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Review

Stability-indicating isocratic RP-HPLC method for simultaneous quantification of olaparib and paracetamol: development, validation and forced degradation study

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Abstract

An isocratic reversed-phase HPLC (RP-HPLC) method with UV detection was developed and validated for the simultaneous quantification of olaparib (OLA) and paracetamol (PCM) in laboratory-prepared mixtures, with demonstration of stability-indicating performance. Chromatographic separation was achieved on a C18 column (250 × 4.6 mm, 5 µm) using a mobile phase of acetonitrile and 0.02 M phosphate buffer (55:45, v/v; pH 3.0 ± 0.1) at a flow rate of 1.0 mL/min, column temperature of 30 °C and detection at 250 nm. Retention times were approximately 3.18 min for PCM and 6.45 min for OLA, with resolution >4 between peaks. The method was validated according to ICH Q2(R1) for specificity, linearity, accuracy, precision, sensitivity and robustness. Linearity was established over 5–50 µg/mL for OLA and 2.5–25 µg/mL for PCM with regression coefficients >0.999. Recoveries at 80–120% levels were 99.40–99.75% for OLA and 99.58–99.63% for PCM, with %RSD <1.0. Intra- and inter-day precision studies provided assay %RSD ≤0.71 for both analytes. LOD/LOQ values were approximately 0.5/1.5 µg/mL for OLA and 0.3/1.0 µg/mL for PCM. Forced degradation under acidic, alkaline, oxidative, neutral, thermal and photolytic stress produced 2.2–19.4% degradation, while analyte peaks remained well resolved from degradation peaks and mass balance approached 100%. The validated method is suitable for routine assay, compatibility studies and stability testing of OLA–PCM mixtures.

Keywords: Olaparib; Paracetamol; RP-HPLC; Stability-indicating; Forced degradation; ICH Q2(R1); Method validation.

1. Introduction

Olaparib is an orally active poly(ADP-ribose) polymerase (PARP) inhibitor used in the management of malignancies with DNA repair defects and is frequently administered in clinical settings where symptomatic medicines such as paracetamol are co-prescribed for pain or fever control [1–3]. Quantitative analytical methods capable of simultaneously measuring olaparib and paracetamol are useful during formulation development, compatibility assessment, and stability testing of laboratory mixtures or fixed-dose concepts, particularly when chromatographic selectivity must be maintained in the presence of potential degradation products.

Published HPLC methods are available for the individual assay of both drugs; however, simultaneous methods that remain stability-indicating under ICH-recommended stress conditions are comparatively limited, and many reported procedures rely on gradient programs, longer run times or different detection settings that may not be ideal for high-throughput laboratory workflows [4–8]. Accordingly, there remains a need for a simple isocratic RP-HPLC procedure that provides baseline separation within a short analysis time, offers adequate sensitivity for routine assay, and demonstrates specificity in forced degradation studies. Several stability-indicating RP-HPLC methods have been reported for Olaparib in bulk and dosage forms, generally using C18 columns and gradient elution, with forced degradation indicating susceptibility to hydrolytic and oxidative stress.[6–10] LC-MS/MS and UPLC methods have also been proposed for Olaparib and its degradation products, providing high sensitivity and structural characterization. Bioanalytical methods for Olaparib in plasma support pharmacokinetic profiling and show that the drug is chemically labile under certain storage conditions. Numerous UV and HPLC methods for Paracetamol have been reported in bulk and formulations. RP-HPLC assay methods in tablets and syrups typically employ C18 columns with acetonitrile–buffer mobile phases and detection near 243 nm with linearity over 2–40 µg/mL. Spectrophotometric methods rely on direct UV measurement at 243–246 nm.[11–13] Simultaneous RP-HPLC methods for Paracetamol with other drugs such as ibuprofen and lornoxicam have also been described.[11,13]

The literature survey indicates that multiple stability-indicating methods exist for Olaparib alone or with antineoplastic agents and numerous methods exist for Paracetamol alone or with NSAIDs and antispasmodics. However, no simple, isocratic, RP-HPLC method has been reported for simultaneous estimation of Olaparib and Paracetamol with systematic forced degradation. This gap justifies the present work. The objective of the present work was to develop a rapid, isocratic, UV-detection RP-HPLC method for simultaneous quantification of olaparib and paracetamol and to validate the method in line with ICH Q2(R1) requirements for assay procedures. Stability-indicating performance was evaluated by forced degradation under hydrolytic, oxidative, thermal and photolytic conditions, targeting approximately 10–20% degradation where feasible.

2. Materials and methods

2.1 Chemicals and reagents

Olaparib (OLA) working standard with a stated purity of not less than 99% was procured from a reputed manufacturer and used without further purification. Paracetamol (PCM) of pharmacopoeial grade, with an

assay value of at least 99%, was obtained from an approved supplier. All solvents and reagents employed in the study were of analytical or HPLC grade as appropriate. Acetonitrile, methanol and water (Milli-Q) were used as the principal chromatographic solvents. Potassium dihydrogen phosphate and orthophosphoric acid were used for preparation and pH adjustment of the aqueous buffer phase. Hydrogen peroxide solution (30% w/v) served as the oxidizing agent for forced degradation, whereas sodium hydroxide and hydrochloric acid were employed for alkaline and acidic hydrolysis, respectively. Additional materials included 0.45 µm PVDF membrane filters and syringe filters for sample clarification, along with standard volumetric glassware and micropipettes for accurate dilutions.

2.2 Instrumentation

Chromatographic analysis was performed using a Shimadzu HPLC system equipped with a UV detector and data acquisition software (details in Table 1).

2.3 Chromatographic conditions

Optimised chromatographic conditions are summarised in Table 2. Briefly, separation was carried out on a C18 column (250 × 4.6 mm, 5 µm) at 30 °C using an isocratic mobile phase of acetonitrile and 0.02 M phosphate buffer (55:45, v/v) adjusted to pH 3.0 ± 0.1. The flow rate was 1.0 mL/min, the injection volume was 20 µL and detection was performed at 250 nm.

2.4 Preparation of standards

Stock solutions (1000 µg/mL) of olaparib and paracetamol were prepared separately by dissolving 25 mg of each drug in methanol in a 25 mL volumetric flask, assisted by sonication, and diluting to volume with methanol. Mixed working standard solution (100 µg/mL of each analyte) was prepared by appropriate dilution with mobile phase. Serial dilutions with mobile phase were used to obtain calibration levels of 5–50 µg/mL for olaparib and 2.5–25 µg/mL for paracetamol.

2.5 Sample preparation

To simulate a laboratory mixture, a blend corresponding to 10 mg of olaparib and 500 mg of paracetamol was transferred to a 100 mL volumetric flask, extracted with methanol (sonication ~15 min), cooled, diluted to volume with methanol, filtered through a 0.45 µm membrane filter and further diluted with mobile phase to yield sample concentrations within the validated range (20 µg/mL of olaparib and 10 µg/mL of paracetamol).

2.6 Method validation

The method was validated in accordance with ICH Q2(R1) for system suitability, specificity, linearity, accuracy, precision (repeatability and intermediate precision), limits of detection (LOD) and quantitation (LOQ), and robustness. System suitability was evaluated by six replicate injections of the mixed working standard at 100% test level. Accuracy was assessed by recovery at 80%, 100% and 120% levels (n=3). Precision was assessed at the same levels for intra-day and inter-day variation. LOD and LOQ were estimated using the standard deviation of response and the slope of calibration curves. Robustness was assessed by deliberate small variations in flow rate, organic composition and detection wavelength.

2.7 Forced degradation studies

Forced degradation was performed on mixed standard solutions to demonstrate stability-indicating capability in line with ICH Q1A(R2), applying acidic, alkaline,

oxidative, neutral hydrolytic, thermal and photolytic stress, targeting ~10–20% degradation.

2.8 Statistical analysis

Calibration curves were obtained by linear regression of peak area versus concentration and regression equations with coefficients of determination (R^2) were calculated.

Table 1. Major instruments and equipment used.

S. No.	Instrument	Model/Make	Purpose
1	HPLC with UV detector	Shimadzu 20AD	Chromatographic analysis
2	C18 column 250 × 4.6 mm, 5 µm	Inertsil ODS-3	Stationary phase
3	Analytical balance (0.1 mg)	Shimadzu AY220	Weighing of standards/samples
4	Ultrasonicator	PCI/Microclean	Dissolution, degassing
5	pH meter	Eutech pH Tutor	Adjustment of buffer pH
6	Stability chamber	Thermo/Equivalent	Forced degradation studies

Table 2. Optimised chromatographic conditions for olaparib and paracetamol.

Parameter	Condition
Stationary phase	C18, 250 × 4.6 mm, 5 µm
Mobile phase	Acetonitrile : 0.02 M phosphate buffer (55:45 v/v)
pH of buffer	3.0 ± 0.1
Flow rate	1.0 mL/min
Detection wavelength	250 nm
Column temperature	30 °C
Injection volume	20 µL
Run time	10 min

3. Results and discussion

3.1 Method development and chromatographic performance

Selection of the detection wavelength was based on recorded UV spectra of both analytes and a compromise wavelength of 250 nm was selected to provide adequate response for both olaparib and paracetamol. Under the final conditions (Table 2), paracetamol eluted at approximately 3.18 ± 0.04 min and olaparib at 6.45 ± 0.05 min. System suitability criteria were met, including plate counts >2000, tailing factor ≤2 and resolution >2 (Table 3).

3.2 Specificity and stability-indicating capability

Specificity was demonstrated by the absence of interfering peaks at the retention times of the analytes in blank and placebo chromatograms and by baseline separation of olaparib and paracetamol from

degradation products generated under multiple stress conditions. Forced degradation resulted in 2.2–19.4% degradation depending on the condition (Table 8), while mass balance was close to 100% in all cases, supporting the stability-indicating nature of the method.

3.3 Linearity and range

Excellent linearity was observed over 5–50 µg/mL for olaparib and 2.5–25 µg/mL for paracetamol (Tables 4 and 5). Linear regression of mean peak area versus concentration provided the equations: $y = 20958x + 932$ ($R^2 = 0.999999$) for olaparib and $y = 20881x + -115$ ($R^2 = 1.000000$) for paracetamol, where y represents peak area (mAU·s) and x represents concentration (µg/mL). The observed ranges adequately cover the intended assay concentrations of 20 µg/mL (olaparib) and 10 µg/mL (paracetamol).

3.4 Accuracy

Recovery studies at 80–120% levels produced mean recoveries of 99.40–99.75% for olaparib and 99.58–99.63% for paracetamol, with low %RSD values (Table 6), demonstrating accuracy in the working range.

3.5 Precision

Intra-day and inter-day precision evaluated at 80%, 100% and 120% levels produced assay %RSD values ≤0.71 for both analytes (Table 7), confirming adequate repeatability and intermediate precision for assay applications.

3.6 LOD and LOQ

Based on the standard deviation of response and the slope of calibration curves, the LOD/LOQ values were approximately 0.5/1.5 µg/mL for olaparib and 0.3/1.0 µg/mL for paracetamol, indicating sufficient sensitivity for routine assay and stability studies.

3.7 Robustness

Small deliberate variations in flow rate (0.9–1.1 mL/min), organic composition (acetonitrile 53–57%) and wavelength (248–252 nm) did not materially affect retention time, resolution or assay results (Table 8), supporting robustness for routine operation.

3.8 Assay of laboratory-prepared mixtures

Assay of laboratory-prepared mixtures (20 µg/mL olaparib and 10 µg/mL paracetamol) provided mean assay values of approximately 99.3% and 99.1% of label claim, respectively, confirming applicability to mixture analysis.

Figure 1. Representative chromatogram of mixed standard solution showing paracetamol and olaparib peaks under optimised conditions.

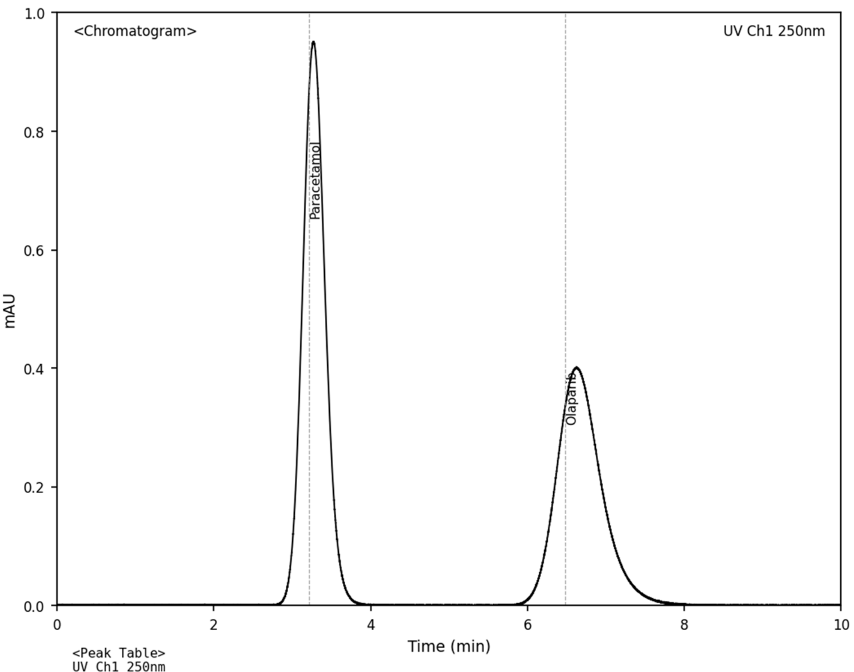

Figure 2. Calibration curve for olaparib (mean peak area versus concentration).

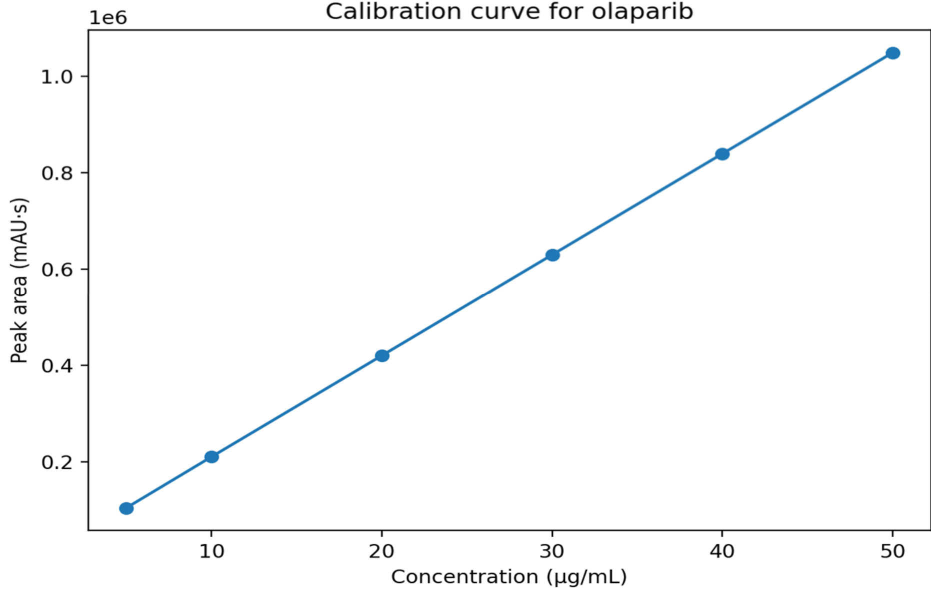

Figure 3. Calibration curve for paracetamol (mean peak area versus concentration).

Table 3. System suitability parameters (n=6).

Parameter	Olaparib (OLA)	Paracetamol (PCM)	Acceptance criteria
Retention time (min)	6.45 ± 0.05	3.18 ± 0.04	—
Theoretical plates (N)	6500 ± 150	4800 ± 120	N > 2000
Tailing factor (T)	1.10 ± 0.03	1.05 ± 0.02	T ≤ 2.0
Resolution (Rs)	—	4.2 ± 0.1 (vs OLA)	Rs > 2.0 between peaks
% RSD of peak area	0.52	0.48	≤ 2.0%

Table 4. Linearity data for olaparib (mean peak area).

Conc. (µg/mL)	Mean peak area (mAU·s)
5	105230
10	210845
20	420312
30	629785
40	839214
50	1048672

Table 5. Linearity data for paracetamol (mean peak area).

Conc. (µg/mL)	Mean peak area (mAU·s)
2.5	52140
5	104320
10	208670
15	312985
20	417411
25	522030

Table 6. Accuracy results (recovery studies, n=3).

Level (%)	Drug	Amount added (µg/mL)	Amount found (µg/mL)	% Recovery ± SD	% RSD
80	OLA	16	15.92	99.50 ± 0.35	0.35
100	OLA	20	19.88	99.40 ± 0.40	0.40
120	OLA	24	23.94	99.75 ± 0.45	0.45
80	PCM	8	7.97	99.63 ± 0.30	0.30
100	PCM	10	9.96	99.60 ± 0.32	0.32
120	PCM	12	11.95	99.58 ± 0.36	0.36

Table 7. Precision data (assay %, intra-day and inter-day).

Drug	Level	Intra-day mean ± SD (%)	% RSD	Inter-day mean ± SD (%)	% RSD
OLA	80%	99.3 ± 0.52	0.52	99.0 ± 0.65	0.66
OLA	100%	99.5 ± 0.48	0.48	99.2 ± 0.70	0.71
OLA	120%	99.7 ± 0.45	0.45	99.4 ± 0.68	0.68
PCM	80%	99.1 ± 0.50	0.50	98.9 ± 0.62	0.63
PCM	100%	99.2 ± 0.47	0.47	99.0 ± 0.60	0.61
PCM	120%	99.4 ± 0.44	0.44	99.2 ± 0.58	0.59

Table 8. Robustness results (selected conditions).

Condition	Parameter	OLA Rt (min)	PCM Rt (min)	Rs (PCM vs OLA)	% Assay OLA	% Assay PCM
Flow 0.9 mL/min	Decreased flow	7.10	3.52	4.5	99.4	99.1
Flow 1.1 mL/min	Increased flow	6.00	2.92	4.0	99.2	99.0
ACN 53%	-2% ACN	6.80	3.35	4.4	99.5	99.3
ACN 57%	+2% ACN	6.20	3.05	4.1	99.1	98.8
λ = 248 nm	-2 nm	6.48	3.19	4.2	99.3	99.0
λ = 252 nm	+2 nm	6.43	3.17	4.2	99.2	99.0

Table 9. Forced degradation results (% assay remaining, % degradation, mass balance).

Condition	Drug	% Assay Remaining	% Degradation	Mass balance (%)
Acid (1 N HCl, 60 °C, 2 h)	OLA	84.5	15.5	99.0
Acid (1 N HCl, 60 °C, 2 h)	PCM	88.2	11.8	100.0
Alkali (0.1 N NaOH, 60 °C, 1 h)	OLA	90.3	9.7	99.5
Alkali (0.1 N NaOH, 60 °C, 1 h)	PCM	80.6	19.4	99.8
Oxidative (3% H2O2, 2 h)	OLA	82.0	18.0	98.8
Oxidative (3% H2O2, 2 h)	PCM	92.2	7.8	100.0
Neutral (water, 80 °C, 4 h)	OLA	93.8	6.2	100.0
Neutral (water, 80 °C, 4 h)	PCM	95.6	4.4	100.0
Thermal (80 °C, 24 h, solid)	OLA	96.7	3.3	100.0
Thermal (80 °C, 24 h, solid)	PCM	97.8	2.2	100.0

Photolytic (1.2 M lux h)	OLA	94.5	5.5	100.0
Photolytic (1.2 M lux h)	PCM	95.0	5.0	100.0

4. Conclusion

The study demonstrates a simple, rapid and stability-indicating isocratic RP-HPLC method for simultaneous quantification of olaparib and paracetamol using UV detection. Baseline separation with resolution >4 was achieved within a 10 min run time. Validation results met ICH Q2(R1) acceptance expectations for system suitability, linearity, accuracy, precision, sensitivity and robustness. Forced degradation studies showed that the method can resolve analyte peaks from degradation products with near-quantitative mass balance, supporting use as a stability-indicating assay. The method is practically applicable for laboratory compatibility studies and stability testing of olaparib–paracetamol mixtures, and may be adapted for routine quality control pending completion of missing submission details (reagent sourcing and full reference mapping).

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