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RESEARCH ARTICLE

Greenness, Whiteness, and Sustainability Profiling of an Ethanol-Based RP-HPLC Method for Atorvastatin and Clopidogrel in Pharmaceutical Tablets

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Abstract: ***Introduction*****:** Atorvastatin calcium and Clopidogrel bisulfate are frequently combined in fixed doses for use in cardiovascular treatment. Traditional HPLC methods often rely on toxic solvents, underscoring the need for environmentally friendly alternatives.

Methods**:** A green Reverse Phase-HPLC method was developed using a Waters Xterra RP18 column with a 10 mM potassium phosphate buffer (pH 4.0) and ethanol (70:30 v/v) at a flow rate of 1.2 mL/min. Detection occurred at 230 nm. The method was validated for linearity, precision, accuracy, and robustness. Greenness and sustainability were assessed using various green metric tools.

Results/Discussion**:** Atorvastatin and Clopidogrel bisulfate were separated at 2.152 and 3.646 minutes, respectively, with a total runtime of less than 6 minutes. The method demonstrated excellent linearity (regression coefficient > 0.999), precision (%Relative standard deviation < 1%), and recovery (99.4-100.2%). Tablet analysis confirmed consistent drug content (99.8%). Greenness metrics indicated high eco-compliance (AGREE: 0.81; whiteness: 63; Need, Quality and Sustainability: 95%).

Conclusion**:** The validated method is rapid, precise, and sustainable, offering a superior alternative for routine quality control of Atorvastatin and Clopidogrel bisulfate formulations while adhering to green analytical principles.

Keywords: AGREE, atorvastatin, clopidogrel, GAPI, green analytical chemistry, NQS, RGB, RP-HPLC.

1. INTRODUCTION

Cardiovascular Diseases (CVDs) are still the major cause of death around the world. Recent health reports show that these conditions lead to about 18 million deaths each year, which shows their continuous impact on global health [1]. Fixed-Dose Combination (FDC) therapies are now used more often in treatment plans because they help reduce the risk of cardiovascular problems in patients. Notably, the combination of Atorvastatin Calcium (ATV), an 'HMG-CoA reductase inhibitor', and Clopidogrel Bisulfate (CLP), an antiplatelet agent targeting the P2Y12 receptor, is extensively prescribed to lower 'low-density lipoprotein' cholesterol levels and prevent thrombosis following 'acute coronary syndromes' or 'percutaneous coronary intervention [2, 3]. The chemical structures of ATV and CLP are shown in Figs. (**1** and **2**), with molecular formulas $C_{33}H_{35}FN_2O_5$ and $C_{16}H_{16}ClNO_6S_2$, respectively.

Analytical methods for the simultaneous quantification of ATV and CLP are crucial for dosage standardization and routine analysis of pharmaceutical products. 'High-Performance Liquid Chromatography (HPLC)', particularly in reverse-phase mode, is considered the gold standard because of its robustness, accuracy, and sensitivity [4, 5]. Numerous Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) techniques are documented for the 'determination' of ATV [6, 7] and CLP [8, 9], as well as their simultaneous estimation [10, 11]. However, these methods predominantly utilize 'solvents such as 'acetonitrile

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and methanol', which are classified as hazardous under the 'Globally Harmonized System (GHS)' because of their flammability, toxicity, and poor biodegradability [12, 13].

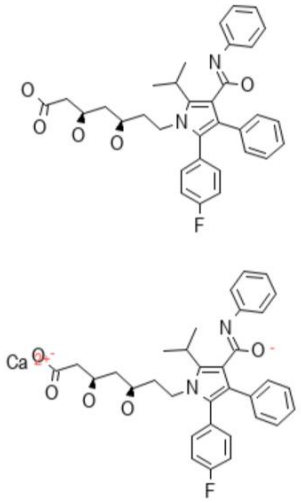


Fig. (1). Chemical structure of atorvastatin calcium. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

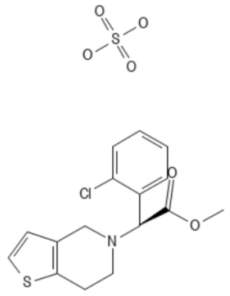


Fig. (2). Chemical structure of clopidogrel bisulfate. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

There is now a strong focus on sustainability in many analytical laboratories, and this has led to the development of Green Analytical Chemistry (GAC). The main idea of GAC is to reduce the environmental load from chemical analysis by using safer solvents, lowering energy use, and cutting down waste, which follows the 12 Principles of Green Chemistry [14-16]. Ethanol is employed as a greener solvent compared to traditional solvents due to its renewability, low toxicity, sensitivity, and easy handling [17, 18]. Literature review suggests that employing ethanol has more environmental benefits apart from retaining the analytical performance [19, 20]. Even though many advantages are there with ethanol, no works were reported using ethanol as a solvent for ATP and CLP. In the existing literature, the quantification of these drugs was carried out mainly by employing non-green solvents. [21, 22]. Apart from solvents, the utilisation of green metric tools is also still limited in the literature [23, 24].

To encourage greener practices and evaluate the greenness of the method, green metric tools are employed to measure the environmental impact. These tools summarize the greenness of the method based on the 12 GAC Green Analytical Chemistry principles. One such tool is Analytical GREEness (AGREE), which indicates the greenness score in a single circle. The score near 1 and green colour indicates the method's environment is safe, whereas yellow indicates moderate, and red indicates hazardous [25]. GAPI, which is the Green Analytical Procedure Index, helps to understand the steps and environmental impact of sample collection to analysis with colour codes of green, yellow, and red [26]. Complex GAPI, an advanced version of GAPI, helps to understand chemical hazard, exposure, reagent hazards ad energy consumption of the complete analytical procedure [27].

Apart from the above tools, the RGB Red-Green-Blue model also evaluates the greenness in terms of practicality, performance, and environmental impact of the method [28]. One more helpful tool is the NQS need, quality, and sustainability index. It provides information about the exact need for greener methods, the quality of the method, and environmental impact with sustainability [29]. Regarding the toxicity measures of the solvent ClorTox Index is employed to understand the toxicity of solvents and the disposal of waste generation [30].

Recent literature review suggests that combining the green metric helps to understand the modern analytical methods that are environmentally safe and sustainable. A few important works include the employment of green solvents instead of conventional solvents for extraction, the evaluation of greenness for current analytical methods, and the assessment of greenness as well as whiteness in analytical methods [31-33]. When used together, these tools provide analysts with a strong, clear basis for designing, improving, and validating environmentally responsible methods aligned with global sustainability goals.

2. RESULTS AND DISCUSSION

2.1. Linearity

The linearity of the RP-HPLC method was checked by preparing six concentration levels for each drug within their calibration ranges. Atorvastatin showed a clear linear response from 5 to 25 µg/mL, and clopidogrel showed linearity from 30 to 187.5 µg/mL. For both drugs, peak area was plotted against concentration, and the data were examined using linear regression. The correlation values and other related results are given in Table **1**. The regression equations obtained were $y = 26793x + 2467$ for atorvastatin ($r^2 = 0.9998$) and $y = 14450x + 25883$ for clopidogrel ($r^2 = 0.9996$), as illustrated in Figs. (**3** and **4**). The method provides a clear and consistent linear response across all tested concentrations, as evidenced by the high r^2 values of 0.999. This agreement implies that the technique is trustworthy for precisely measuring every medication within the authorised analytical range.

2.2. System Suitability

The system suitability was analysed by injecting six standard solutions containing 10 µg/mL of atorvastatin and 75 µg/mL of clopidogrel as per the optimised conditions.

Table 1. Analytical method validation data for atorvastatin and clopidogrel bisulfate.

S. No.	Validation Parameter	Atorvastatin	Clopidogrel Bisulfate
1	USP Plate count	5430	4897
2	USP Tailing	1.12	1.19
3	% RSD (System Precision)	0.38%	0.42%
4	Resolution	9.58	-
5	Accuracy	99.6-100.2%	99.4-100.0%
6	Precision (%RSD)	<1%	<1%
7	Method Precision (n = 6)	10 µg/mL	75 µg/mL
8	Ruggedness	No significant variation across analysts and instruments	No significant variation across analysts and instruments
9	Range of Linearity	5.0-25.0 µg/mL	30.0-187.5 µg/mL
10	Correlation Coefficient (r^2)	0.9998	0.9996
11	Limit of Detection (LOD)	1.51 µg/mL	3.86 µg/mL
12	Limit of Quantification (LOQ)	4.58 µg/mL	11.72 µg/mL
13	Robustness (Tailing Factor)	Flow rate -: 1.18Flow rate +: 1.20More organic phase (ethanol): 1.17Less organic phase (ethanol): 1.21	Flow rate -: 1.21Flow rate +: 1.22More organic phase (ethanol): 1.20Less organic phase (ethanol): 1.24

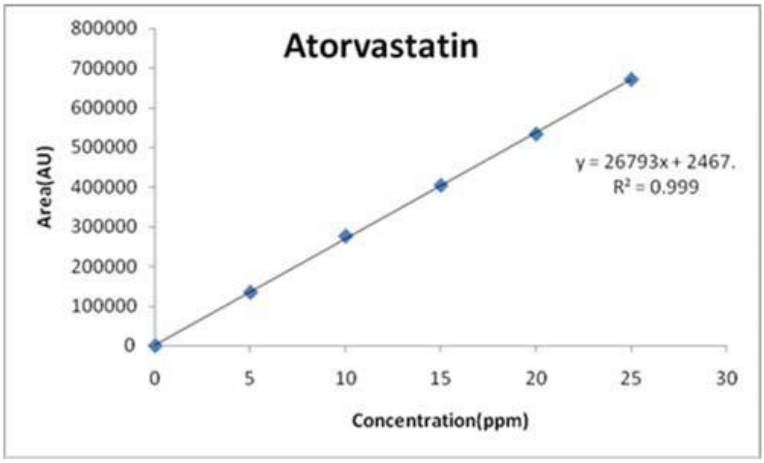


Fig. (3). Calibration plot assessment of atorvastatin. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

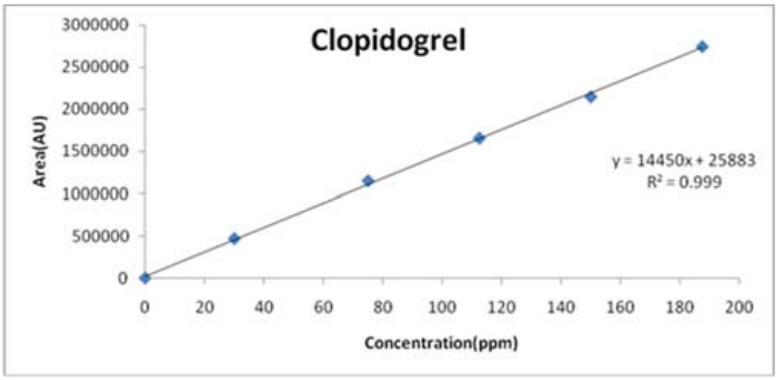
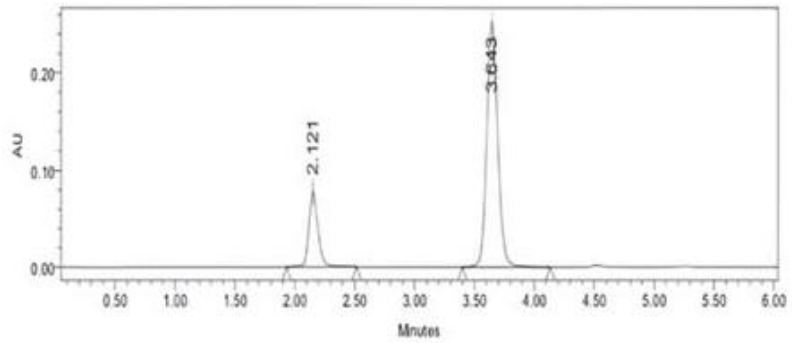
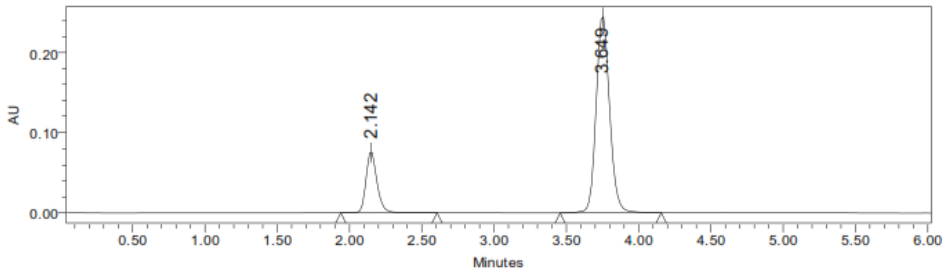


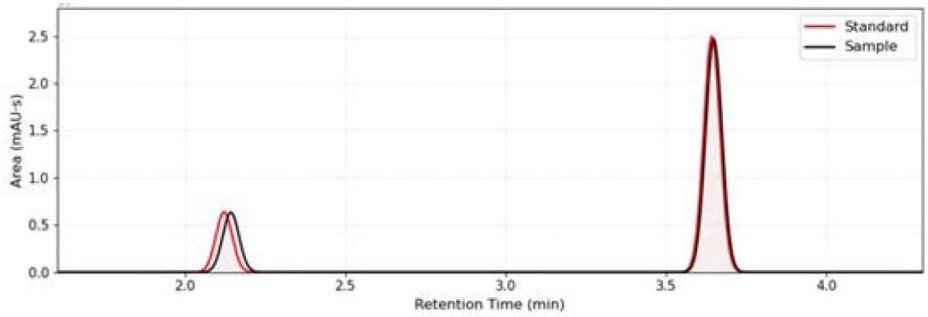
Fig. (4). Calibration plot assessment of clopidogrel. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).



(**a**)



(**b**)



(**c**)

Fig. (5). Optimized RP-HPLC chromatograms and overlay chromatogram for atorvastatin and Clopidogrel. **a**. optimised chromatogram of standard **b**. optimised chromatogram of standard sample. **c**. overlay chromatogram comparing the standard and the sample. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

Table **1** represents the acceptable criteria of the both the formulations as per the ICH guidelines with 5430 and 4897 theoretical plates that suggest the column efficiency, Tailing factors of both the drugs were reported as 1.12 and 1.19 with good peak symmetry and the % RSD for both the drugs are within the limits with 0.38% and 0.42% which represents the good repeatability with a resolution of 9.58 for clear separation.

2.3. Specificity

The specificity of the method was demonstrated by the blank, standard, and sample chromatograms with a retention time of 2.152 min for atorvastatin and 3.646 min for clopidogrel. There is no interference of excipients with the peaks as per Fig. (**5**). These results demonstrate the good selectivity for both drugs in pharmaceutical dosage forms.

2.4. Assay of Standard and Sample

Three replicates of standard and sample solutions were analysed for the assay performance with both drugs. The percentage purity was found to be 99.86% for atorvastatin and 99.96% for clopidogrel for both the standard and sample. The summary of the results was tabulated in Table **1**.

2.5. Precision

2.5.1. Method Precision

Six replicates of standard solution containing 10 µg/mL of atorvastatin and 75 µg/mL of clopidogrel were injected, and % RSD was calculated to analyse the method precision. The % RSD of both the peaks was found to be 0.38% for atorvastatin and 0.42% for clopidogrel, which are tabulated in Table **1**.

2.5.2. Ruggedness

Six replicates of both atorvastatin and clopidogrel concentrations were injected into the system by two different analysts under different conditions, and the % RSD was calculated to analyse the ruggedness of the method. Both the drugs' % RSD were found to be within the limits as tabulated in Table **1**.

2.6. Accuracy

Method's accuracy was assessed by injecting three different concentrations of 50%, 100%, and 150% with the standard addition method, and recovery was calculated. The atorvastatin has 98.6 % and 100.3 % with an average of 99.7% where as for clopidogrel, 98.9 % and 101.6 % with an average of 99.6 %. The % recover values are within the acceptance range of 98-102% as per the ICH guidelines. The results are tabulated in Table **1**.

2.7. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were assessed to understand the sensitivity of the method. The LOD and LOQ were calculated by signal to the signal-to-noise ratio. The results obtained were LOD of 0.5 µg/mL for atorvastatin and 7.2 µg/mL for clopidogrel, whereas LOQ of 1.5 µg/mL and 22.0 µg/mL for atorvastatin and clopidogrel. The results are summarized in Table **1**.

2.8. Robustness

Robustness was analysed to understand the effect of minor changes in the alteration of the mobile phase (±5%) and flow rate (±0.2 mL/min). The peak area, retention time, theoretical plate count, and tailing factor were assessed, and found that there are no consistent changes under altered conditions. The results are tabulated in Table **1** and are within the limits.

2.9. Environmental Assessment (GREEN, BLUE, RED, RGB FACTOR)

The greenness of the developed RP-HPLC method for atorvastatin and clopidogrel was checked using several green assessment tools such as AGREE, GAPI, ComplexGAPI, RGB, BAGI, NQS, and RAPI. Fig. (**6**) shows that the AGREE score was close to one, which indicates good alignment with the 12 principles of green analytical chemistry. The GAPI and ComplexGAPI pictograms also showed mostly green areas in the different steps, meaning the method has less environmental impact than regular methods. The results from RAPI, RGB, and NQS are shown in Fig. (**7**). A high RAPI value showed strong analytical performance with low resource use. The RGB model combined the red, green, and blue factors and gave a balanced view of performance, greenness, and practicality. The NQS score also supported the method by showing good analytical need, quality, and sustainability. Overall, these results show that the developed RP-HPLC method gives better efficiency, safety, and environmental benefits when compared to methods that use acetonitrile or methanol.

2.10. Comparison of Clortox Estimation for the Traditional and Developed Methods

ClorTox or Clarke's Toxicity Index, which is an environmental safety assessment tool, was employed to compare the existing methods with the proposed method for the understanding of the hazards to the environment. ClorTox provides a numerical value that indicates the eco-friendliness of the solvent employed in the method. The toxicity scores of 0.25, 0.50, 0.75, and 1.00 for Phosphate buffer, ethanol, methanol, and acetonitrile were used to calculate the clorTox value. Based on these values, the ClorTox score for the developed method comprising 70% buffer and 30% ethanol was calculated according to the standard equation (1):

'ClorTox '∑ (proportion of the solvent x toxicity score) (1)

∑ Proportion of the solvent.

Proposed HPLC : (0.70 x 0.25)+(0.30 x 0.50)÷1

0.325 ÷1

0.325

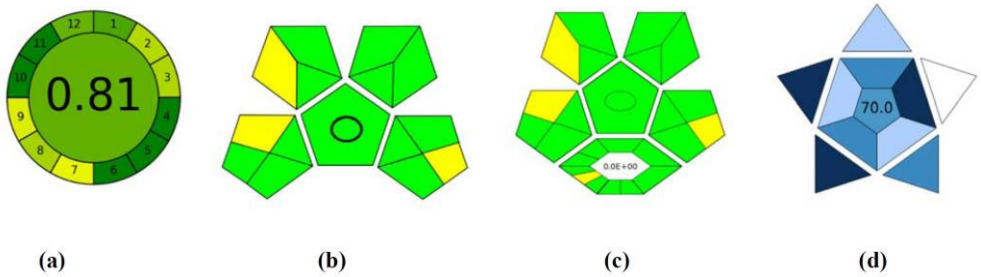
Acetonitrile-based method:

(0.65 x 0.25)+(0.35x1)÷1

0.512÷ 1

0.512

Methanol-based method:



(a) (b) (c) (d)

Fig. (6). Multi-metric evaluation of analytical greenness. (**a**) AGREE score representing the overall greenness index of the method (0-1 scale). (**b**) hexagonal greenness assessment illustrating the contribution of the 12 greenness principles. (**c**) complex pentagonal/hexagonal composite metric showing the balance of environmental factors, including safety, waste, energy, and resource consumption. (**d**) Blue-star greenness profile summarising the practicality of the method. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

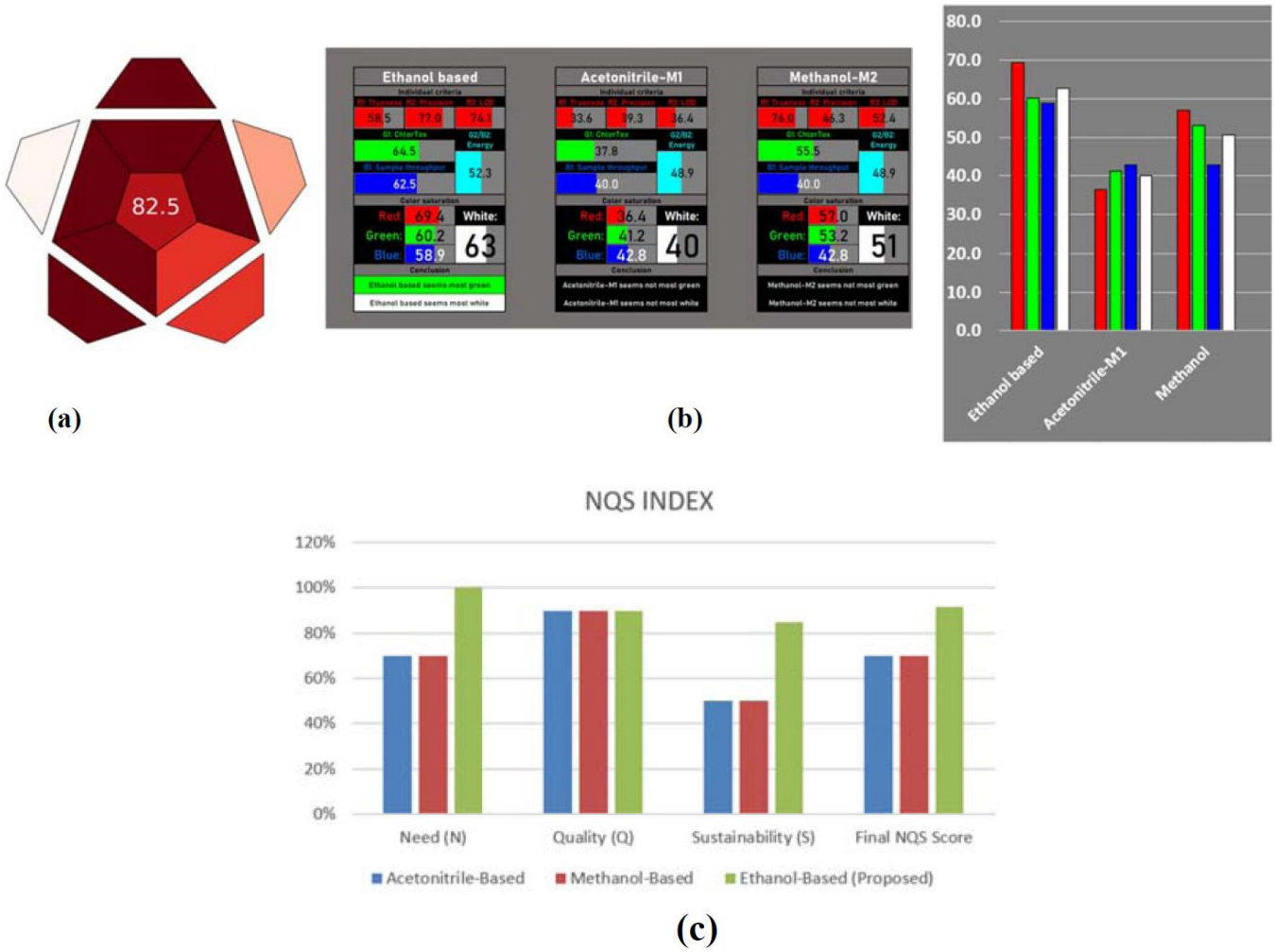


Fig. (7). Comprehensive Sustainability profiling using RAPI, RGB, and NQS frameworks. (**a**) RAPI assessment illustrating the resource use, analytical performance, and environmental impact parameters of the method. (**b**) RGB model showing the balance between analytical efficiency (Red), environmental friendliness (Green), and operational benefits (Blue). (**c**) NQS evaluation providing a numerical quality score summarising the overall sustainability and greenness of the analytical approach. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

(0.65 x 0.25)+(0.35x0.75)÷1

0.425÷1

0.425

The ClorTox value for the proposed method was 0.325, showing much lower toxicity and environmental impact than conventional solvent systems. This low value supports that the method is environmentally safer and fits well with green analytical chemistry principles.

To support this result, the Substitution Hazard Score (CHsub) was also calculated to understand the overall hazard of the solvent mixture. CHsub looks at human toxicity, environmental persistence, bioaccumulation, and exposure risks. The CHsub value for the method was calculated using equation (2) given below:

'CHsub=1×NCat1+0.75×NCat2+0.5×NCat3+0.25×NCat4' (2)

In this evaluation, NCat1-4 refer to the number of solvents placed in each hazard category. Using this method, the ethanol-based mobile phase gave a CHsub value of 0.5, which is much lower than the values for acetonitrile (1.0) and methanol (0.75). This shows that ethanol is a safer and more environmentally friendly solvent because of its low toxicity, fast biodegradability, and renewable origin.

The ClorTox results also explain the RGB model, that the proposed method is much less toxic and environment friendly compared to traditional methods, as per Table **2**. The AGREE, GAPI, and Complex GAPI results demonstrated that the method was developed with good environmental and analytical impact.

The combined results of all the green analytical chemistry tools like ClorTox, CHsub, AGREE, GAPI, Complex GAPI, BAGI, and RGB tools support that the method is a strong, environmentally friendly, and safe. Using ethanol as the main solvent helped to achieve these benefits because it is less toxic, biodegradable, and renewable. These features match the principles of Green Analytical Chemistry and make ethanol a good replacement for harmful solvents used in HPLC.

Table 2. comparative assessment of the ethanol-based RP-HPLC method versus conventional approaches: Analytical efficiency, cost, and environmental impact.

S. No.	Comparative Metric	Green Method Using Ethanol	Traditional ACN Methods	Conventional MeOH Methods
1	Composition of Eluent	Ethanol + Phosphate Buffer (30:70, v/v)	Acetonitrile blended with aqueous buffer	Methanol combined with water (60:40, v/v)
2	RT (Atorvastatin)	2.14 min	3.1 - 5.5 min	3.5 - 6.4 min
3	RT (Clopidogrel)	3.65 min	2.9 - 6.1 min	3.0 - 5.9 min
4	Detection Wavelength	230 nm	220 - 240 nm	220 - 240 nm
5	Flow rate	1.2 mL/min	Between 1.0-1.5 mL/min	Between 0.8-1.2 mL/min
6	Run-Time	6 min	9-10 min	9-12 min
7	Chromatographic Column Specification	'Waters Xterra RP18 (150 × 4.6 mm, 3.5 µm)'	Commercially available C18 columns	'Phenomenex C18 (250 × 4.6 mm, 5 µm)'
8	Analytical Greenness Assessment	0.81	NA	NA
9	GAPI Assessment	Mostly green	Presence of multiple yellow/red segments	Mostly red/yellow areas
10	Solvent Impact	Favorable (low hazard potential and renewable source)	Less favorable (known health and environmental risks)	Intermediate (regulated for laboratory use)
11	ClorTox Index	0.325	0.825	0.66
12	Substitution Hazard (CHsub)	0.5 (lower hazard class)	1.0 (higher hazard classification)	0.75 (moderate hazard level)
13	Estimated Solvent Expenditure (per L)	Economical (₹ 80 - ₹ 120)	Relatively expensive (₹ 200 - ₹ 400)	Moderate (₹ 150 - ₹ 250)
14	Laboratory Safety Measures Required	Minimal precaution; standard handling is sufficient	Requires additional safety protocols and ventilation	Needs standard precautions; flammability noted
15	Waste Disposal Needs	Lower due to benign nature	Requires regulated disposal systems	Requires careful waste segregation
16	Solvent Recovery Feasibility	Easy	Difficult (volatile, hazardous)	Moderate
17	Ecological Burden	Reduced, biodegradable, and low persistence	Elevated; hazardous residues may persist	Moderate ecological considerations

2.11. Evaluation of Sustainability- NQS

The overall robustness of the RP-HPLC method developed was assessed using the Need-Quality-Sustainability (NQS) index. The NQS index is a comprehensive index that combines the quality of analysis, the performance of the method, and the sustainability of the method into a single value. The NQS index is gaining popularity in the development of methods based on the principles of Green Analytical Chemistry.

Need (N): The method fulfills an analytical need, as it is the first green RP-HPLC method using ethanol as a solvent for the simultaneous determination of atorvastatin and clopidogrel in fixed-dose combinations in tablets. In contrast, most of the previous work was carried out using conventional solvent systems and did not involve a full green evaluation, thus adding to the importance of the current method.

Quality (Q): The approach showed positive analytical performance and was validated following the ICH Q2(R2) guidelines. The two analytes showed excellent linearity with r^2 values of over 0.999. The %RSD was less than 1%, which are with in the acceptance range of regulatory requirements. The other validation parameters also showed compliance with the regulatory guidelines, as the results were specific, sensitive, and reproducible in any quality control lab.

Sustainability (S): The method is sustainable in terms of evaluation with different Green metric tools, such as AGREE with a value of 0.81, and GAPI, as well as COMPLEX GAPI, with overall greenness in major parts of the hexagon. Apart from this, the toxicity scores are comparatively less with a ClorTox value of 0.325 and CHsub of 0.5, which represents low toxicity and environmental safety.

The NQS scored was calculated as per the equation:(3):

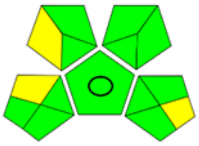
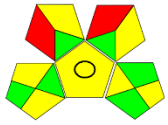
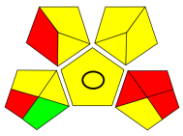
$$\frac{100+95+90}{3} = 95.0\% \quad (3)$$

A very good high score of NQS indicates the strength, applicability, and sustainability of the method. The compli-

Table 3. NQS-based sustainability profiling of proposed green RP-HPLC method versus conventional techniques.

S. No.	Analytical Approach	Need Score (N)	Quality Score (Q)	Sustainability Score (S)	Overall NQS %
1	Traditional ACN method	70.0%	90.0%	50.0%	70.00%
2	Conventional methanol method	70.0%	90.0%	60.0%	73.30%
3	Ethanol-Based (Proposed)	100%	95.0%	90.0%	95.00%

Table 4. Comparative green analytical chemistry assessment of the proposed and conventional RP-HPLC methods for atorvastatin and clopidogrel.

Method	Eco-Safety and Solvent Compatibility	Analytical Greenness Index	Green Analytical Procedure Index	Blue Applicability Grade Index	'ClorTox' Scale	Importance of Scores
Ethanol-Based 'RP-HPLC Method	Employs ethanol as a green solvent, characterised by low toxicity, renewability, and environmental compatibility in line with GAC standards.				0.325	High AGREE and low ClorTox affirm strong environmental safety; favourable GAPI and BAGI reflect practical greenness.
Traditional ACN -based	Utilises acetonitrile, associated with elevated environmental hazards due to its flammability, low degradability, and occupational safety risks.				0.825	Poor AGREE, and high ClorTox indicate a toxic environmental footprint; GAPI suggests poor greenness.
Conventional MeOH -based	Applies methanol as an intermediate option in toxicity and sustainability, ranking between acetonitrile and ethanol.				0.66	Slightly better than ACN but inferior to ethanol in all green metrics and solvent safety.

ance with the regulatory requirements brings the method significantly to the pharmaceutical analysis sector for the regular analysis of this fixed combination. A comparison between the conventional methods and the proposed method elaborates on the performance of the greener method, while the traditional methods score low, indicating the toxicity and environmental impact. In contrast, the ethanol-based method scored a much higher NQS score of 95%, clearly indicating a strong environmental advantage without any compromise in accuracy, robustness, or usability. A comparison of the NQS scores of all the methods considered is presented in Table **3**.

3. DISCUSSION

This study presents a validated and environmentally friendly RP-HPLC method for estimating atorvastatin calcium and clopidogrel bisulfate together in fixed-dose tablets. A review of earlier work showed that no green solvent RP-HPLC method has been developed for this drug pair, which makes this study important.

The method uses ethanol as the solvent because it has low toxicity, is biodegradable, and comes from a renewable source. It is mixed with 10 mM potassium phosphate buffer (pH 4.0) and used as the mobile phase. The separation was done on a Waters Xterra RP18 column (150 × 4.6 mm, 3.5 µm). Clear and separate peaks were obtained with retention times of 2.14 min for atorvastatin and 3.65 min for clopidogrel.

The validation of the method was carried out as per the ICH Q2(R2) guidelines. The method showed a good linearity with (r^2>0.999), precise with %RSD less than 1, and accurate with recoveries 99.4% and 100.2 %, ranging between 98%-102%. The method is suitable, specific, and robust for regular quality control. Employment of Ethanol increased the sustainability of the method with less ClorTox values (0.325), a CHsub score of 0.5, and AGREE 0.82. Using ethanol improved the environmental profile of the method. A low ClorTox value (0.325) and a CHsub score of 0.5 indicate the safety and reliability for the environment.

The replacement of solvent approach helps to understand the replacement of harmful, hazardous solvents like acetonitrile with greener solvents like ethanol for routine analysis. Ethanol is considered a greener solvent due to its renewability, less toxic nature, and ease of disposal [34-36]. This method applies to other fixed-dose combinations that have suitable solubility and UV properties [37, 38].

Even though ethanol is greener, it has limitations in separation, such as higher viscosity, the back pressure in the system, and at low temperatures, it requires a stronger column

[39, 40], and it has a cutoff of 210nm, which limits the drugs with low wavelength ranges [41]. Its stronger elution strength can reduce retention of non-polar compounds, and column equilibration can be slower than with acetonitrile [42]. Because of these points, ethanol should be selected carefully while considering the analyte properties and instrument limits.

3.1. Method Significance and Economic-Environmental Advantage

The proposed method offers various advantages over the conventional methods with strong analytical performance and enhanced environmental safety for the regular routine quality control. This method employs the renewable, greener solvent for the method development and validation of atorvastatin and clopidogrel instead of utilizing hazardous solvents such as acetonitrile. This substitution is in compliance with the 12 Green Analytical Chemistry principles and supports global initiatives for minimising the analytical impact on the environment.

From an analytical perspective, the method stands out for its effective separation with retention times of 2.152 minutes for atorvastatin and 3.646 minutes for clopidogrel. The method complies with all the regulatory validation parameters as per the ICH Q2(R2) guidelines with excellent sensitivity, linearity, precision, and robustness. The shorter run time increases the sample throughput, indicating the high suitability for routine analysis. In addition to these advantages low toxicity of the solvent helps to improve the analyst and environmental safety and performance.

In terms of the expenses for the operation, ethanol provides a clear financial benefit as ethanol is highly economical and much more affordable, ranging from (₹80-₹120/L) compared with acetonitrile (₹200-₹400/L) and methanol (₹150-₹250/L). Apart from the cost difference, ethanol is biodegradable and non-carcinogenic, with easy handling of waste disposal. Its ease of recovery and recyclability further support cost-effective laboratory practices by minimising overall solvent consumption.

The greenness evaluations indicate the method's environmental friendliness wit high AGREE score, Greener GAPI and COMPLEX GAPI profiles, and low ClorTox values and CHsub scores. In conclusion, the method is very good in terms of analytical way for the routine estimation of the dosage forms, also protects the environment and the analyst from toxic solvents. It also advances the utilisation of economic solvents.

3.2. Comparative Evaluation of the Proposed Ethanol-Based Method Against Conventional RP-HPLC Approaches

When this method is compared with the usual RP-HPLC methods that use acetonitrile or methanol, the ethanol method shows clear benefits. It gives good analytical results, is safer to handle, and also reduces the overall cost. Acetonitrile is often used because of its sensitivity, but it is toxic, flammable, expensive, and does not degrade easily, which makes its disposal difficult. Methanol is slightly better but still has toxicity and volatility issues, so it is not considered a green option.

In this method, ethanol is employed instead of traditional solvents. Ethanol is renewable, biodegradable, and much safer. This lowers the environmental impact. The ClorTox and CHsub scores of 0.325 and 0.5 are comparatively lower than those of the conventional methods. The AGREE score of 0.81, Greener profiles of GAPI and COMPLEX GAPI indicate the greenness of the method.

The ClorTox value of 0.325 and the CHsub score of 0.5 are both lower than the values for acetonitrile and methanol. The AGREE score of 0.81 and the mostly green GAPI and ComplexGAPI diagrams also support the greener nature of the method.

The run time was shorter, 6 minutes, and with retention times of 2.152 and 3.646, indicating faster separation and elution. The conventional methods utilise 8-15 minutes, which is comparatively less in proposed method. This helps reduce solvent use, handle more samples, and improve day-to-day workflow.

NQS score indicates that his ethanol-based method is much in need of regular analysis and proves that it has more advantages in terms of quality and sustainability, with the scores of 95 %. This clearly demonstrates the method's balanced performance in terms of analytical need, validation quality, and sustainability. All the results are tabulated in Table **4**.

This study is limited to the simultaneous determination of atorvastatin and clopidogrel in pharmaceutical tablet formulations. The method was not evaluated for biological matrices such as plasma or serum. In addition, comprehensive forced degradation studies were not performed to fully establish the stability-indicating capability of the method. Future work may extend the applicability of the method to biological samples and detailed stability studies.

4. MATERIALS AND METHODS

4.1. Chemicals and Reagents

Atorvastatin calcium (ATV;99.84%) and clopidogrel bisulfate (CLP;99.62%) working standards were kindly provided by Sura Labs, Hyderabad, India. Marketed fixed-dose combination tablets containing '10 mg of ATV' and '75 mg of CLP' were procured from a local retail pharmacy and labeled as Atocor CV 10. Ethanol (HPLC grade, purity ≥ 99.9%) was sourced from Merck (Mumbai, India) and used as a sustainable organic modifier in the mobile phase. Potassium dihydrogen phosphate (KH_2PO_4; purity ≥ 99.5%) and orthophosphoric acid (H_3PO_4; purity ≥ 85%) were obtained from Fisher Scientific (India). 'Milli-Q ultrapure water (resistivity ≥ 18.2 MΩ·cm)' was used to prepare all aqueous solutions and dilutions. All solvents and reagents employed were of analytical or 'HPLC' grade. Prior to use, they were filtered through a '0.45 μm membrane filter' and 'degassed'.

4.2. Instrumentation

Chromatographic separation was conducted utilizing a 'Waters Alliance e2695 HPLC system' with a 'quaternary

solvent delivery pump', 'an autosampler', and a 'column oven, integrated with a Waters 996 PDA detector. The system was managed using Empower 2 chromatography data software to facilitate data acquisition, peak integration, and quantitative calculations. A Lab India 'digital pH meter' (Model: Pico+) was used to 'adjust the pH of the mobile phase'. An 'ultrasonic bath' sonicator (Spectralab, India) was used to prepare the samples and standards. Analytical weighing was performed using a Shimadzu AY-220 electronic balance with a readability of 0.1 mg. All chromatographic measurements were performed at ambient temperature (25 ± 2°C).

4.3. Chromatographic Conditions

Chromatographic separation was conducted utilising a Waters Xterra® RP18 column (150 mm × 4.6 mm, 3.5 µm particle size). The mobile phase comprised a '10 mM potassium dihydrogen phosphate buffer', orthophosphoric acid, and ethanol in a '70:30 v/v ratio'. Filtration through a 0.45 µm membrane followed by ultrasonic degassing was performed to prepare the mobile phase for analysis. The system operated in isocratic mode at a flow rate of '1.2 mL/min', with the column maintained at ambient temperature. The detection wavelength was set at 230 nm, and the 'injection volume' was '15 µL'. The run time was six minutes.

4.4. Preparation of Mobile Phase

To prepare the 10 mM phosphate buffer, 1.36 g of KH_2PO_4 was accurately weighed and dissolved in one liter of Milli-Q water. The pH of this solution was then adjusted to 4.0 using dilute orthophosphoric acid, with the measurement carried out on a Lab India digital pH meter. The mobile phase was prepared by mixing the phosphate buffer with ethanol in a 70:30 (v/v) proportion. Before use, the mixture was passed through a 0.45 µm nylon membrane filter and degassed in an ultrasonic bath for 15 minutes. A freshly prepared mobile phase was used for all chromatographic runs to ensure consistent performance and stable retention characteristics.

4.5. Experimental

4.5.1. Optimized Conditions

Several trial combinations of mobile phase ratios and solvent systems were run to optimise, and the final chromatographic conditions were selected based on their ability to produce well-defined peaks, satisfactory resolution, and a short overall analysis time. The most favourable results were achieved using a 'Waters Xterra® RP18 column' (150 mm × 4.6 mm, 3.5 µm) maintained at ambient temperature, with a mobile phase consisting of '10 mM potassium dihydrogen phosphate buffer (pH 4.0) and ethanol in a 70:30 v/v ratio. A flow rate of 1.2 mL/min was employed under isocratic conditions, and 'UV detection was conducted at 230 nm'. 'The injection volume' was set at 15 µL, and the total runtime was 6 minutes. Under these conditions, Atorvastatin and Clopidogrel were well resolved with retention times of 2.14 minutes and 3.65 minutes, respectively. The system demonstrated high reproducibility and low baseline noise, rendering it suitable for routine analysis.

4.5.2. Linearity

A series of 'standard' stock solutions was prepared separately for atorvastatin calcium and clopidogrel bisulfate using ethanol: water (30:70, v/v) as the diluent. From these stock solutions, appropriate aliquots were again diluted to obtain 'six concentration' levels for each of the drugs. The final concentration range for atorvastatin was 5-25 µg/mL, and for clopidogrel, it was 30-187.5 µg/mL. Calibration standards were freshly prepared on the day of analysis and analysed to generate a linear regression equation based on the mean peak area responses versus their corresponding concentrations.

4.5.3. Preparation of Sample Solution

Twenty tablets containing a fixed-dose combination of atorvastatin calcium (10 mg) and clopidogrel bisulfate (75 mg) were accurately weighed and finely powdered using a mortar and pestle. A portion of the powder, 10 mg of atorvastatin, and 75 mg of clopidogrel were transferred into a 100 mL volumetric flask. To this, 70 mL of ethanol: water (30:70, v/v) was added as a diluent, and the mixture was sonicated in an ultrasonic bath for 20 min. The solution was allowed to cool, diluted to the same quantity with the same diluent. The resulting mixture was filtered through No. 1 Whatman filter paper. An appropriate aliquot of the clear filtrate was further diluted to yield a sample 'concentration of 10 µg/mL' of 'atorvastatin and '75 µg/mL' of 'clopidogrel for chromatographic analysis.

4.5.4. System Suitability

The chromatographic method was fine-tuned for the concurrent determination of Atorvastatin and Clopidogrel. From the prepared mixed standard solution, 15 µL aliquots were introduced into the HPLC system, and the analysis was carried out in six replicates to assess reproducibility. System suitability was established by evaluating key performance indicators such as retention time consistency (±0.1 min), %RSD of peak area and height (<2%), tailing factor (<1.5), and column efficiency (theoretical plates>2000) for each compound. Adequate resolution between the analyte peaks was ensured throughout the optimisation process to confirm satisfactory separation and system performance.

4.5.5. Validation of the Method

The RP-HPLC method was comprehensively validated to confirm its reliability, reproducibility, and appropriateness for routine quality control assessments. The method validation was performed as per ICH Q2(R2) guidelines with parameters of linearity, accuracy, precision, robustness, ruggedness, system suitability, LOQ, and LOD. Each parameter was evaluated with experimental procedures, and the results were analysed with acceptance criteria.

4.5.6. Environmental Impact Assessment

The greenness and sustainability of the developed method were checked using different tools that are commonly used to evaluate environmentally safe analytical methods. The AGREE metric, GAPI pictogram, and ComplexGAPI framework were used to see how well the method follows

the principles of Green Analytical Chemistry. This focus includes solvent safety, energy use, and waste reduction. The environmental impact of the method with regular RP-HPLC methods was also compared. To do this, tools like the Bioanalytical Greenness Index (BAGI), the Red Analytical Performance Index (RAPI), and the Red-Green-Blue (RGB12) model were used. Together, these tools provide a clear view of the method's analytical performance, practicality, and environmental impact. This aided in the understanding of how suitable and sustainable the method is for long-term use.

CONCLUSION

A simple, precise, sensitive, accurate, validated, and green RP-HPLC method was developed to estimate atorvastatin calcium and clopidogrel bisulfate together in fixed-dose tablets. This method employs ethanol as a solvent for the first time instead of traditional solvents to reduce toxicity. The method adapted all ICH Q2(R2) guidelines by showing linear, accurate, precise, sensitive, and robust results. There is no interference of peaks with good separation using ethanol as a solvent. Green assessments like AGREE, GAPI, ComplexGAPI, BAGI, RAPI, ClorTox, CHsub, and NQS showed that the method is more sustainable than regular methods. Ethanol is a reliable environment safe solvent for the routine analysis or quality control for this combination dosage form. It also supports the move toward greener HPLC practices. The same approach can also be tried for other drug mixtures or for stability studies in the future.

AUTHORS' CONTRIBUTIONS

The authors confirm their contribution to the paper as follows: Writing the paper: KA; methodology: RSS; data curation: NS. All authors reviewed the results and approved the final version of the manuscript.

LIST OF ABBREVIATIONS

CVDs	=	Cardiovascular Diseases
FDC	=	Fixed-Dose Combination
ATV	=	Atorvastatin Calcium
GHS	=	Globally Harmonized System
GAC	=	Green Analytical Chemistry

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analysed during this study are included in this published article.

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The authors declare no conflict of interest, financial or otherwise.

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Declared none.

AI DISCLOSURE STATEMENT

I, Dr. Ramya Sri Sura, confirm that AI-assisted tools were used only for limited language refinement and technical assistance during the preparation of the manuscript titled Greenness, Whiteness, and Sustainability Profiling of an Ethanol-Based RP-HPLC Method for Atorvastatin and Clopidogrel in Pharmaceutical Tablets.

No AI tool was used for generating scientific content, interpreting results, drawing conclusions, or drafting the core scientific sections of the manuscript. All scientific ideas, methodology, experimental work, data interpretation, and conclusions are entirely original and authored by me and my co-authors.

The AI-generated content in the manuscript has been substantially revised, edited, and validated by the authors to ensure accuracy, originality, and compliance with ethical standards. The authors take full responsibility for the integrity and authenticity of the manuscript.

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