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AQbD-Assisted Optimisation and Validation of GC-FID Method for Residual Solvents in Remdesivir API

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ABSTRACT: The study used an Analytical Quality by Design approach to develop and validate a GC-FID method for residual solvent analysis in Remdesivir API following ICH Q3C and Q2 guidelines. Several organic solvents are used in remdesivir synthesis and purification, requiring efficient residual solvent analysis. Conventional methods are time-consuming and do not assess variable interactions. Therefore, an Analytical Quality by Design-based GC-FID method was developed and optimised using experimental design techniques. Risk assessment identified critical method variables. A fractional factorial design studied oven temperature, split ratio, carrier gas flow, and injection temperature on resolution, tailing factor, and plate count. Optimisation used Central Composite Design with 27 runs in Design-Expert Pro 13 to establish the method operable design region and robust conditions. The GC-FID method was optimised using an HP-5 column (30 m, 0.32 mm, 0.25 µ) with hydrogen as carrier gas under split injection conditions. The oven temperature program and detector settings ensured efficient separation of residual solvents. The method showed good validation results with acceptable accuracy (% recovery 80–120%), precision (%RSD <2), and linearity ($r^2 > 0.99$). LOD and LOQ were 25.03–31.16 ppm and 75.84–94.42 ppm, respectively. All solvents showed well-resolved peaks meeting system suitability criteria. The method demonstrated ideal greenness scores (MoGAPI 89, AGREE 0.86, BAGI 80, RAPI 90), confirming its suitability for routine analysis and regulatory compliance. The AQbD-based GC-FID method ensured reliable, precise, and green residual solvent analysis of remdesivir API.

1. INTRODUCTION

Remdesivir (RMV) is a broad-spectrum antiviral agent originally developed for the treatment of emerging viral infections, particularly those caused by RNA viruses. It is a nucleotide analogue prodrug that inhibits viral replication by targeting the viral RNA-dependent RNA polymerase, thereby interfering with viral genome synthesis. Remdesivir gained global prominence during the COVID-19 pandemic after demonstrating in vitro and clinical activity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Initially developed by Gilead Sciences for Ebola virus disease, its antiviral potential was later extended to coronaviruses, including SARS-CoV and MERS-CoV [1-2].

Organic solvents are frequently used in the manufacture of excipients, pharmacological ingredients, or the formulation of drug products. Their toxicity, their impact on the medicinal substance's crystal quality, and their potentially disagreeable taste or Odor make them undesirable in the finished product [4]. Several production procedures or methods are employed to get rid of them. Small amounts of solvents remain even after such procedures. These trace amounts of organic solvents are sometimes referred to as residual solvents (RS) or organic volatile impurities (OVIs). Pharmaceutical companies are concerned about the presence of residual solvents in the finished product, as these solvents are harmful [5]. For residual solvents, the ICH has issued guidelines, namely ICH Q3C. This guideline establishes allowable quantities of solvents in drug products and classifies them by toxicity [5]. Class 1 solvents are extremely hazardous and present serious health hazards, such as mutagenicity, teratogenicity,

or carcinogenicity. When making pharmaceuticals, certain solvents should be avoided, and if they must be used, they should be used at the lowest amounts possible. Although Class 2 solvents are less harmful than Class 1 solvents, they can still be harmful to health; it's important to carefully monitor their levels. There is a limit on how much of these solvents can be used in pharmaceutical products. Class 3 solvents are those that have a low potential with PDE, generally 50 mg/day [6].

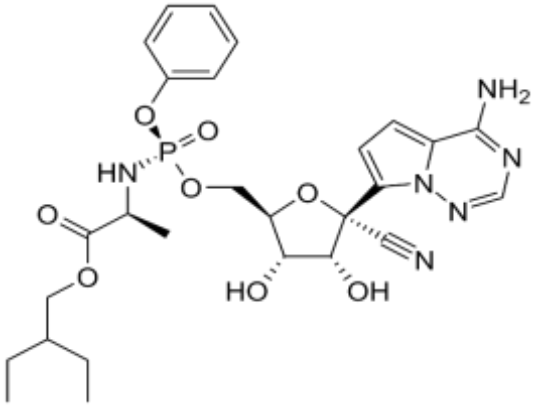


Fig. 01. Chemical structure of remdesivir

Since gas chromatography GC-FID can separate solvents with high accuracy and a very low detection limit, the procedure is validated by ICH Q2 criteria to make sure it satisfies the requirements for LOD and LOQ, linearity, robustness, system suitability, specificity, accuracy, and precision [7]. We believe this method is the best way to detect residual solvents such as methanol, acetone, diethyl ether, dichloromethane, and n-butanol in Remdesivir pharmaceuticals [8]. Although several GC-MS/MS methods [9,10,11,12] have been reported for residual solvent analysis in pharmaceutical drugs, limited studies are available on the optimisation of GC conditions for residual solvent analysis of RMV using the AQbD approach[13,14,15]. Existing methods lack systematic experimental-design-based optimisation to improve resolution, tailing factor, and analysis time. Therefore, the objective of this work is to develop an AQbD-based robust and sustainable analytical method for the estimation of residual solvent content in pharmaceutical drugs (RMV) by GC with FID [16,17].

2. MATERIALS AND METHODS

2.1. Chemicals

Methanol (MET), and diethyl ether (DEE) were purchased from Thermo Fisher Scientific Pvt. Ltd, Mumbai; acetone (ACT), n-butanol (BUT), 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 remdesivir (RMV) pharmaceutical drug was gifted by Aurobindo Pharma Ltd, Hyderabad.

2.2. Instrument details

A Shimadzu 2025 GC autosampler was used to develop an analytical technique in conjunction with FID. The GC system was operated by GC Solutions software. The analysis was conducted using a greyhound HP-5 capillary column, 30 m X 0.32 mm, 0.25 µ film thickness, coated with (5%-phenyl)-methyl polysiloxane.

2.3. Preparation of standard solutions and sample solution

Standard stock and sample solutions were prepared to achieve the concentrations of 60000 ppm (MET), 1000000 ppm (DEE), 100000 ppm (ACT), 10200 ppm (DCM), and 100000 ppm (BUT), and 1000 ppm (sample). All stock and samples were diluted using DMSO as a diluent.

3. METHOD DEVELOPMENT

Chromatographic condition optimisation is essential—such as column selection, oven temperature program, inlet temperature, split ratio, injection volume, carrier gas flow, and detector temperature—to achieve reduced retention time and improve overall performance.

3.1. Selection of column

The selection of the capillary column (5% phenyl–95% dimethylpolysiloxane) was based on the physicochemical characteristics of the target residual solvents and the sample matrix. The investigated solvents exhibited a broad range of boiling points and polarities: DEE (34.6 °C), DCM (39.8 °C), ACT (56.1 °C), MET (64.7 °C), and BUT (117.7 °C). A moderately non-polar HP-5 stationary phase provides separation primarily through volatility differences while maintaining sufficient selectivity for compounds with varying polarities.

3.2. Selection of injection system

Direct liquid injection was selected over headspace GC because it provided higher sensitivity, better peak reproducibility, and simpler sample preparation for the analysis of methanol, diethyl ether, acetone, dichloromethane, and n-butanol in Remdesivir API. DMSO was chosen as the diluent due to its excellent solubilising ability and high boiling point, which prevented interference with the target analytes. A split injection mode minimised matrix loading, inlet contamination, and carryover effects. Method optimisation showed no evidence of backflash, peak distortion, or analyte loss. The optimised method exhibited acceptable system suitability with tailing factors below 2.0, resolution above 2.0, and %RSD below 5%.

3.3. Selection of detector:

FID was selected due to its high sensitivity and wide linear range for volatile organic solvents. Since FID is a non-specific detector, analyte identification was based on retention-time matching with individual standards and confirmed through blank, diluent, API, and spiked chromatograms. No interference was observed, and all solvents were well resolved (Rs > 2.0).

3.4. Analytical quality by design

AQbD uses a risk-based approach to develop robust methods by identifying critical variables affecting performance. As per the ICH Q8 (R2) and Q9, it begins with defining the Analytical Target Profile (ATP). It then identifies critical analytical attributes and evaluates the impact of critical method attributes (CMAs) and critical method parameters (CMPs) using risk management. Finally, optimisation through DoE establishes the Method Operable Design Region. (Table S1)

3.5. Design of experiments

DOE is used to optimise chromatographic parameters by studying high-risk factors via a fractional factorial design with three levels and centre points. It evaluates relationships between factors and responses (Rs, TF and TP) using Response Surface Methodology (RSM). Results were analysed in Design-Expert Pro 13, with models fitted by Least Squares. Model significance and performance are confirmed using ANOVA and statistical indicators.

3.6. Design space and validation of the design space

The MODR was established using Monte Carlo simulation (10,000 iterations, 95% confidence level) in Design-Expert® Pro. The design space was defined as the multidimensional region where the probability of simultaneously meeting all response criteria exceeded 95%. Checkpoint experiments within MODR showed <5% prediction error, confirming method robustness and reliability. (Table S2)

The counter plots demonstrated that initial oven temperature and split ratio significantly influenced the chromatographic performance. High oven temperature and moderate to high split ratio produced maximum desirability and improved resolution. The tailing factor remained within acceptable limits, indicating good peak symmetry. Additionally, theoretical plate counts increased from approximately 4000 to 5500, confirming enhanced column efficiency and robust method performance. (Fig. 2)

The 3D surface plots revealed the combined effects of Initial Oven Temperature and Split Ratio on chromatographic responses. Resolution (Rs) increased slightly with increasing oven temperature and split ratio, while the tailing factor (TF) remained relatively constant within the acceptable range, indicating good peak symmetry. Theoretical plates (TP) showed a significant increase with increasing oven temperature, demonstrating improved column efficiency. The overlay plot identified a broad Method Operable Design Region where all predefined criteria for resolution, tailing factor, and theoretical plates were simultaneously satisfied, confirming the robustness of the optimised GC method. (Fig. 3)

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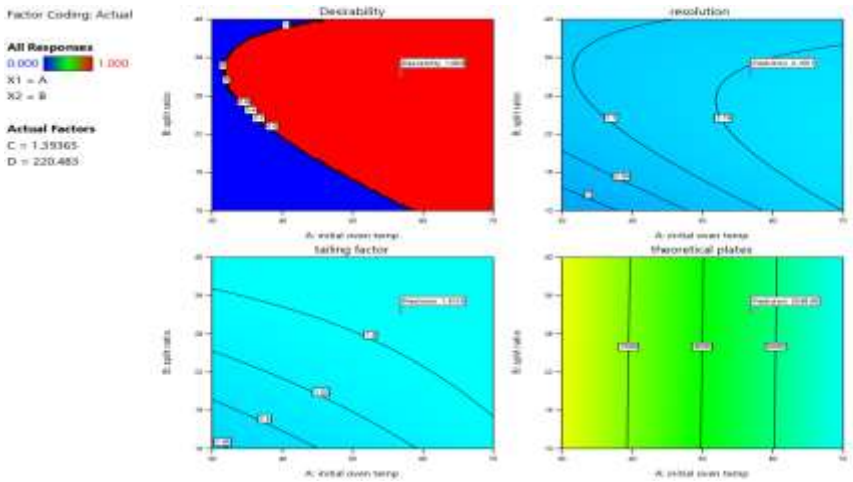
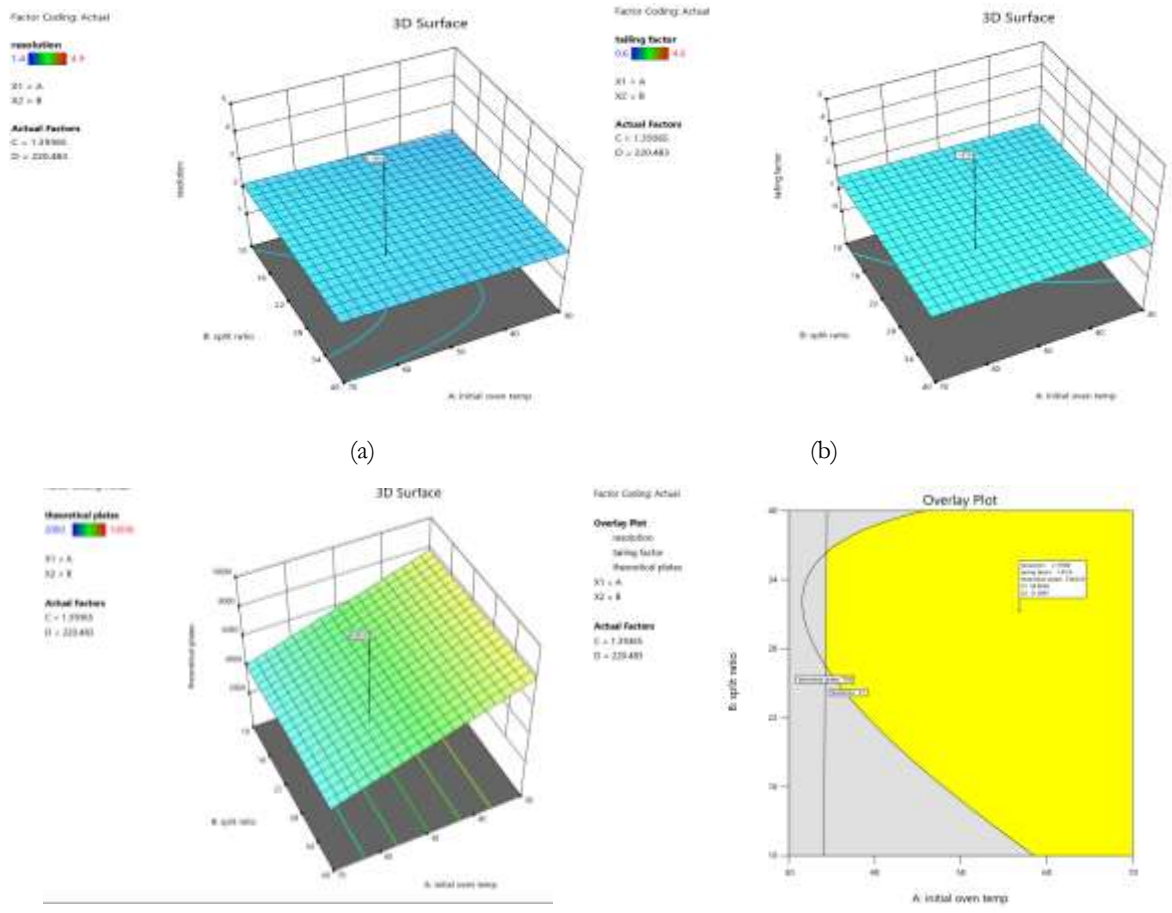


Fig. 02. Contour graphs for responses



(a) (b)

(c) (d)

Fig. 03. 3D surface graph of (a) Rs (b) TF (c) TP and (d) Overlay plot of MODR.

3.7. Method performance control strategy

To ensure the performance of the chromatographic method, many parameters such as system suitability, linearity, limit of detection, limit of quantification, precision (repeatability and intermediate precision), accuracy and robustness were validated as per USP and ICH Q2 (R1)

4. METHOD VALIDATION

Method validation was performed as per ICH Q2(R1) guidelines.

4.1. System Suitability

SST was evaluated using six replicate injections of a 100% standard solution. The acceptance criteria were Rs ≥ 2.0, TF ≤ 2.0, TP ≥ 2000, %RSD of peak areas (≤ 5.0%), and %RSD of retention times (≤ 1.0%). All parameters met the predefined criteria, confirming the suitability of the GC-FID method for residual solvent analysis.

4.2. Specificity

To verify whether the developed method can specifically resolve RS, interference from blank at the Rt of the RS was assessed by injecting three replicates of individual RS and DMSO samples into the GC system.

4.3. Linearity

Linearity is the ability of a method to produce results that are directly proportional to the concentration of the analyte. Residual solvents of MET, DEE, ACT, DCM, and BUT concentrations ranging from 300-7500, 500-12500, 500-12500, 60-1500, and 500-12500 ppm. To create the regression equation, the peak area vs solvent concentrations was displayed.

4.4. Accuracy

Accuracy was evaluated by spiking residual solvent standards at 50%, 100%, and 150% of the specification level. Three replicate preparations (n = 3) were analysed at each concentration level, and the %recovery was calculated by comparing the measured concentration with the known added amount. The recoveries obtained were within the predefined acceptance criteria, demonstrating the accuracy of the developed GC-FID method.

4.5. Precision

Inter and intra-day precision testing was performed by injecting six replicates into GC at the sample solution to determine the ability of the method to produce repeatable results under specified conditions.

4.6. LOD and LOQ

The LOD, commonly described as a signal-to-noise ratio of 3:1, is the lowest concentration of an analyte that can be accurately detected but not always quantified. Conversely, LOQ is the lowest analyte concentration that may be measured with reasonable accuracy and precision; it is usually indicated by a signal-to-noise ratio of 10:1. The following formulas were used to calculate both values statistically.

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

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

Where: σ is the standard deviation, and S is the slope.

4.7. Robustness

The ability of an analytical method to withstand minor but intentional changes in method parameters, such as column oven temperature and flow rate, is known as robustness, and it indicates the method's dependability under typical operating conditions. Generally, in gas chromatography method development, robustness was performed by altering the column oven temperature and the flow rate of the carrier gas.

5. RESULTS

5.1. Analytical quality by design (AQbD)

5.1.1. Analytical target profile (ATP) and critical analytical attributes (CAAs)

The ATP was established to develop a robust and reliable GC-FID method for the determination of residual solvents in Remdesivir. The ATP defined the method's purpose, analytes, matrix, analytical technique, and performance requirements. Critical Analytical Attributes (CAAs), including resolution (Rs), tailing factor (TF), and number of theoretical plates (TP), were

selected to assess chromatographic performance and guide method optimisation. These CAAs served as key indicators of method suitability and robustness.

5.1.2. Risk assessment

Risk assessment, as per ICH Q9, is performed in three steps: risk identification, risk analysis, and risk evaluation. The Ishikawa diagram, also called a cause-and-effect diagram, was used to categorise and review the effect of all the possible factors on CAAs as shown in Fig. 2. To identify these factors, we reviewed the literature.

Failure Mode and Effects Analysis was used to identify and rank potential risks affecting method performance. Risk Priority Numbers were calculated as Severity × Occurrence × Detectability, with higher values indicating greater criticality. Initial oven temperature, split ratio, carrier gas flow rate, and injection temperature show the highest RPN values and were selected as Critical Method Parameters (CMPs) for DoE-based optimisation.

Preliminary studies showed that DMSO provided more consistent peak shapes, shorter retention times, improved tailing factors, and higher theoretical plate counts than DMF. Furthermore, DMSO did not interfere with the retention or quantification of the target residual solvents and was therefore selected as the diluent. Hydrogen was used as the carrier gas, as it provided shorter analysis times and improved chromatographic performance. The critical method parameters identified during preliminary studies were subsequently evaluated using a DoE-based response surface optimisation approach.

5.1.3. Design of experiment

A fractional factorial design was used to evaluate the selected risk factors based on the risk assessment results (X1: Initial oven temp, X2: Split ratio, X3: Carrier gas flow, X4: Injection temperature). All 27 experiments were performed, and data were analysed using the design of Expert Pro software. Multiple linear regression and the least squares method were applied for the creation of mathematical models.

5.1.4. Effect of factors on resolution (Rs)

Resolution in gas chromatography describes how well two components are separated and depends on efficiency, selectivity, and retention. The model F-value (46.26) indicates strong significance, with only a 0.01% chance of noise. Significant terms include D, AC, and D^2 ($p < 0.05$), while values above 0.10 are not significant. The lack of fit ($F = 10.32$) shows a 9.15% probability of noise, indicating potential model inadequacy and the need for improvement. (Table S3)

5.1.5. Effect of factors on tailing factor (TF)

The TF describes peak symmetry in gas chromatography, with an ideal value of 1. The model F-value (30.66) indicates significance, with D and D^2 as significant terms ($p < 0.05$). The lack of fit is not significant ($F = 2.40$), indicating good model adequacy. Predicted R^2 (0.8411) is in reasonable agreement with Adjusted R^2 (0.9411), confirming a reliable and well-fitting model. (Table S4)

5.1.6. Effect of factors on theoretical plates (TP)

The number of theoretical plates (N) indicates GC column efficiency, with higher values giving better separation. The model F-value (238.34) shows strong significance, with A as a significant term ($p < 0.05$). The lack of fit is not significant ($F = 4.24$), indicating good model adequacy. Predicted R^2 (0.9648) agrees well with Adjusted R^2 (0.9733), confirming a reliable and well-fitting model. (Table S5)

5.1.7. Optimised chromatographic conditions:

GC analysis used an HP-5 column with split injection (1:20) at 220 °C and 1.94 mL/min carrier gas flow. The oven was programmed from 30°C to 200 °C, ensuring efficient vaporisation and separation of residual solvents. **(Table 1)**

Table 1:

Chromatographic conditions

Gc parameter	
Column	HP-5 column (30 °C, 0.32 mm, 0.25 µm)
Temperature programming	
Rate (°C/min) Temperature (°C) Hold time (min)	
- 30	5
30 200	5
Inlet	220 °C
Detector	230 °C
Initial oven	30 °C
Final oven	200 °C
Mobile phase	
Carrier gas Flame Pressure Column flow Purge flow Total flow	Nitrogen and hydrogen Zero air 60.7 kPa 1.94 mL/min 3.0 mL/min 43.7 mL/min
Split ratio GC retention time	1:20 18 min

5.2. Method validation

5.2.1. Linearity

A graph of concentration Vs peak area was developed in order to establish a linear relationship between residual solvent concentration and average peak area. The correlation coefficient, y-intercept, and slope of the regression were then calculated. The acceptance criteria for linearity, r^2, must be greater than 0.99 (Fig. S04-S08).

5.2.2. LOD and LOQ

According to the ICH recommendations, the limit of detection and the limit of quantitation were calculated to determine the smallest concentration were detected and accurately quantified. The approach based on the standard deviation of the response and the slope was used for determining the detection and quantitation limits as per equations (Table 2).

Table 2:

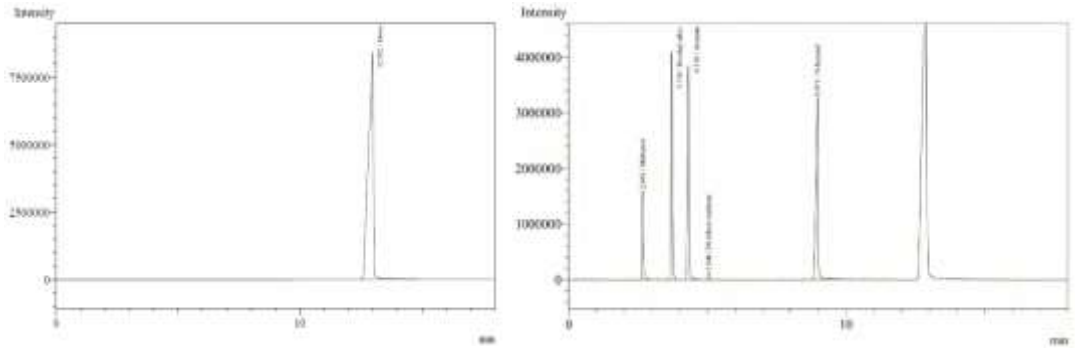
Linearity, LOD, and LOQ values

Solvent	**Linearity range (ppm)**	**(r^2)**	**Slope**	**LOD (ppm)**	**LOQ (ppm)**
MET	300-7500	0.997	2390	25.03	75.84
DEE	500-12500	0.997	8827	29.31	88.82

ACT	500-12500	0.994	7437	26.28	79.64
DCM	60-1500	0.993	183	10.80	31.34
BUT	500-12500	0.996	7807	31.16	94.42

5.2.3. Specificity and system suitability

No interference with solvent or API peaks; all peaks were highly resolved, indicating that the technique was specific, and the system suitability parameter was evaluated and found within the limits (**Fig.** 4, **Table** S6)



(a) (b)

Fig. 4 (a). Blank chromatogram (b). Standard chromatogram

5.2.4. Precision

To determine the precision of the system, the SD and %RSD were estimated using this approach. The %RSD values intraday and interday were found to be 1.15, 0.78 (MET), 1.15, 1.11(DEE), 0.58,1.05 (ACT), 0.87,0.74 (DCM), and 1.26,1.04 (BUT). The values obtained from precision were within 2% (Table 3)

Table 3:

Precision results

Intraday precision					
Solvent	MET	DEE	ACT	DCM	BUT
***Mean peak area**	152863	506818	466985	15289	440327
SD	1765	8849	2711	310	5555
%RSD	1.15	1.15	0.58	0.87	1.26
Inter-day precision					
Solvent	MET	DEE	ACT	DCM	BUT
Mean peak area	152712	502628	470823	15366	440885
SD	1184	9082	9675	114	4567
%RSD	0.78	1.11	1.05	0.74	1.04

*Six replicates (n = 6)

5.2.5. Accuracy

The following formula is used to calculate the % recovery at three distinct levels in triplicate to determine accuracy:

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

Where Asp represents the solvent area in the spiked sample, As represents the solvent area in the sample, and Astd represents the solvent area in the standard solution. The acceptance criteria for %recovery for accuracy are 80-120%. The % recovery was found to be 98,44,98.09,97.70 (MET), 98.41, 98.79, 97.85 (DEE), 98.22,98.59,97.64 (ACT), 97.66,97.61,97.66 (DCM), and 98.73, 98.99,96.90(BUT), respectively. The calculated accuracy values were within acceptable limits, demonstrating the developed method's accuracy. The detailed results are presented in Table 4.

Table 4.

Recovery studies results

*Mean of three replicates

%Spiking*	MET	DEE	ACT	DCM	BUT
50%	98.44	98.41	98.22	97.46	98.73
100%	98.09	98.79	98.59	97.61	98.99
150%	97.7	97.85	97.64	97.66	96.9

5.2.6. Robustness

To perform the robustness test, the flow rate was adjusted to ±0.2 mL/min of optimised chromatographic conditions (1.94 mL/min). The corresponding %RSD values were 1.18%, 1.03%, 1.16%, 1.15%, and 0.61%, all of which were below 2.0%These low %RSD values indicate that the deliberate variation in flow rate had no significant effect on the analytical performance of the method.(Table 5)

Table 5

Results for robustness

***Solvent**	**MET**	**DEE**	**ACT**	**DCM**	**BUT**
Flow rate +0.2 mL/min					
MEAN	137878	423897	444417	22131	548601
SD	2457	8592	7814	431	3364
%RSD	1.18	1.03	1.16	1.15	0.61
Flow rate -0.2 mL/min					
MEAN	120468	444257	491046	25884	562129
SD	1867	6422	7319	228	6548
%RSD	1.15	1.15	1.19	0.88	1.16

* Mean of six replicates (n=6).

5.3. Eco-Friendly Assessment

Green analytical practices aim to minimise or eliminate the use of organic solvents, energy consumption, sample processing steps, and waste to create more sustainable laboratory workflows. While it is not feasible to eliminate solvents from liquid chromatography, sustainability can be enhanced by employing safer, biodegradable solvents, reducing waste, streamlining

procedures, and adopting miniaturised sample preparation methods. Assessing the environmental impact of an analytical technique is complex and necessitates a thorough evaluation of multiple contributing factors.

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.

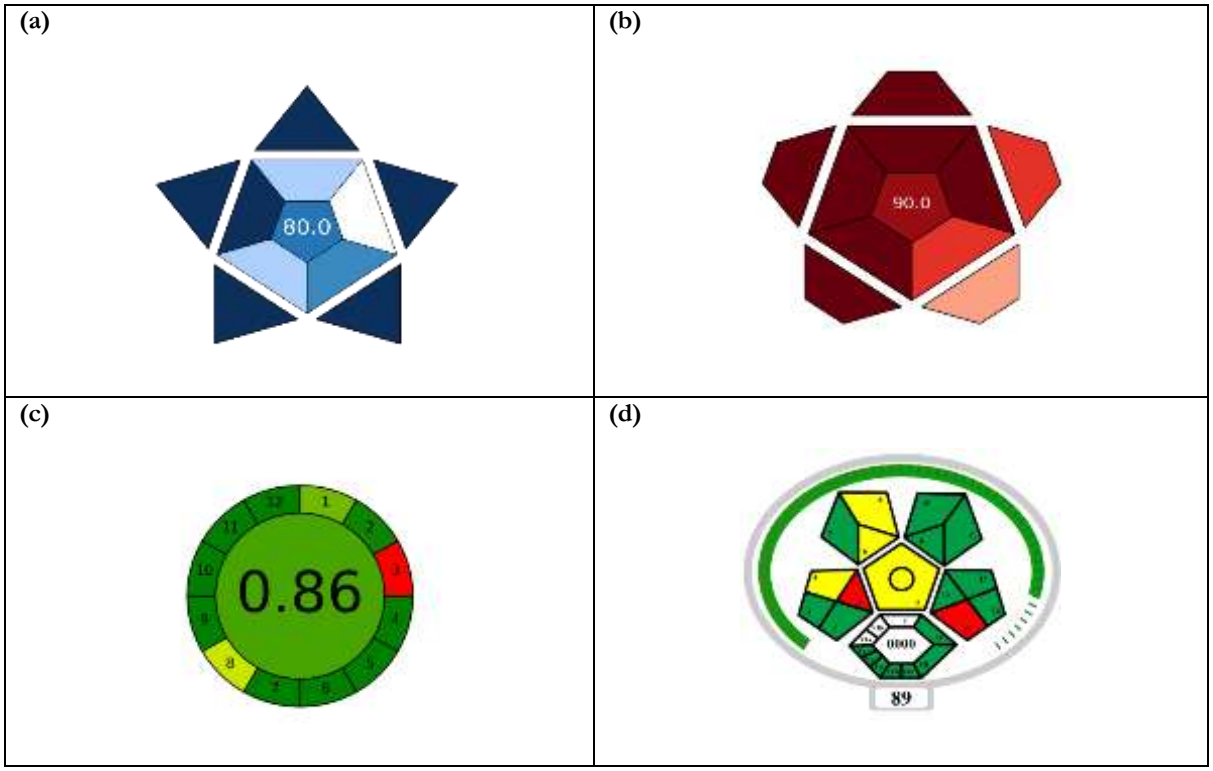


Fig. 5. Evaluation of the Eco-Friendly assessment of the proposed GC method: (a) BAGI (b) RAPI (c) AGREE (d) ComplexMoGAPI.

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

5.3.1. BAGI

This method achieved a score of 80 (Fig. 5), highlighting its suitability for routine applications owing to the accessibility of reagents, solid-state sample preparation, minimal sample volume, high throughput, and widespread instrumental availability. However, modest scores were noted for multi-analyte capability and miniaturised extraction sample preparation, indicating operational aspects that could be refined. The BAGI pictogram and scores provide rapid benchmarking of strengths and limitations, making it easy to compare this protocol with other alternatives. Notably, although BAGI quickly identifies practical aspects, it does not thoroughly account for reagent safety and waste impact [18,19,20,21].

5.3.2. 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].

5.3.3. 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].

5.3.4. 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].

7. DISCUSSION:

No interfering peaks were observed in blank, DMSO diluent, or Remdesivir sample chromatograms at the retention times of the target residual solvents, confirming method specificity. The AQbD approach was employed to develop a robust, reliable, and scientifically sound GC-FID method for the quantification of residual solvents in Remdesivir. An Analytical Target Profile was established to define the method objectives, analytical technique, target analytes, sample matrix, and desired performance criteria. Based on the ATP, critical analytical attributes (CAAs), namely resolution (Rs), tailing factor (TF), and theoretical plates (TP), were identified as key indicators of chromatographic performance and method suitability.

A systematic risk assessment was performed in accordance with ICH Q9 guidelines using the Ishikawa diagram and Failure Mode and Effects Analysis. Potential factors influencing method performance were identified through literature review and experimental knowledge. Risk Priority Numbers were calculated by considering the severity, occurrence, and detectability of each factor. Initial oven temperature, split ratio, carrier gas flow rate, and injection temperature exhibited the highest RPN values and were therefore selected as Critical Method Parameters for further optimisation. Preliminary studies identified DMSO as the optimal diluent and hydrogen as the carrier gas, providing improved chromatographic performance and shorter analysis times.

A DoE strategy employing a fractional factorial design was used to investigate the effects of the selected CMPs. Twenty-seven experimental runs were conducted, and the results were analysed using Design-Expert® software. Mathematical models were generated using multiple linear regression and least-squares analysis to evaluate the influence of process variables on the selected responses.

For resolution (Rs), the model was highly significant with an F-value of 46.26. Significant model terms included D, AC, and D^2 ($p < 0.05$), indicating their strong influence on chromatographic separation. Although the model showed a significant lack of fit, it provided valuable information regarding factor interactions affecting resolution.

The tailing factor (TF) model was also significant ($F = 30.66$), with D and D^2 identified as significant contributors. The non-significant lack of fit confirmed good model adequacy, while the close agreement between predicted R^2 (0.8411) and adjusted R^2 (0.9411) demonstrated satisfactory predictive capability.

For theoretical plates (TP), the model exhibited excellent significance ($F = 238.34$), with initial oven temperature (A) being the most influential factor. The non-significant lack of fit and strong agreement between predicted R^2 (0.9648) and adjusted R^2 (0.9733) confirmed the robustness and reliability of the developed model.

Overall, the AQbD-based approach enabled systematic method understanding, identification of critical factors, and establishment of an optimised and robust GC-FID method for residual solvent analysis in Remdesivir.

Methanol, diethyl ether, acetone, dichloromethane, and n-butanol eluted at 2.66, 3.73, 4.30, 5.05, and 8.87 min, respectively, with adequate resolution. The method showed excellent linearity ($R^2 > 0.99$), acceptable precision (%RSD ≤ 2%), and satisfactory sensitivity with LOD and LOQ values below ICH Q3C limits. AQbD optimisation identified injection temperature and carrier gas flow as critical factors affecting chromatographic performance, while the established MODR ensured method robustness. The direct injection GC-FID method provided a short analysis time (18 min) and is suitable for routine quality control of residual solvents in Remdesivir API and finished products.

8. CONCLUSION

A robust AQbD-based GC-FID method was successfully developed and validated for the simultaneous determination of five residual solvents—methanol, diethyl ether, acetone, dichloromethane, and n-butanol—in Remdesivir API. Critical method

parameters were systematically optimised using Design of Experiments (DoE), and a Method Operable Design Region (MODR) was established to ensure method robustness. The validated method demonstrated excellent specificity, linearity, accuracy, precision, sensitivity, and robustness in accordance with ICH Q2 guidelines. The developed procedure provided efficient separation of all target solvents within an 18 min run time and complied with ICH Q3C requirements for residual solvent analysis. Furthermore, the Eco-Scale assessment indicated acceptable environmental performance of the method. Owing to its reliability, simplicity, and robustness, the proposed GC-FID method is suitable for routine quality control and regulatory assessment of residual solvents in Remdesivir API and finished pharmaceutical products.

Credit authorship contribution statement

Morla Sivaprasd: Methodology, Investigation, Data curation, Formal analysis, Validation, Methodology, Data curation. Subhashini V: Writing – review & editing, Methodology, Conceptualisation.

Declaration of generative AI and AI-assisted technologies in the writing process

Grammarly and Paper pal improved language; authors reviewed, edited, and accept full responsibility.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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