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APPLICATION OF QUALITY BY DESIGN APPROACH IN UV-VIS SPECTROSCOPY METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF SAXAGLIPTIN HCL AND METFORMIN HCL IN ACTIVE PHARMACEUTICAL INGREDIENT

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ABSTRACT

A simple, accurate, precise and sensitive UV-Vis method has been developed for simultaneous estimation of Saxagliptin HCL and Metformin HCL in active pharmaceutical ingredient form by using quality by design (QbD) approach. A UV spectroscopic method was validated in terms of input variable effects on characteristics of UV spectrum were selected for critical parameters selection and parameters like linearity, accuracy, precision, robustness, ruggedness. The ICH guidelines Q8 to Q11 have discussed QbD implementation in API synthetic process and formulation development. Analytical QbD key tools are identification of critical quality attributes (CQA) with risk assessment, control strategy and Risk assessment, Analytical QbD method validation, continuous method monitoring (CMM) and continual improvement. The wavelength of 204.5nm and 232.5 nm are selected as absorbance maxima for Saxagliptin HCL and Metformin HCL respectively.

Keywords: Saxagliptin HCL, Metformin HCL, Quality by design, UV-Vis method

INTRODUCTION

Saxagliptin mainly used for the treatment of type 2 diabetes mellitus. Saxagliptin is in a class of medications called dipeptidyl peptidase-4 (DPP-4) inhibitors. Chemically

Saxagliptin is (1s, 3s, 5s)-2-[(2s)-2-amino-2-(3-hydroxyadamantan-1-yl) acetyl)-2-azabicyclo [3.1.0] hexane-3-carbonitrir le hydrochloride **(Figure 1)**. $C_{18}H_{25}N_3O_2$, H_2O is a molecular formula and the 351.9 g/mol is a molecular weight. In monotherapy or in combination with another drug for the treatment of type 2 diabetes Saxagliptin is used. Saxagliptin and Metformin combination therapy is more effective than monotherapy of Saxagliptin or Metformin **[1]**.

Metformin is the first-line medication for the treatment of type 2 diabetes, particularly in people who are overweight. It's another use is in the treatment of polycystic ovary syndrome which is not associated with overweight. Metformin is chemically N, N-Dimethylimidodicarbonimidic diamide. Its empirical formula is $C_4H_{11}N_5$ and molar mass is 129.164g.mol^{-1}. Metformin is generally a biguanide antihyperglycemic agent. It generally works by increasing the insulin sensitivity of body tissues and decreasing the glucose production by the liver. Quality by design is a essential part of the modern approach and a systemic approach for the pharmaceutical and quality development. Pharmaceutical quality can be assured by understanding, manufacturing variables and controlling formulation by Quality by design **(Figure 2)**.

Figure 1: Structure of Saxagliptin and Metformin

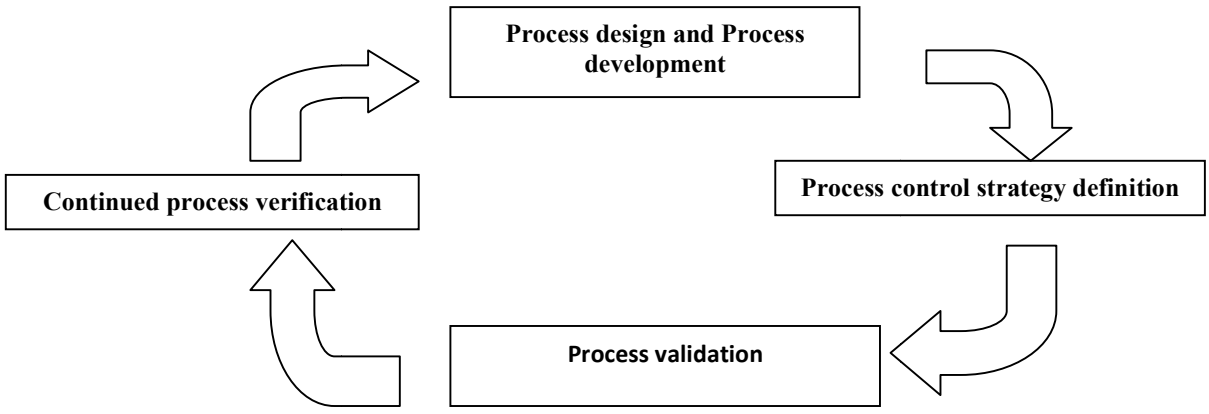

Figure 2: Quality by Design

According to ICH Q8 Quality by Design is a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and control based on sound science and quality risk management. QbD is a systematic approach for the product development by understanding various input variables (e.g. materials, process parameters) on final product. Thus the approach of QbD defines the input parameters of the appropriate ranges within which quality of the product is built into it. Simultaneous estimation of Saxagliptin and Metformin has also been developed using RP HPLC method in bulk and in tablet dosage form. A very precise care and typical reports are developed by using UV spectrophotometric method for the simultaneous estimation of Saxagliptin and Metformin in bulk and in tablet dosage form. In analytical QbD some factors may include the type of analytical technique use, reagents and solvents used instruments and instrumental parameters.

In analytical QbD approach the first stage is to set an analytical target profile (ATP) for the various methods. In UV spectrometric analytical method by implementing QbD approach, the various effects of method input variables on spectral shape, intensity of absorbance and absorbance maxima were studied and also critical parameters were choosen for the proposed method.

MATERIALS AND METHODS

Apparatus

A Shimadzu model 1800 double beam spectrophotometer with wavelength accuracy of ±0.1 nm and spectral width of 1nm and pair of matched quartz cell of 10 mm used to measure the absorbance of solutions. By using UV-Probe system software the spectra were automatically obtained.

Reagents and Materials

Saxagliptin and Metformin were purchased from Vishal Institute of Pharmaceutical Education & Research Centre, Ale. And all other chemicals used from analytical reagent grade.

Preparation of stock and working solution

The solubility of Saxagliptin HCL in different solvents such as distilled water, methanol, DMSO, ethyl acetate was determined. The maximum solubility of Saxagliptin was found to be in distilled water. Hence the distilled water is used for the preparation of standard stock and working solutions. And in case of Metformin HCL, the solubility in different solvents such as ethanol, DMSO, distilled water, 0.1 M HCL. The maximum solubility of Metformin was found to be in distilled water. Hence distilled water is used for the preparation of

standard stock and working solutions. The stock solution was prepared by dissolving 25 mg of Saxagliptin and Metformin and transferred to two separate 25 ml volumetric flask and make the volume upto mark with distilled water and final concentration of solution containing 1000µg/ml concentration. Standard working solution of 10µg/ml was prepared by dilution from stock solution of Saxagliptin and Metformin **[2]**.

Determination of wavelength of maximum absorption

Standard working solution (10µg/ml) of Saxagliptin and Metformin was scanned from 200-400 nm in the UV spectrophotometer for the selection of analytical wavelength. Saxagliptin showed maximum absorbance (λmax) at 204.5 nm and Metformin showed maximum absorbance (λmax) at 232.5 nm **(Figure 3)**. Hence these two wavelengths selected as an analytical wavelengths **[2, 3]**.

Implementation of AQbD approach in the development of the analytical method

For the development of AQbD approach, the Ishikawa diagram was used to study the relationship between different variable input parameters and the characteristics of method performance of the spectrophotometric analytical methods **(Figure 4) [2, 4]**.

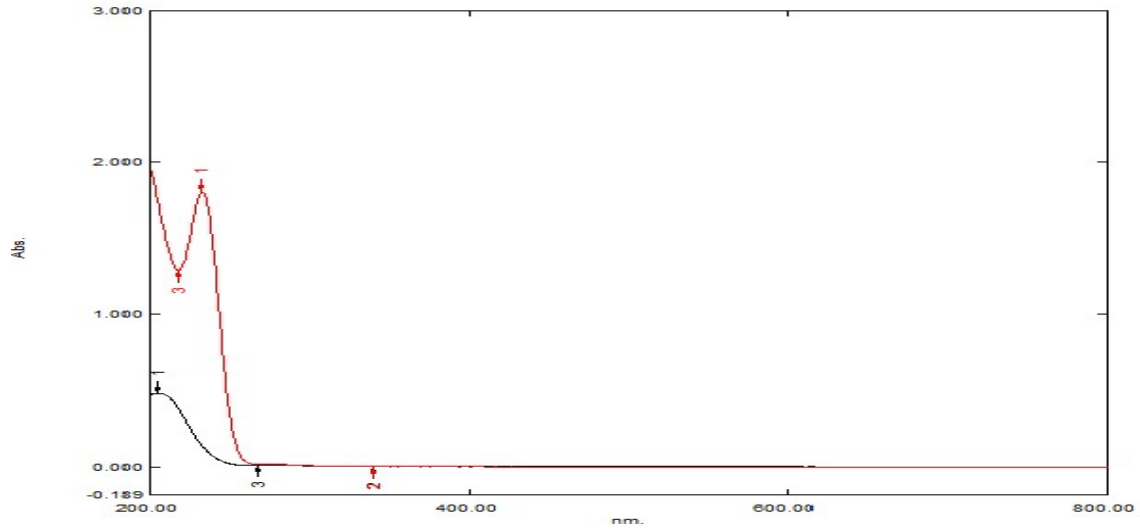

Figure 3: UV spectra of Saxagliptin and Metformin

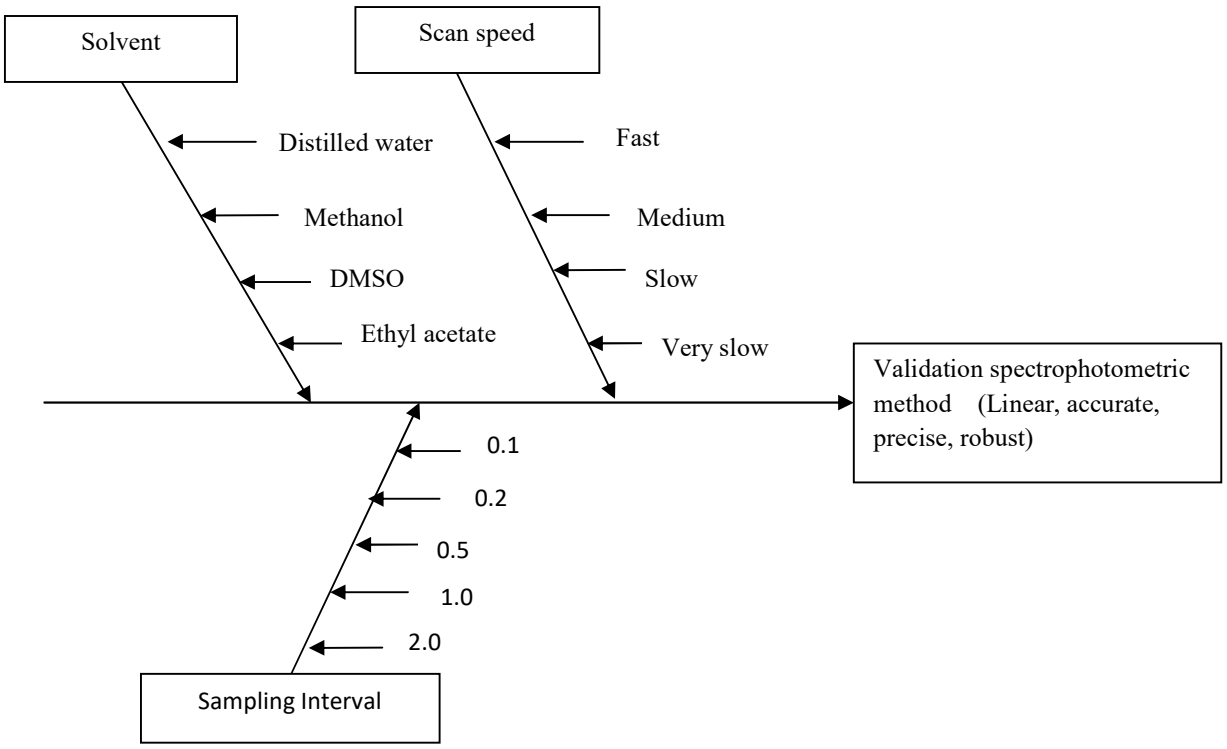

Figure 4: Ishikawa diagram for Saxagliptin showing the relationship between input parameters and the method performance characteristics of spectrophotometric analytical methods

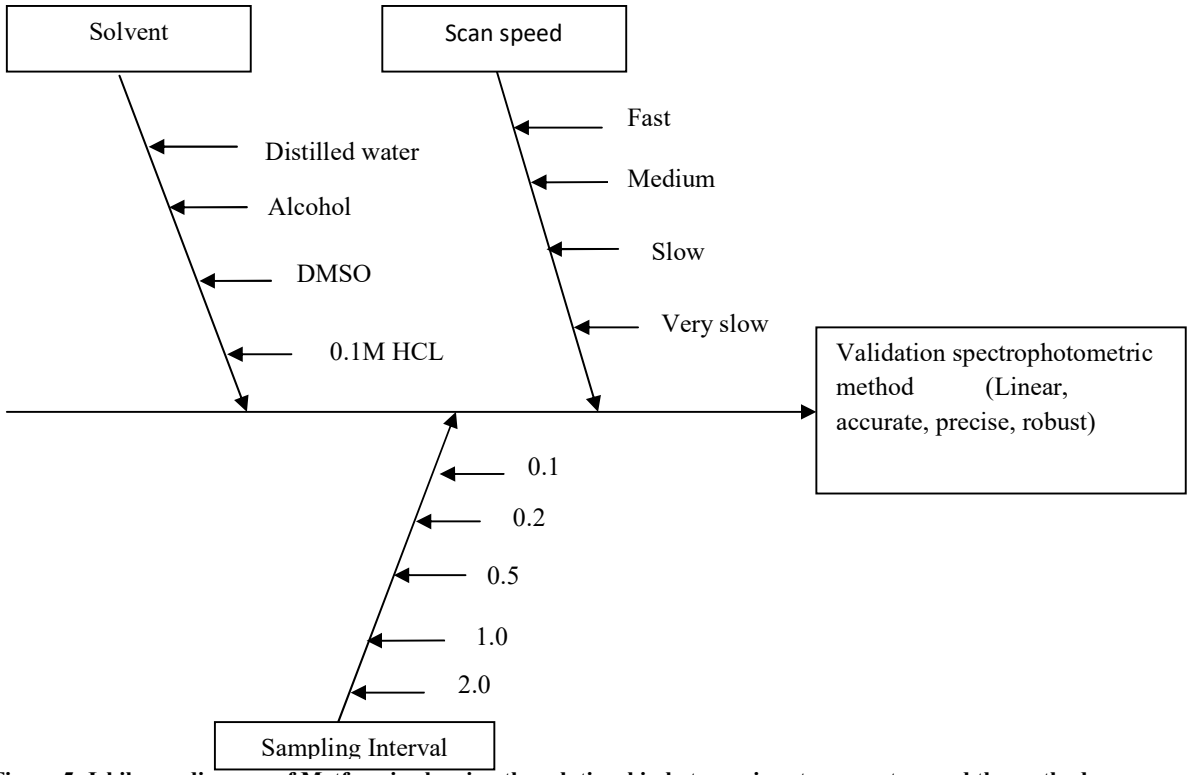

Figure 5: Ishikawa diagram of Metformin showing the relationship between input parameters and the method performance characteristics of spectrophotometric analytical methods

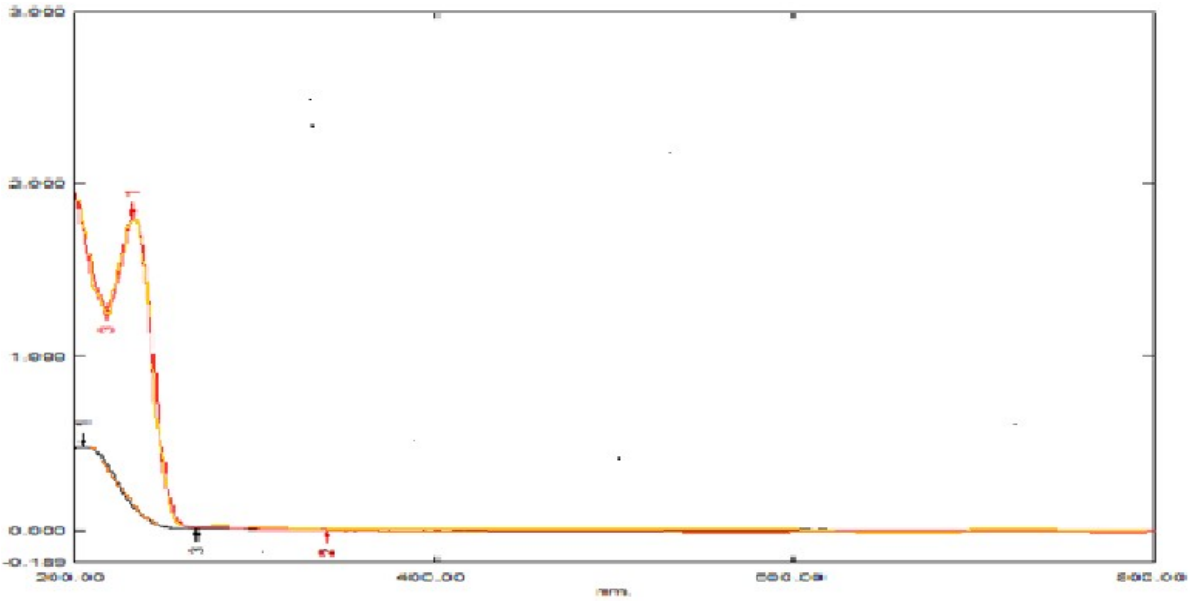

Figure 6: Overlay UV spectrum at varied scan speed (fast, medium, slow, and very slow) and varied sampling interval (0.1, 0.2, 0.5, 1.0, and 2.0 nm)

Simultaneous estimation of Saxagliptin HCL and Metformin HCL

The analysis of simultaneous estimation method is based on the absorption of drugs Saxagliptin and Metformin at their wavelength maxima. Two wavelengths are selected for the development of the simultaneous equations is 204.5 nm and 232.5 nm. The absorptivity values determined are for Metformin are 0.1591 (ax_1), 0.0348 (ax_2) and for Saxagliptin 0.09494 (ay_1), 0.05518 (ay_2) at 232.5 nm and 204.5 nm, respectively. These values are obtained from average of six estimations. A_1 and A_2 are the absorbances of samples at the 232.5 nm and 204.5 nm respectively; Cx and Cy are the concentration of Saxagliptin and Metformin respectively.

$$Cx = A_2ay_1 - A_1ay_2 / ax_2ay_1 - ax_1ay_2 \quad \text{eqn.1}$$

$$Cy = A_1ax_2 - A_2ax_1 / ax_2ay_1 - ax_2ay_1 \quad \text{eqn.2}$$

Validation.

In order to achieve analytical target profile of method, the selected critical parameters should comply with method performance characteristics of an analytical method. For an analytical method in ICH guidelines Q2 (R1), the ICH has laid down various method performance characteristics. According to ICH Q2 (R1) on the selected critical parameters in order to implement AQbD approach, the spectrophotometric analytical method is an appropriate validating method. The characteristics studied were linearity, precision, accuracy, Limit of detection (LOD), Limit of quantification (LOQ), robustness and ruggedness **[2, 5]**.

Linearity

According to guideline of ICH, the linearity of analytical procedures determines that test results are directly proportional to the concentration of an analytical sample. For linearity study, five solutions of different concentrations (2, 4, 6,8,10µg/ml) were prepared in distilled water from stock solutions of Saxagliptin and Metformin. The calibration curve was constructed by plotting the peak of absorbance versus concentration, using linear regression analysis **[2, 6]**.

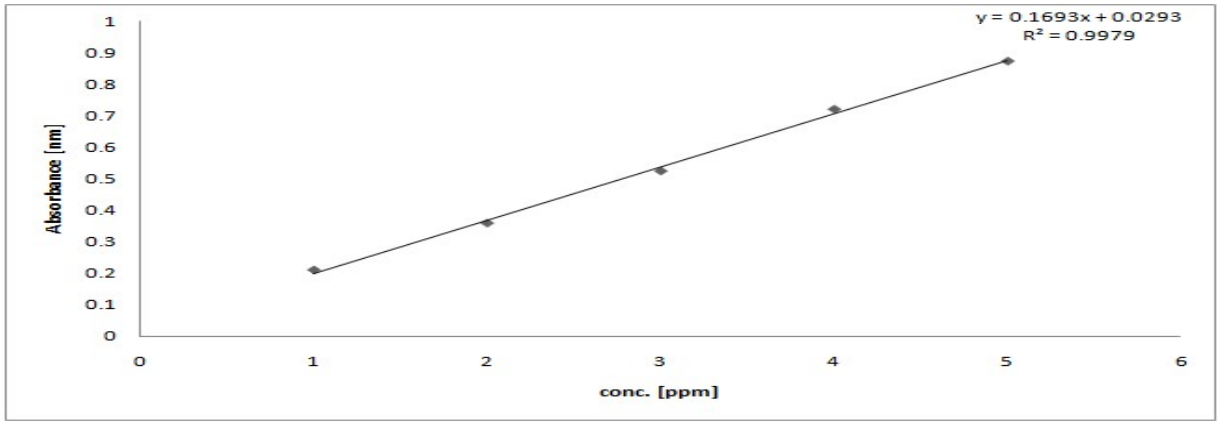

Figure 7: Calibration curve of Saxagliptin HCL

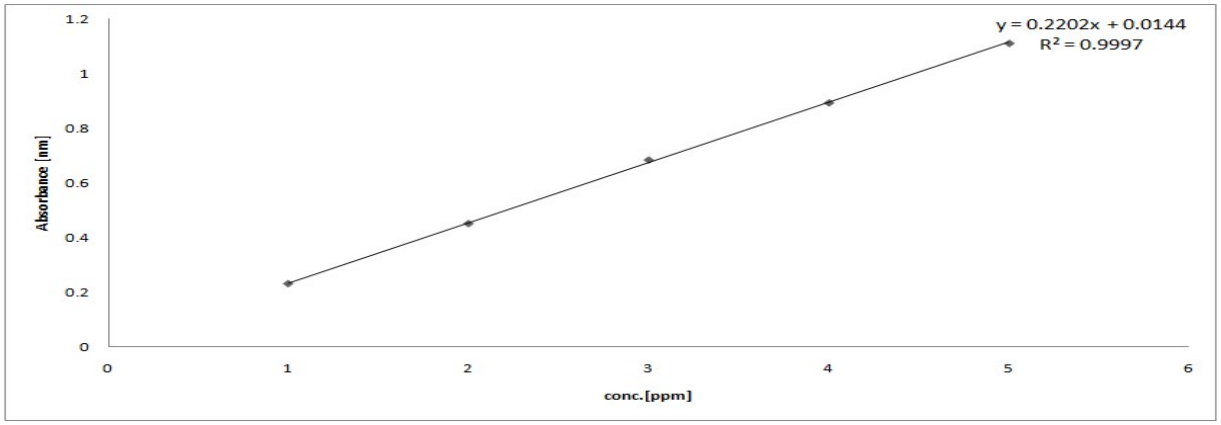

Figure 8: Calibration curve of Metformin HCL

Precision

The repeatability studies were carried out estimating the response of Saxagliptin (6µg/ml) and Metformin (6µg/ml) six times and results are reported in terms of standard deviation. The intra-day studies were carried out by estimating the corresponding responses on the same day for three times, and the results are reported in terms of relative standard deviation **[3]**.

Detection limit

The procedure is the lowest amount of analyte in sample which can be detected but not necessarily quantitated as an exact value

means the detection limit (LOD). It can be determined by:

$$LOD = \frac{3.3\sigma}{S}$$

Where

σ = response of relative standard deviation.

S = slope of calibration curve.

Quantitation limit

The lowest amount of analyte in sample, which can be quantitatively determined with a suitable precision and accuracy, means the analytical procedure of quantitation limit (LOQ). It can be determined by:

$$LOQ = \frac{10\sigma}{S}$$

Where

σ = response of relative standard deviation.

S = slope of calibration curve.

Robustness

As per USP, robustness measures the capacity of an analytical method to remain unaffected by small but deliberate variations in method parameters. It can be determined by using a replicates of solution (8µg/ml) at six times and the results are reported in terms of mean of percentage relative standard deviation.

Ruggedness

The method of ruggedness were determined by carrying out the method by the three different analyst and taking the absorbance and results are reported in terms of mean of percentage relative standard deviation **[3-10]**.

Accuracy

The method of accuracy was carried out by recovery study from API at three different level such as 80%, 100% 120%. Percentage recovery should be within 98%-102% **[4, 11-15]**.

Table 1: Recovery Studies

Amount of API sample used for Metformin	Theoretical amount added(%)	Obtained Metformin (µg)	%Recovery	Amount of API sample used for Saxagliptin	Obtained Saxagliptin (µg)	%Recovery
4 µg	80	3.22	100.62	2 µg	1.58	98.75
4 µg	100	3.98	99.5	2 µg	1.98	99.00
4 µg	120	4.81	100.20	2 µg	2.41	100.41
	MEAN % RECOVERY		100.10	MEAN % RECOVERY		99.38

RESULTS AND DISCUSSION

Parameters	Saxagliptin HCL	Metformin HCL
Wavelength	204.5 nm	232.5 nm
Linearity Range (µg/ml) Slope (m) Intercept (c)	 2-10 0.1693 0.0293	 2-10 0.2202 0.0144
Correlation Coefficient (r^2)	0.9979	0.9997
Slope(m)	0.1693	0.2202
Intercept (C)	0.0293	0.0144
Precision Intra Day	 0.3436	 0.0267

Inter Day	**0.9846**	**0.2698**
LOD (µg)	**9.72**	**7.72**
LOQ (µg)	**29.46**	**23.42**
Robustness	**0.2818**	**0.2991**
Ruggedness	**0.0660**	**0.1670**

CONCLUSION

A simple, rapid, sensitive, accurate, precise and inexpensive spectrophotometric method was developed for estimation of Saxagliptin hydrochloride and Metformin Hydrochloride in bulk by using analytical quality by design" (AQbD) approach. After investigation of the effect of method input variables on absorbance response, the critical parameters have been selected for proposed method and then it was further validated as per the ICH guidelines. The method having economic advantage over a chromatographic method thus the developed method not involves complexity. Therefore, developed spectrophotometric method can be used flexibly and efficiently for the determination of simultaneous estimation of Saxagliptin Hydrochloride and Metformin Hydrochloride either in bulk or in the dosage formulations.

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Conflict of Interest

Author doesn't have any conflict of interest

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