

Analytical Method for Estimation of Enalapril by UV Spectrophotometry Using Hydrotropic Solubilization: AGREE Metric Evaluation

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ABSTRACT

Objectives: The objective of the current study was to design and confirm a green, eco-friendly UV-visible spectrophotometric system of estimating Enalapril maleate using safe and cost-effective solvents. **Materials and Methods:** Several hydrotropic solvents were also screened to improve the solubility of Enalapril and the choice was made on 2 M ammonium acetate, which enhanced the solubility of Enalapril by about 40-fold over distilled water. Its maximum absorbance was registered at 265 nm and was linear at 2-10 µg/mL. The method validation was done according to the ICH guidelines, and the accuracy, precision, sensitivity (LOD and LOQ) and the reliability were evaluated with the help of a marketed formulation (Enam 5 mg). The studies on forced degradation under acidic, basic, neutral, and oxidative environments were conducted to determine stability-indicating character of the method. Method greenness was evaluated using the AGREE software. **Results:** The procedure was proven to be very linear, accurate (mean recovery 98.5%) and precise (%RSD < 2%). LOD was 0.70 µg/mL and LOQ 2.11 µg/mL. The tablet analysis of marketed products depicted 101.7% marking the content. Studies about forced degradation showed stability in neutral and basic media, whereby acidic (14.37%) and oxidative (17.35) conditions were observed to undergo degradation. AGREE score of 0.87 showed that there was remarkable observance of the postulates of green analytical chemistry. **Conclusion:** The designed UV-visible spectrophotometric system is easily obtained, dependable, specific and sustainable to the environment. It can be used in quality control of Enalapril maleate in bulk drug and pharmaceutical preparations of routine quality control of the substance and in promoting green analytical practice.

Keywords: AGREE, Enalapril, Greenness, Stability studies, UV spectroscopy.

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Received: 21-03-2026;

Revised: 08-05-2026;

Accepted: 17-07-2026.

INTRODUCTION

Chemically, enalapril maleate is known as (S)-1-[N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]. A prodrug that is a member of the class of antihypertensive drugs known as Angiotensin-Converting Enzyme (ACE) inhibitors is L-proline maleate. Because of its advantageous pharmacological profile and demonstrated effectiveness, it is frequently used to treat diabetic nephropathy, heart failure, and hypertension (Sweetman, 2011). After oral administration, hepatic esterases hydrolyze enalapril to its active metabolite, enalaprilat, which lowers blood

pressure, reduces peripheral vascular resistance, and inhibits the conversion of angiotensin I to angiotensin II (Burnier & Brunner, 2000; Meredith & Elliott, 1992). The poor aqueous solubility of enalapril maleate (about 0.25 mg/mL at 25°C) despite its clinical significance presents serious difficulties for formulation development and analytical quantification (IPC, 2022). Traditional organic solvents like methanol, ethanol, acetonitrile are used in chromatographic analytical procedures to improve solubility and extraction effectiveness. But according to the current framework of Green Analytical Chemistry (GAC), these solvents are unsustainable due to their toxicity, volatility, and environmental risks (Gałuszka *et al.*, 2013; Płotka-Wasyłka, 2018). Therefore, one of the main concerns in modern pharmaceutical analysis is the need for ecologically friendly analytical techniques that minimize solvent waste, lower toxicity, and conserve resources. Without the use of hazardous organic solvents, hydrotropic solubilization presents a viable environmentally friendly method for increasing



DOI: 10.5530/ijpi.20260258

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the solubility of medications that are not very soluble in water. Hydrotropic agents, such as ammonium salts, sodium benzoate, urea, and nicotinamide, enhance drug solubility through weak non-covalent interactions, including hydrogen bonding, π - π stacking, and molecular aggregation, leading to improved dissolution in aqueous media (Maheshwari, 2006; Etman & Naggar, 1999). By encouraging eco-safety, cost-effectiveness, and operational simplicity, this method not only improves analytical sensitivity but also advances green chemistry principles. Using 2 M ammonium acetate as a hydrotropic solubilizing agent, the current study attempts to develop and validate a straightforward, accurate, and environmentally sustainable UV-Visible spectrophotometric method for the estimation of Enalapril maleate. To guarantee linearity, precision, accuracy, and sensitivity, method validation was carried out in compliance with ICH Q2(R2) guidelines. Additionally, the Analytical GREENness (AGREE[®]) tool, which combines all twelve GAC principles into a single metric assessment, was used to critically assess the method environmental performance (Pena-Pereira *et al.*, 2020; Mundada *et al.*, 2023). In line with the objectives of sustainable pharmaceutical quality control, this study presents an analytical technique that is accurate, repeatable, and environmentally friendly.

MATERIALS AND METHODS

Enalapril as a gift sample by Dr. Reddy's Laboratories, Hyderabad. Analytical grade of Urea and ammonium acetate procured from Lobachemie, Mumbai. Enam 5 tablets purchased from the local market manufactured by Dr. Reddy's Laboratory, Hyderabad. The present study didn't involve in any human participation and animal experiments. Hence, ethical approval from the institution not required.

Methods

Preliminary Studies: Solubility Test

Equilibrium solubility studies were conducted using various hydrotropic agents to determine the most suitable solvent system for Enalapril. Aqueous solutions of different hydrotropic agents, such as urea and ammonium acetate, were prepared individually at specific molar concentrations. A measured volume of each hydrotropic solution was accurately transferred into separate vials, and an accurately weighed quantity of Enalapril (10 mg) was added to each. The vials were placed in a water bath shaker and agitated for 12 hr to ensure saturation, followed by equilibration for 24 hr to attain equilibrium solubility. The mixtures were then filtered, and the solubility of Enalapril in each hydrotropic system was determined in triplicate, Enalapril was found to be freely soluble and stable in 2M ammonium acetate solution as shown in Table 1. The solubility enhancement ratio was calculated accordingly (Joshi *et al.*, 2019; Venkatachalam *et al.*, 2022).

Preparation of Standard Solution

Enalapril (10 mg) was accurately weighed and transferred into a clean, dry 10 mL volumetric flask. About 2 mL of 2 M ammonium acetate solution was added, and the flask was sonicated to dissolve the drug completely. The volume was then made up to the mark with the same solvent to obtain a concentration of 1 mg/mL (Stock Solution I). From this, 5 mL of Stock Solution I was accurately transferred into a 50 mL volumetric flask, and the volume was made up to the mark with distilled water to obtain Stock Solution II. A 1 mL portion of Stock Solution II was further transferred into a 10 mL volumetric flask and diluted to the mark with distilled water to prepare the working standard solution used for analysis. Twenty tablets of Enalapril (Enam 5 mg) were weighed and finely powdered. A quantity of the powder equivalent to 50 mg of Enalapril was accurately transferred into a 100 mL volumetric flask, and about 20 mL of 2 M ammonium acetate solution was added. The mixture was sonicated to dissolve the drug completely, and the volume was made up to the mark with distilled water to obtain the sample stock solution. From this, suitable aliquots were taken and diluted with distilled water to obtain concentrations within the Beer's law range for further analysis.

Determination of the Absorption Maxima ($\lambda_{\max}$) of Enalapril

The working standard solution of Enalapril was scanned using a UV-visible spectrophotometer over the wavelength range of 200-400 nm employing 2 M ammonium acetate as the solvent and blank. The $\lambda_{\max}$ for Enalapril was observed as shown in Figure 1. This wavelength was selected for the preparation of the calibration curve and for subsequent estimation of Enalapril in bulk and tablet dosage forms (Jain *et al.*, 2012).

Preparation of standard solution for Calibration Curve

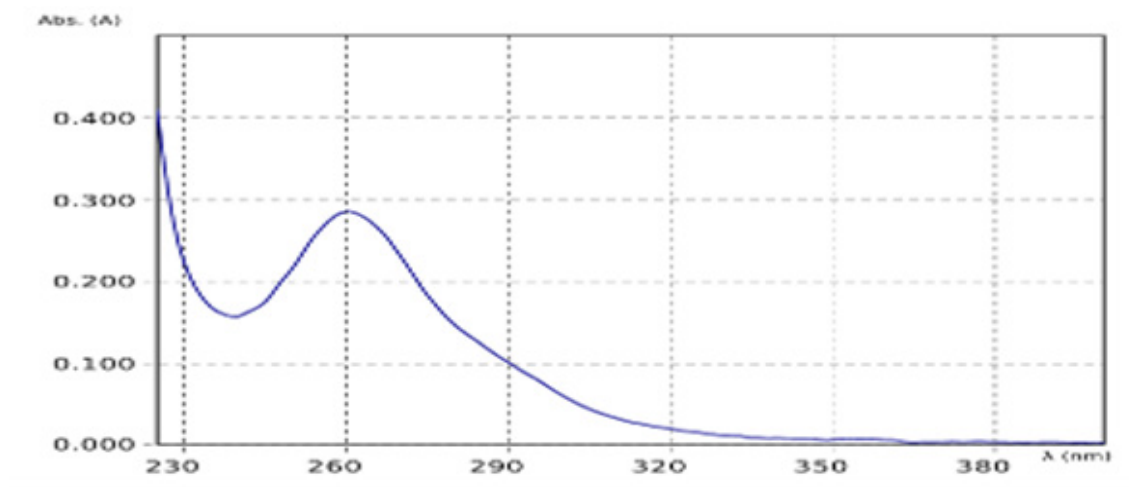
From the working standard solution of Enalapril, aliquots of varying volumes were accurately transferred into a series of 10 mL volumetric flasks and diluted to the mark with distilled water to obtain solutions of different concentrations (2-10 μ g/mL). The absorbance of each solution was measured at the selected wavelength ($\lambda_{\max}$) against the reagent blank. A calibration curve was plotted between absorbance and concentration using excel software. The curve was found to obey Beer-Lambert's law within the studied concentration range.

Preparation and Analysis of Sample Solution

A clean, dry 100 mL volumetric flask containing an accurately weighed quantity of tablet powder equivalent to 10 mg of Enalapril was used for analysis. About 70 mL of 2 M ammonium acetate solution was added and sonicated for 10 min to dissolve the drug completely. The volume was made up to the mark with the same solvent to obtain the sample stock solution (1 mg/mL). From

Table 1: Solubility of Enalapril.

Drug	Description	Solvent Used	Result
Enalapril	Weigh accurately 10.0 mg of the sample into a test tube and add 10 mL of the solvents. Place the lid and shake well; if necessary, sonicate for 10 min.	Water	Slightly soluble
		2 M Urea	Slightly soluble
		5 M Urea	Slightly soluble
		0.5 M Ammonium acetate	Slightly soluble
		2 M Ammonium acetate	Freely soluble

**Figure 1:** UV scan spectrum of Enalapril.

this stock, 5 mL was pipetted into a 25 mL volumetric flask and diluted to volume with distilled water to obtain a working sample solution. An aliquot of 1 mL of this solution was transferred to a 10 mL volumetric flask and diluted to the mark with distilled water to achieve the desired analytical concentration. The absorbance of the sample solution was measured at the selected $\lambda_{\max}$, and the concentration of Enalapril was determined using the calibration curve.

Validation of the Method

The developed UV spectrophotometric method for Enalapril was validated as per ICH guidelines for various parameters, including linearity, accuracy (recovery studies by the spiking method), precision (repeatability and intermediate precision), robustness, and specificity. The results demonstrated that the method is simple, accurate, precise, and reliable for the routine estimation of Enalapril in bulk and tablet dosage forms (Mallikarjuna *et al.*, 2011; Sowjanya *et al.*, 2012; Gherman *et al.*, 2013).

Forced Degradation Studies of Enalapril

Forced degradation studies were performed to evaluate the stability of Enalapril under various stress conditions, including basic, acidic, oxidative, and neutral environments (Saad *et al.*, 2018). A standard drug solution of Enalapril (10 µg/mL) was used for all degradation studies. For basic degradation, 2 mL of the standard drug solution was transferred into a 10 mL volumetric flask, and the volume was made up to the mark with 0.5 M

NaOH. Two separate samples were prepared, one maintained at room temperature and the other at 60°C. The treated samples were analysed spectrophotometrically at 265 nm on the 1st, 3rd and 5th day to monitor the extent of degradation. For acidic degradation, a similar procedure was followed using 0.5 M HCl in place of NaOH. Two dilutions were maintained, one at room temperature and another at 60°C, and the samples were analysed at 265 nm on the 1st, 3rd, and 5th days. In oxidative (peroxide) degradation, 2 mL of the standard drug solution was transferred to a 10 mL volumetric flask, and the volume was made up with 5% hydrogen peroxide solution. The samples were again stored under room temperature and 60°C conditions, and degradation was monitored at 265 nm at specified intervals (1, 3, and 5 days). For neutral degradation, 2 mL of the standard drug solution was diluted to 10 mL with distilled water, and the same storage conditions and analysis schedule were applied. In all cases, the absorbance of the treated samples was measured using a UV-visible spectrophotometer at 265 nm against a suitable blank, and the results were compared with those of the untreated control sample to assess the percentage degradation and stability profile of Enalapril under each condition (ICH, 2003; Blessy *et al.*, 2014).

RESULTS AND DISCUSSION

Solubility studies

In equilibrium solubility studies, a substantial enhancement in the solubility of Enalapril was observed in 2 M sodium acetate hydrotopic solution compared to solubility in distilled water,

were shown in Table 2. Enalapril was found to exhibit maximum solubility in 2 M ammonium acetate solution (2.00% w/v), showing a 40-fold solubility enhancement compared to distilled water. Therefore, 2 M ammonium acetate was selected as the optimised hydrotropic agent for further analytical and method development studies.

Selection of Wavelength

The wavelength for Enalapril estimation in 2 M ammonium acetate solution was selected based on the drug's absorption maxima obtained from the UV spectrum scan. The $\lambda_{\max}$ of Enalapril was found to be 265 nm in 2 M ammonium acetate solution, were shown in Figure 1, indicating optimal sensitivity for quantitative analysis using the hydrotropic method.

The stock standard solution of Enalapril was appropriately diluted with distilled water to obtain concentrations ranging from 2 to 10 $\mu\text{g/mL}$. The absorbance of each solution was recorded at the selected wavelength of 265 nm using a UV-visible spectrophotometer. The calibration curve was then plotted between concentration ($\mu\text{g/mL}$) and absorbance, showing a linear relationship as per Beer-Lambert's law and the result data were shown in Figure 2, Tables 3 and 4. Linearity was observed in the concentration range of 2-10 $\mu\text{g/mL}$ with an excellent correlation coefficient, while accuracy studies indicated recoveries within the acceptable range of 99-107%. The low values obtained for LOD (0.70 $\mu\text{g/mL}$) and LOQ (2.11 $\mu\text{g/mL}$) further emphasised

the sensitivity of the proposed method for routine analysis of Enalapril in bulk and pharmaceutical dosage forms.

UV method validation

The developed method for estimating Enalapril was validated as per ICH guidelines. The accuracy of the developed method for Enalapril was evaluated using the recovery (spiking) method at three different concentration levels 80%, 100%, and 120%, corresponding to 4.8, 6.0, and 7.2 $\mu\text{g/mL}$ solutions, respectively. Each level was analyzed in triplicate, keeping the amount of sample constant at 6 $\mu\text{g/mL}$. The mean percentage recovery was found to be 98.5% with an RSD of 1.54%, which is well within the acceptable limits as per ICH guidelines, confirming the accuracy and reliability of the proposed UV spectrophotometric method for the estimation of Enalapril. All Experiments were performed in triplicate ($n=3$) results are Expressed as mean $\pm$ Standard Deviation (SD). Percentage Relative Standard Deviation (%RSD) was calculated to evaluate method precision. Statistical calculations were performed using Microsoft excel. The detailed results were shown in Table 5. The precision of the methods was studied on the same and different days at 265 nm wavelength, and data are shown in Tables 6 and 7. The robustness, reproducibility, and appropriateness of the developed analytical method for routine quantitative analysis of enalapril maleate were confirmed by its high intraday and inter day accuracy and low percentage RSD values.

Table 2: Solubility ratio calculation of Enalapril.

Drug (mg)	Solvent System	Solubility (% w/v)	Temperature ($^{\circ}\text{C}$)	Solubility Enhancement Ratio
Enalapril	Distilled water	0.05	25 $^{\circ}\text{C}$	1.0
Enalapril	2 M Urea	0.06	25 $^{\circ}\text{C}$	1.2
Enalapril	5 M Urea	0.08	25 $^{\circ}\text{C}$	1.6
Enalapril	0.5 M Ammonium acetate	0.10	25 $^{\circ}\text{C}$	2.0
Enalapril	2 M Ammonium acetate	2.00	25 $^{\circ}\text{C}$	40.0

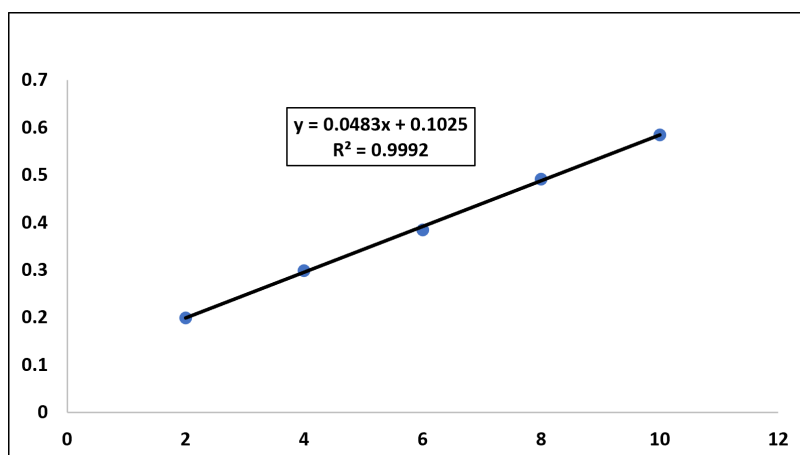


Figure 2: Linearity graph of Enalapril.

Determination of Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined in accordance with ICH Q2(R1) guidelines. The method applied in this study was based on the standard deviation of the response and the slope of the calibration curve. Using this approach, the LOD of Enalapril was calculated to be 0.70 µg/mL, while the LOQ was found to be 2.11 µg/mL. These values indicate that the method was sufficiently sensitive for detecting and quantifying low concentrations of Enalapril.

Estimation of the marketed formulation

The Created and approved method was used to determination of Enalapril foe commercial version. The percentage recovery was found to be 101.2% were shown in Table 8.

Forced degradation studies of Enalapril

The studies on forced degradation demonstrated the stability of the drug across different conditions. Under fundamental conditions, it exhibited a degradation of 5.93% at 60°C and 8.45% at ambient temperature. Under acidic conditions, the level of degradation increased significantly, attaining 9.05% at 60°C and 14.37% at ambient temperature. The oxidative degradation process utilizing hydrogen peroxide achieved a degradation rate of 17.35% at 60°C, in contrast to a mere 3.42% at room temperature. Under neutral conditions, the degradation observed was minimal, recorded at 6.43% for elevated temperatures and 6.85% for room

Table 3: Calibration curve data of Enalapril.

Concentration (µg/mL)	Absorbance (265 nm)
2	0.199
4	0.299
6	0.385
8	0.492
10	0.585

temperature. The drug maintains stability in basic and neutral conditions; however, it shows considerable instability in acidic and oxidative environments, which requires meticulous attention to storage and formulation practices and the results were given in Table 9.

Greenness Evaluation Using AGREE® Score

The greenness evaluation of the developed analytical method was performed using the AGREE® software (Analytical Greenness Metric Approach and Software). The method achieved an overall AGREE® score of 0.87, shown in Figure 3 indicating strong compliance with Green Analytical Chemistry principles. The AGREE pictogram displayed mostly dark green segments, confirming the method's eco-friendly nature. However, minor drawbacks were observed in Principle 3 (limited automation and offline analysis), Principle 5 (relatively high energy consumption), and Principle 10 (inadequate automation leading to operator exposure). Overall, the method demonstrates excellent greenness with minor areas for improvement. The environmental assessment of the method also highlighted its sustainable features,

Table 4: Linearity data of Enalapril.

Sl. No.	Parameters	Enalapril
1.	Working $\lambda_{\max}$	265nm
2.	Linearity range	2 to 10 µg/mL
3.	Regression Equation	$y = 0.0483x + 0.1025$
4.	Slope	0.0483
5.	Intercept	0.1025
6.	Correlation coefficient	$R^2 = 0.9992$
7.	Inter day precision	1.02%
8.	Intraday precision	0.58%
9.	LOD (µg/mL)	0.70 µg/mL
10.	LOQ (µg/mL)	2.11 µg/mL

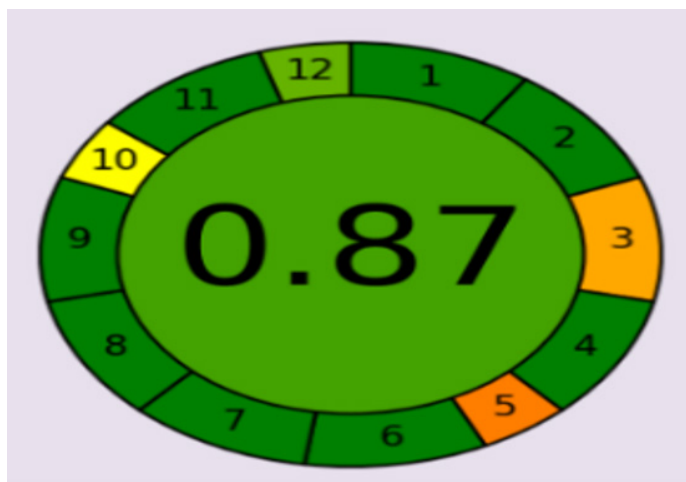


Figure 3: Greenness evaluation of enalapril with proposed method.

Table 5: Accuracy data for Enalapril.

Conc. of drug in tablet sample (µg/mL)	Conc. of drug added to final (µg/mL)	Concentration found (µg/mL)	% Amount recovered
6	4.8	10.64	96.78
6	6	11.95	99.28
6	7.2	13.09	98.07
Mean			98.04
SD			1.25
%RSD			1.28

Table 6: Statistical analysis of intraday precision.

Sl. No.	Concentration (µg/ml)	Absorbance			% RSD
		Day 1	Day 2	Day 3	
1.	2	0.180	0.181	0.181	0.74
2.	6	0.388	0.388	0.388	0.00
3.	10	0.593	0.591	0.5912	0.19
% Average RSD					0.31

Table 7: Statistical analysis of Interday precision.

Sl. No.	Concentration (µg/mL)	Absorbance			% RSD
		Morning	Afternoon	Evening	
1.	2	0.180	0.1805	0.178	0.74
2.	6	0.393	0.393	0.394	0.15
3.	10	0.602	0.592	0.596	0.84
% Average RSD					0.58

Table 8: Assay of Enalapril market formulation.

Trade name	Amount Obtained	Standard deviation	%RSD	Percentage Recovery
Enam 5 mg	5.1 mg	0.001032796	0.183228	101.2

Table 9: Forced Degradation Study Data of Enalapril.

Degradation Type	Experimental Condition	Day 1 (Ao)	Day 3	Day 5 (A)	% Degradation (Ao-A)/ Ao*100
Basic Degradation	At 60°	0.5952	0.5609	0.5599	5.9308%
	At Room temperature	0.4481	0.4247	0.4102	8.4579%
Acidic Degradation	At 60°	0.3977	0.3884	0.3617	9.0520%
	At Room temperature	0.489	0.4678	0.4187	14.3763%
Peroxide Degradation	At 60°	0.4401	0.4102	0.3637	17.3597%
	At room temperature	0.3975	0.3884	0.3839	3.4214%
Neutral Degradation	At 60°	0.5094	0.5053	0.4766	6.4389%
	At room temperature	0.4404	0.4247	0.4102	6.8574%

though some limitations were noted. These challenges could be addressed in future by integrating automated and miniaturized UV platforms and adopting energy-efficient analytical devices, which would further enhance the green credentials of the method (Mehmood *et al.*, 2023).

CONCLUSION

In this study, a simple, reliable, and eco-friendly UV-visible spectrophotometric method was successfully developed for estimating Enalapril maleate using 2 M ammonium acetate as a green hydrotropic solvent. The method showed excellent linearity,

accuracy, and precision, along with a remarkable increase in the drug's solubility. It effectively detected Enalapril in both bulk and tablet forms, demonstrating good recovery and consistency with labelled claims. Stability studies confirmed that the method can identify degradation under acidic and oxidative conditions, proving its stability-indicating ability. With an impressive AGREE® score of 0.87, the method strongly supports the principles of Green Analytical Chemistry, making it a sustainable and practical choice for routine quality analysis.

ACKNOWLEDGEMENT

The authors are thankful to Shri Vishnu College of Pharmacy for providing the necessary facilities.

ABBREVIATIONS

ACE: Angiotensin-Converting Enzyme; **AGREE:** Analytical GREENness; **GAC:** Green Analytical Chemistry; **HCl:** Hydrochloric acid; **ICH:** International Council for Harmonisation; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **NaOH:** Sodium hydroxide; **RSD:** Relative Standard Deviation; **UV-vis:** Ultraviolet Visible.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Lakshmi Prasanthi Nori conceptualized the study and supervised the overall work. Nuziveeti Lakshmi Durga Bhavani conducted the literature review and drafted the initial manuscript. Lankadi Devi performed the hydrotropic solubilization studies. Bolapati Gayathri Priya carried out the UV spectrophotometric method development and analysis. Bikkina Aruna conducted the method validation experiments (accuracy, precision, and linearity studies). Bommina Abhisri performed the statistical analysis of the validation data. G. Yuthika Lakshmi carried out the AGREE metric evaluation for greenness assessment of the developed method. All authors reviewed, revised, and approved the final manuscript.

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Cite this article: Bhavani NLD, Devi L, Priya BG, Aruna B, Abhisri B, *et al.* Analytical Method for Estimation of Enalapril by UV Spectrophotometry Using Hydrotropic Solubilization: AGREE Metric Evaluation. *Int. J. Pharm. Investigation*. 2026;16(4):1550-6.