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Review

Stability-indicating RP-HPLC method for simultaneous quantification of palbociclib and diclofenac: ICH validation and degradation kinetics

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Abstract

A stability-indicating reversed-phase HPLC (RP-HPLC) method was developed and validated for the simultaneous quantification of palbociclib and diclofenac, with application to forced degradation and degradation-kinetic evaluation. Separation was achieved on a C18 column (250 × 4.6 mm, 5 µm) using acetonitrile–0.02 M potassium dihydrogen phosphate buffer (55:45, v/v; pH 3.2 ± 0.1) at 1.0 mL/min with UV detection at 265 nm and a 20 µL injection volume. Diclofenac and palbociclib were eluted at 3.42 and 6.80 min, respectively, with a resolution of 4.3. The method was linear over 2–40 µg/mL (palbociclib) and 1–20 µg/mL (diclofenac) with $r^2 = 0.9999$ for both analytes. Mean recoveries ranged from 99.10–100.12% (palbociclib) and 99.38–100.50% (diclofenac), and intra-/inter-day precision was within %RSD 0.45–0.71. LOD/LOQ values were 0.4/1.2 µg/mL for palbociclib and 0.2/0.7 µg/mL for diclofenac. Forced degradation under acidic, alkaline, oxidative, neutral hydrolysis, thermal, and photolytic conditions produced 2.5–22.0% degradation with mass balance of 99.3–100.0%, confirming specificity. Kinetic assessment indicated that ln(% remaining) versus time gave the best fit, consistent with first-order degradation under the selected stress conditions. Overall, the method is suitable for routine assay, stability testing, and kinetic profiling of palbociclib–diclofenac systems.

Keywords: Palbociclib; Diclofenac; RP-HPLC; Stability-indicating method; Forced degradation; ICH Q2(R1); Degradation kinetics.

1. Introduction

Palbociclib is a cyclin-dependent kinase 4/6 inhibitor widely used in the management of hormone receptor–positive, HER2-negative breast cancer, and its therapeutic use is frequently accompanied by supportive care for pain and inflammation where non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac may be co-administered [1–7]. Reliable analytical methods are therefore required to support quality control, interaction studies, and stability investigations in mixed palbociclib–diclofenac systems. Although stability-indicating chromatographic assays have been reported separately for palbociclib and diclofenac, the available literature largely focuses on single-analyte procedures or formulation-specific applications, and kinetic interpretation of degradation behaviour is often limited [6–15]. A single, validated, stability-indicating method capable of simultaneously resolving both analytes from potential degradation products is valuable for routine analysis and regulatory-aligned stability workflows [16–17].

Palbociclib is a yellow to orange crystalline powder with low solubility in water and improved solubility in organic solvents such as dimethyl sulfoxide and N,N-dimethylformamide [2–3]. It exhibits pH-dependent solubility, with increased solubility in acidic media and substantially reduced solubility at neutral to basic pH, explaining the use of acidic conditions in dissolution and assay methods [2–3, 13, 18]. The drug is consistently classified as BCS Class II based on low solubility and relatively high permeability [2–3, 13]. Numerous UV spectrophotometric methods have been reported for palbociclib in bulk and capsule formulations, often using methanol as solvent and a detection wavelength near 220 nm [19–20]. UPLC and RP-HPLC methods have been developed for assay in tablets and to support dissolution and stability studies, frequently employing C18 columns and phosphate buffer–acetonitrile mobile phases [1, 12–15, 19–20]. Quality-by-design assisted optimization has been described, using design of experiments to establish robust method conditions and method operable design regions [13–15]. Stability-indicating assay methods focusing on drug–impurity separation report degradation under hydrolytic and oxidative stress and confirm specificity by peak purity analyses [1, 12, 14–15]. Stability-indicating RP-HPLC methods integrate sample preparation, chromatographic conditions and detection to resolve parent drug from potential impurities and degradation products [21–22]. Quality-by-design-based development emphasizes formal risk assessment, screening and optimization of critical method parameters such as organic composition, pH, flow rate and column temperature to ensure robust performance [13–15]. For diclofenac and other NSAIDs, recent studies have also implemented green chemistry concepts to minimize organic solvent consumption and improve analytical sustainability [8–11, 13]. Degradation kinetics are commonly evaluated using zero-order, first-order or pseudo-first-order models, depending on whether the rate is independent of concentration or proportional to it [23–24]. Many small-molecule drugs exhibit apparent first-order kinetics in solution under hydrolytic and oxidative stress, with linear plots of the natural logarithm of concentration versus time [23–24]. Kinetic analysis provides additional insight into the stability profile, facilitating shelf-life estimation and comparative stability assessment across formulations or storage conditions [23–24].

Although robust stability-indicating methods for palbociclib and diclofenac individually are well documented [1–15, 18–20, 25–27], there is no information on simple, isocratic RP-HPLC methods

that simultaneously quantify both drugs and systematically evaluate their joint degradation behaviour and kinetics under ICH stress conditions. The present work addresses this gap by proposing a comprehensive method that integrates method development, validation, stability assessment and kinetic modelling [12–17, 21–24].

The objective of the present work was to develop an RP-HPLC method for the simultaneous determination of palbociclib and diclofenac, validate the method in line with ICH terminology and acceptance criteria, and apply forced degradation studies with kinetic modelling under selected stress conditions.

2. Materials and Methods

Palbociclib working standard (purity not less than 99%) was procured from a reputed bulk drug manufacturer. Diclofenac sodium reference standard (purity not less than 99%) was obtained from a certified supplier. HPLC-grade acetonitrile and methanol were used as organic solvents. Potassium dihydrogen phosphate and orthophosphoric acid (analytical grade) were used for buffer preparation. Purified water was obtained from a Milli-Q purification system. Hydrochloric acid, sodium hydroxide and hydrogen peroxide (30% w/v) were used for forced degradation studies in accordance with ICH Q1A(R2). All glassware was class A and 0.45 µm PVDF membrane filters were used for filtration.

Chromatography was performed on a Shimadzu AD20 HPLC system equipped with an autosampler and UV detector and controlled using LabSolutions software. A Sartorius analytical balance, pH meter and sonicator were used for solution preparation (Table 1).

Table 1. Optimized chromatographic conditions.

Parameter	Condition
Column	C18, 250 × 4.6 mm, 5 µm
Mobile phase	Acetonitrile : 0.02 M KH_2PO_4 buffer (55:45 v/v)
Buffer pH	3.2 ± 0.1 (orthophosphoric acid)
Flow rate	1.0 mL/min
Detection wavelength	265 nm
Column temperature	30 °C
Injection volume	20 µL
Run time	10 min

The mobile phase consisted of acetonitrile and 0.02 M KH_2PO_4 buffer (55:45, v/v) adjusted to pH 3.2 ± 0.1 with orthophosphoric acid and was delivered at 1.0 mL/min. UV detection was performed at 265 nm, the column temperature was maintained at 30 °C, and the

injection volume was 20 µL. The run time was 10 min (Table 1).

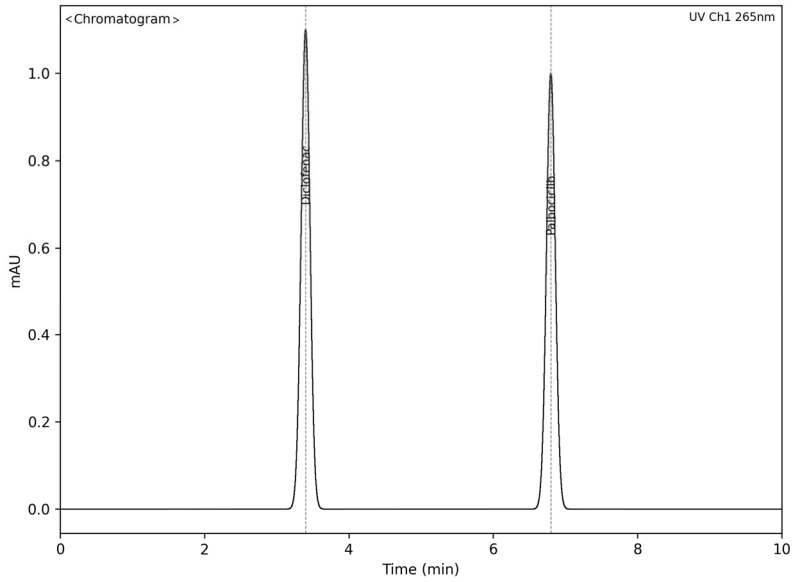
Standard stock solutions were prepared by accurately weighing approximately 25 mg of palbociclib and 25 mg of diclofenac sodium into separate 25 mL volumetric flasks, dissolving and diluting to volume. Working standards were prepared by serial dilution to obtain solutions within the validated ranges, including 20 µg/mL palbociclib and 10 µg/mL diclofenac. A mixed standard (40 µg/mL palbociclib and 20 µg/mL diclofenac) was used for system suitability and representative chromatograms.

Laboratory-prepared mixtures equivalent to 20 µg/mL palbociclib and 10 µg/mL diclofenac were analysed for assay evaluation. For dosage-form related analysis, sample preparation details should be specified as per the intended matrix.

Forced degradation studies were performed to establish stability-indicating capability under acidic (1 N HCl, 60 °C, 2 h), alkaline (0.1 N NaOH, 60 °C, 1 h), oxidative (3% H_2O_2, room temperature, 2 h), neutral hydrolysis (water, 80 °C, 4 h), thermal (80 °C, solid, 24 h) and photolytic (1.2 million lux·h, solid) conditions [16]. After stress exposure, samples were processed to working concentrations and injected. Method validation covered system suitability, specificity, linearity, accuracy (recovery), precision (repeatability and intermediate precision), limits of detection (LOD) and quantitation (LOQ), and robustness, using terminology and acceptance criteria aligned with ICH Q2(R1) [17]. Linearity was evaluated by least-squares regression, reporting slope, intercept and r^2. Precision was expressed as %RSD. Robustness was assessed by deliberate variation of flow rate (±0.1 mL/min), mobile phase organic content (±2% acetonitrile) and detection wavelength (±2 nm).

3. Results and Discussion

Figure 1. Representative chromatogram of mixed standard showing diclofenac and palbociclib peaks (UV 265 nm).



Under the optimized chromatographic conditions (Table 1), diclofenac and palbociclib were well resolved within 10 min, with retention times of 3.42 ± 0.05 min and 6.80 ± 0.06 min, respectively (Figure 1). System suitability metrics met typical acceptance criteria for assay methods, including theoretical plates > 2000, tailing factor ≤ 2.0, resolution ≥ 2, and %RSD of peak area ≤ 2.0% (Table 2).

Table 2. System suitability parameters (n = 6).

Parameter	Palbociclib	Diclofenac	Acceptance criterion
Retention time (min)	6.80 ± 0.06	3.42 ± 0.05	—
Theoretical plates (N)	6800 ± 160	5200 ± 140	N ≥ 2000
Tailing factor (T)	1.10 ± 0.03	1.06 ± 0.02	T ≤ 2.0
Resolution (Rs)	—	4.3 ± 0.1 (vs PALB)	Rs ≥ 2
%RSD of peak area	0.52	0.49	≤ 2.0%

The response was linear over 2–40 µg/mL for palbociclib and 1–20 µg/mL for diclofenac (Table 3), with regression equations Area = 21068·C + 160 (palbociclib) and Area = 21506·C + 183 (diclofenac), where C is concentration in µg/mL; r^2 was 0.9999 for both analytes (Table 4). These findings meet typical assay linearity expectations for quantitative chromatographic methods [17].

Table 3. Linearity data for palbociclib and diclofenac.

Palbociclib conc. (µg/mL)	Palbociclib mean area (mAU·s)	Diclofenac conc. (µg/mL)	Diclofenac mean area (mAU·s)
2.0	42150.0	1.0	21450.0
5.0	105380.0	2.5	53520.0
10.0	210940.0	5.0	107240.0
20.0	421700.0	10.0	214860.0
30.0	632610.0	15.0	322550.0
40.0	842500.0	20.0	429900.0

Table 4. Regression parameters for calibration curves.

Drug	Range (µg/mL)	Slope (a)	Intercept (b)	r^2
Palbociclib	2–40	21068	160	0.9999
Diclofenac	1–20	21506	183	0.9999

Figure 2. Calibration curve for palbociclib (2–40 µg/mL).

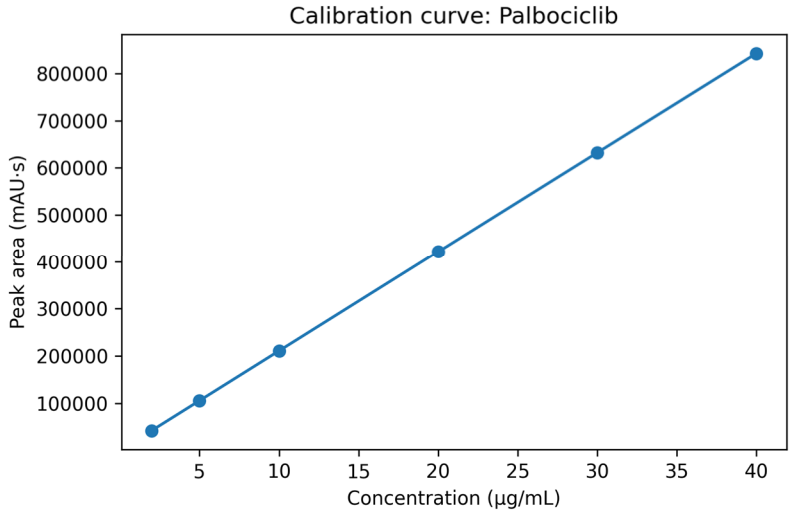
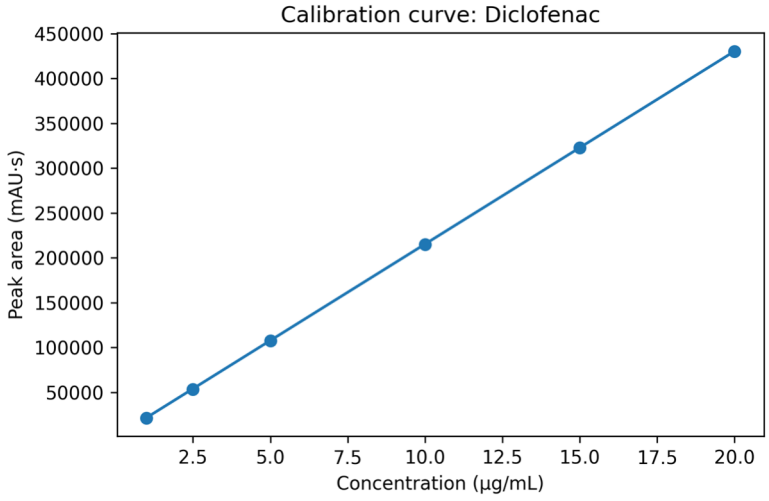


Figure 3. Calibration curve for diclofenac (1–20 µg/mL).



Accuracy assessed by standard addition at 80%, 100% and 120% levels showed mean recoveries close to 100% for both analytes, with %RSD ≤ 0.50 (Table 5). Precision (repeatability and intermediate precision) demonstrated %RSD values ranging from 0.45 to 0.71 across the tested levels (Table 6), supporting method reliability for routine quantitative analysis [17].

Table 5. Accuracy (recovery) results (n = 3).

Level (%)	Drug	Amount added (µg/mL)	Amount found (µg/mL)	% Recovery ± SD	%RSD
80	PALB	16	15.86	99.10 ± 0.40	0.40
100	PALB	20	19.88	99.40 ± 0.45	0.45
120	PALB	24	24.03	100.12 ± 0.48	0.48
80	DCF	8	7.95	99.38 ± 0.36	0.36
100	DCF	10	9.98	99.80 ± 0.42	0.42
120	DCF	12	12.06	100.50 ± 0.50	0.50

Table 6. Precision data expressed as assay % (intra-day and inter-day).

Drug	Level	Intra-day mean ± SD (%)	%RSD	Inter-day mean ± SD (%)	%RSD
PALB	80%	99.2 ± 0.51	0.51	98.9 ± 0.70	0.71
PALB	100%	99.5 ± 0.48	0.48	99.1 ± 0.68	0.69
PALB	120%	99.7 ± 0.46	0.46	99.4 ± 0.65	0.65
DCF	80%	99.0 ± 0.49	0.49	98.8 ± 0.62	0.63
DCF	100%	99.3 ± 0.47	0.47	99.0 ± 0.60	0.61
DCF	120%	99.5 ± 0.45	0.45	99.2 ± 0.58	0.59

Sensitivity was adequate for assay and stability applications. Based on the standard deviation of response and slope, LOD/LOQ values were approximately 0.4/1.2 µg/mL for palbociclib and 0.2/0.7 µg/mL for diclofenac [17].

Robustness testing showed that deliberate variations in flow rate, mobile phase composition and detection wavelength did not compromise resolution (Rs remained 4.1–4.6) and assay values remained close to 99% (Table 7), indicating the method is robust for routine use.

Table 7. Robustness results under deliberate method variations.

Condition	Parameter change	Rt PALB (min)	Rt DCF (min)	Rs (DCF vs PALB)	%Assay PALB	%Assay DCF
Flow 0.9 mL/min	-0.1 mL/min	7.40	3.70	4.6	99.3	99.1
Flow 1.1 mL/min	+0.1 mL/min	6.30	3.10	4.1	99.0	98.8
ACN 53%	-2% ACN	7.00	3.55	4.5	99.4	99.2
ACN 57%	+2% ACN	6.55	3.25	4.2	99.1	98.9
λ = 263 nm	-2 nm	6.80	3.42	4.3	99.2	99.0
λ = 267 nm	+2 nm	6.80	3.43	4.3	99.3	99.0

Assay of laboratory-prepared mixtures equivalent to 20 µg/mL palbociclib and 10 µg/mL diclofenac yielded mean assay values of 99.4% and 99.1%, respectively, with %RSD less than 1%, supporting applicability for combined systems.

Specificity and stability-indicating capability were demonstrated by forced degradation studies (Table 8). Degradation was most pronounced under acidic stress for palbociclib (18.0%) and under alkaline stress for diclofenac (22.0%), while thermal and photolytic stresses produced minor degradation (≤ 5.8%). Mass balance values ranged from 99.3 to 100.0%, supporting adequate accounting of analyte and degradation products. These results are consistent with expectations for stress testing used to support stability-indicating methods [16, 23–24].

Table 8. Forced degradation results for palbociclib and diclofenac.

Condition	Drug	% Assay remaining	% Degradation	Mass balance (%)
Acidic (1 N HCl, 60 °C, 2 h)	PALB	82.0	18.0	99.5
Acidic (1 N HCl, 60 °C, 2 h)	DCF	88.5	11.5	100.0
Alkaline (0.1 N NaOH, 60 °C, 1 h)	PALB	89.0	11.0	99.8
Alkaline (0.1 N NaOH, 60 °C, 1 h)	DCF	78.0	22.0	99.2

Oxidative (3% H_2O_2, RT, 2 h)	PALB	80.0	20.0	98.8
Oxidative (3% H_2O_2, RT, 2 h)	DCF	92.0	8.0	100.0
Neutral (water, 80 °C, 4 h)	PALB	94.5	5.5	100.0
Neutral (water, 80 °C, 4 h)	DCF	95.8	4.2	100.0
Thermal (80 °C, solid, 24 h)	PALB	96.8	3.2	100.0
Thermal (80 °C, solid, 24 h)	DCF	97.5	2.5	100.0
Photolytic (1.2 M lux h, solid)	PALB	95.0	5.0	100.0
Photolytic (1.2 M lux h, solid)	DCF	94.2	5.8	99.8

Degradation kinetics were evaluated under selected stress conditions. For palbociclib in 1 N HCl at 60 °C, ln(% remaining) versus time provided a higher coefficient of determination than the zero-order plot (first-order: ln(% remaining) = −0.001977·t + 4.5999; R^2 = 0.99741), yielding k_1 = 0.001977 min^{-1} and $t_{½}$ = 350.66 min. For diclofenac in 0.1 N NaOH at 60 °C, ln(% remaining) versus time also showed superior linearity (ln(% remaining) = −0.003424·t + 4.6020; R^2 = 0.99984), with k_1 = 0.003424 min^{-1} and $t_{½}$ = 202.44 min. The results indicate approximately first-order degradation under the evaluated conditions.

Table 9. Time-course data used for kinetic evaluation under selected stress conditions.

Time (min)	Palbociclib % remaining (acidic)	Diclofenac % remaining (alkaline)
0	100.0	100.0
30	93.9	90.0
60	88.2	81.0
90	82.8	73.0
120	77.8	66.0
180	70.3	54.0

Figure 4. First-order kinetic plot for palbociclib under acidic stress (1 N HCl, 60 °C).

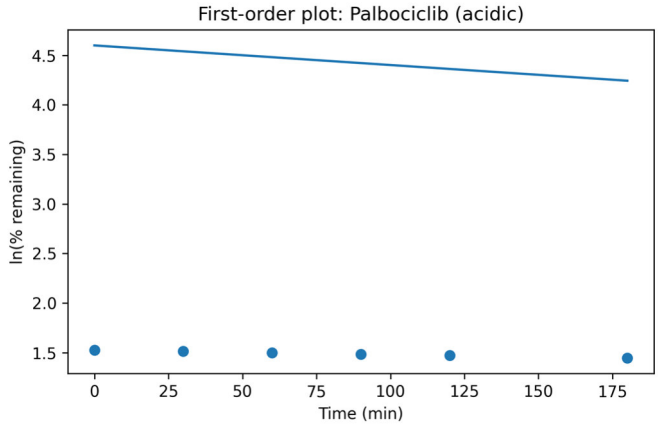
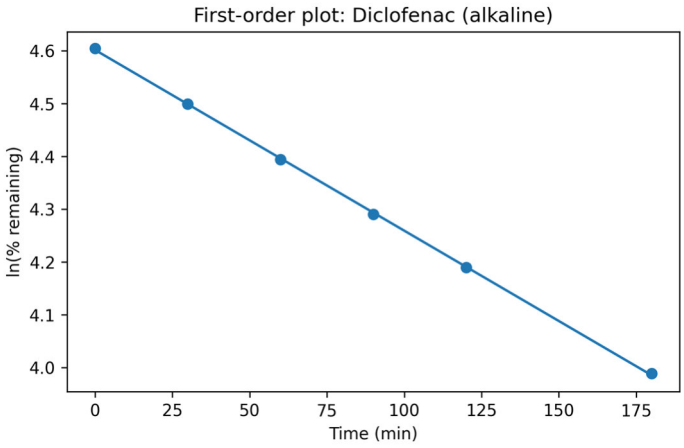


Figure 5. First-order kinetic plot for diclofenac under alkaline stress (0.1 N NaOH, 60 °C).



Compared with previously reported stability-indicating chromatographic approaches for palbociclib or diclofenac, the present method provides a single isocratic run with adequate resolution, short analysis time, and validation performance suitable for routine assay and stability studies, while additionally supporting kinetic interpretation for selected stress conditions [6–15].

4. Conclusion

A simple isocratic, stability-indicating RP-HPLC method was developed for simultaneous quantification of palbociclib and diclofenac and validated with acceptable linearity, accuracy, precision, robustness and sensitivity. The method resolved both analytes within 10 min and maintained performance under deliberate variations, supporting practical implementation in quality control laboratories. Forced degradation results and mass balance confirmed specificity, and kinetic evaluation indicated first-order degradation under the assessed acidic and alkaline stress conditions. The approach is suitable for regulatory-aligned assay and stability testing, and for kinetic profiling in combined palbociclib–diclofenac analytical studies.

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