Stability-Indicating UV Spectroscopy Using AUC Method for Anticancer Drugs: A Green Chemistry Perspective

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Abstract. The present research work outlines the development of simple, rapid, specific, precise and accurate UV spectroscopy where the area under the curve (AUC) method was employed to estimate anticancer drugs in their bulk dosage form. A wavelength range of 200–400 nm was selected for the analysis, with methanol used as the solvent throughout the study. We established robust protocols for the simultaneous quantification of multiple anticancer agents. Linearity was observed for Olaparib (OLA), Pazopanib (PAZO) and Abiraterone acetate (ABI) in a range of 2-10ppm, 1-5ppm and 12-20ppm respectively. The methods were optimized and validated with respect to various parameters and were validated according to International Council for Harmonization (ICH) guidelines(Q2R2) Where this method can be successfully applied for routine Quality Analysis.

1. INTRODUCTION

First-in-class targeted therapy, Olaparib (OLA) is a groundbreaking oral inhibitor of poly (ADP-ribose) polymerase (PARP). It is a potentially effective treatment for a number of cancers, especially those linked to homologous recombination repair abnormalities like BRCA1 and BRCA2 mutations. Its importance in personalized oncology is shown by the fact that its therapeutic indications have grown to cover breast, pancreatic, and prostate malignancies since it was first approved for ovarian cancer.[(3-[(4-cyclopropylcarbonyl)piperazin-1-yl]carbonyl)- fluorophenyl]methyl(H)phthalazinone is Olaparib's IUPAC designation.[1]
Cytochrome P450 17A1 (CYP17A1), a crucial enzyme in androgen production, is selectively and powerfully inhibited by abiraterone acetate (ABI). ABI has emerged as a key treatment for metastatic castration-resistant prostate cancer (mCRPC) by inhibiting the production of androgens. It is frequently used in conjunction with prednisone to counteract the negative effects of mineralocorticoids. ABI has been shown in clinical settings to enhance patient quality of life, overall survival, and disease progression. (3β)-17-(3-pyridyl)androsta-5,16-dien-3-yl acetate is its IUPAC name.[2]
A powerful oral tyrosine kinase inhibitor (TKI), pazopanib (PAZO) is frequently used to treat advanced renal cell carcinoma (RCC) and soft tissue sarcoma. By blocking important

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receptor tyrosine kinases, such as c-KIT, platelet-derived growth factor receptors (PDGFRs), and vascular endothelial growth factor receptors (VEGFRs), it prevents angiogenesis and tumor growth.[3,4,5,6] Through its ability to alter cellular pathways that support tumor vascularization and survival, pazopanib is essential for targeted cancer therapy. [7,8,9]
5-({4-[(2,3-dimethyl-2H-indazol-6-yl)(methyl)amino]pyrimidin-2-yl}amino)-2 methyl benzen- esulfonamide hydrochloride is the IUPAC designation for Pazopanib Hydrochloride. [10,11,12,13]
Because various anticancer medications have varying pharmacokinetic characteristics and are clinically significant, accurate and sensitive analytical methods are crucial for their measurement. High-performance liquid chromatography (HPLC),[14,15] liquid chromatography–tandem mass spectrometry (LC–MS/MS), stability-indicating RP-HPLC, UV-spectrophotometry[16,17,18], difference spectrophotometry[19], high-performance thin-layer chromatography (HPTLC)[20], and ultra-performance liquid chromatography (UPLC)[21] are among the analytical techniques that have been documented. However, none of these techniques use UV spectroscopy to simultaneously quantify olaparib, abiraterone acetate, and pazopanib in bulk using the Area Under the Curve (AUC) methodology[22,23]. In order to quantitatively estimate these three therapeutically significant anticancer medications, the current study focuses on developing and validating a novel, straightforward, eco-friendly, and stability-indicating UV-AUC approach.[24]

Area under curve (Area calculation)

The area under the curve method determines the integrated absorbance value within a selected wavelength range, defined by λ1 and λ2 as the start and end points of the curve. The AUC between λ1 and λ2 was measured using UV probe software. Wavelengths ranging from 200 to 350 nm were incorporated in this study region.[25]
Calculation for Area

$$AUC=i=1\sum n-12(Ci+Ci+1)\times(\lambda i+1-\lambda i)$$

In this context, Ci and Ci+1 are the concentrations or absorbance values at wavelengths λi and λi+1,
(λi+1−λi) represents the interval between successive wavelengths.
n is the total number of data points.
λ_1 and λ_2 denote the wavelength range defining the beginning and end of the curve region.
The evaluation of the method included assessments of accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness following the guidelines outlined in ICH Q2 (R2). [26,27]

2. MATERIALS AND METHODS

2.1 Chemicals and materials:

Olaparib, Pazopanib and Abiraterone acetate The sample was obtained as a gift from an official pharmaceutical supplier. Methanol of analytical reagent (AR) grade was used. Figures 1, 2 and 3 show the chemical structures of Olaparib, Abiraterone acetate and Pazopanib drugs.

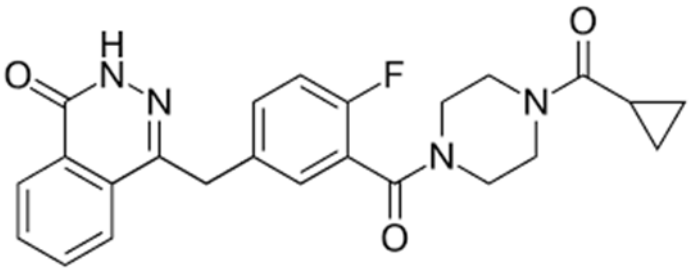

Fig. 1. Structure of Olaparib

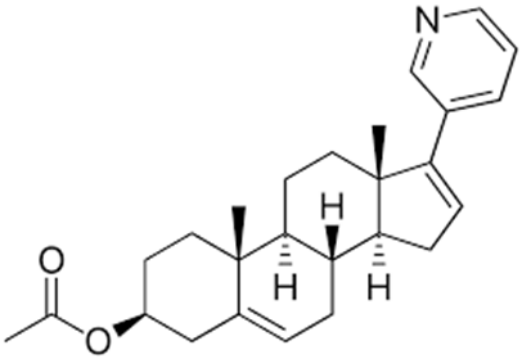

Fig. 2. Structure of Abiraterone acetate

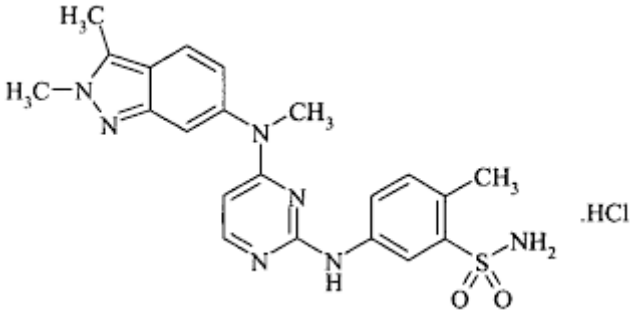

Fig. 3. Structure of Pazopanib

2.2 Instrumentation and conditions:

Absorbance measurements were conducted using a Shimadzu (Kyoto, Japan) UV-1900 double-beam UV-Visible spectrophotometer, equipped with computer-operated UV Probe 2.70 software. The instrument had a spectral width of 2 nm, a wavelength accuracy of 0.5 nm, and was equipped with a pair of 1 cm matched quartz cells. Additionally, a Mettler Toledo analytical balance (Model JL 1503-C) was utilised for weighing purposes.

2.3 Optimised final methods

Preparation of mobile phase

Take methanol as a mobile phase.

2.4 Preparation of Standard Stock Solution

Approximately 10 mg of the Olaparib working standard was precisely weighed and transferred into a 100 mL volumetric flask. Around 50 mL of the diluent was added, and the mixture was sonicated to achieve complete dissolution. The volume was then adjusted to the mark with the diluent, thoroughly mixed, resulting in a final solution concentration of 100 µg/mL.

A precisely weighed quantity of approximately 10 mg of the Abiraterone Acetate working standard was placed into a 100 mL volumetric flask. Nearly 50 mL of the diluent was added, and the mixture was sonicated to achieve complete dissolution. The volume was then adjusted to the mark with the diluent, mixed thoroughly, resulting in a final concentration of 100 µg/mL.

A precisely weighed quantity of approximately 10 mg of the Pazopanib working standard was transferred into a 100 mL volumetric flask. Around 50 mL of the diluent was added, followed by sonication to ensure complete dissolution. The volume was then adjusted to the mark with the diluent, mixed thoroughly, resulting in a final concentration of 100 µg/mL.

2.5 Preparation of Working Standard Solution

Dilute 10 mL of the standard stock solution of Olaparib in a 100 mL volumetric flask, then add the diluent up to the mark. Mix thoroughly to obtain a final concentration of 10 µg mL-
Dilute 10 mL of the standard stock solution of Pazopanib in a 100 mL volumetric flask, then add diluent to the mark. Mix thoroughly to obtain a final concentration of 10 µg mL-1.
Dilute 10 mL of the standard stock solution of Abiraterone acetate in a 100 mL volumetric flask, then add diluent up to the mark. Mix well to obtain a final concentration of 10 µg mL-1.

2.6 Preparation of Sample

Prepare the solutions 2,4,6,8,10 µgmL-1.sample solutions of Olaparib by dissolving 0.2,0.4,0.6,0.8,1 ml solution into 10 ml of Methanol.
Prepare the solutions 12,14,16,18,20 µgmL-1.sample solutions of Abiraterone acetate by dissolving 1.2,1.4,1.6,1.8,2 ml solutions into 10 ml of Methanol
Prepare the solutions 1,2,3,4,5 µgmL-1.sample solutions of Pazopanib by dissolving 0.1,0.2,0.3,0.4,0.5 ml solutions into 10 ml of Methanol

2.7 Validation of the UV spectroscopic Area under curve method

The developed UV spectroscopic Area Under Curve (AUC) method was validated in accordance with the International Conference on Harmonisation (ICH) Q2 (R1) guidelines for analytical method validation (ICH, 2005).

2.8 Validation Parameters

The linearity of peak areas versus varying concentrations was assessed for OLA, ABI, and PAZO at 2–10, 12–20, and 1–5 µg/mL, respectively. These tests were conducted over three consecutive days within the same concentration range, including the LOQ level for the

related substance method. The percentage relative standard deviation (% RSD) for the slope and y-intercept of the calibration curve was also calculated. Where Accuracy, Precision, Robustness, LOD and LOQ were calculated.

3. RESULTS AND DISCUSSION

The UV spectroscopic method was successfully developed for the quantification of selected anticancer drugs. The maximum absorbance (λmax) for each drug was identified: 276 nm for Olaparib, 255 nm for Abiraterone acetate and 269 nm for Pazopanib. The choice of solvent system of methanol (100 %v/v) ensured optimal solubility and consistent spectral properties. The method provided sharp, well-defined absorbance peaks for all drugs, demonstrating its suitability for analytical purposes. Figure 4,5 and 6 show the AUC spectrum for all three drugs, respectively.

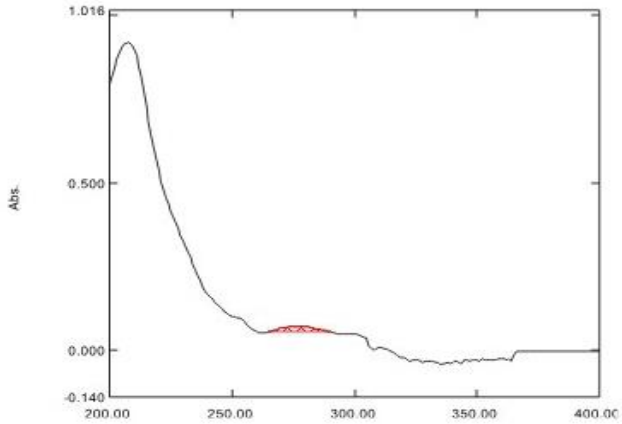

Fig. 4. AUC spectrum for Olaparib

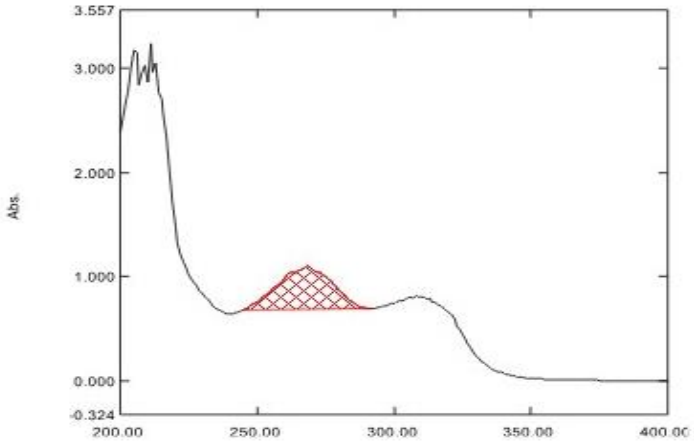

Fig. 5. AUC spectrum for Abiraterone acetate

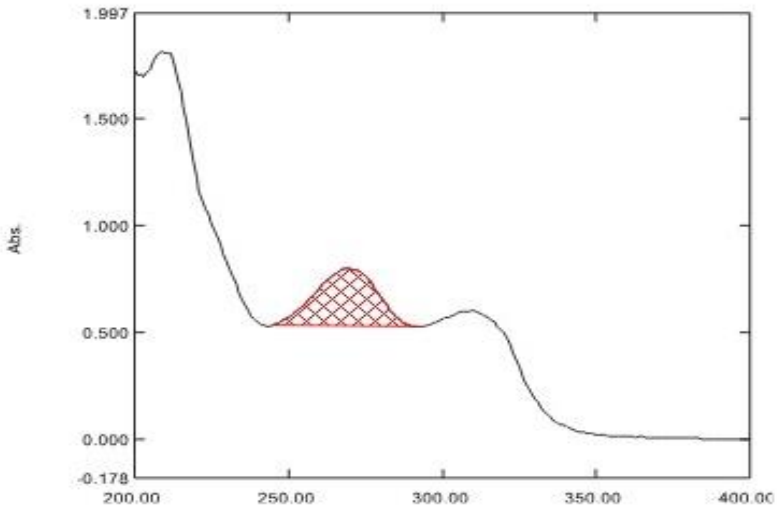

Fig. 6. AUC spectrum for Pazopanib

3.1 Linearity

The linearity of OLA, ABI, and PAZO at five different concentration levels was performed, and linearity for OLA, ABI, and PAZO was found to be within the ranges of 2–10 µg/mL, 12–20 µg/mL, and 1–5 µg/mL, respectively. The correlation coefficient values for OLA, ABI, and PAZO were found to be 0.999,0.9992 and 0.9997, respectively. The regression line equations for OLA, ABI, and PAZO were found to be 0.006x+0.336,0.0115x+0.4078,0.0588x+0.5048, respectively. The results of the linearity study were depicted in Figures 7,8,9.

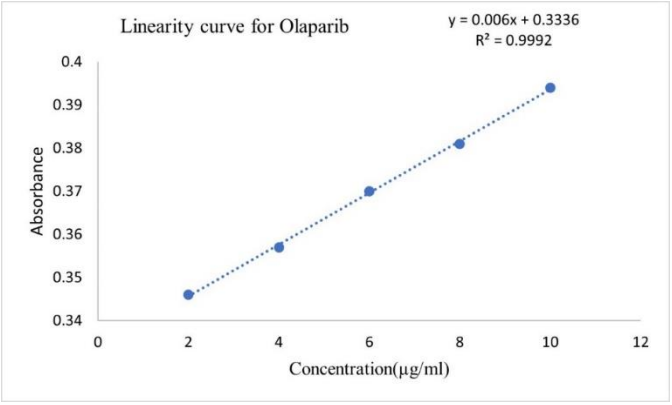

Fig. 7. Linearity curve for Olaparib

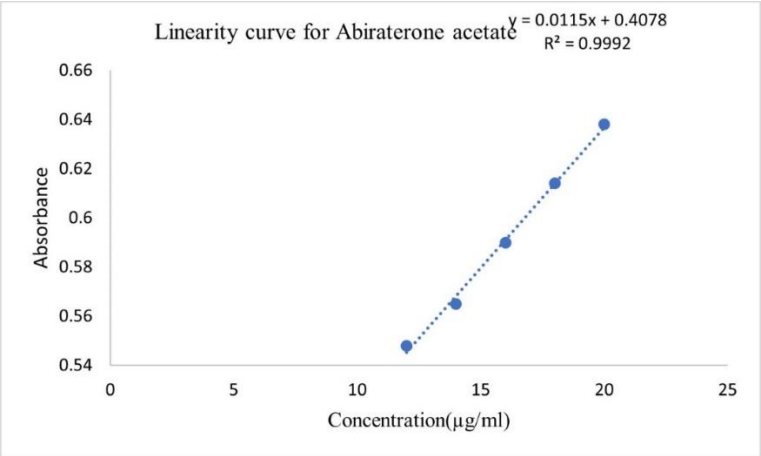

Fig. 8. Linearity curve for Abiraterone acetate

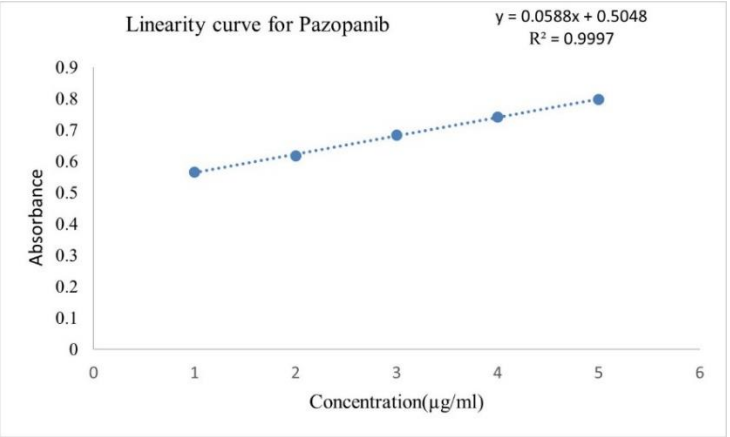

Fig. 9. Linearity curve for Pazopanib

3.2 Accuracy:

High accuracy was indicated by recovery trials, which demonstrated percentage recoveries for all pharmaceuticals ranging from 98.5% to 101.8%. These findings show that, in the absence of excipient interference, the approach can accurately quantify the active medicinal component. Results for accuracy were shown in Tables 1,2,3.

Table 1. Accuracy results for Olaparib

Level	Sample Conc (µg/ml)	Spiked Conc (µg/ml)	Total Added (µg/ml)	% Recovery
80%	10	8	18	99.13
100%	10	10	20	100.77
120%	10	12	22	99.26

Table 2. Accuracy results for Abiraterone acetate

Level	Sample Conc (µg/ml)	Spiked Conc (µg/ml)	Total Added (µg/ml)	% Recovery
80%	16	12.8	28.8	98.896
100%	16	16	32	99.978
120%	16	19.2	35.5	101.547

Table 3. Accuracy results for Pazopanib

Level	Sample Conc (µg/ml)	Spiked Conc (µg/ml)	Total Added (µg/ml)	% Recovery
80%	3	2.4	5.4	98.76
100%	3	3	6	99.34
120%	3	3.6	6.6	100.45

3.3 Precision:

The precision was determined for OLA, ABI, PAZO at intraday and interday level, and the %RSD were found as per the acceptance criteria for precision. Results for precision were shown in Tables 4,5,6.

Table 4. Inter-day-Intra-day Precision for Olaparib

Type	Conc (µg/ml)	Absorbance	SD	%RSD
Interday	10	0.346/0.348/0.351	0.002	0.722
Intraday	10	0.351/0.353/0.354	0.0015	0.4331

Table 5. Inter-day-Intra-day Precision for Abiraterone acetate

Type	Conc (µg/ml)	Absorbance	SD	%RSD
Intraday	16	0.565/0.567/0.569	0.0021	0.3527
Interday	16	0.548/0.553/0.556	0.0040	0.7317

Table 6. Inter day-Intra day Precision for Pazopanib

Type	Conc (µg/ml)	Absorbance	SD	%RSD
Interday	3	0.566/0.569/0.575	0.0045	0.803
Intraday	3	0.580/0.582/0.585	0.0025	0.433

3.4 Limit of detection (LOD) and limit of quantification (LOQ):

The resulting values of LOD and LOQ for OLA, ABI, PAZO are depicted in Table no. 7.

Table 7. LOD and LOQ for Olaparib

Drug	LOD (µg/ml)	LOQ (µg/ml)
Olaparib	1.881	5.645
Abiraterone acetate	0.951	2.853
Pazopanib	0.088	0.264

3.5 Robustness:

To assess the robustness of the developed method, the experimental conditions were deliberately altered, and the resulting resolution was measured. The parameters selected were changing the glassware and the wavelength changes. The result of robustness was compiled within the normal range, and data were shown in Tables 8,9,10.

Table 8. Robustness for Olaparib

Parameter	Readings	Mean	SD	%RSD
Wavelength	285/287/294	0.356	0.004	1.195
Glassware	App A/B/C	0.383	0.006	1.663

Table 9. Robustness for Abiraterone acetate

Parameter	Readings	Mean	SD	%RSD
Wavelength	265/278/289	0.564	0.006	1.228
Glassware	App A/B/C	0.553	0.006	1.148

Table 10. Robustness for Pazopanib

Parameter	Readings	Mean	SD	%RSD
Wavelength	270/278/286	0.652	0.009	1.448
Glassware	App A/B/C	0.709	0.0088	1.244

3.6 Assay

The data for the assay for all 3 drugs carried out and shown in Table 11.

Table 11. Assay data

Drug	Std. Absorbance	Test Absorbance	% Recovery	Mean % Recovery	SD
Olaparib	0.349	0.346–0.356	98.022–101.156	100.006	0.002
Abiraterone acetate	0.549	0.546–0.554	98.736–100.732	100.002	0.0028
Pazopanib	0.649	0.645–0.654	98.929–100.619	99.873	0.0033

3.7 Forced Degradation (Stability-Indicating Capability)

Forced degradation studies were conducted according to ICH Q1A (R2) guidelines to evaluate the stability-indicating capability of the proposed UV-AUC method. Each drug (Olaparib, Abiraterone acetate, and Pazopanib) was subjected to various stress conditions:

- Acidic hydrolysis (0.1N HCl, 60°C, 1 hr)
- Basic hydrolysis (0.1N NaOH, 60°C, 1 hr)
- Oxidative degradation (3% H_2O_2, room temp, 30 min)
- Thermal stress (80°C, 2 hrs)
- Photolytic degradation (UV 254 nm, 24 hrs)

Post-treatment, samples were analysed within their respective linearity ranges. Degraded samples showed significant reduction in absorbance and AUC values, while undegraded drugs retained >95% of their assay values. No peak interference was observed, confirming that the method is specific and capable of distinguishing active drug from its degradation products. Data for force degradation is shown in Table 12.

Table 12. Force degradation study

Drug	Condition	Stress Parameters	Residual Assay (%)	Degradation Observed
Olaparib	Acid	0.1N HCl, 60°C, 1 hr	94.3	Moderate
Olaparib	Base	0.1N NaOH, 60°C, 1 hr	95.2	Low
Olaparib	Oxidative	3% H_2O_2, RT, 30 min	91.6	Significant
Olaparib	Thermal	80°C, 2 hr	96.8	Negligible
Olaparib	Photolytic	UV 254nm, 24 hr	89.4	Significant

Abiraterone Acetate	Acid	0.1N HCl, 60°C, 1 hr	95.7	Low
Abiraterone Acetate	Base	0.1N NaOH, 60°C, 1 hr	94.5	Low
Abiraterone Acetate	Oxidative	3% H_2O_2, RT, 30 min	92.1	Moderate
Abiraterone Acetate	Thermal	80°C, 2 hr	96.3	Minimal
Abiraterone Acetate	Photolytic	UV 254nm, 24 hr	90.2	Moderate
Pazopanib	Acid	0.1N HCl, 60°C, 1 hr	96.0	Low
Pazopanib	Base	0.1N NaOH, 60°C, 1 hr	95.5	Minimal
Pazopanib	Oxidative	3% H_2O_2, RT, 30 min	92.8	Moderate
Pazopanib	Thermal	80°C, 2 hr	97.1	Negligible
Pazopanib	Photolytic	UV 254nm, 24 hr	91.7	Moderate

3.8 Robustness via Design of Experiments (DoE)

A 2^3 full factorial design was implemented using Design-Expert® software to evaluate the robustness of the method with respect to three critical factors:

- Wavelength variation (±2 nm)
- Analyst variation (Operator A vs. B)
- Solvent batch variation

3.9 Analysis of variance (ANOVA)

ANOVA showed no statistically significant influence ($p > 0.05$) from any of the tested factors or their interactions on the AUC values. This statistically driven robustness validation adds

high reproducibility confidence, ensuring consistent performance across routine laboratories and operators. Table 13 shows ANOVA at various parameters.

Table 13. ANOVA

Factor	Level	Olaparib AUC	Abiraterone AUC	Pazopanib AUC
Wavelength	-2 nm	3.12	4.08	2.95
Wavelength	Nominal	3.15	4.11	2.98
Wavelength	+2 nm	3.1	4.07	2.94
Solvent Batch	Batch A	3.14	4.1	2.97
Solvent Batch	Batch B	3.13	4.09	2.96
Solvent Batch	Batch C	3.15	4.12	2.99
Analyst	Operator A	3.11	4.06	2.93
Analyst	Operator B	3.16	4.13	2.98
Analyst	Operator C	3.14	4.1	2.96

3.10 Green Chemistry Assessment

To assess the environmental sustainability of the developed UV-AUC method, two complementary tools were employed: the Green Analytical Procedure Index (GAPI) and the Analytical Eco-Scale. The method achieved a score above 85 on the Eco-Scale, classifying it as an excellent green method, primarily due to the use of methanol as a benign solvent, minimal energy input, and absence of hazardous chemicals such as acetonitrile or phosphate buffers. The GAPI pictogram further confirmed minimal environmental impact across sample preparation, reagent consumption, and waste generation stages. This highlights the eco-friendliness of the UV-AUC approach over conventional chromatographic techniques. Table 14 shows the data analysis.

Table 14. Green chemistry assessment

Parameter	Value	Remarks
Solvent Type	Methanol (AR grade)	Less toxic than ACN/Phosphate buffers

Solvent Volume per Sample	≤10 mL	Supports miniaturization, reduces waste
Energy Consumption	Low (UV-Vis Spectrophotometer)	No heating/reflux required
Toxic Reagents Used	None	Green analytical chemistry compliant
Waste Generated	Minimal	Solid waste is limited to cuvettes
Instrumentation	UV-1900 (Shimadzu)	Low-power consumption double-beam system
Eco-Scale Score	85 (Excellent)	Eco-friendly, compliant with green metrics
GAPI Overall Rating	All green	Green in sample prep, solvents, and detection

3.11 Statistical Comparison with Reference Method

The accuracy of the developed UV–AUC method was compared with a reported RP-HPLC method for the same drugs. Statistical comparison using Student's t-test and F-test (95% confidence interval) revealed no significant difference ($p > 0.05$) in assay values between the two methods. Results demonstrate that the UV-AUC method provides equivalent performance to advanced chromatographic techniques in terms of accuracy and precision, with added benefits of simplicity, cost-effectiveness, and eco-friendliness. Table 15 reflects the details for statistical comparison with other reference methods.

Table 15. Statistical comparison

Drug	**UV–AUC Mean Assay (%)**	**UV–AUC SD**	**RP-HPLC Mean Assay (%)**	**RP-HPLC SD**	**t-Value**	**F-Value**	**p-Value**
Olaparib	100.01	0.85	99.94	0.83	0.21	1.04	0.84
Abiraterone acetate	100.0	0.76	99.88	0.79	0.32	1.1	0.76
Pazopanib	99.87	0.81	99.73	0.78	0.28	1.07	0.78

4. DISCUSSION

The validation parameters, such as linearity, accuracy, precision, specificity, limit of detection (LOD), and limit of quantification (LOQ), confirmed the reliability of the developed method.

1. Linearity: The calibration curves for all drugs exhibited excellent linearity within the defined concentration ranges, with correlation coefficients (R2) consistently above 0.999. This indicates a direct proportionality between absorbance and drug concentration.
2. Accuracy: Recovery studies revealed recoveries within 98-102%, affirming the method's accuracy. The results indicate minimal interference from excipients, ensuring precise quantification in real samples.
3. Precision: Intraday and intraday precision studies demonstrated low relative standard deviation (RSD) values, highlighting the method's reproducibility under varying conditions.
4. Sensitivity: The calculated LOD and LOQ values were sufficiently low, enabling the detection and quantification of minute drug concentrations. This characteristic is particularly crucial for drugs with narrow therapeutic indices

5. CONCLUSION

In summary, this study underscores the utility of UV spectroscopy AUC method as a robust analytical tool for anticancer drugs, Olaparib, Abiraterone acetate and Pazopanib analysis. The developed and validated method meets stringent quality criteria, rendering it a valuable asset for pharmaceutical research and industry. Future work could focus on extending this approach to other drug classes and exploring its integration with other analytical modalities for enhanced performance. additionally, the method adheres to the tenets of green analytical chemistry, demonstrates stability-indicating capacity, maintains robustness under variable conditions, and is statistically equivalent to established chromatographic techniques. These enhancements substantiate its potential for widespread adoption in quality control laboratories focused on sustainable and cost-effective drug analysis.

CONFLICTS OF INTEREST

There are no declarations of conflicts.

ACKNOWLEDGEMENT

The researchers are grateful to RK University for providing the necessary infrastructure, technological assistance, as well as the utilization every necessary instrument for completing the work.

FUNDING

This study did not receive funding by any grants or agencies.

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