

QbD-Driven Green and White Analytical UPLC–CAD Method for Comprehensive Profiling of Delgocitinib and Its Non-Chromophoric Impurities in Pharmaceutical Formulations

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İlaç Formülasyonlarındaki Delgocitinib ve Kromoforik Olmayan Safsızlıklarının Kapsamlı Profillendirilmesi için Qbd Odaklı Yeşil ve Beyaz Analitik UPLC–CAD Yöntemi

SUMMARY

A robust, rapid, and environmentally sustainable QbD-driven UPLC–CAD method was developed for the simultaneous determination of Delgocitinib and its non-chromophoric impurities. Some of the impurities of delgocitinib don't have chromophores, and hence, conventional UV-based methods are inadequate for the quantification of these impurities. Hence, this study was planned to propose a method with charged aerosol detection (CAD) for the sensitive detection of these impurities. The initial method screening demonstrates the unsuitability of formic acid-based mobile phases, whereas ammonium acetate/formate buffers significantly improve the chromatographic performance. The developed quadratic models exhibit excellent statistical significance and predictive power with R^2 values of 0.9839, 0.9760, and 0.9717 for resolution between impurities 3 and 2, resolution between impurity 2 and Delgocitinib, and the tailing factor of impurity 3, respectively. The model produces highly significant F-values ($p < 0.0001$) and an insignificant lack of fit. The method produces excellent linearity ($r^2 \geq 0.999$), precision (%RSD $\leq 1.24\%$), accuracy (recoveries 98.05–100.63%), and high sensitivity (LOD 0.03 $\mu\text{g/mL}$; LOQ 0.10 $\mu\text{g/mL}$ for impurities). The environmental sustainability assessment using AGREE (0.85), GAPI, and White Analytical Chemistry metrics confirms the low solvent consumption, short run time (5 min), and green solvent usage in the proposed method. The cumulative of these results produces an overall method brilliance score of 81.3% (white). The proposed method was statistically robust, environmentally responsible, and well suited for routine quality control and comprehensive impurity profiling of Delgocitinib.

Keywords: Delgocitinib, impurity profiling, non-chromophoric impurities, charged aerosol detection, green method, method whiteness.

ÖZ

Delgocitinib ve kromofor içermeyen safsızlıklarının eş zamanlı olarak belirlenmesi için sağlam, hızlı ve çevre dostu bir QbD tabanlı UPLC–CAD yöntemi geliştirilmiştir. Delgocitinib'in bazı safsızlıkları kromofor içermediğinden, geleneksel UV tabanlı yöntemler bu safsızlıkların nicel tayini için yetersiz kalmaktadır. Bu nedenle, bu çalışmada bu safsızlıkların hassas tespiti için yüklü aerosol dedektörü (CAD) ile bir yöntem önerilmesi planlanmıştır. İlk yöntem taraması, formik asit bazlı mobil fazların uygunluğunu gösterirken, amonyum asetat/format tamponları kromatografik performansı önemli ölçüde iyileştirmektedir. Geliştirilen ikinci dereceden modeller, safsızlık 3 ve 2 arasındaki çözünürlük, safsızlık 2 ve Delgocitinib arasındaki çözünürlük ve safsızlık 3'ün kuyruk faktörü için sırasıyla 0,9839, 0,9760 ve 0,9717 R^2 değerleriyle mükemmel istatistiksel anlamlılık ve tahmin gücü sergilemektedir. Model, oldukça anlamlı F değerleri ($p < 0,0001$) ve önemsiz uyumsuzluk üretir. Yöntem, mükemmel doğruluk ($r^2 \geq 0,999$), hassasiyet (%RSD $\leq 1,24\%$), doğruluk (geri kazanımlar %98,05–100,63), ve yüksek duyarlılık (LOD 0,03 $\mu\text{g/mL}$; safsızlıklar için LOQ 0,10 $\mu\text{g/mL}$) üretir. AGREE (0,85), GAPI ve White Analitik Kimya metrikleri kullanılarak yapılan çevresel sürdürülebilirlik değerlendirmesi, önerilen yöntemde düşük çözücü tüketimini, kısa çalışma süresini (5 dk) ve yeşil çözücü kullanımını doğrular. Bu sonuçların toplamı, genel yöntem mükemmellik puanını %81,3 (beyaz) olarak vermektedir. Önerilen yöntem istatistiksel olarak sağlam, çevreye duyarlı ve Delgocitinib'in rutin kalite kontrolü ve kapsamlı safsızlık profillemesi için oldukça uygundur.

Anahtar Kelimeler: Delgocitinib, safsızlık profillemesi, kromoforik olmayan safsızlıklar, yüklü aerosol tespiti, yeşil yöntem, yöntem beyazlığı.

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INTRODUCTION

Delgocitinib is a potent topical Janus kinase inhibitor class medical drug that emerges as an effective therapeutic agent for the management of atopic dermatitis and other inflammatory dermatological disorders (Dhillon, 2020; Margitta et al., 2022). It blocks the JAK-STAT-mediated cytokine pathways involved in atopic dermatitis and thereby suppresses inflammatory signaling. Delgocitinib generally exhibits low systemic adverse effects due to its minimal percutaneous absorption (Nakagawa, Osamu, Atsuyuki, & Hironobu, 2020). Its common side effects include the irritation at the application-site, erythema, and pruritus (Amano, Saeko, Hayato, & Mutsuyoshi, 2015). The stringent control of the impurity profile was very essential for delgocitinib, similar to many small-molecule kinase inhibitors, to ensure its product quality, safety, and regulatory compliance.

Notably, several impurities of delgocitinib don't have strong chromophores and exhibit poor absorbance in the UV region (Tanja Vehovec, Aleš Obreza, 2010). Hence, conventional LC-UV methods are inadequate to detect these impurities for reliable quantification. This methodological limitation underscores the need for an alternative, universal detection strategy that has the capability to detect both chromophoric and non-chromophoric species within a single analytical run (Neha, et al., 2023). The charged aerosol detection (CAD) gained wide acceptance for the detection of analytes with weak or absent UV absorption (Imad et al., 2021). The LC-CAD offers a consistent response factor, is compatible with gradient elution, and is robust across diverse chemical classes. Hence, this technique emerged as an attractive tool for impurity profiling of complex pharmaceutical matrices (Liu, Lu, Peng, Shanjun, & Dan, 2023). However, the development of a CAD-based analytical method requires careful optimization of analytical parameters to achieve adequate sensitivity and resolution.

In recent years, the QbD approach has transformed analytical method development by introduc-

ing systematic risk assessment, design of experiments (DoE), and assessment of method operable design region (Saniye, Serkan, & Nafiz, 2023). The regulatory agencies also encourage the QbD-driven analytical procedures due to enhanced method robustness, predictive performance, and lifecycle manageability (Prasad, Kasimela, & Anna, 2021; Simão, Chaudhary, & Ribeiro, 2023). Simultaneously, the contemporary push towards Green Analytical Chemistry (GAC) and White Analytical Chemistry (WAC) emphasizes the environmental sustainability and operational efficiency throughout the analytical workflow. The GAC promotes the elimination of hazardous solvents with minimal waste and energy-efficient practices (Bhupatirajou, Peddi, Edla, Rekha, & Kasimala, 2024). The WAC integrates the ecological, economic, and practical criteria to evaluate the overall analytical method's greenness, whiteness, and sustainability. The combination of QbD principles with GAC and WAC fosters the development of modern pharmaceutical assays that are not only scientifically rigorous but also environmentally conscious and resource-efficient (Veerendra, Pradeep Kumar, Sharad & Vinod Kumar, 2024).

The early studies reported in literature for the quantification of delgocitinib mainly utilize the conventional LC-UV methods for assay determination and stability assessments. One HPLC method is reported for quantification of residual benzene in the delgocitinib API. This method demonstrates adequate sensitivity for quantification of residual benzene in delgocitinib, and the method doesn't address the broader impurity analysis (Nomura, Ito, Takegami, & Tatsuya, 2019). One phase 3 long-term study that assessed the safety, efficacy, and pharmacokinetics of delgocitinib ointment in infants with atopic dermatitis was reported (Hidemi et al., 2024). This study offers selective and sensitive assessment of delgocitinib in plasma and is not applicable for impurity profiling. The regulatory assessment documents, such as the EMA Public Assessment Report, provide limited

chromatographic specifications but do not disclose complete validated methods or universal detection strategies for impurities (EMA, 2024). The literature lacks a fully validated, green, and QbD-driven UPLC–CAD method capable of profiling both UV-active and non-chromophoric impurities in a single run. Hence, the present study addresses these critical gaps by developing and validating a QbD-driven, green, and white analytical UPLC–CAD method for the comprehensive profiling of delgocitinib and its non-chromophoric impurities in pharmaceutical formulations.

The impurity limits for delgocitinib were established in accordance with the ICH Q3A(R2) guideline for new drug substances (ICH, 2006). There was

no specific pharmacopeial monograph or regulatory limits were currently available for delgocitinib. The identification and qualification thresholds were considered at 0.10%, based on general ICH recommendations (ICH, 2006). These thresholds were used as acceptance criteria for impurity profiling and method validation. The developed analytical method was deemed to be adequate sensitivity and selectivity to detect and quantify impurities at levels below the specified threshold was considered as suitable for regulatory-compliant impurity analysis. The structure and systemic details of delgocitinib and its non-chromophoric impurities in the study were presented in Figure 1.

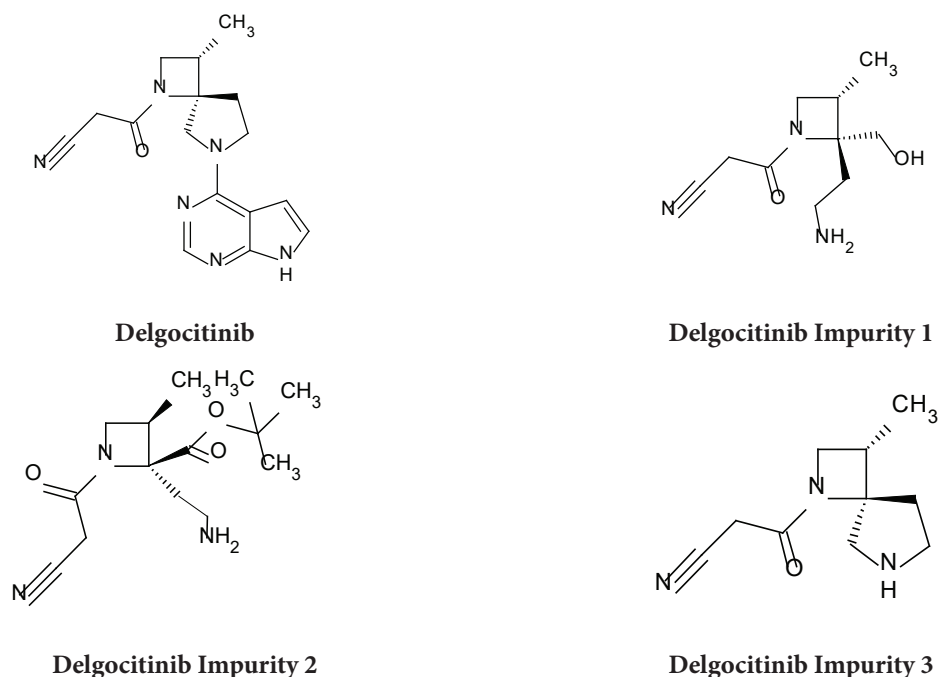


Figure 1. Molecular structure of delgocitinib and its non-chromophoric impurities

MATERIALS AND METHODS

Chemicals and reagents

Delgocitinib active pharmaceutical ingredient with a purity of 98.47%, its non-chromophoric impurities with more than 98 % purity were generously provided by Biophore India Pharmaceuticals Pvt. Ltd., Hyderabad, Telangana, India. The marketed delgocitinib topical cream (ANZUPGO® 2%) was procured from a local pharmacy and used directly for analysis.

The UPLC-grade ethanol (LiChropur™) and formic acid were obtained from Merck Chemicals, Mumbai, India. The ultrapure water was produced through the Millipore Direct-Q 3UV purification system (Merck Millipore, Darmstadt, Germany) and was used throughout the study. All chemicals, solvents, and reagents employed in the study were of analytical or chromatographic grade and were filtered through 0.22 µm membrane filters before use.

Instrumentation and analytical conditions

The ACQUITY™ (Waters Corporation, Milford, MA, USA) UPLC system was utilized for the analysis of delgocitinib and its non-chromophoric impurities. This system includes the Binary Solvent Manager for mobile-phase delivery, the Flow Through Needle Sample Manager for precise sample injection, and the column module to accommodate the column. The Charged Aerosol Detector (CAD) was used for the sensitive detection of both the chromophoric analyte (delgocitinib) and its non-chromophoric impurities. The Empower™ 3 (Waters, USA) software was utilized for instrument control, chromatographic data acquisition, and peak processing during the analysis. All experiments were conducted under controlled laboratory conditions at $25 \pm 2^\circ\text{C}$.

The chromatographic separation was achieved on an ACQUITY BEH C18 column (50 mm $\times$ 2.1 mm, 1.7 μm). The mobile phase consists of 10 mM ammonium formate at pH 3.0 (adjusted with formic acid) and ethanol in a 60:40 (v/v) ratio at an isocratic delivery of 0.3 mL/min. The 2 μL portion of the sample was injected, and the analytes were monitored. The CAD was operated using nitrogen as the nebulizing gas at 35 psi, with an evaporator temperature of 35°C , a power function set to 2.0, and a data collection filter of 10 Hz.

Preparation of standard and calibration solutions

The stock solutions of delgocitinib and each of its non-chromophoric impurities were prepared separately at a concentration of 1000 $\mu\text{g/mL}$. In the preparation, 10 mg of each reference standard was accurately weighed and dissolved in 10 mL of a diluent (equal volumes of ethanol and water). Then, a 100 $\mu\text{g/mL}$ solution was prepared from these stock solutions by appropriate dilution and was used as a 100% reference level solution for quantification. The linear standard solutions of delgocitinib were freshly prepared within the 10 to 60 $\mu\text{g/mL}$ (representing 10% to 60% of the target concentration) concentration range. The

non-chromophoric impurity working solutions were diluted in a similar manner to obtain levels equivalent to 0.1% of the delgocitinib test concentration. Equal volumes of the respective delgocitinib and impurity solutions were mixed to prepare combined calibration samples. Each calibration level was injected in triplicate ($n = 3$) under the optimized UPLC conditions.

Test solution preparation

The cream formulation contains 2% of delgocitinib and was used for the preparation of the formulation. In this, 20 mg of cream formulation was transferred into a 50 mL volumetric flask. It was mixed with about 50 mL of the diluent and sonicated for 15 min to ensure complete extraction of the drug. The solution was then diluted to volume with the same diluent and filtered through a 0.22 μm membrane filter. An aliquot of this filtrate was further diluted to obtain the test solution that corresponds to the 100% level (40 $\mu\text{g/mL}$). The spiked sample was also prepared by adding each non-chromophoric impurity at 1% of the test concentration to the 100% level solution. The unspiked and spiked samples were analyzed under the optimized UPLC conditions to evaluate resolution, recovery, and the method's ability to accurately quantify non-chromophoric impurities.

Method development

The method development process was beginning with the assessment of solubility and selection of solvent of delgocitinib and its non-chromophoric impurities. Different column lengths of the Acquity BEH C18 (100 mm and 50 mm) were evaluated at temperatures between 25 and 35°C to obtain good separation, peak shape, and efficiency. The mobile phase composition was then optimized using ethanol as the organic solvent to support green analytical chemistry practices (Tummala & Amgoth, 2022a, b). Various aqueous components such as 0.1% formic acid and ammonium formate buffer across a wide pH range of 3.0-6.5 were examined to achieve sharp, symmetrical peaks and clear baseline separation of delgocitinib and its non-chromophoric impurities.

QbD approach in method optimization

The clear separation and reliable quantification of delgocitinib and its non-chromophoric impurities was the primary goal in this QbD-guided method development. This study aimed to obtain baseline resolution ($R_s > 2$), and a short run time with less solvent usage to support green analytical practices (Tummala, Gorrepati, & Tatapudi, 2024). The key performance characteristics such as retention time, peak separation, and peak symmetry were defined as critical method attributes (CMAs). The factors that influence CMAs such as percentage of organic solvent, buffer strength, flow rate, and column temperature were identified as critical method parameters (CMPs). A central composite design (CCD) within the Design of Experiments (DoE) approach was employed to evaluate these variables systematically. The effects of the composition of organic solvent, pH of the mobile phase and column temperature (25–40 °C) were assessed to determine the optimal chromatographic conditions for consistent separation of delgocitinib and its non-chromophoric impurities.

Method validation

The suitability of the proposed method for routine quality assessment of delgocitinib and its non-chromophoric impurities was confirmed by performing validation studies as per ICH Q2(R2) guidelines (ICH, Q2(R2), 2022). The linearity range of the proposed method was assessed over 10-60% of the target concentration for delgocitinib with 0.1% of its non-chromophoric impurities. The calibration plots were prepared by relating peak area against analyte concentration. The corresponding correlation coefficient (r^2) of at least 0.999 was accepted as evidence of good linearity. The system precision, repeatability (intra-day), and intermediate precision (inter-day) were evaluated using the 100% level solution. The %RSD values for peak areas and retention times within 2.0% were considered satisfactory for the reproducibility of the proposed method. The accuracy was assessed through recovery studies at 50%, 100%, and 150% levels by spiking the

delgocitinib topical matrix with known amounts of non-chromophoric impurities. The recovery results falling in between 98.0% and 102.0% were considered an acceptable recovery of the proposed method. The sensitivity of the proposed method for quantification of non-chromophoric impurities was proved by evaluating the limit of detection (LOD) and quantification (LOQ) using the signal-to-noise ratio (S/N) approach (Bairam et al., 2024). The method robustness was assessed by introducing small, deliberate variations in proposed analytical conditions such as flow rate (± 0.05 mL/min), pH (± 0.5), and mobile phase ratio ($\pm 5\%$). The chromatographic results were verified for minimal change in peak response and system suitability values. The stability studies were performed on the 100% level solutions stored at room temperature (25 ± 2 °C) and under refrigeration (2-8 °C) for 24 hours to confirm the stability of delgocitinib and its non-chromophoric impurities.

Analytical sustainability assessment

The sustainability of the developed UPLC method for the determination of delgocitinib and its non-chromophoric impurities was comprehensively evaluated using the AGREE, GAPI tools, and RGB 12 algorithm (White Analytical Chemistry model). These tools are widely recognized to assess the compliance of analytical methods with the principles of Green Analytical Chemistry (GAC). The AGREE metric provides the numerical greenness score based on the twelve principles of GAC and generates a value between 0.00 (poor compliance) and 1.00 (excellent compliance). This metric offers an integrated, objective interpretation of the proposed method and aligns with modern sustainability requirements. The Green Analytical Procedure Index (GAPI) was utilized to examine each stage of the analytical workflow, such as sample preparation, solvent and reagent selection, instrument energy demand, and waste generation. The resultant color-coded pentagonal GAPI pictogram highlights the relative environmental burden of each step and helps identify areas of reduced ecological impact.

The overall methodological sustainability of the proposed method was further assessed through the RGB 12 algorithm. This tool combines three essential dimensions, such as analytical performance (red), environmental friendliness (green), and operational/economic practicality (blue). The parameters, such as method simplicity, analysis time, solvent safety, energy consumption, waste output, cost, and robustness, are scored and integrated into a single “whiteness” value. This whiteness score reflects the holistic balance of the proposed method. This study result confirms the analytical efficiency, environmental responsibility, and routine applicability of the proposed method.

RESULTS AND DISCUSSION

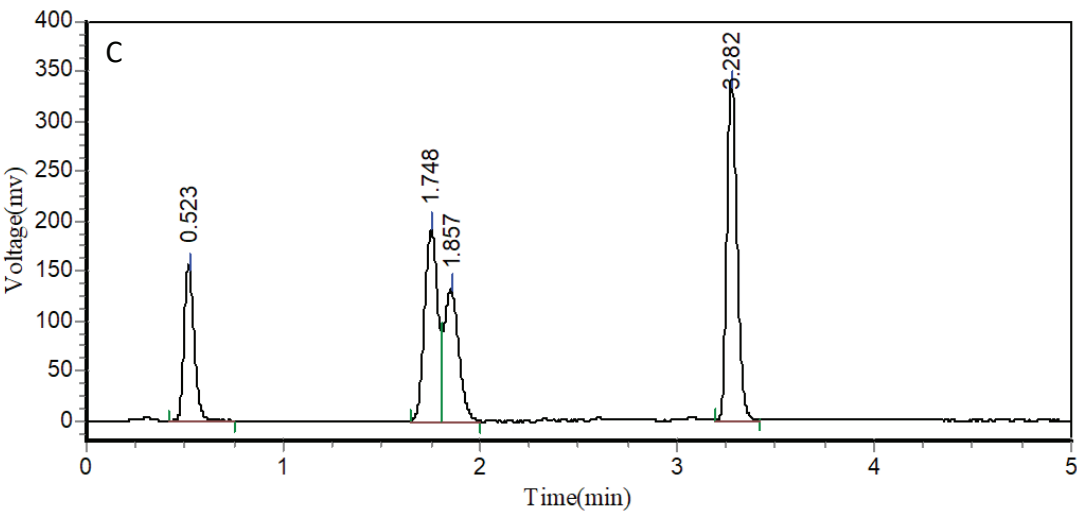
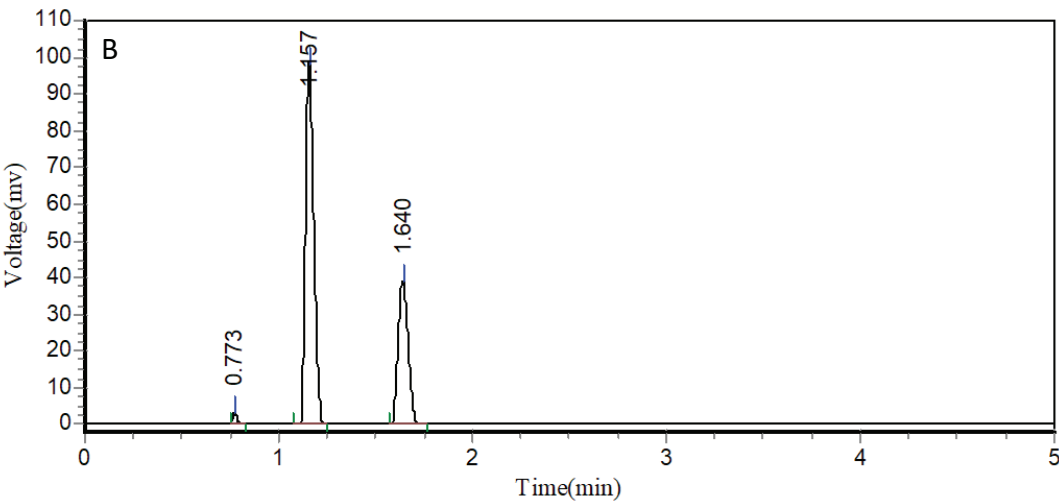
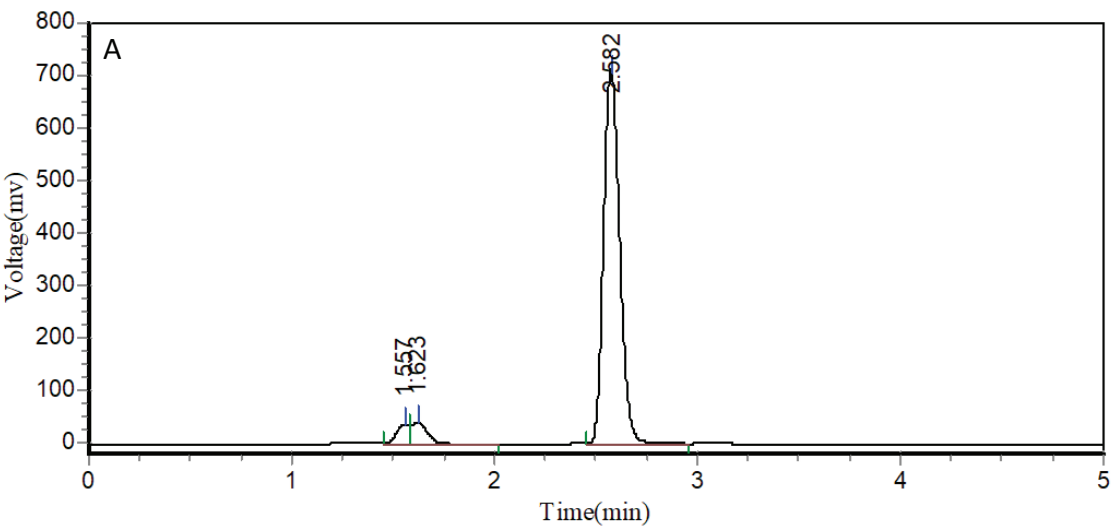
The main goal of this research was to develop a reliable, fast, and environmentally friendly UPLC method for the simultaneous assessment of delgocitinib and its associated non-chromophoric impurities in both the active pharmaceutical ingredient and finished dosage forms. As no fully sustainable or green analytical procedure was currently available to resolve and quantify non-chromophoric impurities of delgocitinib, this study was designed to fulfill the gap noticed in literature.

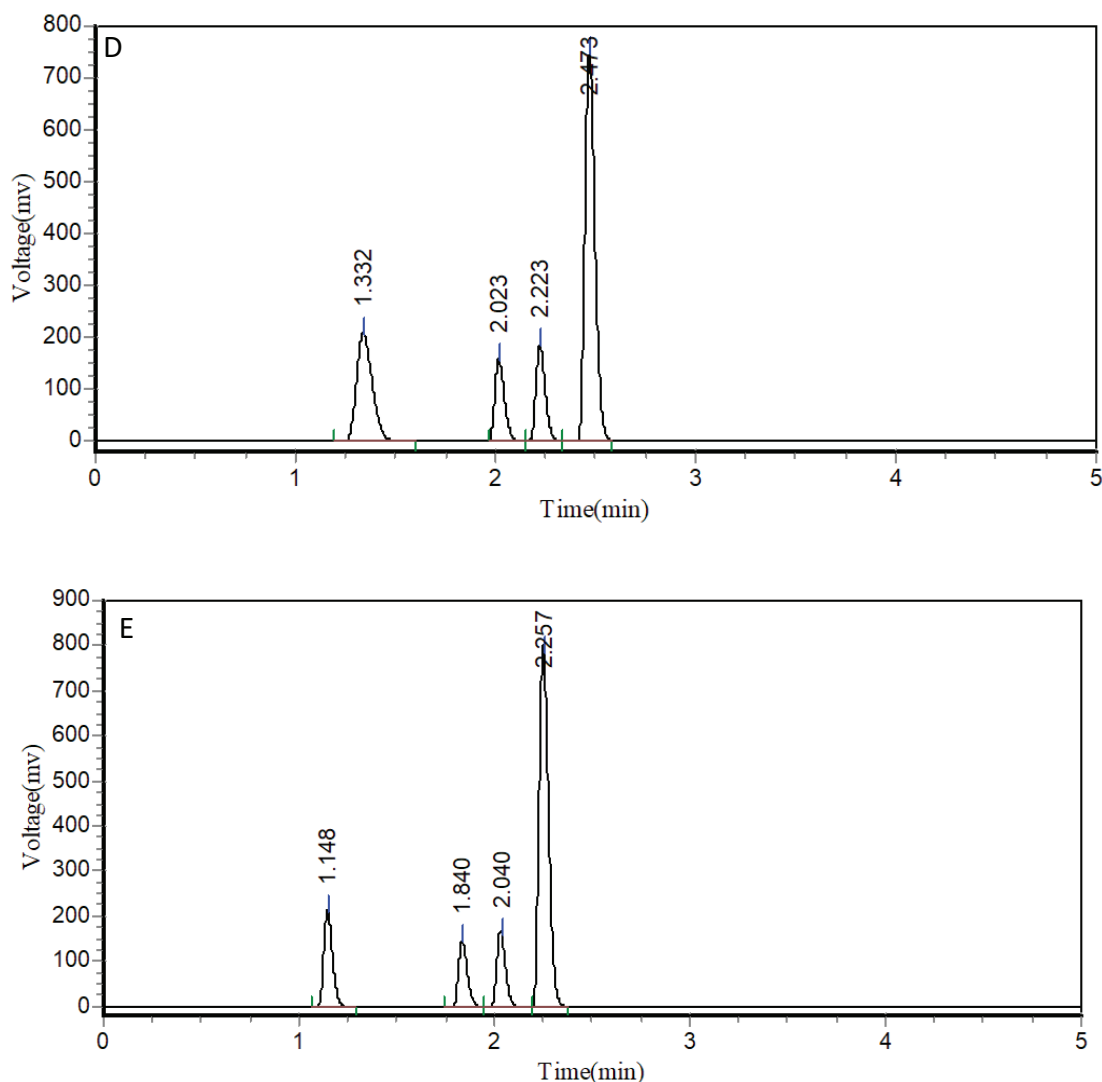
The power function value in the CAD detector was optimized to achieve a balance between detector sensitivity and linearity of response for delgocitinib and its non-chromophoric impurities. In method development process, different power function settings in the range from 1.0 to 2.0 were systematically evaluated. The low power function values produce high sensitivity but display slight non-linearity at higher concentrations. The high values produce better linearity with consistent response across the working range. Hence, a power function of 2.0 was selected as it produces optimal linearity (correlation coefficient > 0.999), acceptable signal-to-noise ratio, and reproducible peak areas for both the analyte and impurities. This setting facilitates the reliable quantification over the studied concentration range without compromising method robustness.

The chromatographic conditions were carefully optimized to obtain the best possible separation, symmetric peak shapes, and high detection sensitivity needed for accurate impurity profiling. The chromatographic trails performed and their corresponding results observed during method optimization were summarized in Table 1, and corresponding chromatographic chromatograms were presented in Figure 2.

Table 1. Experimental Summary of UPLC Method Optimization for the resolution of delgocitinib and its non-chromophoric impurities.

Trail No.	Trail condition	Result	Remark
1	0.1% aqueous formic acid and ethanol in 60:40 (v/v) at 0.3 mL/min; ACQUITY BEH C18 (150 mm) column	All analytes were not observed, and hence the method was not suitable for the analysis (Figure 2A.).	Method rejected
2	0.1% aqueous formic acid and ethanol in 50:50 (v/v) at 0.3 mL/min; ACQUITY BEH C18 (100 mm) column	Only three analytes were noticed in the chromatogram (Figure 2B.).	Method rejected
3	0.1% aqueous formic acid and ethanol in 65:35 (v/v) at 0.3 mL/min; ACQUITY BEH C18 (50 mm) column	Four analytes were observed. The chromatographic results compared to the previous trail suggest that the formic acid in the mobile phase was not suitable for the resolution of analytes (Figure 2C.).	Method rejected
4	5 mM ammonium acetate at pH 3.8 and ethanol in 30:70 (v/v) at 0.3 mL/min; ACQUITY BEH C18 (50 mm) column	Poor separation of analytes was observed with a stable baseline (Figure 2D.).	Method rejected
5	10 mM ammonium acetate at pH 3.5 and ethanol in 65:35 (v/v) at 0.3 mL/min; ACQUITY BEH C18 (50 mm) column	The resolution, symmetry, and suitability were observed to be improved from the previous condition. A Stable base line was observed.	Accepted and utilized for QbD studies





[A] Trail 1; [B] Trail 2; [C] Trail 3; [D] Trail 4; and [E] Trail 5

Figure 2. Chromatograms observed during the method optimization steps for the UPLC analysis of delgocitinib and its non-chromophoric impurities.

QbD-aided method optimization

The QbD approach was utilized to optimize the chromatographic conditions for the separation of delgocitinib and its non-chromophoric impurities. Based on preliminary method optimization studies, the resolution between impurity 3 and 2 (Response 1), the resolution between impurity 2 and delgocitinib (Response 2), and the tailing factor of impurity 3 (Response 3) were selected as the critical response factors in the study. The effects of critical method parameters (CMPs) such as

pH of the mobile phase (Factor A), composition of organic solvent (ethanol, Factor B), and flow rate of the mobile phase (Factor C) on the said response factors were studied. The minimum and maximum levels CMPs such as pH of the mobile phase (2.7–3.3), organic solvent composition (32–48%), and flow rate (0.22–0.38 mL/min) were chosen for DoE study. The results from twenty experimental runs were studied and were tabulated in Table 2. The experimental design consists of 20 experimental runs with 6 center points (runs 3,

4, 9, 10, 16, and 19) to assess experimental variability and model reproducibility. The experimental design, data analysis, and optimization were performed using Design-Expert® software (Stat-Ease Inc., USA). The reproducibility of the center points was also evaluated and shows highly consistent results, with minimal vari-

ability proves the excellent repeatability of the method under optimized conditions. These results were assessed through response surface methodology (RSM) and a quadratic regression model to elucidate the individual, interaction, and quadratic effects of CMPS on chromatographic resolution.

Table 2. Experimental design and chromatographic responses observed during the QbD-based method optimization for the analysis of delgocitinib and its non-chromophoric impurities.

Run	Factor 1: pH	Factor 2: Organic Solvent (%)	Factor 3: Flow Rate (mL/min)	Response 1: Resolution (impurities 3 and 2)	Response 2: Resolution (impurity 2 and Delgocitinib)	Response 3: Tailing Factor (impurity 3)
1	2.8	35	0.35	2.6	3.4	1.35
2	3.2	35	0.35	2.8	3.6	1.3
3	3	40	0.3	3.18	4.05	1.21
4	3	40	0.3	3.2	4.1	1.2
5	3.2	45	0.35	2.4	3.1	1.38
6	2.8	45	0.35	2.2	3	1.42
7	3.2	45	0.25	2.9	3.7	1.28
8	2.8	45	0.25	2.7	3.5	1.32
9	3	40	0.3	3.19	4.08	1.22
10	3	40	0.3	3.17	4.02	1.23
11	2.8	35	0.25	2.9	3.8	1.27
12	3.2	35	0.25	3	3.9	1.25
13	3	40	0.22	3.3	4.3	1.24
14	3	48	0.3	2.5	3.2	1.4
15	3	32	0.3	3	4	1.26
16	3	40	0.3	3.21	4.12	1.2
17	2.7	40	0.3	2.6	3.3	1.36
18	3	40	0.38	2.4	3.1	1.44
19	3	40	0.3	3.2	4.09	1.21
20	3.3	40	0.3	2.7	3.5	1.31

The developed quadratic model demonstrates excellent statistical significance and predictive capability. The model exhibits a high coefficient of determinations (R^2) of 0.9839. The value indicates that approximately 98.39% of the variability in the resolution between impurity 3 and impurity 2 was explained by the selected model. The adjusted R^2 value was observed to be 0.9637, which was in close agreement with the R^2 value confirming the robustness of the model. Fur-

thermore, the model F-value of 48.80 and the associated p-value < 0.0001 clearly demonstrate that the quadratic model was statistically highly significant and suitable to describe the chromatographic behavior.

The three-dimensional response surface plots and their corresponding contour plots reveal a pronounced curvature confirming the presence of significant quadratic and interaction effects among the CMPs. The

resolution was observed to increase at a mobile phase pH of pH 3.0, highlighting the critical role of ionization control on chromatographic selectivity. Similarly, an ethanol composition of approximately 40% (v/v) provides an optimal balance between retention and resolution. The interaction between pH and flow rate further demonstrates that the moderate flow rate (0.30 mL/min) was essential to achieve sufficient analyte–stationary phase interaction without compromising peak shape or analysis time.

The cube plots confirm that the maximum resolution was achieved near the center of the experimental region that demonstrates the robustness of the optimized conditions. The perturbation plot indicates that the mobile phase pH displays the greatest influence on resolution. The predicted versus actual plot displays the close agreement between experimental and predicted values with minimal variability at center points. These results, Figure 3, confirm the model adequacy and insignificant lack of fit.

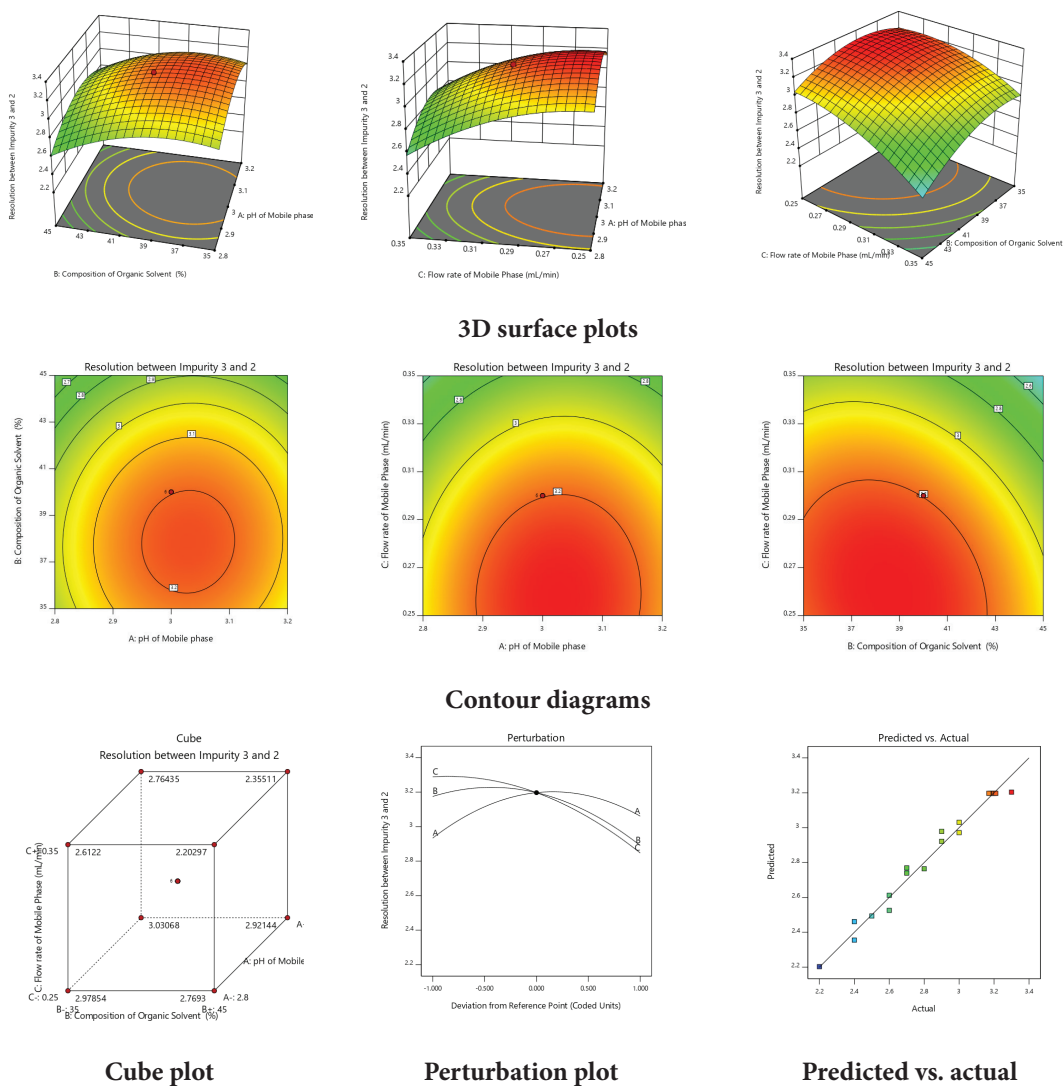


Figure 3. QbD-DoE model graphs observed during the assessment of the impact of organic solvent composition, pH, and flow rate on the resolution between impurity 3 and 2

The resolution between impurity 2 and delgocitinib was identified as Response 2 in the DoE model to evaluate the influence of critical chromatographic parameters on the separation of these impurities. The statistical analysis demonstrates the excellent model fit with R^2 of 0.9760. The high R^2 value indicates that approximately 97.60% of the variation in resolution could be explained by the selected variables. The adjusted R^2 value was found to be 0.9461. These results are in agreement and confirm the high predictive accuracy and minimal model bias. The model F-value (32.59) and the associated p-value (< 0.0001) indi-

cate that the model was highly significant and that the studied factors had a pronounced influence on the separation of impurity 2 and delgocitinib. The experimental result (Table 2.) shows that the resolution between impurity 2 and delgocitinib was observed to be in the range of 3.00 to 4.12. The 3D response surface and contour plots (Figure 4.) reveal a clear non-linear relationship between the two critical factors, such as organic solvent percentage, pH, and flow rate. The model graphs observed in the DoE evaluation of CMPs on resolution between impurity 2 and delgocitinib were presented in Figure 4.

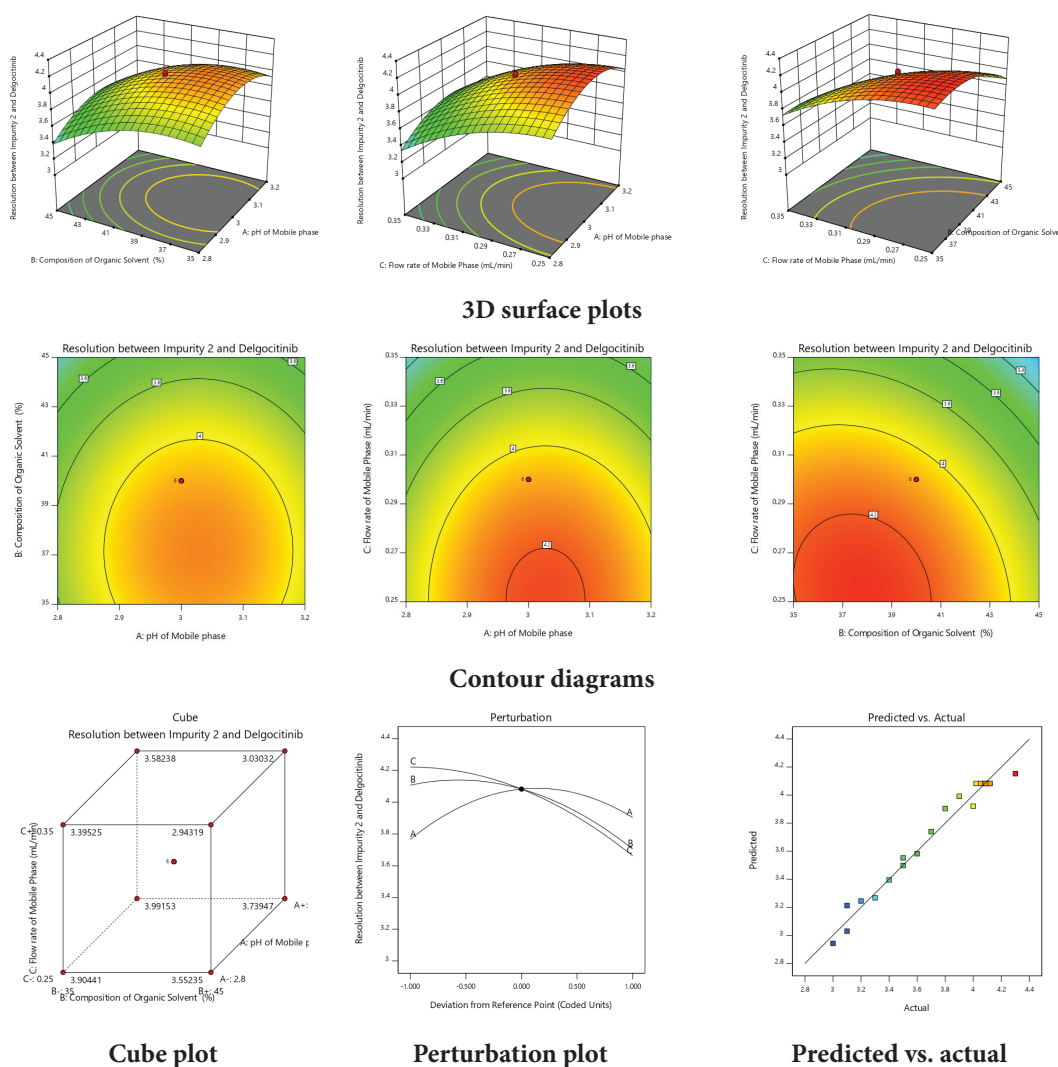


Figure 4. QbD-DoE response surface illustrating the combined influence of organic solvent composition, pH, and flow rate of mobile phase on the resolution between impurity 2 and delgocitinib

The tailing factor was considered a critical chromatographic parameter that reflects peak symmetry and column performance. This factor directly influences the quantitative accuracy and method robustness. Hence, in the QbD-based optimization, the tailing factor of impurity 3 was evaluated as response 3. This result provides the effects of organic solvent composition, flow rate, and pH on peak shape characteristics. The results reveal a well-behaved quadratic model with strong predictive capacity. The R^2 value was observed to be 0.9717 with an adjusted R^2 of 0.9364 indicating that the majority of variation in peak symmetry was explained by the studied factors. The significant model F-value (27.51) and corresponding p-value of 0.0001 confirm that the combined effect of organic solvent composition, mobile-phase pH, and flow rate displays meaningful influence on the tailing behavior.

The 3D response surface and contour plots demonstrate a clear quadratic relationship between the tailing factor and the studied variables. Based on results (Figure 5.), minimum tailing was consistently observed near the center of the design space at pH 3.0, ~40% ethanol, and a flow rate of 0.30 mL/min. This indicates that the peak displays optimal chromatographic symmetry under these conditions. The perturbation plot reveals that the mobile phase pH exhibits the most pronounced influence on the tailing factor, highlighting the importance of ionization control and solvent strength in minimizing peak asymmetry. The cube plot further supports the robustness of the optimized conditions. Additionally, the predicted versus actual plot displays the excellent agreement between experimental and model-predicted values with minimal dispersion. This result demonstrates good model adequacy and insignificant lack of fit.

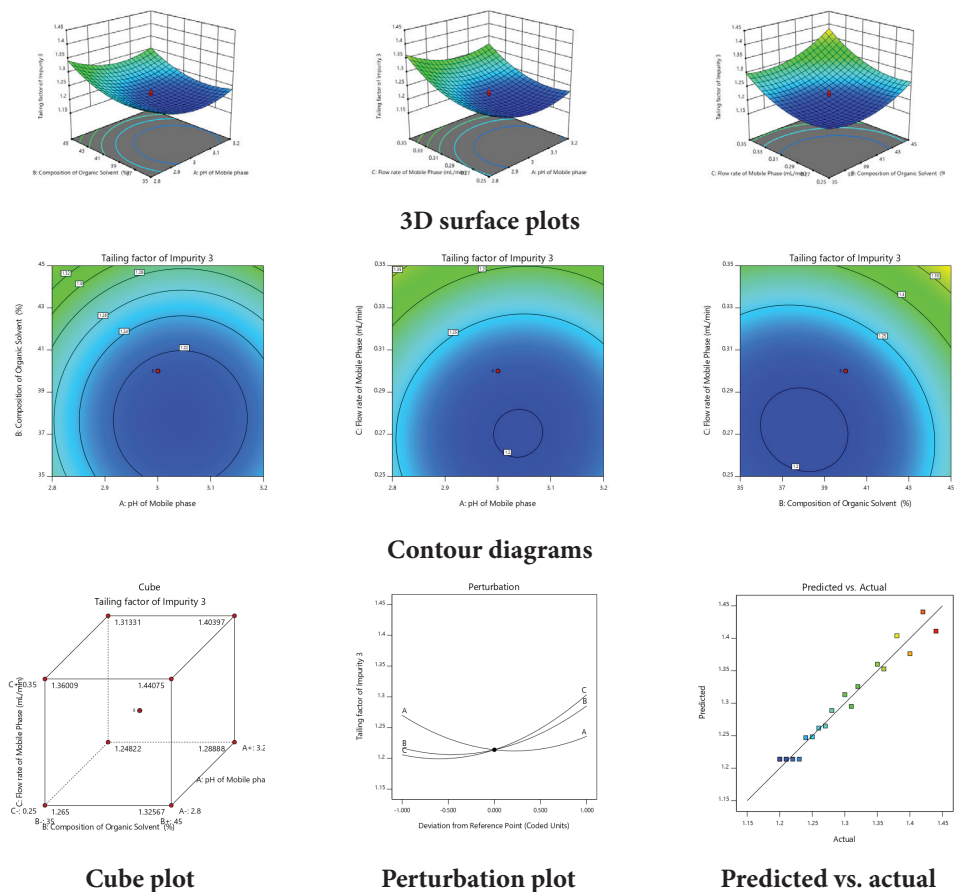


Figure 5. QbD-DoE response surface illustrating the combined influence of organic solvent composition, pH, and flow rate of mobile phase on tailing factor of impurity 3.

The lack-of-fit results were noticed to be statistically significant ($p < 0.05$) for Response 1 and Response 2. This indicates that minor variability between predicted and experimental values exists and this variability was commonly noticed in complex chromatographic systems. However, the models still demonstrate high significance with strong predictive capability. In contrast, the lack-of-fit for Response 3 was observed to be not significant ($p = 0.081$) proves good agreement between the model and experimental data. Additionally, the low residual values and consistent pure error across all responses support the adequacy and reliability of the developed models for method optimization.

The fitted regression models were noticed to be $R_1 = \beta_0 + \beta_1A + \beta_2B + \beta_3C + \beta_{12}AB + \beta_{13}AC + \beta_{23}BC +$

$\beta_{11}A^2 + \beta_{22}B^2 + \beta_{33}C^2$ for R_1 , $R_2 = \beta_0 + \beta_1A + \beta_2B + \beta_3C + \beta_{12}AB + \beta_{13}AC + \beta_{23}BC + \beta_{11}A^2 + \beta_{22}B^2 + \beta_{33}C^2$ for R_2 and $R_3 = \beta_0 + \beta_1A + \beta_2B + \beta_3C + \beta_{12}AB + \beta_{13}AC + \beta_{23}BC + \beta_{11}A^2 + \beta_{22}B^2 + \beta_{33}C^2$ for R_3 . The regression coefficients (β values) correspond to each term were derived from ANOVA and were provided in the manuscript (Table 3.). The models were found to be statistically significant ($p < 0.001$) with strong contributions from linear and quadratic terms, particularly flow rate and organic composition. The regression coefficients indicate that factor C and B exert the most significant influence on resolution and peak shape whereas quadratic terms confirm the curvature effects within the design space. These equations enable prediction of responses within the design space and support method optimization and robustness evaluation.

Table 3: Regression coefficients of quadratic models for responses (coded factors)

Term	R ₁	R ₂	R ₃
Intercept (β_0)	3.19	4.06	1.22
A (pH)	+0.117	+0.126	+0.045
B (Organic %)	-0.263	-0.371	+0.089
C (Flow rate)	-0.408	-0.516	+0.128
AB	+0.012	0.000	+0.004
AC	+0.012	0.000	+0.006
BC	+0.088	+0.071	+0.025
A ²	-0.379	-0.469	+0.168
B ²	-0.312	-0.335	+0.157
C ²	-0.245	-0.268	+0.173

The DoE analysis confirms the optimized combination of 60% 10 mM ammonium formate at pH 3.0, 40% of ethanol, and a flow rate of 0.30 mL/min produces the most favorable responses. This condition yields acceptable system suitability results (Table 4.) for delgocitinib and its non-chromophoric impurities in the study. In these optimized conditions, the method produces resolution values of 3.17 for non-chromophoric Impurities 3 and 2 and 4.26 for non-chromophoric Impurities 2 and delgocitinib, with acceptable tailing

factor and theoretical plates. The system suitability chromatogram (Figure 6) further demonstrates the clear and well-resolved elution of all components, with retention times of 1.148 min (Impurity 1), 2.048 min (Impurity 3), 2.323 min (Impurity 2), and 2.623 min (delgocitinib). The DoE findings and system suitability results confirm that the optimized UPLC method was robust, precise, and capable of reliable separation of delgocitinib and its non-chromophoric impurities for routine quality control applications.

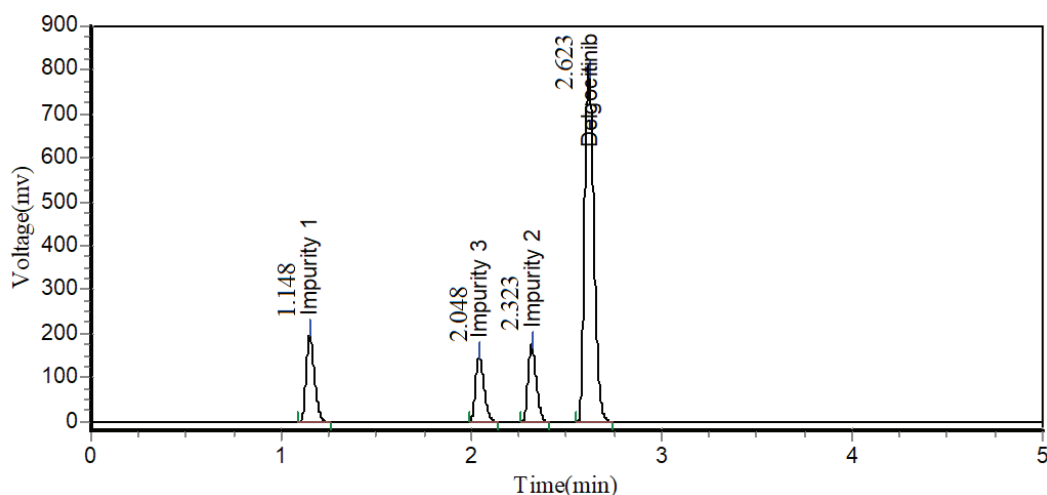


Figure 6. System suitability chromatogram showing baseline separation of Delgocitinib and non-chromophoric Impurities 1, 2, and 3 obtained using the optimized UPLC conditions (60% ethanol, pH 3.0, 0.30 mL/min)

Table 4. System suitability results of delgocitinib and its non-chromophoric impurities in the optimized method

Parameter	Delgocitinib	Impurity 1	Impurity 2	Impurity 3	Acceptance criteria
t_R in min	2.623	1.148	2.323	2.048	-
Resolution	4.26	---	3.17	5.92	> 2
Theoretical plates	13049	4981	9572	7663	> 2000
Tailing factor	1.01	0.99	1.02	0.98	< 2

Method validation

The proposed method was validated in accordance with the guidelines of ICH Q2(R2) to prove the applicability of the method for the routine analysis of Delgocitinib and its non-chromophoric impurities. The method demonstrates excellent linearity for over 10–60 $\mu\text{g/mL}$ for Delgocitinib and 0.1–0.6 $\mu\text{g/mL}$ for its non-chromophoric impurities. The correlation coefficient (r^2) of the linearity range was observed to be 0.9998 for delgocitinib and higher than 0.999 for all non-chromophoric impurities. This result establishes the strong linear relationship between concentration and detector response over the studied concentration range. The linearity range selected based on the intended application of the method for impurity quantification in the commercial formulation of delgocitinib (2% w/w). The chosen range was adequately covers

the specification limits and expected impurity levels in the formulation that ensures reliable quantification within the relevant working range. It is important to note that the LOQ for the impurities was established separately during method validation and demonstrates acceptable precision and accuracy at that level. Therefore, while calibration from LOQ was generally recommended but the selected linearity range was considered appropriate and practically relevant for routine quality control of the formulation.

The intra-day (repeatability), inter-day (intermediate precision), and ruggedness studies were performed to assess the precision of the method. The % RSD in intra-day precision was observed to be in the range of 0.36–1.24%; the inter-day precision was found to be 0.19–0.97%, whereas ruggedness was reported within 0.51–1.17%. The %RSD values in all

these studies were noticed to be within the acceptance limit of 2% for delgocitinib and its non-chromophoric impurities confirming the reproducibility and robustness of the method. The recovery studies at 50%, 100%, and 150% spiked levels were conducted to assess the method accuracy. The mean recovery values were observed to be within the range of 98.05% and 100.63% for delgocitinib and its non-chromophoric impurities. These results meet the acceptance criteria of 98–102% and prove the accuracy and reliability of the method for impurity quantification.

The LOD and LOQ for the non-chromophoric impurities of delgocitinib was assessed as per ICH recommendation based on the standard deviation of the response (σ) and the slope (S) of the calibration curve. The LOD and LOQ were calculated using

the equations $LOD = 3.3 \times (\sigma/S)$ and $LOQ = 10 \times (\sigma/S)$, respectively, where σ was standard deviation of y-intercepts of regression lines and S represents the slope of the calibration curve. The LOD values were noticed to be in the range of 0.03 $\mu\text{g/mL}$ and LOQ values between 0.10 $\mu\text{g/mL}$ and 0.15 $\mu\text{g/mL}$ for the non-chromophoric impurities. The calculated LOQ values were further verified experimentally with replicate analysis of solutions at LOQ level. The results demonstrate acceptable precision and signal-to-noise ratio proves the sensitivity and reliability of the method. The low LOD and LOQ values confirm the capability of the method to detect and quantify trace levels of non-chromophoric impurities with high precision. A summary of the validation results is presented in Table 5.

Table 5. Validation results of the developed UPLC method for the quantification of delgocitinib and its non-chromophoric impurities in accordance with ICH Q2(R2) guidelines.

S. No.	Test	Parameter	Results observed			
			Delgocitinib	Impurity 1	Impurity 2	Impurity 3
1	Linearity	Range ($\mu\text{g/mL}$)	10-60	0.1-0.6	0.1-0.6	0.1-0.6
2		Slope	44916	992918	885222	769195
3		Intercept	3447.1	1678.8	5561.8	292.49
4		Correlation coefficient	0.9998	0.9994	0.9992	0.9994
5	Repeatability (%RSD) ^s	Intra-day precision	0.36	0.23	0.91	1.24
6	Intermediate Precision (% RSD) ^s	Inter-day precision	0.53	0.81	0.19	0.97
7		Ruggedness	1.17	1.12	0.51	0.63
8	Recovery/ accuracy (% recovery) ^{ss}	50% spiked level	99.36 $\pm$ 0.15	99.04 $\pm$ 0.11	100.28 $\pm$ 0.19	98.99 $\pm$ 0.17
9		100% spiked level	98.91 $\pm$ 0.23	98.52 $\pm$ 0.46	99.61 $\pm$ 0.32	100.09 $\pm$ 0.34
10		150% spiked level	100.21 $\pm$ 0.54	99.94 $\pm$ 0.31	98.09 $\pm$ 0.23	99.58 $\pm$ 0.59
11	Sensitivity ($\mu\text{g/mL}$)	LOD	-	0.03		
12		LOQ	-	0.10		

%RSD values for n = 3 (\$); average $\pm$ SD for n=6

The robustness assessment results summarized in Table 6 demonstrate that the proposed method remains stable and reliable under small, deliberate variations in critical method parameters. The adjustments

in mobile phase ratio composition ($\pm 5\%$) produce negligible changes in peak area ($<0.6\%$) and maintain adequate resolution for both impurity pairs. The resolution was observed to be in the range of 3.10 to 3.29

for non-chromophoric impurities 3 and 2 and 4.11 to 4.31 for non-chromophoric impurities 2 and delgocitinib. The similar trend was observed with pH modification of ± 0.5 units. In this, the peak area deviation was observed to be less than 0.5%, and the resolution values were within the acceptance criterion of > 2.0 . This indicates that the ionization-dependent selectivity of the analytes does not significantly impact this pH change. The variations in flow rate (± 0.05 mL/min) also display minimal influence on both peak area and chromatographic separation. The consistency of all responses across these stress conditions highlights the ruggedness of the method and validates that none of the tested parameters exert a critical effect on quantification or resolution.

Table 6. Influence of minor changes in mobile phase ratio, mobile-phase pH, and flow rate on the chromatographic performance of delgocitinib and its non-chromophoric impurities under optimized UPLC conditions

S. No.	Parameter	Change	% change in peak area of delgocitinib	Resolution between nonchromophoric impurities 3 and 2	Resolution between non-chromophoric impurity 2 and Delgocitinib
1	Mobile phase ratio	+5% change	0.13	3.15	4.19
2		-5% change	0.28	3.20	4.28
3	pH	+0.5 units	0.31	3.12	4.22
4		-0.5 units	0.29	3.25	4.15
5	Flow rate	+0.05 mL/min	0.11	3.21	4.13
6		-0.05 mL/min	0.47	3.19	4.27

The solution stability of delgocitinib and its non-chromophoric impurities was evaluated and results demonstrate that no significant changes in assay values or impurity levels were noticed in the study. The % variations were noticed to be within $\pm 2\%$ of initial values for delgocitinib and impurities. Further, the chromatogram shows no additional peaks observed in the chromatograms. The system suitability parameters were noticed to be within acceptable limits proves the absence of co-eluting degradants. These findings indicate that both the analyte and its impurities were stable under the tested conditions proves the reliability of the proposed method for routine analysis and sample handling over the studied period.

The relationship between DoE optimization and robustness was clearly established in this study. The DoE approach was used to identify CMPS and define a design space within which the method consistently meets performance criteria. Then, robustness test was performed with the application of small, deliberate variations around the optimized conditions ($\pm 5\%$ organic composition, ± 0.5 pH units, and ± 0.05 mL/min flow rate) within this design space. The results demonstrate negligible changes in peak area ($< 0.6\%$) and consistently acceptable resolution proves that the method operates reliably within the defined region and validate the robustness of the DoE-optimized conditions for delgocitinib.

Green, white, and sustainable analytical evaluation

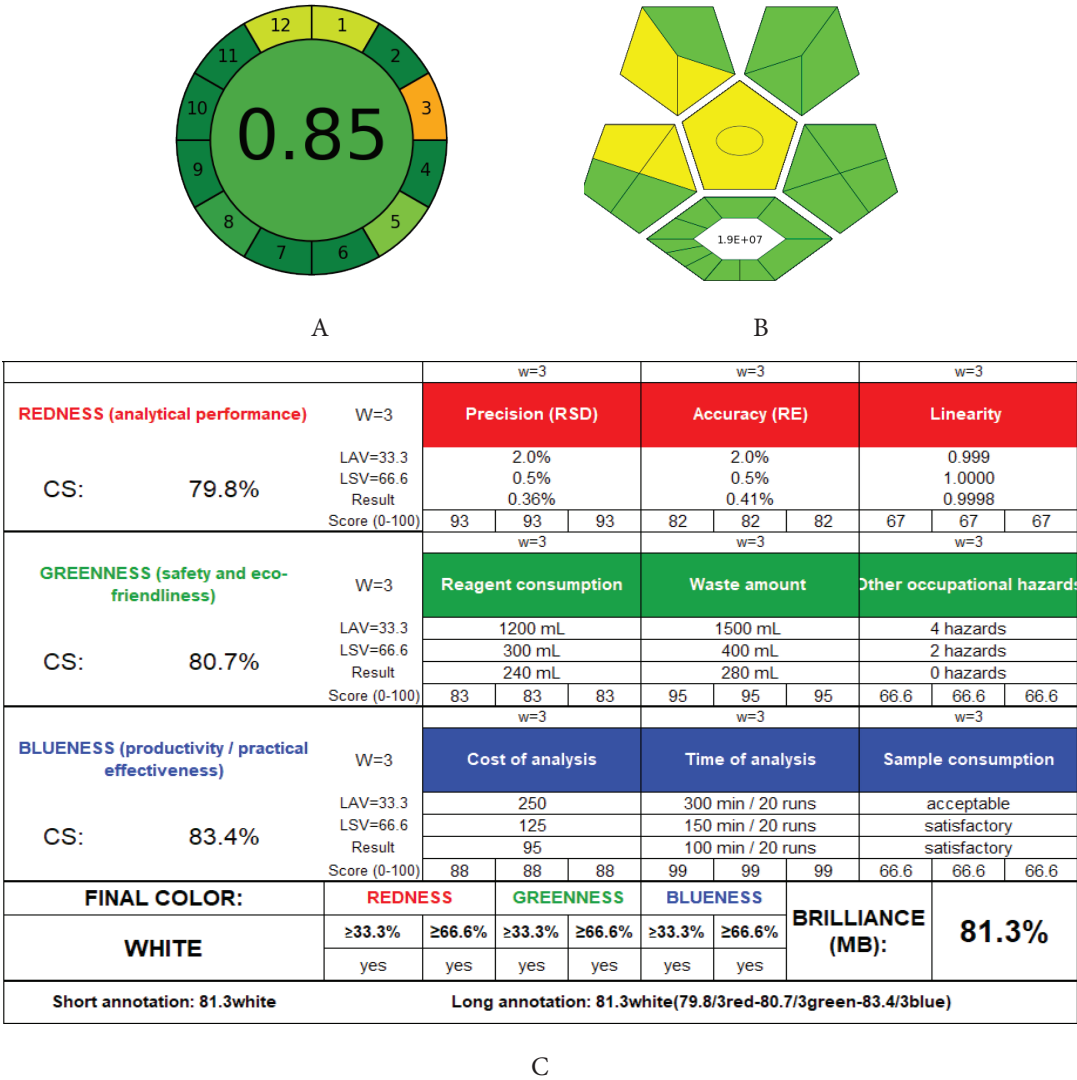
The environmental sustainability and practical efficiency of the proposed QbD-driven HPLC-CAD method were comprehensively evaluated using AGREE, GAPI, and WAC metrics. The AGREE assessment yields the high overall score of 0.85, and the score reflects excellent compliance with the twelve principles of GAC (Figure 7A.). The AGREE score (0.85) was derived through standard software with default weighting of the 12 GAC principles. This tool considers the experimental inputs such as ethanol as the organic solvent, low solvent consumption per run, short analysis time, minimal sample preparation, and

absence of highly hazardous reagents. The cumulative of these factors yields significantly best score for the proposed method.

The GAPI evaluation was performed based on defined criteria such as sample preparation steps, reagent type, instrumentation, and waste generation. In this, predominantly green zones were assigned due to simple sample preparation, use of a relatively safer solvent, and moderate waste generation. The yellow zones observed in the pictogram were due to attributed to solvent flammability and chromatographic waste that was commonly reported for chromatography based analytical methods. The calculated numerical E-factor (1.9×10^7) was intrinsically high due to microgram-level analyte usage (Figure 7B.). This value was typical for analytical-scale methods and does not reflect poor environmental performance. The E-factor was defined as the ratio of total waste generated to the mass of analyte, and in trace-level pharmaceutical analysis (μg -level analyte concentration). This factor inherently leads to disproportionately high values despite low absolute solvent consumption. Therefore, the E-factor was not fully representative metric for evaluating greenness in chromatographic methods. This limitation was addressed by interpreting the E-factor result with complementary metrics such as AGREE, GAPI, and WAC that consider solvent type, hazard, energy consumption, and overall method efficiency.

The holistic evaluation using the WAC framework demonstrates balanced performance across analytical

efficiency (red), environmental friendliness (green), and practical applicability (blue). In this, the calculation was performed through established parameters such as Least Acceptable Value (LAV) and Least Significant Value (LSV). The LAV and LSV values considered in this evaluation along with observed results were presented in figure 7C. The method achieves 79.8% in analytical performance (redness) and was supported by excellent precision ($\text{RSD} \leq 0.36\%$), high accuracy (relative error $\leq 0.41\%$), and strong linearity ($R^2 \geq 0.999$). The greenness score of 80.7% reflects minimum reagent consumption, low waste generation per analysis, and the absence of highly hazardous reagents. Furthermore, the method exhibits strong practical effectiveness (blue-ness) of 83.4% and was due to low analysis cost, short total analysis time, and acceptable sample consumption. In an instrumental perspective, the proposed HPLC-CAD method operates under moderate energy conditions without the need for vacuum systems or high gas consumption like LC-MS/MS that requires additional energy for ionization and vacuum generation. This contributes to high practical efficiency (blueness) and less environmental burden that support overall WAC evaluation. Collectively, these contributions result in the overall method brilliance (MB) score of 81.3%, and the score classifies the method as “white”. This score confirms the method suitability as a sustainable, efficient, and environmentally responsible analytical procedure for routine quality control and impurity profiling of delgocitinib in pharmaceutical formulations.



(A) The AGREE pictogram showing an overall greenness score of 0.85, (B) the GAPI pictogram illustrating low waste generation and minimal environmental risk, and (C) the White Analytical Chemistry assessment highlighting balanced red, green, and blue with an overall method brilliance of 81.3%.

Figure 7. Green and WAC evaluation of the developed HPLC–CAD method for delgocitinib and its non-chromophoric impurities

CONCLUSION

A robust, rapid, and environmentally sustainable QbD-driven UPLC method was successfully developed and validated for the simultaneous determination of delgocitinib and its non-chromophoric impurities in bulk drug and finished dosage forms. The application of RSM enables the systematic understanding of the individual, interaction, and quadratic effects of critical method parameters. The RSM produces

statistically significant and highly predictive quadratic models ($R^2 \geq 0.97$; $p < 0.0001$) with an insignificant lack of fit. The optimized chromatographic conditions produce excellent resolution between critical impurity pairs and acceptable peak symmetry, ensuring reliable impurity profiling. Method validation in accordance with ICH Q2(R2) was confirmed to produce excellent linearity, precision, accuracy, robustness, and sensitivity. The validation demonstrates the suitability

of the method for routine quality control. Furthermore, a comprehensive sustainability assessment using AGREE, GAPI, and WAC frameworks classifies the method as “white” and highlights its low solvent consumption, short run time, and use of ethanol as a green organic solvent. Collectively, these results establish the proposed method as a scientifically rigorous, regulatory-compliant, and environmentally responsible analytical tool for comprehensive impurity profiling of delgocitinib. The developed QbD-driven approach can be readily extended to pharmaceuticals containing non-chromophoric or weakly UV-absorbing impurities, overcoming limitations of conventional UV-based methods. Future work may integrate mass spectrometry for structural elucidation of unknown or trace impurities and support analytical lifecycle management in line with ICH Q14.

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AUTHOR CONTRIBUTION STATEMENT

NUR: project administration, writing – original draft, writing – review & editing. NUR, SVSRKB, BKT, DR, UN: The conceptualization, methodology, investigation, literature review, data curation, formal analysis, and visualization. NUR, SVSRKB, BKT, DR, and UN authors reviewed manuscript and approved it for publication.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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