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QUALITY BY DESIGN APPROACH FOR THE DEVELOPMENT AND VALIDATION OF SAXAGLIPTIN BY RP-HPLC WITH APPLICATION TO FORMULATED FORMS

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ABSTRACT: Quality by Design (QbD) is a philosophy that refines the level of knowledge associated with a product that uses process understanding to deliver a product with the desired critical quality attributes. The objective of this present study was to develop and demonstrate an integrated multivariate approach to develop and quantify the constituent concentrations of saxagliptin drug in its pure and formulated forms. The method was developed using a mobile phase acetonitrile: water (pH3), (20:80 v/v) on an Agilent, TC C18 (250 × 4.6 mm) 5μm column and flow rate 1.0 ml/min which was optimized with help of design expert software and validated according to ICH Q2 guideline and application of this method to different stress condition of saxagliptin. The detector linearity was established in concentrations ranging from 10-90μgmL-1, the regression coefficient was 0.9993. The method fulfilled validation criteria and was shown to be sensitive, with limits of detection (LOD) and quantitation (LOQ) of 0.003 and 0.010μgmL-1, respectively. The percentage relative standard deviations for robustness and ruggedness were observed within the range of 0.28 and 0.57. The drug was subjected to acidic, alkali, neutral, oxidative, heat and photo degradation stress. Drug shows significantly degraded in acidic and alkali condition while remain stable in other conditions. The high recovery and low relative standard deviation confirm the suitability of these methods can be employed for the routine analysis of tablet containing saxagliptin.

INTRODUCTION: Quality by design is a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management [1].

The objective of the QbD initiative is to demonstrate both understanding and control of pharmaceutical processes to deliver high quality pharmaceutical products while affording opportunities for continuous improvement. QbD delivers a better understanding of method capabilities and limitations and ensures a superior chance of successful downstream method validation and transfer. It has become an important paradigm in the pharmaceutical industry since its introduction by the US Food and Drug Administration [2–4]. The QbD concept can be extended to analytical methods [5–8]. The analytical

methods used for the analysis of active pharmaceutical ingredients (API) and drug products are an integral part of the QbD.

Saxagliptin (**Fig.1**) is chemically (1S, 3S, 5S) -2- [(2S) -2-amino-2- (3-hydroxy -1-adamantyl)acetyl] -2-azabicyclo [3.1.0]hexane-3-carbonitrile is oral hypoglycemic (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. DPP-4 inhibitors represent a new therapeutic approach to the treatment of type 2 diabetes mellitus. They inhibit the inactivation of incretins, resulting in the stimulation of glucose-dependent insulin release and the reduction of glucagon levels [9].
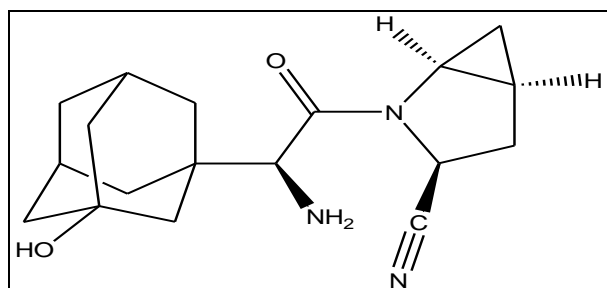

FIG. 1: CHEMICAL STRUCTURE OF SAXAGLIPTIN

Several analytical methods have been developed for quantitative analysis of Saxagliptin in bulk and pharmaceutical formulations like UV-spectrophotometric method[10-13], Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method [14-18], RP-HPLC with fluorescence detection method in human plasma [19], High Performance Thin Layer Chromatographic Method [20]. Ultra performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS) method has been developed for quantification of Saxagliptin in rat plasma [21]. Liquid chromatography and tandem mass spectrometry (LC-MS/MS) method has been developed and validated for simultaneous analysis of Saxagliptin and its major metabolite [22].

LC-ESI-MS/MS method has been developed for estimation of saxagliptin and its forced degradation products [23]. Method development approach with RP-HPLC specifically focused on pharmaceutical development in a QbD environment for saxagliptin has not been reported anywhere. Therefore, there is an unmet need to investigate a systematic HPLC method development approach for pharmaceutical

development using QbD principles to ensure the quality of the method throughout the product lifecycle. The primary objective of this study was to implement QbD approach to develop and validate an RP-HPLC method that could separate drug in the bulk and formulated forms from its potential related substances and to establish an in depth understanding of the method and build in the quality during the method development to ensure optimum method performance over the lifetime of the product with a suitable degradation data.

MATERIALS AND METHODS:
Chemical and reagents: A sample of saxagliptin was obtained as a gift from Neul and laboratories ltd., Hyderabad, Telangana, India. Saxagliptin containing tablets; Onglyza (5 mg) (AstraZeneca UK Limited), were procured from the local market. All HPLC-grade acetonitrile was obtained from Merck, Mumbai, India. Hydrochloric acid, sodium hydroxide and hydrogen peroxide (30%) were obtained from Merck, India. Doubly distilled water was used throughout the investigation.

Instrumentation and chromatographic conditions:
The HPLC system, Agilent 1120 compact with manual Rheodyne injector facility operates at 20μL capacity per injection was used. The instrumentation was controlled by use of EZChrom Elite Compact software. The column used was Agilent TC C18 (250 X 4.6 mm) 5μm and the detector consisted of UV/VIS. Optimization of chromatographic conditions was carried out using water (pH3 with ortho phosphoric acid): acetonitrile (80:20 v/v) as mobile phase. The analysis was carried out under gradient conditions using flow rate 1.0 mLmin^{-1} at 18^{0}C and chromatograms were recorded at 211 nm.

Preparation of standard stock solution:
Weighed accurately 10 mg of saxagliptin and transferred to 100 ml volumetric flask, add 25 mL of mobile phase and sonicate for 15 min and volume was made up to mark with mobile phase (100μgmL-1).

Procedure for preparation of calibration curve
Working solutions containing 10–90 μgmL-1 saxagliptin were prepared by serial dilutions of

aliquots of the stock solution. Aliquots of 20 μL were injected (six injections) and eluted with the mobile phase under the reported chromatographic conditions. The average peak area versus the concentration of saxagliptin in μg/ml was plotted. Alternatively, the corresponding regression equation was derived using mean peak area concentration data and the concentration of the unknown was computed from the regression equation.

Preparation of Sample Solutions:
Twenty tablets of ONGLYZA were weighed and finely powdered. An accurately weighed powder equivalent to 10mg was taken to 100 mL volumetric flask and dissolved in 50 ml diluent and finally volume was made up to the mark with the diluent to obtain a concentration of 100 μgmL−1. This solution was sonicated for 10 min and filtered. Filtration is done to remove the excipients and to avoid clogging of sample applicator syringe. The amount of drug present in the sample solutions were determined using calibrated curves of standard saxagliptin in **Table 3**.

Method validation: [24]
Accuracy and Precision:
To determine the accuracy and intra-day precision, pure saxagliptin solutions at three different concentrations were analyzed in six replicates during the same day. The same procedure was followed in different days for the inter day precision. Mobile phase was injected as blank solution before sample injection and the RSD (%) values of peak area and retention time were calculated.

Limits of Detection (LOD) and Quantification (LOQ):
The LOD and LOQ were obtained by signal to noise (S/N) ratio method. LOQ and LOD were obtained by a series of dilutions of the saxagliptin stock solution. Precision study was performed at LOQ level also. LOQ solution was injected six times and calculated the % RSD values for the obtained peak area and retention time.

Linearity:
Linearity of test solutions for the assay method was prepared from saxagliptin stock solution at nine concentration levels from 10 to 90% of assay analyte concentration (10, 20, 30, 40, 50, 60, 70, 80 and 90μgmL−1). The peak area versus concentration data was treated by least-squares linear regression analysis. Linearity test solutions for the method were prepared by diluting stock solution to the required concentrations.

Robustness and Ruggedness:
To determine the robustness of the method the experimental conditions were deliberately changed. The flow rate of the mobile phase (1.0 ± 0.2mLmin^{-1}), column oven temperature (25 ±2℃), mobile phase composition (actual ±10%), and detection wavelength (211 ± 1nm) were the varied parameters. In each case the %RSD values were calculated for the obtained peak area and retention time. The number of theoretical plates and tailing factors were compared with those obtained under the optimized conditions. Three different columns of same dimensions were used for the analyses. The studies were performed on the same day and on three different days by three different analysts for three different concentrations of saxagliptin (triplicate injections). The area obtained from each concentration was compared with that of the optimized one. The relative standard deviation values were evaluated for each concentration.

Force Degradation Studies:
All stress decomposition studies were performed at drug concentration of 10μgmL−1 in mobile phase. Acid hydrolysis was performed in 0.1M HCl at 80℃ for 2 h. The study in alkaline condition was carried out in 0.1M NaOH at 80℃ for 3 h. The study in neutral condition was carried out in water at 80℃ for 3h. Oxidative studies were carried out at 80℃ and 3% hydrogen peroxide for 2 h. For photolytic degradation studies, pure drug in solid state was exposed to 1.2 million lux hours in a photo stability chamber [33]. Additionally, the drug powder was exposed to dry heat at 105℃ for 2h. Samples were withdrawn at appropriate time and subjected to HPLC analysis after suitable neutralisation and dilution.

RESULTS:
The method development consisted of two phases: the first is screening and the second is optimisation.

Screening: Solubility of the drug is the main criteria for any method to develop and then the UV response of the product at varying wavelengths. Saxagliptin was found soluble in an acetonitrile. A $50\mu gmL^{-1}$ solution showed excellent response at 211nm when it was scanned from 400 to 200 nm in a UV spectrophotometer. In HPLC, variables such as mobile phase, buffer, temperature, sample concentration, and pH were varied by keeping one parameter (stationary phase) constant. Summarized observations are made as per the **Table 1**.

TABLE 1: SCREENING: SUMMARY OF METHOD SELECTION

| S. no.	Column	Observations	Remarks
1.	ZORBAX C18 (250 × 4.6 mm),5μm	Asymmetrical peak	Not satisfactory
2.	ZORBAX C8 (150 × 4.6 mm),5μm with tailing	Asymmetrical peak	Not satisfactory
3.	ZORBAX Cyano (150 × 4.6 mm),5μm	Split peak	Not satisfactory
4.	Eclipse XDB-C18 (150 × 4.6 mm),5μm	Asymmetrical peak	Not satisfactory
5.	Agilent TC-C18 (250 × 4.6 mm),5μm	Symmetrical peak	satisfactory

Optimisation:
Agilent TC C18 (250 X 4.6 mm) 5µm column, was found suitable and the fine tuning of the method was performed with suitable adjustment of pH. All the trials are as shown in **Table 2** and chromatograms are as shown in **Fig.2**.

TABLE 2: FINAL OPTIMIZATION OF pH WITH AGILENT TC C18 (250 × 4.6 MM) 5MM COLUMN
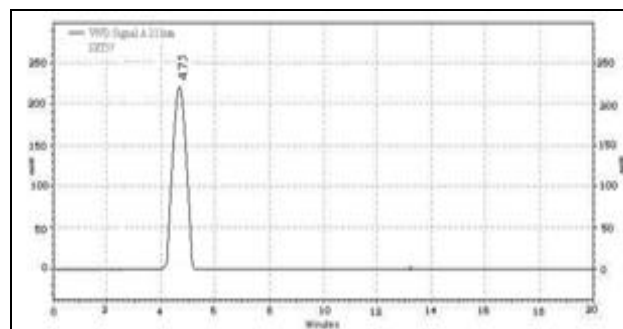
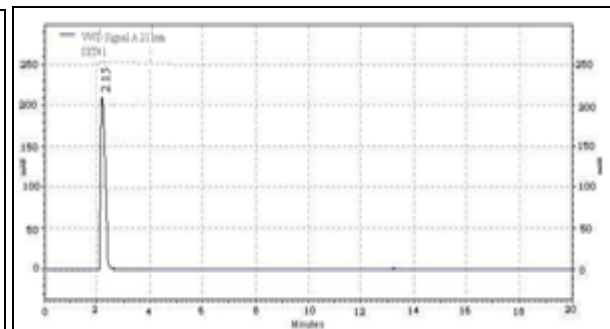| Sr. No.	Trails Taken	Observations	Remarks
1.	ACN: water (pH3) (20:80v/*v*)	Peaks found symmetrical	Satisfactory
2.	ACN: water (pH3.5) (20:80v/*v*)	Broad peak	Not satisfactory
3.	ACN: water (pH4) (20:80 v/*v*)	Broad peak and late elution	Not satisfactory
4.	ACN: water (pH5) (20:80 v/*v*)	Broad Peak and elute before1min	Not satisfactory
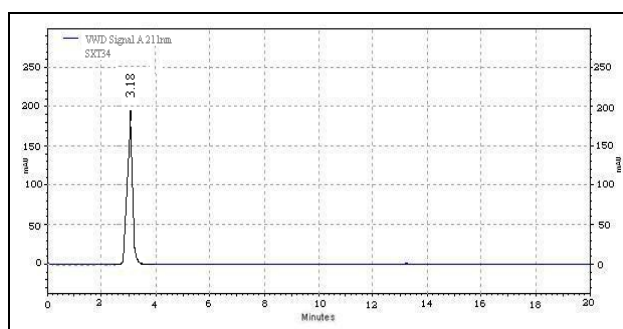
(a) ACN:Water (pH3) (20:80%v/v)
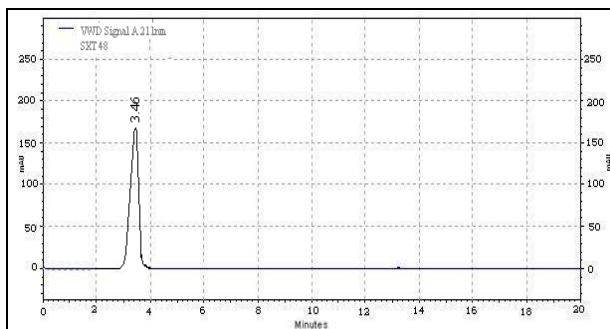
(b) ACN: Water (pH3.5) (20:80%v/v)

(c) ACN: Water (pH4) (20:80%v/v)

(d) ACN: Water (p5) (20:80%v/v)

FIG.2: METHOD OPTIMIZATION AT DIFFERENT pH CONDITIONS.

Final Method Conditions:

Column: Agilent TC C18 (250 X 4.6 mm) 5µm, Oven temp.: 25ºC, Mobile phase: ACN: Water (pH3 with ortho phosphoric acid) (20:80%v/v), Runtime: 10min, Flow rate: 1mL/min, Wavelength: 211nm.

TABLE 3: ASSAY DATA OF MARKETED FORMULATION

Component	Formulation	Amount present (mg)	Amount found (mg)	% Estimated	%RSD
Saxagliptin	Onglyza	5	4.98	99.60	0.57
		5	5.02	100.40	1.98
		5	5.01	100.20	1.83

Validation of the Method:

The described method for the assay of saxagliptin was validated as per the current ICH Q2 (R1) guidelines.

TABLE 4: LINEARITY AND REGRESSION PARAMETERS WITH PRECISION DATA

Parameters	Values
Linear range, μgmL^{-1}	10-90
Limit of quantification(LOQ), μgmL^{-1}	0.003
Limit of detection(LOD), μgmL^{-1}	0.010
Regression equation	$Y = 384792x + 421527$
Slope(b)	384792
Intercept (a)	421527
Correlation coefficient (r^2)	0.9993

Linearity:

A calibration curve was obtained for saxagliptin stock solution. There sponse of the drug was found to be linear in the investigation concentration range from 10 to 90μg mL-1 and the linear regression equation was $Y=384792x+ 421527$ with correlation coefficient of 0.9993, where Y is the mean peak area. The LOD and LOQ values and their standard deviations were evaluated and presented in **Table 4**. These results confirm the linear relation between the mean peak area and concentration as well as the sensitivity of the method.

Accuracy and Precision:

The percentage relative error which is an index of accuracy is ≤0.259andisindicativeofhigh accuracy. The calculated percentage relative standard deviation (%RSD) can be considered to be satisfactory. The results obtained for the evaluation of precision and accuracy of the method is compiled in **Tables 5** and **6.**

TABLE 5: RESULTS OF ACCURACY STUDY ($n = 6$).

Concentration Injected (µg mL^{-1})	Concentration found (µg mL^{-1}) ± SD	Recovery (%)	RSD (%)	SEM
10	10.01 ± 0.026	100.10	0.259	0.0086
18	18.04 ± 0.020	100.22	0.110	0.0066
20	19.99 ± 0.036	99.95	0.180	0.0120
22	22.03 ± 0.050	100.14	0.228	0.0167

TABLE 6: RESULTS OF PRECISION STUDY

Conc^n (µg mL^{-1})	Repeatability (intra-day precision)			Intermediate precision (inter-day)		
	Mean area ± SD (n = 3)	SEM	RSD (%)	Mean area ± SD (n = 3)	SEM	RSD (%)
10	3496023.3±2099.5	1212.10	0.07	3490987.1 ± 11189.8	686.92	0.04
50	18887993.1±1288.4	734.86	0.02	18879743.3 ± 1505.3	869.0	0.02
90	34815838.1 ±1154.7	666.65	0.01	34799984.6 ± 1969.0	1136.8	0.02

Robustness and Ruggedness:
Analytical methods need to be robust so that they can be used routinely without problems and can be easily transferred for use in another laboratory, if necessary. The intrinsic robustness of the HPLC method depends on a range of variables related to the method parameters, such as mobile phase composition (actual ±10%), flow rate (1.0 ± 0.2mL), temperature (25 ±2ºC), wavelength (211 ± 1nm), and so forth. No significant effect was observed on system suitability parameters such as

theoretical plates, tailing factor, capacity factor, and % RSD of, when small but deliberate changes were made to chromatographic conditions. The RSD values ranged from0.28 to 0.57% resume the robustness of the proposed method.

In method ruggedness, different columns (different lots with the same manufacturer), days, and analysts (*n*=3) were performed. There results were summarized in **Table 7**.

TABLE 7: RESULTS OF METHOD ROBUSTNESS AND RUGGEDNESS STUDY.

Mobile phase composition (acetonitrile–water)			Mean area ± SD (n = 3)	SEM	Mean tR ± SD (min)	%RSD
Original	Used	Level				
	18:82	-2	3495022.6 ±1165.5	388.50	3.15±0.011	0.34
20:80	20:80	0	3382742.6 ± 1627.4	542.46	3.18±0.017	0.53
	22:78	+2	3456744.3 ±1907.1	635.70	3.26±0.021	0.64
			Flow rate (mL min−1)			
	0.8	-2	3498833.3 ±1573.3	524.43	3.17±0.012	0.37
1.0	1.0	0	3394801.3 ±1151.9	383.96	3.18±0.015	0.47
	1.2	+2	3554425.0 ±1847.4	615.80	3.21±0.010	0.31
			Temperature(^{0}C)			
	23	-2	3413937.5±1562.0	1104.5	3.22±0.014	0.43
25	25	0	3275772.5±1373.9	971.50	3.18±0.009	0.28
	27	+2	3452723.5±2406.3	1701.5	3.25 ±0.011	0.34
			Wavelength(nm)			
	210	-1	3509060.5±1299.0	918.50	3.12±0.015	0.48
211	211	0	3425812.5±1317.3	931.50	3.18±0.012	0.37
	212	+1	3482523.5±1977.8	1398.5	3.32±0.019	0.57

Selectivity:
Selectivity of the method was evaluated by injecting the mobile phase, placebo blank, pure drug solution and tablet extract. No peaks were

observed for mobile phase and placebo blank and no extra peaks were observed for tablet extracts (**Fig.3 (a) and 3 (b)**).


(a) Placebo blank   (b) Tablet extract
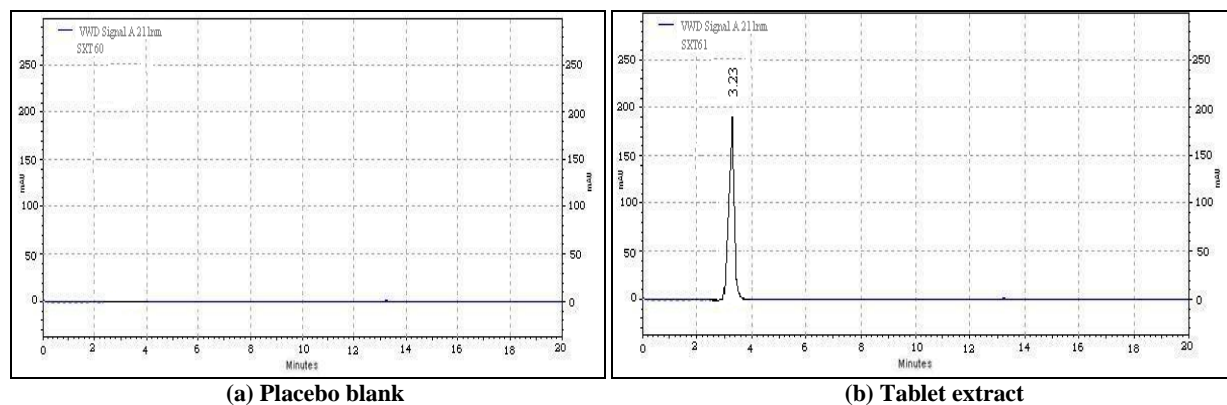
FIG. 3: CHROMATOGRAMS OBTAINED FOR PLACEBO BLANK AND TABLET EXTRACT

Stress Study:
The degradation was performed as per the current practice of ICH guidelines [33]. Saxagliptin was found to be sensitive towards acid and alkali

hydrolysis. The drug degraded up to 23% with acid (0.1N HCl) and 15% with base (0.1N NaOH). The drug was found to be more stable under oxidation (5% H_2O_2), thermal (105^{0}C for 3 hours), neutral

degradation (water), and photolytic (1200K lux hours) conditions rather than under alkali stress conditions. No significant changes (<1%) were observed for the chromatographic responses for the solutions analyzed with other stress conditions. Following are the % degradation pattern of saxagliptin in oxidation, thermal, neutral, and photolytic conditions: 0.12, 0.05, 0.01, and 0.32. **Fig.4** shows the degradation chromatograms of saxagliptin with the corresponding solvent as blank.

(a) 0.1N HCl degradation   (b) 0.1N NaOH degradation
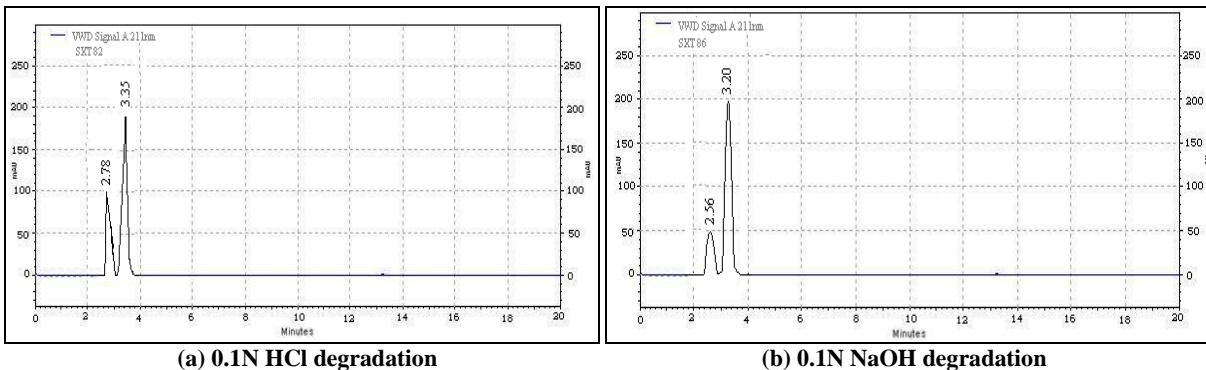
FIG. 4: CHROMATOGRAMS OBTAINED FOR FORCED DEGRADATION

CONCLUSION: A gradient RP-HPLC method was successfully developed for the estimation of saxagliptin in pharmaceutical dosage form. The QbD project aims to encourage debate about quality in the complete development of the drug in a systematic manner. From a solid-state point of view, QbD implementation involves the design of manufacturing processes based on a thorough scientific understanding of the properties and stability of the components of the drug at critical points throughout the development. The experimental design describes the scouting of the key HPLC method components including column, temperature, pH, and mobile phase. The inter-relationships are studied and the preliminary optimized conditions are obtained for each combination. Moreover, this approach ensures better design of products with fewer problems in development, reduces the number of trials required for post market changes, relies more on process and understanding and mitigation of risk, allows implementation of new technology to improve manufacturing without regulatory scrutiny, and enables possible reduction in overall costs of manufacturing resulting in less waste.

The validated method is specific, linear, precise, accurate, robust, rugged, and stable for 24 hours and can be applied for the determination in formulated form. The drug is stable in oxidative, thermal, photolytic and hydrolytic conditions and degrades in acidic, basic conditions. The potential of this QbD approach lies for the simultaneous determination of multiple forms of saxagliptin like pure drug, dosage forms and thus it should be implemented.

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