RASĀYAN *J. Chem.*
Vol. 19 | No. 3 | 1366-1373 | July-September | 2026
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

STATISTICAL METHOD OPTIMIZATION AND VALIDATION OF A ROBUST AND EFFICIENT RP-HPLC METHOD FOR SAXAGLIPTIN USING BOX-BEHNKEN DESIGN

R. Venkatraj[1,✉], M. V. Kumudhavalli[2], M. Kumar[3], and J. S. K. Nagarajan[4]

[1]Research Scholar, Vinayaka Mission's College of Pharmacy, Vinayak Mission's Research Foundation (DU), Salem- 636008 (Tamilnadu), India.

[2]Department of Pharmacognosy, Vinayaka Mission's College of Pharmacy, Vinayak Mission's Research Foundation (DU), Salem-636008 (Tamilnadu), India.

[3]Department of Pharmaceutical Chemistry, Vinayaka Mission's College of Pharmacy, Vinayak Mission's Research Foundation (DU), Salem- 636008 (Tamilnadu), India.

[4]Department of Pharmaceutical Analysis, JSS College of Pharmacy, JSS Research Foundation (DU), Ooty- 643001(Tamilnadu), India

✉Corresponding author: venkatramasamy9002@gmail.com

ABSTRACT

This research work aimed to investigate a novel method of Quality by Design (QbD) approach, by applying a Box–Behnken design (BBD) to statistically validate an RP-HPLC method for the estimation of Saxagliptin. The selected method is helpful for structured optimisation by reducing experimental trial and error. This structured optimisation resulted in consistent performance, producing a sharp, symmetrical peak with a short retention time. The method showed satisfactory validation results, with system suitability parameters within acceptable limits. It exhibited excellent precision (%RSD 0.07%) and good accuracy, with a mean recovery of around 99.50%. Sensitivity was also adequate, with LOD and LOQ values of 0.23 µg/mL and 0.71 µg/mL, respectively. These findings confirm that the method is reliable for routine quantitative analysis. Validation of this method, carried out in compliance with ICH Q8(R2) guidelines, supports its suitability for regulatory and quality control applications. From a broader perspective, this work emphasises the importance of incorporating QbD principles into analytical method development. Such an approach improves method robustness, reproducibility, and overall understanding, while supporting efficient lifecycle management. The strategy presented in this study can also be adapted for other pharmaceutical compounds, offering a systematic and cost-effective pathway for method development. Overall, the study contributes to analytical research by demonstrating how statistical optimisation techniques can improve method performance, ensure regulatory compliance, and support reliable pharmaceutical analysis in both academic and industrial applications.

Keywords: Saxagliptin, Quality by Design (QbD), Box–Behnken Design, Response Surface Methodology, ICH Q8 (R2), Analytical Method Development, Pharmaceutical Analysis

RASĀYAN *J. Chem.*, Vol. 19, No.3, 2026

INTRODUCTION

Saxagliptin is an oral anti-diabetic formulation that improves glycemic control by selective inhibition of DPP-4 and increases hormone secretion by stimulating pancreatic beta cells, increasing glucose-dependent insulin. Chemically,[1] Saxagliptin"(1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile" Molecular formula "(C18H25N3O2)". Molecular weight 315.41. This compound partially dissolved in Water rapidly dissolved in methanol, ethanol, DMSO, and similar organic solvents. Both vildagliptin and Saxagliptin have a cyanopyrrolidine ring as a moiety to contribute activity.[2]

This method is fast and reliable, reproducible. The analytical methods having acritical component of quality control analysis for accurate determination of pharmaceutical compounds. Method development is frequently by one-factor-at a time.[3] In which each parameter differs in dependably, the Quality by Design improves the scientific based risk- oriented method development by applying systematic experimentation, quality risk management. It ensures the method is robust, accurate, fast, reliable and reproducible. This

work describes the method development of a QbD- applied RP-HPLC method for Saxagliptin, optimised through a BB design to achieve consistent and reliable performance.[4]

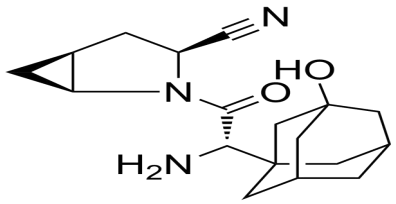

Fig.-1: Structure of Saxagliptin

This method demonstrated good precision, accuracy, and sensitivity while reducing run time and solvent usage. Compliance with ICH Q8(R2) guidelines supports its suitability for routine quality control applications. The use of statistical design improves method understanding and reduces experimental variability. The present work related to SDG-3 Good Health and Well-being by enabling dependable analysis and more sustainable laboratory practices.

EXPERIMENTAL

Chemicals used

Saxagliptin standard procured from Metrochem Pvt. Ltd, India. Marketed formulation 5 mg dose obtained from the local market. Acetonitrile (ACN), Ethanol, water, HPLC grade purchased from Molychem Pvt. Ltd., Mumbai. Ammonium citrate and citric acid, analytical grade, purchased from Merck. HPLC-grade water was used throughout the study.

Instrumentation

The entire study was carried out using Waters Reverse Phase-High Pressure Liquid Chromatography coupled with a binary pump system and photodiode array (PDA) detector.

Methods[5]

Standard Preparation

Carefully weighed 5 mgs of the standard was taken into a 25 ml of volumetric flask. Approximate amount of 10 ml of ethanol was used. sonicate up to 10 minutes. Make up the final volume.

Preparation of Test Substance

Ten tablets, each containing a labelled claim of 5mg of the drug, were accurately weighed to determine the average mass and then triturated into a fine, homogeneous powder. From this comminuted mass, a quantity equivalent to 5 mg of the active ingredient was quantified carefully and transferred into a 100 mL volumetric flask. The drugs were extracted using ethanol with sonication until complete dissolution was achieved. After filtration, 5 millilitres of the clear solution were diluted to 100 mL with ethanol. The prepared sample was finally filtered through a 0.45 μm membrane filter before injection.

Buffer Preparation

To prepare a 20 mM ammonium citrate buffer, 4.85 g of ammonium citrate dibasic was weighed precisely and dissolved in about 800 millilitres of deionised water. The pH was then adjusted to 3.0 with citric acid, and the solution volume was made up to 1000 millilitres using deionised water.

Movable Phase Preparation

Mobile phase contains buffer pH3.0, ACN, and MeOH in the ratio of 50: 40:10. This mixture sonicate up to until gasses evolved.

Method Optimisation by using QbD

"Quality by Design (QbD)" approaches the application of "Box Behnken" experimental design utilizing to optimise the chromatographic method. This experiment to evaluate the interference of critical method parameters (CMPs) on Retention Time (RT), which considers the primary response.[6]

Independent Variable Selected

X_1- pH of buffer, X_2 - Proportion of mobile phase, X_3- Flow rate

In this study conducted using three- factor, two-level design was used; it consists of 20 experimental runs was generated using the State Easy 360 software. This data is expressed in a second-order polymer equation:

$$Y=\beta0 +\beta1 X1 +\beta2 X2 +\beta3 X3 +\beta12 X1 X2 +\beta13 X1 X3 +\beta23 X2 X3 +\beta11 X12 +\beta22 X22 +\beta33 X32$$

Where: Y = measured response (retention time), β_0= intercept, β_1, β_2, β_3= linear coefficients, β_{12}, β_{13}, β_{23} = interaction coefficients, β_{11}, β_{22}, β_{33} = quadratic coefficients' The surface modelling enabled the effect of pH, Moveable Phase ratios and Flow rate on chromatographic conditions. Statistical analysis demonstrates the ruggedness and robustness with a percentage deviation of less than 2%. It confirms the reliability of this method.[7]

Method Validation (ICH Q8(R2))

The developed analytical procedure was validated in accordance with accordance of "International Council for Harmonisation (ICH) guideline Q8(R2)".

System Suitability

To confirm the system suitability of the analytical setup, the experiment was performed with a standard solution that was injected six times consecutively. System suitability performance was confirmed by analysing critical chromatographic parameters, retention time, peak area, tailing factor, theoretical plates, and %RSD, which were examined to ensure consistent system performance. The values are within the acceptable limit range based on the International Council for Harmonisation (ICH) guideline. It shows the adequacy of the chromatographic system.[8]

Specificity

The method's specificity was verified by assessing the analyte in combination with excipients, impurities, and degradation products, where no overlapping or interfering peaks were detected.

Linearity

The linearity was done by preparing different injection concentrations of standard '20,40,60,80,100,120 µg/mL'. It represents a linear relationship between the concentration of the sample and the detector response, which confirms the proportionality of this method.

Precision

Precision was validated by weighing carefully about 5 mg of the bulk drug taken in 100 ml of a volumetric flask, dissolved in ethanol. 10 ml of this stock is transferred into a 100 ml volumetric flask and diluted. The final solution was injected into six replications. Intermediate precision was assessed by the different days and different analysts. It demonstrates % RSD values within an acceptable limit.

Accuracy

Accuracy was estimated through the recovery studies by spiking known quantities of the standard into the placebo at different concentration levels. This method ensures that the method provides accurate quantification of the analyte.

Ruggedness

It is evaluated by a small variation in analytical conditions, changing the analyst, instruments, and reagent batches. It confirms the robustness and reliability of this method under different conditions.

RESULTS AND DISCUSSION

Table-1 shows the mobile phase ratio considered as a critical method parameter X_2 in this experimental design. The optimised chromatographic condition is pH 3.0, mobile phase Buffer: ACN: MeOH (50:40:10) and volumetric flow rate 0.9 ml/min. The RT is 3.378 minutes. with a symmetric peak and system suitability parameters.

Table-1: Response of Box-Behnken design

Run	X_1 (pH)	X_2(Mobile Phase Ratio)	X_3(Flow)	Predicted RT
1	2.8	48:42:10	0.9	3.52
2	3.2	48:42:10	0.9	3.68
3	2.8	52:38:10	0.9	3.18
4	3.2	52:38:10	0.9	3.34
5	2.8	50:40:10	0.8	3.96
6	3.2	50:40:10	0.8	4.12
7	2.8	50:40:10	1.0	2.98
8	3.2	50:40:10	1.0	3.14
9	3.0	48:42:10	0.8	4.02
10	3.0	52:38:10	0.8	3.72
11	3.0	48:42:10	1.0	3.05
12	3.0	52:38:10	1.0	2.82
13	3.0	50:40:10	0.9	3.38
14	3.0	50:40:10	0.9	3.37
15	3.0	50:40:10	0.9	3.39
16	3.0	50:40:10	0.9	3.38
17	3.0	50:40:10	0.9	3.37

Table-2: Regression Coefficient Table (Quartic model)

Term	Beta Coefficient (β)	Standard Error (SE)	t-Statistic	Probability Value	Inference Result
Intercept (β_0)	3.378	0.012	281.5	<0.0001	Significant
X_1 (pH)	+0.120	0.018	6.67	0.0003	Significant
X_2(Mobile phase ratio)	−0.185	0.021	−8.81	<0.0001	Significant
X_3(Flow rate)	−0.465	0.017	−27.35	<0.0001	Significant
X_1X_2	+0.045	0.015	3.00	0.020	Significant
X_1X_3	−0.032	0.014	−2.28	0.048	Significant
X_1X_3	−0.041	0.016	−2.56	0.037	Significant
X_{12}	+0.082	0.013	6.31	0.0004	Significant
X_{22}	+0.065	0.014	4.64	0.002	Significant
X_{32}	+0.090	0.012	7.50	0.0002	Significant

R^2 = 0.986, Adjusted R^2 = 0.971, Predicted R^2 = 0.948, Adequate Precision = 22.4, CV (%) = 1.38”

Regression Equation:
RT=3.378+0.120X1 −0.185X2 −0.465X3 +0.045X1 X2 −0.032X1 X3 −0.041X2 X3 +0. 082X12 +0.065X22 +0.090X32

This regression analysis shows that the flow rate X_3 apply the significant negative effect on RT with the mobile phase ratio X_2. The positive coefficient indicates a curve in the response surface, which shows the suitability of the quadratic model. It demonstrates strong interaction and a nonlinear effect among chromatographic variables.
Figure-2 Image (A) represents the effect on X_1 and X_2, image (B) shows the effects on X_1 and X_3, image (C) shows the effect of X_2 and X_3. This response shows the flow rate has a more significant effect on retention time. Followed by the mobile phase composition.
Increasing the organic phase higher than acetonitrile reduced the retention Time. The flow rate has the strongest negative effect when increasing. Increasing the organic portion and flow rate together cause the sharply decreases the retention time.[9]

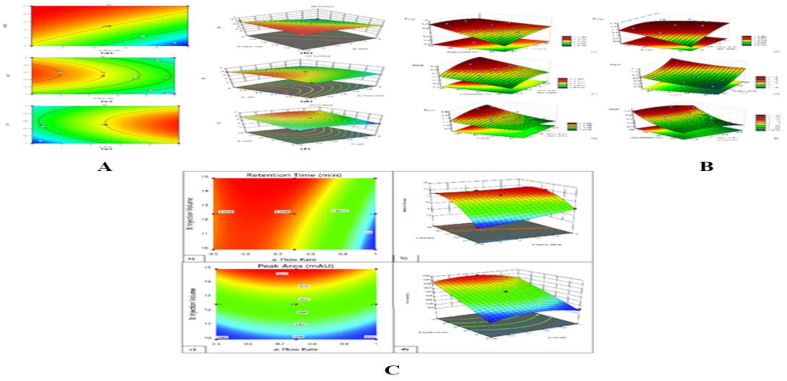

Fig.-2: 3D Response Surface Plot

Optimization - Separation was done by using RP- HPLC with 220nm. The retention time is 3.378min. The sharp and symmetric peak with good resolution was achieved

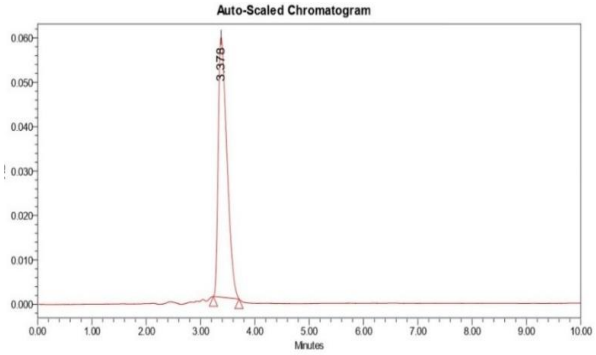

Fig.-3: Bulk Drug Chromatogram of Saxagliptin

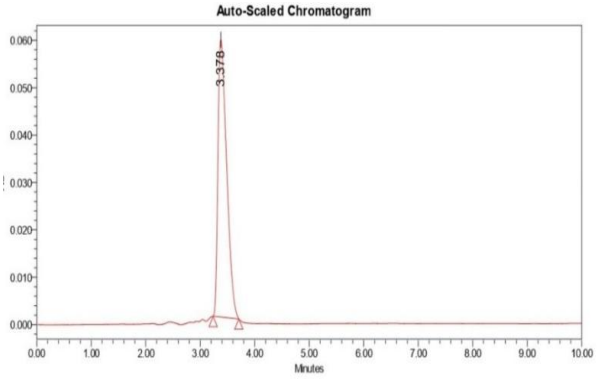

Fig.-4: Sample Chromatogram of Saxagliptin

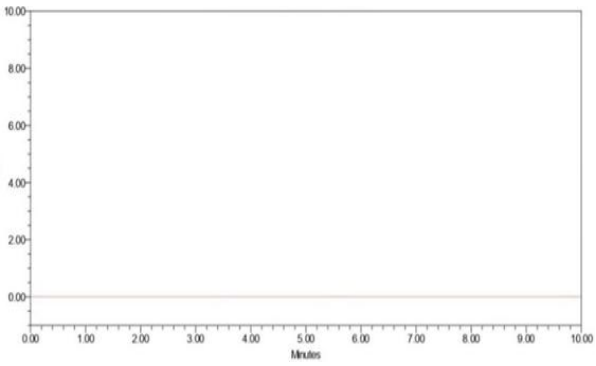

Fig.-5: Blank

Validation[10,11]

System Suitability

The RT range from 3.376 to 3.381 min, the mean value is 3.378 min. The area of the peak is 64952. The deviation of %RSD for RT and peak area was 0.05% and 0.07%.

Table-3: System Suitability

No of Injection	RT (min)	Area of Peak
1	3.378	649242
2	3.381	648910
3	3.376	650115
4	3.379	649875
5	3.377	649420

System suitability shows the %RSD values are the comes under the within the limit. The acceptable limit range is ≤ 2%.

Linearity

Linearity was done by 20-120 µg/ml. concentration range.

Table-4: Linearity Data

Injection	Concentrations (µg/ml)	Area
1	20	129845
2	40	259632
3	60	389410
4	80	519285
5	100	649242
6	120	779105

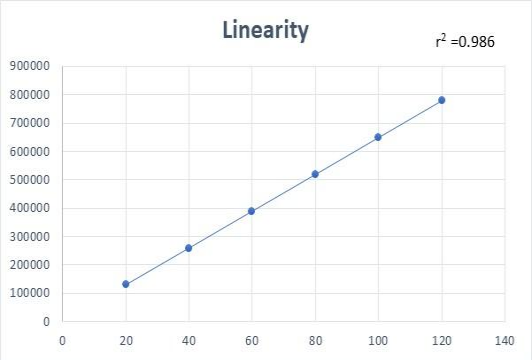

Fig.-6: Linearity

Figure-6 shows the correlation coefficient is 0.986, indicating excellent linearity across the different concentration range.

Table-5: Precision Data

No of Injection	Peak
1	649242
2	648910
3	650115
4	649875
5	649420
6	649690
Mean	649542
Standard Deviation	451.8
%RSD	0.07%

The % RSD area was 0.07%. It shows the excellent precision of the method. The values below the acceptable limit. Acceptance criteria ≤ 2 %.

Accuracy (Recovery Study)
Accuracy was performed by 80%,100%, and 120% concentration levels.

Table-6: Accuracy (Recovery Study) Data

Accuracy Level	Amount added (µg/ml)	Amount Found (µg/mL)	% Recovery
80%	80	79.45	99.31
100%	100	99.62	99.62
120%	120	119.48	99.57
Mean % Recovery			99.50%
%RSD			0.15%

Accuracy (Recovery Study) data shows the percentage values range from 99.31 %, 99.62% and 99.57%. foe 80%. 100% and 120% concentration ranges. Mean value is 99.50 %, and the percentage of recovery is coming under the limit. The acceptable recovery rage 98%-102%.

Limit of Detection and Limit of Quantification (LOD and LOQ)
In line with ICH recommendations, the detection of quantification limit was determined based on the standard deviation of the response (σ = 451.8) along with the slope of the calibration plot (S = 6492.3).

LOD = 0.23 µg/ml and LOQ = 0.71 µg/ml

It indicates that the method has a high sensitivity.

Robustness
The deviation of flow rate, pH, and mobile phase variation the percentage deviation comes under the limit.[11]

Table-7: Robustness

Parameter Varied	Condition	%RSD
Volumetric Flow Rate	0.9 mL/min	0.32
Volumetric Flow Rate	1.1 mL/min	0.28
pH	±0.2	0.41
Mobile Phase	±2% organic	0.35

There is no significant variation observed. The limit range is less than 2%.

CONCLUSION

Using BBD within a QbD framework, a robust RP-HPLC method for the quantification of Saxagliptin was successfully developed. Critical parameters, Volumetric flow rate, mobile phase composition, and buffer pH, were systematically studied to define a robust operating space, resulting in a method that is linear, precise, reproducible, and dependable. The method met the requirements of ICH Q8(R2) guidelines, supporting its use in routine quality control of bulk drug and finished formulations. Application of response surface methodology improved understanding of factor interactions and reduced experimental variability, thereby limiting reliance on trial-and-error.[12] The findings support the use of QbD-driven statistical optimisation to achieve efficient and consistent analytical performance. In addition, the method contributes to Good Health and Well-being by enabling reliable assessment of drug quality, and to Responsible Consumption and Production through reduced solvent use and more resource-efficient laboratory practices.

ACKNOWLEDGMENTS

The authors express their gratitude to Prof. Dr. M. Kumar, Principal, VMCP, VMRF (DU), for providing continuous support to carry out this research work.

CONFLICT OF INTERESTS

The authors do not have any conflicts of interest

AUTHOR CONTRIBUTIONS

The present work related to *SDG-3: Good Health and Well-being* significantly in the drafting of the manuscript in terms of reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

R. Venkat Raj http://orchid.org/0009-0001-7660-325X,
S. M.V. Kumudahvalli http://orchid.org/0000-0001-6675-8883
Kumar https://orcid.org/0000-0002-3513-2383.
J.S.K. Nagarajan https://orcid.org/0000-0003-4569-1071

Cite this article as: R. Venkatraj *et al.*, *Rasayan J. Chem.*, 19(3), 1366-1373(2026), https://doi.org/10.31788/RJC.2026.1939799

REFERENCES

1. K. Bidlan , K. Chaitanya Prasad, K. Nagasree, and K. Sharvan Kumar, I*nternational Journal of Chemistry and Pharmaceutical Sciences*, **14**, 23(2026), https://doi.org/10.30904/j.ijcps.2026.4915
2. S. Gurrala, S. Raj, C. V. S. Subrahmanyam, D. P. Anumolu, S. Naraparaju, and H. Nizampet, *Turkish Journal of Pharmaceutical Sciences*, **19**, 9(2022), https://doi.org/10.4274/tjps.galenos.2021.93195
3. K. R. Vankalapati, P. Alegete, and S. Boodida, *Biomedical Chromatography*, **36**, e5384(2022), https://doi.org/10.1002/bmc.5384
4. Narender Boggula and P. Shanmuga Pandiyan, *International Journal of Pharmaceutical Sciences and Research*, **12(1)**, 314(2021), http://dx.doi.org/10.13040/IJPSR.0975-8232.12(1).314-20
5. P. P. Bhakhar, H. S. Popaniya, K. V. Rathod, and P. N. Vaja, *Asian Journal of Pharmaceutical Analysis,* **16**, 11(2026), https://doi.org/10.52711/2231-5675.2026.00021
6. M. K. Shinde, B. W. Chopade, and V. V. Patil, *Chromatographia*, **85**, 341(2022), https://doi.org/10.1007/s10337-022-04145-z
7. A. Gandi, S. Mojjada, M. Puttagunta, S. S. Manikyam, V. R. Kollabathula, and S. R. Yaraguntla, *Accreditation and Quality Assurance*, **30**, 625(2025), https://doi.org/10.1007/s00769-025-01675-5
8. M. Manasa and V. M. Aanandhi, *Research Journal of Pharmacy and Technology*, **14**, 1045(2021), https://doi.org/10.5958/0974-360X.2021.00187.6
9. K. Patel, U. A. Shah, and C. N. Patel, *Future Journal of Pharmaceutical Sciences*, **9**, 1(2023), https://doi.org/10.1186/s43094-023-00509-w
10. R. S. Tambare, S. R. Shahi, V. C. Gurumukhi, S. M. Kakade, and G. G. Tapadiya, *Heliyon*, **10**, e39172(2024), https://doi.org/10.1016/j.heliyon.2024.e39172
11. S. N. H. Azmi, W. A. N. Al Rawahi, A. I. Al Yahyai *et al.*, *Journal of Chromatography B*, **1234**, Article 124035(2024), https://doi.org/10.1016/j.jchromb.2024.124035
12. P. Ilayaraja, M. Manivannan, and P. Parthiban, *Rasayan Journal of Chemistry*, **19(1)**, 192(2026), http://doi.org/10.31788/RJC.2026.1919511

[RJC-9799/2026]