

Article

Development, Validation, and Greenness Assessment of Eco-Friendly Analytical Methods for the Determination of Abiraterone Acetate in Pure Form and Pharmaceutical Formulations

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Abstract: This study presents the development and validation of environmentally friendly analytical methods for quantifying Abiraterone Acetate (AA) in both its pure form and commercial pharmaceutical formulations. An optimized High-Performance Liquid Chromatography (HPLC) method was developed using an Agilent Extend C18 column (250 mm × 4.6 mm, 5 µm) at 25 °C. The mobile phase consisted of formic acid and ethanol in isocratic mode, with a flow rate of 1.0 mL min^{−1}, and detection was performed at 253 nm. The spectrophotometric method involved a comprehensive evaluation of AA's spectral properties in various solvents, with ultrapure water providing the most suitable spectra for analysis at 253 nm. Both methods were validated according to ICH guidelines, demonstrating selectivity, linearity, accuracy, precision, detection and quantification limits, and robustness, with correlation coefficients exceeding 0.999 across the 5–30 µg mL^{−1} concentration range. Comparative statistical analysis using Student's *t*-test and Fisher's *F*-test showed no significant differences between the two methods. The environmental impact of both methods was assessed using AGREE and GAPI software, confirming their sustainability. These validated methods offer reliable and eco-friendly approaches for the quantitative analysis of AA in tablet formulations, promoting safer and greener laboratory practices in pharmaceutical analysis.



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Keywords: Abiraterone Acetate; green analytical methods; HPLC; spectrophotometry; pharmaceutical validation; sustainable laboratory practices

1. Introduction

In the face of today's growing environmental concerns, the shift towards more sustainable analytical methodologies is paramount. This trend holds particular relevance for liquid chromatography techniques, which are widely used but often lack alignment with green chemistry principles. Specifically, principles 1, 5, 6, 11, and 12 of green chemistry are pertinent to analytical methods. These principles advocate for safer chemistry to minimize waste, energy efficiency, real-time analysis to prevent pollution, and accident prevention [1]. When developing a new analytical method for detecting a specific analyte, two crucial factors must be addressed. First, the method's metrological value must be validated to ensure accuracy, precision, and reproducibility. Second, environmental sustainability must be prioritized by incorporating safer, less harmful chemicals and processes, and adhere to the guidelines for green chemistry. The application of these principles is critical yet still underutilized in many fields, including pharmaceutical analysis [2].

High-Performance Liquid Chromatography (HPLC) remains a cornerstone technology in pharmaceutical quality control, owing to its robust and reliable performance. Traditionally, reverse-phase HPLC utilizes a polar mobile phase combined with a hydrophobic stationary phase. The mobile phase often consists of a mixture of water, modified with additives, and organic solvents like acetonitrile or methanol. In reverse phase separation mode, mobile phases primarily composed of water and aqueous buffers may be utilized,

depending on the analyte's solubility. However, using only water and aqueous buffers in the mobile phase can lead to prolonged analysis times. Consequently, mobile phases that combine aqueous buffers with organic modifiers are preferred for more efficient separation. These organic solvents are preferred due to their favorable chromatographic characteristics, such as miscibility with water, low viscosity, and chemical stability, which ensure high-quality analytical performance [3]. Despite these advantages, both acetonitrile and methanol pose significant environmental and health risks. Acetonitrile is highly volatile, flammable, and toxic, while methanol, though less toxic and biodegradable, is still classified as hazardous [4]. The widespread use of these solvents in HPLC has resulted in substantial amounts of waste, leading to significant environmental and disposal challenges [5].

As HPLC usage expands, so does the generation of solvent waste, amplifying the need for more sustainable approaches. This necessity has pushed researchers toward the development of greener analytical methods, focusing on the reduction or elimination of toxic chemicals in favor of safer, environmentally benign alternatives. One such alternative is ethanol, which has emerged as a promising candidate for use in green HPLC methods. Ethanol's lower toxicity, biodegradability, and cost-effectiveness make it an attractive option, particularly for laboratories with limited resources or those in developing regions [6]. Moreover, ethanol offers comparable chromatographic performance to acetonitrile and methanol, exhibiting similar selectivity and retention times, making it a viable green alternative without compromising analytical efficiency [7]. Additionally, the eluent strengths for a C18 column are comparable: acetonitrile and ethanol both have strengths of 3.1, while methanol has a strength of 1.0 [8]. These properties further substantiate ethanol's potential as a substitute for methanol and acetonitrile in HPLC applications.

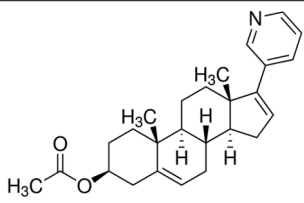
However, the implementation of ethanol in HPLC is not without its challenges. Ethanol has a higher ultraviolet (UV) cutoff value than acetonitrile and methanol, which can lead to increased background noise and reduced sensitivity in UV detection, particularly in gradient elution systems [9]. Additionally, ethanol's higher viscosity compared to acetonitrile and methanol can result in elevated column pressures, potentially affecting both the efficiency and lifespan of chromatographic columns. These issues can be mitigated by optimizing the HPLC method, such as by adjusting column temperature to reduce ethanol's viscosity, thus maintaining system performance and longevity [10].

In this study, I aim to address the growing need for environmentally sustainable analytical techniques by developing a green liquid chromatography (Green LC) method utilizing ethanol as the mobile phase solvent. Ethanol's favorable environmental profile and chromatographic performance make it an ideal candidate for promoting greener analytical practices [11]. Furthermore, I introduce a Simplified Spectrophotometric Method for the analysis of AA, an anti-androgen drug used primarily for treating prostate cancer [12]. AA is also being explored for its potential use in treating ovarian and breast cancers [13]. Its chemical properties and growing therapeutic applications highlight the need for reliable, sustainable analytical methods for its quantification in pharmaceutical formulations [14]. The chemical features of AA are presented in Table 1, as already detailed elsewhere [15].

The literature reveals a wide range of analytical methods for quantifying AA in pharmaceutical and biological samples, including spectrofluorimetric, spectrophotometric, and liquid chromatography techniques [16]. Many different analytical methods have been developed for the quantification of AA in biological fluids, bulk, and dosage forms. For example, the Liquid chromatographic-tandem mass spectroscopy method for the quantification of AA in human plasma [17]; the RP-HPLC/UV technique for the quantification of AA in rat plasma and its application to a pharmacokinetic study in rats [18]; the LC-MS/MS-ESI method for the determination of AA in rat and human plasma and its application to a pharmacokinetic study [19]; Liquid chromatography coupled with ultraviolet detection and electron ionization mass spectrometry method for the analysis of AA stress degradation behavior [20]; AA in human plasma was determined using an ultra-high-performance liquid chromatography-tandem mass spectrometry technique [21]; and AA in human plasma was determined using an HPLC-fluorescence technique [22]. While effective, these methods

often require expensive equipment, toxic solvents, and complex procedures, limiting their accessibility and increasing their environmental impact [15]. The introduction of greener methods for AA analyses is, therefore, timely and necessary. In this context, our study presents a novel, environmentally friendly Green LC method using ethanol, as well as a Simplified Spectrophotometric Method that employs ultrapure water as the solvent [23]. These methods aim to reduce the environmental burden associated with traditional HPLC techniques while maintaining robust analytical performance [24].

Table 1. The physicochemical properties of AA.

Property	Value
Name	17-(pyridin-3-yl)androsta-5,16-dien-3 β -yl acetate
Formula	C ₂₆ H ₃₃ NO ₂
Structure	
Molecular weight	391.55 Daltons
Melting point	144–145 °C
Log P	3.97
pKa/Strongest acidic	18.32
pKa/Strongest basic	4.81
Solubility	Soluble in organic solvents such as ethanol (28 mg/mL at 25 °C), dimethylformamide (~16 mg/mL), and dimethylsulfoxide (~2 mg/mL). Very slightly soluble in aqueous buffers (<1 mg/mL at 25 °C).

2. Materials and Methods

2.1. Instruments

Chromatographic analyses were performed using an Agilent 1260 system (Agilent Technologies, Palo Alto, CA, USA), which includes a UV-Vis Detector, a Quaternary Pump, a Vacuum Degasser, a Column Oven, and ChemStation Software (B.04.03 (November, 2010)) for data acquisition and analysis. The chromatographic separation was achieved with an Agilent Extend C18 column (250 mm × 4.6 mm, 5 μ m). Spectrophotometric analyses were conducted with a UV-1800 dual-beam spectrophotometer (Shimadzu, Kyoto, Japan), using 1.0 cm quartz cuvettes and UV-Probe Software (version 2.70) for data analysis. pH measurements were made with a Mettler-Toledo pH meter (Mettler Toledo, Greisensee, Switzerland) equipped with a glass electrode. Ultrapure water for the experiments was produced using a Millipore Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Reagents and Chemicals and Materials

All solvents utilized were of gradient-grade purity suitable for liquid chromatography, including acetonitrile ($\geq 99.9\%$), methanol ($\geq 99.0\%$), ethanol ($\geq 99\%$), and formic acid ($\geq 99.0\%$). These solvents were sourced from Sigma-Aldrich Chemie GmbH (Istanbul, Turkey). The United States Pharmacopeia (USP) reference standard of Abiraterone Acetate (AA) used in the study had a purity of $\geq 99.5\%$. AA tablets (Zytiga, 250 mg) were obtained from a local pharmacy in Afyonkarahisar, Turkey. Ultrapure water, with a resistivity of 0.080 μ S cm^{−1}, was used for the preparation of all solutions and the mobile phase. The mobile phase was filtered through a 0.20 μ m membrane filter using a vacuum pump and was sonicated prior to the analyses to ensure optimal purity and performance during chromatography.

2.3. Pharmaceutical Solutions

The Stock Standard Solution ($500 \mu\text{g mL}^{-1}$) was prepared by accurately weighing 25 mg of the USP reference standard AA and transferring it to a 50 mL volumetric flask. Ultrapure water (20 mL) was added, and the mixture was sonicated for 6 min to achieve complete dissolution. The volume was then adjusted to 50 mL with ultrapure water. Standard solutions (5, 10, 15, 20, 25, and $30 \mu\text{g mL}^{-1}$) were prepared by performing serial dilutions of the stock standard solution with ultrapure water to achieve the desired concentrations within the range of 5 to $30 \mu\text{g mL}^{-1}$. The sample solution ($25 \mu\text{g mL}^{-1}$) was prepared by accurately weighing ten Zytiga tablets (250 mg each) and recording the average mass per tablet. The tablets were crushed in a dry, clean mortar and ground to a fine powder, which was thoroughly mixed. A 50 mg portion of the powdered tablet, equivalent to AA, was transferred to a 100 mL volumetric flask. Approximately 40 mL of ultrapure water was added, and the mixture was shaken on a rotary shaker for 30 min to ensure complete dissolution. The volume was adjusted to 100 mL with ultrapure water, and the solution was sonicated for 10 min. Finally, the solution was filtered through a $0.45 \mu\text{m}$ membrane filter to obtain the stock sample solution, which was diluted with ultrapure water to achieve a final concentration of $25 \mu\text{g mL}^{-1}$.

2.4. Determination of λ_{Max}

Six standard solutions (5, 10, 15, 20, 25, $30 \mu\text{g mL}^{-1}$) were prepared from the stock standard solution in the concentration range of 5– $30 \mu\text{g mL}^{-1}$, each with three parallels. These solutions were analyzed using a UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan) in the wavelength range of 200 to 400 nm. The spectrophotometer was calibrated using a blank solution (solvent only) before each scan. The maximum absorbance (λ_{Max}) for AA was determined by analyzing the absorbance spectra and identifying the wavelength at which the highest absorbance occurred. All measurements were conducted at room temperature, and each concentration was scanned in triplicate to ensure accuracy and reproducibility.

2.5. Development of Methods and Optimization of Conditions

Chromatographic conditions were carefully optimized to achieve superior peak parameters, including well-defined peak shapes, minimal tailing factors, reduced retention times, and high theoretical plate numbers. Initial experiments involved the evaluation of various mobile phases with different buffer systems, but none met the necessary system compatibility requirements. Additionally, several types and lengths of columns were tested, but these also failed to provide the desired system compatibility parameters.

Optimal peak parameters were obtained using an Extend C18 column (250 mm $\times$ 4.6 mm, $5 \mu\text{m}$). Several mobile phase compositions were evaluated, including mixtures of water/methanol, water/acetonitrile, and water/ethanol. Initially, a mobile phase composed of acetonitrile and ultrapure water (20/80, *v/v*) resulted in excessively prolonged analysis times. To shorten the analysis time, the water component was acidified with formic acid to a pH of 2.0. This pH was selected because it is significantly below the pKa of the analyte, ensuring that the analyte remains protonated and soluble, which is crucial for achieving optimal retention and separation on the C18 column. Under these optimized conditions, the sample solution was injected to assess potential impurities that could interfere with the analyte peak or residual drug matrix components that might persist on the column. Sequential sample injections were performed, with a total analysis time of 6 min. No carryover of impurities was observed between injections, confirming the suitability of the 6 min analysis time. The column temperature was maintained at 25°C , selected for its ability to enhance column efficiency, maintain low column pressure, improve peak shape, and ensure cost-effectiveness. Spectral characterization was performed during the calibration process, focusing on critical factors that influence the accuracy of spectrophotometric methods, including wavelength accuracy, spectral bandwidth, stray light, and linearity. These characteristics are essential for achieving reliable and reproducible analytical results.

For spectrophotometric analysis, the spectral characteristics of AA were assessed in various solvents, including ultrapure water, ethanol, methanol, and isopropyl alcohol. Ultrapure water was selected as the solvent as it provided the most favorable spectral properties for AA. Standard solutions of AA exhibited maximum absorbance at 253 nm, and absorbance measurements for both the standard and sample solutions were recorded at this wavelength.

2.6. Validation of Analytical Methods

The analytical methods developed for the quantification of AA were rigorously validated according to the ICH Q2 (R1) guidelines [6,25]. The validation process included the following parameters: (1) Selectivity was assessed to ensure the method's ability to accurately identify and quantify AA in the presence of other substances. (2) System suitability was verified to confirm that the chromatographic system functioned properly and met predefined criteria. (3) Linearity was evaluated to determine the method's capability to produce results proportional to the concentration of AA within the specified range. (4) Precision was analyzed by examining both repeatability and reproducibility under consistent conditions. (5) Sensitivity was measured by identifying the lowest concentration of AA that could be reliably detected and quantified. (6) Robustness was tested to evaluate the method's reliability under minor variations in operational conditions.

2.7. Selectivity and System Suitability of Methods

To evaluate the selectivity of the chromatographic method, solutions of standard, sample, and mobile phase were injected into the HPLC system. The retention time (R_t) of AA in the chromatograms of both commercial formulations and sample solutions was compared to that of the standard solution to ensure accurate identification and quantification of AA. The system suitability of the chromatographic method was assessed by injecting a $25 \mu\text{g mL}^{-1}$ standard solution of AA into the system at six regular intervals. The following parameters were recorded from the chromatograms: peak area, retention time, tailing factor, and number of theoretical plates. The relative standard deviation (RSD) values for peak area and retention time were calculated to evaluate the precision and consistency of the method.

The selectivity of the spectrophotometric method was assessed by scanning the spectra of standard solutions, sample solutions, and the solvent (ultrapure water) within the wavelength range of 200 to 400 nm. The spectra obtained were analyzed to identify any potential interfering bands and to confirm the absence of overlaps or interferences. For evaluating the system's suitability, absorbance values of a $25 \mu\text{g mL}^{-1}$ standard solution of AA were recorded in six separate measurements. The RSD of these absorbance values was computed to determine the precision and reliability of the method.

2.8. Evaluation of Linearity of Chromatographic and Spectrophotometric Methods

The linearity of the chromatographic method was evaluated by injecting six standard solutions with concentrations ranging from 5 to $30 \mu\text{g mL}^{-1}$ into the HPLC system. This procedure was conducted in triplicate over three separate days. Peak areas corresponding to each concentration were recorded, and calibration curves were constructed by plotting concentrations on the x -axis against peak areas on the y -axis. Regression analysis was performed using the least squares method. Linearity was assessed based on the absolute mean recovery, RSD, and the R -value of the calibration curve.

Similarly, the linearity of the spectrophotometric method was determined by measuring the absorbance values of six standard solutions with concentrations ranging from 5 to $30 \mu\text{g mL}^{-1}$. These measurements were taken in triplicate across three different days, and absorbance values were recorded for each concentration. Calibration curves were plotted with concentrations on the x -axis and absorbance values on the y -axis. Regression analysis was carried out using the least squares method. Linearity was evaluated by analyzing the absolute mean recovery, RSD, and R -value of the calibration curve.

2.9. Evaluation of Sensitivity

The sensitivity of the chromatographic and spectrophotometric methods was evaluated by calculating the limits of detection (LOD) and limits of quantification (LOQ). These limits were established using the standard deviations of the intercept and slope of the calibration curves, following the methodologies outlined by according to Zhao et al. [26] and Shetty and Shah [27]. The calculations were performed using the following equations:

$$\text{LOD} = (3.3 \times \sigma_{\text{intercept}}) / \text{Slope}$$

where $\sigma_{\text{intercept}}$ is the standard deviation of the intercept of the calibration curve.

The LOD and LOQ were calculated for both methods to assess their ability to detect and quantify low concentrations of AA reliably.

2.10. Precision

The precision of the analytical methods was evaluated by examining both intra-day and inter-day variability. A standard solution of AA at a concentration of $20 \mu\text{g mL}^{-1}$ was analyzed multiple times within a single day. Three replicates ($n = 3$) were conducted for each method (chromatographic and spectrophotometric). Peak areas and retention times were recorded, and RSD values for these parameters were computed. Absorbance values were also measured, and their RSD values were calculated.

Additionally, a standard solution of AA at a concentration of $25 \mu\text{g mL}^{-1}$ was analyzed on three separate days. In total, nine replicates ($n = 9$) were performed for each method. Peak areas and retention times were recorded, and RSD values for these measurements were determined. Absorbance values were measured, and their RSD values were calculated. This evaluation confirmed that the methods yielded consistent and reproducible results both within a single day and across multiple days.

2.11. Accuracy

The accuracy of the analytical methods was evaluated using the standard addition method to determine the percentage recovery of AA. The procedure included the following steps: Preparation of Solutions, where a pre-analyzed AA solution at a concentration of $10 \mu\text{g mL}^{-1}$ was utilized. Additional AA standards were then introduced to achieve final concentrations of $25 \mu\text{g mL}^{-1}$, corresponding to 75%, 100%, and 125% of the analyte added. Analysis was conducted by re-quantitatively analyzing the spiked solutions using the developed chromatographic and spectrophotometric methods. The amount of AA recovered from each spiked solution was determined, and the percentage accuracy was calculated using the following formula:

$$\text{Percent Recovery} = \left(\frac{\text{Amount Recovered} - \text{Amount Initially Present}}{\text{Amount added}} \right)$$

The process was repeated six times ($n = 6$) for each concentration level to ensure reliability.

2.12. Robustness

The robustness of the chromatographic method was evaluated by introducing minor, deliberate modifications to the method conditions. Adjustments were made to the mobile phase flow rate ($\pm 0.1 \text{ mL min}^{-1}$), variations in the organic modifier content of the mobile phase were tested ($\pm 2\%$), and the detection wavelength was altered ($\pm 2 \text{ nm}$). Following each modification, a standard solution of AA ($25 \mu\text{g mL}^{-1}$) was injected into the chromatographic system. System suitability parameters, including peak area, retention time, tailing factor, and number of theoretical plates, were recorded and compared with results obtained under the original conditions. Each modification was tested with three replicate analyses of the standard solution to ensure consistency.

The robustness of the spectrophotometric method was assessed by implementing minor changes, including substituting ethanol with isopropyl alcohol and adjusting the detec-

tion wavelength (251 and 253 nm). Absorbance values of a standard solution ($25 \mu\text{g mL}^{-1}$) were measured under each modified condition, and results were compared with those obtained under the original spectrophotometric conditions. Each modification was tested with three replicate analyses of the standard solution to ensure accuracy. This evaluation confirmed that the methods maintained their performance despite minor changes in experimental conditions.

2.13. Application of Analytical Methods to Commercial Formulations

To validate the applicability of the proposed HPLC and spectrophotometric methods for real-world samples, commercial formulations of AA were analyzed. Specifically, the tablet form of AA (Zytiga, 250 mg) was examined using both methods to assess their performance in a practical setting. The chromatographic method involved the analysis of the commercial tablets using the developed HPLC technique. Similarly, the spectrophotometric method involved the analysis of the same tablets using UV spectrophotometry. The parameters evaluated included assay, purity, and content uniformity of the formulations.

2.14. Greenness Evaluation of Analytical Methods

Various techniques are being developed to assess the sustainability of newly established analytical methods in line with the recent advancements in green analytical chemistry (GAC) principles. Among these tools, the Analytical GREEnness (AGREE) metric and the Green Analytical Procedure Index (GAPI (2018)) have gained prominence. Both metrics use a color-coded system (red, yellow, and green) to evaluate and communicate the environmental impact of different analytical approaches [28–30].

2.15. Statistical Analysis

The results from both analytical methods were statistically compared to evaluate their consistency, accuracy, and reliability. A Student's *t*-test was employed to compare the mean values obtained from each method, assessing any significant differences between them. Additionally, the variability of the results was evaluated by comparing their standard deviations, providing insights into the precision of each method. This statistical evaluation demonstrated the effectiveness and robustness of the proposed methods for quantifying AA in commercial pharmaceutical formulations.

3. Results

3.1. Determination of the Wavelength (λ_{Max})

The overlaid UV-Vis absorption spectra AA standard solutions in the concentration range of $5\text{--}30 \mu\text{g mL}^{-1}$ are presented in Figure 1. The spectra exhibited a clear absorption maximum (λ_{max}) at 253 nm for all concentrations. This peak intensity increased proportionally with higher concentrations of AA, confirming the detection wavelength at 253 nm. This wavelength was identified as the most suitable for further analyses due to its consistent and distinct absorbance across all tested concentrations.

3.2. Development of Methods

The absorbance spectra of AA in different solvents (ultrapure water, ethanol, methanol, and isopropyl alcohol) are illustrated in Figure 2. Ultrapure water displayed the highest clarity with the most distinct spectral profile, maintaining strong absorbance with minimal background noise. In contrast, ethanol exhibited a lower absorbance and significant baseline interference. Methanol and isopropyl alcohol also produced clear spectra but with slightly reduced absorbance compared to ultrapure water. Based on these observations, ultrapure water was selected as the optimal solvent for subsequent UV spectrophotometric analysis due to its superior absorbance and minimal interference across the tested wavelengths.

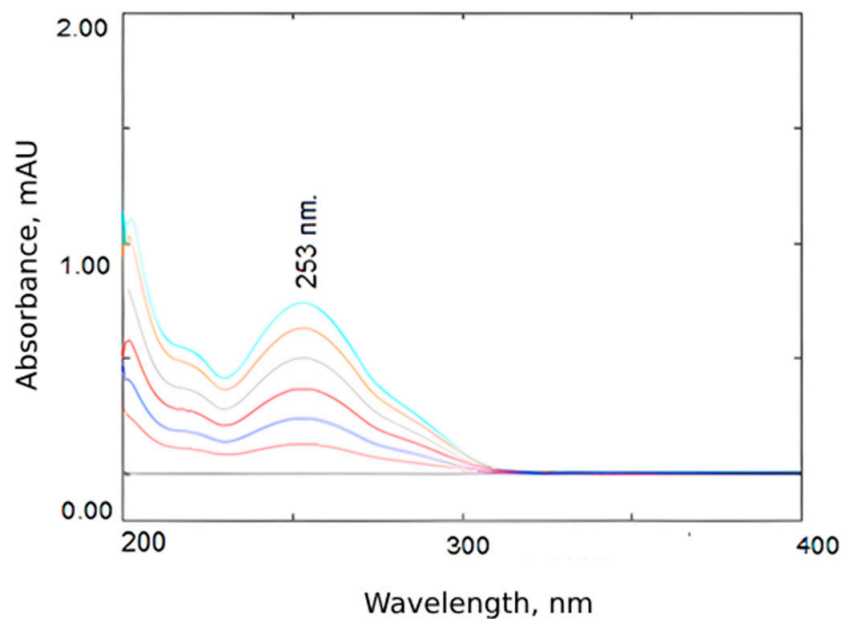


Figure 1. The overlaid spectrum of AA standard solutions in the concentration range of 5–30 $\mu\text{g mL}^{-1}$.

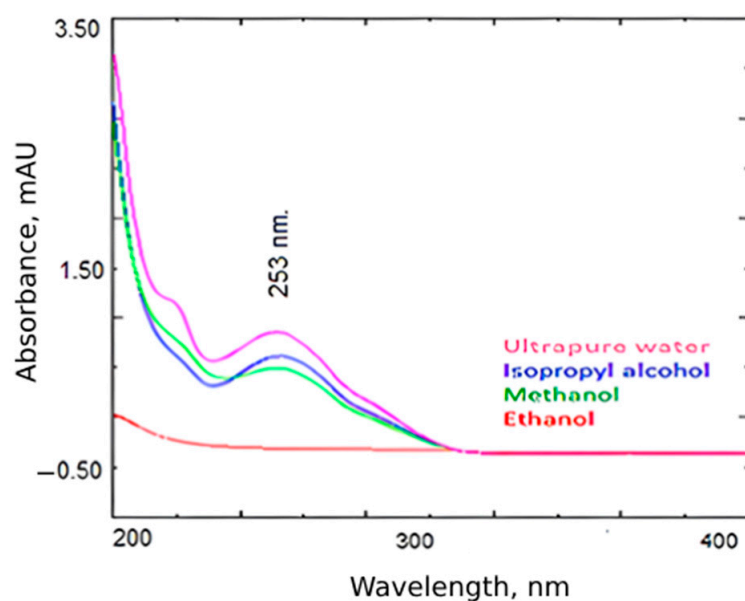


Figure 2. The overlaid spectrum obtained using different solvents (ultra-pure water, ethanol, methanol, and isopropyl alcohol).

3.3. Selectivity Evaluation for the Chromatographic Method

The chromatograms of the standard solution ($30 \mu\text{g mL}^{-1}$), sample solution ($25 \mu\text{g mL}^{-1}$), placebo, and ultra-pure water are shown in Figure 3. The standard and sample solutions exhibited a sharp and well-resolved peak at approximately 4.0 min retention time. The standard solution displayed a higher peak intensity (approximately 80 mAU) compared to the sample solution (~ 60 mAU), indicating a higher concentration of the analyte. In contrast, the placebo and ultra-pure water chromatograms showed no peaks, confirming the specificity of the method and the absence of interference from excipients or solvents. The peak symmetry and retention time consistency between the standard and sample solutions demonstrate the reliability and reproducibility of the analytical method employed.

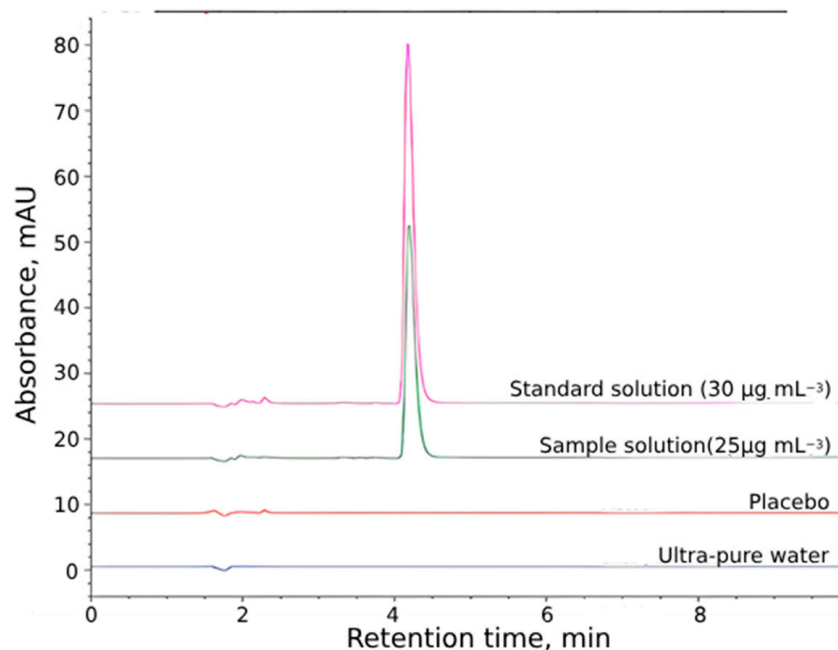


Figure 3. Chromatogram of standard, sample, placebo, and ultra-pure Water.

3.4. Selectivity Evaluation for the Spectrophotometric Method

Figure 4 presents the UV absorption spectra of three solutions: a standard solution ($30 \mu\text{g mL}^{-1}$), a sample solution ($25 \mu\text{g mL}^{-1}$), and ultra-pure water, measured over a wavelength range of 200 to 400 nm. Both the standard and sample solutions display similar spectral profiles, characterized by two distinct absorption peaks. The first and most pronounced peak occurs within the range of 215–220 nm, followed by a broader and less intense peak between 260–270 nm. The standard solution, with its higher concentration, exhibits marginally greater absorbance values than the sample solution throughout the spectrum. Below 210 nm, both the standard and sample spectra show a sharp increase in absorbance, indicative of the presence of the analyte. In contrast, the spectrum for ultra-pure water remains consistently flat, with absorbance values close to zero across the entire wavelength range, effectively serving as a reference baseline.

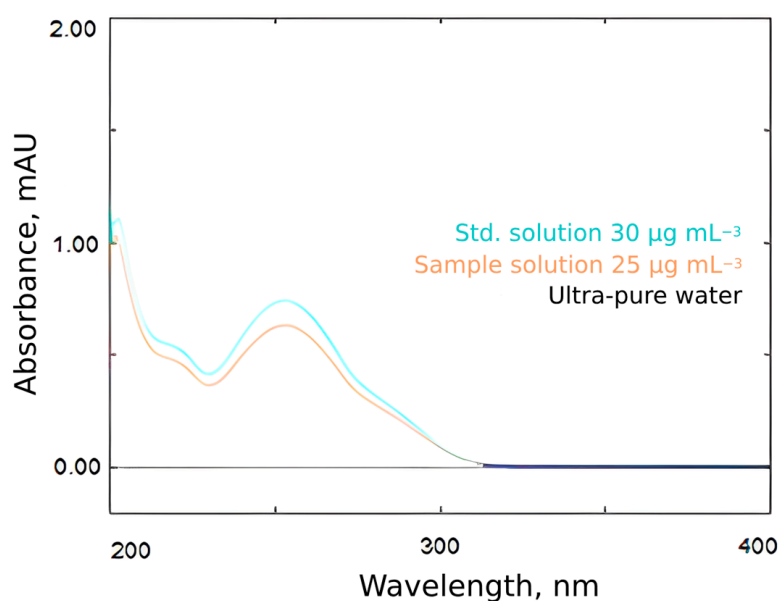


Figure 4. Spectrum of standard, sample, and ultra-pure water.

3.5. System Suitability

Table 2 shows the results of the system suitability test for both liquid chromatography and UV spectrophotometry techniques. The average retention time for the liquid chromatography was 4.197 min, with minimal deviation (RSD = 0.211%), ensuring consistent chromatographic performance. The peak area had an average of 341.02, and the theoretical plate count averaged 5671, indicating high column efficiency. UV spectrophotometry showed an average absorbance of 0.626 with an RSD of 1.094%, showing good consistency and precision.

Table 2. The results of the system suitability test.

Sample	LCT			UVST	
	PA	RT (min)	PT	TPC	Absorbance
1	340.08	4.204	1.542	5666	0.632
2	339.72	4.194	1.558	5657	0.619
3	339.85	4.207	1.545	5674	0.620
4	341.61	4.186	1.548	5683	0.631
5	342.84	4.189	1.548	5656	0.621
6	342.04	4.204	1.561	5687	0.634
Average value	341.02	4.197	1.550	5671	0.626
SD	1.315	0.009	0.008	13.065	0.007
RSD	0.386	0.211	0.484	0.230	1.094

LCT, liquid chromatography technique; UVST, UV spectrophotometry technique; PA, peak area; RT, retention time; PT, peak tailing; TPC, tropic plate count; SD, standard deviation; RSD, relative standard deviation.

3.6. Linearity and Sensitivity

Table 3 summarizes the regression data for both analytical methods. The liquid chromatography technique had a linearity range of 5–30 µg/mL with a slope of 13.793 and a coefficient of determination (R^2) of 0.9999. The UV spectrophotometry technique also displayed strong linearity ($R^2 = 0.9998$) with a slightly lower slope of 0.0252. The lower LOD and LOQ were better for the liquid chromatography method (LOD = 0.40 µg/mL, LOQ = 1.10 µg/mL) compared to UV spectrophotometry (LOD = 0.70 µg/mL, LOQ = 2.00 µg/mL).

Table 3. Regression data of analytical methods.

Parameter	LCT	UVST
Linearity range/µg mL ⁻¹	5–30	5–30
Regression equation ($y = mx + n$)		
Slope (m)	13.793	0.0252
Intercept (n)	−3.187	−0.0022
Coefficient of determination (r^2)	0.9999	0.9998
LOD/µg mL ⁻¹	0.40	0.70
LOQ/µg mL ⁻¹	1.10	2.00
Recovery % [$n = 6$]	99.59–100.58	98.57–100.48

For both analytical methods, a calibration graph was constructed by plotting absorbance values (for the spectrophotometric method) and peak areas (for the chromatographic method) against the concentration of the standard solution. The linearity of each method was evaluated using regression analysis; LCT, liquid chromatography technique; UVST, UV spectrophotometry technique; LOD, limits of detection; LOQ, limits of qualification.

3.7. Precision

Table 4 provides precision results for both methods. The intraday precision for liquid chromatography had an average retention time of 4.201 min and a peak area of 341.15, with RSDs of 0.101% and 0.225%, respectively. Similarly, UV spectrophotometry displayed an intraday absorbance RSD of 0.236%. Inter-day precision for both methods was also high, indicating robustness and repeatability.

Table 4. Precision results of analytical methods.

Precision		LCT		UVST		
		RT (min)	PA	Assay (%)	Absorbance	Assay (%)
Intraday	Average value	4.201	341.15	100.04	0.624	100.11
	SD	0.004	0.766	0.225	0.001	0.236
	RSD	0.101	0.225	0.225	0.236	0.236
Inter day	Average value	4.203	341.08	100.12	0.933	100.24
	SD	0.006	1.199	0.352	0.002	0.327

UVST, UV spectrophotometry technique; PA, peak area; RT, LCT, liquid chromatography technique; retention time; SD, standard deviation; RSD, relative standard deviation.

3.8. Accuracy

Table 5 highlights the accuracy results. For liquid chromatography, the average recovery rates across different standard addition levels (75%, 100%, 125%) ranged from 99.49% to 99.86%, with low RSD values (<0.3%). The UV spectrophotometry method also demonstrated good recovery rates, although the RSD values were slightly higher than those of liquid chromatography, especially at the 75% addition level (RSD = 0.476%).

Table 5. Accuracy results of analytical methods.

Technique	Standard Addition Level (%)	Standard Addition Amount ($\mu\text{g mL}^{-1}$)	Average Recovery (%)	SD	RSD
LCT	75	15	99.49	0.281	0.282
	100	20	99.72	0.189	0.190
	125	25	99.86	0.131	0.131
UVST	75	15	99.31	0.473	0.476

LCT, liquid chromatography technique; UVST, UV spectrophotometry technique; SD, standard deviation; RSD, relative standard deviation.

3.9. Robustness Evaluation

Table 6 outlines the robustness of both analytical methods under various system conditions. For liquid chromatography, recovery percentages remained within 99.44% to 100.01% across different flow rates, ethanol content, and detection wavelengths. Similarly, the UV spectrophotometry method maintained high recovery percentages (99.09%–99.82%) across different solvents and detection wavelengths, demonstrating both methods' robustness.

Table 6. The results of robustness tests for analytical methods (n = 3).

Method	System Conditions	Values	Average Recovery (%)	RSD
LCT	Normal conditions		100.01	0.47
	The high flow rate (mobile phase)	1.10 mL min ^{−1}	99.59	0.54
	The low flow rate (mobile phase)	0.90 mL min ^{−1}	99.44	0.58
	High detection wavelength	255 nm.	99.62	0.40
	Low detection wavelength	251 nm.	99.67	0.50
	The high ethanol content (mobile phase)	62%	99.64	0.43
	The low ethanol content (mobile phase)	58%	99.51	0.46
UVST	Normal conditions		99.82	0.64
	High detection wavelength	255 nm.	99.45	0.62
	Low detection wavelength	251 nm.	99.26	0.73
	Solvent	Ethanol	99.37	0.68
	Solvent	Isopropyl alcohol	99.09	0.66

LCT, liquid chromatography technique; UVST, UV spectrophotometry technique; RSD, relative standard deviation.

3.10. Application of Analytical Methods to Pharmaceutical Formulations

Table 7 provides a statistical evaluation of AA tablet analysis using both methods. Both techniques yielded consistent assay results, with average values near 100% for both methods (liquid chromatography: 100.00%, UV spectrophotometry: 100.00%). The t-test and F-test values indicated no significant differences between the two methods.

Table 7. Statistical evaluation of analysis results of AA tablets (Zytiga, 250 mg).

Sample	LCT		UVST	
	mg in Tablet	Assay (%)	mg in Tablet	Assay (%)
1	249.770	99.93	250.27	100.10
2	249.620	99.87	249.95	99.97
3	250.030	100.04	250.17	100.06
4	249.990	100.02	249.98	99.98
5	249.930	100.00	250.03	100.00
6	250.310	100.15	249.74	99.89
Average value	249.94	100.00	250.02	100.00
SD	0.236	0.094	0.184	0.074
RSD	0.094	0.094	0.074	0.074
$t_{\text{value}}/t_{\text{table}}$	2.51/6.39			
$F_{\text{value}}/F_{\text{table}}$	0.013/2.78			

To evaluate the consistency and reliability of the two methods, statistical tests were performed: Student's *t*-test Used to compare the mean results of the chromatographic and spectrophotometric methods. The calculated *t*-values were compared with tabulated values at a significance level of 0.05. The results indicated that the means of both methods were not significantly different from each other. Fisher's F-Test Applied to assess the variability in results (standard deviations) between the two methods. The calculated *F*-values were compared with tabulated values to determine if there were significant differences in precision. LCT, liquid chromatography technique; UVST, UV spectrophotometry technique; SD, standard deviation; RSD, relative standard deviation.

3.11. Greenness Evaluation of Analytical Methods

Figure 5 illustrates the AGREE pictograms comparing the environmental impact of three analytical methods: the USP official method (A), the chromatographic method (B), and the spectrophotometric method (C). The AGREE score for the USP official method (0.58) indicates a lower adherence to green chemistry principles, primarily due to the use of hazardous solvents and higher waste generation. In contrast, the chromatographic method, with an AGREE score of 0.78, demonstrates significant improvement in environmental performance, largely due to the substitution of ethanol as a more sustainable solvent. The spectrophotometric method achieves the highest AGREE score (0.81), reflecting its use of ultrapure water as the solvent, which minimizes waste and exposure to harmful chemicals.



Figure 5. AGREE pictograms comparing the environmental impact of three analytical methods. (A) AGREE pictogram of the USP official method, (B) AGREE pictogram of the chromatographic method, (C) AGREE pictogram of the spectrophotometric method. In (C), Operation 3 is represented by the green color, reflecting the use of ultrapure water as an environmentally friendly solvent.

Figure 6 shows a comparison of two green analytical chemistry pictograms, labeled A (88) and B (89), which appear to represent GAPI assessments for two different analytical methods—a chromatographic method and a spectrophotometric method for AA determination. Both pictograms feature a similar structure with pentagon shapes arranged around a central red hexagon, enclosed by a green circular outline. The key difference between

the two is that pictogram A contains an additional yellow pentagon segment compared to pictogram B. The numerical values (88 and 89) likely represent greenness scores for each method, with the spectrophotometric method (B) scoring slightly higher, suggesting it may be marginally more environmentally friendly or sustainable according to GAPI criteria. These visualizations effectively allow for quick comparison of the relative “greenness” of the two analytical procedures.



Figure 6. (A) GAPI pictogram of the chromatographic method, (B) GAPI pictogram of the spectrophotometric method.

4. Discussion

The findings of this study demonstrate the efficacy and environmental benefits of the proposed Green LC and Simplified Spectrophotometric Methods for the quantification of AA in pharmaceutical formulations. These methods were rigorously validated, showing strong consistency with established techniques such as reverse-phase HPLC and LC-MS/MS, which are commonly used for AA analysis [23,31]. However, what sets this study apart is the integration of green chemistry principles, which prioritize sustainability alongside analytical performance.

The use of ethanol as the organic solvent in the Green LC method proved highly effective, with chromatographic performance comparable to that of traditional solvents like acetonitrile and methanol [3]. This is supported by previous research that highlights ethanol’s acceptable elutropic strength and reduced environmental footprint, making it a suitable alternative for HPLC applications [9]. Despite ethanol’s slightly higher viscosity, the method remained efficient, with column pressure easily managed through minor temperature adjustments [10]. Furthermore, the retention times were notably shorter compared to conventional methods, a significant improvement in reducing solvent consumption and waste generation. These findings underscore ethanol’s potential as a sustainable solvent without sacrificing analytical quality. These results underscore the superior environmental sustainability of the chromatographic and spectrophotometric methods compared to the conventional USP approach, positioning them as viable green alternatives for pharmaceutical analysis [32].

The Simplified Spectrophotometric Method, employing ultrapure water as the solvent, provided excellent sensitivity for AA quantification despite ethanol’s higher UV cutoff relative to acetonitrile and methanol. The use of ultrapure water eliminates the need for hazardous organic solvents, addressing both environmental and safety concerns. This eco-friendly approach aligns with the principles of green analytical chemistry, emphasizing reduced environmental impact without compromising method sensitivity. By utilizing methods that minimize the use of harmful solvents, we enhance the sustainability of our analytical practices. Additionally, the ability to achieve reliable quantification of AA at lower wavelengths adds further merit to this green analytical approach [7,24].

One of the most significant advantages of these methods is the reduction in hazardous waste. By excluding acetonitrile and methanol, the developed techniques not only lower disposal costs but also reduce the environmental hazards associated with conventional solvents [9]. This makes the methods particularly attractive for resource-limited laboratories, where access to high-purity organic solvents can be restricted. Furthermore, ethanol’s low

cost and availability, especially in developing countries, further support its widespread adoption [9].

The statistical comparison using Student's t-test confirmed the consistency and reliability of the proposed methods. Both the Green Liquid Chromatography and Simplified Spectrophotometric Methods produced results comparable to those obtained using traditional methods, with no statistically significant differences in the mean values [23]. The standard deviations were also similar, indicating that the variability of the results was well within acceptable limits, further validating the precision of the methods [31]. These findings reinforce the feasibility of integrating greener solvents into routine analytical procedures without compromising accuracy or reproducibility.

5. Conclusions

In conclusion, the methods developed in this study provide sustainable and efficient alternatives for the quantification of AA in pharmaceutical formulations, significantly reducing the environmental impact compared to conventional techniques. By incorporating ethanol and ultrapure water as greener solvents, the chromatographic and spectrophotometric methods demonstrated excellent analytical performance while adhering to the principles of green chemistry. These advancements offer safer, more eco-friendly solutions for pharmaceutical quality control, aligning with global efforts to minimize hazardous chemical usage and laboratory waste. Furthermore, the high AGREE scores achieved by the new methods highlight their environmental benefits, proving them to be valuable tools in advancing sustainability within analytical chemistry. As the demand for green methodologies continues to grow, these techniques offer a promising direction toward reducing the ecological footprint of pharmaceutical analysis without compromising accuracy or reliability, thereby contributing to safer laboratory practices and the broader goals of environmental preservation.

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