Int. J. Pharm. Investigation, 2020;10(4):512-518 *Original Article*

Quality by Design based Development and Validation of RP-HPLC Method for Simultaneous Estimation of Sitagliptin and Metformin in Bulk and Pharmaceutical Dosage Forms

Balamurugan Krishnan*, Kirtimaya Mishra
Department of Pharmacy, Annamalai University, Chidambaram, Tamil Nadu, INDIA.

ABSTRACT

Objectives: This paper portrays a recently developed, optimized and validated isocratic RP-HPLC strategy for the separation of two anti-diabetic drugs (sitagliptin and metformin) in bulk and pharmaceutical formulations with the aid of quality by design and multi-criteria decision making approach. **Methods:** The effective chromatographic separation was accomplished by utilizing the Monolithic C_{18} segment (100×4.6 mm id, 5µm molecule size) and PDA-UV- detection at 210nm.The scope of independent variables utilized for the streamlining were methanol: 40-50% v/v, pH: 3.5-4.5 and flow rate:0.3-0.5ml/min. **Results:** Ideal conditions decided for assay were methanol, acetonitrile, pH 3.5±0.5 balanced with the diluted orthophosphoric acid solution and flow rate of 0.484ml/min and pH 3.946. 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 metformin and sitagliptin eluting with retention times of 3.3 and 4.4 min respectively. The optimized assay circumstance was validated as per ICH guidelines and applied for the quantitative analysis of marketed tablets containing sitagliptin and metformin. **Conclusion:** The validation study upheld the determination of the assay conditions by affirming that the assay was specific, accurate and linear, precise and robust. Therefore, this RP-HPLC method can be used as a routine quality control analysis of gliptin derivative like sitagliptin in combination with metformin.

Key words: Multi-Criteria Decision-Making Approach, Quality by Design, RP- HPLC, Sitagliptin, Metformin.

Correspondence

Dr. Balamurugan Krishnan, M. Pharm.,
Associate Professor, Department of Pharmacy, Annamalai University, Chidambaram, Tamil Nadu-608002, INDIA.
Phone no: +91 94861 50867
Email: krishnanbalamurugan7@gmail.com

DOI: 10.5330/ijpi.2020.4.89

INTRODUCTION

Defects in insulin secretion, insulin activity, or both make diabetes which is described by hyperglycemia. Characterization of Diabetes mellitus was broadly acknowledged as insulin-dependent diabetes mellitus or Type 1 and non-insulin dependent diabetes mellitus or Type 2 which was distributed by world health organization (WHO) in 1980.[1] Dipeptidyl peptidase-4 (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 isn't 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 sitagliptin, vildagliptin, saxagliptin, alogliptin and linagliptin.[4] linagliptin is as yet incorporated into dark triangle scheme, while sitagliptin, saxagliptin and vildagliptin were expelled from the dark triangle list in 2012.[5] DPP-4 inhibitors include saxagliptin, linagliptin, alogliptin, sitagliptin and vildagliptin.

Sitagliptin (SIT) Figure 1, chemically (S)-3-amino-1-(3-(trifluoromethyl)-5,6-dihydro-[1,2,3] triazolo[4,3-a]pyrazin-7(8H)-yl)-4-(2,4,5-trifluorophenyl)butane-1-one. Sitagliptin is a white to off-white powder. The solubility of drug substance is soluble in water and N, N-diethyl formamide, marginally soluble in methanol, somewhat soluble in ethanol, acetone and acetonitrile, insoluble in isopropanol and Isopropyl acetate is an intense oral hypoglycaemic drug of the dipeptidyl peptidase 4 (DPP4) inhibitor.[6] Literature review reveals that some analytical methods have been reported for estimation of sitagliptin and simvastatin,[7] Sitagliptin and Gliclazide[8] and Sitagliptin in single pharmaceutical formulation.[9] Few reports also available for the estimation of Sitagliptin in a biological sample.[10] Some analytical methods by UV-spectroscopy[11,12] also present.

Metformin (MET) Figure 2, chemically N, N-diethyl imidodicarbonimidic diamide hydrochloride. It is a white powder, freely soluble in water, slightly soluble in ethanol (95% v/v), practically insoluble in acetone, ether and chloroform. Metformin is the bi-guanide class of anti-diabetic drug. Recently reverse phase high performance chromatography (RP-HPLC)[13] methods have been reported for the simultaneous determination of SIT and MET in pharmaceutical dosage forms and biological fluids which are either tedious or expensive methods.

To the best of our insight, at present, there is no high performance liquid chromatography (HPLC) strategy utilizing advancement strategies utilizing multiple criteria decision-making approach have been accounted for the concurrent estimation of SIT and MET. Consequently, the synchronous assurance of these analytes winds up empowering and essential. Creating and upgrading an isocratic HPLC technique is a mind-boggling system that requires concurrent estimation of a few components, viz., the sort and synthesis of the natural stage, stream rate, pH, kind of stationary stage, section temperature and so on. For a considerable length of time HPLC detachment depended on an experimentation philosophy yet utilizing a tedious experimentation

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

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 chemometric techniques which incorporate the covering determination maps multiple criteria decision making (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 20µl 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 mobile phase was degassed using Branson sonicator (Branson Ultrasonic Corporation, USA). Absorbance spectra were recorded using an UV-double beam spectrophotometer (Systronices 2202 Model UV-1601PC, Japan) employing quartz cell of 1 cm of path length. 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 by the use of Microsoft Excel 2007 software (Microsoft, USA).

Chemicals and reagents

Working standards of SIT and MET were purchased from Biotech Solutions, New Delhi. Methanol (MeOH), acetonitrile (ACN) of HPLC grade and potassium dihydrogen orthophosphate (KH_2PO_4) 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 tablets were purchased from Medicine Chamber, Park Town, Chennai, India.

Standard solutions

Stock standard solutions of SIT and MET (1mg/ml) were prepared in the mobile phase. The readied stock arrangements were put away at 4°C ± 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 SIT and MET were built up in the range of 2.5-12.5µg/ml. A standard solution prepared for the optimization procedure constituted SIT and MET at 10.0 µg/ml and 10.0 µg/ml respectively.

Sample preparation

Weigh and powder 10 tablets of (SIT-50mg and MET-500mg) and transfer the crushed tablet powder equivalent to 1mg of Sitagliptin 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 0.2µm 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 C_{18} Monolithic column (100mm× 4.5mm i.d., 5µm) connected with an C_{18} guard cartridge (4mm×3mm i.d., 5µm). 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 20µl. The HPLC system was used in an air-conditioned laboratory atmosphere (20±2°C).

Validation

Validation studies were conducted utilizing the optimized assay conditions in light of the standards of approval portrayed in the ICH guidelines " Text on validation of Analytical Procedures"[14] and "Q2B, Validation of Analytical Procedure: Methodology".[15] Key analytical parameters, including, accuracy, precision, linearity, detection limit, quantisation limit were evaluated. The calibration curves were tested utilizing one- way analysis of variance (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

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

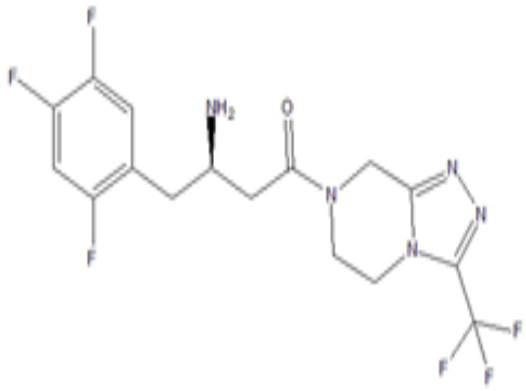

Figure 1: Sitagliptin.

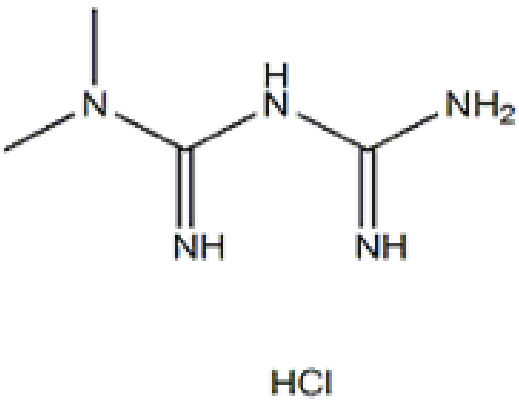

Figure 2: Metformin.

second request prescient model, central composite design (CCD) is utilized, which is an outline write under response surface methodology (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 ($R_{s\,(1,2)}$, $\alpha_{2(1,2)}$), 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.

All experiments were led in a randomized order to limit the impacts of uncontrolled variables that may present an inclination on the estimations. Replicates (n=6) of the central points were performed to evaluate the experimental error. (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),

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_1 X_1 X_2 + \beta_1 X_1 X_3 + \beta_2 X_2 X_3 + \beta_1 X_1^2 + \beta_2 X_2^2 + \beta_3 X_3^2 \quad (1)$$

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

Where Y is the response to the model, ß 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 modelling 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 an 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 general 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[16] of residuals and (b) plot of residuals versus predicted values[17] were analyzed for response K_1, $R_{s\,(1,2)}$ and $\alpha_{(1,2)}$. 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]

As can be found in (Table 2), the interaction term with the largest absolute coefficient among the fitted models is AC (+ 0.107) of tR_2 model. The positive interaction between A and C is statistically momentous (<0.0001) for tR_2. The study reveals that changing the fraction of MeOH from low to high results in a rapid decline in the retention time of SIT 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 has to be at its highest level to shorten the runtime. Particularly this connection is synergistic, as it led to a decrease in runtime.

In (Figure 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 (Figure 3d) shows that MeOH (factor A) had the most important effect on a retention time tR_2 followed by factor B and C. In (Figure 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 (Figure 3a) and (Figure 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.

Response surfaces plots for K_1, $R_{s\,(1,2)}$ and $\alpha_{(1,2)}$ and tR_2 are illustrated in (Figure 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 noteworthiness.

Global optimization

In the present study, the distinguished criteria for the optimization were: resolution between two critical peaks, capacity factor, separation and retention time of the last peak. Derringers desirability function was utilized to optimize three responses with various targets.[19] The

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	$Rs_{(1,2)}$	$\alpha_{(1,2)}$	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

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:

$$D = \left[d_1^{p1} \times d_2^{p2} \times d_3^{p3} \times \ldots\ldots \times d_n^{pn}\right]^{1/n}$$

Where *pi* is the weight of the response, *n* the number of responses and *di* 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 than1 gives less significance to the objective, whereas a weight 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. On the other hand, $R_{s\ (1,\ 2)}$ maximized to allow baseline separation SIT and MET. In order to separate the first eluting peak (MET) from the solvent front, K_1 was in range. Importance can range from 1to 5, which gives emphasis to a target value. 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 (Figure 5). The predicted response values corresponding to the latter value of D were: K_1=1.500, $R_{s\ (1,2)}$=2, $\alpha_{(1,2)}$ = 1.537and tR_2= 4.331min . The prediction efficiency of the model was confirmed by performing the experiment under the optimal condition and the corresponding chromatogram is shown in (Figure 6).

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.

Predicted Error= Experimental- Predicted/ Predicted *100 (4)

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

Assay method validation

The last step of the study was to check method validation for specificity, linearity, intra/between day precision and robustness. The optimized HPLC technique was particular in connection to the placebo utilized as a part of the investigation. All placebo chromatograms demonstrated no interference peaks. An amazing linearity was set up at five levels in the range of 2.5-12.5 µg/ml for SIT and MET with R^2 of more than 0.998 for all the analytes. The slope and intercept of the calibration curve were 99945x + 11581 and 89971x + 53798 for SIT 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 limit of detection (LODs) and limit of quantification (LOQs) for SIT and MET are 0.013, 0.039 µg/ml and 0.013,

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
$Rs_{(1,2)}$	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	$Rs_{(1,2)}$	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

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^2B+1.68* A^2C-0.8678* AB^2+0.0000* AC^2+0.0000* B^2C+0.0000* BC^2+0.0000* A^3+0.0000* B^3+0.0000* C^3	0.7892	<0.0500	42.55	10.8981
$Rs_{(1,2)}$	+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

Model *P* values are statistically significant ($P < 0.05$)

0.039 µg/ml respectively. Accuracy (n=9), assessed by spike recovery, were found to be 101.69, 100.99, 99.91 for SIT and 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.

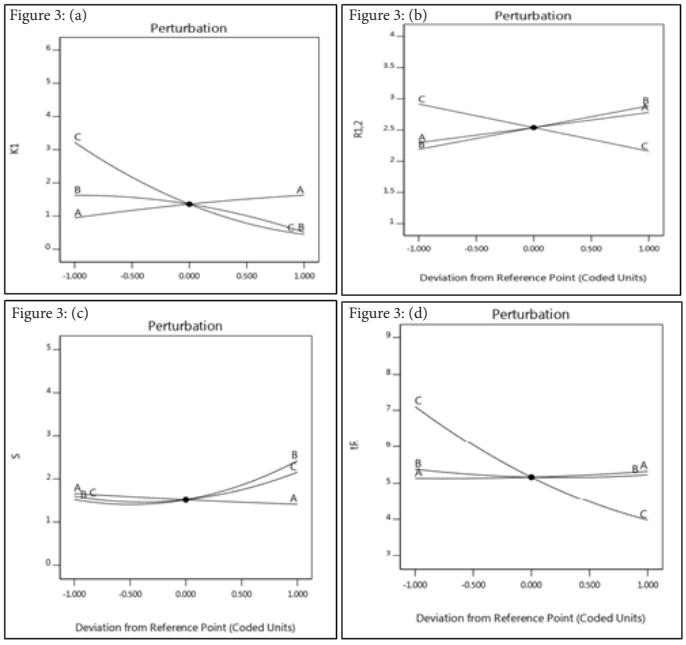

Figure 3: Perturbation plots showing the effect of the each independent variables on (a) K_1, (b) $Rs_{(1,2)}$ (c) $\alpha_{(1,2)}$, (d) tR_2 Where A is the MeOH concentration, B the pH buffer, C the flow rate.

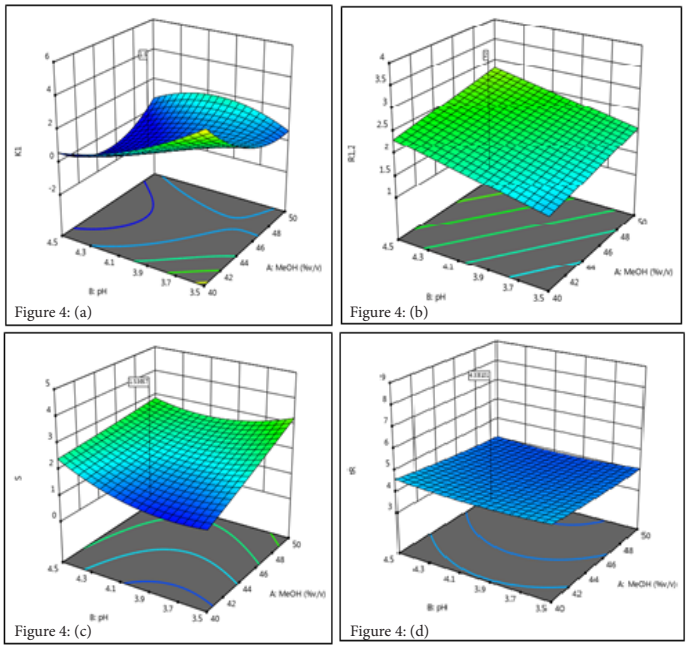

Figure 4: Response surfaces related to MeOH (A) pH of buffer (B) Flow rate of mobile phase (C): (a) capacity factor first peak (K_1), (b) resolution of the critical pair $Rs_{(1,2)}$ (c) separaton of $\alpha_{(1,2)}$ (d) retention time of the last peak (tR_2)

Application of the method

As a final step, commercial tablet product containing 50mg of SIT and 500mg of MET were assayed by the proposed RP-HPLC method. Representative chromatograms are presented (Figure 6). The results achieved when analysing marketed pharmaceutical tablets was 98.45% (49.2mg) of SIT 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.

DISCUSSION

The preliminary chromatographic conditions (stationary phase, pH-range, choice of buffer and wavelength) were chosen based on experience and prior knowledge from literature. The optimization goal was to increase the resolution and decrease the analysis time. For the optimization, central composite design (CCD) was preferred as it is ideal for chromatographic trailing and allows relatively controlled range of experiments to outline the factors that have an effect on the chromatographic behaviour of investigated substances. The method was optimized by developing the experimental methodology, which also provided a detailed understanding of the relation between factor and response and the underlining interaction between them. Numerical optimization by "trading" different variables to achieve the desired objectives, i.e. optimizing the top area and theoretical plate and reducing

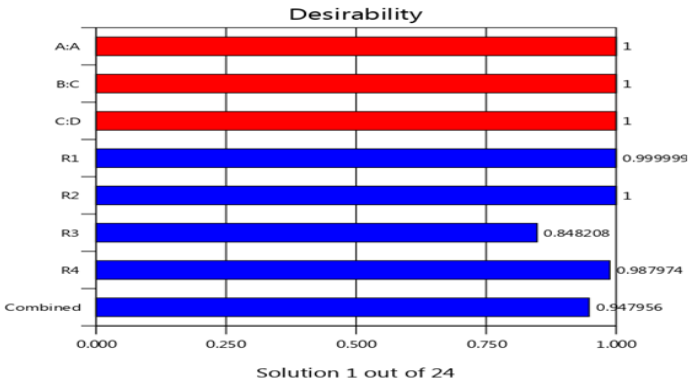

Figure 5: Graphical representation of the overall desirability function D (D=0.947) where MeOH Conc.(a) of 41.227, pH of buffer (b) 3.94 and Flow rate (c) 0.484mL/min and individual desirability of the four responses and three factors.

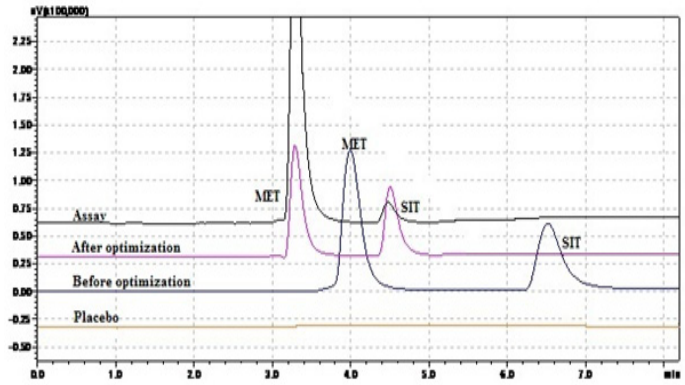

Figure 6: Chromatograms corresponding to (a) a Placebo solution; (b) Synthetic mixture of SIT 10 µg/ml), MET (10 µg/ml) before optimization; (c) Synthetic mixture of SIT (10 µg/ml), MET (10 µg/ml) after optimization; (d) a Real sample of JANUMET tablet (2 µg/ml SIT and 20 µg/ml MET).

retention times and the height to obtain a target feature near to 1 min, has been carried out in the search for optimum condition. The graphical optimization also yielded the optimum.[20] In this study, Analytical quality by design (AQbD) concept was used in the development of RP-HPLC method for the simultaneous estimation of SIT and MET. On the basis of risk priority number, mobile phase parameters were found to be most critical for the given analysis. Therefore, three parameters, pH, flow rate and % MeOH in the mobile phase were selected as Critical material attribute (CMA). A Central Composite experimental design with three independent variables at four levels was employed to optimize critical method parameters.[21] The design space presents the operable method region where the changes will not affect the quality of analysis. Specificity was assessed by percent recovery of both the drugs when analysed in combination. Percent recoveries of both the drugs were within statistical limits. It was observed that the peaks of each of the drugs were well separated and not interfering. Thus, it can be said that the method is specific to each of the two drugs in combination. The estimated limit of detection (LOD) and limit of quantification (LOQ) values confirmed that the methods are sufficiently sensitive. Moreover, percent recoveries of the drugs were found to be acceptable.[22] Hence, the developed method can be suitable, utilized for concurrent, quantitative analysis of SIT 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 SIT and MET in pharmaceutical dosage forms. The method was highly advantageous vis-à-vis in terms of time economy for determination of SITA and MET formed during stress conditions, as is evident from low Rt values of the DPs. The developed method was found to be sensitive which was evaluated in terms of LOD and LOQ. Further, the Rt of SITA and MET in all the dosage forms was similar with respect to the standard SITA and MET without any significant difference in the standard solution. Other parameters, like theoretical plates and peak tailing were found to be within the acceptable limits. This is a corroborated high degree of utility of developed method for routine estimation of SITA and MET in pharmaceutical formulations. The method was optimized by design of expert (DOE) technique using different variables and the method shown to be precise, accurate and linear over the concentration range. The lower solvent consumption along with the short analytical run time leads to a cost effective procedure.

CONCLUSION

An efficient isocratic reversed-phase high-performance liquid chromatography technique was developed, optimized and validated for the simultaneous estimation of SIT and MET in bulk and pharmaceutical formulations utilizing chemometric 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. Time of analysis, resolution and quality of the peaks was all the while optimized by applying helpful tools of Chemometric: central composite design and Derringers desirability function. The validation study upheld the determination of the assay conditions by affirming that the assay was specific, accurate and linear, precise and robust. Therefore, this RP-HPLC method can be used as a routine quality control analysis of gliptin derivative like SIT in combination with MET.

ACKNOWLEDGEMENT

Our thanks to Department of Pharmacy, Annamalai University, Chidambaram for the laboratory facilities provided to an outcome of this investigation. We are thankful to UGC for the financial support through UGC BSR Fellowship.

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.

Role of the Funding Source

The author Kirtimaya Mishra received grants from UGC-BSR (F.25-1/2014-15(BSR)/7-269/2009(BSR), dated 07.10.2015).

ABBREVIATIONS

RP-HPLC: Reverse Phase High Performance Liquid Chromatography; **MCDM:** Multi-Criteria Decision-Making; **CMA:** Critical Method Attributes; **ACN:** Acetonitrile; **DPP-4:** Dipeptidyl peptidase-4; **RSM:** Response Surface Methodology; **CCD:** Central Composite Design; **QbD:** Quality by Design; **DOE:** Design of Expert; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **SIT:** Sitagliptin; **MET:** Metformin.

REFERENCES

1. Siddiqui MR, Alothman ZA, Nafisur R. Analytical techniques in pharmaceutical analysis: A review. Arab J Chem. 2017;10:1409-21.
2. Prashant BM, Rishikesh VA, Rajesh JO. Antidiabetic Drugs: An Overview. Int J Ph and Ch Sc. 2012;1(1):301-6.
3. Balamurugan K, Kirtimaya M, Suresh R. Optimization of the simultaneous determination of sitagliptin and metformin, in human plasma by a rapid hplc method. J Glob Pharma Technol. 2018;10(12):7-12.
4. Monteagle Medical Center, San Francisco CA, USA. Overview of the gliptin class, Dipeptidyl peptidase-4 inhibitors in clinical practice. J Postgraduate Med. 2009;121(1):40-5.
5. Chu ST, Liège B. Gliptins, dipeptidyl peptidase-4 inhibitors and risk of acute pancreatitis. J Expert Opin Drug Saf. 2013;12(4):545-57.
6. Herman G, Bergman A, Liu F, Stevens C, Wang A, Zeng W, *et al.* Pharmacokinetics and pharmacodynamic effects of the oral DPP-4 inhibitor sitagliptin in middle-aged obese subjects. J Clin Pharmacol. 2006;46(8):876-86.
7. Ramalingam P, Udaya BV, Padmanabha RY, Vinod KK. Stability-indicating rp-hplc method for the simultaneous determination of sitagliptin and simvastatin in tablets. Ind J Pharm Sci. 2014;76(5):407-14.
8. Rubina RC, Gopal RM. Development and validation of rp-hplc method for simultaneous estimation of gliclazide and sitagliptin phosphate monohydrate in bulk and tablet dosage form. Int J Pharm Pharm Sci. 2015;7(6):372-6.
9. Karimulla SK, Vasanth PM, Ramesh T, Ramesh M. Method development and validation of Sitagliptin and Metformin using reverse phase HPLC method in bulk and tablet dosage form. Der Pharm Lett. 2013;5(5):168-74.
10. Ramalingam P, Chandra SG, Padmanabha RY, Krishna KP. Stability-indicating rp-hplc method for simultaneous determination of metformin hydrochloride and sitagliptin phosphate in dosage forms. Chromatographia. 2013;76(8):1153-62.
11. Jeyabalan G, Narendra N. Analytical method development and validation of Sitagliptine phosphate monohydrate in pure and tablet dosage form by derivative spectroscopy. J Appl Pharm. 2013;3(1):95-8.
12. Raja T, Lakshmana RA. Validated rp hplc method for simultaneous estimation of metformin hydrochloride and sitagliptin phosphate in bulk drug and pharmaceutical formulation. Int J Ph Ch Bio Sc. 2012;2(4):696-702.
13. Proceedings of the International Conference on Harmonization ICH. Q2B: Text on Validation of Analytical Procedures: Definitions and Terminology. US FDA Federal Register. 1995.
14. Proceedings of the International Conference on Harmonization ICH. Q2B: Text on Validation of Analytical Procedures: Methodology. US FDA Federal Register. 1997.
15. Wai-Bun LBP. Composition optimization of extruded starch using response surface methodology. Packag Technol Sci. 2004;17(6):295-305.
16. Barmpalexis P, Kanaze FI, Georgarakis E. Developing an optimizing a validated isocratic reversed phase HPLC separation of nimodipine and impurities in tablet using experimental design methodology. J Pharmaceut Biomed. 2009;49(5):1192-202.
17. Yogita BW, Dipak DP. An experimental design approach for optimization of spectroscopic method for estimation of cefixime trihydrate using ninhydrin as derivatizing reagent in bulk and pharmaceutical formulation. J Saudi Chem Soc. 2017; 21(11): S101-11.
18. Derringer G, Suich R. Simultaneous optimization of several response variables.

J Qual Technol. 1980;12(4):214-9.
19. Kleinschmidt G, Ermer J, Miller JHM. Method Validation in Pharmaceutical Analysis A Guide to Bes Practice. Wiley-VCH Verlag Gmbhand Co, KGaA. Weinheim. 2005.
20. Christian JR, Kalpana P, Gandhi TR. Validation and experimental design assisted robustness testing of rplc method for the simultaneous analysis of brinzolamide and brimonidine tartrate in an ophthalmic dosage form. Indian J Pharm Sci. 2016;78(5):631-40.
21. Buralla KK, Parthasarathy V. Quality by design based developed and validation of rp-hplc method for simultaneous estimation of pazopanib in bulk and pharmaceutical dosage forms. Int J Pharm. 2019;9(3):135-40.
22. Suresh R, Manavalan R, Valliappan K. Developing and optimizing a validated rp-hplc method for the analysis of amlodipine and ezetimibe with atrovastatin in pharmaceutical dosage forms applying response surface methodology. Int J Pharm Pharm Sci. 2012;4(3): 550-8.

Article History: Submission Date : 06-06-2020; Revised Date : 31-07-2020; Acceptance Date : 02-10-2020.
Cite this article: Krishnan B, Mishra K. Quality by Design based Development and Validation of RP-HPLC Method for Simultaneous Estimation of Sitagliptin and Metformin in Bulk and Pharmaceutical Dosage Forms. Int. J. Pharm. Investigation, 2020;10(4):512-8.