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Development and Validation of an RP-HPLC Method for Simultaneous Estimation of Saxagliptin and Metformin in Bulk and Pharmaceutical Dosage Forms Based on Quality by Design

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ABSTRACT

Using a quality by design (QBD) and multi-criteria decision making (MCDM) approach, this paper describes a recently developed, optimized, and validated isocratic RP-HPLC strategy for the separation of two anti-diabetic drugs Saxagliptin (SAX) and Metformin (MET) in bulk and pharmaceutical formulations. The effective chromatographic separation was accomplished by utilizing the onyx monolithic C18 segment (100×4.6 mm id, 5µm molecule size) and PDA-UV- detection at 210nm. MeOH was used at a concentration of 40–50% v/v, pH was 3.5–4.5, and flow rate was 0.3–0.5 ml/min for the streamlining. Methanol (MeOH), acetonitrile (ACN), pH 3.50.5 balanced with the diluted orthophosphoric acid solution, flow rate of 0.484 ml/min, and pH 3.946 were chosen as the ideal assay conditions. Peak area ratio of the analyte was utilized for the evaluation of pharmaceutical formulation tests. Total chromatographic analysis time per sample was approximately 4.33 min with MET and SAX eluting with retention times of 3.3 and 4.4 minutes respectively. For the quantitative analysis of (RIAX-M) tablets containing Saxagliptin-SAX and Metformin-MET, the optimal assay scenario was utilized after being validated in accordance with ICH recommendations. The validation investigation confirmed that the test was specific, accurate, linear, precise, and robust, supporting the determination of the assay conditions. Therefore, this RP-HPLC method can be used as a routine quality control analysis of gliptin derivative like SAX in combination with metformin.

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INTRODUCTION

Defects in insulin secretion, insulin activity, or both make diabetes which is described by hyperglycemia. Characterization of Diabetes mellitus was broadly acknowledged as IDDM or Type 1, and NIDDM or Type 2 which was distributed by WHO in 1980 [1]. DPP-4 inhibitors are the most recent medications which work by hindering the activity of DPP-4, an enzyme which destroys the hormone incretin and enables the body to deliver more insulin just when it is required and diminish the measure of glucose being created by the liver when it is not required [2]. The adjustment in glucagon connects straightly with the change in glucose tolerance. The gliptin derivatives enhance insulin secretion in response to hyperglycemia in people, it appears to be suitable to match them with drugs that have an alternate component of the activity, for example, insulin sensitizers or Metformin [3]. During short-term clinical trials, no increased risk of acute pancreatitis has been seen with Saxagliptin, Vildagliptin, Saxagliptin, Alogliptin, and Linagliptin [4]. Linagliptin (Trajenta) is as yet incorporated into dark triangle scheme, while Sitagliptin (Januvia), Saxagliptin (Onglyza) and Vildagliptin (Galvus) were expelled from the dark triangle list in 2012 [5]. DPP-4 inhibitors (Gliptins) include Saxagliptin, Linagliptin, Alogliptin, Saxagliptin, and Vildagliptin.

Saxagliptin (SAX), chemically (1S, 3S, 5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile. Saxagliptin is a white to off-white powder. The substance is soluble in water, and very soluble at low pH showing a minimum solubility of 17.6 mg/ml over the pH range 1.2 to 8.7 and also freely soluble in methanol, acetonitrile, acetone, polyethylene glycol, ethanol, Isopropyl Alcohol and slightly soluble in ethyl acetate. Literature review reveals that some analytical methods have been reported for estimation of Saxagliptin and Simvastatin, Saxagliptin and Gliclazide and Saxagliptin in single pharmaceutical formulation [6-9]. Few reports also available for the estimation of Saxagliptin in a biological sample [10]. Some analytical methods by UV-spectroscopy also present [11, 12].

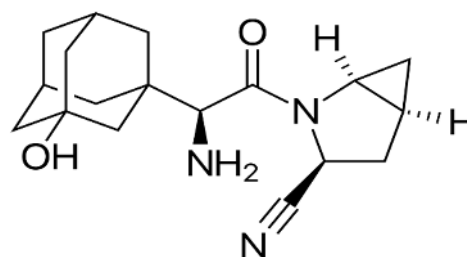
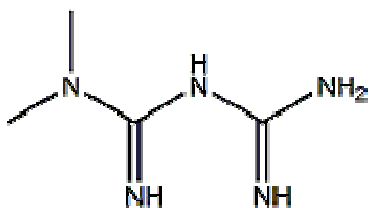


Fig 1: Saxagliptin

Metformin (MET), chemically N, N-diethyl imido-dicarbonimidic diamide Hydrochloride. It is a white powder that dissolves readily in water, only marginally in ethanol (95% v/v), and almost completely in acetone, ether, and

chloroform. Metformin is the biguanide class of anti-diabetic drug. Recently RP-HPLC methods have been reported for the simultaneous determination of SAX and MET in pharmaceutical dosage forms and biological fluids which are either tedious or expensive methods [13].



HCl

Fig 2: Metformin

To the best of our insight, at present, there is no HPLC strategy utilizing advancement strategies utilizing MCDM approach have been accounted for the concurrent estimation of SAX and MET. As a result, the simultaneous assurance of these analytes turns out to be both beneficial and crucial. The development and improvement of an isocratic HPLC technology is a complex process that calls for the simultaneous estimate of a number of factors, including the type and synthesis of the natural stage, stream rate, pH, type of stationary stage, section temperature, and others. For a considerable length of time HPLC detachment depended on an experimentation philosophy yet utilizing a tedious experimentation approach coming about just in an evident ideal and data concerning the affectability of the elements

on the analytes partition and communication between factors isn't accessible. To accomplish this target any of the Chemometrics techniques which incorporate the covering determination maps MCDM, factorial outline and reaction surface system can be connected. The best test configuration approach to model and advancement are the reaction surface design.

MATERIALS AND METHODS

Apparatus

Chromatographic measurements were made on a RP-HPLC Shimadzu (Tokyo, Japan) model which consisted of a LC-20AD solvent delivery module, SPD-M20A prominence diode array detector, a Rheodyne injector (model 7125, USA) valve fitted with a 20 μ l loop. The system was controlled through a system controller (SCL-10A) and a personal computer using a Shimadzu chromatographic software (LC Solution, Release 1-11SP1) installed on it. The Branson sonicator was used to degas the mobile phase (Branson Ultrasonic Corporation, USA). Using a quartz cell with a 1 cm path length and a UV-double beam spectrophotometer (Systronics 2202 Model UV-1601PC, Japan), absorbance spectra were captured.

Softwares

Experimental design, data analysis, and desirability function calculations were performed by using a trial of version 11 of

Design-Expert® Software 2017. The calculations for the analysis were performed using Micro soft Excel 2007 software (Microsoft, USA).

Chemicals and reagents

Working standards of SAX and MET were purchased from Biotech Solutions, New Delhi. MeOH of HPLC grade and potassium dihydrogen orthophosphate and orthophosphoric acid was of analytical- reagent grade supplied by M/S SD Fine Chemicals, Mumbai, India. The HPLC grade water was prepared by using Milli-Q Academic, Millipore, and Bangalore, India. The pharmaceuticals RIAX-M tablet manufactured by Dr Reddy's Laboratories Ltd (Batch No' 17JX009A, MFG: NOV 2021, EXP: OCT 2023) were purchased from Medicine Chamber, Park Town, Chennai, India.

Standard solutions

Stock standard solutions of SAX and MET (1mg/ml) were prepared in the mobile phase. The readied stock arrangements were put away at $4^{\circ}\text{C} \pm 0.05$, subsequently shielded from light. Working standard solutions were freshly arranged by diluting the stock solutions with mobile phase during analysis day. Calibration curves revealing peak area ratios of SAX and MET were built up in the range of 2.5-12.5 $\mu\text{g/ml}$. A standard solution prepared for the optimization procedure constituted SAX and MET at 10.0 $\mu\text{g/ml}$ and 10.0

$\mu\text{g/ml}$ respectively.

Sample preparation

Weigh and powder 10 tablets of RIAX-M (SAX-5mg and MET-500mg) and transfer the crushed tablet powder equivalent to 1mg of Saxagliptin and 10mg of Metformin into a 10mL of volumetric flask, add 8mL of mobile phase and sonicate for not less than 30min with occasional shaking. Make up the volume to 10mL with mobile phase (MeOH, ACN, 0.01mM KH_2PO_4 at pH 3.5 ± 0.5 (42.135:10:47.865 %v/v) and mix. Filter the solution through the 0.2 μm membrane filter (Gelman-Science, India). Transfer 1mL of above solution into a 10mL volumetric flask, dilute to volume with mobile phase and mix.

Chromatographic procedure

Chromatographic separations were carried out on an Onyx C_{18} Monolithic column (100mm \times i.d., 5 μm) connected with an Onyx C_{18} guard cartridge (4mm \times 3mm i.d., 5 μm). The mobile phase consisted of MeOH, ACN, 0.01mM KH_2PO_4 (pH 3.5 ± 0.5), adjusted with freshly prepared 10 % orthophosphoric acid. A wavelength of 210 nm was selected for detection. The injection volume of the sample was 20 μl . The HPLC system was used in an air-conditioned laboratory atmosphere ($20\pm 2^{\circ}\text{C}$).

Validation

Validation studies were conducted utilizing the optimized assay conditions considering the standards of approval portrayed in the ICH guidelines "Text on validation of Analytical Procedures" and "Q2B, Validation of Analytical Procedure: Methodology" [14, 15]. Key analytical parameters, including, accuracy, precision, linearity, detection limit, quantitation limit was evaluated. The calibration curves were tested utilizing one- way ANOVA at a 5 % significance level. Calibration curves were built in a low region of 10-50% of the target analyte concentration for the limit of detection and quantification. Additionally, robustness of the proposed technique was evaluated as for little modifications in the MeOH concentration, pH, and buffer concentration.

RESULTS AND DISCUSSION

Optimization design and analysis

Amid the procedure of streamlining technique, it is obligatory to research the shape term utilizing Factorial design in the center points. ANOVA made for 2k Factorial outline shows that arch is significant for all the responses (K_1 , $R_s(1,2)$ and $\alpha(1,2)$, tR_2) and the p-value is under 0.05. This infers a quadratic model and additionally, cubic models ought to be considered to demonstrate the separation procedure. For resolution and separation models we chose cubic and for retention

time, a capacity factor we chose quadratic models. Keeping in mind the end goal to get second request prescient model, central composite design (CCD) is utilized, which is an outline write under RSM. CCD is picked because of its adaptability and can be connected to upgrade a RP-HPLC separation gaining better comprehension of variables fundamental and communication impacts. The choice of key elements analyzed for improvement depended on preparatory trials and earlier information from the literature. The variables chose for enhancement process were MeOH concentration (A), pH of buffer (B) and flow rate. The limit factor for first eluted peak (K_1), the resolution and separation of the second peak ($R_s(1,2)$, $\alpha(1,2)$), the retention time of last peak (tR_2), were chosen as reactions. In the preparatory investigation, the resolution between two peaks ($R_s(1,2)$), were observed to be near 0 and were merging, consequently, these two peaks were considered as critical peaks and included as one of the responses for the global optimization.

To reduce the effects of uncontrolled variables that could have an influence on the estimations, all tests were conducted in a random order. To assess the experimental error, replicates ($n = 6$) of the key points were run. **(Table 1)**, summarizes the conducted experiments and responses. The quadratic and cubic mathematical

model for the independent factors is specified in Eq. (1) and (2),

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (1)$$

$$y = \beta_0 x^3 + \beta_1 x^2 + \beta_2 x + \beta_3 \quad (2)$$

Where Y is the response to the model, β is the regression coefficient and X1, X2, and X3 represents factors A, B, and C, individually. Statistical parameters obtained from ANOVA for the compact models are given in (Table 2). The insignificant terms ($P > 0.05$) were eliminated from the model through a backward elimination process to get a simple and realistic model. Since R_2 always decreases when a regress or variable is eliminated from a regression model; in statistical modeling adjusted R_2 which takes the quantity of regress or variables into account is usually selected.

In the present study, the adjusted R_2 was well within the acceptable limits of $R_2 = 0.80$ which uncovered that the experimental data demonstrate a good fit with the second- order polynomial equations. For all the reduced models, the P value of < 0.05 is obtained, implying these models are significant. The adequate

precision value is assess of the signal (response) to noise (deviation) ratio. A ratio greater than 4 is desirable. In this study, the ratio was observed to be in the range of 6.631-17.235, which demonstrates an adequate signal and thusly the model is significant for the separation procedure. The coefficient of variation (C.V.) is a measure of reproducibility of the model and as a rule, a model can be considered reasonably reproducible if it is less than 10%. The C. V. for all the models was found to be less than 10% except for K_1 (42.55), $R_s (1, 2)$ (21.33), $\alpha (1, 2)$ (37.06). Hence, the diagnostic plots, (a) normal probability plots of residuals and (b) plot of residuals versus predicted values were analyzed for response K_1 , $R_s (1,2)$ and $\alpha (1, 2)$ [16, 17]. Since the assumptions of normality and constant variance of residuals were observed to be satisfied, the fitted model for the K_1 , $R_s (1, 2)$, $\alpha (1, 2)$ was accepted [18].

Table 1: Experimental design and results of a rotatable central composite design

Design points	Factor levels			Responses			
	A MeOH % v/v	B pH	C Flow rate mL min ⁻¹	K ¹	R _s (1,2)	α _(1,2)	tR ₂
1	45	4	0.4	1.482	2.724	5.014	1.32
2	45	4	0.4	1.506	2.81	5.08	1.27
3	50	4.5	0.5	0.5	2.21	3.97	2.39
4	40	4.5	0.5	0.285	1.16	4.54	1.37
5	40	3.5	0.5	4.46	1.225	4.459	1.298
6	50	3.5	0.3	0.68	2.32	8.108	1.421
7	50	3.5	0.5	0.45	1.994	3.981	3.351
8	36.591	4	0.4	1.078	3.342	5.21	2.08
9	45	4	0.231821	5.534	3.295	8.807	1.313
10	45	4	0.4	1.136	2.594	4.98	1.354
11	45	4	0.4	1.145	2.734	5.013	1.345
12	45	4	0.4	1.145	2.644	4.988	1.454
13	45	4.8409	0.4	0.097	3.299	5.399	4.541
14	40	3.5	0.3	0.103	2.19	6.981	2.345
15	45	4	0.4	1.45	2.264	4.64	2.318
16	45	4	0.5681	0.87	2.735	3.53	4.123
17	45	4	0.4	1.482	2.393	6.46	1.52
18	53.409	4	0.4	2.227	3.455	5.35	1.423
19	50	4.5	0.3	0.786	3.731	7.636	1.423
20	40	4.5	0.3	1.778	2.57	6.666	4.144
21	45	3.159	0.4	1.965	1.621	5.601	1.44

As can be found in (Table 2), the interaction term with the largest absolute coefficient among the fitted models is AC (+ 0.107) of tR₂ model. The positive interaction between A and C is statistically momentous (<0.0001) for tR₂. The study reveals that changing the fraction of MeOH from low to high results in a rapid decline in the retention time of SAX and MET both at the low and high level of pH. Further at a

low level of factor A, an increase in the pH results in a marginal decrease in the retention time. Therefore, when the MeOH concentration must be at its highest level to shorten the runtime. Particularly this connection is synergistic, as it led to a decrease in runtime.

In (Fig.3) perturbation plots are exhibited for predicted models in order to gain an effect of an independent factor on a specific

response with all other factor held constant at a reference point. A steepest slope or curvature shows affectability of the response to a particular factor (**Fig 3d**) shows that MeOH (factor A) had the most important effect on a retention time tR_2 followed by factor B and C. In (**Fig 3C**) the factors (pH and flow rate) had significant

effect on $R_s (1, 2)$ and $\alpha (1, 2)$ and only one factor A had significant effect on K_1 . In (**Fig 3a**) and (**Fig 3b**), K_1 and $R_s (1, 2)$ values increased as the level of MeOH concentration (factor A) decreased and $R_s (1, 2)$ values increased at the level of buffer pH (factor B) and Flow rate (factor C) are at midpoint.

Table 2: Models and statistical parameters obtained from ANOVA for CCD

Responses	Regression model	Adjusted R^2	Model P value.	%C.V	Adequate precision
K_1	+1.36+0.3416* A-0.5554* B-1.39* C+0.3320* AB-0.4225* AC-0.7382* BC-0.0700* A ² -0.2897* B ² +0.4779* C ² +0.7243* ABC+0.2624* A ² B+1.68* A ² C-0.8678* AB ² +0.0000* AC ² +0.0000* B ² C+0.0000* BC ² +0.0000* A ³ +0.0000* B ³ +0.0000* C ³	0.7892	<0.0500	42.55	10.8981
$R_s(1,2)$	+2.54+0.2416* A+0.3488* B-0.3781* C	0.3759	<0.0500	21.33	8.1975
tR_2	+5.16+0.0941* A-0.0774* B-1.56* C-0.0311* AB-0.3931* AC+0.1071* BC+0.0635* A ² +0.01413* B ² +0.3776* C ²	0.9009	<0.0500	7.96	17.2352
$\alpha (1,2)$	+1.52-0.1231* A+0.4494* B+0.2791* C-0.3543* AB+0.8393* AC-0.3350* BC+0.0164* A ² +0.4544* B ² +0.3581* C ²	0.4873	<0.0500	37.06	6.6318

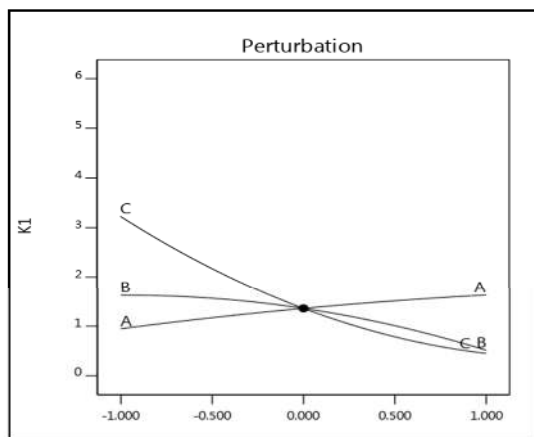


Fig 3 (a)

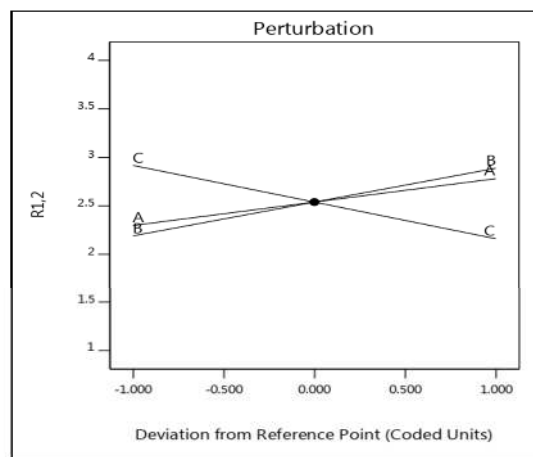


Fig 3 (b)

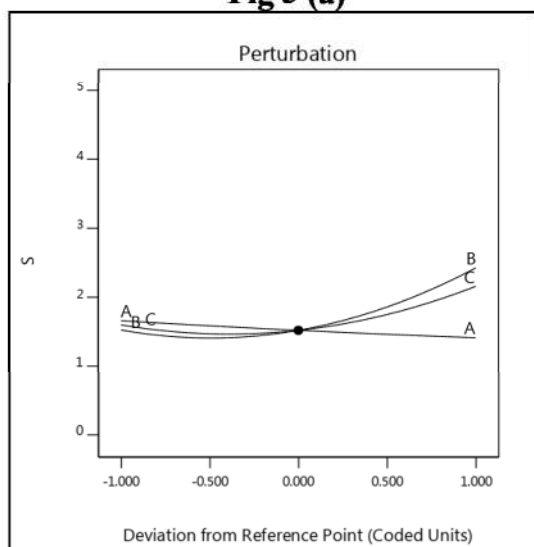


Fig 3 (c)

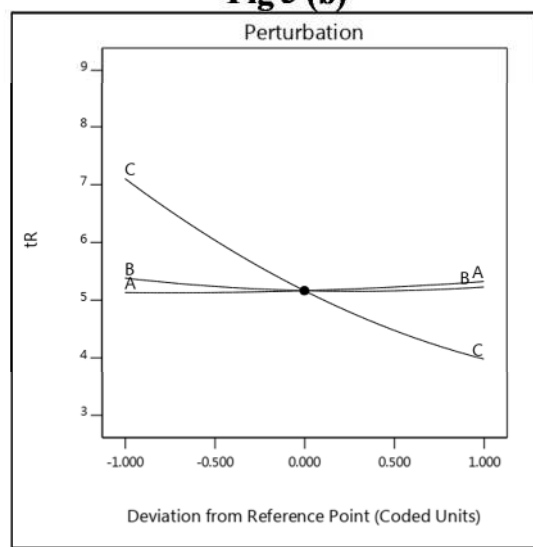


Fig 3 (d)

Figure 3: Perturbation plots showing the effect of each independent variables on (a) K_1 , (b) $R_s(1,2)$ (c) $\alpha(1,2)$, (d) tR_2 Where A is the MeOH concentration, B the pH buffer, C the flow rate.

Response surfaces plots for K_1 , $R_s(1,2)$ and $\alpha(1,2)$ and tR_2 are illustrated in (Fig. 4) (% Methanol concentration is plotted against the pH Flow rate held at constant at the center value). Analysis of perturbation

plots and response plots of optimization models uncovered that factor A and B had the huge impact on a separation of the analytes, whereas the factor C i.e. the Flow rate, is of little noteworthy.

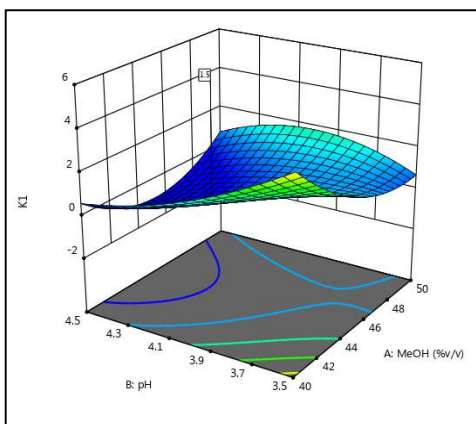


Fig 4 (a)

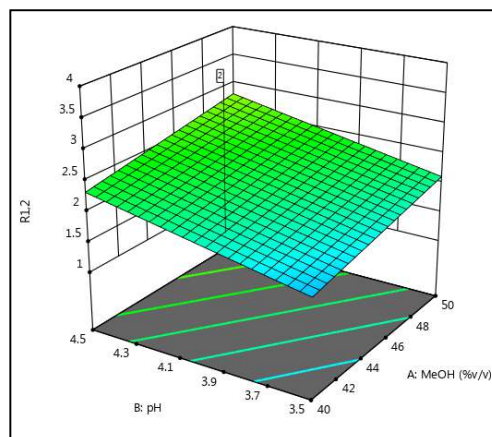


Fig 4 (b)

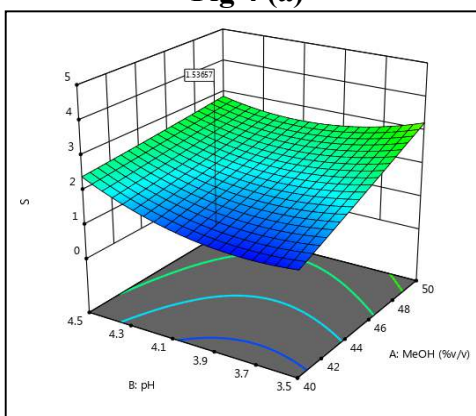


Fig 4 (c)

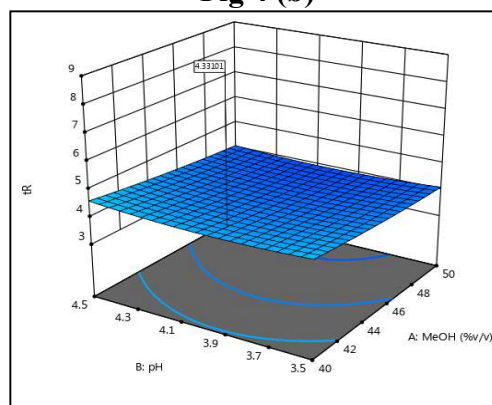


Fig 4 (d)

Figure 4: Response surfaces related to MeOH (A) pH of buffer (B) Flow rate of mobile phase (C): (a) capacity factor first peak (K₁), (b) resolution of the critical pair R_{s(1,2)} (c) separation of α (1, 2) (d) retention time of the last peak (t_{R2}).

Global optimization

Resolution between two key peaks, capacity factor, peak separation, and peak retention duration were the distinguishing factors for optimization in the current study. Derringers desirability function was utilized to optimize three responses with various targets [19]. The Derringers desirability function, D, is characterized as the geometric mean, weighted, or something else, of the individual desirability functions. The expression that

characterizes the Derringers desirability function is:

$$D = [d_1^{p_1} \times d_2^{p_2} \times d_3^{p_3} \times \dots \times d_n^{p_n}]^{1/n}$$

Where p_i is the weight of the response, n the number of responses and d_i is the individual desirability function of every response. Desirability task (D) can take values from 0 to 1. Weights can extend from 0.1 to 10. Weights lower than 1 gives less significance to the objective, whereas weights more than 1 gives more

significance to the objective. In the present study, pi values were set 1 for K_1 , $R_s (1, 2)$ and $\alpha (1, 2)$ responses and pi for tR_2 was set to 5. A value of D close up to 1 indicates that the amalgamation of the different criteria is matched in a global optimum (**Table 3**). Criteria I have been wished-for selecting an optimum experimental circumstance for analyzing schedule quality control samples. As can be seen under criteria I, the responses tR_2 was minimized, in order to shorten the analysis time. $R_s (1, 2)$, on the other hand, maximized to enable baseline separation between SAX and MET. K_1 was within range to differentiate the initial eluting peak (MET) from the solvent front. A target value can be given significance by using the importance scale

of 1 to 5. The significance for retention time is 5 to trim down the time of analysis. Following the conditions and restrictions above, the optimization procedure was carried out. The Graphical representation of the overall desirability function D ($D=0.948$) where MeOH Conc. (A) of 41.227, pH of buffer (b) 3.946 and Flow rate (c) 0.484mL/min and individual desirability of the four responses and three factors. (**Fig.5**). The predicted response values corresponding to the latter value of D were: $K_1=1.500$, $R_s (1, 2) = 2$, $\alpha (1, 2) = 1.537$ and $tR_2= 4.331$ min. The prediction efficiency of the model was confirmed by performing the experiment under the optimal condition and the corresponding chromatogram is shown in (**Fig.6**).

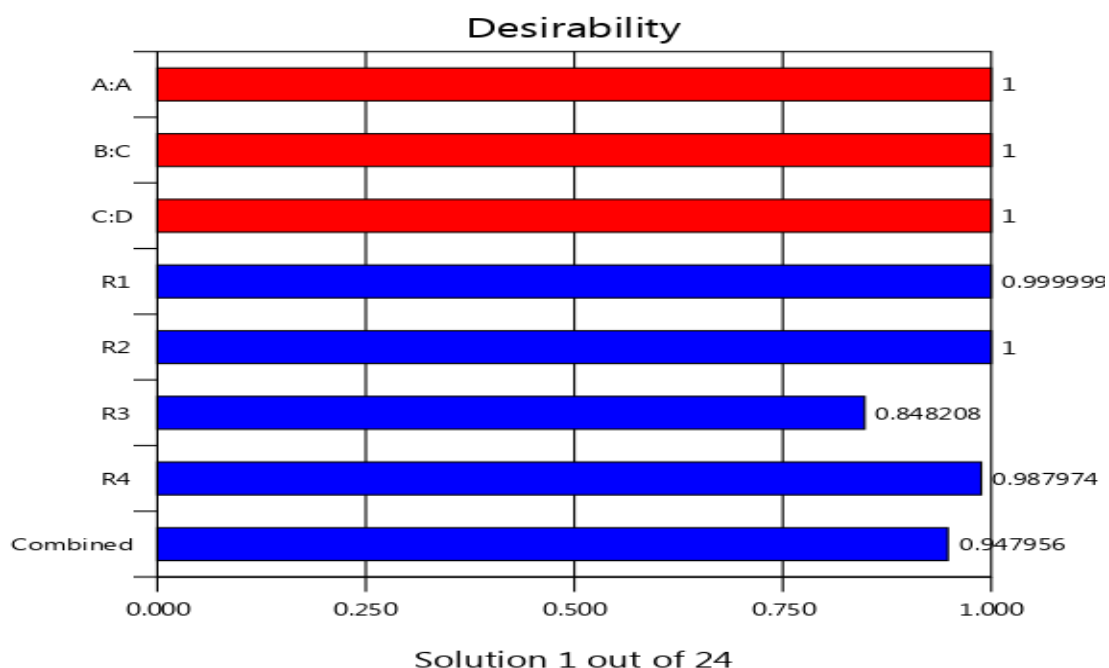


Figure 5: Graphical representation of the overall desirability function D ($D=0.947$) where MeOH Conc. (a) of 41.227, pH of buffer (b) 3.94 and Flow rate (c) 0.484mL/min and individual desirability of the four responses and three factors.

In order to study the predictability of the projected model, the agreement between experimental and predicted responses for the predicted optimums I are shown in (Table 4). The Percentage of prediction

inaccuracy was calculated by Eq. (3). The average error for $K_1= 6.6$ $R_{s(1,2)} = 5$ $\alpha_{(1,2)} = 7$ and $tR_2 = 2.32$ were respectively, indicating good correlation between the experimental and predicted responses.

$$\text{Predicted Error} = \frac{\text{Experimental} - \text{Predicted}}{\text{Predicted}} * 100 \quad (4)$$

$$D = \left[d_1^{p1} \times d_2^{p2} \times d_3^{p3} \times \dots \times d_n^{pn} \right]^{1/n} \quad (2.14)$$

Table 3: Criteria for the optimization of the individual responses for the analysis of quality control samples (Criteria I)

Responses	Lower limit	Upper limit	Criteria I		
			Goal	Importance	Weights
K_1	0.097	5.534	Target	1.5	4
$R_{s(1,2)}$	1.16	3.731	Target	2	2
$\alpha_{(1,2)}$	1.27	4.541	Target	1.5	3.5
tR_2	3.53	8.807	Minimize	4	3.5

Table 4: Comparison of observed and predictive values of different objective functions under optimal conditions

Optimum conditions	MeOH(%)	pH	Flow (ml/min)	K_1	$R_{s(1,2)}$	tR_2	$\alpha_{(1,2)}$
For Formulation	Desirability Value (D) = 0.947						
	41.227	3.94	0.484				
	Experimental value			1.6	2.1	4.4	1.6
	Predicted value			1.500	2.000	4.331	1.537
	Average % error			6.66	5	2.32	7

Assay method validation

The last step of the study was to check method validation for specificity, linearity, intra/between day precision, and robustness. In comparison to the placebo

used in the experiment, the improved HPLC method was unique. Every placebo chromatogram showed no interference peaks. For SAX and MET, a remarkable linearity was established at five levels in

the 2.5-12.5 g/ml range with R² values of greater than 0.998 for all the analytes. The slope and intercept of the calibration curve were 99945x + 11581 and 89971x + 53798 for SAX and MET respectively. Since the correlation coefficients are not good indicators of linearity performance of an analytical procedure a one-way ANOVA was performed. For all the analytes, the calculated F- Value (F calculated) was found to be less than the theoretical F-value (F critical) at 5% significance level, indicating that there was no significant difference between replicate determinations for each concentration level. The LODs and LOQs for SAX& MET are 0.013, 0.039 µg/ml and 0.013, 0.039 µg/ml respectively. Accuracy (n=9), assessed by spike recovery, were found to be 101.69, 100.99, 99.91 for SAX& 100.47, 99.9, 100.44 for MET, with were within acceptable ranges of 100± 2%. The intra and inter-assay

precision (n=6) was established since, the % CV were well within the target criterion of ≤ 2 and ≤3. Respectively. Robustness revision reveals that small changes did not alter the retention times, retention factor, and resolution and therefore it would be concluded that the method conditions are robust.

Application of the method

As a final step, commercial tablet product containing 5mg of SAX and 500mg of MET were assayed by the proposed RP-HPLC method. Representative chromatograms are presented (Fig.6). The results achieved when analyzing RIAX-M tablets was 98.45% (4.9 mg) of SAX and 97.58% (487.94mg) of MET. Good conformity was found between the assay results and the label claim of the product. The % C.V. for the tablet is < 2, indicating the precision of the analytical methodology.

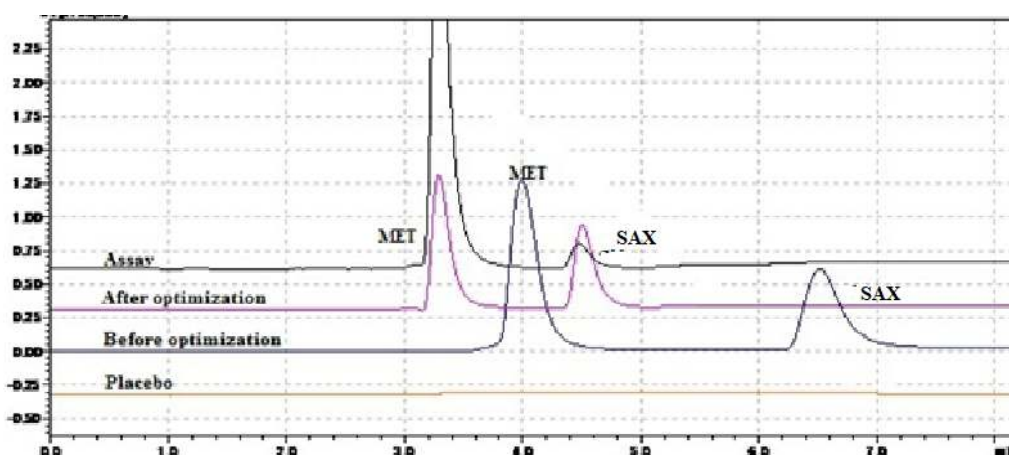


Figure 6: Chromatograms corresponding to (a) a Placebo solution; (b) Synthetic mixture of SAX 10 µg/ml, MET (10 µg/ml) before optimization; (c) Synthetic mixture of SAX (10 µg/ml), MET (10 µg/ml) after optimization; (d) a Real sample of RIAX-M tablet (2 µg/ml SAX and 20 µg/ml MET)

DISCUSSION

In this study, AQbD concept was used in the development of RP-HPLC method for the simultaneous estimation of SAX and MET. Mobile phase characteristics were discovered to be the most crucial for the specified analysis based on risk priority number. Consequently, three CMA parameters were chosen: pH, flow rate, and percentage of MeOH in the mobile phase. A Central Composite experimental design with three independent variables at four levels was employed to optimize critical method parameters. All of the 21 experiments were performed randomly and chromatograms obtained were evaluated for capacity factor, resolution, separation and retention time for peaks of both the drugs. The factors A and B had a considerable impact on the separation of the analytes, whereas the factor C, or flow rate, is of less consequence, according to the analysis of perturbation plots and response plots of optimization models. The precise CMA combination used to identify the point is a mobile phase made up of MeOH, ACN, and 0.01mM KH_2PO_4 at pH 3.50.5 (42.135:10:47.865%v/v) at room temperature. The operable method region is shown in the design space where adjustments won't impair the accuracy of the research. The percentage of both medications recovered after being examined together served as a measure of

specificity. The percentages of both medications recovered were within statistically acceptable ranges. It was noted that the peaks of each drug were clearly distinct from one another and did not interfere. Thus, it can be said that the method is specific to each of the two drugs in combination. The estimated LOD and LOQ values confirmed that the methods are sufficiently sensitive. Moreover, percent recoveries of the drugs were found to be acceptable. Hence, the developed method can be suitable, utilized for concurrent, quantitative analysis of SAX and MET. The method was validated for linearity, precision, accuracy, sensitivity, system suitability, as well as robustness. The developed method is convenient and effective for quality control as well as simultaneous routine analysis of SAX and MET in pharmaceutical dosage forms.

CONCLUSIONS

An efficient isocratic reversed-phase high-performance liquid chromatography technique was developed, optimized and validated for the simultaneous estimation of SAX and MET in bulk and pharmaceutical formulations utilizing Chemometrics Multi-Criteria Decision-Making Approach. This technique decreases overall assay development time and gives fundamental data with respect to the affectability of different chromatographic factors and their

interaction effects on the attributes of separation. By utilizing two useful chemometrics tools—the central composite design and Derringer’s desirability function analysis time, resolution, and peak quality were continuously enhanced. The validation investigation confirmed that the test was specific, accurate, linear, precise, and robust, supporting the determination of the assay conditions. Therefore, this RP-HPLC method can be used as a routine quality control analysis of gliptin derivative like SAX in combination with MET.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest. The article does not contain any studies with animals or human participants performed by any of the authors.

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