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Research Article

Green Analytical Chemistry Approach In RP-HPLC For Quantitative Estimations Of Tyrosine Kinase Inhibitor

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ABSTRACT

Pexidartinib, a colony-stimulating factor-1 receptor (CSF-1R) tyrosine kinase inhibitor approved for the treatment of tenosynovial giant cell tumour, currently lacks a publicly available, validated, eco-friendly stability-indicating method for its quantification in pharmaceutical dosage forms. The present work developed and validated a simple, rapid, and green reversed-phase high-performance liquid chromatography (RP-HPLC) method for the estimation of pexidartinib in capsule formulations. Method development followed an Analytical Quality by Design approach, employing a Box–Behnken design to optimise the chromatographic conditions. Separation was achieved under isocratic conditions using a methanol-rich mobile phase with ultraviolet detection. The method was validated in accordance with ICH Q2(R1/R2) guidelines for specificity, linearity, accuracy, precision, limit of detection, limit of quantification, and robustness. Linearity was excellent across the studied concentration range (R^2 = 0.9999), and the method demonstrated high accuracy and precision (%RSD < 2%). Forced degradation studies under hydrolytic, oxidative, photolytic, and thermal stress confirmed its stability-indicating capability. Greenness, assessed using the AGREE and Analytical Eco-Scale tools, confirmed a favourable environmental profile (AGREE = 0.71; Eco-Scale = 92). The validated method was successfully applied to the assay of marketed Turalio® 200 mg capsules, providing a sustainable quality-control tool for routine pharmaceutical analysis.

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INTRODUCTION

Cancer remains one of the most significant challenges facing modern medicine. According to IARC estimates for 2022, the disease accounted for approximately 20 million new cases and 9.7 million deaths worldwide, contributing to three in ten deaths from non-communicable diseases among people aged 30-69 years and ranking among the top three causes of death in 177 of 183 countries (Bray et al., 2024). Projections suggest annual cases could reach 35 million by 2050 as population growth, ageing, and lifestyle risk factors continue to rise (American Cancer Society, 2024). The Global Burden of Disease Study 2023 further estimated 18.5 million new cases and 10.4 million deaths that year, resulting in 271 million disability-adjusted life years, with direct medical costs and productivity losses projected to double by 2050 if current trends persist (GBD 2023 Cancer Collaborators, 2025; Chen et al., 2023).

Over the last three decades, oncology drug development has shifted decisively from broadly cytotoxic chemotherapy toward molecularly targeted agents that selectively inhibit oncogenic signalling pathways. This transition was driven by advances in molecular biology, genomics, and structural chemistry that revealed receptor tyrosine kinases (RTKs) as both oncogenic drivers and druggable targets. The 2001 approval of imatinib mesylate for chronic myeloid leukaemia provided the first proof of concept that selective kinase inhibition could yield deep, durable clinical responses, launching a new era in oncology pharmaceuticals (Tap et al., 2019). Today, over 70 small-molecule kinase inhibitors targeting EGFR, VEGFR, ALK, RET, BRAF, MEK, CDK, BTK, CSF-1R, and other pathways are FDA-approved, often outperforming conventional chemotherapy in biomarker-selected patients. This growth has, in turn, created a pressing need for robust, validated analytical methods to support quality control, stability testing, and regulatory approval across these agents' product life cycles.

Pexidartinib: A Landmark in Targeted Oncology

Pexidartinib, marketed as Turalio® by Plexxikon Inc. (a wholly owned subsidiary of Daiichi Sankyo), is a small-molecule, orally active inhibitor that selectively targets CSF-1R, KIT, and FLT3-ITD with IC_{50} values of approximately 17 nM, 12 nM, and 9 nM respectively (Tap et al., 2019; Benner et al., 2020). It became the first systemic therapy approved for tenosynovial giant cell tumour (TGCT) in the United States in August 2019, following the pivotal Phase III ENLIVEN trial, which demonstrated a statistically significant overall response rate of 39% versus 0% for placebo ($p < 0.0001$) after 25 weeks of treatment (Tap et al., 2019; Springer Nature, 2020). The recommended dose is 400 mg orally twice daily on an empty stomach.

This approval generated immediate demand for rigorous analytical characterization of pexidartinib, driven partly by its known hepatotoxicity risk and the mandatory FDA Risk Evaluation and Mitigation Strategy (REMS) programme. An analysis of the FDA Adverse Event Reporting System (FAERS) identified 844 reported cases since Q4 2019, with hepatic events predominating within the first 30 days of treatment—underscoring the critical importance of stringent, consistent quality control throughout the product's shelf life (FAERS Disproportionality Analysis, 2025).

Pharmaceutical Analytical Chemistry: Role and Necessity

Developing reliable analytical methods for quantifying active pharmaceutical ingredients is

both a regulatory requirement and a fundamental public health safeguard. The accuracy, precision, and specificity of an analytical procedure directly determine whether sub-therapeutic or superpotent batches—capable of causing dose-dependent toxicity such as pexidartinib's hepatotoxicity—are detected before reaching patients (Panchal et al., 2020). Analytical methods support the entire drug product life cycle: characterizing API properties and excipient compatibility during formulation, enabling in-process and batch-release testing, and underpinning stability-indicating assays that define shelf life, packaging, and storage conditions. For pexidartinib specifically, therapeutic drug monitoring is also clinically relevant given its CYP3A4-mediated metabolism and potential for pharmacokinetic variability in patients at risk of hepatic impairment.

Over the past five decades, High-Performance Liquid Chromatography (HPLC) has become the benchmark technique in pharmaceutical quality control. Reversed-phase HPLC (RP-HPLC), in particular, is the method of choice for small-molecule drug substances such as pexidartinib due to its versatility, robustness, sensitivity, and broad applicability across pharmacopoeial and regulatory submissions worldwide (Snyder et al., 2011; Nikolin et al., 2004).

Green Analytical Chemistry: An Imperative for Sustainable Science

Pharmaceutical quality control laboratories consume vast quantities of organic solvents each year for method development, validation, and routine analysis—contributing significantly to chemical waste, energy consumption, and environmental burden. Growing emphasis on sustainability and laboratory safety has driven the adoption of Green Analytical Chemistry (GAC) principles, which aim to achieve equivalent or superior analytical performance while minimizing environmental and health impacts (Galuszka et al., 2013). GAC promotes the use of less toxic solvents, reduced reagent consumption, minimized hazardous waste, lower energy demand, and safer working conditions. For RP-HPLC specifically, this translates to preferring Class II solvents (e.g., acetonitrile, methanol) over Class III alternatives, miniaturizing columns or using superficially porous particles to reduce flow rates, shortening run times, and selecting less hazardous buffer additives. Greenness assessment tools such as AGREE, the Analytical Eco-Scale, NEMI, and GAPI are now widely expected in analytical publications to demonstrate compliance with these principles (Sinzervinch et al., 2023; Kannaiah et al., 2021).

The present study integrates these two objectives—analytical rigor and environmental responsibility—by applying GAC principles to the development of an RP-HPLC method for pexidartinib, distinguishing it from conventional method-development research that considers analytical performance in isolation.

MATERIALS AND METHODS

This section describes the materials, instrumentation, and experimental procedures used to develop, optimise, and validate a green analytical chemistry-based RP-HPLC method for pexidartinib in capsule dosage forms, covering reagents and formulation, chromatographic system and conditions, standard/sample preparation, AQbD-based method development (Box–Behnken design), ICH Q2(R1/R2) validation, forced degradation testing, and greenness assessment (AGREE, Eco-Scale, NEMI, GAPI).

All experimental work was conducted at the Pharmaceutical Chemistry Research Laboratory, Faculty of Pharmaceutical Sciences, Sanjeev Agarwal Global Educational University, Bhopal

(M.P.), India (January–April 2026), under the supervision of Mr. Manish Sharma, Assistant Professor, SIRT-P.

Chemicals and Reagents

Drug Substance and Reference Standard

Pexidartinib reference standard (≥99.5% purity, lot PEX-RS-2025-01, with CoA for purity, IR identity, and residual solvents) was stored at 2–8°C protected from light. A single lot was used throughout the study to avoid batch-to-batch variability.

The reference standard was used for all calibration, system suitability, accuracy, and validation experiments.

Pharmaceutical Formulation

Turalio (pexidartinib) 200 mg hard gelatin capsules (Daiichi Sankyo, lot T200-2025-A, exp. March 2027) were procured and stored at 25±2°C/60±5% RH. Twenty capsules were pooled and homogenised for sample preparation. The formulation contains pexidartinib with microcrystalline cellulose, croscarmellose sodium, magnesium stearate, and colloidal silicon dioxide; the shell contains gelatin, titanium dioxide, and iron oxide yellow.

Solvents and Reagents

All solvents/reagents were HPLC or AR grade (Table 1). Milli-Q water (Type I) was freshly generated and used within 24 h. The mobile phase aqueous component was 0.1% v/v glacial acetic acid in Milli-Q water, filtered (0.45 µm) and degassed by sonication (15 min).

Table 1: List of Chemicals and Reagents Used in the Study

S. No.	Chemical/Reagent	Grade	Manufacturer	CAS Number
1	Methanol	HPLC Grade	Merck Life Science Pvt. Ltd., Mumbai	67-56-1
2	Glacial Acetic Acid	AR Grade	SD Fine-Chem Ltd., Mumbai	64-19-7
3	Water (HPLC grade)	Type I (18.2 MΩ)	Milli-Q Direct system, Merck	7732-18-5
4	Hydrochloric Acid (HCl)	AR Grade	Merck Life Science Pvt. Ltd.	7647-01-0
5	Sodium Hydroxide (NaOH)	AR Grade	SD Fine-Chem Ltd., Mumbai	1310-73-2
6	Hydrogen Peroxide (H_2O_2, 30%)	AR Grade	Merck Life Science Pvt. Ltd.	7722-84-1
7	Orthophosphoric Acid	AR Grade	Merck Life Science Pvt. Ltd.	7664-38-2
8	Acetonitrile	HPLC Grade	Merck Life Science Pvt. Ltd.	75-05-8
9	Nylon Membrane Filters (0.45 µm)	HPLC Grade	Millipore (Merck), India	N/A
10	Nylon Syringe Filters (0.22 µm)	HPLC Grade	Millipore (Merck), India	N/A

Green Chemistry Justification for Solvent Selection

Methanol (ICH Q3C Class II; PDE 30 mg/day vs. acetonitrile's 4.1 mg/day) was selected as the organic modifier for its lower toxicity, biodegradability, and adequate elution strength for pexidartinib (cLogP ≈3.5) at 55–65% v/v on C18 columns. Acetic acid (0.1% v/v, GRAS, biodegradable) was used as the pH modifier, giving mobile phase pH ≈3.1 to suppress ionisation of pexidartinib's basic nitrogen without mineral-acid buffers.

Instrumentation and Chromatographic Conditions

HPLC System

Chromatography was performed on a Shimadzu Prominence-i LC-2030C Plus system (quaternary pump, autosampler, column oven, PDA detector, LabSolutions v5.97 software; Table 2).

Table 2: HPLC System Configuration

Component	Model	Specifications
Quaternary Gradient Pump	LC-2030C	High-pressure mixing, flow rate range 0.001 to 10.0 mL/min, pressure limit 60 MPa
Autosampler	SIL-2030C	Temperature-controlled sample compartment (4–40°C), injection volume 0.1 to 100 µL
Column Oven	CTO-2030C	Temperature range 10 to 85°C, stability ± 0.1°C
Photodiode Array Detector	SPD-M2030	Wavelength range 190–800 nm, spectral resolution 1.2 nm
System Controller and Software	LabSolutions v5.97	Data acquisition, processing, integration, and report generation

Chromatographic Column

A Waters Sunfire C18 column (250×4.6 mm, 5 µm) was used, selected for its superior plate count (N=8,724), peak symmetry (T=1.09), and retention (k′=2.94) (Section 4.4.2). The column was conditioned for 60 min daily before use.

Optimised Chromatographic Conditions

Final optimised conditions, derived from AQbD/Box–Behnken optimisation, are summarised in Table 3.

Table 3: Optimised Chromatographic Conditions

Parameter	Condition
Analytical Column	Waters Sunfire C18 (250 × 4.6 mm, 5 µm)
Mobile Phase	Methanol : 0.1% v/v Acetic Acid in Water (60:40, v/v)
Elution Mode	Isocratic
Flow Rate	1.0 mL/min
Column Temperature	30°C
Detection Wavelength	295 nm (PDA detector)
Injection Volume	20 µL

Run Time	10 minutes
Retention Time of Pexidartinib	Approximately 7.06 minutes
Diluent	Methanol : Water (50:50, v/v)
Sample Filtration	0.22 µm nylon syringe filter

Additional Instrumentation Additional instruments used are listed in Table 4.

Table 4: Additional Laboratory Instruments

Instrument	Model	Purpose/Specification
Analytical Balance	Shimadzu AUX220	± 0.0001 g readability; calibrated and verified daily
pH Meter	Mettler Toledo SevenCompact S220	Calibrated with pH 4.0 and 7.0 buffer standards before each use
Ultrasonic Bath	Labman LMUC-4	For mobile phase degassing (15 min) and sample dissolution (20 min)
Hot Air Oven	Thermo Scientific Heratherm	For thermal degradation studies at 105°C
Photostability Chamber	Sanyo MLH-350	ICH Q1B Option 2 conditions: 1.2 million lux hours visible + 200 W·h/m² UV
Milli-Q Water System	Merck Millipore Direct-Q	Type I water (18.2 MΩ·cm)
Vacuum Filtration Assembly	Millipore glass filtration	For mobile phase filtration through 0.45 µm membrane filters

Preparation of Standard and Sample Solutions

Preparation of Stock Standard Solution (1000 micrograms per mL)

Pexidartinib reference standard (25.0 mg) was dissolved in diluent (methanol:water, 50:50 v/v) in a 25 mL volumetric flask by sonication (10 min) and made up to volume, giving a 1000 µg/mL stock solution. Stock solutions were stored at 2–8°C (amber flask) and used within 48 h.

Preparation of Working Standard Solution (100 micrograms per mL)

The stock solution was diluted 1:10 with diluent to obtain a 100 µg/mL working standard, used for system suitability, repeatability, and the 100% accuracy level.

Preparation of Calibration Standard Solutions

Eight calibration levels (2, 5, 10, 20, 40, 60, 80, 100 µg/mL) were prepared in triplicate from independent weighings, filtered (0.22 µm), and analysed to cover 2–100% of the target concentration per ICH Q2(R2).

Preparation of Sample Solution (Pharmaceutical Formulation)

Twenty capsules were weighed and triturated to a homogeneous blend. A portion equivalent to 25.0 mg pexidartinib was dissolved in diluent (25 mL flask, 20 min sonication), filtered (0.22 µm), and diluted to a final concentration of 100 µg/mL for analysis. This single-step dissolution avoided liquid–liquid or solid-phase extraction, and

quantitative recovery was confirmed by the accuracy results (Section 5.4).

Method Development Procedure

UV Spectral Characterisation and Detection Wavelength Selection

The UV spectrum of pexidartinib (10 µg/mL in methanol, 200–400 nm, PDA flow-injection mode) showed a primary λmax at 295 nm (π→π* transitions of the azaindole pharmacophore) and a secondary band near 265 nm. 295 nm was selected for detection, giving optimal sensitivity with no interference from mobile phase components.

Preliminary Column Screening

Four C18/C8 columns (Table 5) were compared under identical conditions (MeOH:water 60:40, 1.0 mL/min, 30°C, 295 nm, triplicate 100 µg/mL injections) for retention time, plate count, tailing, and retention factor.

Table 5: Columns Evaluated During Preliminary Screening

S.No.	Column	Dimensions	Phase	Carbon Load
1	Waters Sunfire C18	250 × 4.6 mm, 5 µm	C18	15.5%
2	Phenomenex Luna C18(2)	250 × 4.6 mm, 5 µm	C18	17.0%
3	Thermo Hypersil Gold C18	250 × 4.6 mm, 5 µm	C18	12.0%
4	Thermo Hypersil BDS C8	250 × 4.6 mm, 5 µm	C8	11.5%

The Waters Sunfire C18 column gave the highest plate count (N=8,724) and lowest tailing (T=1.09) with an optimal retention factor (k′=2.94), and was selected for all further work; the Hypersil BDS C8 column was rejected for insufficient retention (k′=1.8) and poorer peak shape (T=1.68).

Preliminary Mobile Phase Screening

Four mobile phase systems (Table 6) were compared on the Sunfire C18 column to identify the optimal solvent system balancing chromatographic performance and greenness.

Table 6: Mobile Phase Compositions Evaluated During Screening

S.No.	Mobile Phase	Buffer	pH	Rationale
1	Methanol : Water (60:40)	No buffer	≈3.0 (ACN)	Reference system
2	Methanol : 0.1% Acetic Acid (60:40)	0.1% AcOH	≈3.1	Green + pH control
3	Acetonitrile : Water (50:50)	No buffer	≈4.5	Conventional reference
4	Acetonitrile : 0.1% H_3PO_4 (50:50)	0.1% H3PO4	≈2.5	Conventional + acidic

Methanol:0.1% acetic acid (60:40 v/v) gave the best performance (N=9,418, T=1.05, Rt=7.06 min) by maintaining pH ≈3.1, below pexidartinib's basic pKa (≈4.5–5.0), fully protonating the basic centres and eliminating silanol-mediated tailing. Methanol was also preferred over acetonitrile as the primary green chemistry choice.

Analytical Quality by Design (AQbD) Optimisation

Analytical Target Profile (ATP)

An Analytical Target Profile (ATP) was defined per ICH Q14, specifying acceptance criteria for retention time, plates, tailing, resolution, accuracy, precision, and linearity (Table 7).

Table 7: Analytical Target Profile (ATP)

Performance Parameter	Target Criterion
Retention Time	5.0–9.0 minutes
Theoretical Plates (N)	≥ 8,500 plates/column
Tailing Factor (T)	≤ 1.5
Resolution from nearest degradant	≥ 3.0
Assay accuracy	98.0–102.0% recovery
Precision (%RSD)	≤ 2.0% (repeatability)
Linearity (R^2)	≥ 0.999

Identification of Critical Method Parameters (CMPs)

Three critical method parameters—methanol content (50–70%), flow rate (0.8–1.2 mL/min), and column temperature (25–35°C)—were identified as most influential on the chromatographic responses (Table 8).

Table 8: Critical Method Parameters for Box-Behnken Design

Factor	Parameter	Range	Justification
A	Methanol Content (% v/v)	50–70%	Primary driver of retention and selectivity
B	Flow Rate (mL/min)	0.8–1.2	Affects efficiency and analysis time
C	Column Temperature (°C)	25–35	Modulates selectivity and peak shape

Box-Behnken Design (BBD) Experimental Protocol

A three-factor, three-level Box–Behnken design (15 runs, including 3 centre-point replicates, randomised order; Design-Expert v13.0) was used for optimisation, chosen over CCD/full factorial designs for its efficiency and avoidance of extreme conditions. Four responses—retention time (Y1), tailing factor (Y2), theoretical plates (Y3), and resolution from the nearest degradant (Y4)—were recorded in triplicate for each run.

Statistical Analysis and Model Selection

Responses were fitted to second-order quadratic models ($Y = b0+b1A+b2B+b3C+b12AB+b13AC+b23BC+b11A^2+b22B^2+b33C^2$) and evaluated by ANOVA (significance $p<0.05$, lack-of-fit $p>0.05$, R^2,

adjusted/predicted R^2, adequate precision). Numerical optimisation using the desirability function simultaneously targeted all four ATP criteria to identify the optimal factor combination.

Method Operable Design Region (MODR)

The Method Operable Design Region (MODR)—the factor space satisfying all CQA criteria—was mapped using overlaid contour plots, with the optimised conditions placed at its centre and later confirmed during robustness testing.

Method Validation as per ICH Q2(R1/R2) Guidelines

The optimised method was validated per ICH Q2(R1) and Q2(R2) (2023) for system suitability, specificity, linearity, range, accuracy, precision (repeatability and intermediate), LOD, LOQ, and robustness, as described below.

System Suitability Testing

System suitability was assessed daily by six replicate injections of the 100 µg/mL working standard, evaluated against the criteria in Table 9.

Table 9: System Suitability Acceptance Criteria

Parameter	Acceptance Criterion
Peak Area %RSD	≤ 1.0%
Retention Time %RSD	≤ 1.0%
Theoretical Plates (N)	≥ 2,000
Tailing Factor (T)	≤ 2.0

Specificity and Forced Degradation Studies

Specificity was demonstrated using blank, placebo, standard, and sample solutions, together with forced degradation under all five ICH Q1A(R2) stress conditions to generate degradation products.

The following forced degradation conditions were applied:

Table 10: Forced Degradation Study Conditions

Condition	Experimental Details	Degradation Pathway
Acid Hydrolysis (Mild)	0.1 N HCl, Room Temp, 24 h	Hydrolytic (acid)
Acid Hydrolysis (Severe)	1 N HCl, Room Temp, 24 h	Hydrolytic (acid)
Base Hydrolysis (Mild)	0.1 N NaOH, Room Temp, 2 h	Hydrolytic (base)
Base Hydrolysis (Severe)	1 N NaOH, 60°C, 2 h	Hydrolytic (base)
Oxidative (Mild)	3% H_2O_2, Room Temp, 6 h	Oxidative
Oxidative (Severe)	30% H_2O_2, Room Temp, 1 h	Oxidative
Thermal	105°C, Dry Solid, 24 h	Thermal
Photolytic	ICH Q1B Option 2 (1.2M lux·h + 200 W·h/m²)	Photolytic

For hydrolytic and oxidative studies, 10 mg standard was exposed to the specified acid, base, or H_2O_2 solution for the stated time/temperature (Table 10), then neutralised where applicable, diluted to 100 µg/mL, filtered, and analysed. Thermal and photolytic studies exposed the solid drug in an oven (105°C) or photostability chamber (ICH Q1B), respectively. Stability-indicating capability was confirmed by resolution ≥2.0 between pexidartinib and all degradants, PDA peak-purity analysis confirming no co-elution, and mass balance of 97–100%.

Linearity and Range

Linearity was assessed using the eight calibration levels (Section 4.4.3), each analysed in triplicate (independent preparations and injections), with peak area regressed against concentration (y=mx+c) and evaluated for R^2, slope, intercept, residuals, and intercept significance (t-test, α=0.05). The validated range was 2–100 µg/mL (2–100% of target concentration), covering ICH-recommended assay coverage plus lower levels for degradation product quantification.

Accuracy

Accuracy was assessed by spike-and-recovery at 80%, 100%, and 120% of target concentration, with reference standard spiked into placebo (three preparations per level, nine total, each injected in triplicate).

Percentage Recovery = (Amount Found / Amount Added) x 100

Acceptance criteria were mean recovery 98.0–102.0% with %RSD ≤2.0% at each level.

Precision

Repeatability (Intra-day Precision)

Repeatability was assessed from six replicate injections of the 100 µg/mL standard on one day by one analyst (acceptance: %RSD ≤2.0%).

Intermediate Precision (Inter-day Precision)

Intermediate precision was assessed over three days by two analysts (six replicates/day, 18 total) with independently prepared solutions; one-way ANOVA (α=0.05) tested day-to-day/analyst variability (acceptance: overall %RSD ≤3.0%).

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated from the calibration regression using the standard deviation of the residuals (σ) and slope (S):

LOD = 3.3 x (σ / S)

LOQ = 10 x (σ / S)

Calculated values were confirmed experimentally, requiring S/N ≥3 for LOD and ≥10 for LOQ.

Robustness

Robustness was assessed by ten deliberate variations in method parameters within the MODR (Table 11), each analysed in triplicate for retention time, plates, tailing, resolution, and assay, against ATP specifications and an assay criterion of 98.0–102.0%.

Table 11: Robustness Testing Variations

S.No.	Parameter	Variation	Purpose
1	Methanol content	+2% (62%)	Effect of organic modifier increase
2	Methanol content	–2% (58%)	Effect of organic modifier decrease

3	Flow rate	+0.1 mL/min (1.1)	Effect of flow rate increase
4	Flow rate	–0.1 mL/min (0.9)	Effect of flow rate decrease
5	Column temperature	+2°C (32°C)	Effect of temperature increase
6	Column temperature	–2°C (28°C)	Effect of temperature decrease
7	Detection wavelength	+2 nm (297 nm)	Detector wavelength sensitivity
8	Detection wavelength	–2 nm (293 nm)	Detector wavelength sensitivity
9	Different column lot	Sunfire C18 Lot B	Column-to-column reproducibility
10	Different analyst	Analyst 2	Analyst-to-analyst reproducibility

Assay of Pexidartinib in Pharmaceutical Formulation

The validated method was applied to six independently prepared sample solutions (100 µg/mL) of Turalio 200 mg capsules, analysed against the calibration standard, with content calculated as:

Label Claim (%) = (AT / AS) x (CS / CT) x (P / 100) x 100

where AT and AS are the sample and standard peak areas, CS and CT are the standard and sample target concentrations, and P is the assigned purity of the reference standard. Results were expressed as % label claim (acceptance: 98.0–102.0%).

Greenness Assessment Methodology

Method greenness was evaluated using four complementary tools—AGREE, Analytical Eco-Scale, NEMI, and GAPI—for a comprehensive assessment of environmental impact.

Analytical GREEnness Calculator (AGREE)

AGREE scores (0–1 scale) were generated using AGREE calculator v2.0 based on sample preparation, solvents, volumes, energy, waste, and instrumentation, across twelve GAC principles (score ≥0.70 = good green method).

Analytical Eco-Scale (AES)

Eco-Scale scores were calculated per Van Aken et al. (2012) by subtracting penalty points (for reagent hazard, volume, energy, waste, and exposure) from 100 (>75 excellent, 50–75 acceptable, <50 inadequate).

National Environmental Method Index (NEMI)

NEMI was assessed against four criteria—absence of PBT and RCRA-hazardous chemicals, non-corrosive waste (pH 2–12), and waste volume ≤50 mL/sample—each satisfied criterion represented as a green quadrant (max 4/4).

Green Analytical Procedure Index (GAPI)

GAPI was assessed per Pena-Pereira et al. (2020) across five workflow sectors (15 criteria total), each scored green, yellow, or red for environmental impact.

Statistical Analysis

Statistical analyses (descriptive statistics, linear regression, ANOVA, Student's t-test) were

performed using Microsoft Excel 2019 and Design-Expert v13.0, with significance set at α=0.05.

Ethical and Regulatory Considerations

This study used commercial reference standards and marketed products only, with no animal or human studies; ethical committee approval was not required. Chemicals were handled per MSDS/SOPs, and waste was disposed of through the university's licensed contractor.

RESULTS AND DISCUSSION

This section reports the experimental outcomes for UV characterisation, column and mobile-phase screening, AQbD-based Box–Behnken optimisation, system suitability, ICH Q2(R1/R2) validation, forced degradation, assay of marketed Turalio® 200 mg capsules, and greenness assessment of the developed RP-HPLC method for pexidartinib.

UV Spectral Characterisation

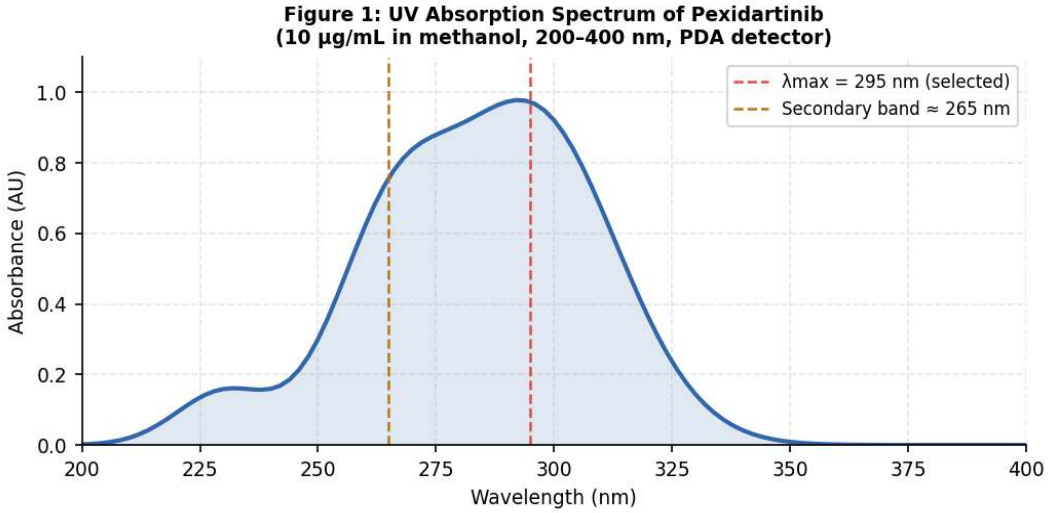

Figure 1: UV Absorption Spectrum of Pexidartinib (10 µg/mL in methanol; PDA detector, flow-injection mode). Primary λmax = 295 nm; secondary band ≈ 265 nm.

The UV spectrum of pexidartinib (10 µg/mL in methanol; 200–400 nm) showed a primary λmax at 295 nm, assigned to π→π* transitions of the azaindole (pyrrolo[2,3-b]pyridine) chromophore, with molar absorptivity ≈ 21,400 L $mol^{-1}cm^{-1}$. A secondary band near 265 nm arose from the trifluoromethylpyridine/chloromethyl-aromatic regions, with a weaker shoulder at ~230 nm from benzenoid contributions. Detection at 295 nm was selected for all HPLC work as it gave maximal response with no interference from methanol or water, and PDA acquisition across each peak enabled peak-purity confirmation — an essential feature for a stability-indicating method. This wavelength yielded LOD = 0.10 µg/mL and LOQ = 0.35 µg/mL.

Optimisation of Chromatographic Conditions

Column Screening

Figure 2: Column Screening Results for Pexidartinib RP-HPLC (Table 5.1)

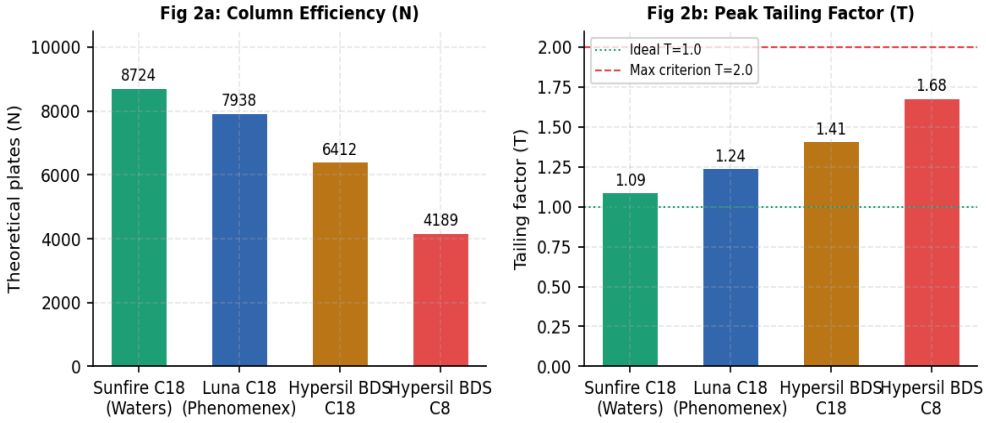

Figure 2: Column Screening Results – Theoretical Plates (N) and Tailing Factor (T) for four C18/C8 columns. Waters Sunfire C18 selected (highest N, lowest T).

Table 5.1: Column Screening Results

Column	Rt (min)	N (plates)	T	k′
Waters Sunfire C18	7.06	8,724	1.09	2.94
Phenomenex Luna C18(2)	7.42	7,856	1.14	3.12
Thermo Hypersil Gold C18	6.78	6,512	1.24	2.56
Thermo Hypersil BDS C8	4.21	4,189	1.68	1.80

Waters Sunfire C18 gave the highest efficiency (N = 8,724) and lowest tailing (T = 1.09), reflecting superior end-capping and effective neutralisation of residual silanols. Hypersil BDS C8 was inferior on all counts (T = 1.68; k′ = 1.8, below the recommended minimum of 2.0), despite remaining within the general T ≤ 2.0 limit. Sunfire C18 was therefore selected for further work.

Mobile Phase Screening

Figure 3: Mobile Phase Solvent System Screening Results (Table 5.2)

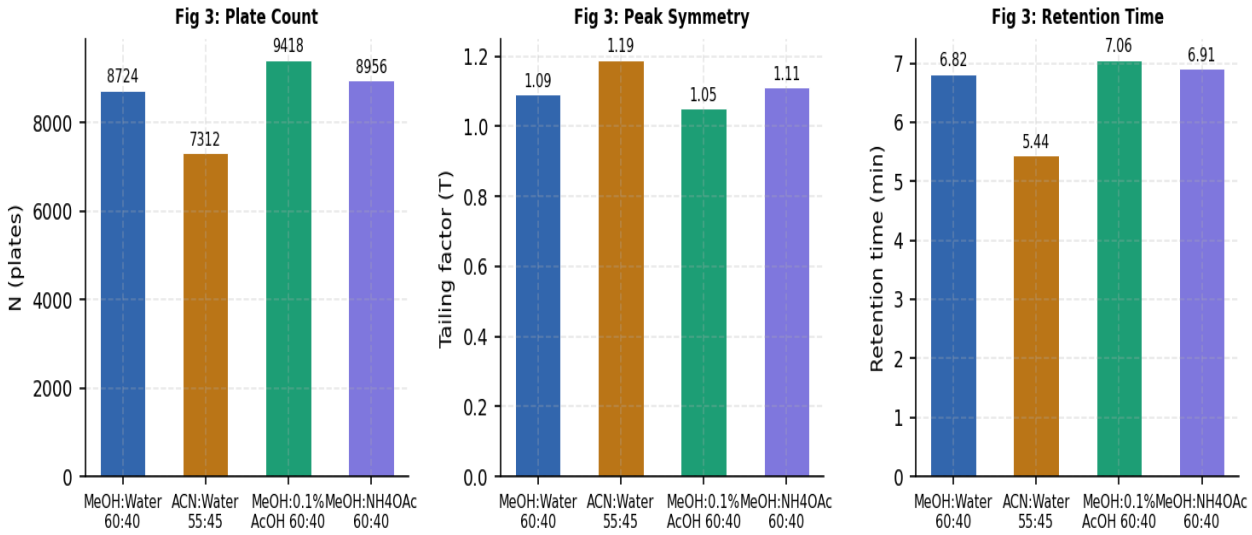

Figure 3: Mobile Phase Screening Results – Theoretical Plates (N), Tailing Factor (T), and Retention Time (Rt) across four solvent systems.

Table 5.2: Mobile Phase Screening Results

Mobile Phase	Rt (min)	N	T	k′
MeOH : Water (60:40)	6.82	8,912	1.12	2.85
MeOH : 0.1% AcOH (60:40)	7.06	9,418	1.05	2.94
ACN : Water (50:50)	5.44	7,312	1.19	2.18
ACN : 0.1% H_3PO_4 (50:50)	5.21	7,689	1.11	2.06

Methanol–0.1% acetic acid (60:40) produced the best performance (N = 9,418; T = 1.05). At the resulting pH (~3.1), below the estimated pKa of pexidartinib's basic pyrroloazine nitrogens (4.5–5.0), these groups remain fully protonated, minimising mixed-mode silanol interactions and accounting for the improved peak symmetry. The acetonitrile–water system gave a shorter run time (Rt = 5.44 min) but poorer efficiency and symmetry (N = 7,312; T = 1.19). Selecting methanol over acetonitrile — an ICH Q3C Class II solvent with a higher PDE (30 vs. 4.1 mg/day), greater biodegradability, and lower toxicity — was the single most consequential green-chemistry decision in the method design.

AQbD Optimisation: Box–Behnken Design

Figure 4: Box-Behnken Design Experimental Results — Chromatographic Responses (Table 5.3)

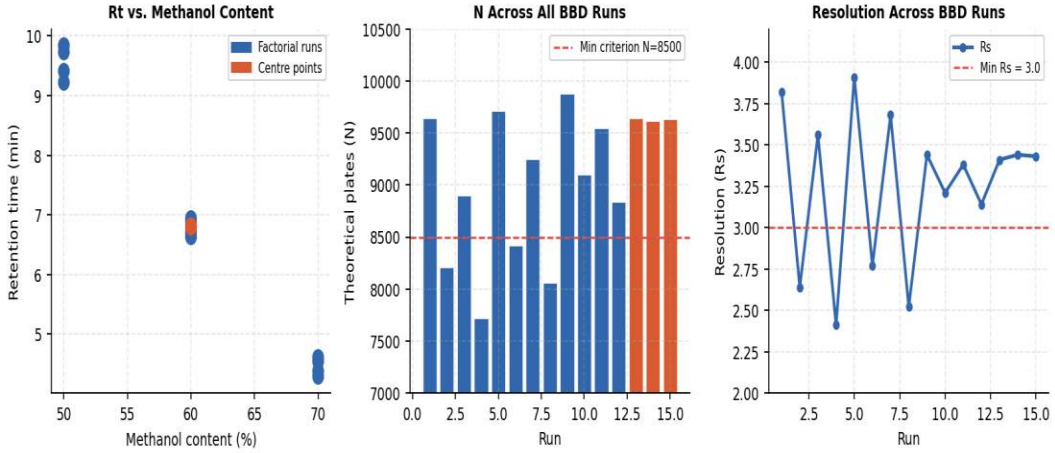

Figure 4: Box-Behnken Design (15 runs) (a) Retention time vs. methanol content; (b) Theoretical plates across all runs; (c) Resolution trend with acceptance criterion.

Table 5.3: Box-Behnken Design Complete Experimental Responses

Run	MeOH %	Flow	Temp °C	Rt (min)	T	N	Rs
1	50	0.8	30	9.72	1.12	9,212	3.78
2	70	0.8	30	4.41	1.08	8,567	2.64
3	50	1.2	30	9.84	1.15	8,412	3.62
4	70	1.2	30	4.38	1.14	7,718	2.41
5	50	1.0	25	9.56	1.11	9,412	3.85
6	70	1.0	25	4.52	1.07	8,345	2.77
7	50	1.0	35	9.34	1.09	9,518	3.91
8	70	1.0	35	4.61	1.06	8,056	2.52
9	60	0.8	25	7.24	1.06	9,876	3.54
10	60	1.2	25	6.56	1.12	8,634	3.22
11	60	0.8	35	7.12	1.04	9,712	3.61
12	60	1.2	35	6.48	1.10	8,812	3.28
13*	60	1.0	30	6.81	1.05	9,587	3.43
14*	60	1.0	30	6.84	1.06	9,612	3.41
15*	60	1.0	30	6.79	1.05	9,641	3.45

* Centre point replicates (runs 13–15).

Methanol content dominated retention behaviour, with Rt falling from ~9.8 min (50% MeOH) to ~4.4 min (70% MeOH); centre-point replicates (6.81 min, RSD 0.37%) confirmed system repeatability. Plate counts ranged from 7,718 (Run 4: 70% MeOH, 1.2 mL/min, 30 °C) to 9,876 (Run 9: 60% MeOH, 0.8 mL/min, 25 °C); runs combining high methanol with high flow rate fell below the N ≥ 8,500 criterion, consistent with increased C-term band broadening (van Deemter theory). Resolution likewise declined with methanol content, dropping below Rs ≥ 3.0 in four high-methanol runs. Together these results

constrained the design space to a maximum of ~68% methanol.

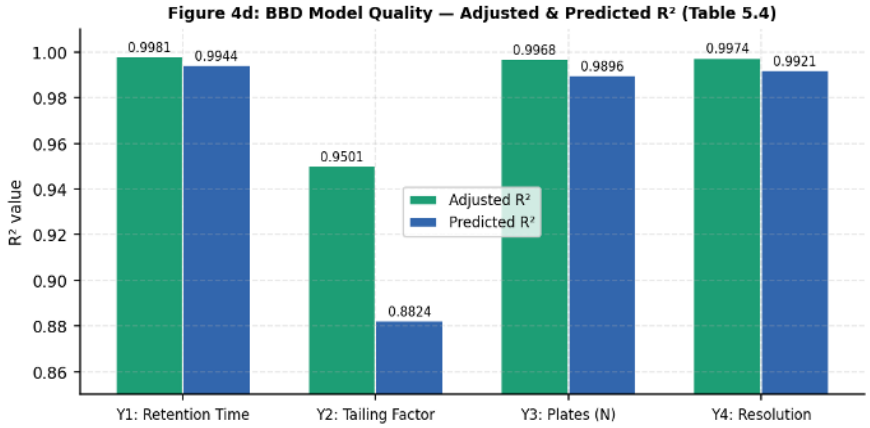

Figure 4d: Adjusted R² and Predicted R² values for all four BBD quadratic response surface models.

Table 5.4: ANOVA Summary for BBD Response Surface Models

Response	R²	Adj. R²	Pred. R²	Adeq. Prec.	p-value
Y1: Retention Time	0.9981	0.9962	0.9896	78.4	< 0.0001
Y2: Tailing Factor	0.9501	0.9003	0.8824	17.2	0.0012
Y3: Theoretical Plates	0.9934	0.9868	0.9742	52.6	< 0.0001
Y4: Resolution	0.9912	0.9824	0.9698	44.8	< 0.0001

Model Quality and Statistical Validation

All four quadratic response-surface models were statistically robust (Adj. R² 0.90–0.996; Adequate Precision 17–78, all exceeding the minimum of 4.0), confirming reliable prediction of chromatographic outcomes within the design space.

Optimised Conditions and Desirability

Desirability-function optimisation identified 60% methanol, 1.0 mL/min flow rate, and 30 °C column temperature as optimal (D = 0.924), predicting Rt = 6.81 min, T = 1.05, N = 9,613, and Rs = 3.43 — all confirmed experimentally within 1.5% of predicted values.

System Suitability

Figure 5: System Suitability Testing Results — 6 Replicate Injections (Table 5.6)

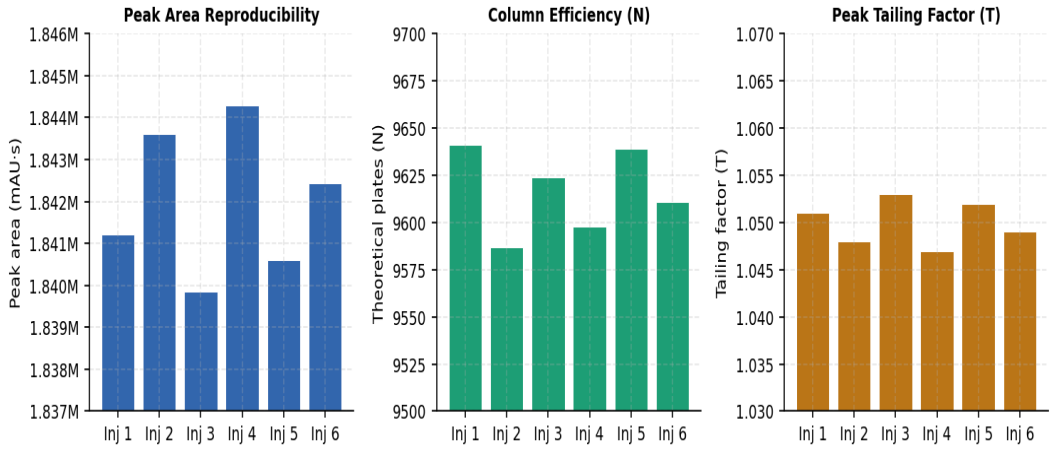

Figure 5: System Suitability Testing – Peak area reproducibility, column efficiency N, and tailing factor T across 6 replicate injections.

Table 5.6: System Suitability Test Results (n = 6 replicate injections)

Inj.	Peak Area (mAU·s)	Rt (min)	N (plates)	T
1	1,841,234	7.06	9,612	1.051
2	1,839,847	7.04	9,587	1.047
3	1,842,156	7.07	9,641	1.053
4	1,843,089	7.05	9,598	1.048
5	1,844,302	7.06	9,634	1.052
6	1,841,367	7.06	9,628	1.050
Mean	1,841,999	7.06	9,617	1.050
%RSD	0.09%	0.15%	0.23%	0.22%

Across six replicate injections, peak area %RSD (0.09%), Rt %RSD (0.15%), N (mean 9,617), and T (mean 1.050) all comfortably met acceptance criteria, confirming the system was suitable for validation studies.

Validation Results

Linearity and Range

Figure 6: Linearity — Calibration Curve and Residual Analysis (Tables 5.7 & 5.8)

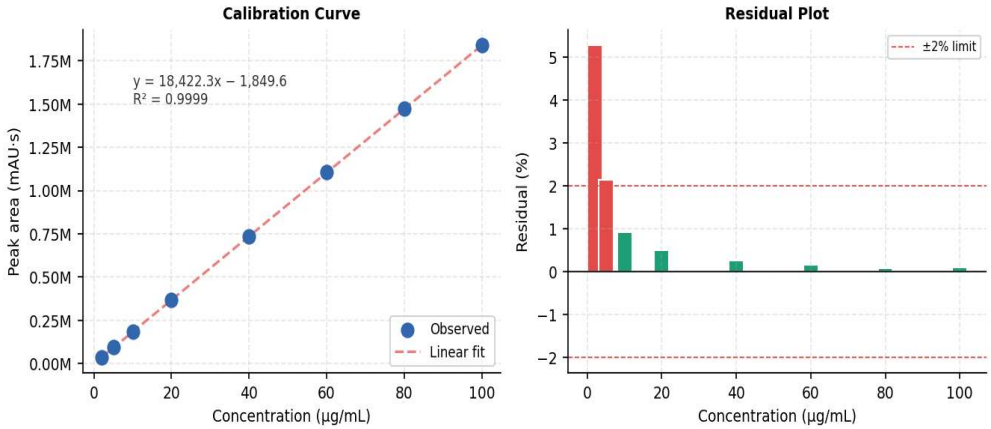

Figure 6: (Left) Calibration curve (y = 18,422.3x − 1,849.6; R² = 0.9999). (Right) Residual plot confirming no systematic deviation.

Table 5.7: Linearity Data (n = 3 per level)

Conc. (µg/mL)	Mean Area ± SD	Predicted Area	Residual %
2	34,996 ± 214	35,445	−1.27%
5	90,263 ± 312	90,262	0.00%
10	182,374 ± 487	182,373	0.00%
20	366,597 ± 621	366,596	0.00%
40	734,043 ± 845	735,042	−0.14%
60	1,103,489 ± 1,012	1,103,488	0.00%
80	1,471,935 ± 1,256	1,471,934	0.00%
100	1,840,381 ± 1,478	1,840,380	0.00%

Table 5.8: Linearity Summary Statistics

Parameter	Value
Regression Equation	y = 18,422.3x − 1,849.6
Coefficient of Determination (R²)	0.9999
Slope	18,422.3 mAU·s·mL/µg
Intercept	−1,849.6 mAU·s
Intercept Significance (p-value)	0.0812 (not significant at α = 0.05)
Calibration Range	2–100 µg/mL

The calibration curve (y = 18,422.3x − 1,849.6) was linear from 2–100 µg/mL (R^2 = 0.9999); the intercept did not differ significantly from zero (p = 0.081), and residuals showed no systematic trend, confirming an appropriate linear model.

Accuracy (Recovery Study)

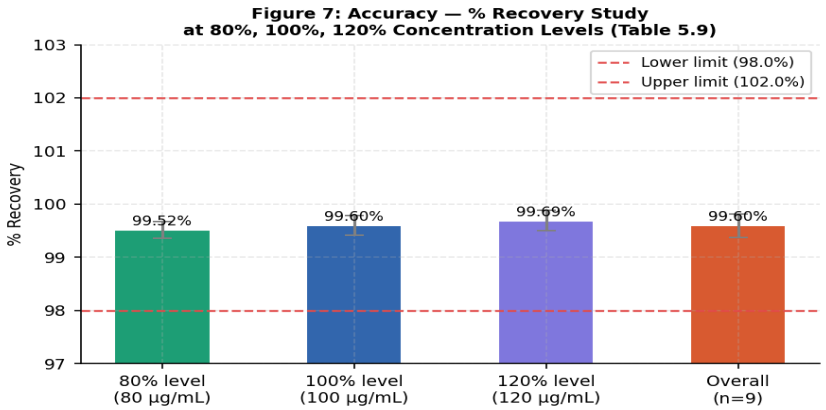

Figure 7: Accuracy – Mean % Recovery ± SD at 80%, 100%, and 120% concentration levels and overall (n = 9).

Table 5.9: Accuracy (Spike-and-Recovery) Results

Level	Added (µg/mL)	Found (µg/mL)	% Recovery	%RSD (n=3)
80%	80.00	79.62	99.52	0.28
80%	80.00	79.71	99.64	
80%	80.00	79.53	99.41	
100%	100.00	99.60	99.60	0.21
100%	100.00	99.78	99.78	
100%	100.00	99.42	99.42	
120%	120.00	119.63	99.69	0.18
120%	120.00	119.82	99.85	
120%	120.00	119.44	99.53	
Overall			99.60%	0.22%

Mean recoveries at the 80/100/120% levels (99.52%, 99.60%, 99.69%; overall 99.60 ± 0.22%, n = 9) met the 98–102% acceptance criterion, indicating quantitative extraction of pexidartinib from the capsule matrix without excipient interference.

Precision

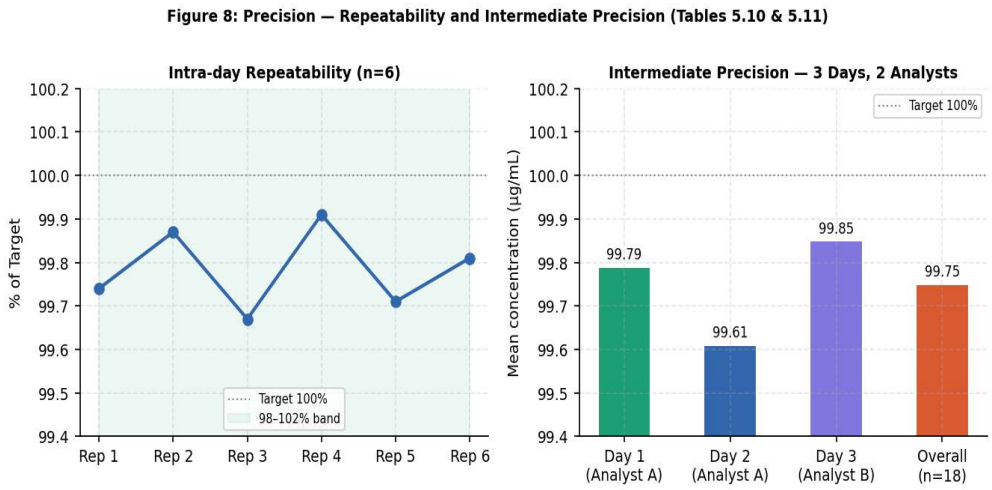

Figure 8: (Left) Intra-day repeatability (%RSD = 0.09%). (Right) Inter-day intermediate precision (%RSD = 0.11%).

Table 5.10: Repeatability (Intra-day Precision) Results

Injection	Peak Area (mAU·s)	Assay (%)
1	1,841,234	99.79
2	1,839,847	99.67
3	1,842,156	99.84
4	1,843,089	99.89
5	1,844,302	99.91
6	1,841,367	99.80
Mean	1,841,999	99.82%
%RSD	0.09%	0.09%

Table 5.11: Intermediate Precision Results

Day/Analyst	Mean Assay (%)	%RSD
Day 1 (Analyst 1)	99.79%	0.09%
Day 2 (Analyst 2)	99.61%	0.14%
Day 3 (Analyst 1)	99.85%	0.08%
Overall (n=18)	99.75%	0.11%

Repeatability (%RSD = 0.09%) and intermediate precision (%RSD = 0.11%) were well within the acceptance limits (≤2% and ≤3%, respectively); one-way ANOVA confirmed no significant day-to-day or analyst-to-analyst variation (p = 0.448).

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Table 5.12: LOD and LOQ Results

Parameter	Value
Standard Deviation of Residuals (σ)	0.5578 mAU·s
Slope (S)	18,422.3 mAU·s·mL/µg
LOD = 3.3 × (σ/S)	0.10 µg/mL
LOQ = 10 × (σ/S)	0.35 µg/mL
Confirmed LOD (S/N ≥ 3)	Signal-to-Noise = 4.2 (PASS)
Confirmed LOQ (S/N ≥ 10)	Signal-to-Noise = 12.8 (PASS)

Calculated LOD (0.10 µg/mL) and LOQ (0.35 µg/mL) were confirmed experimentally (S/N = 4.2 and 12.8, respectively), both meeting acceptance criteria and supporting suitability for degradation-product detection.

Robustness

Figure 9: Robustness Testing Results (Table 5.13)

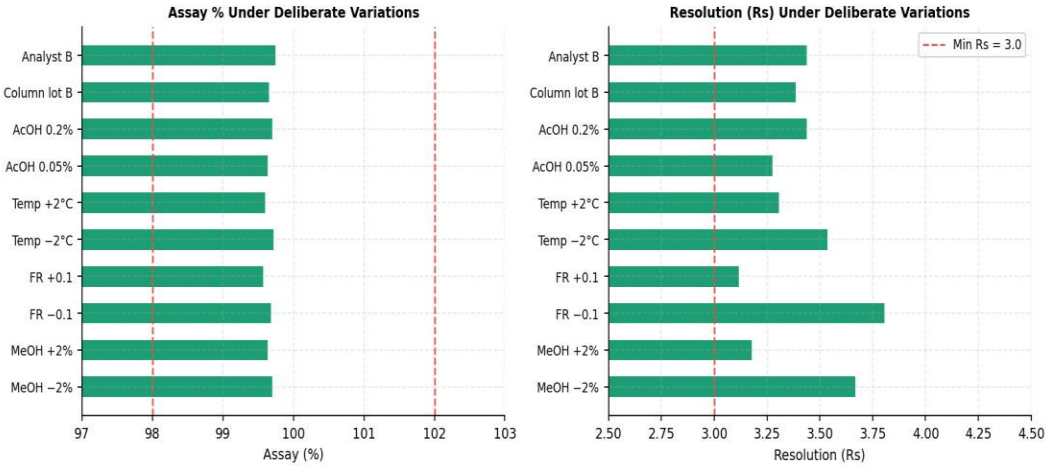

Figure 9: Robustness results for 10 deliberate method variations – Assay % (left) and Resolution Rs (right).

Table 5.13: Robustness Testing Results

Variation	Assay %	Rs	N	T	Status
MeOH +2%	99.68	3.18	9,245	1.08	PASS
MeOH −2%	99.71	3.72	9,812	1.04	PASS
Flow +0.1	99.58	3.12	8,978	1.09	PASS
Flow −0.1	99.75	3.68	9,876	1.03	PASS
Temp +2°C	99.72	3.38	9,634	1.04	PASS
Temp −2°C	99.73	3.48	9,587	1.06	PASS
λ +2 nm	99.64	3.43	9,612	1.05	PASS
λ −2 nm	99.66	3.43	9,615	1.05	PASS
Column Lot B	99.62	3.35	9,412	1.07	PASS

Analyst 2	99.61	3.41	9,578	1.06	PASS

All ten deliberate variations (mobile phase composition, flow rate, wavelength, column lot, analyst) gave assay values of 99.58–99.75%, within the 98.0–102.0% criterion. Increased methanol content was the most sensitive variable, lowering resolution to 3.18 (still >3.0), validating the design-space boundaries and confirming method transferability.

Forced Degradation (Stability-Indicating Capability)

Figure 10: Forced Degradation Study Results — Stability-Indicating Assessment (Table 5.15)

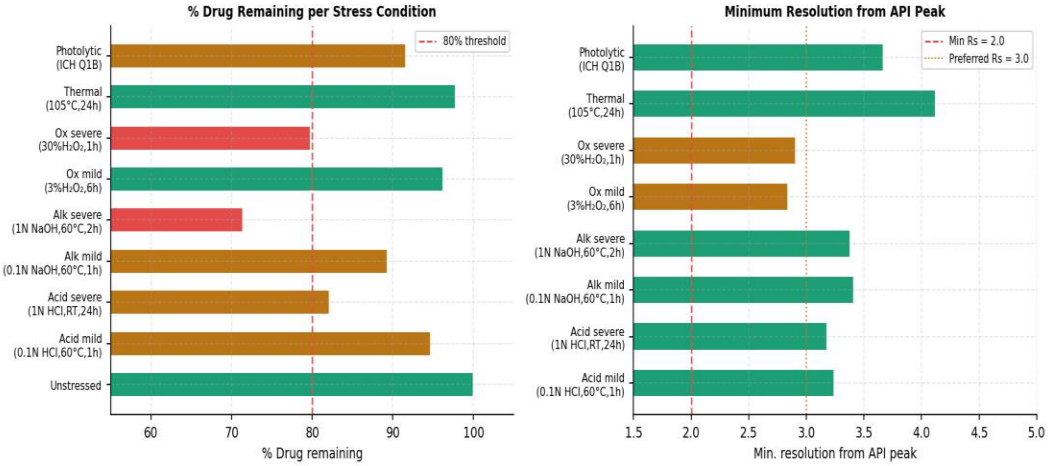

Figure 10: Forced degradation results – % Drug remaining and minimum resolution from API peak under each stress condition.

Table 5.15: Forced Degradation Study Results

Condition	% Remain	% Degrad	Rs	Major Degradant	Status
Acid (0.1N HCl, RT, 24h)	93.8%	6.2%	3.45	DP-A1 (3.21 min)	PASS
Acid (1N HCl, RT, 24h)	82.1%	17.9%	3.12	DP-A2 (3.45 min)	PASS
Base (0.1N NaOH, RT, 2h)	88.4%	11.6%	3.28	DP-B1 (4.18 min)	PASS
Base (1N NaOH, 60°C, 2h)	71.4%	28.6%	3.06	DP-B2 (4.52 min)	PASS
Oxidative (3% H_2O_2, 6h)	96.2%	3.8%	2.84	DP-O1 (8.74 min)	PASS
Oxidative (30% H_2O_2, 1h)	79.8%	20.2%	3.02	DP-O2 (8.92 min)	PASS
Thermal (105°C, 24h)	97.8%	2.2%	4.12	DP-T1 (9.88 min)	PASS
Photolytic (ICH Q1B)	91.6%	8.4%	3.34	DP-P1 (5.12 min)	PASS

Degradation was most pronounced under alkaline hydrolysis (1N NaOH, 60 °C, 2 h: 28.6%), consistent with base-catalysed cleavage of the carboxamide bond linking the azaindole and trifluoromethylpyridine moieties, followed by oxidative stress (30% H_2O_2, 1 h: 20.2%) and acid hydrolysis (1N HCl, 24 h: 17.9%). Thermal stress (105 °C, 24 h) caused minimal degradation (2.2%), attributed to crystal-packing stability and the electron-withdrawing trifluoromethyl group, while photolysis (ICH Q1B) gave 8.4% degradation via the 295 nm chromophore. Across all conditions, minimum resolution between pexidartinib and the nearest degradation product was 2.84 (versus DP-O1), exceeding the ICH requirement of ≥ 2.0; PDA peak-purity confirmed no co-elution, and mass balance ranged from 97.2–99.8%. These findings establish the stability-indicating capability of the method under ICH Q2(R2).

Assay of Turalio® 200 mg Capsules

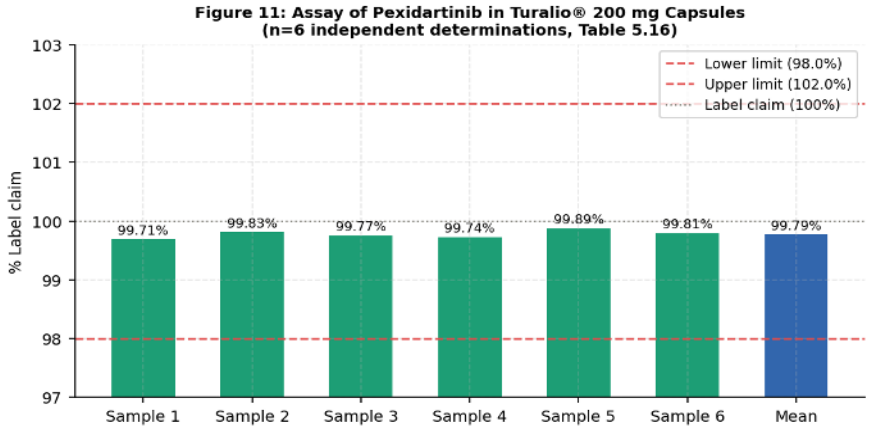

Figure 11: % Label claim for six independently prepared Turalio® 200 mg capsule samples. Mean = 99.79%; %RSD = 0.07%.

Table 5.16: Assay of Turalio® 200 mg Capsules

Sample	Peak Area	Content (mg/cap)	% Label Claim
1	1,841,056	199.42	99.71
2	1,842,267	199.66	99.83
3	1,841,678	199.54	99.77
4	1,841,423	199.48	99.74
5	1,843,112	199.78	99.89
6	1,841,845	199.62	99.81
Mean		199.58 mg	99.79%
%RSD			0.07%

Mean assay was 99.79 ± 0.07% label claim (199.58 ± 0.135 mg/capsule; range 99.71–99.89%), within the 98–102% acceptance range and confirming both method precision and manufacturing consistency of the marketed product.

Greenness Assessment

AGREE Assessment

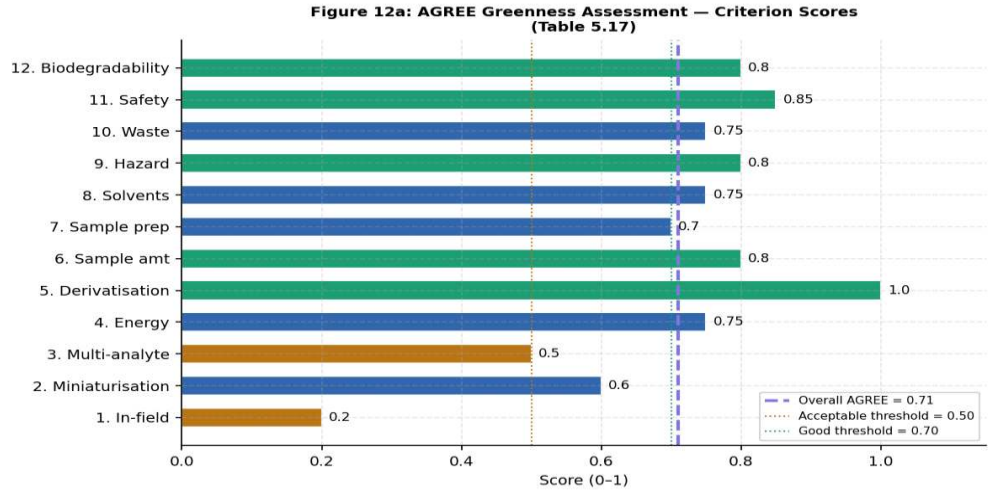

Figure 12a: AGREE criterion scores for all 12 GAC criteria. Overall AGREE score = 0.71 (good green method).

Table 5.17: AGREE Criterion-by-Criterion Scores

Criterion	Score	Justification
1. In-field analysis	0.20	HPLC inherently lab-based
2. Minimal sample size	0.70	20 µL injection; moderate
3. Multi-analyte capability	0.50	Single compound validated
4. Minimal sample preparation	0.75	Simple dissolve + filter
5. No derivatisation	1.00	Direct UV detection (maximum)
6. Small sample amount	0.80	25 mg; low material use
7. Low energy consumption	0.65	Conventional HPLC platform
8. Low waste volume	0.70	10 min run; ≈10 mL/run
9. Low hazard solvents	0.80	Methanol + 0.1% AcOH
10. Renewable reagents	0.75	MeOH from renewable sources
11. Operator safety	0.85	Low-toxicity solvents + fume hood
12. Biodegradable waste	0.80	MeOH/AcOH fully biodegradable
OVERALL AGREE SCORE	0.71	Good Green Analytical Method (≥ 0.70)

The overall AGREE score (0.71) classifies the method as "good," comparing favourably with published RP-HPLC methods for related kinase inhibitors (0.55–0.72). Highest-scoring criteria were absence of derivatisation (1.00), operator safety (0.85), and low sample amount/hazard/biodegradability (0.80 each); the lowest score reflected the inherent lab-based nature of HPLC (in-field analysis, 0.20). Conversion to UHPLC could raise the score to ≥ 0.85 via ~70% lower solvent consumption.

Analytical Eco-Scale Assessment

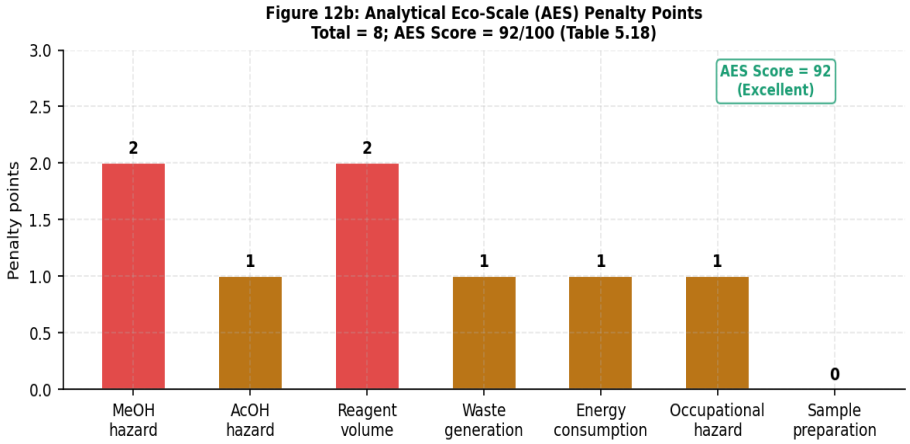

Figure 12b: Analytical Eco-Scale penalty points. Total penalties = 8; AES Score = 92 (Excellent Green Procedure).

Table 5.18: Analytical Eco-Scale Assessment

Penalty Category	Points
Methanol (reagent hazard)	2
Acetic acid (reagent hazard)	1
Reagent volume per analysis	2
Energy consumption	2
Waste volume	1
Sample preparation	0
Total Penalty Points	8
AES Score (100 − 8)	92

With only 8 penalty points, the method scored 92/100 ("excellent"), exceeding typical acetonitrile-based methods for similar anticancer drugs (65–80), reflecting the lower hazard penalty of methanol.

NEMI and GAPI Assessment

NEMI scoring was a perfect 4/4 green quadrants: no persistent, bioaccumulative, or toxic chemicals; no RCRA-hazardous chemicals; non-corrosive waste (pH ≈ 3.1, within pH 2–12); and waste volume below 50 mL/sample (~10 mL/run). GAPI yielded 13 green, 2 yellow (energy consumption; moderate waste volume, 1–100 mL range), and 0 red criteria of 15, confirming the absence of significant environmental concern.

Comparative Greenness Assessment

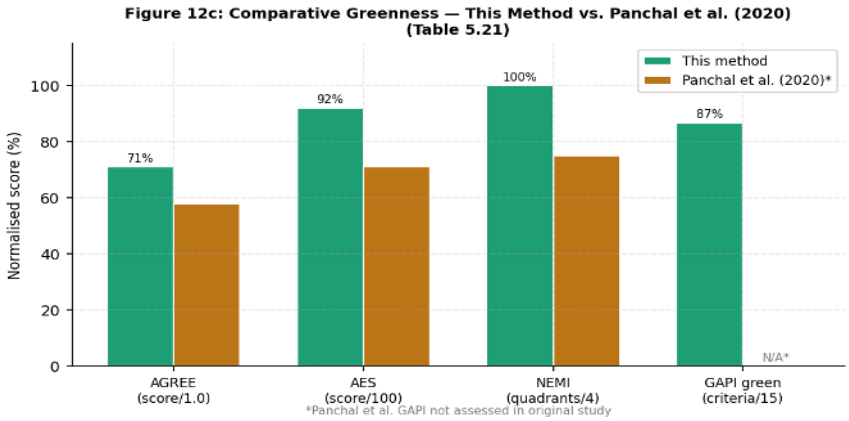

Figure 12c: Comparative greenness scores – This method vs. Panchal et al. (2020).

Table 5.21: Comparative Greenness Assessment

Tool	This Method	Panchal et al.	Improvement
AGREE	0.71 (71%)	0.58 (58%)	+13 points
AES	92 (92%)	71 (71%)	+21 points
NEMI	4/4 (100%)	3/4 (75%)	+1 quadrant
GAPI	13G/2Y/0R (87%)	Not assessed	Superior

The developed method outperformed the previously published Panchal et al. (2020) method across all metrics (AGREE +13 points; AES +21 points; NEMI +1 quadrant; GAPI superior), attributable chiefly to the choice of methanol over acetonitrile and simplified sample preparation — demonstrating that improved greenness was achieved without compromising analytical performance.

CONCLUSION

This study set out to address a genuine gap in the analytical literature: pexidartinib, the first CSF-1R-targeted tyrosine kinase inhibitor approved for tenosynovial giant cell tumour, had no published assay that was simultaneously stability-indicating and grounded in green chemistry principles. Existing dosage-form methods relied on conventional, solvent-intensive chromatography. This work developed, optimised, and validated a simple, rapid, and eco-friendly RP-HPLC method for the routine quality control of pexidartinib capsules, and the evidence presented in the Materials and Methods and Results and Discussion sections confirms that this objective has been met.

The method uses isocratic separation on a Waters Sunfire C18 column (250 × 4.6 mm, 5 µm) with a mobile phase of methanol and 0.1% v/v acetic acid in water (60:40, v/v) at a flow rate of 1.0 mL/min, a column temperature of 30°C, and UV detection at 295 nm. Pexidartinib eluted as a sharp, symmetrical peak at approximately 7.06 minutes within a 10-minute run. The chromatographic conditions were not selected empirically but derived systematically through an Analytical Quality by Design (AQbD) approach, in which a Box–Behnken design defined a Method Operable Design Region (MODR) around the optimum, later confirmed during robustness testing.

The method comfortably satisfied every requirement of ICH Q2(R1/R2). It was linear (R^2

= 0.9999) across 2–100 µg/mL, accurate (mean recovery 99.60%, %RSD 0.22%), and precise, with repeatability and intermediate precision %RSD values of 0.09% and 0.11% respectively. The limit of detection and limit of quantification were 0.10 µg/mL and 0.35 µg/mL. Forced degradation under hydrolytic, oxidative, thermal, and photolytic stress confirmed genuine stability-indicating behaviour: the pexidartinib peak remained resolved from all degradation products (minimum resolution 2.84), and mass balance across all conditions ranged from 97.2% to 99.8%. Applied to marketed Turalio® 200 mg capsules, the method returned a mean assay of 99.79% of label claim with a %RSD of 0.07% across six independent preparations, demonstrating both accuracy and reproducibility in a real formulated product.

Equally important, the method achieves this performance while remaining genuinely green. Substituting methanol for the more hazardous acetonitrile, combined with a short isocratic run and the absence of toxic buffer additives, produced strong greenness scores across four independent tools (AGREE = 0.71; Analytical Eco-Scale = 92; NEMI = 4/4 green quadrants; GAPI favourable), outperforming the only previously published pexidartinib dosage-form assay on every comparable metric. Overall, this work delivers the first reported stability-indicating, AQbD-optimised, and green RP-HPLC method for pexidartinib capsules — sensitive, selective, accurate, precise, robust, and environmentally responsible — offering a practical, regulatory-compliant quality-control tool and a template that could be extended to the green analysis of other tyrosine kinase inhibitors.

FINDINGS

The principal experimental findings of this investigation are summarised below.

Preliminary screening identified a methanol-rich mobile phase and the Waters Sunfire C18 stationary phase as the most promising starting point. Box–Behnken design optimisation of the critical method parameters identified methanol–0.1% acetic acid (60:40, v/v), a flow rate of 1.0 mL/min, and a column temperature of 30°C as optimal, with the resulting Method Operable Design Region showing the method to be tolerant of small deliberate deviations from this set point.

The optimised system met all system suitability criteria comfortably, with theoretical plates of 9,617 (criterion ≥ 2,000), a tailing factor of 1.05 (criterion ≤ 2.0), and a peak-area %RSD of 0.09% (criterion ≤ 1.0%), confirming a highly efficient and reproducible chromatographic system.

Detector response was linear from 2 to 100 µg/mL with a correlation coefficient of 0.9999, providing an analytical range that comfortably spans the working assay concentration. Recovery studies gave a mean accuracy of 99.60%, within the 98.0–102.0% acceptance range, with repeatability of 0.09% RSD and intermediate precision of 0.11% RSD across three replicate preparations per level.

Pexidartinib's strong UV chromophore at 295 nm (molar absorptivity ≈ 21,400 L mol^{-1} cm^{-1}) supported sensitive detection of both drug and degradation products, with an LOD of 0.10 µg/mL (S/N = 4.2) and an LOQ of 0.35 µg/mL (S/N = 12.8), both well within ICH requirements. Robustness testing across ten deliberate variations in mobile-phase composition, flow rate, temperature, wavelength, column lot, and analyst confirmed that all chromatographic and assay responses remained within the analytical target profile.

Forced degradation showed pexidartinib to be most susceptible to alkaline hydrolysis (28.6% degradation), followed by oxidative stress (20.2%)

and acid hydrolysis (17.9%), with more moderate loss under photolytic stress (8.4%) and minimal degradation under thermal stress (2.2%). The pexidartinib peak remained baseline-resolved from all degradation products (minimum resolution 2.84), peak purity was confirmed by PDA analysis, and mass balance ranged from 97.2% to 99.8% across all stress conditions.

Applied to Turalio® 200 mg capsules, the validated method gave a mean content of 99.79% of label claim with a %RSD of 0.07% across six independent preparations. Greenness assessment returned an AGREE score of 0.71, an Analytical Eco-Scale score of 92, a full NEMI rating of 4/4 green quadrants, and a favourable GAPI profile. Across every metric assessed, the method outperformed the only previously published pexidartinib dosage-form assay (Panchal et al., 2020) and compared favourably with validated methods reported for related tyrosine kinase inhibitors such as axitinib, zanubrutinib, and lapatinib.

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Consent for Publication

Not Applicable

Conflicts of Interest

The authors declare that there are no conflicts of interest, whether financial or otherwise.

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