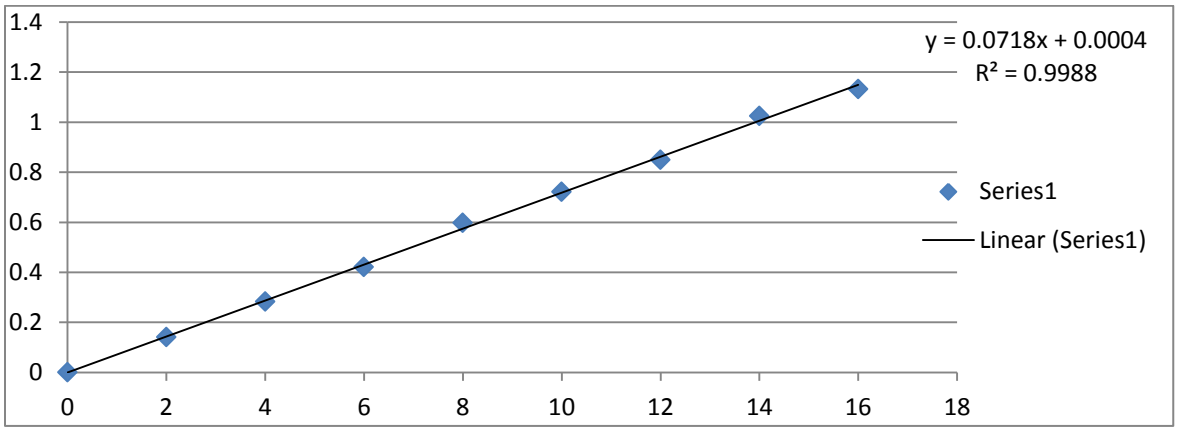

Fig. 4: Linearity range graph of Metformin Hydrochloride at 210nm

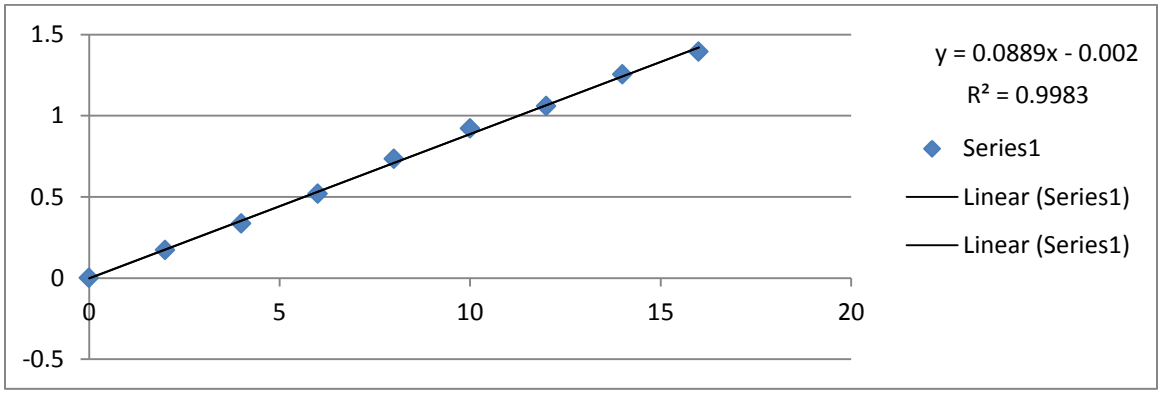

Fig. 5: Linearity range graph of Metformin Hydrochloride at 227nm

Table 4: Linearity Range Data of Saxagliptin

Volume of stock solution(ml)	Volume adjusted(ml)	Concentration µg/ml	Absorbance at 210 nm	Absorbance at 227 nm
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

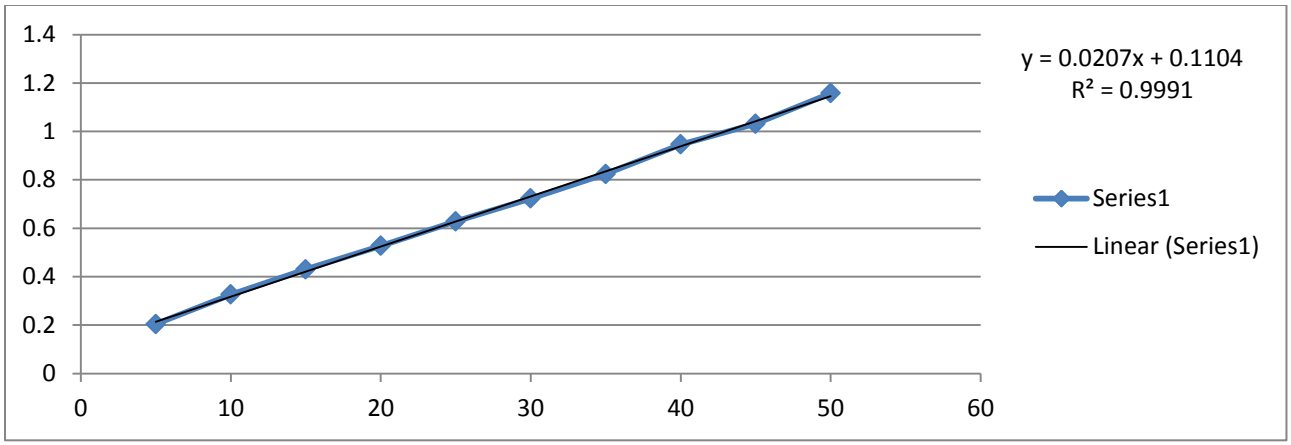

Fig. 6: Linearity range graph of Saxagliptin at 210nm

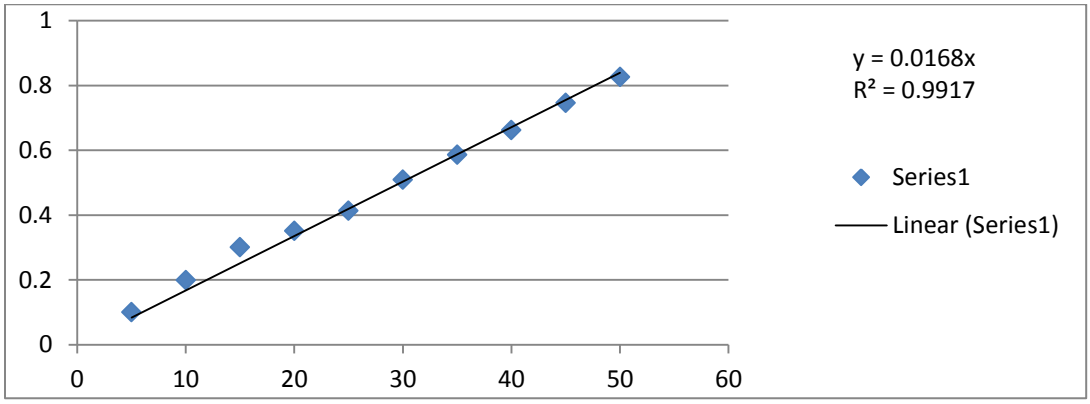

Fig. 7: Linearity range graph of Saxagliptin at 227nm

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	0.09974	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(µg/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.

Precision

System Precision

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

Method Precision

The absorbance of six determinations of working solution was taken at wavelengths 210 and 227 nm. 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.

Report

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

Table 7: Recovery Data of Standard Mixture

Level (%)	Sample conc. (µg/ml)		Total Conc. (µg/ml)		Amount of std recovered(µg/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

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

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

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

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

DISCUSSION

A combination of Metformin Hydrochloride and Saxagliptin is mainly used as Antihypertensive drugs. However, no method is so far reported for simultaneous estimation of these drugs in combined dosage form by Q- absorbance method.

- The linearity was determined of working standard solution and found to be in the concentration range of 5-50µg/ml for both saxagliptin and 2-16µg/ml of metformin hydrochloride . The regression equation for linearity was found to be Y = 0.0718x + 0.0004 for Metformin Hydrochloride and Y =0.0207x + 0.1104 for Saxagliptin. The linearity graph for both the drugs was satisfactory as observed from the correlation coefficient values which were 0.9988% and 0.9991 % for Metformin Hydrochloride and Saxagliptin respectively. The slope of the linearity graph was found to be 0.071 and 0.020 for Metformin Hydrochloride and Saxagliptin.
- The precision of method and system was determined of standard solution. In method precision the % RSD of the assay was found to be 0.0290% and 0.1248% for Metformin Hydrochloride and Saxagliptin. In system precision the % RSD was found to be 0.4356% and 0.4341% for Metformin Hydrochloride and Saxagliptin. For intraday precision the % RSD of the assay was found to be 0.0273% and 0.1269% for Metformin Hydrochloride and Saxagliptin. For inter-day precision the % RSD of the assay was found to be 0.1214%and 0.1451% for Metformin Hydrochloride and Saxagliptin. As all the values of % RSD for precision study obtained was within the acceptance criteria of less than 2%, the proposed method was found to be providing good degree of precision.
- The accuracy was determined through recovery study of the drug by spiking the standard drug of Metformin Hydrochloride and Saxagliptin at three different levels of 80 %, 100 % and 120 % with previously analyzed samples of known fixed concentration.
- The percentage recovery was found to be 99.43% to 99.62% for metformin hydrochloride and 99 % to 102.25% for

saxagliptin . The percentage recovery was in total agreement with acceptance criteria of 90 %-110 %.

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 5-50 µg/ml and 2-16 µg/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 90 – 110 %. 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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