RESEARCH PAPER

Multi-Response Optimisation of Gas Chromatographic Method for Residual Solvent Quantification in Sitagliptin API via DoE

[1*]M. Siva Prasad and [2]Dr Subhashini V

[1]Research Scholar, School of Pharmaceutical Sciences, VISTAS, Chennai-600117.
[2]Department of Pharmacology, School of Pharmaceutical Sciences, VISTAS, Pallavaram, Chennai, India-600117
*[*]**Corresponding Author:** sivaprasad1155@gmail.com*

Received: 28th Feb, 2026; Revised: 6th March 2026; Accepted: 7th April, 2026; Available Online: 20th April, 2026

ABSTRACT

Introduction: A sitagliptin-tailored approach for residual solvent analysis offering enhanced specificity and efficiency was developed in this study, addressing the literature gap in the generic ICH Q3C of anti-diabetic residual solvent profiling.

Objective: A Quality by Design-optimised and validated method for the quantification of six residual solvents, including methanol, acetone, acetonitrile, ethyl acetate, toluene, and THF, in accordance with ICH 2/3, was developed by gas chromatography with a flame ionisation detector.

Methods: the DoE-driven Central Composite Design (CCD, α=1.68, 13 runs) was systematically optimised and validated as per ICH Q2 for the Column temperature (220–240°C) and Hold Time (2–10 min) in Design-Expert® 13. The non-polar greyhound HP-5 column, FID detector, and splitless injection were selected for targeting the solvents' physicochemical diversity (boiling points 56–111°C), Resolution (Rs≥2.0), Tailing Factor (TF 0.5–1.5), and Theoretical Plates (TP≥2000).

Results: The study established optimal conditions (CT=232.1, HT=6.2min; desirability=0.98) that yielded Rs=4.8, TF=0.6, TP=2850. ANOVA confirmed linear Rs model ($p<0.0001$, Adequate Precision=34.69; Pred R^2=0.9256). Validation demonstrated $r^2>0.999$, %RSD<1.5%, recovery 98.5–101.5%, LOQ below PDE limits and robustness under ±5°C/±0.2 mL/min variations. This Complete baseline resolution was achieved in <20 min without matrix interference.

Keywords: Sitagliptin, residual solvents, gas chromatography, Design of Experiments, Quality by Design, ICH Q3C, method validation

How to cite this article: Prasad MS, Subhashini V, Multi-Response Optimisation of Gas Chromatographic Method for Residual Solvent Quantification in Sitagliptin API via DoE. Int J Drug Deliv Technol. 2026;16(6): 591-600. DOI: 10.25258/ijddt.16.6.67

Source of support: Nil.

Conflict of interest: None

1. INTRODUCTION

Residual solvents as defined by ICH Q3 guidelines, are chemicals incorporated and generated during the fabrication of Active Pharmaceutical Ingredient or excipients or into a dosage form that are not wholly eliminated from the final product.[1,2] Owing to their lack of pharmacological effect, potential toxicity, and effect on the physicochemical properties of pharmaceutical products, guidelines have been prescribed to limit and specify solvent levels in drug products. There are 3 distinct classes of residual solvents based on their toxicological profile. Class 1 solvents are strictly prohibited; class 2 solvents (limited carcinogenicity and reversible toxicity) have rigid concentration limits for their use; class 3 solvents, hence, have high permissible limits. [2,3]

The multi-step synthesis of sitagliptin involves various solvents, including methanol, acetonitrile, acetone, tetrahydrofuran, toluene and ethyl acetate. For their simultaneous residual quantification, a specialised analytical method is required, given their varied boiling points and Permitted Daily Exposures. [3,4,5]

Despite the growing therapeutic importance, the existing studies focused only on the liquid chromatographic technique for assay, dissolution and impurity profiling of sitagliptin; a published method for residual solvent analysis is not established. The generally approached gas chromatography method for a wide range of pharmacological ingredients and drugs is easily adaptable from broader ICH Q3C guidelines [5,6,7,8,9]. Furthermore, there is notable gravity for a systematically developed and validated robust GC method tailored to the unique RS profile of sitagliptin. [10,11,12,13,14,15]

Addressing this gap, an sitagliptin-specific residual solvent method was developed, optimised and validated by integrating a QbD approach using DoE for defining a Method Operable Design Range, centred on Column Temperature and Ramp time as Critical Method Parameters.[16,17] This study offers a novel, practical,

**Author for Correspondence: sivaprasad1155@gmail.com*

scientifically rigorous, fully sitagliptin-anchored GC-RS procedure, providing a transparent, statistically sound method for matrix effects, enhancing reliability and transferability for routine Quality Control and Regulatory complaint batch release.

2. EXPERIMENTAL

2.1. Equipment

A Shimadzu 2025 GC auto sampler equipped with an FID was used for performing all experimental trials. GC Solutions software was used for data processing and interpretation. The analysis was performed using a 30m long Greyhound column (HP-5) coated with (5% phenyl-methylpolysiloxane), which has a 0.32 mm internal diameter and a 0.25 µm thick stationary phase.

Statistical analysis of the experimental data, including ANOVA and optimisation, was executed using Design-Expert® 13 software from Stat-Ease Inc. A Central Composite Design (CCD) with two factors (The column temperature and ramp time) at two levels.

2.2. Chemicals and reagents

Methanol (MET), and Ethyl acetate (EA) were purchased from Thermo Fisher Scientific Pvt. Ltd, Mumbai; acetone (ACT), Acetonitrile (ACN), tetrahydrofuran (TDF), toluene (TOU) N, N-dimethyl sulfoxide (DMSO), and acetone were purchased from Sisco Research Laboratories, Mumbai; Dichloromethane (DCM) were purchased from Avantor Performance Materials Pvt. Ltd, Mumbai. All chemicals were used in GC grade, and sitagliptin (SGP) pharmaceutical drug was gifted by Aurobindo Pharma Ltd, Hyderabad.

2.3. Blank, standard and sample preparation

DMSO was used as a diluent to dissolve the analytes. To formulate a combination of standard stock solutions, each RS was prepared to obtain concentrations of 60000 ppm (MET), 1000000 ppm (EA), 100000 ppm (ACT), 10200 ppm (ACN), 100000 ppm (TDF), and 100000 ppm (TOU).

3. METHOD DEVELOPMENT

3.1. Instrumental parameters selection

To strategically match the physicochemical properties of the 6 target solvents (boiling points 56–111°C) and the sitagliptin matrix, the Greyhound HP-5 column (5-phenyl-methylpolysiloxane), FID and Spitless injection were selected, leveraging volatility-based separation by non-polar HP-5 stationary phase. The low-boiling-point polar solvents, for instance, acetone and methanol, elute early via weak interactions, while the high-boiling-point solvents retain longer owing to their necessary π-π affinity of phenyl groups.

The FID was selected because it responds proportionally to the carbon content of organic solvents; this sensitivity ensures clean quantification of target solvents, offering universal sensitivity at ppm levels as required by ICH Q3C. spitless injection further enhances this sensitivity, ensuring full sample transfer, which is critical for capturing trace amounts of early-eluting solvents. Together, this setup yields high thermal stability, well-resolved peaks with no interference of matrix and solvents in a rapid runtime. [Fig 01]

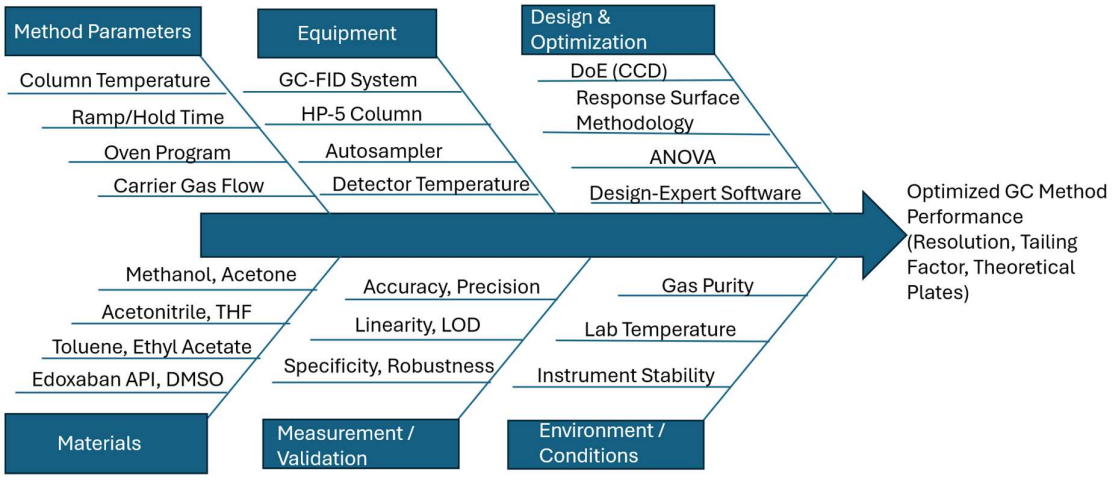


Fig. 01 Fish bone diagram depicting the quality material attributes and critical quality parameters.

3.2. QbD approach

A structured QbD strategy was employed to implement an optimised GC method for RSA in sitagliptin API. The CCD (α=1.68) matrix comprised of 13runs (8 factorial points, 4 centre points and 1 axial point) was generated and analysed using Design-Expert® version 13 (Stat-Ease Inc., Minneapolis, MN, USA). The targeted Critical Quality Attributes (CQA) were resolution (RS ≥1.5), tailing factor (TF-0.5–1.5) and theoretical plates (TP ≥2000) feature for accurate integration and column efficiency [Figure 1]. The design space spanned Column temperature (CT: 200-240°C) and Ramp Time (HT: 2–10 min) captures both linear and curvature effects of the independent variables. This ensured comprehensive statistical coverage with minimal resource expenditure, critical for New Chemical Entities [Fig 02].

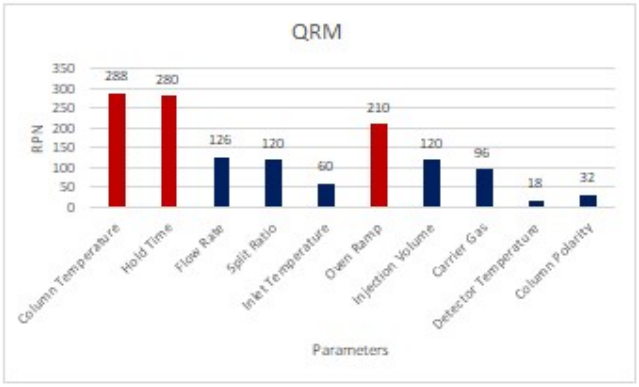
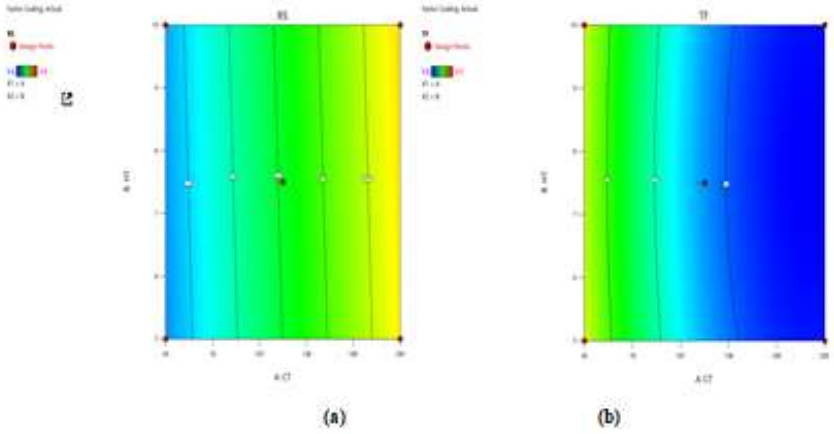


Fig.S3: FMEA risk assessment for critical parameters of GC

The numerical optimisation through ANOVA showed a linear model for RS, and quadratic models for TF and TP with excellent predictability and efficiency. The method yielded a CT=232.1°C, ramp time=6.2 min, (desirability=0.98), achieved factors Rs=4.8, TF=0.6, and TP=2850. The visual 3D response surface plots confirmed the robustness of the established design space with the optimal 'sweet spot' as per ICH Q2 (R2) validation guidelines [Figure 3].



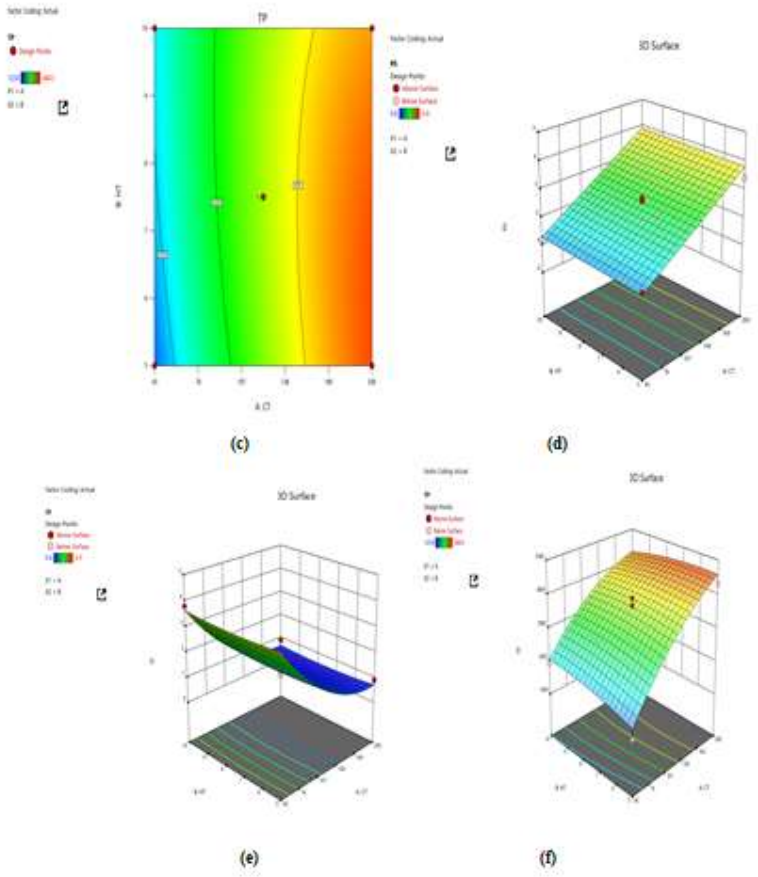


Fig.3: Contour and 3D surface plot of Resolution, tailing factor, Theoretical plates

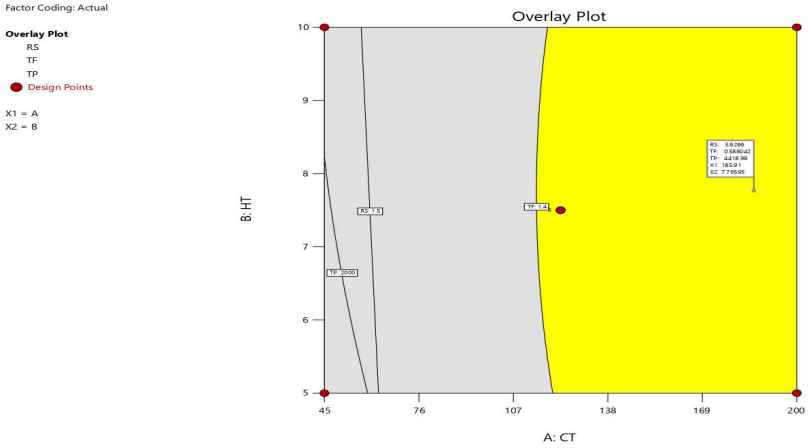


Fig.4: Overlay Plot depicting the Optimised Design Space for Column

3.3. Method Optimisation Details

A 30 m, 0.32 mm, and 0.25 µm HP-5 column was used for GC analysis. With a flow rate of 1.94 ml/min and a split ratio of 1:20, a microliter (µL) of standard and sample solutions was injected into an injection port at 220°C. The inlet temperature exceeded the boiling point of the mixture. The column oven's initial temperature was 40°C, which was maintained for 5 min, after which it increased steadily to 200°C at 30°C/min. [Table 1].

GC parameter	Condition
Column	HP-5 column (30m, 0.32mm, 0.25µm)
Temperature programming	

Rate (°C/min)	Temperature (°C)	Hold time (min)
-	45	5
20	145	10
Inlet temperature	220°C	
Detector temperature	230°C	
Initial oven temperature	45°C	
Final oven temperature	145°C	
Carrier gas Flame Pressure Column flow Purge flow Total flow	Nitrogen and Hydrogen Zero-air 99 kPa 3.94 mL/min 1.0 mL/min 53.7 mL/min	
Split ratio GC cycle time	1:20 20 min	

4. METHOD VALIDATION

The analytical Method validation was performed to examine linearity, precision, accuracy, specificity, LOD, LOQ, and robustness in accordance with the ICH Q2 (R2) criteria.

4.1 System Suitability

The system suitability of the optimised method was assessed by injecting six duplicates of the standard solution (100%) to determine whether the method was appropriate for its intended use.

4.2 Specificity

To assess whether the new method could precisely resolve residual solvents (RS), three replicates of separate RS and DMSO samples were injected into the GC system to test the interference from blanks to the RS.

4.3 Linearity

To establish method linearity, known concentrations of MET, ACT, ACN, EA, TOU, and THF were spiked in triplicate at 300-7500, 500-12500, 500-12500, 60-1500, and 500-12500 ppm. A peak area vs. drug concentration plot was used to develop the regression equation.

4.4 Accuracy

Accuracy testing was performed by injecting three quality RS samples of 50%, 100%, and 150% in triplicate into the GC to quantify the degree to which the observed value and the value regarded as an approved reference value agreed. The percentage of recovery was calculated and confirmed to meet the approval standards.

4.5 Precision

Inter-day and intra-day precision analyses were performed by injecting six replicates into the GC at 1 standard solution (100%) to assess the method's capacity to deliver reproducible results under defined conditions.

4.6 LOD and LOQ

The limit of detection (LOD) is also known as the signal-to-noise ratio of 3:1. The LOQ, in contrast, is typically represented by a signal-to-noise ratio of 10:1. Both numbers were empirically calculated by the following formulae.

$$\boldsymbol{LOD = 3.3 \times (\sigma/S)}$$

$$\boldsymbol{LOQ = 10 \times (\sigma/S)}$$

Where σ represents the standard deviation, and S denotes the slope.

4.7 Robustness

The ability of an analytical method to cope with slight but intentional variations in method parameters reflects its dependability under normal operating conditions. Conventionally, in gas chromatographic method development, robustness is achieved by adjusting the column oven temperature and carrier gas flow rates.

5. RESULTS

5.1 Design of Experiments

The DoE developed polynomial models that elucidate clear, quantifiable relationships between factors and chromatographic performance as follows:

Resolution (RS) increased linearly with both Column Temperature (CT, coefficient +0.017) and Hold Time (HT, +0.019). This indicates that elevating CT from its low level (-1) enhances peak separation more effectively than HT. This is due to the acceleration of analytes' partitioning by CT on the non-polar HP-5 phase

RS: 0.347598 + 0.0168382 * CT + 0.0191421 * HT

Tailing Factor (TF) exhibited strong negative linear dependence on both factors, dominated by HT (-0.229 >> CT -0.054), reflecting improved peak symmetry as extended HT. This minimises band broadening of lower boiling point solvents (e.g., acetone/methanol)

TF: 6.57795 + (-0.0535294 * CT) + (-0.229071 * HT) + 1.35234e-17 * (CT)(HT) + 0.000140271 * CT^2 + 0.0148 *HT^2

Theoretical Plates (TP) showed pronounced positive linear response to HT (+397, primary driver), promoting column efficiency and stabilized equilibration, by moderate CT gain (+40); negative quadratic terms (CT^2 - 0.065, HT^2 -17.13) and (CT) (HT) interaction (-1.00) revealed an optimal plateau before efficiency declined at

extremes, thus defining the method operable design region.

TP: (-1413.8) + 40.2499 * CT + 396.875 * HT + (-1.00129 * (CT) (HT) + (-0.0651946 * CT^2) + (-17.132 * HT^2)

These relationships are validated by ANOVA ($p<0.0001$ across models), pinpointing CT=232°C and HT=6.2 min as the robust "sweet spot" giving optimal desirability of 0.98, where Rs maximizes while TF/TP remain optimal [Table 2,3]

Table 2 Central Composite Design matrix for GC method optimisation (n=13 runs)

S.No	Factor 1	Factor 2	Response 1	Response 2	Response 3
Run	A: CT	B: HT	RS	TF	TP
3	45	5	1.3	3.8	1293
6	200	5	3.4	0.9	4327
7	45	10	1.2	3.8	1946
11	200	10	3.6	0.9	4204
9	12.8984	7.5	0.6	4.9	1234
8	232.102	7.5	4.8	0.6	4843
10	122.5	3.96447	2.5	1.3	3673
2	122.5	11.0355	2.7	1.2	3542
13	122.5	7.5	2.6	1.3	3895
1	122.5	7.5	2.6	1.1	3543
12	122.5	7.5	2.6	1.2	3567
5	122.5	7.5	2.6	1.3	3478
4	122.5	7.5	2.7	1.3	3674

Table 3: ANOVA for response surface models.

Response	Model	p-value	Adjusted R2	Predicted R2	Adeq Precision
Resolution (RS)	Linear	< 0.0001	0.9583	0.9258	34.6897
Tailing Factor (TF)	Quadratic	< 0.0001	0.9783	0.9178	31.5147
Plates (TP)	Quadratic	< 0.0001	0.9477	0.8219	20.9973

5.2 Linearity

To ascertain the linear relationship between the residual solvent concentration and average peak area, a concentration vs. peak area graph was created. The regression's slope, y-intercept, and correlation coefficient were computed. The acceptance parameter for linearity, r^2, must be greater than 0.99.

Table 4 Linearity, LOD, and LOQ values

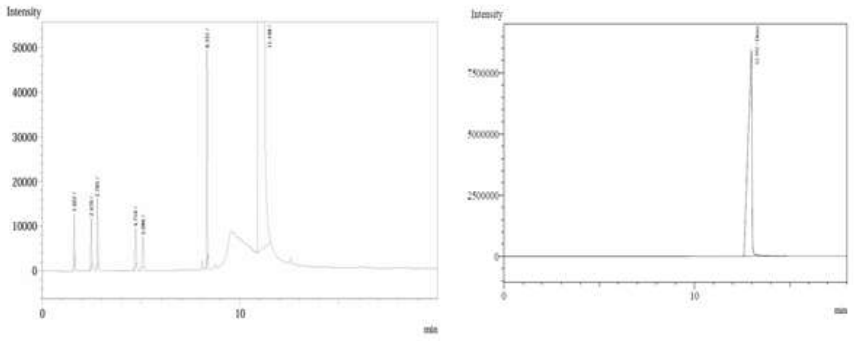
Solvent	Linearity range (ppm)	(r^2)	LOD (ppm)	LOQ (ppm)
Acetone	500-12500	0.9999	64	194
Methanol	300-7500	0.9999	99	301
THF	500-12500	0.9998	93	283
Ethyl Acetate	60-1500	0.9999	108	328
Acetonitrile	500-12500	0.9998	76	230
Toluene	500-12500	0.9998	71	215

5.3 LOD and LOQ

According to the ICH recommendations, The response's standard deviation (SD) and slope were used to calculate the limit of detection (LOD) and limit of quantitation (LOQ). This approach was used to establish the detection and quantitation limits [Table 4].

5.4 Specificity and system suitability

The specificity stipulated no interference between the solvent or API peaks, and all peaks were well-resolved. After evaluation, it was found that the system suitability parameters fell within the constraints for individual responses.i.e RS ≤ 15%, TF ≤10% and TP >1.5. [Table 5 and Figure 5].



(a) (b)

Fig 5: (a) standard chromatogram, (b) Blank chromatogram

Table 5 specificity, and system suitability parameters

Solvent name	Rt (min)	Theoretical plates	Tailing factor	Resolution
Acetone	1.601	7850	1.05	2.6
Methanol	2.470	8120	1.15	2.1
THF	2.785	7950	1.02	2.9
Ethyl acetate	4.716	8425	1.13	2.5
Acetonitrile	5.086	8650	1.14	2.3
Toluene	8.331	9100	1.16	3.2

5.5 Precision

The optimised method was applied to measure the SD and % RSD of the system. The %RSD values were established as follows: 0. 44 for acetone, 0.36 for methanol, 0.80 for THF, 0.47 for ethyl acetate, 0.25 for acetonitrile, and 1.22 for toluene. After examining the following results, [Table 6].

Table 6: Precision results

Intra-day precision						
Solvent	Acetone	Methanol	THF	Ethyl Acetate	Acetonitrile	Toluene
Parameter	Peak area	Peak area	Peak area	Peak area	Peak area	Peak area
	(n=6)	(n=6)	(n=6)	(n=6)	(n=6)	(n=6)
Mean	55452	51684	79765	58610	53791	188026
SD	243.751	184.541	639.629	273.546	133.254	2289.76
%RSD	0.44	0.36	0.8	0.47	0.25	1.22
Inter-day precision						
Solvent	Acetone	Methanol	THF	Ethyl Acetate	Acetonitrile	Toluene
Parameters	Peak area	Peak area	Peak area	Peak area	Peak area	Peak area
	(n=6)	(n=6)	(n=6)	(n=6)	(n=6)	(n=6)
Mean	55068	51883	79720	58762	53993	185605
SD	230.108	183.756	164.999	218.229	179.344	2272.32
%RSD	0.42	0.35	0.21	0.37	0.33	1.22

5.6 Accuracy

To assess accuracy, the percentage recovery at three separate levels was computed in triplicate using the formula below:

$$\%\boldsymbol{Recovery} = [(\boldsymbol{Asp} - \boldsymbol{As})/\boldsymbol{Astd}] \times \mathbf{100}$$

where Astd stands for the solvent area in the reference solution, Asp for the solvent area in the spiked sample, and As for the solvent area in the sample. The range of acceptable accuracy recovery percentages was 80–120%.

The accuracy calculations were within the bounds and are shown in the table below. [Table 7]

Table 7: Recovery studies results

Average %recovery						
% Spiking	Acetone	Methanol	THF	Ethyl Acetate	Acetonitrile	Toluene
50%	99.23	99.77	100.18	99.56	100.41	100.08
100%	99.96	99.28	100.44	99.17	99.68	100.41
150%	100.03	99.35	99.41	99.92	99.71	99.99

5.7 Robustness

The robustness test was conducted by varying the flow rate by 0.2 mL/min above and below the usual value. Table 10 shows the% RSD values for acetone, methanol, THF, acetonitrile, ethyl acetate, and toluene for three independent injections. The robustness limit is <10, and the method adhered to this limit.

Table 8 Results for robustness

Solvent	Acetone (n-6)	Methanol (n-6)	THF (n-6)	Ethyl Acetate (n-6)	Acetonitrile (n-6)	Toluene (n-6)
Flow rate +0.2 mL/min						
MEAN	55009	51389	58901	189259	79731	53372
SD	161.2203	141.4214	62.9325	428.5067	230.5168	121.6224
%RSD	0.29	0.28	0.11	0.23	0.29	0.23
Flow rate -0.2 mL/min						
MEAN	55009	51389	58901	189259	79731	53372
SD	161.2203	141.4214	62.9325	428.5067	230.5168	121.6224
%RSD	0.29	0.28	0.11	0.23	0.29	0.23

5.8 Eco-Friendly Assessment

Green analytical practices promote sustainable workflows by minimising solvent use, energy consumption, sample preparation, and waste. Although solvents cannot be eliminated in liquid chromatography, sustainability can be improved through safer biodegradable solvents, simplified procedures, miniaturised sample preparation, and comprehensive environmental impact assessment.

The Eco-Friendly of the method was assessed using the following tools:

1. Methods' practicality (Blueness) assessment: Blue Applicability Grade Index (BAGI) tool.
2. Analytical performance assessment (redness): Red analytical performance index (RAPI).
3. Analytical methodology greenness assessment: Analytical Greenness (AGREE) metric & ComplexMoGAPI.

The GC method, assessed using multiple modern analytical sustainability and performance tools, showed strong overall applicability, excellent analytical performance, and significant environmental sustainability.

5.8.1 BAGI

The method scored 80, demonstrating suitability for routine use due to accessible reagents, solid-state preparation, low sample volume, high throughput, and widely available instrumentation. BAGI effectively benchmarks practicality but inadequately addresses reagent safety and waste impact, highlighting areas for improvement. [18,19,20,21].

5.8.2 RAPI

The method receives very high RAPI scores—typically 10/10 for core parameters—illustrating its outstanding repeatability, precision, recovery, and robustness with a wide working range. Minor deductions till indicate strong reliability but allow room for minor optimisation. The RAPI score of 90.0 (Fig. 5) reflects the exceptional analytical quality and reproducibility [22,23,24].

5.8.3 Complex MoGAPI

This tool demonstrates the method's operational advantages (low sample/solvent use, concise steps, reduced energy demand), but lack of waste treatment and partial compliance with Green Chemistry principles. An overall score of 89 (Fig. 5) indicates respectable sustainability, but the yellow pictogram icons highlight notable limitations compared to ideal eco-friendly protocols [23,24].

5.8.4 AGREE

The score of 0.86 (Fig. 5) demonstrates better—but not outstanding—green chemistry compliance. The method's greenness benefits from sample minimisation, automation, derivatisation avoidance, and energy efficiency, but gaps remain in comprehensive operator safety and in situ analysis opportunities. Pictograms assist in quickly identifying opportunities for greener practices [23,24].

6. DISCUSSION

The study successfully corroborated a novel sitagliptin-characteristic GC method for simultaneous determination of all six ICH class 2 and 3 RS, optimised by DoE-driven QbD. The Analytical Quality by Design (AQbD) approach was applied to develop a robust, reliable, and scientifically justified GC-FID method for the determination of residual solvents in Remdesivir. Initially, an Analytical Target Profile (ATP) was established to clearly define the

intended purpose of the method, including the analytical technique, target residual solvents, sample matrix, and predefined performance requirements. Based on the ATP, critical analytical attributes (CAAs), namely resolution (Rs), tailing factor (TF), and theoretical plates (TP), were identified as essential indicators of chromatographic efficiency, peak symmetry, and overall method suitability.

Subsequently, a structured risk assessment was conducted in accordance with ICH Q9 guidelines using the Ishikawa fishbone diagram and Failure Mode and Effects Analysis (FMEA). Potential sources of variability affecting method performance were identified through scientific literature and prior experimental knowledge. Risk Priority Numbers (RPNs) were calculated by evaluating the severity, occurrence, and detectability associated with each factor. Initial oven temperature, split ratio, carrier gas flow rate, and injection temperature demonstrated the highest RPN values and were therefore designated as Critical Method Parameters (CMPs) for optimization. Preliminary investigations further established dimethyl sulfoxide (DMSO) as the most suitable diluent and hydrogen as the preferred carrier gas, owing to their superior chromatographic performance and reduced analysis time.

The MODR for stability was refined with the best condition (CT=232.1°C, ramp time=6.2 min), yielding Rs=4.8, TF=0.6, and TP=2850. All the validation results remain within acceptance limits across this MODR as per ICH Q2 (R2) with confirmed linearity (r^2>0.999), precision (%RSD<1.5), accuracy (98.5–101.5% recovery), specificity and sensitivity (LOQ below PDE limits, e.g., methanol 301 ppm vs. 410 ppm limit), stability under ±5°C CT/±0.2 mL/min flow rate further proved the model reliability.

The direct injection GC-FID method provided a short analysis time and is suitable for routine quality control of residual solvents in Sitagliptin API and finished products.

7. CONCLUSION

A QbD-validated GC method was accordingly devised for the determination of RS in sitagliptin API. This method fully adheres to ICH Q3 and Q2 guidelines. The study logically defended the results with data-driven DoE for an efficient method engineered for regulatory compliance for a drug-specific method. Further optimisation of the method may be acquired by automation with headspace sampling for more complex matrices beyond the sitagliptin API. This substantial methodology strengthens pharmaceutical quality assurance across manufacturing and regulatory industries, advancing with QbD application.

9. REFERENCES

1. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. (2021). ICH Q3C(R9): Impurities: Guidelines for residual solvents. https://database.ich.org/sites/default/files/ICH_Q3C(R9)_Guideline_MinorRevision_2024_2024_Approved.pdf
2. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. (2022). ICH Q2(R2): Validation of analytical procedures—Step 2 draft guideline. https://database.ich.org/sites/default/files/ICH_Q2R2_Document_Step2_Guideline_2022_0324.pdf
3. Akhila, V., Keerthana, T., Soujanya, C., Devi, B., Madhuri, L., Kumar, A., & Swathi, N. (2025). From limits to detection: Gas chromatography as a tool for residual solvent analysis. International Journal of Pharmaceutical Research and Applications, 10(5), 492–504. https://doi.org/10.35629/4494-1005492504
4. Ebru Uçaktürk., Development of a gas chromatography–mass spectrometry method for the analysis of sitagliptin in human urine. Journal of Pharmaceutical and Biomedical Analysis, 2013, 71-76, Volume 74. https://doi.org/10.1016/j.jpba.2012.10.012
5. B'Hymer, C. (2003). Residual solvent testing: A review of gas chromatographic and alternative techniques. Pharmaceutical Research, 20(3), 337–344. https://doi.org/10.1023/A:1022692224151
6. Reddy, S. K., & Rao, G. S. (2019). Analytical method development and validation for the estimation of residual solvents in pharmaceutical substances using gas chromatography. Journal of Pharmaceutical Analysis, 9(5), 357–364. https://doi.org/10.1016/j.jpha.2019.03.002
7. Teglia, C. M., Montemurro, M., De Zan, M. M., & Cámara, M. S. (2015). Multiple response optimization in the development of a headspace gas chromatography method for the determination of residual solvents in pharmaceuticals. Journal of Pharmaceutical Analysis, 5(5), 296–306. https://doi.org/10.1016/j.jpha.2015.05.003
8. Iuliani, P., Carlucci, G., & Marrone, A. (2010). Investigation of the HPLC response of NSAIDs using fractional experimental design and multivariate regression analysis. Journal of Pharmaceutical and Biomedical Analysis, 51(1), 46–55. https://doi.org/10.1016/j.jpba.2009.07.022
9. Myers, R. H., Montgomery, D. C., & Anderson-Cook, C. M. (2016). Response surface methodology: Process and product optimization using designed experiments (4th ed.). John Wiley & Sons.
10. Gandhi, P. J., & Lee, M. W. (2014). Sitagliptin: Review of pharmacology and clinical trials. American Journal of Health-System Pharmacy, 71(23), 2023–2033. https://doi.org/10.2146/ajhp130654
11. Kirchhof, P., Toennis, T., Goette, A., Camm, A., Diener, H., Becher, N., ... & Zapf, A. (2023). Anticoagulation with sitagliptin in patients with atrial high-rate episodes. The New England Journal of

Medicine, 389(13), 1167–1179. https://doi.org/10.1056/NEJMoa2303910

12. Stacy, Z., Call, W., Hartmann, A., Peters, G., & Richter, S. (2016). Sitagliptin: A comprehensive review of the pharmacology and clinical data for the management of atrial fibrillation and venous thromboembolism. Cardiology and Therapy, 5(1), 1–18. https://doi.org/10.1007/s40119-016-0058-2

15. Mundhe, B. S., Salve, H. D., Shelke, A., & Jadhav, A. (2024). Analytical method development and validation of assay of anticoagulant drug sitagliptin by RP-HPLC method. Degrés, 9(1). https://doi.org/10.21477/ijapsr.v2i3.8099

16. Ogata, K., Mendell, J., Tachibana, M., Masumoto, H., & Oguma, T. (2010). Clinical safety, tolerability, pharmacokinetics, and pharmacodynamics of sitagliptin, an oral, direct factor Xa inhibitor, in healthy volunteers. Journal of Clinical Pharmacology, 50(7), 743–753. https://doi.org/10.1177/0091270009352087

17. Gode, S. G., & Gollapalli, V. L. (2022). Quantitative determination of residual solvents in palonosetron API by HS-GC method. Asian Journal of Pharmaceutical and Clinical Research, 15(2), 75–81. https://doi.org/10.22159/ajpcr.2022.v15i2.43542

18. Aesha Bhalodia, Sonal Desai.Application of seven tools for evaluation of greenness of chromatographic methods for Remdesivir analysis. Green Analytical Chemistry Volume 11, December (2024), 100149. https://doi.org/10.1016/j.greeac.2024.100149

19. N. Manousi, W. Wojnowski, J. Płotka-Wasylka, V. Samanidou, Blue applicability grade index (BAGI) and software: a new tool for the evaluation of method practicality, Green Chem. 25 (2023) 7598–7604. https://doi.org/10.1039/D3GC02347H.

20. R. Mahdavi, Z. Talebpour, M. Noori, Application of new green evaluation tools to assess the environmental impact of analytical procedures based on solid phase microextraction techniques, Green Anal. Chem. 10 (2024) 100144. https://doi.org/10.1016/j.greeac.2024.100144.

21. P.M. Nowak, W. Wojnowski, N. Manousi, V. Samanidou, J. Płotka-Wasylka, Red analytical performance index (RAPI) and software: the missing tool for assessing methods in terms of analytical performance, Green Chem. 27 (2025) 5546–5553. https://doi.org/10.1039/D4GC05298F.

22. F.R. Mansour, K.M. Omer, J. Płotka-Wasylka, A total scoring system and software for complex modified GAPI (ComplexMoGAPI) application in the assessment of method greenness, Green Anal. Chem. 10 (2024) 100126. https://doi.org/10.1016/j.greeac.2024.100126.

23. J. Płotka-Wasylka, W. Wojnowski, Complementary green analytical procedure index (ComplexGAPI) and software, Green Chem. 23 (2021) 8657–8665. https://doi.org/10.1039/D1GC02318G.