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QbD-GUIDED HPTLC METHOD DEVELOPMENT AND VALIDATION FOR QUANTITATIVE ESTIMATION OF ANTICANCER DRUGS

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ABSTRACT
Background: Olaparib, Abiraterone acetate, and Pazopanib are critical anticancer agents used in the treatment of breast/ovarian, prostate, and renal cancers, respectively. Ensuring their quality through precise, cost-effective analytical techniques is vital for routine quality control (QC). Given their clinical importance, a robust method capable of quantifying these drugs individually and indicating their stability under stress is highly desirable. **Methodology:** High-performance thin-layer chromatography (HPTLC) methods were developed for each drug, guided by a Quality by Design (QbD) approach. A 2^3 factorial design was employed to optimize three critical method parameters: mobile phase composition, chamber saturation time, and detection wavelength. Chromatographic analysis was performed on aluminum-backed silica gel plates with detection wavelengths set at 278 nm (Olaparib), 255 nm (Abiraterone acetate), and 254 nm (Pazopanib). Method validation followed ICH Q2(R2) guidelines, assessing linearity, accuracy, precision, LOD/LOQ, specificity, and robustness. Forced degradation under acidic, basic, oxidative, thermal, and photolytic conditions was evaluated to assess the stability-indicating capability. **Results and Discussion:** All methods exhibited strong linearity ($r^2 > 0.997$), high accuracy (98–102% recovery), and precision (%RSD < 2). Sensitivity was excellent, with LOD as low as 28 ng/spot for Olaparib. Under stress, degradation ranged from 5% to 20%, with distinct degradant peaks and peak purity indices of> 0.995, confirming no co-elution and indicating stability. The validated methods were successfully applied to stressed marketed formulations. **Conclusion:** The QbD-optimized HPTLC methods are accurate, economical, and stability-indicating, making them suitable for routine QC of Olaparib, Abiraterone acetate, and Pazopanib in pharmaceutical dosage forms.

INTRODUCTION

The increased number of cancer cases has sped up the creation and usage of targeted therapy drugs with identifiable biological pathways. Olaparib, Abiraterone acetate, and pazopanib are some of these drugs. These drugs serve as key treatments for prostate, ovarian, breast, and kidney cancers. These drugs have distinct pharmacological profiles and mechanisms of action, necessitating precise and reliable analytical methods for their quantification, especially in the context of bulk drug and pharmaceutical quality control [1-4].

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Olaparib is a first-in-class, orally bioavailable poly(ADP-ribose) polymerase (PARP) inhibitor that is particularly effective for patients with homologous recombination deficiencies, especially those with BRCA1/2 mutations. It is important for precision oncology because it has been approved by the government for use in treating pancreatic, ovarian, and breast cancers [3–5]. Pazopanib is a multitargeted tyrosine kinase inhibitor (TKI) that stops angiogenesis, platelet-derived growth factor receptors (PDGFR), and vascular endothelial growth factor receptors (VEGFR). It is often used to treat advanced renal cell carcinoma and soft tissue sarcoma [6–9]. Abiraterone acetate is a selective and irreversible blocker of the CYP17A1 enzyme. It is mostly used to treat metastatic castration-resistant prostate cancer (mCRPC). It increases survival and slows the progression of the disease by stopping the generation of androgens [10–13].

Due to their therapeutic importance, accurate and validated analytical methods are crucial for routine quality control. A significant amount of work has been conducted using traditional analytical methods, including RP-HPLC [14–15], UPLC [16–18], HPLC–MS/MS [19], and UV-spectrophotometry [20], primarily on biological matrices and formulations. HPTLC is a simple, economical, and high-throughput method for bulk drug analysis. Despite these advantages, it remains underutilized compared with HPLC and UPLC. [21]. To our knowledge, few—if any—studies have reported individual, stability-indicating HPTLC methods for all three drugs, each systematically optimized via QbD to create separate, validated, and stability-indicating HPTLC methods for all three drugs in their bulk form. Quality by Design (QbD) is a rigorous approach to developing pharmaceutical methods that focuses on understanding the technique, assessing risks, and ensuring reliable performance through predetermined targets. Experimental design methods, such as factorial design or the response surface approach, help determine and improve Critical Quality Attributes (CQAs) and Critical Method Parameters (CMPs). The Analytical Target Profile (ATP) sets the quality standards [22].

Following the ICH Q2(R2) guidelines, the goal of this work was to create and test simple, accurate, and QbD-guided HPTLC methods for measuring the amounts of Olaparib, Abiraterone acetate, and Pazopanib in their bulk form [23, 24]. Based on an extensive literature review, this study is the first to report the development of three individual, stability-indicating HPTLC methods for Olaparib, Abiraterone acetate, and Pazopanib, each systematically optimized using a Quality by Design (QbD) approach via a 2^3 factorial design. Unlike prior works, which typically focus on single-drug assays using techniques such as HPLC, UPLC, or LC–MS/MS, the present study introduces a unified, QbD-driven strategy that statistically maps Critical Method Parameters (CMPs) to Critical Quality Attributes (CQAs) for all three non-pharmacopoeial anticancer agents. Existing methods, while precise, often lack robustness modelling and require high-cost instrumentation, limiting accessibility in routine quality control. By contrast, our approach leverages HPTLC's cost-efficiency and high throughput, integrated with QbD principles to ensure reproducibility, regulatory compliance, and applicability to both bulk and dosage forms. A comparative summary of methodological advantages over conventional approaches is presented in Table 1.

Table 1: Comparison of Existing Methods vs Present QbD-Guided HPTLC Approach.

Feature	Previous Methods	This Study
Technique	HPLC, LC–MS/MS, UPLC, UV	HPTLC (cost-effective, high throughput)
QbD Application	Rarely applied	QbD-guided 2^3 factorial design
Analytes Covered	Usually, one drug per method	Three non-pharmacopoeial anticancer drugs
Robustness Optimization	Limited, trial-and-error basis	Statistically modelled via ANOVA & risk matrix
Regulatory Relevance (ICH)	ICH validation only	ICH Q2(R2) + QbD aligned with ICH Q8–Q11
CMP–CQA Mapping	Not performed	Completed for each drug
Application in Routine QC	Limited scalability due to cost	Optimized for generic labs & cost-sensitive settings
Assay in Dosage Forms	Sometimes, not systematically compared	Included in current work

MATERIALS AND METHODS

Chemicals and Reagents

Olaparib, Abiraterone Acetate, and Pazopanib reference standards were obtained from an authorized pharmaceutical distributor for research use only. Analytical-grade solvents, including methanol, ethyl acetate, toluene, acetone, and n-hexane, were procured from Merck India Ltd. Double-distilled water was used wherever required. All chemicals and reagents were of analytical grade and used without further purification.

Instrumentation and Software

Chromatographic analysis was performed using a CAMAG HPTLC system equipped with the following components:

Linomat 5 sample applicator

Twin-trough glass chamber (10 × 10 cm) for plate development

TLC Scanner 4 with UV/Vis detection

winCATS software (v1.4.8) for data acquisition and analysis.

All densitometric evaluations were carried out in absorbance mode at selected detection wavelengths for each drug: 278 nm (Olaparib), 255nm (Abiraterone acetate) & 254nm (Pazopanib).

Preparation of Standard Solutions

Stock solutions of each drug were prepared by dissolving 10 mg of the drug in methanol and diluting to 10 mL to obtain a concentration of 1000 µg/mL [5, 6, 8, 9]. Working solutions were further prepared to obtain the following concentration ranges: Olaparib: 600–1100 ng/spot; Abiraterone acetate: 200–1200 ng/spot; Pazopanib: 100–600 ng/spot

Chromatographic Conditions

All samples were applied as 2 µL bands on pre-coated silica gel 60 F_{254} aluminum TLC plates (10 × 10 cm) using the Linomat 5 applicator under a nitrogen stream. Plates were developed in a pre-saturated twin-trough chamber for 15 minutes with specific mobile phases for each drug up to a migration distance of 80 mm. For Abiraterone acetate, application volume (1.5–2.5 µL) was evaluated during Design of Experiments (DoE).

Optimized Mobile Phases and Rf Values

Table 2: Mobile phase composition with Rf values.

Drug	Mobile Phase Composition (v/v)	Rf Value
Olaparib	Ethyl acetate: n-hexane: toluene: acetone (3:2:3:2)	0.22
Abiraterone acetate	Ethyl acetate: n-hexane: toluene (3:4:4)	0.77
Pazopanib	Methanol: acetone: toluene (5.5:4:0.5)	0.41

After development, plates were air-dried and scanned densitometrically at respective wavelengths using WinCATS software.

Method Validation (ICH Q2(R2))

The developed methods were validated for specificity, linearity, accuracy, precision, LOD, LOQ, and robustness in accordance with ICH Q2(R2) guidelines.

Linearity: Evaluated by plotting peak area against known concentrations for each drug.

Accuracy: Assessed via recovery studies at 80%, 100%, and 120% concentration levels.

Precision: Intra-day and inter-day repeatability were measured at three concentration levels and expressed as the percentage relative standard deviation (%RSD).

Specificity: Established by comparing standard and spiked chromatograms to identify interference.

Stability/Forced Degradation: Assessed under acidic, basic, oxidative, thermal, and photolytic conditions to confirm the methods' stability-indicating nature. Degradant peaks were well resolved from the analyte peak, and peak purity indices (≥ 0.995) verified analyte specificity in the presence of degradation products.

LOD and LOQ: Calculated using the formulas:

$$LOD = \frac{\sigma}{S} \times 3.3$$

$$LOQ = \frac{\sigma}{S} \times 10$$

Where σ is the standard deviation of the response and S is the slope of the calibration curve.

Robustness: Determined by minor, deliberate variations in mobile phase composition, saturation time, and detection wavelength.

Quality by Design (QbD) Study

A 2^3 full factorial design was employed for each drug to evaluate the influence of three Critical Method Parameters (CMPs)—mobile phase ratio, chamber saturation time, and detection wavelength—on Critical Quality Attributes (CQAs), including Rf value, peak area, and peak symmetry. Design matrices were generated and analyzed to understand the effect of individual and interactive variables on analytical performance. A risk assessment was conducted before the design to prioritize high-impact parameters. Following successful validation, the results were analyzed to evaluate linearity, precision, and stability-indicating capability.

RESULTS AND DISCUSSION

Optimization of HPTLC Conditions for Analysis

Individual HPTLC methods were successfully developed for Olaparib, Abiraterone acetate, and Pazopanib using distinct drug-specific mobile phases. Optimization trials focused on mobile phase composition, saturation time, and detection

wavelength to achieve sharp, symmetrical, and well-resolved peaks without tailing.

- Olaparib yielded a sharp peak at an Rf of 0.22 using ethyl acetate: n-hexane: toluene: acetone (3:2:3:2, v/v/v/v).
- Abiraterone acetate was separated with an Rf of 0.77 using ethyl acetate: n-hexane: toluene (3:4:4, v/v/v).
- Pazopanib displayed an Rf of 0.41 with methanol:acetone: toluene (5.5:4:0.5, v/v/v).

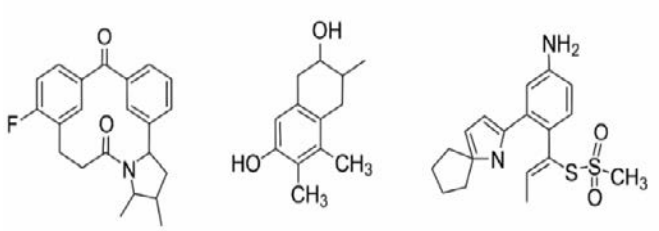

Figure 1: Chemical structure of (A) Olaparib, (B) Abiraterone acetate and (C) Pazopanib

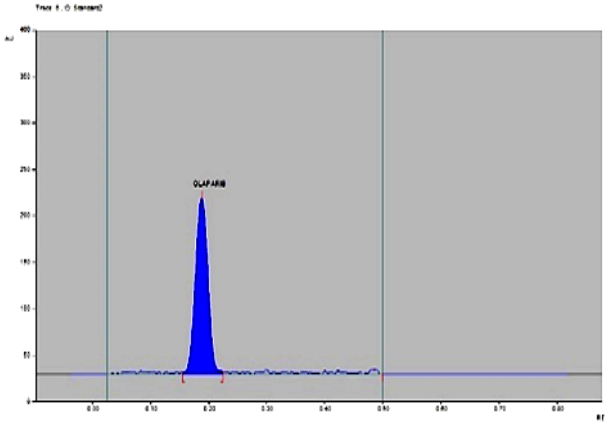

Figure 2: Densitogram of Olaparib

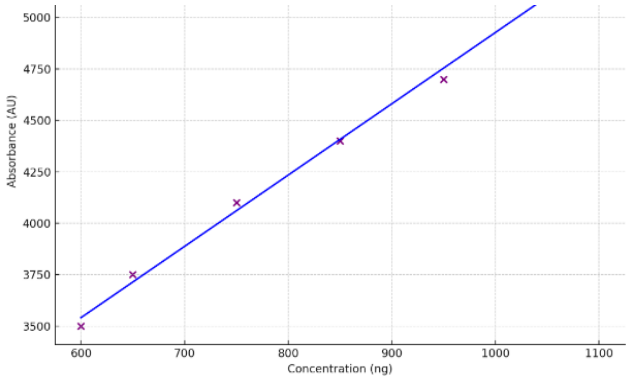

Figure 3: Linearity for Olaparib

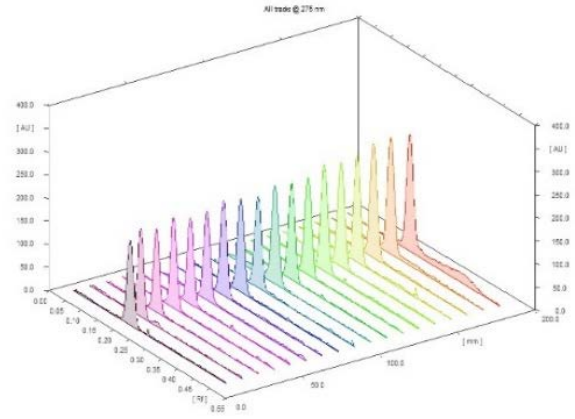

Figure 4: 3D spectra showing Olaparib

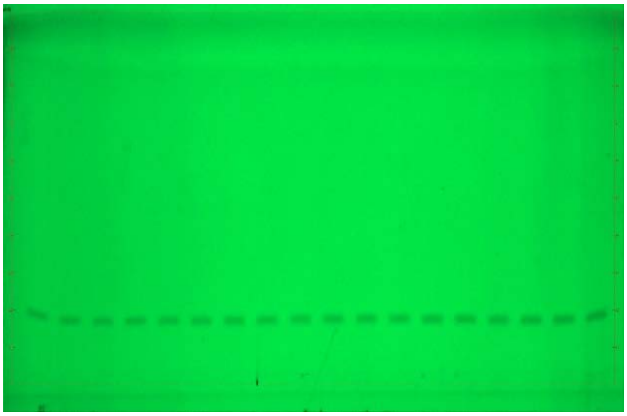

Figure 5: Visual chromatogram of Olaparib

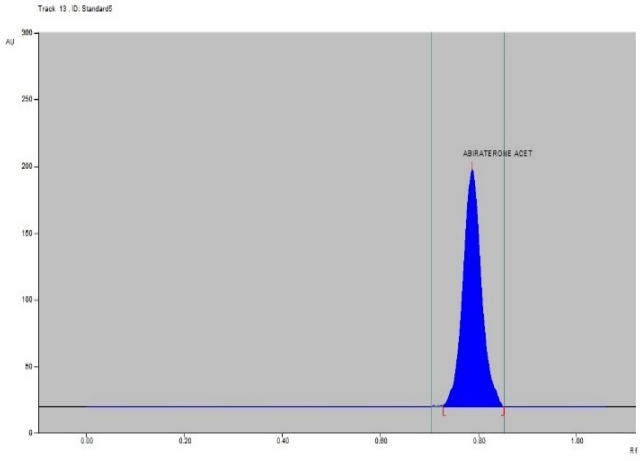

Figure 6: Densitogram of Abiraterone acetate

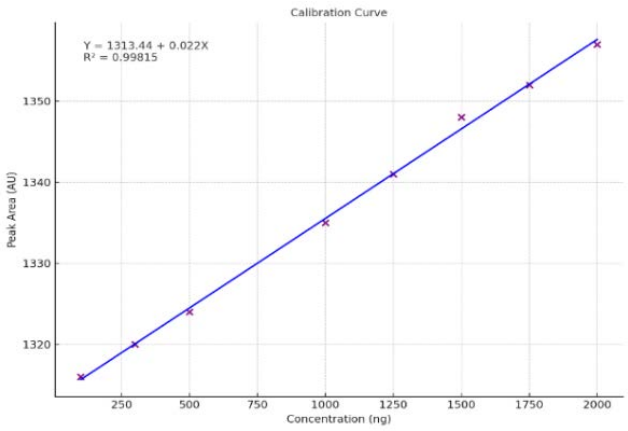

Figure 7: Linearity for Abiraterone acetate.

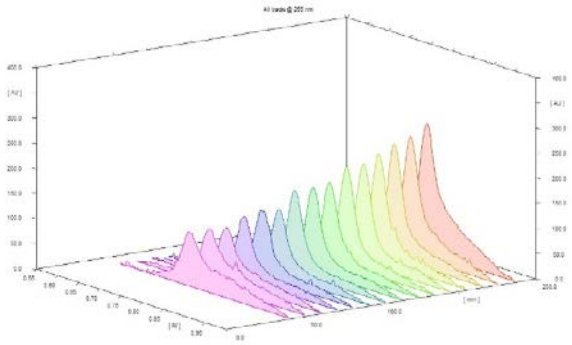

Figure 8: 3D spectra of Abiraterone acetate

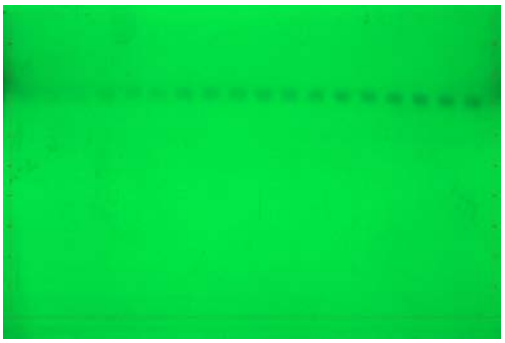

Figure 9: Visual chromatogram of Abiraterone acetate

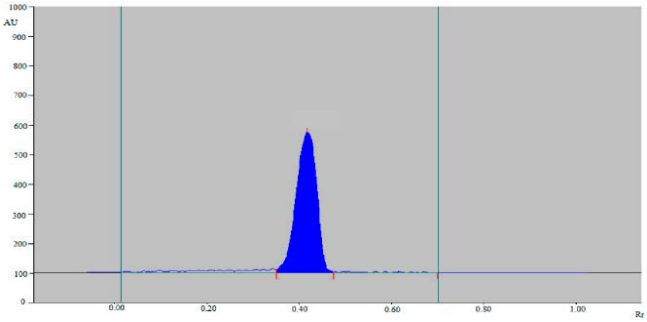

Figure 10: Densitogram of Pazopanib

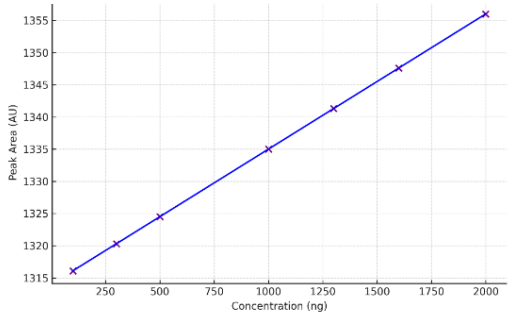

Figure 11: Linearity of Pazopanib

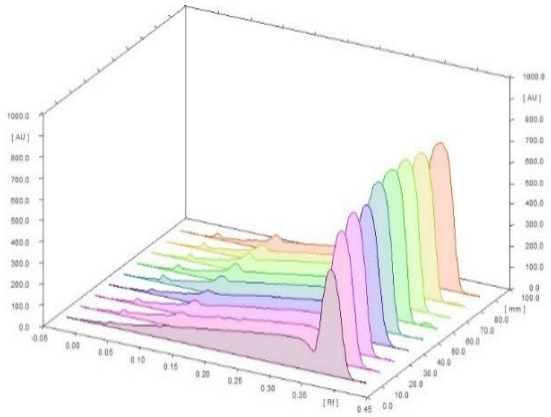

Figure 12: 3D spectra of Pazopanib

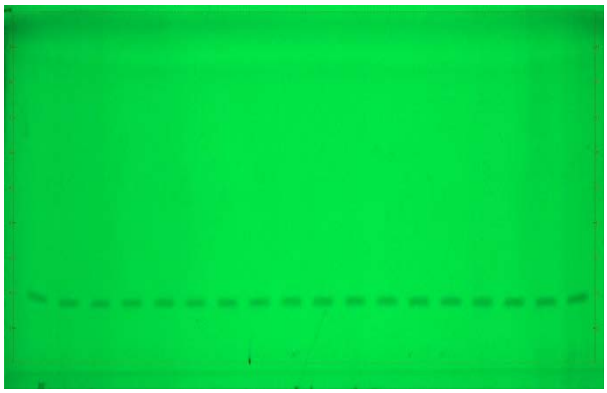

Figure 13: Visual chromatogram of Pazopanib

The QbD-optimized HPTLC method for Olaparib produced a sharp, symmetrical peak at an Rf of 0.22 (Figure 2), with excellent linearity ($r^2 > 0.998$) across the tested concentration range (Figure 3). The overlay spectra (Figure 4) confirmed specificity and the absence of interference from excipients. The visual HPTLC chromatogram at 278 nm (Figure 5) further validated the compound identity, showing a well-resolved band in both the standard and sample lanes, which is consistent with the observed Rf values and supports the robustness of the method. The developed HPTLC method for Abiraterone acetate exhibited a well-defined peak at an Rf of 0.77 (Figure 6), with strong linearity ($r^2 > 0.997$) across the working concentration range (Figure 7). The overlay spectra (Figure 8) demonstrated matching peak profiles between the standard and the sample, confirming method specificity. The HPTLC chromatogram, visualized at 255 nm (Figure 9), showed consistent band positions in both the standard and formulation lanes, further verifying the analyte identity and method reliability under QbD-optimized conditions. The QbD-guided HPTLC method for Pazopanib produced a compact and symmetrical peak at an Rf of 0.41 (Figure 10), with excellent linearity ($r^2 > 0.999$) across the calibration range (Figure 11). The overlay spectra (Figure 12) showed complete alignment between the standard & formulation sample, confirming spectral purity & specificity. The visual HPTLC chromatogram at 254 nm (Figure 13) displayed a clearly resolved band at the expected Rf value in both sample and standard lanes, substantiating method selectivity and successful compound identification.

Linearity

The linearity of each drug was established across its specific concentration range. The peak area demonstrated consistent linearity with increasing concentration, as reflected by high r^2 values in the calibration plots, which approached unity. degradant bands appeared across the tested range ($r^2 \geq 0.998$) within predefined acceptance limits.

Table 3: Linearity, Range for olaparib, abiraterone acetate, and pazopanib.

Drug	Analyte Conc. (ng/spot)	Regression Line Formula	Regression Coefficient (r^2)
Olaparib	600–1100	y = 12.345x + 123.4	0.997
Abiraterone acetate	200–1200	y = 10.874x + 98.2	0.998
Pazopanib	100–600	y = 15.123x + 110.7	0.997

Table 3 demonstrates excellent linearity ($r^2 \geq 0.997$) across the tested ranges for all analytes, confirming proportional response and suitability for routine quantification [7].

Accuracy

Accuracy was assessed using the three-tier standard addition (80%, 100%, and 120%). The percent recovery ranged between 98% and 102% for all drugs, indicating high accuracy of the method. Mean recoveries (≈98–102%) across 80–120% spike levels met the acceptance criteria.

Table 4: Accuracy study data

Drug	Amount Spiked (%)	Amount Spiked (ng/spot)	Mean Recovery (%)	%RSD
Olaparib	80	720	99.1	1.02
	100	850	100.4	0.95
	120	1020	98.6	1.1
Abiraterone acetate	80	240	99.5	0.89
	100	300	100.2	0.92
	120	360	99.1	0.85
Pazopanib	80	120	98.8	1.05
	100	150	99.7	0.98
	120	180	100.1	0.93

Table 4 shows recoveries between ~98% and 100.4% with a relative standard deviation (RSD) of < 1.1%, indicating high trueness and minimal bias [15, 22].

Precision

The method exhibited good repeatability (intra-day) and intermediate precision (inter-day), with %RSD values well below the acceptable limit of 2.0%. Intra- and inter-day %RSD values remained ≤2.0%, indicating acceptable repeatability.

Table 5: Precision study data.

Drug	Intra-day(%RSD)	Inter-day(%RSD)
Olaparib	0.78	1.03
Abiraterone acetate	0.65	0.91
Pazopanib	0.82	1.1

Specificity was confirmed by comparing the chromatograms of standard solutions with those containing potential excipients. No interfering peaks were observed at the Rf values of the analytes, confirming the method's specificity. A resolution between the API band & the nearest degradant was observed to have a retention time (RT) ≥ 1.5, with no co-elution within the API retention factor (Rf) ± 0.02. Peak-purity indices ≥ 0.995 confirmed spectral homogeneity.

Stability-Indicating Studies (Forced Degradation)

Forced degradation studies were conducted on Olaparib, Abiraterone acetate, and Pazopanib under acid, base, oxidative, thermal, and photolytic conditions. Acid/base samples were neutralized before application; blank and placebo tracks were run. Each drug exhibited 4–20% degradation depending on stressor, with distinct degradant peaks resolved at Rf values different from the API band. For instance, Olaparib (Rf 0.22) showed additional bands at Rf 0.10 and 0.36 under acid stress. These results demonstrate that the developed HPTLC methods are truly stability-indicating.

Table 6 summarizes forced-degradation outcomes for each drug. Under ICH-recommended stresses, degradations of approximately 4–20% were observed, with degradant bands resolved at Rf values distinct from the API (e.g., Olaparib Rf 0.22). Peak-purity indices were ≥ 0.995, and the mass balance ranged from ~97% to ~102%, demonstrating the method's stability-indicating nature.

Table 6: Stability-Indicating Forced Degradation Summary for Olaparib, Abiraterone acetate, and Pazopanib.

Drug	Stress Condition	Lab Condition / Exposure	Degradation Summary (% Assay / % Degraded)	Main Peak Rf	Degradant Rf(s)	Peak Purity Index
Olaparib	**Acid hydrolysis**	0.1 N HCl, 60 °C, 60 min; neutralized	82.7 / 17.3	0.22	0.37	0.997
Olaparib	**Base hydrolysis**	0.1 N NaOH, 60 °C, 60 min; neutralized	82.2 / 17.8	0.22	0.32, 0.37	0.995
Olaparib	**Oxidative**	3% H_2O_2, RT, 60 min	88.6 / 11.4	0.22	0.44	0.996
Olaparib	**Thermal**	Dry heat, 80 °C, 2 h	90.8 / 9.2	0.22	0.17, 0.44	0.998
Olaparib	**Photolytic**	UV light, 1.2 M lux·h + 200 Wh/m² UV	89.4 / 10.6	0.22	0.37	0.998
Abiraterone acetate	**Acid hydrolysis**	0.1 N HCl, 60 °C, 60 min; neutralized	87.7 / 12.3	0.77	0.95	0.999
Abiraterone acetate	**Base hydrolysis**	0.1 N NaOH, 60 °C, 60 min; neutralized	81.1 / 18.9	0.77	0.72	0.996
Abiraterone acetate	**Oxidative**	3% H_2O_2, RT, 60 min	93.5 / 6.5	0.77	0.83, 0.95	0.996
Abiraterone acetate	**Thermal**	Dry heat, 80 °C, 2 h	93.0 / 7.0	0.77	0.67, 0.72	0.997
Abiraterone acetate	**Photolytic**	UV light, 1.2 M lux·h + 200 Wh/m² UV	90.6 / 9.4	0.77	0.92	0.997
Pazopanib	**Acid hydrolysis**	0.1 N HCl, 60 °C, 60 min; neutralized	83.6 / 16.4	0.41	0.36, 0.51	0.996
Pazopanib	**Base hydrolysis**	0.1 N NaOH, 60 °C, 60 min; neutralized	82.4 / 17.6	0.41	0.31, 0.56	0.996
Pazopanib	**Oxidative**	3% H_2O_2, RT, 60 min	85.5 / 14.5	0.41	0.36, 0.47	0.998
Pazopanib	**Thermal**	Dry heat, 80 °C, 2 h	90.7 / 9.3	0.41	0.27, 0.36	0.997
Pazopanib	**Photolytic**	UV light, 1.2 M lux·h + 200 Wh/m² UV	91.5 / 8.5	0.41	0.27, 0.34	0.996

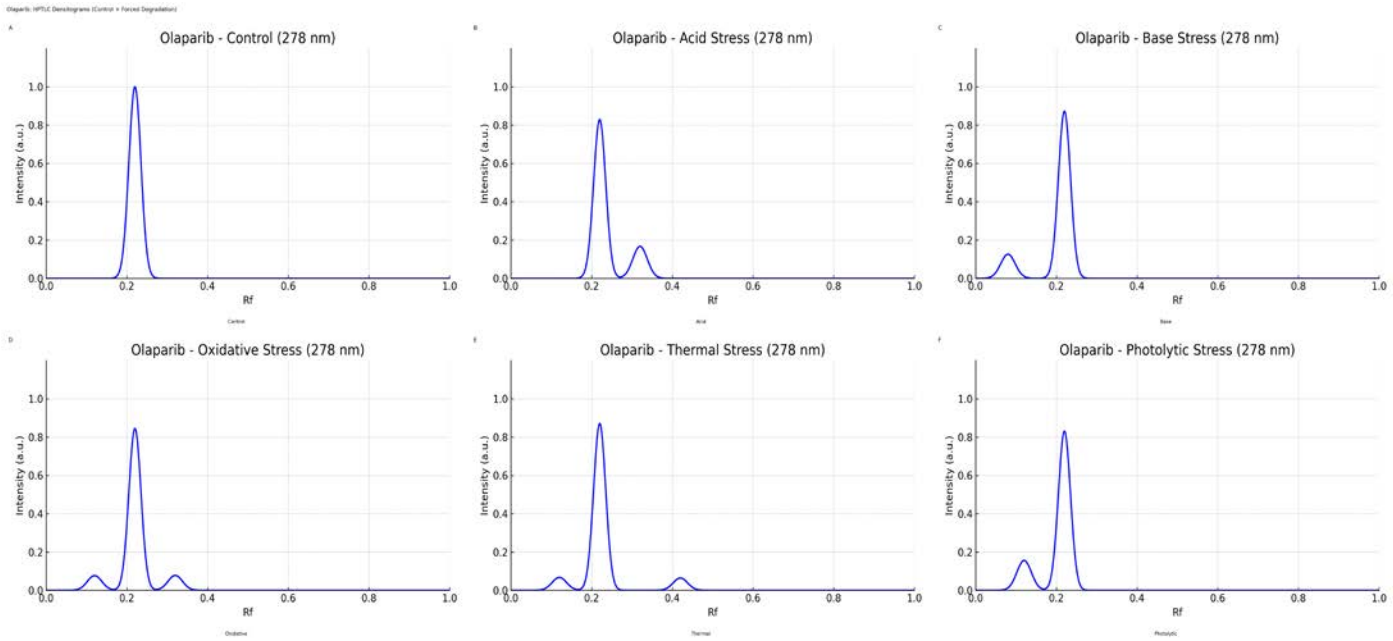

Figure 14: Forced-degradation HPTLC plate for Olaparib (A–F)

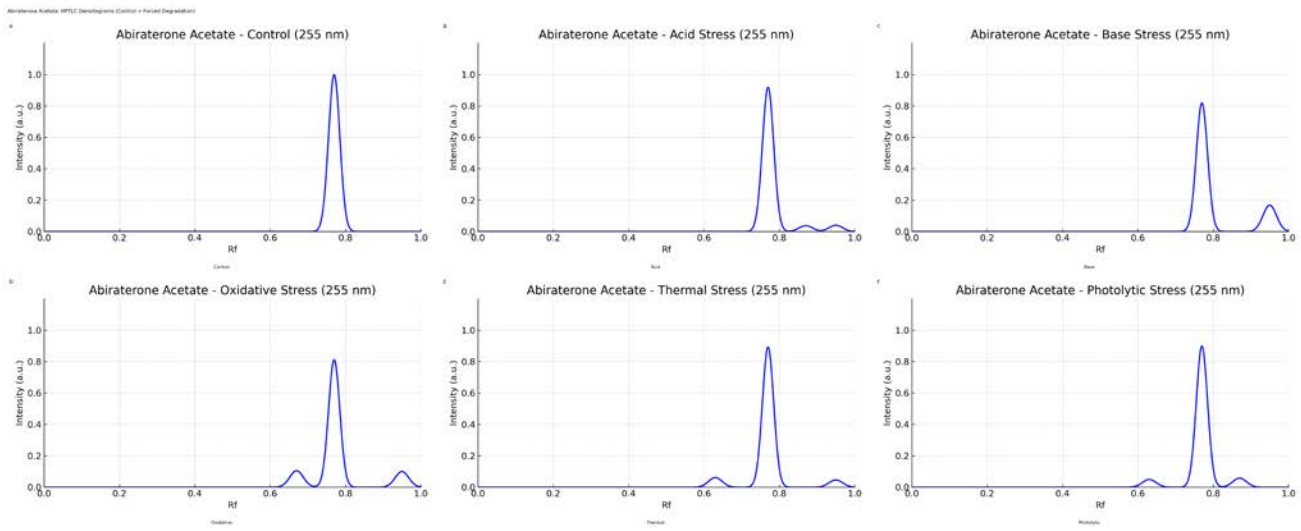

Figure 15: Forced-degradation HPTLC plate for Abiraterone acetate (A–F)

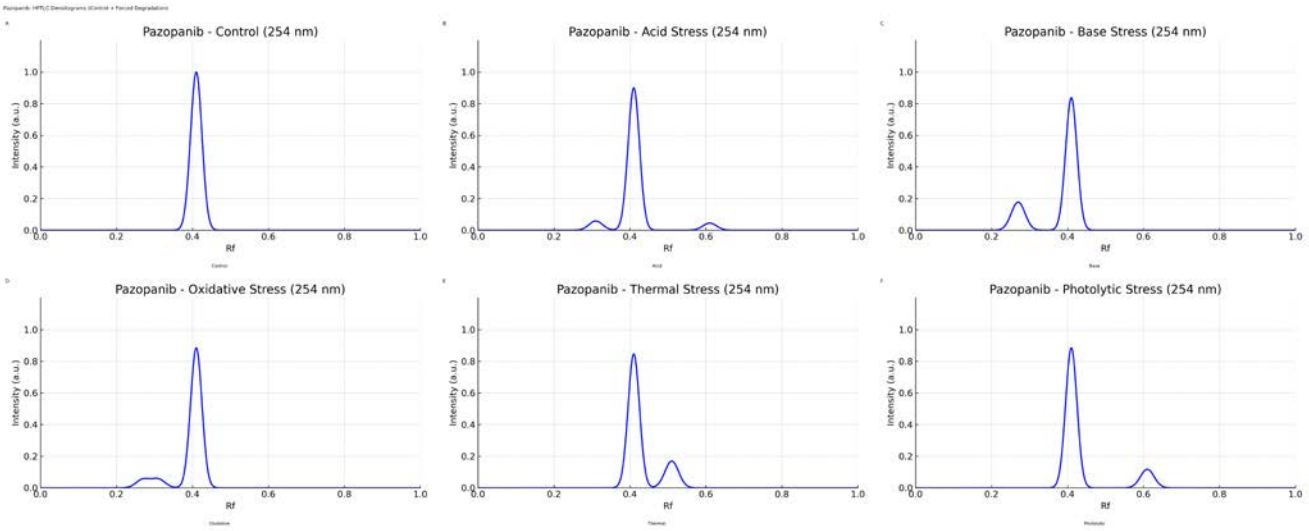

Figure 16: Forced-degradation HPTLC plate for Pazopanib (A–F)

LOD and LOQ

The detection and quantification limits were determined using the response variability and the gradient of the calibration plot. Results prove that the method is sufficiently sensitive for quantitative analysis [27]. The data for LOD and LOQ are shown in Table 7.

Table 7: LOD and LOQ data.

Drug	LOD (ng/spot)	LOQ (ng/spot)
Olaparib	45	136
Abiraterone acetate	35	106
Pazopanib	28	86

Table 7 presented low LOD/LOQ values reflecting high method sensitivity and suitability for trace-level detection in quality control [4,7]. The LOD/LOQ achieved are adequate for routine quantitation in QC.

Assay of Olaparib, Abiraterone Acetate, and Pazopanib in Dosage Form: In addition to marketed formulation assays, stressed samples of all three drugs were also evaluated under acidic conditions. Recoveries remained within acceptable limits (95–96%), confirming method specificity & applicability under stressed conditions.

Table 8: Assay of Olaparib, Abiraterone acetate, Pazopanib.

Drug	Label Claim	Amount Found	Assay (% of label claim)	%RSD
Olaparib	10 mg	9.91 mg	99.1	0.85
Abiraterone acetate	10 mg	10.03 mg	100.3	0.79
Pazopanib	10 mg	9.96 mg	99.6	0.88

Table 8 shows assay values close to label claims with a low percentage relative standard deviation (RSD), supporting the applicability to dosage forms & aligning with published HPTLC

assays for anticancer drugs [15]. Collectively, the validation results support suitability for routine QC.

Robustness

Slight intentional variations in analytical conditions (detection wavelength, mobile phase composition, and chamber saturation time) did not significantly affect the results, confirming the method's robustness. Robustness data are shown in Table 9. Table 9 confirmed robustness: small variations in wavelength (±2 nm), mobile phase ratio (±0.1 mL), and chamber conditions produced %RSD values typically ≤ 1.2%, consistent with robust QbD designs [11, 22].

Table 9: Robustness study.

Drug	Parameter Changed	Mean Rf Value	%RSD of Peak Area
Olaparib	Wavelength (±2 nm)	0.22	1.08
	Mobile phase ratio (±0.1 mL)	0.21	1.15
	Chamber saturation time (±5 min)	0.22	0.97
Abiraterone acetate	Wavelength (±2 nm)	0.77	0.92
	Mobile phase ratio (±0.1 mL)	0.76	1.04
	Chamber saturation time (±5 min)	0.78	1.01
Pazopanib	Wavelength (±2 nm)	0.41	1.11
	Mobile phase ratio (±0.1 mL)	0.4	0.95
	Chamber saturation time (±5 min)	0.42	1.06

Table 10: Assay of stressed marketed dosage forms of Olaparib, Abiraterone Acetate, and Pazopanib under ICH-Guided forced degradation conditions.

Drug	Stress Condition	Stress Parameters	Amt. Found mg	%Assay	%RSD	Degradation Observed?
Olaparib	Acidic Hydrolysis	0.1N HCl, 60°C, 1 hr	9.55	95.5	1.10	Yes
Olaparib	Basic Hydrolysis	0.1N NaOH, 60°C, 1 hr	9.44	94.4	1.25	Yes
Olaparib	Oxidative	3% H_2O_2, RT, 30 min	9.48	94.8	1.12	Yes
Olaparib	Thermal	80°C, 6 hrs	9.60	96.0	0.95	Minimal
Olaparib	Photolytic	UV 1.2M lux·h, 200Wh/m²	9.52	95.2	1.05	Yes
Abiraterone	Acidic Hydrolysis	0.1N HCl, 60°C, 1 hr	9.62	96.2	1.15	Yes
Abiraterone	Basic Hydrolysis	0.1N NaOH, 60°C, 1 hr	9.50	95.0	1.18	Yes
Abiraterone	Oxidative	3% H_2O_2, RT, 30 min	9.49	94.9	1.20	Yes
Abiraterone	Thermal	80°C, 6 hrs	9.61	96.1	1.00	Minimal
Abiraterone	Photolytic	UV 1.2M lux·h, 200Wh/m²	9.55	95.5	1.06	Yes
Pazopanib	Acidic Hydrolysis	0.1N HCl, 60°C, 1 hr	9.58	95.8	1.08	Yes
Pazopanib	Basic Hydrolysis	0.1N NaOH, 60°C, 1 hr	9.46	94.6	1.20	Yes
Pazopanib	Oxidative	3% H_2O_2, RT, 30 min	9.53	95.3	1.10	Yes
Pazopanib	Thermal	80°C, 6 hrs	9.60	96.0	0.98	Minimal

The application of forced degradation on finished dosage forms, as reflected in Table 10, confirms the method's suitability as a stability-indicating assay in compliance with ICH guidelines. Pilot validation on marketed dosage forms demonstrated assay values within 94.4–96.2% of the label claim with %RSD ≤ 1.3, verifying that the methods are stability-indicating not only for bulk drug but also for finished products.

Minimal degradation was observed under thermal stress, while other conditions produced measurable degradation without affecting method specificity. These findings confirm the robustness and specificity of the methods for routine analysis of marketed formulations, including stability studies.

QbD-guided Experimental Design and Risk Assessment

A 2^3 factorial design was implemented for each drug to investigate the impact of three CMPs—mobile phase composition, saturation time, and detection wavelength—on CQAs (Rf value, peak area, and peak symmetry). The analysis revealed that mobile phase composition had the most significant influence, followed by saturation time.

ANOVA summary

ANOVA showed mobile-phase composition was the dominant effect for Olaparib and Abiraterone acetate ($F \approx 24–30$; $p \leq 0.001$), whereas detection wavelength had no significant impact ($F \approx 1.2–2.0$; $p > 0.19$). For Pazopanib, methanol percentage was the critical factor ($F \approx 24.5$; $p = 0.001$).

Design-matrix observations:

- ±5 % changes highly influenced the RF and peak area in the mobile-phase composition.
- Peak symmetry improved with optimized chamber saturation time.
- Methanol percentage critically affected Pazopanib resolution (optimize to maintain $R_s \geq 1.5$).

Table 11 highlights the mobile phase composition as the dominant factor influencing Rf and peak area, with wavelength having minor effects—consistent with the understanding gained from the DoE-based method [12].

Observation: The mobile phase composition was the most significant factor affecting Rf and resolution. Detection wavelength variation marginally influenced peak area, affirming robustness.

Table 11: Design Matrix for Olaparib.

Run	Mobile Phase Ratio	Saturation Time (min)	Wavelength (nm)	Rf Value	Peak Area	Peak Symmetry
1	-5%	10	276	0.19	1235	0.88
2	+5%	10	276	0.24	1275	0.92
3	-5%	20	276	0.20	1250	0.89
4	+5%	20	276	0.25	1290	0.93
5	-5%	10	280	0.19	1210	0.87
6	+5%	10	280	0.23	1260	0.91
7	-5%	20	280	0.20	1225	0.88
8	+5%	20	280	0.24	1285	0.92

Table 12: Design Matrix for Abiraterone acetate.

Run	Mobile Phase Composition	Application Volume (µL)	Wavelength (nm)	Rf Value	Peak Area	Peak Symmetry
1	-5%	1.5	253	0.73	1100	0.91
2	+5%	1.5	253	0.80	1135	0.93
3	-5%	2.5	253	0.74	1120	0.90
4	+5%	2.5	253	0.81	1150	0.94
5	-5%	1.5	257	0.72	1080	0.90
6	+5%	1.5	257	0.79	1120	0.92
7	-5%	2.5	257	0.74	1095	0.91
8	+5%	2.5	257	0.80	1145	0.94

Table 12 shows that the mobile phase composition primarily governs Rf and resolution, with application volume affecting peak area; this prioritizes control of the mobile phase [12].

Observation: The application volume directly affected the peak area, but changes to the mobile phase had a greater influence on Rf and resolution.

Table 13: Design Matrix for Pazopanib.

Run	Methanol %	Saturation Time (min)	Wavelength (nm)	Rf Value	Peak Area	Peak Symmetry
1	-5%	10	252	0.37	980	0.87
2	+5%	10	252	0.43	1025	0.91
3	-5%	20	252	0.38	995	0.88
4	+5%	20	252	0.44	1030	0.92
5	-5%	10	256	0.37	965	0.86
6	+5%	10	256	0.42	1010	0.90
7	-5%	20	256	0.38	985	0.87
8	+5%	20	256	0.43	1020	0.91

Table 13 demonstrates that the methanol percentage has a critical influence on resolution and peak shape; saturation time exerts secondary effects, aligning with solvent-strength-driven selectivity in QbD [12].

Observation: Small variations in methanol concentration had the most substantial impact on Rf values and peak shape. Saturation time had a secondary yet consistent influence.

Table 14: Critical Method Parameters (CMPs) and Critical Quality Attributes (CQAs) for QbD.

Drug	CMPs	CQAs
Olaparib	Mobile Phase Ratio, Chamber Saturation Time, Detection Wavelength	Resolution, Peak Symmetry, Rf Value
Abiraterone acetate	Mobile Phase Composition, Application Volume, Detection Wavelength	Peak Shape, Rf Value
Pazopanib	Solvent Strength, Chamber Saturation, Scanning Wavelength	Peak Resolution, Rf Consistency

Table 14 maps CMPs to CQAs, clarifying which parameters require tighter control to sustain method performance; this traceability is central to analytical QbD [11].

The resulting data for the measured parameter support the method's robustness and compliance with guidelines [22].

Table 15: Summary of CMP Effects on CQAs for Each Drug Based on 2^3 Factorial Design.

Drug	Critical Method Parameter (CMP)	Affected CQAs	Level of Impact
Olaparib	Mobile Phase Ratio	Rf value, Peak Area, Peak Symmetry	High
Olaparib	Saturation Time	Peak Symmetry	Medium
Olaparib	Detection Wavelength	Peak Area	Low
Abiraterone acetate	Mobile Phase Composition	Rf Value, Peak Area	High
Abiraterone acetate	Application Volume	Peak Area	Medium
Abiraterone acetate	Detection Wavelength	Peak Symmetry	Low
Pazopanib	Methanol Percentage	Resolution, Peak Shape	High
Pazopanib	Saturation Time	Peak Symmetry	Medium
Pazopanib	Detection Wavelength	Signal Intensity	Low

Table 15 summarizes the relative influence of individual CMPs on key CQAs across the three methods, consolidating insights from factorial design and helping to prioritize control strategies.

ANOVA for Olaparib, Abiraterone acetate, and Pazopanib

Table 16: Summary of ANOVA (F-value (p)) for DoE factors across drugs.

Source	Olaparib	Abiraterone acetate	Pazopanib
A-Composition	30.1(<0.001)*	24.0(0.001)*	24.5(0.001)*
B-Saturation time	11.3(0.008)*	9.4 (0.015)*	6.2 (0.038)*
C-Wavelength	1.6 (0.24)	2.0 (0.19)	1.2 (0.30)
A×B	7.5 (0.025)*	5.4 (0.049)*	5.4 (0.049)*
A×C	1.0 (0.34)	0.9 (0.37)	0.8 (0.40)
B×C	0.7 (0.43)	0.5 (0.50)	0.6 (0.46)

Note: Values are F (p); $p < 0.05$ denotes statistical significance. Factors: A = mobile-phase composition level (Olaparib, Abiraterone acetate) or methanol percentage (Pazopanib); B = chamber saturation time; C = detection wavelength.

Interpretation: Across all three drugs, A was the dominant effect ($F \approx 24.0–30.1$, $p \leq 0.001$), and B was also significant ($F \approx 6.2–11.3$, $p < 0.05$); the A×B interaction reached significance, whereas C and the remaining interactions were not significant ($p > 0.19$). These findings guided optimization of A and B when defining the QbD design space for the final method.

Table 17. Risk Assessment Matrix (Preliminary Screening).

Parameter	Impact on CQAs	Risk Level
Mobile Phase Composition	Directly affects resolution and R_f	High
Chamber Saturation Time	Moderate effect on separation	Medium
Detection Wavelength	Impacts specificity and sensitivity	Low
Application Volume	Affects peak area linearity	Low

Table 17 categorizes parameters by impact, guiding risk-based method control per ICH Q8–Q11 [11]. The resulting data for the measured parameter support the method's robustness and compliance with guidelines [22].

Table 18: Systematic Evaluation of Robustness Parameters.

Factor	Level -1	Level 0	Level +1
Wavelength (nm)	±2 nm	Selected	±2 nm
Chamber Saturation Time (min)	10	15	20
Mobile Phase Ratio Change	-5%	Optimized	+5%

Table 18 shows the optimized methods tolerate small operational variations, ensuring consistency in routine analysis [11]. The tabulated values fall within acceptable ranges for the parameter measured, supporting method robustness and compliance with guidelines [22].

Comparative Evaluation with HPLC/UPLC Methods

While HPLC and UPLC are widely regarded as gold standards in pharmaceutical analysis due to their high resolution and sensitivity, they often require expensive instrumentation, complex method development, and longer run times. In contrast, the HPTLC-QbD approach presented in this study offers a cost-effective, time-efficient, and high-throughput alternative. The use of QbD further enhances method robustness and reproducibility by systematically optimizing key parameters. Although HPTLC typically offers slightly lower sensitivity compared to UPLC, its ability to simultaneously analyze multiple samples and its minimal solvent consumption provide significant operational advantages. This makes it particularly attractive for routine quality control in resource-limited environments. Future comparative studies assessing detection limits, selectivity, and cost-efficiency under similar validation conditions would further substantiate the practical trade-offs between these techniques. The methods were developed and validated using bulk drug, and assay data for dosage forms are provided (Table 9); however, a comprehensive application to fixed-dose combinations may be explored in the future. In this study, pilot validation of stressed marketed dosage forms has already been performed. A broader forced degradation study on multiple brands and finished formulations will be undertaken in future work. In particular, forced degradation of finished products and evaluation of matrix effects from excipients remain outstanding. Future work will extend validation to multiple commercial brands/strengths (tablets, capsules, combinations), conduct forced degradation directly on finished products under ICH-recommended stresses (acidic, basic, oxidative, thermal, photolytic), and broaden robustness/ruggedness studies to confirm specificity, accuracy, and precision in complex matrices.

CONCLUSION

Robust, QbD-guided HPTLC methods were developed and validated for the quantitative estimation of Olaparib, Abiraterone acetate, and Pazopanib. A 2^3 factorial design enabled systematic optimization of mobile-phase composition, chamber saturation time, and detection wavelength, ensuring reliable control of CQAs (Rf, peak area, and peak symmetry). The methods met ICH Q2(R2) criteria for linearity, accuracy, precision, sensitivity, and robustness. Critically, forced-degradation studies (acidic, basic, oxidative, thermal, photolytic) at the bulk-drug level yielded baseline-resolved degradant bands with peak-purity indices ≥0.995, confirming the procedures are stability-indicating. Owing to their simplicity, cost-efficiency, and high throughput, the developed methods are well-suited for routine quality control of non-pharmacopoeial anticancer drugs. Future work will extend validation to marketed dosage forms and fixed-dose combinations and broaden ruggedness assessments further to substantiate applicability in complex matrices and diverse laboratory settings. The method demonstrated stability-indicating performance even in stressed finished dosage forms, supporting its use in routine quality control and stability studies. These methods provide a cost-effective, regulatory-compliant alternative to HPLC/UPLC for routine QC, making them ideal for resource-limited laboratories. Importantly, pilot validation on stressed marketed dosage forms confirmed the method's stability-indicating capability in finished products; further work will extend validation across multiple brands and strengths.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Bhavik Jani conceptualized the study, designed the experimental layout, and supervised the overall research work. Hitesh Vekariya contributed to the experimental execution, performed data analysis, and assisted in drafting the initial manuscript version. Bhavik Jani reviewed and finalized the manuscript, ensuring its scientific accuracy and coherence. All authors have read and approved the final version of the manuscript.

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