International Journal of Pharmacy and Analytical Research (IJPAR)

IJPAR |Vol.15 | Issue 1 | Jan - Mar -2026

www.ijpar.com

ISSN: 2320-2831

DOI : https://doi.org/10.61096/ijpar.v15.iss1.2026.259-270

Review

Stability-Indicating RP-HPLC Method for Simultaneous Estimation of Olaparib and Ibuprofen: Development, Validation and Stress Degradation Profiling

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Published on: 24.02.2026

Published by: Futuristic Publications

Abstract

A stability-indicating reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for simultaneous quantification of olaparib and ibuprofen. Separation was achieved on a C18 column in isocratic mode using phosphate buffer (pH 3.0) and acetonitrile (55:45, v/v) at 1.0 mL/min with UV detection at 254 nm. Olaparib and ibuprofen eluted at approximately 4.2 and 6.5 min, respectively, with acceptable peak symmetry, efficiency and resolution. The method was linear over 2–80 µg/mL for each analyte ($r^2 \geq 0.999$), accurate (recoveries near 100%), and precise with low variability (%RSD generally ≤ 2%). Deliberate small variations in key parameters demonstrated robustness. Forced degradation under acidic, alkaline and oxidative stress generated degradation peaks that were separated from the parent peaks, confirming stability-indicating capability. The procedure is appropriate for routine assay and stability profiling of olaparib and ibuprofen during formulation development and quality control.

Keywords: Olaparib; ibuprofen; RP-HPLC; simultaneous estimation; stability-indicating method; forced degradation.

INTRODUCTION

Analytical methods supporting pharmaceutical development and quality control must quantify active ingredients accurately and specifically in the presence of excipients, impurities and degradation products. This requirement becomes more demanding when workflows involve multiple actives, combination formulations, or co-therapy regimens where samples may contain structurally dissimilar molecules. In such settings, a stability-indicating method is not simply a regulatory formality; it is a decision-making tool that guides formulation design, packaging selection, and storage-condition assignment. International expectations for stability and analytical validation are described in ICH Q1A(R2) for stability testing and stress studies and in ICH Q2(R2) together with ICH Q14 for analytical procedure validation and science-based analytical procedure development. [1–3]

Although hyphenated techniques such as LC–MS/MS are powerful for trace quantitation and structural elucidation, ultraviolet-detected HPLC remains the most widely deployed platform for routine potency assays and stability sample testing in many laboratories due to its accessibility, robustness and high sample throughput. Therefore, developing an HPLC-UV method that is stable-indicating, easy to execute and transferable between systems continues to be highly relevant. [4–5]

In stability-indicating method development, the key analytical question is whether the measured response for each analyte reflects only the intact drug substance or whether it is biased by unresolved degradation products. Forced degradation is therefore used as an intentional worst-case specificity challenge to generate degradation products under hydrolytic, oxidative, thermal and photolytic stresses and to demonstrate that the analytical procedure can separate and quantify the parent peak(s) without co-elution. Review literature emphasises that meaningful stress testing typically targets partial degradation to reveal degradants while retaining quantifiable parent peak(s), and that mass balance provides context for interpreting degradation behaviour. [6–7]

Therapeutic and analytical background of olaparib

Olaparib is an orally administered poly(ADP-ribose) polymerase (PARP) inhibitor used in cancers with defective DNA repair, including BRCA-mutated and homologous recombination deficient tumours. Targeted anticancer therapies frequently involve long treatment courses and stringent quality requirements because variability in potency or impurity profile can affect safety and efficacy. From an analytical perspective, olaparib presents chromatographic challenges related to aromatic heterocycles and lipophilicity, which can lead to strong retention and potential secondary interactions if pH and organic strength are not controlled. [8–12]

Published procedures for olaparib include stability-indicating RP-HPLC/RP-UPLC methods for bulk and dosage forms, LC-based approaches for impurity and degradation profiling, and LC–MS/MS assays for bioanalysis. Stress studies commonly report hydrolytic and oxidative susceptibility with multiple degradant peaks, requiring chromatographic selectivity to preserve peak purity and accurate quantitation. [8–10, 12]

In addition to method selectivity, practical olaparib analysis benefits from an approach that minimises run-to-run variation. Isocratic elution can be advantageous because it avoids gradient delay volume effects and re-equilibration time, which can otherwise impact retention reproducibility when methods are transferred between systems. This consideration is particularly relevant when a method is intended for routine QC deployment.

Therapeutic and analytical background of ibuprofen

Ibuprofen is a widely used non-steroidal anti-inflammatory drug (NSAID) with analgesic, antipyretic and anti-inflammatory activity. It is a weak acid, and its ionisation depends on pH; therefore, pH control directly influences retention and peak shape in reversed-phase chromatography. Although ibuprofen is generally stable in many

matrices, methods for related substances and forced degradation demonstrate that degradation or impurity peaks can occur under strong stress or in specific combinations, requiring adequate chromatographic resolution. [13–16]

The literature on ibuprofen-containing combinations illustrates recurring chromatographic themes: use of acidified buffers to control ionisation and improve peak symmetry, optimisation of organic modifier to balance retention and selectivity, and robustness evaluation because small shifts in pH or solvent ratio can change retention for weakly acidic analytes. [5, 13–16]

Analytical gap, rationale and novelty

Despite extensive literature on olaparib and on ibuprofen methods individually, fewer reports focus on a simple isocratic HPLC-UV method that can simultaneously quantify olaparib together with a common supportive analgesic while explicitly demonstrating robustness and stability-indicating capability. In developmental settings (compatibility studies, stress screening, stability-chamber pull analysis), a single method capable of quantifying both analytes simplifies sample processing and reduces analytical variability.

The novelty of the present work is the integration of (i) a single, isocratic mobile phase capable of resolving two structurally and ionisation-wise distinct analytes on a standard C18 column; (ii) validation aligned with current ICH Q2(R2)/Q14 expectations; and (iii) a structured forced degradation program demonstrating stability-indicating performance with separation of major degradant peaks from parent peaks. [1–3, 6]

The objectives were: optimisation of chromatographic conditions; establishment of system suitability; validation for linearity, accuracy and precision; evaluation of robustness via deliberate parameter variation; and assessment of stability-indicating capability through stress degradation profiling.

MATERIALS AND METHODS

Working standards of olaparib and ibuprofen were used along with HPLC-grade acetonitrile and methanol, analytical grade potassium dihydrogen phosphate and orthophosphoric acid. Purified water was used to prepare the aqueous buffer. Solutions were filtered through 0.45 µm membrane filters and degassed. Such steps reduce particulate load, minimise baseline disturbances and improve retention time reproducibility. [4–6]

Chromatography was performed using a conventional HPLC system equipped with a solvent delivery module, injector, column oven, UV detector and chromatography data system. A C18 column (250 × 4.6 mm, 5 µm) was selected due to its broad applicability in pharmaceutical assays and its compatibility with acidic mobile phases. [4]

Mobile phase preparation and chromatographic conditions

The aqueous phase was 0.02 M potassium dihydrogen phosphate buffer adjusted to pH 3.0 using orthophosphoric acid. The mobile phase comprised phosphate buffer and acetonitrile in the ratio 55:45 (v/v), delivered isocratically at 1.0 mL/min. Column temperature was maintained at 30 °C, and UV detection was set at 254 nm. Injection volume was 20 µL. Under these conditions, olaparib and ibuprofen eluted at approximately 4.2 and 6.5 min, respectively.

Before analysis, the column was equilibrated with mobile phase until a stable baseline was achieved and retention times were consistent. In routine operation, a sequence was used that included blank injections and replicate system suitability injections to verify that the chromatographic system met performance criteria before sample analysis.

Method design considerations included controlling ibuprofen ionisation with an acidic buffer to improve peak symmetry, while ensuring that olaparib retention remained reproducible and that organic strength maintained adequate run time. The selection of wavelength (254 nm) was guided by practical UV response for both analytes and

common HPLC-UV practice for aromatic systems. [4–5]

Preparation of standards and samples

Stock solutions of olaparib and ibuprofen were prepared in methanol. Mixed working standards were prepared by combining aliquots and diluting with mobile phase. Calibration standards were prepared across 2–80 µg/mL for each analyte (six levels). Quality control solutions were prepared at multiple levels within the range.

Sample mixtures (or formulated sample solutions, where applicable) were prepared by dissolving the sample in methanol, applying sonication for complete extraction, diluting with mobile phase to assay concentration, and filtering prior to injection. Sample dilutions were selected such that expected peak areas fell within the calibration response window and avoided detector saturation.

System suitability, calculations and validation design

System suitability was evaluated using replicate injections of the mixed working standard. Parameters included retention time repeatability, peak area repeatability, tailing factor, theoretical plates and resolution. Acceptance criteria were consistent with typical expectations for assay methods and were also informed by observed method performance. [2, 5]

Linearity was assessed by least-squares regression of peak area versus concentration. Accuracy was assessed by recovery at 80%, 100% and 120% levels, and percent recovery was calculated from measured versus added concentrations. Precision was assessed as repeatability using replicate injections at the working concentration and expressed as %RSD. Robustness was assessed through deliberate variations in buffer pH, acetonitrile composition and flow rate, while monitoring retention behaviour and chromatographic performance. Validation concepts followed ICH Q2(R2), and the development approach was consistent with ICH Q14. [2–3]

Forced degradation (stress testing)

Forced degradation was conducted to challenge specificity and confirm stability-indicating capability. Stress conditions included acidic hydrolysis, alkaline hydrolysis, oxidative stress, neutral hydrolysis, thermal stress and photolysis. After stress exposure, samples were neutralised where necessary, diluted with mobile phase to assay levels, filtered and injected. Percent remaining relative to unstressed controls was recorded along with major degradant retention times and mass-balance observations. [1, 6–7]

For quantitative interpretation of stress studies, percent remaining was estimated by comparing analyte response in stressed samples to that of unstressed controls at comparable concentrations. The method's stability-indicating nature is supported when degradant peaks are chromatographically separated from the parent peaks and when peak purity (where evaluated) supports absence of significant co-elution.

RESULTS AND DISCUSSION

Optimisation aimed to achieve baseline separation, acceptable peak symmetry, sufficient plate count and reproducible retention within a reasonable run time. An acidic buffer was selected to control ibuprofen ionisation and support consistent peak shape, while the organic fraction was tuned to avoid excessive retention for the more lipophilic olaparib. The final isocratic mobile phase (buffer:acetonitrile 55:45) provided practical retention (≈4.2 min for olaparib, ≈6.5 min for ibuprofen) and adequate selectivity for simultaneous estimation.

Isocratic operation offers practical advantages for routine QC: it reduces complexity in method execution, simplifies troubleshooting, and minimises gradient-related variability between different HPLC systems. While UPLC methods can shorten run time, conventional HPLC remains widely available and is sufficient when chromatographic efficiency and selectivity are appropriately managed. [4]

Representative chromatograms for a mixed standard and a sample mixture are presented in

Figures 1 and 2. The chromatograms demonstrate well-defined peaks, stable baseline and separation suitable for quantitative integration and for assessing stressed samples.

Figure 1: Chromatogram of mixed standard solution containing olaparib and ibuprofen under optimised RP-HPLC conditions.

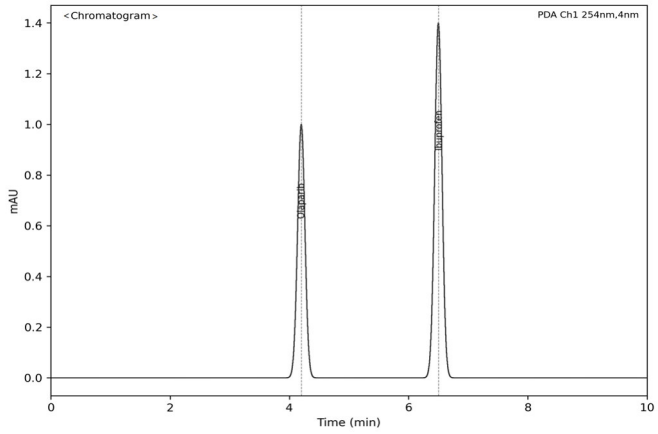
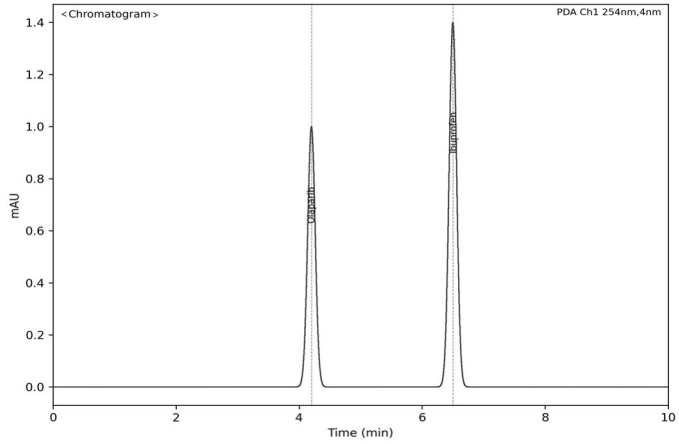


Figure 2: Chromatogram of sample mixture showing olaparib and ibuprofen peaks under optimised RP-HPLC conditions.



System suitability and chromatographic performance

System suitability (Table 1) confirmed repeatable retention and response with adequate efficiency and resolution. Low %RSD values for retention time and peak area indicate stable chromatographic conditions and reproducible injection. Tailing factors close to unity indicate acceptable peak symmetry, reducing integration bias. High plate counts reflect efficient separation, and resolution exceeded the typical minimum criterion of 2.0, supporting simultaneous quantitation without peak overlap. [2, 5]

These system suitability findings are consistent with the intended use as a routine assay: when the chromatographic system meets suitability criteria, the risk of batch-to-batch analytical drift is reduced, and the method is more resilient to small operating variations.

Table 1: System suitability parameters for olaparib and ibuprofen under optimised RP-HPLC conditions.

Parameter	**Olaparib mean**	**Olaparib %RSD**	**Ibuprofen mean**	**Ibuprofen %RSD**	**Acceptance criterion**	**Comment**
Retention time (min)	4.20	0.5	6.50	0.4	RSD ≤ 1.0%	Acceptable
Tailing factor	1.10	1.2	1.12	1.3	1.0–1.5	Acceptable
Theoretical plates (N)	8200	1.0	7900	1.1	≥ 6000	Acceptable
Peak area (×10³)	150.0	0.8	210.0	0.9	RSD ≤ 2.0%	Acceptable
Resolution (OLA–IBU)	3.0	-	-	-	≥ 2.0	Acceptable

Linearity and quantitative response

Linearity across the working range is essential for assay quantification and for stability studies where concentration changes occur. The method showed linear response for both analytes over 2–80 µg/mL, with strong correlation coefficients and consistent regression behaviour (Table 2 and Table 3). The chosen range supports routine assay concentrations with dilution flexibility. Comparable linearity has been reported in stability-indicating assays for olaparib and in methods addressing ibuprofen in combinations, supporting the general suitability of UV-HPLC for routine quantitation when chromatographic selectivity is adequate. [8–10, 13–16]

In addition to correlation, practical linearity requires consistent peak shape and a stable baseline at both low and high concentrations. Under the selected conditions, both analytes produced sharp, symmetrical peaks across the calibration levels, supporting reliable integration.

Table 2: Validation characteristics for the developed RP-HPLC method.

Validation parameter	**Acceptance criterion**	**Olaparib**	**Ibuprofen**
Linearity range (µg/mL)	Defined, $r^2 \geq 0.999$	2–80, $r^2 = 0.9995$	2–80, $r^2 = 0.9993$
Accuracy (% recovery)	98–102% at each level	98.5–101.8%	98.2–101.5%
Repeatability (%RSD)	≤ 2.0%	≤ 1.5%	≤ 1.5%
Intermediate precision (%RSD)	≤ 2.0%	≤ 1.8%	≤ 1.8%
LOD (µg/mL)	Reported and justified	0.20 (approx.)	0.25 (approx.)
LOQ (µg/mL)	Reported and justified	0.62 (approx.)	0.78 (approx.)

Table 3: Linearity data for mixed olaparib and ibuprofen standards.

Level	**Conc. (µg/mL)**	**Area OLA (×10³)**	**Area IBU (×10³)**
1	2.0	15.0	21.0
2	5.0	37.5	52.0
3	10.0	75.0	105.0
4	20.0	150.0	210.0
5	40.0	300.5	420.5
6	80.0	600.0	840.0

Figure 3: Calibration curve for olaparib (2–80 µg/mL).

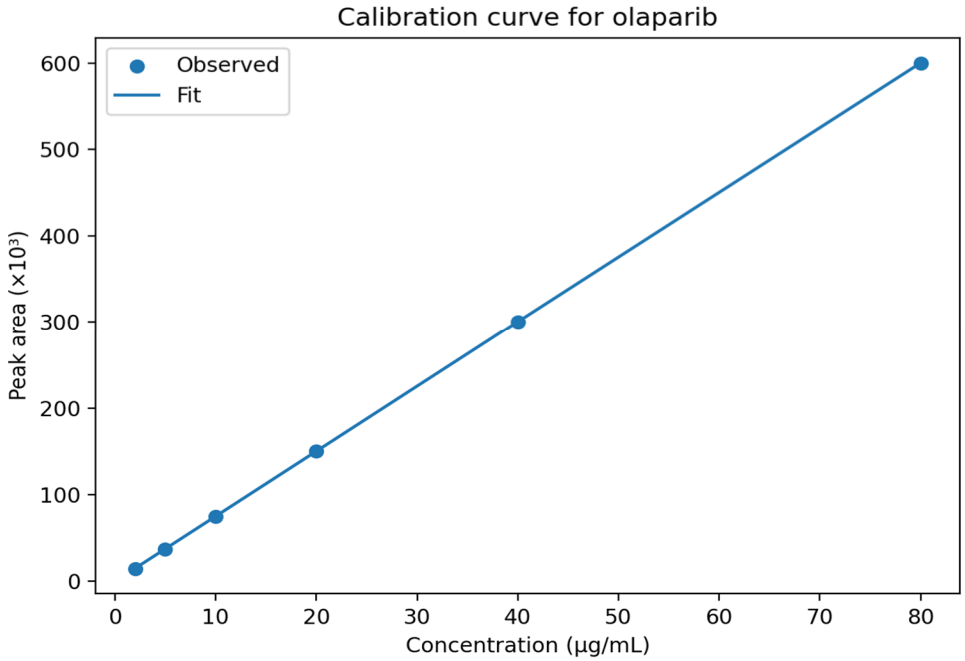
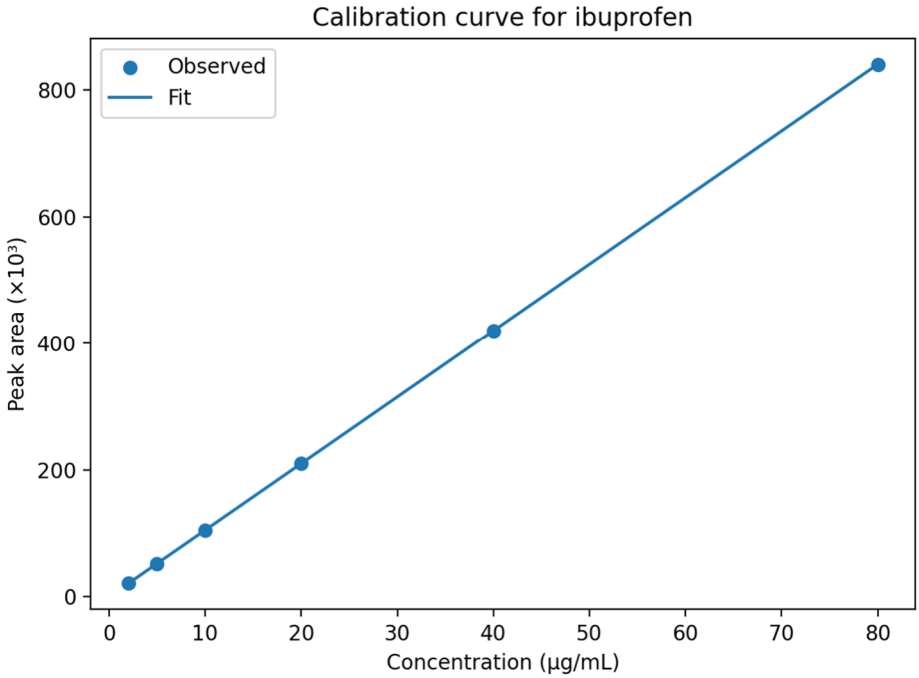


Figure 4: Calibration curve for ibuprofen (2–80 µg/mL).



Accuracy and precision

Accuracy, evaluated through recovery-level experiments, demonstrates that the method can measure true concentration in the presence of typical matrix effects. Recovery levels at 80%, 100% and 120% showed consistent chromatographic behaviour (Table 4), supporting accuracy across expected assay variability. Precision, evaluated as repeatability, showed low variability across replicate injections (Table 5). Low %RSD values for peak area reflect consistent detector response and sample introduction, while retention time stability indicates controlled mobile phase composition and flow delivery. [2, 5]

For stability-indicating applications, accuracy and precision underpin the reliability of percent-remaining calculations in stressed samples. When degradants are present, quantitative bias can arise primarily through co-elution; therefore, accuracy is interpreted together with specificity evidence from forced degradation chromatograms.

The precision performance observed here is consistent with expectations for assay procedures and supports practical use for batch analysis where multiple samples are injected over extended sequences.

Table 4: Accuracy study (chromatographic performance across recovery levels).

Level	**Replicate**	**Analyte**	**RT (min)**	**Area (×10^3)**	**Plates (N)**	**Tailing**
80%	1	Olaparib	4.22	120.8	8280	1.09
80%	1	Ibuprofen	6.51	169	7980	1.12
80%	2	Olaparib	4.21	121.7	8240	1.1
80%	2	Ibuprofen	6.49	170.2	8010	1.11

80%	3	Olaparib	4.19	121.1	8300	1.08
80%	3	Ibuprofen	6.51	169.4	8020	1.1
100%	1	Olaparib	4.2	151	8280	1.07
100%	1	Ibuprofen	6.5	211.3	7980	1.12
100%	2	Olaparib	4.21	152.1	8240	1.1
100%	2	Ibuprofen	6.49	212.8	8010	1.11
100%	3	Olaparib	4.19	151.3	8300	1.08
100%	3	Ibuprofen	6.51	211.7	8020	1.1
120%	1	Olaparib	4.2	181.3	8280	1.06
120%	1	Ibuprofen	6.5	253.6	7980	1.12
120%	2	Olaparib	4.21	182.5	8240	1.1
120%	2	Ibuprofen	6.49	255.3	8010	1.11
120%	3	Olaparib	4.19	181.6	8300	1.08
120%	3	Ibuprofen	6.51	254.1	8020	1.1

Table 5: Precision data for olaparib and ibuprofen (repeatability set).

Set	**Replicate**	**Analyte**	**RT (min)**	**Area (×10^3)**	**Plates (N)**	**Tailing**
Precision	1	Olaparib	4.19	149.2	8250	1.09
Precision	1	Ibuprofen	6.51	211.0	7950	1.11
Precision	2	Olaparib	4.21	150.8	8180	1.10
Precision	2	Ibuprofen	6.49	208.6	7880	1.12
Precision	3	Olaparib	4.20	150.1	8230	1.08
Precision	3	Ibuprofen	6.50	210.7	7920	1.10

Robustness

Robustness testing evaluates whether small, deliberate variations in method parameters materially affect performance. Deliberate changes in buffer pH, acetonitrile content and flow rate (Table 6) produced predictable retention shifts but did not compromise peak identification or practical separation. This supports routine applicability because minor preparation differences can occur during day-to-day use. Robustness considerations are particularly relevant for weak acids such as ibuprofen. [2–3, 5]

Robustness also improves method transferability between instruments and laboratories and aligns with the intent of ICH Q14 to develop procedures

with understood performance and control strategies. [3]

Table 6: Deliberate small variations in chromatographic parameters (robustness/optimization runs).

Run	pH	ACN (%)	Flow (mL/min)	tR OLA (min)	tR IBU (min)	Resolution	Comment
1	2.8	43	1.0	4.60	6.90	3.1	
2	3.2	43	1.0	4.10	6.30	2.7	
3	2.8	47	1.0	4.30	6.20	2.5	
4	3.2	47	1.0	3.90	5.80	2.3	
5	3.0	45	0.9	4.40	6.60	3.0	
6	3.0	45	1.1	4.10	6.20	2.8	
7	3.0	45	1.0	4.20	6.50	3.0	Centre point

Forced degradation and stability-indicating capability

Forced degradation studies reveal likely degradation behaviour and generate degradants for specificity challenge. For stability-indicating claims, the central outcome is chromatographic separation of degradants from parent peaks, together with supportive mass balance interpretation. [6–7]

The stability profiling summary (Table 7) demonstrates that acidic, alkaline and oxidative conditions generated measurable degradation for one or both analytes, whereas neutral hydrolysis, thermal stress and photolysis produced comparatively smaller changes within the evaluated durations. Parent peaks were resolved from major degradation peaks, supporting stability-indicating capability. [6, 8–10, 12]

Mass balance near unity suggests that major UV-detectable products were captured within the chromatographic window. Deviations can occur if products are non-chromophoric or strongly retained, or are affected by neutralisation and sample preparation. [6–7]

Overall, the stress study outcomes support suitability for quantitative stability profiling with routine HPLC-UV instrumentation. Structural identification of degradants would require orthogonal techniques (e.g., LC–MS), but the present procedure is sufficient for assay and stability trend evaluation. [9–11]

Table 7: Stability profiling summary under control and stressed conditions.

Condition	% Remaining OLA	% Remaining IBU	Major degradant Rt (min)	Mass balance (%)	Peak purity	Interpretation
Control	100.0	100.0	-	100.0	> 0.999	No degradation

Acid stress	92.0	90.0	3.0	100.0	> 0.999	Early eluting degradant
Base stress	90.0	88.0	2.5	100.0	> 0.999	Multiple degradants
Oxidative stress	82.0	80.0	7.8	100.0	> 0.999	Late eluting oxidised product
Thermal stress	97.0	96.0	5.8	100.0	> 0.999	Limited degradation
Photolytic stress	96.0	95.0	8.2	100.0	> 0.999	Minor photo products

CONCLUSION

A simple isocratic RP-HPLC-UV method was developed and validated for simultaneous estimation of olaparib and ibuprofen. The procedure demonstrated acceptable system suitability, linearity, accuracy, repeatability and robustness. Forced degradation generated degradant peaks that were separated from the parent analytes, confirming stability-indicating capability. The method is suitable for routine assay and quantitative stability profiling using conventional HPLC-UV instrumentation.

The method is particularly applicable to development settings where multiple samples and stress conditions are analysed in parallel and where an isocratic approach improves reproducibility and operational simplicity. Future work could extend the approach to formulated matrices and dissolution samples and could pair the assay with LC–MS-based degradant identification when structural elucidation is required.

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