Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(1), 2214-2227 | ISSN:2251-6727

Green, Blue and White Assessment of an Environmentally Conscious RP-HPLC Method for Simultaneous Estimation of Paracetamol and Promethazine Hydrochloride Using AQbD and Degradation Studies

Vineetha Rosireddy[1]*, Manikandan Krishnan[1] and Atyurmila Chakraborty[2]

*[1]Department of Pharmaceutical Analysis, SRM College of Pharmacy SRM Institute of Science and Technology Kattankulathur, Tamil Nadu, 603203, India

[2]Anand College of Education, Department of pharmacy Kabilpur, Debra, Paschim, Medinipur, West Bengal-721126, India

(Received: 05 December 2025 Revised: 15 January 2026 Accepted: 10 February 2026)

KEYWORDS

Analytical Quality by Design, Blueness Greenness, Reverse Phase-High Performance Liquid Chromatography,

and Whiteness.

ABSTRACT:

Introduction: Paracetamol and promethazine are widely used pharmaceutical agents, often co-formulated for the management of pain, fever, and allergic conditions. The development of analytical methods for their simultaneous determination is essential for quality control and stability assessment.

Objectives: The objective of this research was to develop and validate a novel, eco-friendly reversed-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of paracetamol and promethazine, including forced degradation studies, while adhering to the principles of green, blue, and white analytical chemistry.

Results Chromatographic separation was achieved using gradient elution with ethanol and water, modified with 0.1% acetic acid, on an Inertsil ODS-3 column (250 mm × 4.6 mm, 5 µm) at 35 °C and a flow rate of 0.8 mL/min. Detection was performed at 250 nm. Retention times were 6.097 min for paracetamol and 8.850 min for promethazine. The method exhibited excellent linearity with correlation coefficients (R^2) of 0.999 across concentration ranges of 10–200 µg/mL and 1–10 µg/mL, respectively. Limits of detection and quantification were 2.6 and 7.9 µg/mL for paracetamol, and 0.60 and 1.82 µg/mL for promethazine. Sustainability assessment revealed high greenness, blueness, and whiteness scores.

Conclusions: The developed RP-HPLC method is simple, sensitive, robust, and environmentally sustainable, making it suitable for routine quality control and stability studies while promoting green, blue, and white analytical chemistry principles.

1. Introduction

N-(4-hydroxyphenyl) acetamide, commonly known as paracetamol (PCM), is a white, crystalline, odourless compound with limited solubility in water. Widely recognized for its analgesic and antipyretic properties, it is used to manage mild to moderate pain and fever. PCM exerts its pharmacological effects through both central and peripheral mechanisms, displaying antipyretic activity and weak anti-inflammatory properties. Various pharmacopoeias provide detailed monographs on PCM and its combination formulations. Owing to its widespread use in pharmaceutical products, there is growing interest in developing robust, rapid, and precise analytical methods for the determination of PCM, both as a single component and in combination formulations [1-3].

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(1), 2214-2227 | ISSN:2251-6727

N,N-dimethyl-1-(10H-phenothiazin-10-yl) propan-2-amine, known as promethazine (PMZ), is a water and ethanol soluble crystalline powder ranging in colour from white to pale yellow. It is a first-generation H_1 receptor antagonist primarily used for the prevention and treatment of nausea, vomiting, and motion sickness. Additionally, PMZ exhibits antipsychotic properties and is occasionally employed as an adjunct in the management of certain mental health condition. Beyond its antihistaminic action, PMZ may exert antiemetic and sedative effects rough interactions with other neurotransmitter systems, including cholinergic pathways as well as via direct action on specific brain regions. The drug is available in multiple dosage forms, including tablets, suppositories, and oral syrups.

The estimation of PMZ, alone or alongside other drugs in pharmaceutical formulations, has been achieved using various reported HPLC methods [4-7]. The rising global demand for these drugs highlights their growing therapeutic significance. The Indian PCM market is projected to grow from USD 1.36 billion in 2024, while the global PMZ market is valued at USD 1.2 billion in 2024.This surge necessitates reliable and rapid analytical methods for quality control.

Various analytical techniques, including UV spectrophotometry and HPLC, have been employed for the quantification of PMZ either individually or in combination with PCM [8–10]. The present study marks the first application of eco-friendly solvents for this drug combination, developed within the AQbD framework using a Central Composite Design. AQbD emphasizes a science- and risk-based approach to method development, beginning with predefined objectives and focusing on a deep understanding of both the product and the process to ensure robust control and quality assurance.

In response to the increasing regulatory and industrial emphasis on sustainability, the proposed method has been rigorously evaluated using the principles of Green Analytical Chemistry (GAC), Blue Analytical Chemistry (information-richness), and White Analytical Chemistry (holistic assessment) [11-18]. Unlike conventional techniques that often neglect environmental considerations, this method integrates environmental metrics with analytical robustness, efficiency, and degradation profiling making it highly applicable for contemporary pharmaceutical quality control and regulatory standards.

By replacing hazardous solvents with greener alternatives and reducing resource consumption, the method directly contributes to minimizing the ecological footprint of analytical laboratories. Its validated performance, combined with a strong alignment to sustainable practices, underscores its potential for widespread routine use in industrial settings bridging the gap between analytical precision and environmental responsibility.

Ultimately, this study aims to establish a rapid, accurate, cost-effective, and eco-conscious RP-HPLC method for the simultaneous estimation of PCM and PMZ. Through the integration of green, white, and blue analytical chemistry principles, it not only enhances scientific rigor but also promotes a forward-thinking approach to pharmaceutical analysis grounded in sustainability and holistic quality.

Current chromatographic techniques for dual drug analysis often overlook safer, eco-compatible solvent systems, limiting their applicability in sustainable laboratory environments. Conventional methods largely omit holistic evaluation frameworks that consider environmental, operational, and informational criteria. Risk-based method development strategies remain underutilized, particularly for multi-analyte systems requiring precise control. Additionally, few studies have adopted systematic design tools to enhance method robustness and predictability. Given these limitations, there is a pressing need to develop a novel, sustainability-driven analytical

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2214-2227 | ISSN:2251-6727

approach that integrates green solvent usage, risk-based optimization, and comprehensive method evaluation for the simultaneous estimation of pharmaceutically relevant drug combinations. A comprehensive, sustainability-aligned approach for the concurrent assessment of these analytes using greener alternatives is yet to be fully established.

2. Materials and Methods

2.1 Chemicals and reagents

Certified pharmaceutical grade samples of paracetamol (PCM) and promethazine (PMZ) were received as a courtesy from Aurobindo Pharma Ltd., Hyderabad, Telangana, India. Ethanol was obtained from Fisher Scientific India, Mumbai. Sodium hydroxide (NaOH) was sourced from SD Fine-Chem Ltd., Mumbai, and concentrated hydrochloric acid (HCl) was procured from Loba Chemie Pvt. Ltd., Mumbai, India. A 3% v/v hydrogen peroxide (H_2O_2) solution was obtained from Central Drug House (CDH), New Delhi. Milli-Q grade water was prepared using a purification system from Millipore Sigma, USA. Acetic acid was obtained from Thomas Baker Chemicals, Mumbai. A 0.45 µm membrane filter was procured from Axiva Sichem Biotech, New Delhi. A commercial syrup formulation containing PCM (625 mg) and PMZ (25 mg) per 25 mL was purchased from a nearby pharmacy.

2.2 Analytical System and Operating Software

The analysis was performed using the Agilent 1220 Infinity II HPLC system, which includes a binary pump, autosampler, and PDA detector. The software employed for HPLC analysis is Agilent Open Lab CDS chemstation version 2.6. For the evaluation of Central Composite Design in AQbD Design-Expert® Software version 22.0, was employed. The Green Analysis was conducted using the GAPI Chart Maker Version 0.1 Beta software, the AMGS tool was utilized using the AMGS calculator, and AGREE tool was employed to calculate the AGREE metrics. Baggi was utilized to calculated the practicability and RBG algorithm excel-spreadsheet was used to calculate the whiteness.

2.3 Standard and sample solution preparation

2.3.1 PCM and PMZ standard preparation

A stock solution containing a combination of PCM and PMZ (1000 µg/mL each) was prepared using ethanol as the diluent. The mixture was sonicated for 15 minutes, filtered through a 0.45 µm membrane filter, and then appropriately diluted to achieve a final concentration of 10 µg/mL.

2.3.2 PCM and PMZ sample preparation

An accurately measured volume of syrup equivalent to 625 mg of PCM and 25 mg of PMZ (corresponding to 25 mL of syrup) was transferred into a clean, dry 25 mL volumetric flask. Ethanol was added to aid in the dissolution of the active pharmaceutical ingredients, followed by sonication for 20 minutes to ensure complete extraction and obtain a homogeneous solution.

After sonication, cool the solution to ambient temperature and filter it through a 0.45 µm membrane filter to remove any excipients. The clean filtrate was diluted with ethanol to achieve the requisite concentration of PCM (250 µg/mL) and PMZ (10 µg/mL) for quantitative analysis.

2.4 Chromatographic conditions

Chromatographic separation was carried out using an Inertsil ODS-3 column (250 mm × 4.6 mm, 5 µm). The mobile phase consisted of ethanol and 0.1% v/v acetic acid, selected to avoid the use of hazardous solvents or buffers, thereby promoting a greener analytical approach. The flow rate was maintained at 0.8 mL/min, with an injection volume of 10 µL, and detection was performed at 250 nm. The column oven temperature was set at 35 °C to ensure consistent performance. All solutions were filtered through a 0.45 µm membrane filter (Pall Life Sciences, Bengaluru, India), and solvents were degassed using an ultrasonic bath (Lab Man,

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2214-2227 | ISSN:2251-6727

Maharashtra, India) prior to use. A gradient elution program was employed to ensure efficient separation of the analytes.

Table 1 presented the mobile phase composition in gradient elution.

Time in [min]	Mobile phase	
	Mobile phase A: ethanol	Mobile phase B: water [with 0.1% acetic acid]
0 .00	10	90
5 .00	45	55
9 .00	45	55
10 .00	10	90

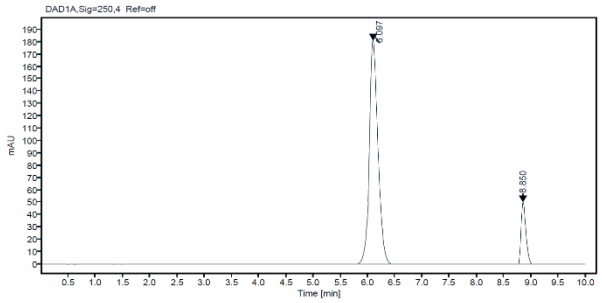
3. Results

3.1 System suitability studies

To assess the system appropriateness parameters, six identical standard PCM and PMZ solutions were injected. All % relative standard deviations (RSD) have been calculated for retention time (Rt), peak area, and theoretical plates. **Table 2 shows the details of system suitability parameters. Figure1 shows the standard chromatogram of PCM and PMZ.**

Para meters	Retention time		Resolution		Theoretical plates	
	PCM	PMZ	PCM	PMZ	PCM	PMZ
Mean	6.82	8.7	12.28		7809.65	19133.27
SD	0.08	0.13	0.20		79.03	256.04
% RSD	1.22	1.49	1.66		1.01	1.33

Figure 1: Standard chromatogram of PCM and PMZ showing retention times of 6.097 minutes for PCM and 8.850 minutes for PMZ.



3.2 Degradation conditions

A range of stress conditions were imposed on PCM and PMZ stock solutions, as indicated below. A level of degradation was evaluated periodically. Degradation was defined as a decrease in peak area or the appearance of new peaks. The percentage recovery determined the level of degradation.

3.3 Forced degradation

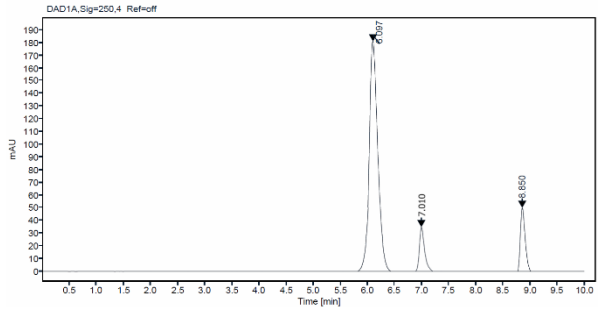
Forced degradation was carried out with the intention of purposely degrading the active drugs. These tests took place to evaluate the extent to which a technique of analysis would work to quantify an active ingredient along with its breakdown products without causing interference. The drug is exposed to an acid, a base, an oxidizing agent, and photolytic and thermal degradations.

3.3.1 Acid degradation

For acid degradation, 0.1 M HCl solution was used. 1 mL of the stock solution (100 µg/mL) was pipetted out, and the resulting mixture was kept undisturbed at room temperature for 60 min. Then neutralized using a base (NaOH) and subsequently diluted with ethanol. Keep it for 8 hours at room temperature. Subsequently, it was filtered and loaded into a vial for chromatographic injection.

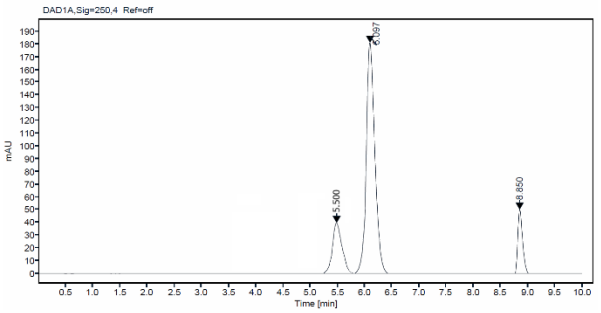
Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2214-2227 | ISSN:2251-6727

Figure 2 shows the chromatogram resulting from the acid degradation of PCM and PMZ.



3.3.2 Alkali degradation

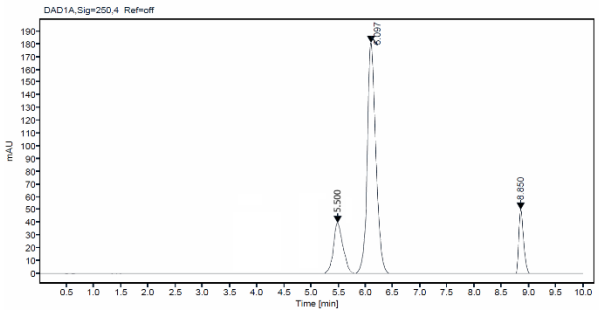
For alkali degradation, a 0.1 M sodium hydroxide (NaOH) solution was used. 1mL of the stock solution (100 µg/mL) was pipetted out, and the resulting mixture was kept undisturbed at room temperature for 60 minutes. Then neutralized using an acid (HCL] and subsequently diluted with ethanol. Keep it for 8 hours at room temperature. Subsequently, it was filtered and loaded into a vial for chromatographic injection. **Figure 3 shows the chromatogram resulting from the alkali degradation of PCM and PMZ.**



3.3.3 Oxidative degradation

To carry out oxidative degradation, 3% (v/v) hydrogen peroxide (H_2O_2) was utilized. 1 mL of the stock solution (100 µg/mL) was pipetted, and 1 mL of H_2O_2 was added to it, followed by dilution with ethanol. The mixture was then left at 8 hours at room temperature. Subsequently, it was filtered and loaded into a vial for chromatographic injection.

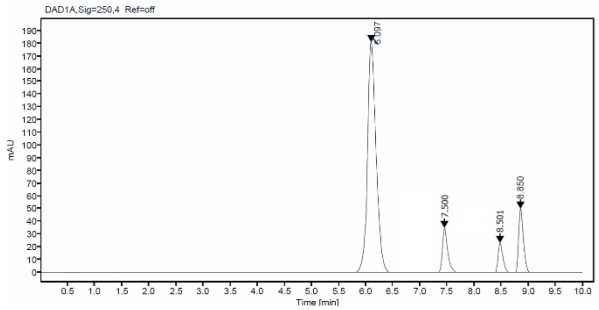
Figure 4 shows the chromatogram resulting from the oxidative degradation of PCM and PMZ.



3.3.4 Photolytic degradation

For photolytic degradation, 1 mL of the stock solution (100 µg/mL) was pipetted and diluted with ethanol. The resulting mixture was placed in a photostability chamber and exposed to UV light at 254 nm for 8 hours. Subsequently, it was filtered and loaded into a vial for chromatographic injection.

Figure 5 shows the chromatogram resulting from the photolytic degradation of PCM and PMZ.

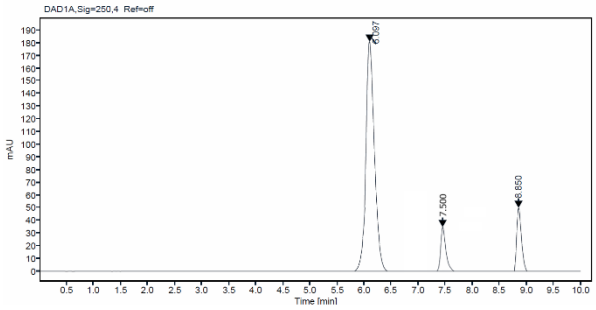


3.3.5 Thermal degradation

For thermal degradation, 1 mL of the stock solution (100 µg/mL) was pipetted and diluted with ethanol. The mixture was then kept at room temperature for eight hours. Subsequently, it was filtered and loaded into a vial for chromatographic injection.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2214-2227 | ISSN:2251-6727

Figure 6 shows the chromatogram resulting from the thermal degradation of PCM and PMZ.



The appearance of additional peaks beyond the standard retention times of 6.097 minutes for PCM and 8.850 minutes for PMZ indicates the formation of degradation products. These peaks are not present in the standard chromatogram, confirming their origin from forced degradation studies. Each newly observed peak corresponds to specific stress conditions such as acidic, alkaline, oxidative, thermal, or photolytic degradation.

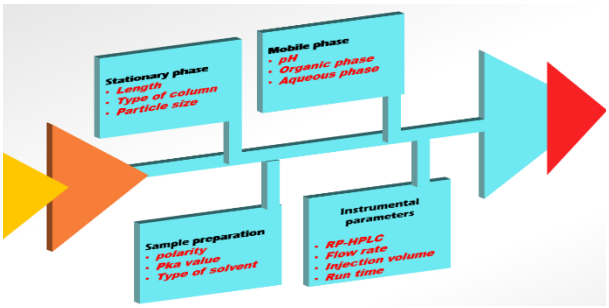
Table: 3 shows the results of degradation.

S.no	Factors	Drug recovered		Drug degradation	
		PCM	PMZ	PCM	PMZ
1	**NaOH** [0.1M NaOH]	98.8	99.0	1.2%	1%
2	**HCL** [0.1M HCL]	97.6	97.5	2.4%	2.5%
3	**H_2O_2** [3% v/v]	95.1	98.3	4.9%	1.7%
4	**Photolytic**	95	91	5%	9%
5	**Thermal**	87	85	13%	15%

3.6 Analytical Quality by Design (AQbD) Approaches

An RP-HPLC method was developed and optimized for the simultaneous estimation of PCM and PMZ using the AQbD framework, ensuring enhanced method stability and robustness. A key initial step in this strategy involved defining the Method Performance Goals, which emphasized the use of greener solvents and reagents to establish a method that is not only precise, accurate, and specific but also environmentally sustainable. To optimize the method, preliminary experiments were conducted using a systematic trial-and-error approach to identify critical independent variables and their influence on the dependent analytical responses. Careful selection of these variables and responses is central to AQbD methodology, enabling the development process to progress from simple screening experiments to a thorough risk assessment. Complementing this, the Ishikawa fishbone diagram was employed as a powerful tool to visualize and prioritize factors with the greatest potential impact on method performance. Subsequently, a structured experimental design was implemented to fine-tune the method parameters, ensuring that each aspect operated at its optimal level within the defined design space [19-20].

Figure 7 shows the Ishikawa Fish Bone Diagram.



3.6.1 Central Composite Design (CCD)

A central composite design with axial points positioned at the face center was developed to investigate the absorption process, applying the principles of response surface methodology (RSM). This design enables the construction of a second-order (quadratic) model for the response variable, while avoiding the extensive experimental runs required by a full three-level factorial design. Model parameters are typically estimated using

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2214-2227 | ISSN:2251-6727

linear regression, which may be applied iteratively after completion of the experiments. To simplify data analysis and enhance interpretability, coded variables are often used in such optimization strategies.

3.7 Development and optimization using AQbD

3.7.1 Comprehensive method performance

The integration of perturbation, contour, and 3D surface plots enabled a thorough evaluation of the effect critical method variables upon quality attributes of retention time, tailing factor, and resolution. This multi-dimensional approach ensured a harmonized method performance by visualizing the balance between efficiency, peak shape, and separation.

Table 4 presents the selected factors and their corresponding responses used in the QbD study.

Std	Run	Factors		Responses		
		A: Mobile Phase A	B: Flow rate	1. Retention time (min)	2. Tailing	3. Resolution
4	1	15	1	6.99	1.9	11.7
12	2	10	0.85	5.7	1.9	11.9
13	3	10	1	6	1.8	11.1
11	4	10	1.14	6.2	2	12
9	5	10	1	6.9	2	11.5
5	6	2.92	1.2	5.9	1.9	11.1
8	7	10	0.85	5.9	1.8	11.1
2	8	15	1	8	1.8	12.2
7	9	10	1	6.6	1.6	12.2
10	10	10	1.14	7.1	1.9	12.2
1	11	5	0.8	6	1.8	11.2
6	12	17.0711	1	7.8	1.91	12.2
3	13	5	1	6.8	1.9	11

Table 5 provides the ANOVA results for the quadratic model applied in the response surface methodology.

Factors	P value	F value	S. D	Mean	%c. V	R2	Adjusted r2	Predicted r2	Adequate Precision
Retention time	0.038	17.41	0.587	6.61	8.89	0.477	0.373	0.173	6.112
Tailing	0.046	4.12	0.069	1.86	3.71	0.746	0.565	0.365	6.241
Resolution	0.004	9.89	0.314	11.65	2.70	0.664	0.597	0.397	9.109

3.7.2 Perturbation

The plots were instrumental in revealing the sensitivity of each response to variations in mobile phase composition. The perturbation plots for retention time, tailing factor, and resolution all displayed a distinct cross-shaped pattern. This indicates that the two factors, ethanol (mobile phase A) and 0.1% acetic acid (mobile phase B) exert opposing effects as the level of one factor increases, the response is counterbalanced by a decrease due to the other. Such a pattern highlights strong interactive effects between the two components of the mobile phase, suggesting that the method is highly sensitive.

3.7.2 Contour Plots

The perturbation plots effectively illustrated the sensitivity and interactive behaviour of the chromatographic responses to variations in mobile phase composition. For retention time, the colour gradient ranged from blue (lower values) to green (moderate values), indicating a transition in response primarily influenced by ethanol concentration (mobile phase A). The tailing factor plot was dominated by yellow, with minimal green, signifying higher tailing values and a pronounced sensitivity to changes in mobile phase composition, particularly 0.1%v/v acetic acid (mobile phase B). The resolution plot showed a wide green region (moderate values), bordered by blue (lower resolution) and red (higher resolution) zones, emphasizing to maintain optimal separation.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2214-2227 | ISSN:2251-6727

3.7.2 3D Surface Plots

The 3D surface plots provided a comprehensive visualization of the combined effects of ethanol (mobile phase A) and 0.1% acetic acid (mobile phase B) on key chromatographic responses. These plots revealed clear interaction patterns between the two variables, highlighting their collective influence on method performance. For retention time, a sloping surface was observed, indicating a strong linear and possibly quadratic dependence on ethanol concentration. The tailing factor surface exhibited curvature, suggesting nonlinear effects and significant interaction between the two mobile phase components. The resolution plot displayed a well-defined peak, illustrating an optimal zone where the balance of mobile phase composition led to maximum resolution. Overall, the 3D surface plots underscored the importance of both individual and interactive effects of the mobile phase components, guiding the optimization strategy for a robust and efficient chromatographic method.

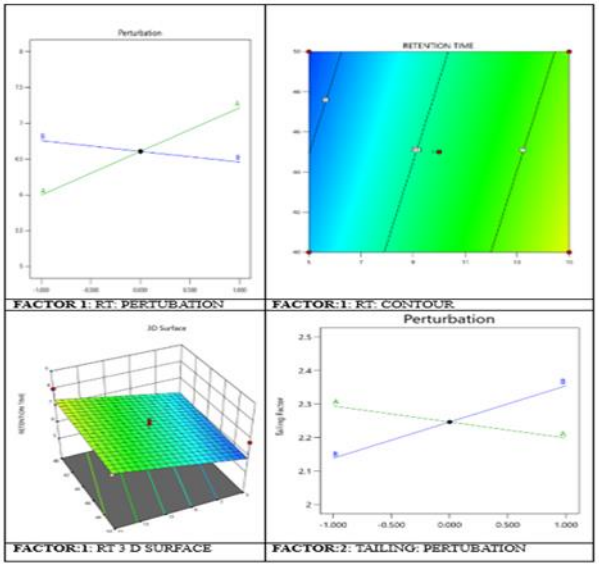
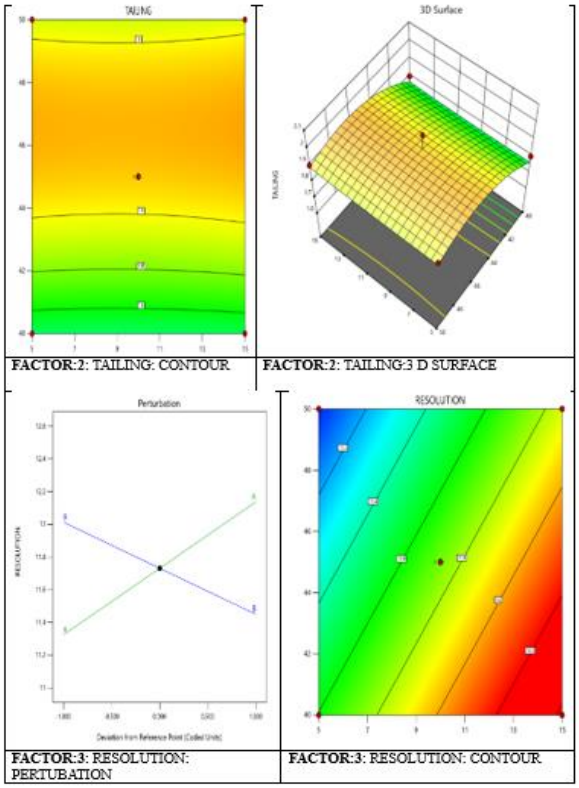


Figure 8 illustrates the Quality by Design (QbD) outputs, including perturbation plots, contour plots, 3D response surface plots, and the design space overlay, based on the three critical factors: retention time (Rt), tailing factor, and resolution.

Table 4 presents the selected factors and their corresponding responses used in the QbD study.



4. Discussion

4.1 Stability of the solutions

The standard PCM and PMZ solutions were checked for stability at room temperature for 72 h. The standard drug solution was compared with the test samples of PCM and PMZ, to find out the percentage assay value.

4.2 Validation parameters

The developed method was validated in accordance with ICH guidelines and demonstrated reliable linearity, accuracy, precision, and sensitivity for both PCM and PMZ. Linearity was observed over the concentration ranges of 10–200 µg/mL for PCM and 1–10 µg/mL for PMZ, with correlation coefficients (R^2) of 0.998 for both, indicating strong linear responses. The regression equations were $y = 19000x + 10000$ for PCM and $y = 55000x + 5000$ for PMZ. The method exhibited good sensitivity,

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2214-2227 | ISSN:2251-6727

with LOD and LOQ values of 2.6 µg/mL and 7.9 µg/mL for PCM, and 0.60 µg/mL and 1.82 µg/mL for PMZ, respectively. Accuracy was confirmed through recovery studies, with values ranging from 98.2% to 99.6% for PCM and 98.1% to 99.8% for PMZ, falling well within the acceptable limits. Precision was assessed through interday and intraday studies, with %RSD values of 0.58% and 0.48% for PCM, and 0.56% and 0.46% for PMZ, respectively well below the 2% threshold indicating consistent and reproducible results. Overall, the obtained validation results comply with standard regulatory requirements, confirming the method's suitability for routine analysis.

Tables 6 illustrates the results of validation parameters.

S.No	Parameters	PCM	PMZ
1	Concentration[µg/ml]	10- 200	1-10
2	Regression equation	y = 19000x+10000	y = 55000x+5000
3	Correlation coefficient[R^2]	0.998	0.998
4	LOD [µg/ml]	2.6	0.60
5	LOQ [µg/ml]	7.9	1.82
6	Accuracy [in %]	98.2-99.6%	98.1-99.8%
7	Interday [in % RSD]	0.58	0.56
8	Intraday [in % RSD]	0.48	0.46

4.3 Analysis of greenness

Ethanol emerges as a smart, eco-conscious substitute for acetonitrile and methanol in chromatography. Its low toxicity and minimal vapor pressure mean safer handling and reduced exposure. With wide availability and low cost, it's a practical fit for budget-sensitive labs worldwide. Water, the ultimate green solvent, is clean, abundant, and free of disposal concerns. Its UV transparency down to 190 nm allows precise detection of weakly absorbing compounds. Low Flame Ionization Detector [FID] response makes water compatible with non-traditional

Methods. Together, ethanol and water form a powerful green duo for sustainable separations. This method was crafted with a green vision and strong analytical integrity. The approach effectively demonstrates the incorporation of Green and White Analytical Chemistry principles within contemporary method development. There are several green assessment tools that can be employed in the context of GAC.

4.3.1 Green analytical procedure index (GAPI)

In the proposed method, GAPI [21-25] assessment indicates a predominantly **green** pictogram, reflecting the use of environmentally benign substances. Ethanol, employed as the main solvent, is classified as a green solvent based on the National Fire Protection Association (NFPA) ratings, which categorize chemicals into red (most hazardous), yellow (moderately hazardous), and green (least hazardous) zones. Since GAPI is a qualitative tool relying on visual interpretation through colour coding, the green depiction confirms minimal ecological and health hazards. The method also aligns with the E-Factor principles, which emphasize reduced chemical waste relative to product yield. This combined assessment using both GAPI and E-Factor underscores the method's sustainability and confirms that it avoids perilous reagents while maintaining efficiency.

4.3.2 Analytical Eco-Scale (AES) [26-27]

The proposed method received an AES score of **95**, indicating a high level of environmental compliance. This score suggests that the method involves minimal use of hazardous substances, generates low waste, and operates under relatively mild conditions. The small number of penalty points likely results from standard procedural steps such as sample handling or solvent use. Since a score above 75 is considered acceptable for green analytical procedures, a value of 95 confirms that

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2214-2227 | ISSN:2251-6727

the method is environmentally favourable and adheres closely to green chemistry principles.

4.3.3 Analytical Greenness (AGREE) [28-29]

The proposed method yielded an AGREE score of **0.84**, indicating a strong alignment with the principles of green analytical chemistry. This high score reflects favourable performance across various criteria, including low reagent toxicity, reduced waste generation, energy efficiency, and minimal procedural steps. The visual output a circular pictogram highlights consistent greenness across most parameters, suggesting that the method is both environmentally responsible and analytically reliable. The score, close to the maximum value of 1.0, demonstrates that the method is well-optimized for sustainability without compromising analytical performance.

4.3.4 Analytical Method Greenness Score (AMGS) [30-31]

An AMGS of 88 was attained by the proposed method, reflecting its strong environmental sustainability. Scores within the range of 80 to 89 are considered green and sustainable, indicating efficient solvent use, reduced energy consumption, and minimal environmental health and safety risks. This relatively high composite score demonstrates effective minimization of waste and energy demand, contributing to the overall sustainability of the method. The evaluation was performed using the ACS Green Chemistry Institute's green evaluation calculator, ensuring a standardized and reliable assessment. Accordingly, the method is categorized as an environmentally conscious chromatographic technique.

4.3.5 Carbon Footprint Analysis

The developed RP-HPLC method demonstrates reduced carbon dioxide emissions primarily due to its low energy consumption, approximately **1.5 kWh per analysis**, which is significantly lower than typical HPLC methods that often exceed 2–3 kWh per run. Additionally, the use of ethanol, a renewable solvent with a lower carbon footprint than commonly used acetonitrile or methanol, further reduces environmental impact. The method's shorter run times and moderate flow rate minimize solvent consumption and waste generation, thereby decreasing the overall carbon emissions linked to solvent production, use, and disposal. This confirms that the method is greener with a smaller carbon footprint.

4.4 Blueness assessment using Blue Applicability Grade Index (BAGI) [32-33]

The developed method achieved a high BAGI score of **85.0**, indicating strong practical applicability across key criteria such as overall functionality, risk reduction, time efficiency, and cost-effectiveness. This score reflects the method's relevance and utility in analytical applications. However, while BAGI effectively evaluates practical aspects, it does not fully address environmental sustainability. To provide a more comprehensive evaluation covering utility, analytical performance, and green metrics, the RGB12 algorithm was also applied. Together, these assessments confirm that the method balances operational efficiency with environmental considerations, supporting its suitability for routine analysis.

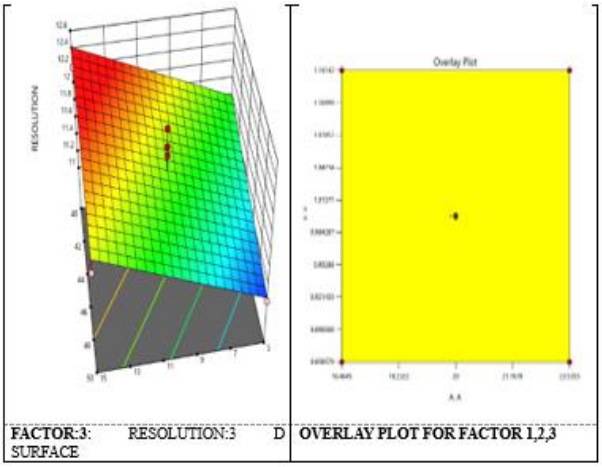


Figure 9 shows the results of Baggi.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2214-2227 | ISSN:2251-6727

4.5 Analysis of whiteness [34]

The Green Analytical Chemistry (GAC) framework, while foundational, has been critiqued for not fully addressing the broader dimensions of sustainability in analytical practices. To overcome these limitations, the concept of White Analytical Chemistry (WAC) has been introduced. WAC represents a progressive evolution of GAC, retaining its core environmental principles while expanding its scope to incorporate a more holistic, balanced approach that integrates environmental, economic, and analytical performance criteria. This comprehensive perspective positions WAC as a more complete model for truly sustainable analytical science.

4.5.1 RGB Algorithm

The developed method achieved an impressive RGB (WAC) score of **95.5**, indicating strong compliance with the principles of White Analytical Chemistry. This high score reflects an optimal balance across the three pillars: excellent analytical performance (Blue), low environmental impact (Green), and strong practical and economic feasibility (Red). With a score well above the 66.6% threshold, the method is considered highly sustainable and well-aligned with modern analytical standards. The high Method Brilliance and Colour Score further confirm its efficiency, eco-friendliness, and suitability for widespread analytical implementation. **Figure:10 shows the RGB algorithm for developed RP-HPLC method.**

A comparison of the proposed HPLC method with previously published methods was performed using various sustainability assessment tools, including AGREE, GAPI, Eco-Scale, BAGGI, and the RGB algorithm. The earlier methods exhibited moderate sustainability scores across these tools. However, the proposed method demonstrated improvements in reagent safety and environmental impact. It achieved the highest Eco-Scale score of 91, reflecting better overall greenness. GAPI and AGREE visual representations also supported its superior sustainability profile. These findings confirm that the proposed method is more sustainable and better aligned with green analytical chemistry principles.

Table 7 shows a comparison of the suggested HPLC method versus the previously published HPLC method in terms of sustainability assessment.

S.No	Reference	Conditions	GAPI	AES	AGREE	BAGI	RGB Model
1	Borkar D, Godre V P (9)	HPLC, Methanol: water: Triethyl amine [90:10:0.1]		Reagent=14 Instrument =1 Occupational hazard=0 Waste =0 Total =100-13 AES= 87			
2	Chaudhary J, Jain A, Saini V. (8)	HPLC, Methanol: water: acetic acid [79:20:1]		Reagent=16 Instrument =1 Occupational hazard=0 Waste =0 Total =100-17 AES= 83			
3	Kakadiya J,Parmar N (10)	HPLC, Methanol: water: Acetic acid [20:79:1]		Reagent=10 Instrument =1 Occupational hazard=0 Waste =0 Total =100- AES = 89			
4	Proposed Method	HPLC, Ethanol: 0.1 % v/v acetic acid		Reagent=8 Instrument =1 Occupational hazard=0 Waste =0 Total =100-5 AES = 91			

5. Conclusion

This study presents a novel and scientifically significant approach by integrating AQbD, GAC,

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(1), 2214-2227 | ISSN:2251-6727

and WAC principles to develop a robust and eco-friendly RP-HPLC method for the simultaneous quantification of PCM and PMZ in bulk and pharmaceutical formulations. The application of AQbD, through CCD, enabled precise identification and graphical representation of critical method parameters, resulting in optimized chromatographic conditions that ensure method robustness and reproducibility. Compared to conventional methods relying on acetonitrile or methanol, the substitution of ethanol as the organic solvent not only minimized environmental hazards but also enhanced method greenness, as confirmed by multiple green evaluation metrics showing a reduction in ecological impact.

The method demonstrated excellent analytical performance, with linearity coefficients (R^2 > 0.999), high accuracy (recoveries within 98.5–101.5%), and precision (RSD < 2%). These results confirm the method's reliability and suitability for routine quality control and stability testing with minimal revalidation, thereby supporting regulatory compliance and operational efficiency.

The integrative strategy employed in this work addresses critical gaps in pharmaceutical analysis by combining sustainability, quality, and efficiency in a single validated protocol. This approach not only advances analytical methodology for PCM and PMZ but also establishes a reference for future environmentally conscious method development in pharmaceutical sciences, facilitating widespread adoption in industrial laboratories aiming for greener and more sustainable practices.

6.REFERENCES

1. Mahaparale, S., Telekone, R. S., Raut, R. P., Damle, S. S., Kasture, P. V. 2010. Spectrophotometric determination of paracetamol and promethazine in combined dosage form. Indian J. Pharm. Sci., 72, 133–136.

2.Abdel Rahman, M. A., Elghobashy, M. R., Zaazaa, H. E. 2022. Green and stability-indicating HPLC determination of pharmaceutical mixtures. BMC Chem., 16, 108.

3.bdelaleem, E. A., Abdelwahab, N. S. 2013. Validated analytical methods for pharmaceutical analysis. Anal. Methods, 5, 541–545.

4.Thumma, S., Zhang, S.-Q., Repka, M. A. 2008. Development of pharmaceutical formulations containing antihistamines. Pharmazie, 63, 562–567.

5.Patil, A. S., Amrutkar, S. V., Nalwade, S. 2024. Eco-friendly chromatographic method development. J. Chromatogr. Sci., 62, 273–280.

6.Shree, V. K., Nagaraju, P. 2025. Analytical method development and validation of pharmaceutical dosage forms. J. Innov. Appl. Pharm. Sci., 10, 16–20.

7.Takale, N., Kaliyaperumal, N., Krishnan, G., Mannathusamy, M., Govindasamy, R. R. 2020. Development of chromatographic methods for drug analysis. Oriental J. Chem., 36, 889–896.

8.Chaudhary, J., Jain, A., Saini, V. J. 2019. Simultaneous determination of drugs using spectrophotometric techniques. Res. Pharm., 23, 476–483.

9.Al-Saidi, K. H., Hammza, R. A. 2014. Analytical applications in pharmaceutical sciences. Al-Nahrain J. Sci., 17, 15–22.

10.Sawant, R. L., Ahmed, R., Supriya, S. R., Sheetal, R. R. 2013. Spectroscopic analysis of pharmaceutical compounds. Spectrochim. Acta A, 111, 235–240.

11.Rosireddy, V., Krishnan, M. 2024. White analytical chemistry-based chromatographic method development. Anal. Chem. Lett., 14, 406–425.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(1), 2214-2227 | ISSN:2251-6727

12.Chakraborty, A., Jayaseelan, K. 2025. Green analytical method assessment strategies. Green Anal. Chem., 12, 100183.

13.Attimarad, M., Venugopala, K. N., SreeHarsha, N., Aldhubiab, B. E., Nair, A. B. 2020. Green analytical procedures for pharmaceutical compounds. Microchem. J., 152, 104365.

14.Dharuman, N., Lakshmi, K. S., Krishnan, M. 2023. Environmentally benign chromatographic methods. Green Chem. Lett. Rev., 16, 2214176.

15.Chakraborty, A., Jayaseelan, K. 2025. Analytical quality by design-based method development. J. AOAC Int., 108, 10–22.

16.Rosireddy, V., Krishnan, M. 2024. Sustainable pharmaceutical process development. Green Process. Synth., 13, 20240003.

17.Katamesh, N., Abbas, A. E. F., Mahmoud, S. A. 2024. Eco-friendly pharmaceutical analysis methods. BMC Chem., 18, 54.

18.Wadie, M., Tantawy, M. A., Goda, Z. M. 2024. Fluorimetric determination of drugs. J. Fluoresc., 34, 1–10.

19.Peraman, R., Bhadraya, K., Reddy, Y. P. 2015. Analytical techniques in pharmaceutical sciences. Int. J. Anal. Chem., 2015, 1–9.

20.Rathaur, S., Dadge, S., Gayen, J. R. 2025. Emerging analytical tools in pharmaceutical research. Future J. Pharm. Sci., 11, 125. https://doi.org/10.1186/s43094-025-00885-5

21.Mohamed, D., Fouad, M. M. 2020. Green spectrophotometric methods. Microchem. J., 157, 104873.

22.Ashraf, A., Saad, A., Ibrahim, M., Khaled, E., Hassan, W. 2024. Sustainable chromatographic methods for drug analysis. BMC Chem., 18, 133.

23.Al-Rimawi, F., Al-Najjar, A., Al-Momani, I., Khoury, H., Qaraman, A. 2024. Greenness assessment of analytical methods. BMC Chem., 18, 177.

24.Płotka-Wasylka, J. 2018. A new tool for green analytical method evaluation. Talanta, 181, 204–209.

25.Rosireddy, V., Krishnan, M. 2024. White analytical chemistry-based eco-friendly method design. Green Anal. Chem., 11, 100171.

26.Mohamed, H. M., Lamie, N. T. 2016. Validated green spectrophotometric determination of drugs. J. AOAC Int., 99, 1260–1265.

27.Gałuszka, A., Migaszewski, Z., Namieśnik, J. 2012. The 12 principles of green analytical chemistry. TrAC Trends Anal. Chem., 37, 61–72.

28.Saleh, S. S., Lotfy, H. M., Tiris, G., Erk, N., Rostom, Y. 2020. Eco-friendly spectrophotometric pharmaceutical analysis. Microchem. J., 159, 105557.

29.Pena-Pereira, F., Wojnowski, W., Tobiszewski, M. 2020. The RGB model in analytical chemistry. Anal. Chem., 92, 10076–10082.

30.Hicks, M. B., Farrell, W., Aurigemma, C., Lehmann, L., Weisel, L., Nadeau, K., Lee, H., Moraff, C., Wong, M., Huang, Y., Ferguson, P. 2019. Green solvent selection and evaluation strategies. Green Chem., 21, 1816–1826.

31.Power, F., Ferguson, P., Herbert, A., Ryan, S., Osborne, M., Trezise, L. 2025. Sustainable solvent systems in chromatography. Green Chem., 27, 14088–14100.

32.Manousi, N., Wojnowski, W., Płotka-Wasylka, J., Samanidou, V. 2023. Analytical greenness metrics and assessment tools. Green Chem., 25, 7598–7604.

33. Mahdavi, R., Talebpour, Z., Noori, M. 2024. Advances in green analytical chemistry. Green Anal. Chem., 10, 100144.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(1), 2214-2227 | ISSN:2251-6727

34.Nowak, P. M., Wietecha-Posłuszny, R., Pawliszyn, J. 2021. Modern trends in analytical sample preparation. TrAC Trends Anal. Chem., 138, 116223.

Conflicts of interest

There are no conflicts to declare

Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

Acknowledgement

The authors thankful to the SRM college of pharmacy, SRM institute science and Technology, Kattankulathur for their constant support and encouragement.

Funding Statement

There was no funding received by the authors.

Authors contributions

Vineetha Rosireddy*: Writing original draft, writing review & editing, methodology, software, formal analysis, data curation, and validation.

Manikandan Krishnan[1]: Conceptualization, methodology, visualization, validation, and project administration.

Atyurmila Chakraborty[2]**:** Software and formal analysis.