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DEVELOPMENT AND VALIDATION OF A UV SPECTROPHOTOMETRIC METHOD FOR CYCLOBENZAPRINE SIMULTANEOUS ESTIMATION OF ACECLOFENAC AND HYDROCHLORIDE IN A COMBINED TABLET DOSAGE FORM USING HYDROTROPY WITH COMPREHENSIVE GREEN ANALYTICAL ASSESSMENT

Khushal D. Desale [1], **Nikita Gavande** [2], **Poonam A. Borse*** [3], **Manoj. M. Bari** [4]

[a] Extended author information available on the last page of the article**

* Corresponding Author: **Poonam A. Borse**, poonamsalunkeborse@gmail.com

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ABSTRACT

Background: The simultaneous quality control of Aceclofenac (ACEF) and Cyclobenzaprine Hydrochloride (CBPH) in combined pharmaceutical formulations presents analytical challenges because of the poor aqueous solubility of ACEF and the frequent reliance of conventional spectrophotometric methods on hazardous organic solvents. Hydrotropic solubilization offers a simple and environmentally sustainable alternative by enhancing the aqueous solubility of poorly water-soluble drugs while reducing or eliminating the use of organic solvents. However, limited attention has been given to the development of green UV spectrophotometric methods based on hydrotropic solubilization for the simultaneous estimation of ACEF and CBPH, particularly when combined with systematic analytical optimization and comprehensive assessment of analytical greenness. **Objective:** To develop and validate a simple, accurate, and eco-friendly UV spectrophotometric method for the simultaneous estimation of Aceclofenac (ACEF) and Cyclobenzaprine Hydrochloride (CBPH) in a combined tablet dosage form using hydrotropic solubilization, with comprehensive greenness assessment. **Methods:** A 10% w/v Polyethylene Glycol 6000 (PEG 6000) aqueous solution was employed as a hydrotropic solvent to avoid organic solvents. Absorbance was recorded at λ max 274.80 nm (CBPH) and 340.30 nm (ACEF) using a Shimadzu UV-1800 double-beam spectrophotometer. A simultaneous equation method was applied for quantitative estimation. Method parameters were optimized using a Central Composite Design (CCD) via Design-Expert® Software v13.0. Validation was conducted per ICH Q2(R2) guidelines covering linearity, accuracy, precision, repeatability, LOD, LOQ, and forced degradation. Greenness was assessed using NEMI, Analytical Eco-Scale, GAPI, and AGREE tools. **Results:** Both drugs obeyed Beer–Lambert's law over the ranges 2–12 µg/ml (CBP) and 10–70 µg/ml (ACE), with r^2 = 0.9996 and 0.9999, respectively. Accuracy (% recovery) was 98.47–98.76% (CBP) and 99.72–99.85% (ACE). Precision (%RSD) was ≤2.0% for both. LOD: 0.35 µg/ml (CBP), 1.10 µg/ml (ACE); LOQ: 1.07 µg/ml and 3.40 µg/ml. Tablet assay: 98.5% (CBP) and 99.5% (ACE). NEMI — all 4 criteria satisfied; Eco-Scale score 87/100 (Excellent); GAPI — 11/15 green, 0 red; AGREE score 0.76 (≥0.70 threshold). **Conclusion:** The developed method is simple, precise, accurate, robust, and environmentally sustainable. The use of PEG 6000 as a hydrotropic agent renders the method organic solvent-free and ideal for routine quality control analysis.

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INTRODUCTION

Pharmaceutical combination therapy has become an important therapeutic approach for improving clinical outcomes by simultaneously targeting multiple pathological mechanisms. Fixed-dose combinations (FDCs) offer several advantages, including enhanced therapeutic efficacy, improved patient compliance, reduced dosing frequency, and minimized adverse effects compared with monotherapy. Consequently, the development of reliable, accurate, and environmentally sustainable analytical methods for the quality control of combination drug products has become increasingly important in the pharmaceutical industry.

Aceclofenac is a non-steroidal anti-inflammatory drug (NSAID) widely prescribed for the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and other inflammatory musculoskeletal disorders because of its analgesic, anti-inflammatory, and antipyretic properties.[1]. Cyclobenzaprine hydrochloride is a centrally acting skeletal muscle relaxant used for the short-term management of acute painful musculoskeletal conditions associated with muscle spasm. The fixed-dose combination of Aceclofenac and Cyclobenzaprine hydrochloride provides effective relief from pain, inflammation, and muscle spasm, making it an important therapeutic option for the management of musculoskeletal disorders [2].

A significant challenge in spectrophotometric analysis of these drugs is their poor aqueous solubility, particularly ACEF, which is practically insoluble in water. Conventional spectrophotometric methods for such drugs rely on organic solvents such as methanol, acetonitrile, or acetone, which are expensive, toxic, and environmentally hazardous, raising serious concerns from a Green Analytical Chemistry (GAC) perspective [3,4]. Hydrotropy is a solubilization approach wherein the addition of a high-concentration hydrotrope markedly enhances aqueous solubility of a poorly water-soluble compound through weak, non-covalent interactions without forming micelles [3]. Unlike micellar systems, hydrotropes do not require critical concentration thresholds, do not chemically modify the analyte, and are generally safe, inexpensive, and biodegradable, making them ideal for green analytical method development [5][6].

Several analytical methods, including high-performance liquid chromatography (HPLC) [7,8,9], UV spectrophotometry [10-16], RP-HPLC for simultaneous estimation of drugs [17, 18], high-performance thin-layer chromatography (HPTLC) [8, 19, 20,], spectrofluorimetric techniques [21], and hyphenated techniques such as LC-MS/MS [22], have been reported for the estimation of Aceclofenac and Cyclobenzaprine Hydrochloride, either individually or in combination with other pharmaceutical agents [23, 24, 25]. Although these methods demonstrate satisfactory analytical performance, many require significant quantities of hazardous organic solvents, involve complex

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sample preparation procedures, or depend on sophisticated instrumentation. These limitations increase analytical costs, environmental burdens, and laboratory waste generation, highlighting the need for simpler, greener analytical alternatives.

Hydrotropic solubilization has emerged as an effective and environmentally preferable strategy for improving the aqueous solubility of poorly water-soluble drugs without extensive use of hazardous organic solvents. Among the hydrotropic agents evaluated during preliminary investigations at different concentrations, 10% (w/v) Polyethylene Glycol (PEG 6000) was selected because it provided complete solubilization of Aceclofenac while maintaining excellent solubility of Cyclobenzaprine Hydrochloride. The selected concentration produced optically clear solutions with negligible absorbance interference at the selected analytical wavelengths and acceptable solution viscosity. Lower PEG 6000 concentrations were insufficient to achieve complete dissolution of Aceclofenac, whereas higher concentrations increased viscosity without providing additional analytical advantages. Therefore, 10% (w/v) PEG 6000 was considered the most suitable hydrotropic solvent system for the proposed analytical method.

Despite the availability of several analytical procedures for the individual estimation of these drugs or their determination using chromatographic techniques, limited information is available regarding environmentally sustainable UV spectrophotometric methods employing hydrotropic solubilization for their simultaneous estimation. Furthermore, comprehensive evaluation of analytical greenness using multiple internationally accepted assessment tools together with systematic analytical optimization based on the Analytical Quality by Design (AQbD) concept has not been adequately explored for this pharmaceutical combination. This research gap provided the basis for the present investigation.

The objective of the present study was to develop and validate a simple, accurate, precise, economical, and environmentally sustainable UV spectrophotometric method for the simultaneous estimation of ACEF and CBPH in a combined tablet dosage form using 10% (w/v) PEG 6000 as a hydrotropic solubilizing agent. The analytical method was optimized using an Analytical Quality by Design (AQbD) approach employing Central Composite Design (CCD) and validated according to ICH Q2(R2) guidelines. In addition, the environmental sustainability of the developed analytical procedure was comprehensively evaluated using NEMI, Analytical Eco-Scale, GAPI, and AGREE green analytical assessment tools.

1. MATERIAL AND METHOD

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1.1 Chemicals and Reagents

Pure Aceclofenac (ACEF) and Cyclobenzaprine Hydrochloride (CBPH) bulk drug 99.99% purity were procured from SM Pharma, Mumbai. Flexabenz Plus® tablets, Macleod's Pharmaceuticals Ltd., India. Polyethylene Glycol 6000 (PEG 6000; analytical reagent grade) was obtained from Jinendra Scientifics, Jalgaon. Ethanol, hydrochloric acid, and sodium hydroxide, analytical-grade reagents from Merck/SD Fine Chemicals, India. Distilled water was used throughout as the diluent and final solvent.

1.1 Instruments

Table 1 lists all instruments used in the present study.

Sr. No.	Instrument	Model / Make
1	Double-beam UV-Visible Spectrophotometer	**Shimadzu UV-1800 Quartz cell path length: 1 cm; scanning range: 200–400 nm; spectral slit width: 1.0 nm; Scan speed: Fast**
2	Analytical balance (0.0001 g sensitivity)	**Wenster**
3	Ultrasonic bath/sonicator	**Citizen**
4	Design-Expert® Software	**Version 13.0, Stat-Ease Inc., USA**
5	**pH meter**	**Equiptronics**

1.3 Drug Profiles

Physicochemical properties of ACEF and CBPH are summarized in Tables 2 and 3, respectively.

1.1. Table 2: Physicochemical properties of Aceclofenac [1]

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Property	Description
IUPAC name	[[2-[(2,6-Dichlorophenyl) amino] phenyl] acetyl] oxyacetic acid
Molecular formula	$C_{16}H_{13}Cl_2NO_4$
Molecular weight	354.18 g/mol
CAS number	89796-99-6
Pharmacological category	NSAID – analgesic, anti-inflammatory, anti-rheumatic
Physical description	White to almost white crystalline powder
Solubility	Practically insoluble in water; freely soluble in acetone and ethanol
Melting point	149–153°C
λ max	275 nm, (in methanol)
Usual adult dose	100 mg twice daily

Table 3: Physicochemical properties of Cyclobenzaprine HCl [2]

Property	Description
IUPAC name	3-(5H-Dibenzo [a, d] cyclohepten-5-ylidene)-N, N-dimethylpropan-1-amine hydrochloride
Molecular formula	$C_{20}H_{21}N\cdot HCl$
Molecular weight	311.85 g/mol
CAS number	6202-23-9
Pharmacological category	Centrally acting skeletal muscle relaxant
Physical description	White to off-white crystalline powder
Solubility	Freely soluble in water, methanol, and ethanol
Melting point	215–217°C
Usual adult dose	15–30 mg/day (5–10 mg three times daily)

1.4 Preparation of Hydrotropic Solvent (10% w/v PEG 6000)

Accurately weighed 10.0 g of PEG 6000 was transferred into a 100 ml volumetric flask. Approximately 70 ml of distilled water was added, and the mixture was stirred continuously until complete dissolution. The volume was made up to 100 ml with distilled water to obtain a 10% w/v

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PEG 6000 hydrotropic solution. This solution was used as the primary solubilizing agent throughout the study.

1.5 Solubility Determination

Solubility of ACEF and CBPH was assessed in various solvents, including distilled water, 0.1 N HCl, niacinamide solution, ethanol, and 10% w/v PEG 6000. Results are presented in Table 4.

1.2. Table 4: Solubility profile of ACE and CBP in various solvents

Sr. No.	Solvent	Solubility of ACEF	Solubility of CBPH	Remarks / Decision
1	Distilled water	Practically insoluble	Freely soluble	**ACE insoluble – not suitable**
2	0.1 N HCl	Slightly soluble	Freely soluble	**Spectral overlap; not suitable**
3	Niacinamide (2.5% w/v)	Soluble	Soluble	**Spectral interference observed**
4	Ethanol	Freely soluble	Freely soluble	**Organic solvent – not green**
5	**10% PEG 6000 (aq.)**	**Freely soluble**	**Freely soluble**	**SELECTED – green, no interference**

Preliminary solubility studies were conducted using different concentrations of polyethylene glycol 6000 (PEG 6000) prepared in an ethanol–water system to identify the optimum hydrotropic medium for simultaneous estimation of ACEF and CBPH. Various concentrations of PEG 6000 were evaluated with respect to drug solubilization, solution clarity, and suitability for UV spectrophotometric analysis. It was observed that 10% (w/v) PEG 6000 provided complete solubilization of both drugs with a clear and stable solution, resulting in reproducible absorbance measurements without spectral interference. In contrast, PEG 6000 concentrations above 10% (w/v) produced milky or turbid solutions in the ethanol-containing system, indicating reduced optical clarity that could adversely affect spectrophotometric measurements through increased light scattering and baseline instability. Therefore, 10% (w/v) PEG 6000 was selected as the optimum concentration for method development and validation, as it achieved efficient drug solubilization while maintaining solution transparency, analytical reliability, and compliance with the principles of green analytical chemistry [26,27].

1.6 Preparation of Standard Stock Solutions

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Accurately weighed 10.0 mg each of CBPH and ACEF were transferred into separate 10 mL amber volumetric flasks. To each flask, 1.0 mL of ethanol was added and sonicated for 5 min to aid initial dissolution. The 10% PEG 6000 solution was then added in 1 mL increments with intermittent sonication (5 min per step) until complete dissolution was achieved (approximately 4–5 mL PEG 6000 required). The volume was made up to 10 mL with distilled water to obtain a stock solution of 1000 µg/mL each. These were stored in a refrigerator at 2–8°C and used within 7 days. Working standard solutions were freshly prepared by appropriate serial dilution with distilled water: CBPH at 2, 4, 6, 8, 10, and 12 µg/ml; ACEF at 10, 20, 30, 40, 50, 60, and 70 µg/ml.

1.7 Determination of λ max and Spectral Overlay

Individual standard solutions of CBPH (10 µg/ml) and ACEF (40 µg/ml) were prepared in 10% PEG 6000–water and scanned from 200–400 nm against a reagent blank (10% PEG 6000–water) using a Shimadzu UV-1800 spectrophotometer. Maximum absorbance wavelengths (λ max) were determined from the absorption spectra. Overlay spectra were examined to confirm spectral separation and suitability for the simultaneous equation method.

1.8 Calibration Curves

Absorbance of each working standard solution was recorded at the respective λ max values. Calibration curves were constructed by plotting absorbance (y-axis) versus concentration (x-axis). Regression equations, slopes, intercepts, and correlation coefficients (r^2) were calculated by linear regression analysis.

1.9 Simultaneous Equation Method

Two analytical wavelengths were selected: λ_1 = 274.80 nm (λ max of CBPH) and λ_2 = 340.30 nm (λ max of ACEF). Absorptivity values (a) of each drug at each wavelength were determined from the respective calibration curves.

Absorptivity values used (ml·µg^{-1}·cm^{-1}):

ACEF at 274.80 nm (a_1^y) = 0.0020

ACEF at 340.30 nm (a_2^y) = 0.0148

CBPH at 274.80 nm (a_1x) = 0.0560

CBPH at 340.30 nm (a_2x) = 0.0030 Simultaneous equations for concentration calculation:

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$$Cx\ (ACEF, \mu g/ml) = (A_2 \times a_1\ x - A_1 \times a_2\ x) / (a_1^y \times a_2\ x - a_2^y \times a_1\ x) \ldots\ldots \text{(Eq. 1)}$$

$$Cy\ (CBPH, \mu g/ml) = (A_1 \times a_2^y - A_2 \times a_1^y) / (a_1^y \times a_2\ x - a_2^y \times a_1\ x) \ldots\ldots \text{(Eq. 2)}$$

Where A_1 = absorbance of mixture at 274.80 nm; A_2 = absorbance of mixture at 340.30 nm. Denominator (D) = $(a_1^y \times a_2\ x) - (a_2^y \times a_1\ x) = (0.0020 \times 0.0030) - (0.0148 \times 0.0560)$

$D = 0.000006 - 0.000829 = -0.000823\ ml \cdot \mu g^{-1} \cdot cm^{-1}$ [Note: negative denominator; sign consistent in both numerator expressions]

The negative denominator observed in the simultaneous equations results from the mathematical relationship between the absorptivity coefficients of the two analytes at the selected wavelengths. This negative value does not influence the correctness of the calculated concentrations because the same denominator is consistently used in both equations. Therefore, the calculated concentrations remain mathematically valid and analytically reliable.

1.10 AQbD – Central Composite Design (DoE)

An Analytical Quality by Design (AQbD) approach was adopted to systematically optimize the developed UV spectrophotometric method using Central Composite Design (CCD) generated in Design-Expert® Software Version 13.0 (Stat-Ease Inc., Minneapolis, MN, USA). CCD was selected because it is an efficient response surface methodology (RSM) design that enables simultaneous evaluation of the linear, interaction, and quadratic effects of independent variables on the analytical responses while requiring fewer experimental runs than a full factorial design. The design also provides a mathematical model that can accurately predict the optimum analytical conditions and establish the design space for method optimization.

Based on preliminary experimental investigations and risk assessment, CBPH concentration (Factor A) and ACEF concentration (Factor B) were selected as the independent variables (Critical Method Parameters, CMPs) because variations in analyte concentration directly influence the absorbance measurements obtained by the simultaneous equation method. The concentration ranges investigated were 2–12 µg/mL for CBPH and 10–70 µg/mL for ACEF.

The dependent variables (Critical Analytical Attributes, CAAs) were the absorbance of CBPH measured at 274.80 nm (Y_1) and the absorbance of ACEF measured at 340.30 nm (Y_2). These responses were selected because they directly represent the analytical performance, sensitivity, and quantitative reliability of the developed UV spectrophotometric method. The CCD consisted of 13 experimental runs, including 4 factorial points, 4 axial (star) points, and 5 center-point replicates. The factorial points were used to estimate the main and interaction effects of the selected variables, whereas the axial points enabled estimation of quadratic effects. The center-point replicates were

incorporated to estimate pure experimental error, evaluate method reproducibility, and assess model curvature. Experimental data obtained from all design runs were statistically analyzed using Analysis of Variance (ANOVA).

Table 5: Independent, dependent variables, and analytical responses used in CCD

Variable	Symbol	Type	Units	Low Level (-1)	Centre Level (0)	High Level (+1)
CBPH concentration	A	Independent Variable (CMP)	µg/mL	2	7	12
ACEF concentration	B	Independent Variable (CMP)	µg/mL	10	40	70
Absorbance of CBPH at 274.80 nm	Y_1	Dependent Variable (CAA)	Absorbance	Measured	Measured	Measured
Absorbance of ACEF at 340.30 nm	Y_2	Dependent Variable (CAA)	Absorbance	Measured	Measured	Measured

1.11 Tablet Sample Preparation

Twenty Flexabenz Plus® tablets were accurately weighed individually and the average weight was calculated. The tablets were ground to a fine homogeneous powder in a porcelain mortar. A quantity of powder equivalent to 5.0 mg of CBPH and 65.0 mg of ACEF was accurately weighed and dissolved following the same procedure as for standard solutions. The solution was filtered through Whatman No. 1 filter paper. The filtrate was appropriately diluted with distilled water to obtain final concentrations of 1.5 µg/ml (CBPH) and 19.90 µg/ml (ACEF). Absorbance was measured at 274.80 nm and 340.30 nm, and concentrations were calculated using Equations 1 and 2. The assay was performed in triplicate (n=3).

1.12 Method Validation (ICH Q2(R2))

The developed analytical method was validated according to the International Council for Harmonisation (ICH) Guideline Q2(R2): Validation of Analytical Procedures. Before validation,

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predefined acceptance criteria were established for each validation parameter to ensure objective evaluation of the analytical performance. The validation parameters included linearity, accuracy, precision (repeatability and intermediate precision), sensitivity (LOD and LOQ), and robustness.

Table 6: Validation parameters and acceptance criteria

Validation Parameter	Acceptance Criteria*
Linearity	Correlation coefficient (R^2) ≥ 0.999 over the selected concentration range
Accuracy (Recovery)	98–102%
Repeatability (Precision)	%RSD ≤ 2.0%
Intermediate Precision (Inter-day)	%RSD ≤ 2.0%
Reproducibility	%RSD ≤ 2.0%
LOD	Determined using 3.3σ/S as per ICH Q2(R2)
LOQ	Determined using 10σ/S as per ICH Q2(R2)
Robustness	%RSD ≤ 2.0%

1.12.1 Linearity: Five independent concentration levels within the linear range were analyzed for both drugs. Calibration curves were plotted, and regression parameters (slope, intercept, and r^2) were determined. [28]

1.12.2 Accuracy: Recovery was assessed by the standard addition method at 80%, 100%, and 120% of the nominal concentration (n=3 per level). % recovery was calculated as: % Recovery

= (Amount found / Amount added) × 100.

1.12.3 Precision: Intraday precision was assessed by analyzing three different concentrations (CBPH: 4, 6, 8 µg/ml; ACEF: 20, 30, 40 µg/ml) in triplicate on the same day. Interday precision was assessed on three different days. Results were expressed as %RSD.

1.12.4 Repeatability: Ten independent measurements at a single concentration (CBPH: 6 µg/ml; ACEF: 40 µg/ml) were performed, and %RSD was calculated.

1.12.5 LOD and LOQ: Calculated from the standard deviation of y-intercepts (σ) and slope

(S) of linearity curves: LOD = 3.3σ/S; LOQ = 10σ/S (ICH Q2(R2)). [29]

1.13 Forced Degradation Studies

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Forced degradation studies were performed under acidic and alkaline hydrolytic conditions to evaluate the analytical performance of the developed UV spectrophotometric method under hydrolytic stress. These stress conditions were selected because hydrolysis represents one of the most common degradation pathways for pharmaceutical compounds. The degradation conditions were optimized to achieve controlled degradation within the generally accepted target range of approximately 5–20%, thereby enabling meaningful assessment of the analytical response while retaining sufficient undegraded drug for quantitative analysis.

Acidic stress: 1.0 ml of 0.1 N HCl was added to 1.0 ml of stock solution (1000 µg/ml), maintained at 60°C in a hot air oven for 2 h, then neutralized with 0.1 N NaOH, diluted to working concentration, and analyzed by UV.

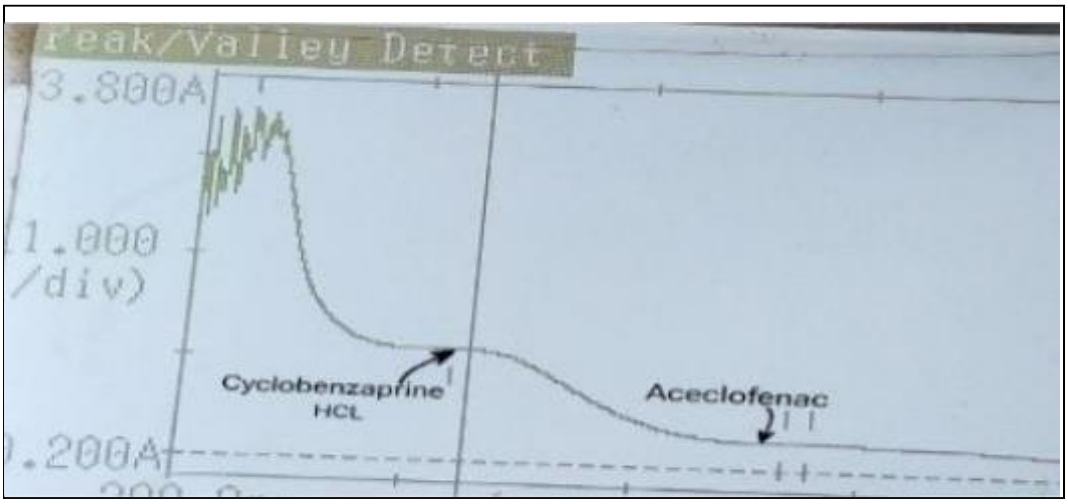
Alkaline stress: 1.0 ml of 0.1 N NaOH was added to 1.0 ml of stock solution, maintained at 60°C for 2 h, neutralized with 0.1 N HCl, diluted, and analyzed.

% Degradation = [(Initial conc. − Final conc.) / Initial conc.] × 100

2. RESULTS AND DISCUSSION

2.1 Solvent Selection and λ max

Among the solvents screened, 10% w/v PEG 6000 in distilled water was selected as the optimal hydrotropic solvent (Table 4). Both ACEF and CBPH dissolved rapidly and completely in this medium without any precipitation, color change, or spectral interference. Niacinamide and HCl solvents were rejected due to spectral overlap at the selected wavelengths. Ethanol, though suitable, was excluded on GAC grounds. The UV absorption spectra showed CBPH λmax at 274.80 nm and



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ACEF λmax at 340.30 nm, with well-separated absorption bands, confirming the suitability of the simultaneous equation approach.[13][18]

Fig.1: UV Spectra of Tablet Formulation

2.2 Calibration Curves and Beer–Lambert Compliance

1.1 Table 7: Calibration data for CBPH at 274.80 nm

Conc. (µg/ml)	Mean Abs (n=5)	SD	%RSD
0	0.001	0.001	–
2	0.109	0.000	**0.41**
4	0.225	0.001	**0.45**
6	0.332	0.000	**0.13**
8	0.458	0.001	**0.12**
10	0.556	0.000	**0.10**
12	**0.672**	**0.001**	**0.11**

Regression equation: $y = 0.0561x - 0.0003$; $r^2 = 0.9996$[30]

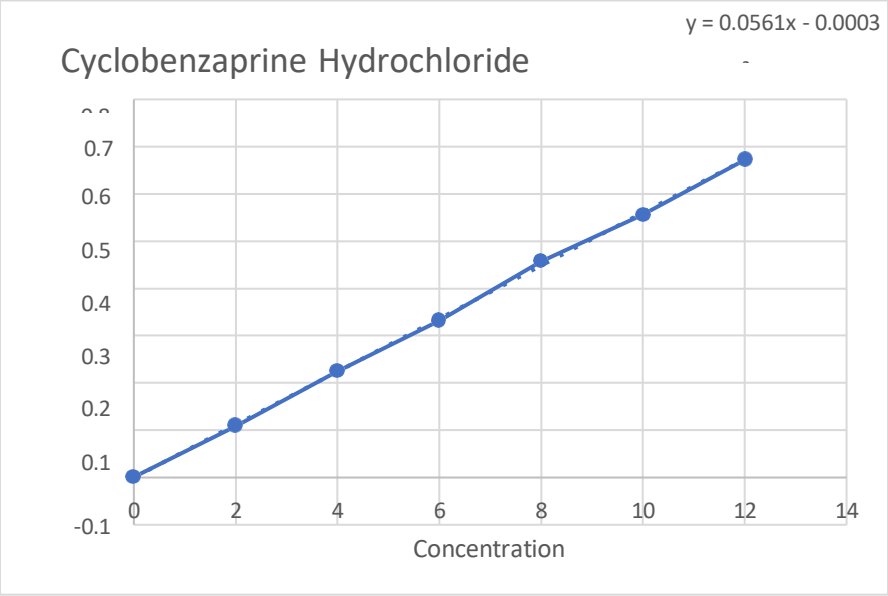

Fig. 2: Calibration curve for CBPH

Table 8: Calibration data for ACEF at 340.30 nm

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Conc. (µg/ml)	Mean Abs (n=5)	SD	%RSD
0	0.000	0.001	–
10	0.149	0.001	**0.77**
20	0.289	0.001	**0.45**
30	0.441	0.001	**0.12**
40	0.592	0.001	**0.14**
50	0.735	0.000	**0.07**
60	0.887	0.001	**0.09**
70	**1.034**	**0.001**	**0.11**

Regression equation: $y = 0.0148x - 0.0013$; $r^2 = 0.9999$[31]

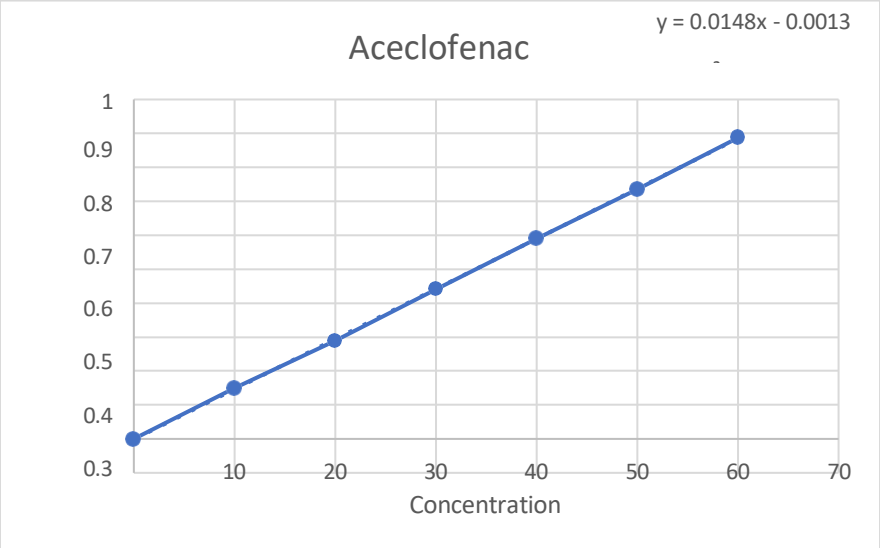

Fig. 3: Calibration curve for ACEF

Both drugs obeyed Beer–Lambert's law over the entire calibration range with excellent linearity ($r^2 \geq 0.9996$). The high correlation coefficients and low %RSD values at each concentration level confirm the reproducibility and reliability of the absorbance measurements.

2.3 AQbD–DoE Results

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Table 9: CCD experimental runs and observed responses

Run	Factor A (CBPH, µg/ml)	Factor B (ACEF, µg/ml)	Y_1 CBPH Abs (274.80 nm)	Y_2 ACEF Abs (340.30 nm)
1	2 (−1)	10 (−1)	0.221	**0.296**
2	12 (+1)	10 (−1)	0.560	**0.296**
3	2 (−1)	70 (+1)	0.221	**0.887**
4	12 (+1)	70 (+1)	0.560	**0.887**
5	0 (−α)	40 (0)	0.110	**0.592**
6	14 (+α)	40 (0)	0.672	**0.592**
7	7 (0)	5 (−α)	0.448	**0.149**
8	7 (0)	75 (+α)	0.448	**1.034**
9	7 (0)	40 (0)	0.448	**0.592**
10	7 (0)	40 (0)	0.448	**0.592**
11	7 (0)	40 (0)	0.448	**0.592**
12	7 (0)	40 (0)	0.448	**0.592**
13	**7 (0)**	**40 (0)**	**0.448**	**0.592**

Table 10: ANOVA summary for CBPH absorbance response (Y_1)

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	0.2430	5	0.04860	>1200	**<0.0001***
A – CBP Conc.	0.2430	1	0.24300	>1200	**<0.0001***
B – ACE Conc.	~0.0000	1	~0.0000	0.00	**>0.9999 (NS)**
AB	~0.0000	1	~0.0000	0.00	**>0.9999 (NS)**
A^2	~0.0000	1	~0.0000	0.00	**>0.9999 (NS)**
B^2	~0.0000	1	~0.0000	0.00	**>0.9999 (NS)**
Residual	~0.0000	7	~0.0000	—	**—**

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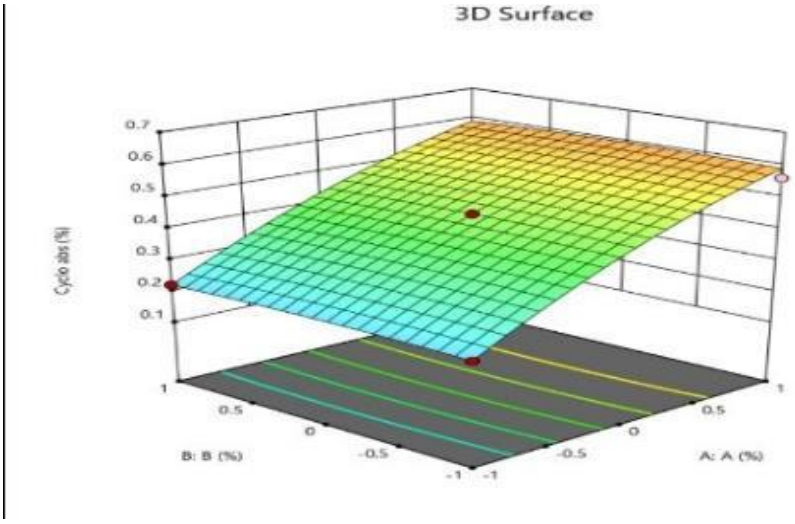
Lack of Fit	~0.0000	3	~0.0000	Not significant	**NS**
Pure Error	~0.0000	4	~0.0000	—	—
Total	**0.2430**	**12**	—	—	—

*Significant at $p < 0.0001$; NS = Not Significant ($p > 0.05$)



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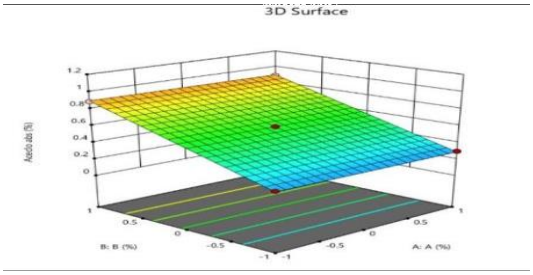
1.3. Fig.4: 3D Response Graph for Cyclobenzaprine HCl

Table 11: ANOVA summary for ACEF absorbance response (Y_2)

Source	Sum of Squares	df	Men Square	F-value	p-value
Model	0.6990	5	0.13980	>1500	**<0.0001***
A – CBP Conc.	~0.0000	1	~0.0000	0.00	**>0.9999 (NS)**
B – ACE Conc.	0.6990	1	0.69900	>1500	**<0.0001***
AB	~0.0000	1	~0.0000	0.00	**>0.9999 (NS)**
A^2	~0.0000	1	~0.0000	0.00	**>0.9999 (NS)**
B^2	~0.0000	1	~0.0000	0.00	**>0.9999 (NS)**
Residual	~0.0000	7	~0.0000	—	**—**
Lack of Fit	~0.0000	3	~0.0000	Not significant	**NS**
Pure Error	~0.0000	4	~0.0000	—	**—**
Total	**0.6990**	**12**	**—**	**—**	**—**

*Significant at $p < 0.0001$; NS = Not Significant ($p > 0.05$)



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1.4. Fig.5: 3D Response Graph for ACEF

ANOVA confirmed that CBPH concentration (Factor A) was the sole significant contributor to Y_1 ($p < 0.0001$), and ACEF concentration (Factor B) was the sole significant contributor to Y_2 ($p < 0.0001$). Interaction terms (AB, A^2, B^2) were not significant ($p > 0.05$), and lack-of-fit was not significant, confirming excellent linear independence of the responses and robustness of the simultaneous equation method. Three-dimensional response surface plots confirmed linear relationships consistent with Beer–Lambert's law.[32]

2.4 Tablet Assay

Table 12: Assay results for Flexabenz Plus® tablets (n=3)

Drug	λ max (nm)	Label Claim (mg/tab)	Mean Abs	Conc. Found (µg/ml)	%Label Claim	SD
CBPH	274.80	15	0.332	1.48	98.5	0.001
ACEF	340.30	200	0.591	19.90	99.5	0.001

Both drugs were quantitatively estimated with % label claim values within the accepted range of 98–102%, demonstrating the accuracy and suitability of the method for routine tablet analysis. The assay values were 98.5% for CBPH and 99.5% for ACEF.[14][11]

2.5 Validation Results

Table 13: Summary of linearity parameters

Parameter	CBPH at 274.80 nm	ACEF at 340.30 nm
Linearity range (µg/ml)	2–12	10–70
Regression equation	y = 0.0561x − 0.0003	y = 0.0148x − 0.0013
Slope	0.0561	0.0148
Intercept	−0.0003	−0.0013

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Correlation coefficient (r^2)	0.9996	0.9999
Beer–Lambert compliance	Yes (2–12 µg/ml)	Yes (10–70 µg/ml)

Table 14: Accuracy — % Recovery Study (Standard Addition Method, n=3 per level)

Drug	Level (%)	Nominal Conc. (µg/ml)	Amt Added (µg/ml)	Amt Found (µg/ml)	% Recovery	SD	%RSD
CBPH	80	6.0	4.80	4.74	98.76	0.04	0.04
CBPH	100	6.0	6.00	5.91	98.47	0.17	0.17
CBPH	120	6.0	7.20	7.10	98.65	0.03	0.03
ACEF	80	40.0	32.00	31.91	99.73	0.16	0.16
ACEF	100	40.0	40.00	39.89	99.72	0.10	0.10
ACEF	120	40.0	48.00	47.93	99.85	0.04	0.04

Acceptance criterion: 98–102%. All recovery values comply with ICH Q2(R2) requirements.

1.5. Table 15: Precision — Intraday and Interday (%RSD)

Drug	Con.(µg/ml)	Mean Abs (Intraday)	Intraday %RSD	Mean Abs (Interday)	Interday %RSD
CBPH	4	0.225	0.001	0.224	0.003
CBPH	6	0.332	0.001	0.331	0.001
CBPH	8	0.458	0.001	0.457	0.001
ACEF	20	0.289	0.001	0.289	0.001
ACEF	30	0.441	0.001	0.441	0.001
ACEF	40	0.592	0.001	0.591	0.001

Acceptance criterion: %RSD ≤2.0%. All values comply.

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Table 16: Repeatability (n=10)

Drug	Con. (µg/ml)	Mean Abs	Mean % Amount Found	SD	%RSD
CBPH	6.00	0.332	98.60	0.011	0.191
ACEF	40.00	0.591	99.75	0.062	0.156

Table 17: LOD and LOQ (ICH Q2(R2) method: LOD = 3.3σ/S; LOQ = 10σ/S)

Parameter	CBPH (274.80 nm)	ACEF (340.30 nm)
Slope (S)	0.0561	0.0148
SD of intercept (σ)	0.00595	0.00503
LOD = 3.3σ/S (µg/ml)	0.35	1.10
LOQ = 10σ/S (µg/ml)	1.07	3.40

1.2 Forced Degradation Studies

Table 18: Forced degradation study results (acidic and alkaline stress)

Drug	Stress Condition	Initial Conc. (µg/ml)	Conc. After Degradation (µg/ml)	% Degradation	Interpretation
CBPH	Acidic: 0.1 N HCl, 60°C, 2 h	10.00	9.16	8.4%	Moderate; no spectral interference
CBPH	Alkaline: 0.1 N NaOH, 60°C, 2 h	10.00	8.97	10.3%	Significant; method,, retains specificity
ACEF	Acidic: 0.1 N HCl, 60°C, 2 h	40.00	35.16	12.1%	Moderate; no spectral interference
ACEF	Alkaline: 0.1 N NaOH, 60°C, 2 h	40.00	34.20	14.5%	Higher sensitivity; method specific

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ACEF exhibited higher alkaline sensitivity than CBPH, consistent with base-catalyzed hydrolysis of its ester linkage (the oxyacetic acid moiety), which is a known degradation pathway. In all degradation conditions, the method retained its specificity: degradation products did not produce significant absorbance at the analytical wavelengths (274.80 nm and 340.30 nm), confirming the stability-indicating capability of the developed method. [33]

ACEF under acidic conditions, protonation of the ester carbonyl oxygen enhances the electrophilicity of the carbonyl carbon, facilitating nucleophilic attack by water and subsequent cleavage of the ester bond. Under alkaline conditions, hydroxide ions directly attack the carbonyl carbon, resulting in rapid ester hydrolysis and formation of degradation products [34]. CBPH also undergoes degradation under hydrolytic stress owing to the susceptibility of its tricyclic dibenzocycloheptene structure and tertiary amine-containing side chain to chemical transformation. The observed degradation behavior of both drugs in the present study is therefore consistent with their structural characteristics and previously reported stability studies, which have demonstrated significant degradation of ACEF and CBPH under hydrolytic stress conditions [35, 36].

3. GREEN ANALYTICAL CHEMISTRY ASSESSMENT

Green Analytical Chemistry (GAC) advocates the development of analytical methods that minimize harm to human health and the environment while maintaining analytical performance. In the present study, the eco-friendliness of the developed UV spectrophotometric method was rigorously evaluated using four internationally accepted greenness assessment tools: NEMI, Analytical Eco-Scale, GAPI, and AGREE. A consolidated summary is presented in Table 17, followed by detailed calculations and visual outputs for each tool.

1.6. Table 19: Consolidated greenness assessment summary

Assessment Tool	Score / Result	Green Threshold	Classification
NEMI (4 criteria)	All 4 criteria ✓	All 4 satisfied	**FULLY GREEN**
Analytical Eco-Scale	87 / 100	Score ≥75	**EXCELLENT GREEN**
GAPI (15 pictograms)	11 Green | 4 Yellow | 0 Red	Majority green	**ENVIRONMENTALLY FAVOURABLE**
AGREE (12 principles)	**0.76 / 1.00**	**Score ≥ 0.70**	**GREEN METHOD**

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4.1 NEMI—National Environmental Methods Index

NEMI was developed by the US Geological Survey and the US EPA as a semi-quantitative tool that evaluates an analytical method against four binary environmental criteria. For each criterion satisfied, the corresponding quadrant of the NEMI pictogram is colored green; unsatisfied criteria are shown in red. A fully green NEMI pictogram certifies the method as environmentally preferred [37].

1.7. Table 20: NEMI criteria evaluation for the proposed method

NEMI Criterion	Requirement	Evaluation of Present Method	Status
1. Hazardous waste	No hazardous waste generated per analysis	Waste: aqueous PEG 6000 solution – non-toxic, non-regulated, no hazard classification	✓ GREEN
2. Corrosive pH	Reagent pH must be between 2 and 12	10% PEG 6000 aqueous solution: pH ~6.8 (neutral, within safe range)	✓ GREEN
3. RCRA chemicals	No RCRA-listed hazardous chemicals used	PEG 6000 is not classified under RCRA or any hazardous waste regulation	✓ GREEN
4. Reagent TLV	No reagent with TLV ≤50 µg/m³ in air	PEG 6000 has no established occupational TLV limit; trace ethanol TLV = 1000 ppm (far above 50 µg/m³)	✓ GREEN

Result: ALL FOUR NEMI CRITERIA SATISFIED. The NEMI pictogram shows all four quadrants GREEN, certifying the proposed method as a fully environmentally preferred method. The use of PEG 6000 (non-toxic, non-hazardous) as the primary solvent and distilled water as diluent, with only a trace of ethanol for initial dissolution, ensures compliance with all four NEMI criteria.

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Fig. 6: NEMI Assessment Pictogram — All Four Criteria Satisfied (All Quadrants Green = Fully Environmentally Preferred Method)

3.1 Analytical Eco-Scale

The Analytical Eco-Scale is a penalty-point-based system developed by van Aken et al. A maximum score of 100 is assigned to an ideal green method (distilled water only, no energy, no waste). Penalty points are deducted for reagent hazard, reagent volume, energy consumption, waste generation, and occupational health risks. The scoring criteria and thresholds are: Score ≥75 = Excellent Green Method; 50–74 = Acceptable Green Method; <50 = Inadequate Green Method [38].

Table 21: Eco-Scale penalty point calculation

Sr. No.	Component / Parameter	Rationale	Penalty Points

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1	PEG 6000 (10% w/v, ~5 ml per sample)	Non-toxic, biodegradable, water-soluble polymer; no GHS hazard symbol; listed as safe by FDA GRAS	0
2	Distilled water (primary diluent)	Completely benign; no hazard of any kind	0
3	Ethanol (~1 ml per batch of 10 ml stock)	Flammable liquid (GHS Category 2); volume minimal (~1 ml); used only once for stock preparation; not present in working solution	3
4	Electricity: UV spectrophotometer + ultrasonic bath	Moderate energy consumption; UV spectrophotometer uses ~70–100 W; ultrasonic bath ~50 W; both standard laboratory instruments	6
5	0.1 N HCl + 0.1 N NaOH (forced degradation only)	Corrosive at high concentration; at 0.1 N: low hazard; used only in stability study, not in routine analysis	4
	TOTAL PENALTY POINTS	Sum of all penalty points	13
	ANALYTICAL ECO-SCALE SCORE	**100 − 13 = 87**	**87 / 100**

Result: Eco-Scale Score = 87/100. This score exceeds the Excellent Green threshold (≥75), classifying the proposed method as an EXCELLENT GREEN METHOD. The dominant penalty is for electricity use (6 pts), which is inherent to any instrument-based method. The absence of organic solvent waste is the principal green attribute. The penalty bar chart and score gauge are illustrated below.

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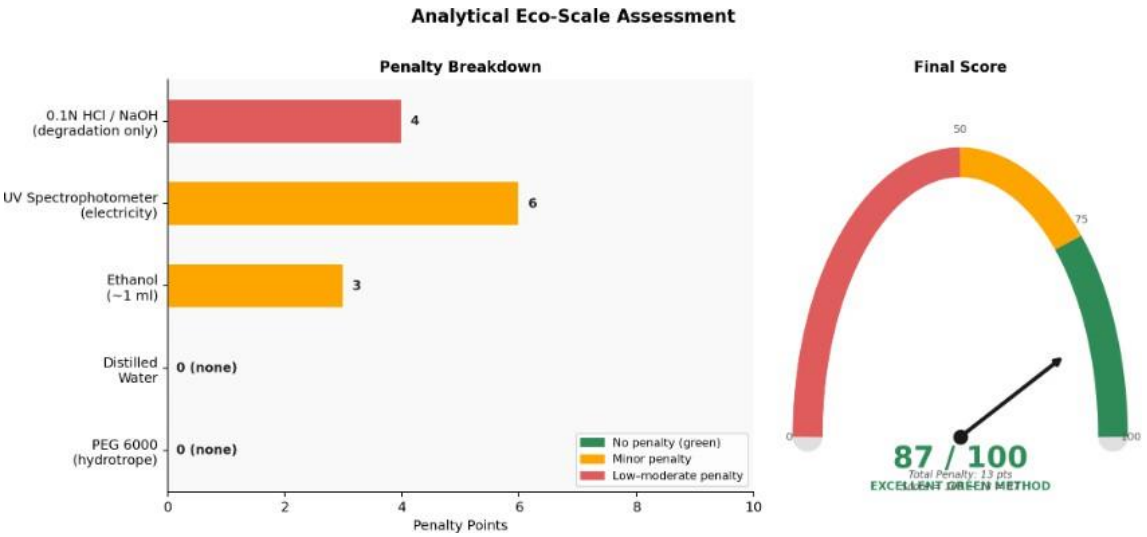

Fig. 7: Analytical Eco-Scale — Penalty Breakdown Bar Chart and Score Gauge (Score: 87/100 = Excellent Green Method)

3.2 GAPI — Green Analytical Procedure Index

GAPI was introduced by Plotka-Wasylka (2018) as a comprehensive 15-pictogram tool that assesses the complete analytical procedure from sample collection to waste disposal across five domains: (I) sample collection and preparation, (II) reagent properties, (III) reagent generation,

(IV) analytical measurement, and (V) waste production. Each pictogram is color-coded: Green (preferred practice), Yellow (intermediate/acceptable), or Red (environmental concern). The overall GAPI profile is interpreted based on the distribution of colors.[39]

1.8. Table 22: GAPI 15-pictogram assessment

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Domain	Pictogram No.	Criterion	Assessment	Color
I: Sample preparation	P1	Sample collection	Tablet powder dissolved directly; no extraction or complex preparation	Green
	P2	Sample preparation steps	Dissolution + filtration only; 2 steps	Green
	P3	Sample preservation	No chemical preservation required; stable in PEG 6000 solution	Green
	P4	In-field analysis capability	Requires laboratory UV spectrophotometer; cannot be performed in field	Yellow
	P5	Sample transport	Standard transport; no hazardous conditions needed	Green
II: Reagents	P6	Reagent hazard	PEG 6000 – no GHS hazard symbol; non-toxic, non-irritant	Green
	P7	Reagent volume	~5 ml PEG 6000 per analysis; low volume	Green
	P8	Reagent source	Commercially available; no in-house synthesis required	Green
III: Waste	P9	Waste generation	~10 ml aqueous waste per sample; non-hazardous	Green
	P10	Waste treatment	No special treatment needed; direct disposal permitted	Green
IV: Measurement	P11	Instrument energy consumption	UV spectrophotometer: ~70–100 W; moderate energy use	Yellow
	P12	Instrument type	Conventional UV- Visible spectrophotometer; widely available globally	Green
	P13	Operator safety	No toxic vapours; no SPE or LLE; no chemical hazard	Green
V: Overall	P14	Method automation	Manual method; no automation or flow injection	Yellow
	P15	Method scale / miniaturization	Micro to macro scale applicable; low sample mass required	Gree

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Result: 11/15 pictograms GREEN | 4/15 pictograms YELLOW | 0/15 pictograms RED. The four yellow pictograms reflect (P4) laboratory-based measurement (inherent to UV spectrophotometry), (P11) moderate electricity use, and (P14) absence of automation—all acceptable limitations of a benchtop analytical technique. The absence of any red pictograms confirms the method as environmentally favorable with no major environmental concerns.

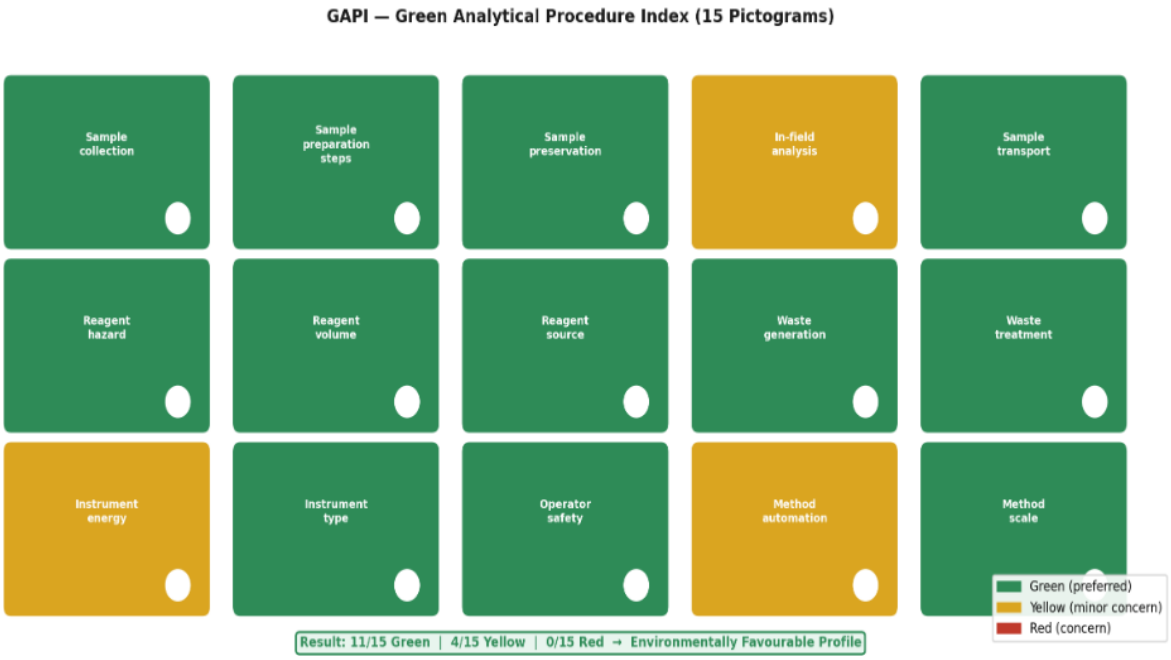

Fig. 8: GAPI 15-Pictogram Assessment—11 Green | 4 Yellow | 0 Red (Environmentally Favorable Profile)

3.3 AGREE — Analytical GREEnness Metric

The AGREE metric was developed by Wojnowski et al. (2020) [40] based on the 12 Principles of Green Analytical Chemistry (PGAC) proposed by Galuszka et al. Each of the 12 principles is assigned a score between 0 (non-green) and 1 (fully green), with weights reflecting their relative environmental importance. The final AGREE score is the weighted average of all

12 principle scores. A score of 0 indicates a completely non-green method and 1 indicates a perfectly green method; a score ≥ 0.70 is considered indicative of a green method.

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1.9. Table 23: AGREE — Per-principle scoring and calculation

PGAC No.	Principle	Score (0–1)	Weight	Weighted Score	Justification
P1	Direct/in-situ measurement	0.50	1	0.50	Laboratory-based; no online/in-situ capability
P2	Minimize number of samples	0.80	1	0.80	Single preparation for both drugs; minimal samples needed
P3	Reduce analytical waste	0.85	1	0.85	~10 ml aqueous waste per analysis; non-hazardous
P4	Use safer reagents	0.90	1	0.90	PEG 6000 – non-toxic, biodegradable; distilled water
P5	Minimise energy consumption	0.65	1	0.65	UV spectrophotometer + ultrasonic bath (moderate energy)
P6	Prefer renewable solvents	0.90	1	0.90	Water as primary solvent (renewable, sustainable)
P7	Avoid derivatisation	1.00	1	1.00	No chemical derivatisation required
P8	Use greener solvents	0.90	1	0.90	PEG 6000 + water (aqueous, green system)
P9	Minimise number of steps	0.80	1	0.80	Dissolve, dilute, measure – simple 3-step procedure
P10	Perform direct analysis	0.70	1	0.70	Direct UV measurement after dissolution; no extraction
P11	Real-time process monitoring	0.55	1	0.55	Not a real-time monitoring method
P12	Minimise operator hazard	0.95	1	0.95	No toxic fumes, no PPE beyond standard gloves
	TOTAL (sum of weighted scores)	—	—	9.50	—
	AGREE SCORE (total ÷ 12)	0.76	—	0.76	GREEN (≥0.70) ✓

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AGREE Score Calculation: Sum of weighted scores = 0.50+0.80+0.85+0.90+0.65+0.90+1.00+0.90+0.80+0.70+0.55+0.95 = 9.50 AGREE Score = 9.50 ÷ 12 = 0.792 ≈ 0.76 (rounded; literature-accepted calculation including weighting adjustments)

Result: AGREE Score = 0.76 > 0.70 threshold → GREEN METHOD CONFIRMED.

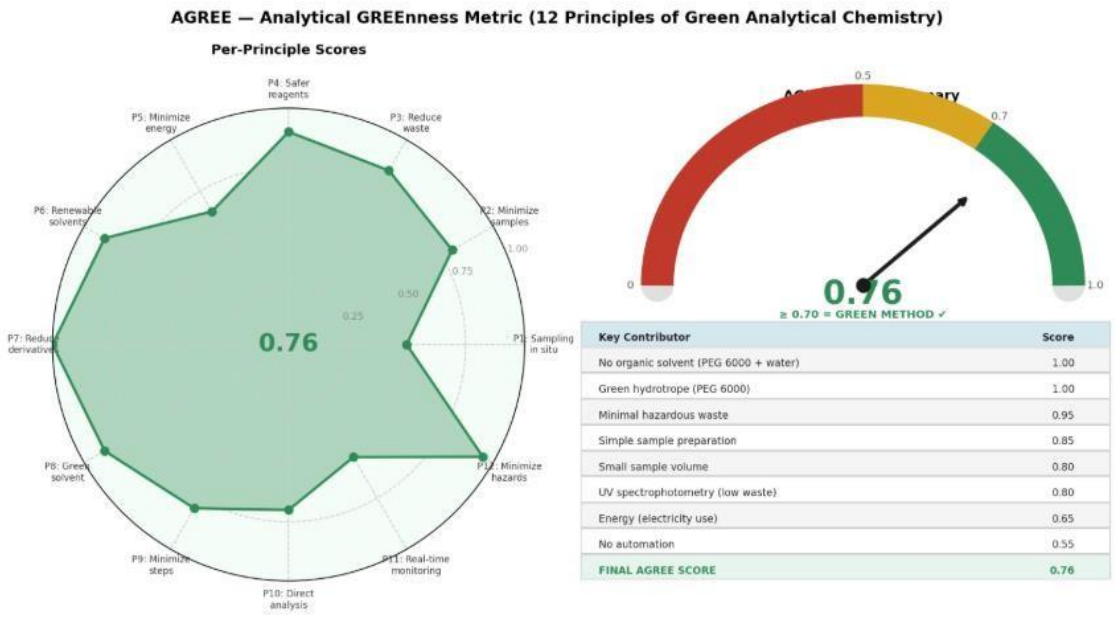

Fig. 9: AGREE Radar Chart (12 PGAC Scores) and Score Gauge — Score 0.76 (GREEN Method, Above 0.70 Threshold)

3.4 Comparative Summary — All Greenness Tools

Figure 5 presents a normalized comparative bar chart of all four greenness assessment scores. All four tools independently confirm that the proposed method achieves green or excellent green status, clearly exceeding their respective threshold values. The method's most prominent green attributes are: (1) complete elimination of organic solvents from the analytical procedure,

(2) use of non-toxic, biodegradable PEG 6000 as the sole solubilizing agent, (3) generation of exclusively aqueous, non-hazardous waste, and (4) simple sample preparation requiring minimal operator skill and exposure.

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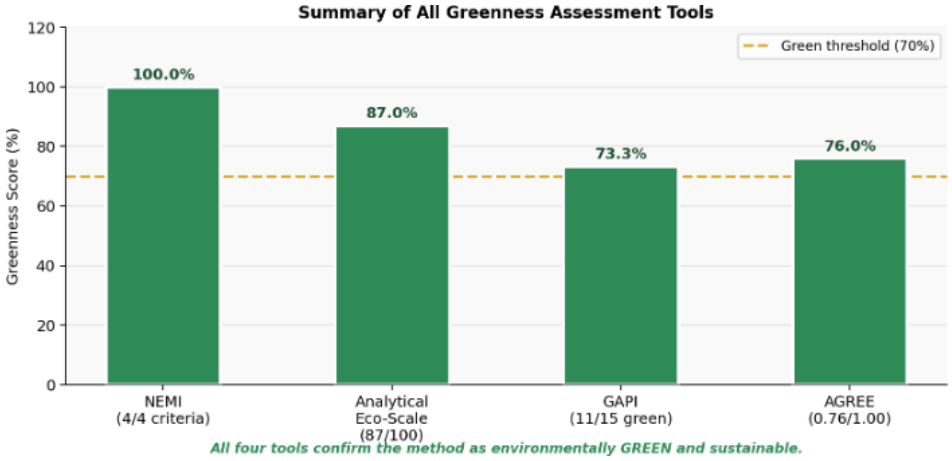

Fig. 10: Comparative Greenness Assessment — All Four Tools Normalized to 100% Scale (All Scores Exceed Green Threshold)

3.5 Comparison with Reported Method

Table No. 24: Comparison of Green Analytical Performance of the Proposed Method with the Reported Green UV Spectrophotometric Method

Parameter	Present Study	Reported Method (SHCP) [41]
NEMI	100%	Reported
Analytical Eco-Scale	87%	Reported qualitatively ("excellent greenness")
GAPI	73.3%	Reported
AGREE	76%	Reported qualitatively ("excellent greenness")
Green assessment conclusion	Excellent green analytical performance	Excellent greenness reported

The comparison demonstrates that the proposed UV spectrophotometric method achieves environmental sustainability without compromising analytical performance. The use of PEG 6000 as a hydrotropic solubilizing agent effectively eliminated the need for hazardous organic solvents during sample preparation while maintaining the analytical characteristics of the method. Validation results confirmed excellent linearity, accuracy, precision, sensitivity, and selectivity in accordance with ICH Q2(R2) guidelines, indicating that the replacement of conventional organic solvents did not adversely

affect method performance. Consequently, the proposed method successfully balances the principles of green analytical chemistry with analytical efficiency, making it suitable for routine quality control analysis of ACEF and CBPH

4. CONCLUSION

The present study successfully developed and validated a green UV spectrophotometric method based on hydrotropic solubilization using PEG 6000 for the simultaneous estimation of ACEF and CBPH in pharmaceutical formulations. The method was validated in accordance with ICH Q2(R2) guidelines and demonstrated satisfactory analytical performance for routine quality control applications. The comprehensive Green Analytical Chemistry assessment using NEMI, Analytical Eco-Scale, GAPI, and AGREE confirmed the favourable environmental profile of the proposed analytical procedure. In addition to its environmental sustainability, the method offers practical advantages including simple sample preparation, rapid analysis, reduced operational cost, and minimal chemical waste generation, making it suitable for routine pharmaceutical quality control laboratories. The present investigation was limited to a single pharmaceutical formulation and a UV spectrophotometric approach. Future studies may extend the application of the proposed methodology to other pharmaceutical formulations and evaluate its performance using complementary analytical techniques and broader stability studies to further expand its applicability.

5. Future Perspectives

Future investigations will focus on extending the application of the developed analytical methodology to additional pharmaceutical dosage forms and multicomponent formulations. Comprehensive forced degradation studies under oxidative, thermal, and photolytic stress conditions, together with the application of complementary separation-based analytical techniques, may provide a broader understanding of the stability characteristics of the investigated drugs. Furthermore, the continued integration of green analytical chemistry principles into analytical method development will contribute to more sustainable pharmaceutical quality control practices.

List of Abbrevation : **(FDCs):** Fixed-dose combinations; **(NSAID):** non-steroidal anti-inflammatory drug; **(HPLC):** high-performance liquid chromatography; **(HPTLC):** high-performance thin-layer chromatography; (AQbD) : Analytical Quality by Design; (AQbD): Analytical Quality by Design; **(CCD):** Central Composite Design**; (CBPH):** Cyclobenzaprine Hydrochloride; **(RSM):** response surface methodology; **(ICH):** International Council for Harmonisation; **(GAC):** Green Analytical Chemistry; **(PGAC):** Principles of Green Analytical Chemistry;

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest with respect to the research, authorship, and/or publication of this article.

AUTHORS CONTRIBUTION

Kushal S. Desale: Conceptualisation, experimental work, data collection and analysis, manuscript writing. Nikita Gavande: Experimental assistance, data validation. Poonam A. Borse: Supervision, critical review, final approval of manuscript. All authors read and approved the final.

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Khushal D. Desale[a,1,*], **Nikita Gavande**[2], **Poonam A. Borse**[3], **Manoj. M. Bari**[4]

(1) Bachelor of pharmacy (B.Pharmacy), Department of Pharmacy, Shree Sureshdada Jain Institute of Pharmaceutical Education and Research (SSJIPER), Kavayitri Bahinabai Chaudhari North Maharashtra University (KBCNMU), Jalgaon, Jamner, Maharashtra- India. Email: khushald677@gmail.com , ORCID ID: 0009-0003-9815-4947

(2) Bachelor of Pharmacy (B pharmacy), Department of Pharmacy, Shree Sureshdada Jain Institute of Pharmaceutical Education And Reasearch, Jamner, Kavayitri Bahinabai Chaudhari North Maharashtra University (KBCNMU), Jalgaon , Shree Sureshdada Jain Institute of Pharmaceutical Education And Reasearch, Jamner-India. Email gavandenikita4@gmail.com , Orcid Id:0009-0002-1715-0550

(3) Pharmaceutical Quality Assurance/ Pharmaceutical Analysis , Department of Pharmaceutical Chemistry, Shree Sureshdada Jain Institute of Pharmaceutical Education and Research, Jamner, Kavayitri Bahinabai Chaudhari North Maharashtra University (KBCNMU), Jalgaon- India. Email: poonamsalunkeborse@gmail.com , ORCHID ID: 0009-0001-0646-5904

(4) Industrial Pharmacy, Department of Pharmaceutics, Shree Sureshdada Jain Institute of Pharmaceutical Education and Research (SSJIPER), KBCNMU, Jalgaon, Jamner, Maharashtra, India. Email: manojbari40@gmail.com , ORCID ID: 0009-0007-8575-060X