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Design of experiments and white analytical chemistry-driven green and sensitive spectrofluorimetric estimation of pregabalin in its pharmaceutical dosage forms and spiked human plasma

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Abstract

Background Pregabalin (PGB) is a medication with anticonvulsant, analgesic and anxiolytic properties, employed in the treatment of epilepsy, neuropathic pain, fibromyalgia, restless leg syndrome, opioid withdrawal syndrome and generalized anxiety disorder. Several spectrofluorimetric techniques have been documented for the determination of PGB in pharmaceutical dosage forms. However, these published methods typically involve the use of expensive and toxic organic solvents and reagents, as well as high reaction temperatures for PGB analysis. These components pose risks to aquatic life and the environment, making them less environmentally friendly and user-friendly. A recent advancement in analytical chemistry has introduced a white analytical approach, providing an economical, eco-friendly and user-friendly method for the development of analytical procedures.

Objectives Therefore, a green and sensitive spectrofluorimetric determination of PGB, guided by white analytical chemistry principles, has been conducted utilizing distilled water as an environmentally friendly solvent.

Methods The establishment of the spectrofluorimetric method involved employing the design of experiments approach to ensure a robust, precise and accurate estimation of PGB. Response surface analysis and optimization of critical procedural variables and responses were carried out using the central composite design. The validation of the developed method adhered to the guidelines outlined in ICH (International Council for Harmonization) Q2 (R1) and M10.

Results The established spectrofluorimetric method was utilized to determine the PGB content in commercially available formulations and human plasma samples spiked with PGB. The obtained results were in accordance with the labeled claim of PGB in the formulations. The recovery of PGB in the spiked human plasma samples ranged from 85 to 90% of the spiked amount.

Conclusions The greenness profiles of the published and suggested spectrofluorimetric methods for PGB estimation were evaluated and compared using the AGREE calculator, GAPI software and ESA tool. The suggested method demonstrated sensitivity, robustness, environmental friendliness and user-friendliness.

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Keywords Green and sensitive spectrofluorimetric method, Pregabalin, White analytical chemistry, Design of experiments, Central composite design

Background

Recently introduced into the literature, white analytical chemistry is an approach for the development and evaluation of accurate, sensitive, precise, environmentally friendly and cost-effective analytical methods for estimating drugs and their dosage forms. White analytical chemistry incorporates the key principles of green analytical chemistry in method development. It involves an RGB (red, green and blue) model-based assessment of the developed analytical method. The red (R) model-based assessment comprises four principles: R1—scopes and applications, R2—accuracy, R3—precision and R4—linearity and sensitivity. The green (G) model-based assessment includes the four major principles of green analytical chemistry: G1—environmental impact, G2—energy consumption, G3—waste generation and G4—health impact of the analytical method. The blue (B) model-based assessment includes four major principles: B1—time efficiency, B2—cost efficiency, B3—simplicity of the method and B4—instrument handling for the assessment of the analytical method [1].

Within the pharmaceutical industry, chromatographic analytical methods are frequently employed for the quality control and routine analysis of both drug substances and drug products. In the course of chromatographic analysis of drug samples, toxic organic solvents like chloroform, acetonitrile, methanol, toluene, etc., have traditionally been utilized and subsequently left as organic waste in the environment. Adhering to standards outlined in the Indian Pharmacopoeia, ICH (International Council for Harmonization) Q3C guidelines and published solvents selection guides, these organic solvents are recognized as harmful to aquatic animal life and environmentally hazardous. In line with the principles of green and white analytical chemistry, there is a concerted effort to reduce, eliminate and recycle the consumption and wastage of such toxic organic solvents during the development and life cycle management of analytical methods, aiming to preserve the environment [2–5].

The design of experiments (DoE) is a systematic methodology employed in the development of analytical methods to ensure robustness, precision and accuracy. As outlined in the ICH Q14 guideline, the incorporation of the design of experiments approach is a regulatory requirement for the registration and approval of new drug substances and products. Additionally, the design of experiments is a valuable tool for the implementation of white analytical chemistry, aiding in the development of analytical methods that are both robust and precise. Furthermore, the application of the design of experiments proves beneficial in minimizing the consumption of organic solvents during the development of analytical methods. Recent literature reveals the emergence of numerous published analytical methods that integrate hybrid principles of design of experiments, green analytical chemistry and white analytical chemistry [6–10].

Pregabalin (PGB) is a medication with anticonvulsant, analgesic and anxiolytic properties, prescribed for conditions such as epilepsy, neuropathic pain, fibromyalgia, restless leg syndrome, opioid withdrawal syndrome and generalized anxiety disorder [11]. Numerous methods, including spectrophotometric, RP-HPLC (reversed-phase high-pressure liquid chromatographic) and LC–MS/MS, have been documented in the literature for estimating PGB in pharmaceutical dosage forms and conducting bioanalysis [12–22]. However, these analytical approaches involve the use of toxic organic solvents, posing environmental risks. Spectrofluorimetric methods, recognized for their sensitivity, specificity, accuracy and precision in drug sample estimation, offer a more environmentally friendly alternative with reduced organic solvent usage, time and cost. In the context of implementing the principles of white analytical chemistry, the spectrofluorimetric method stands out as an ideal choice for green, accurate, sensitive, precise and economical analysis of drug samples. Notably, PGB is a nonfluorescent drug molecule, necessitating derivatization for the development of a spectrofluorimetric method. Recent literature has featured various spectrofluorimetric methods utilizing different derivative reagents as fluorescent probes for PGB estimation [23–28]. However, some published spectrofluorimetric methods employ toxic organic solvents, high reaction temperatures and toxic reagents, generating over 100 mL of organic waste. Additionally, the sensitivity of certain published spectrofluorimetric methods is limited to the microgram level.

Therefore, a sensitive, robust and environmentally friendly spectrofluorimetric method has been developed for the estimation of PGB, utilizing safe solvents and eliminating the need for derivative reagents. The development of this spectrofluorimetric method followed a comprehensive strategy based on the principles of white analytical chemistry and the design of experiments. The design of experiments approach was implemented using

a central composite design, which facilitated response surface analysis and optimization of the spectrofluorimetric method. The developed method underwent validation in accordance with ICH Q2 (R1) and M10 guidelines. Application of the developed spectrofluorimetric method included the assay of both marketed formulations and spiked human plasma samples. To assess and compare the greenness and whiteness profiles of the published and developed spectrofluorimetric methods, various tools and models such as the AGREE (Analytical Greenness) calculator, ESA (Eco-scale Assessment) tool, GAPI (Green Analytical Procedure Index) and RGB (red, green and blue) models were employed.

Methods

Instruments and softwares

The determination of PGB was conducted utilizing a Spectrofluorometer Shimadzu RF-5301 PC model, obtained from Toshvin Analytical Private Limited in Vapi, Gujarat, India. The spectrum of PGB was recorded using a UV–Visible spectrophotometer 3092 model (Lab India Private Limited, Mumbai, Maharashtra, India). For precise weighing, a single pan digital analytical balance, acquired from Shimadzu Scientific Instruments (India) Private Limited in Bangalore, India, was employed. The response surface modeling based on Design of Experiments (DoE) was carried out using MINITAB 18 software (trial version). To evaluate the environmental friendliness of the method, the AGREE calculator (accessible at https://mostwiedzy.pl/AGREE) was employed. Additionally, the greenness profile of the method was assessed using Complex GAPI software, which can be accessed at mostwiedzy.pl/complexgapi.

Reagents and materials

The PGB API (Active Pharmaceutical Ingredient) with 99.98%W/W purity was received as a complimentary gift sample from Vapi Care Pharma Private Limited in Gujarat, India. High-quality double-distilled water was meticulously prepared in the Quality Assurance Laboratory at our institute. To facilitate filtration, membrane filters (0.45 µm) and sample syringe filters (0.22 µm) were acquired from Pall India Private Limited, located in Andheri, Mumbai, India. The pharmaceutical dosage forms of PGB (Pregazo 75 capsule—contains 75 mg PGB, Pregal 75 capsule—contains 75 mg PGB, and Pregadd tablets—contains 50 mg PGB) were procured from the local market in Surat, Gujarat, India.

Establishing calibration curve for assay of marketed formulations

A standard solution of PGB (1000 µg/ml) was prepared by dissolving 10 mg of PGB in distilled water in a 10-mL volumetric flask. Subsequent dilutions were carried out with distilled water to generate concentrations ranging from 1.0 to 25 µg/ml. Consequently, 100 µL from the working solution (10 to 25 µg/ml) of PGB was transferred into a series of 10-mL volumetric flasks and topped up to the mark with borate buffer of pH 10.0, resulting in the final concentration range of 10–250 ng/mL. The fluorescence intensity was measured at an emission wavelength of 410 nm using an excitation wavelength of 230 nm. A blank experiment was conducted in a similar manner without the presence of PGB.

Establishing a calibration curve through the spiking of PGB to human plasma samples

In each 100 µL aliquot of human plasma, a 10 µL portion from distinct working standard solutions (100–2500 µg/mL) was individually introduced, thoroughly mixed and vortexed for 15 min. To extract PGB from the human plasma, 3.0 mL of methanol was added as a precipitating agent and mixed for an additional 15 min. Following complete precipitation, the resulting samples were diluted to a final volume of 10 mL with methanol. Subsequently, each sample underwent centrifugation at 4000 RPM for 15 min. A 1.0 mL aliquot from the supernatant of each solution was filtered through a 0.22 µm syringe filter and transferred into a series of 10-mL volumetric flasks, completed to the mark with borate buffer of pH 10.0, resulting in the final concentration range of 10–250 ng/mL. The fluorescence intensity was measured at an emission wavelength of 410 nm using an excitation wavelength of 230 nm. A blank experiment was conducted similarly without the presence of PGB.

Implementing design of experiments approach in method development

Following preliminary experiments, critical procedural variables and method performance attributes (responses) were identified for the development of the intended method. These critical method variables were examined for their main effects, two-way interactions and quadratic effects on selected responses using central composite design through Minitab 18 software. Navigating the analytical design space was allowed for the optimization of responses in the targeted spectrofluorimetric method for estimating PGB. Mean response surface analysis was conducted to delineate the analytical design space and validate the model.

Analysis of pregabalin in marketed formulations

A sample equivalent to 10 mg of PGB was taken from each pharmaceutical dosage form of PGB. This sample was dissolved, diluted to 10 mL with distilled water and then filtered. Subsequently, a 1.0 mL aliquot was further diluted to 10 mL with distilled water. From this solution, another 1.0 mL aliquot was diluted to 10 mL with distilled water. A 100 µL aliquot of the sample was then transferred into a 10-mL volumetric flask and completed to the mark with borate buffer at pH 10.0. The fluorescence intensity was measured at an emission wavelength of 410 nm using an excitation wavelength of 230 nm. A blank experiment was conducted in a similar manner, excluding the presence of PGB sample.

Results

Defining the analytical target profile for the design of experiments approach

The spectrofluorimetric method was developed to estimate PGB in its pharmaceutical dosage forms and spiked human plasma samples, employing environmentally safe and sustainable solvents. The developed method was designed to yield optimal fluorescence intensity and % recoveries of PGB, ensuring sensitive, selective and specific estimation in the proposed matrices. Consequently, fluorescence intensity and % recovery of PGB have been identified as crucial responses in the development of the targeted spectrofluorimetric method (Fig. 1).

Utilizing response surface analysis for method optimization

The Minitab 18 software proposed thirteen experimental runs (refer to Table 1), which were subsequently conducted in the laboratory. The recorded responses from these experimental runs were analyzed through ANOVA, multiple regression analysis and response surface analysis using the software. According to the ANOVA results (refer to Table 2), the main effects of critical procedure variables A and B were found to be significant for responses R1 and R2, along with significant quadratic effects of critical procedure variables A and B. However, the two-way interactions of critical procedure variables A and B were determined to be nonsignificant for responses R1 and R2. The multiple regression analysis revealed R-squared, adjusted R-squared and predicted R-squared values for

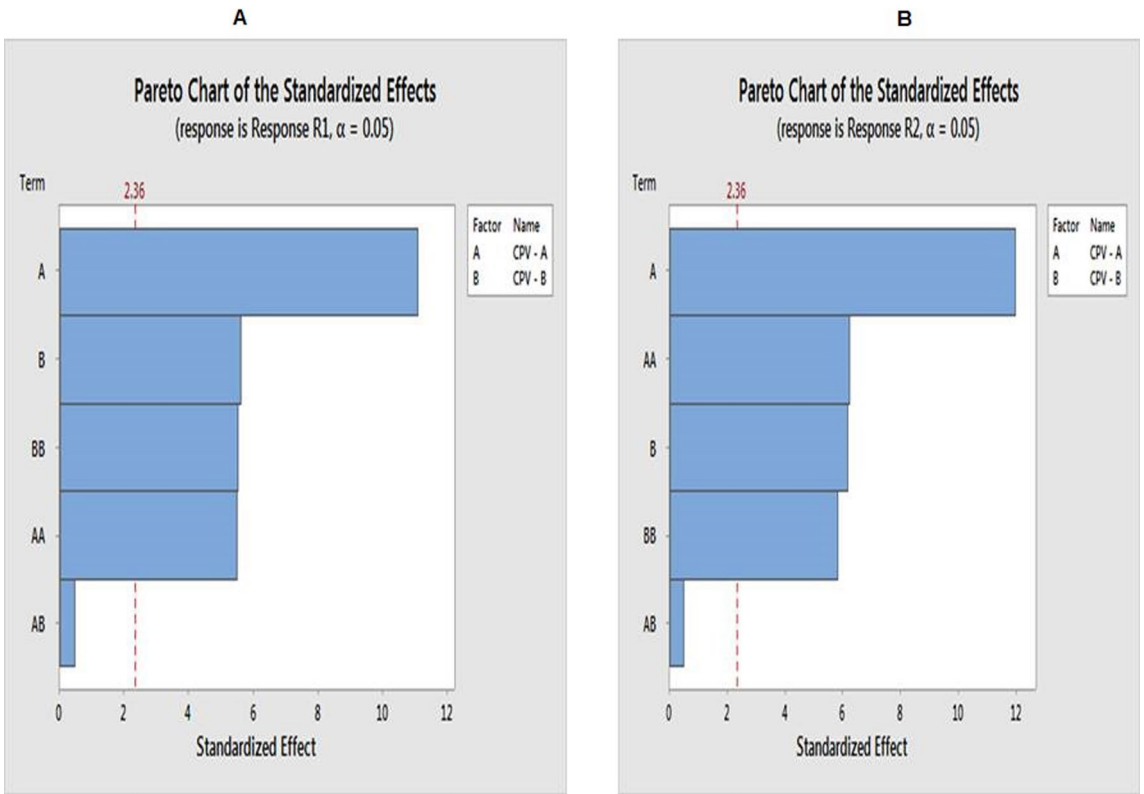

Fig. 1 Pareto chart analysis using central composite design and Minitab 18 software. **A** Pareto chart showing significant main effects and quadratic effects of critical procedure variables on fluorescence intensity—Response R1 and **B** Pareto chart showing significant main effects and quadratic effects of critical procedure variables on % recoveries—Response R2

Table 1 Design metrics and measured responses for DoE-based response surface analysis and optimization of critical procedure variables (CPVs) using central composite design by Minitab 18 software

Standard run order	Random run order	Point type	Blocks	CPV—A	CPV—B	Response R1	Response R2
3	1	Factorial	1	−1	+1	260.14	90.14
7	2	Axial	1	0	−α	170.12	75.14
12	3	Center	1	0	0	230.41	85.49
13	4	Center	1	0	0	230.74	85.78
5	5	Axial	1	−α	0	240.12	87.14
2	6	Factorial	1	+1	−1	110.14	65.11
4	7	Factorial	1	+1	+1	160.11	73.18
11	8	Center	1	0	0	230.47	85.71
9	9	Center	1	0	0	230.74	85.88
1	10	Factorial	1	−1	−1	200.11	80.41
8	11	Axial	1	0	+α	210.45	83.14
10	12	Center	1	0	0	230.11	85.42
6	13	Axial	1	+α	0	140.77	70.12

CPV—A (Excitation wavelength in nm): Axial point (−α)—225.85, low level (−1)—230 nm, medium level (0)—240 nm, high level (+1)—250nm and axial point (+*α*)—254.142

CPV—B (pH of medium): Axial point (−*α*)—7.59, low level (−1)—8.0, medium level (0)—9.0, high level (+1)—10.0 and axial point (+*α*)—10.41

Table 2 Analysis of variances (ANOVA) for study of significant main effect, two-way interactions and quadratic effects of critical procedure variables using central composite design and Minitab 18 software

Source	Degree of freedom (DF)	Adjusted sum of square	Adjusted mean square	*F*-Value	*P*-Value
ANOVA for Response R1—Fluorescence intensity of PGB					
Model	5	23,123.5	4624.7	41.66	0.000
Linear	2	17,141.6	8570.8	77.21	0.000
CPV—A	1	13,654.0	13,654.0	123.01	0.000
CPV—B	1	3487.6	3487.6	31.42	0.001
Square	2	5956.7	2978.3	26.83	0.001
CPV—A*CPV—A	1	3354.6	3354.6	30.22	0.001
CPV—B*CPV—B	1	3379.1	3379.1	30.44	0.001
Two-way interaction	1	25.3	25.3	0.23	0.648
CPV—A*CPV—B	1	25.3	25.3	0.23	0.648
ANOVA for Response R2—%recovery of PGB					
Model	5	681.653	136.331	49.17	0.000
Linear	2	502.583	251.292	90.64	0.000
CPV—A	1	396.632	396.632	143.07	0.000
CPV—B	1	105.951	105.951	38.22	0.000
Square	2	178.381	89.190	32.17	0.000
CPV—A*CPV—A	1	107.538	107.538	38.79	0.000
CPV—B*CPV—B	1	94.042	94.042	33.92	0.001
Two-way interaction	1	0.689	0.689	0.25	0.633
CPV—A*CPV—B	1	0.689	0.689	0.25	0.633

responses exceeding 0.9, with the difference between adjusted R-squared and predicted R-squared being less than 0.2. These outcomes suggest that the selected models were well suited for predicting responses R1 and R2. The 2D and 3D response surface contour plots (refer to Figs. 2A, B, 3A, B) illustrate multidimensional interactions and the quadratic relationship between critical procedure variables (A and B) and responses

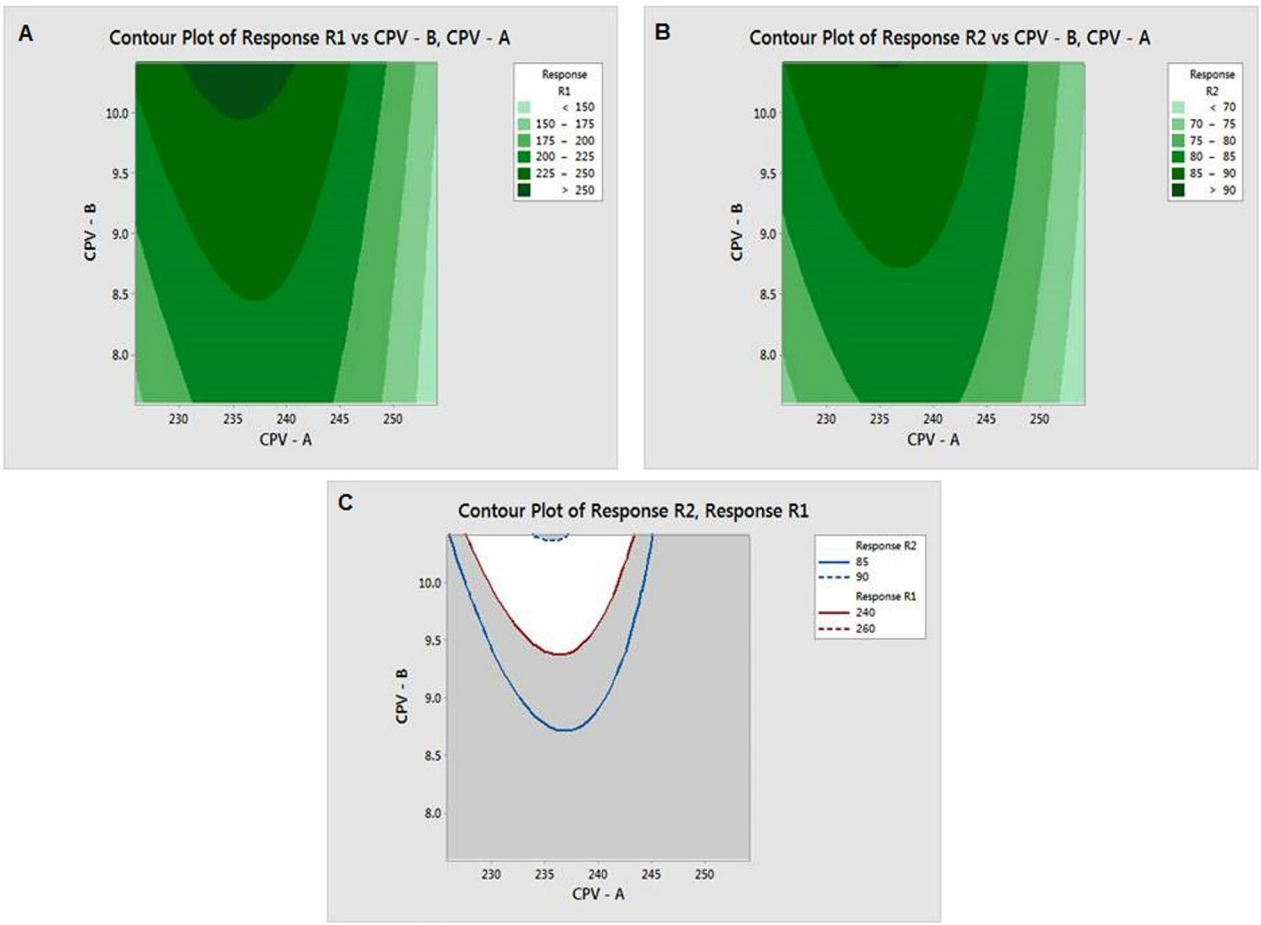

Fig. 2 DoE-based response surface analysis of critical method variables and responses using central composite design. **A** 2D contour plot showing multidimensional interactions between critical procedure variables A and B with fluorescence intensity—Response R1. **B** 2D contour plot showing multidimensional interactions between critical method variables A and B with % recoveries—Response R2. **C** Analytical design space for desirable maximum fluorescence intensity and %recovery of PGB

R1 and R2. The analytical design space was navigated to achieve maximum fluorescence intensity and % recovery of PGB, aligning with the analytical target profile of the method, using overlaid response surface plots.

Employing the mean response surface method to define the analytical design space

Following an examination of the overlaid response surface plots, the analytical design spaces, directed toward achieving maximum fluorescence intensity and % recovery of PGB, were scrutinized for accuracy and precision in predicting responses R1 and R2 using the mean response surface method. The visual representation of these navigated analytical design spaces is depicted in Figs. 2C and 3C, highlighted in white shades. Subsequently, the experimental runs recommended by the software were executed in the laboratory, and the actual responses were analyzed for their %RSD ($n=3$). The %RSD for all experimental runs was determined to be less than 2%, indicating the accurate and precise prediction of responses R1 and R2 by the suggested models.

Spectrofluorimetric method validation

The method's specificity was confirmed by comparing fluorescence spectra among various samples, including blank and standard PGB. Blank samples exhibited no interference from excipients or human plasma matrices (Refer Additional file 1: Fig. S2). Linearity was established through five repetitions of calibration curve procedures, demonstrating a linear relationship within the 10–250 ng/mL concentration range, with a correlation coefficient exceeding 0.995 (refer to Figs. 4 and 5). Additionally, % recoveries of PGB from spiked human plasma samples (at 50%, 100% and 150% levels) ranged from 85 to 90%. Evaluations for sample carryover and sample dilution in the estimation of PGB were conducted within spiked human plasma samples. The method's

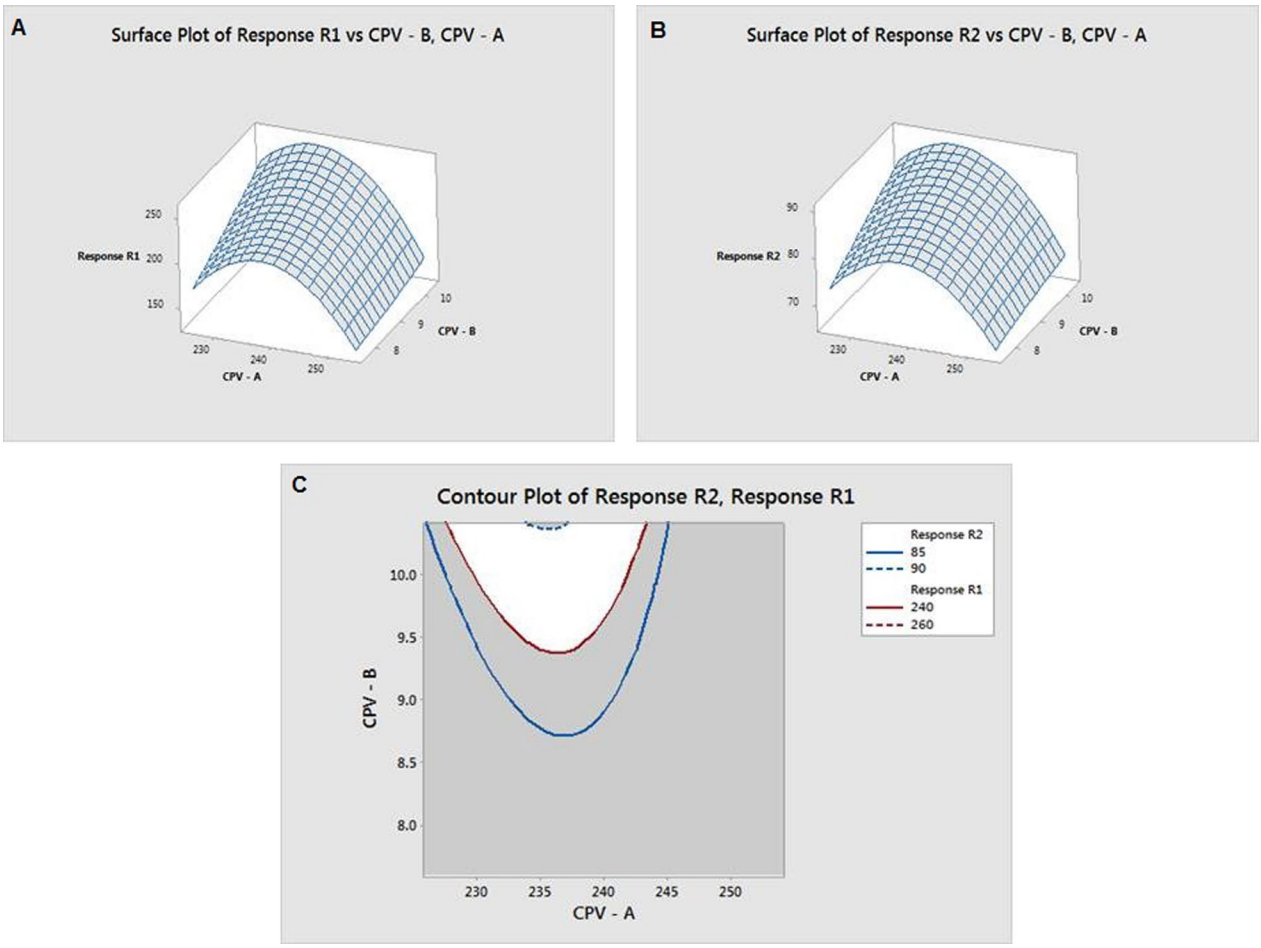

Fig. 3 DoE-based response surface analysis of critical method variables and responses using central composite design and Minitab 18 software. **A** 3D contour plot showing multidimensional interactions between critical procedure variables A and B with fluorescence intensity—Response R1. **B** 3D contour plot showing multidimensional interactions between critical method variables A and B with % recoveries—Response R2. **C** Analytical design space for desirable maximum fluorescence intensity and %recovery of PGB

robustness was assessed by varying scanning speed and excitation wavelength, yielding consistent results. Precision studies for both intra-day and inter-day analyses demonstrated the method's reliability in estimating PGB. The method exhibited remarkable sensitivity, with the lowest limit of detection and quantitation determined to be 5.0 ng/mL and 10 ng/mL, respectively, underscoring its capability to detect PGB at nanogram-level concentrations. A summary of the validation parameters is presented in Table 3.

Formulations assay and spiked human plasma samples analysis

The established method was employed to assay various market formulations of PGB, revealing % assay values for PGB in the formulations within the range of 95% to 105% of the labeled claim of PGB. The presence of excipients did not cause interference in the estimation of PGB. In spiked human plasma samples, the % recovery of PGB was determined to be in the range of 85% to 90%. The matrices of blank human plasma samples did not pose interference in the estimation of PGB.

Discussion

The UV spectrum of PGB in distilled water exhibited two peaks at 210 nm and 230 nm (Refer Additional file 1: Figure S1 for UV spectrum of PGB), respectively. The 210 nm peak corresponded to the π–π^* transition, while the 230 nm peak was attributed to the $n - \pi^*$ transition of the primary amine functional group in PGB. Upon excitation at 230 nm, the nitrogen of the amino group led to a fluorescence emission at 410 nm. The fluorescence intensity at 410 nm was employed for a linearity study, revealing a linear relationship at nanogram-level concentrations of PGB. This fluorescence intensity was further examined in buffer solutions spanning a pH range of 1.0 to 12.0,

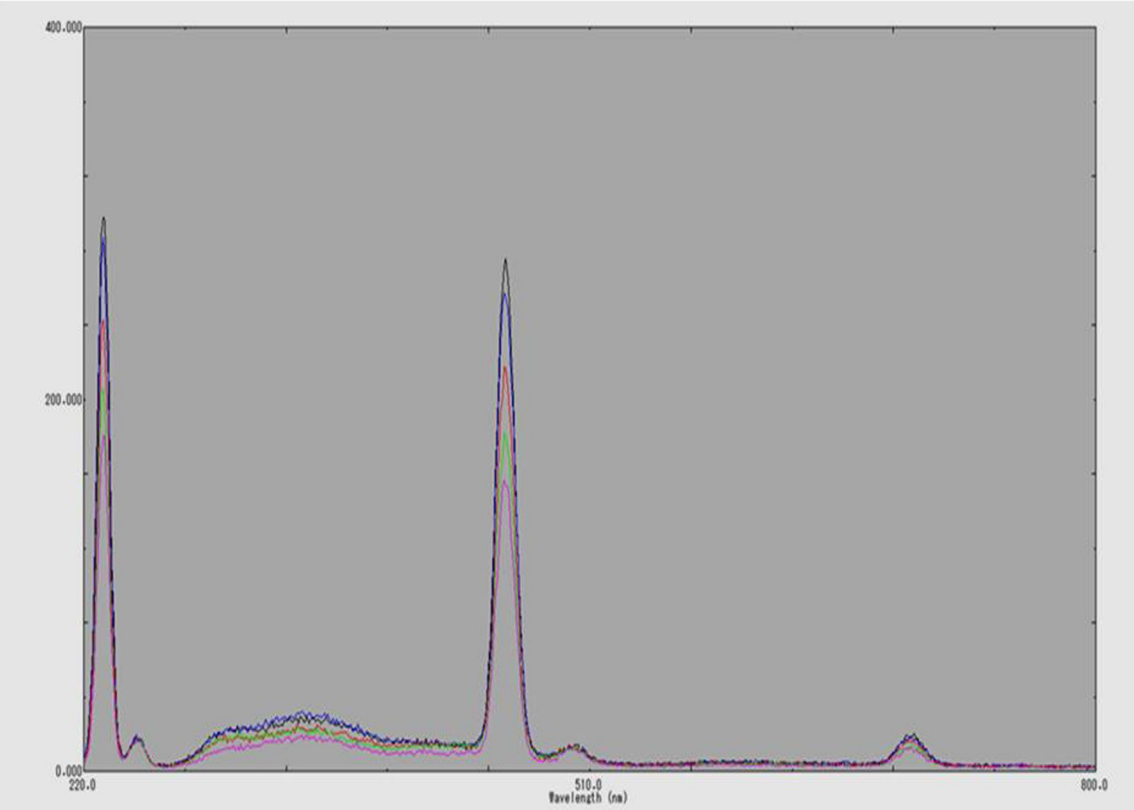

Fig. 4 Overlaid fluorescence spectrum showing linearity of PGB over concentration range of 10–50 ng/mL in spiked human plasma at emission wavelength of 410 nm and excitation wavelength of 230 nm

with the highest intensity observed in borate buffer at pH 8–10. The existing literature has detailed spectrofluorimetric methods involving derivatization, often utilizing toxic organic solvents and reagents, and exhibiting sensitivity at microgram levels. Additionally, these methods necessitated high reaction temperatures during the derivatization of PGB, rendering them less user-friendly. Notably, no spectrofluorimetric method in the literature has been reported for the detection and quantification of PGB without the need for derivatization.

A recent development in analytical chemistry advocates for a white analytical chemistry approach, emphasizing the development of green, economical and user-friendly analytical methods. Following the ICH Q14 guideline, the implementation of a design of experiments approach is a regulatory requirement for the registration of new drug products and substances. Despite this, the literature lacks an analytical method for estimating PGB using the white analytical chemistry and design of experiments approach. In response, a robust, green and user-friendly spectrofluorimetric method has been devised for the estimation of PGB in pharmaceutical dosage forms and spiked human plasma samples. This method integrates the principles of white analytical chemistry and the design of experiments approach, aligning with the contemporary trend toward environmentally conscious and efficient analytical methodologies, as suggested by recent developments in analytical chemistry.

Design of experiments using Minitab 18 software

After conducting initial experiments, it was determined that the excitation wavelength (designated as critical procedure variable A) and the pH of the medium (designated as critical procedure variable B) played pivotal roles. These variables were subsequently investigated to establish their graphical relationships with selected responses using response surface methodology. The fluorescence intensity and percentage recovery of PGB were chosen as critical responses for the development of a targeted spectrofluorimetric method. To analyze the identified critical procedure variables, a central composite design was employed, allowing the examination of main effects, two-way interactions and quadratic effects of the variables on the selected responses (R1 and R2). The analytical design space was explored to achieve maximum

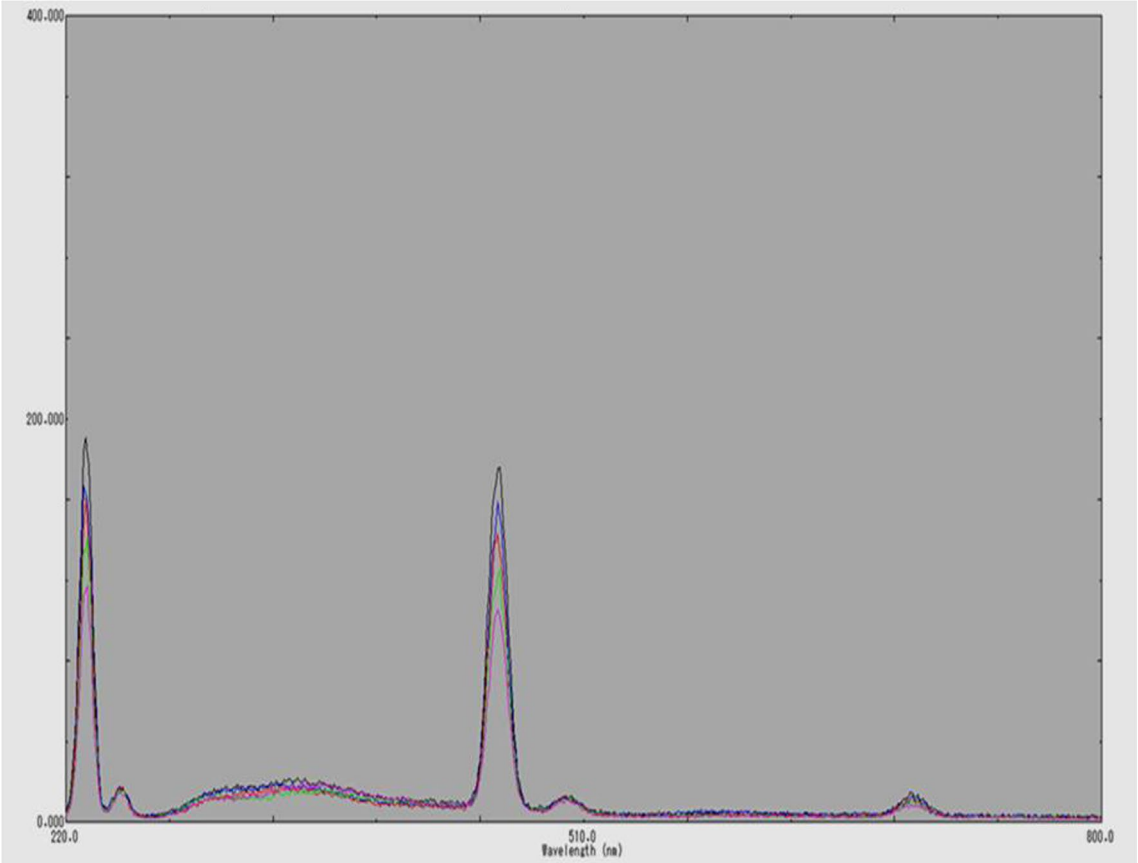

Fig. 5 Overlaid fluorescence spectrum showing linearity of PGB over concentration range of 50–250 ng/mL at emission wavelength of 410 nm and excitation wavelength of 230 nm

Table 3 Validation summary of developed spectrofluorimetric method as per ICH guidelines

Validation as per ICH Q2 (R1) guideline for assay of PGB in pharmaceutical dosage forms		**Validation as per ICH M10 guideline for analysis of spiked human plasma samples of PGB**	
Validation parameter	**Results**	**Validation parameters**	**Results**
Linearity range	10 to 250 ng/mL	Linearity range	10 to 250 ng/mL
Regression line equation	$Y = 1.7606x + 85.838$	Regression line equation	$Y = 0.4468x + 154.41$
Correlation coefficient	0.9955	Correlation coefficient	0.9976
Repeatability of sample measurement	0.88% (RSD)	Stability study (at 4 °C , 25 °C and −20 °C for 24 h)	6.77–8.65% (RSD)
Repeatability of sample preparation	0.98% (RSD)	Carryover effect and matrix effect	5.87–8.23% (RSD)
Intra-day precision	0.95–1.45% (RSD)	Intra-day precision	6.33 – 7.45% (RSD)
Inter-day precision	1.23–1.98% (RSD)	Inter-day precision	7.99–8.78% (RSD)
%recovery	98–102%	%recovery	85–90%
Limit of detection (LOQ)	5.0 ng/mL	Lowest limit of detection (LLOD)	5.0 ng/mL
Limit of quantitation	10.0 ng/mL	Lowest limit of quantitation (LLOQ)	10.0 ng/mL
Robustness	1.77–1.99% (RSD)	Robustness	8.77–9.32% (RSD)
Specificity	specific	Specificity	Specific

fluorescence intensity and percentage recovery of PGB in alignment with the analytical target profile of the method, as illustrated through overlaid response surface plots. Following a thorough examination of these plots, the navigated analytical design spaces for maximum fluorescence intensity and percentage recovery of PGB were assessed for accuracy and precision in predicting responses R1 and R2 using the mean response surface method. The optimized range for the excitation wavelength (critical procedure variable A) was determined to be 230 to 240 nm, while the optimized range for the pH of the medium (critical procedure variable B) was identified as 9.0 to 10.0. Consequently, the spectrofluorimetric estimation of PGB was conducted with an excitation wavelength of 230 nm at a pH of 9.0 in a borate buffer.

The developed spectrofluorimetric method has undergone validation in accordance with the guidelines of ICH Q2 (R1) for the assay of pharmaceutical dosage forms, encompassing specificity, accuracy, linearity, precision, LOD, LOQ and robustness. Additionally, the validation of the developed spectrofluorimetric method has been conducted following the ICH M10 guideline for estimating PGB in spiked human plasma samples. The results indicate that the developed method is accurate, precise, robust, sensitive, specific and linear in the estimation of PGB in both its pharmaceutical dosage forms and spiked human plasma samples. All validation parameters align with the standards outlined in the ICH Q2 (R1) and M10 guidelines.

The conventional technique was utilized to assay different commercial formulations of PGB (Pregazo 75 capsule—contains 75 mg PGB, Pregal 75 capsule—contains 75 mg PGB, and Pregadd tablets—contains 50 mg PGB), uncovering assay values for PGB in these formulations falling between 95 to 105% of the stated PGB content. Interestingly, the addition of other substances did not affect the measurement of PGB. When PGB was intentionally added to human plasma samples, its recovery ranged from 85 to 90%. Remarkably, the absence of any interfering substances in blank human plasma samples did not hinder the determination of PGB levels (refer supplementary files for fluorescence spectrum of blank human plasma and reagents). According to the reported LC–MS/MS method [20], the reported linearity range and Cmax value of PGB was found to be 0.1 to 15 µg/mL and 6.925 to 7.477 µg/mL in human plasma. The proposed spectrofluorimetric method was found to be complied with the reported LC–MS/MS method for the estimation of PGB in spiked human plasma samples. The developed method can be extended as sensitive and green analytical tool for pharmacokinetic and bioequivalence study of PGB in human and rat plasma.

Evaluation of spectrofluorimetric methods through white analytical chemistry principles

The comparative analysis of the existing and proposed methods for estimating PGB has been conducted through white analytical chemistry and an RGB model-based scoring system, as detailed in Table 4. The spectrophotometric methods published in the literature were evaluated against the proposed spectrofluorimetric method using both the RGB and WAC scoring systems. The red model-based assessment focused on evaluating the validation efficiency, sensitivity, scope and applicability of spectrofluorimetric methods. Some published spectrofluorimetric methods [24, 26, 27] exhibited sensitivity at the microgram level, earning a Rrd model-based score of 95 out of 100 (refer to Table 4). In contrast, our proposed method and other published spectrofluorimetric methods [22, 23] achieved a perfect score of 100, attributed to their nanogram-level sensitivity and applicability for PGB estimation in marketed formulations and spiked human plasma. This high score reflects their implementation of the design of experiments approach, exceptional sensitivity, % recoveries and applicability for PGB estimation.

The green model-based scoring and greenness profile assessment of analytical methods utilized the AGREE calculator, GAPI software, NEMI standards and ESA tool. However, the literature suggests that NEMI standards for greenness assessment provide the least accurate information. Therefore, AGREE and GAPI scores were considered for the calculation of the green model-based score. The green model-based scoring system assessed environmental friendliness, with published methods scoring less than 65 out of 100 based on AGREE and GAPI software criteria. The proposed method earned a score of 80 due to its use of environmentally friendly solvents (distilled water and methanol only), reduced organic waste generation and lower power consumption for PGB estimation. In contrast, the published spectrofluorimetric methods required high reaction temperatures and costly organic solvents and derivative reagents.

Assessing time, cost efficiency and user-friendliness through the blue model-based scoring system, published spectrophotometric methods scored 90 out of 100, respectively. The proposed method, which does not require high temperatures or costly derivative reagents and solvents for PGB estimation, achieved a perfect score of 100 for its economical, rapid, user-friendly and straightforward approach. The overall WAC (white analytical chemistry) score, calculated by averaging RGB model scores, for published spectrofluorimetric methods

Table 4 Comparison and assessment of published and proposed spectrofluorimetric for the estimation of PGB using principles of green and white analytical chemistry

Types of spectrofluorimetric method	Analytical conditions	Sensitivity, whiteness and greenness profile assessment of methods
Method 1—Spectrofluorimetric estimation of PBG using 7-chloro-4-nitrobenzofurazon (NBD-Cl) as fluorescent probe [22]	Linearity—40 to 400 ng/mL Derivatization agent—7-chloro-4-nitrobenzofurazon (NBD-Cl) Excitation wavelength—460 nm Emission wavelength—558 nm Extraction solvent—Chloroform (More than 10 mL) Reaction temperature—60 to 80˚C Application—Assay of marketed formulations	The method includes toxic organic solvents and reagents. The reaction temperature is more than room temperature The method has sensitivity as nanogram level AGREE score: 0.55 GAPI e-factor: 0.5 ESA penalty points: 08 Red model s score—100/100 Green model score—55/100 Blue model score—90/100 Whiteness score—(100 + 55 + 90)/3 = 81.66
Method 2—Spectrofluorimetric estimation of PGB using fluorescamine, 2,4-dinitrofluorobenzene and chloranil as fluorescent probes [23]	Linearity—20 to 280 ng/mL Derivatization agent—Fluorescamine, 2,4-dinitrofluorobenzene and chloranil Excitation wavelength—385 nm Emission wavelength—485 nm Extraction solvent—Water Reaction temperature—60 to 80˚C Application—Assay of marketed formulations and bioanalysis of spiked urine samples	The method needs toxic derivatization reagents and high temperature for reaction The method has sensitivity as nanogram level AGREE score: 0.60 GAPI e-factor: 0.50 ESA penalty points: 06 Red model s score—100/100 Green model score—60/100 Blue model score—90/100 Whiteness score—(100 + 60 + 90)/3 = 83.33
Method 3—Spectrofluorimetric estimation of PGB using fluorescamine as fluorescent probe [24]	Linearity—0.01 to 0.3 µg/mL Derivatization agent—Fluorescamine Excitation wavelength—390 nm Emission wavelength—487 nm Extraction solvent—Water Reaction temperature—60 to 80˚C Application—Assay of marketed formulations	The method needs toxic derivatization reagents and high temperature for reaction The method has sensitivity as micro and nanogram level AGREE score: 0.65 GAPI e-factor: 0.35 ESA penalty points: 06 Red model s score—95/100 Green model score—65/100 Blue model score—90/100 Whiteness score—(95 + 65 + 90)/3 = 83.33
Method 4—Spectrofluorimetric estimation of PGB using carbon quantum dots [26]	Linearity—4 to 100 µg/mL Derivatization agent—Carbon quantum dots from ascorbic acid Excitation wavelength—356 nm Emission wavelength—524 nm Extraction solvent—Water Reaction temperature—160˚C, 70 min Application—Assay of marketed formulations and bioanalysis of spiked urine sample	The method needs toxic reagents and very high temperature for reaction The method has sensitivity as microgram level only AGREE score: 0.65 GAPI e-factor: 0.35 ESA penalty points: 06 Red model s score—95/100 Green model score—65/100 Blue model score—90/100 Whiteness score—(95 + 65 + 90)/3 = 83.33

Table 4 (continued)

Types of spectrofluorimetric method	Analytical conditions	Sensitivity, whiteness and greenness profile assessment of methods
Method 5—Spectrofluorimetric estimation of PGB using acetylbutyrolactone as fluorescent probe [27]	Linearity—10 to 100 µg/mL Derivatization agent—Acetylbutyrolactone Excitation wavelength—380 nm Emission wavelength—440 nm Extraction solvent—Water Reaction temperature—95˚C Application—Assay of marketed formulations	The method needs toxic derivatization reagents and high temperature for reaction The method has sensitivity as microgram level only AGREE score: 0.65 GAPI e-factor: 0.35 ESA penalty points: 06 Red model s score—95/100 Green model score—65/100 Blue model score—90/100 Whiteness score—(95 + 65 + 90)/3 = 83.33
Proposed spectrofluorimetric estimation of PGB	Linearity—10 to 250 ng/mL Derivatization agent—No reagent Excitation wavelength—230 nm Emission wavelength—410 nm Extraction solvent—Water Reaction temperature—25˚C (Room Temperature) Application—Assay of marketed formulations	No chemical reaction required and water is used as solvent The method has sensitivity at nanogram level. The method does not require heating. Hence, it is more user-friendly AGREE score: 0.80 GAPI e-factor: 0.25 ESA penalty points: 03 Red model s score—100/100 Green model score—80/100 Blue model score—100/100 Whiteness score—(100 + 80 + 100)/3 = 93.33

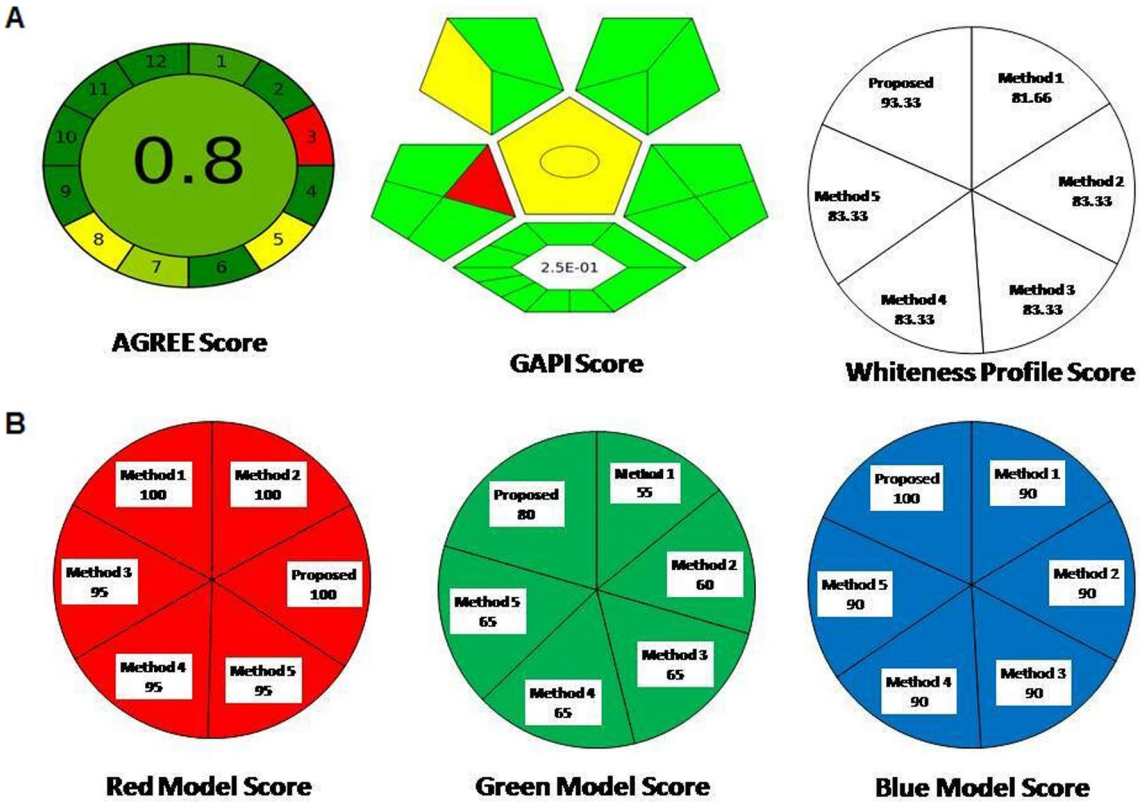

Fig. 6 **A** Greenness profile study of proposed spectrofluorimetric method using AGREE and GAPI software. **B** Whiteness profile assessment and comparison of published and proposed spectrofluorimetric methods for the estimation of PGB using RGB model score

was found to be less than 84 out of 100. In contrast, the proposed method received a score of 93.33 due to its validation efficiency, environmental friendliness, speed, cost-effectiveness and user-friendliness in PGB estimation. A comprehensive comparison of the greenness profile, WAC and RGB model-based scoring is depicted in Fig. 6A, B.

Conclusions

A hybrid approach, combining principles from white analytical chemistry and design of experiments, was employed for the spectrophotometric estimation of PGB. The resulting method incorporates safe, environmentally friendly and cost-effective solvents. Validation of the developed method encompassed specificity, sensitivity, selectivity, precision, accuracy and linearity in the estimation of PGB. Application of the method to assess PGB in various marketed formulations yielded results in accordance with the labeled claim. Furthermore, the method's applicability was extended to the estimation of PGB in spiked human plasma samples, demonstrating validation in accordance with ICH M10 guidelines. Both the developed and published methods were evaluated for their greenness and whiteness profiles in PGB estimation using RGB model scoring system, AGREE and GAPI software. The proposed method proved to be sensitive, robust, environmentally friendly, economical and user-friendly in estimating PGB across diverse samples. This developed method holds potential for use in pharmacokinetic and pharmacodynamic studies of PGB, presenting an economical and eco-friendly analytical tool for bioanalysis and quality control of PGB pharmaceutical dosage forms in the pharmaceutical industry.

Abbreviations

ADS	Analytical Design Space
AGREE	Analytical Greenness
ANOVA	Analysis of Variance
DoE	Design of Experiments
ESA	Eco-Scale Assessment
GSK	GlaxoSmithKline
GAC	Green Analytical Chemistry
GAPI	Green Analytical Procedure Index
HPLC	High-Pressure Liquid Chromatography
HPTLC	High-Performance Thin-Layer Chromatography
ICH	International Council for Harmonization
LOD and LOQ	Limit of Detection and Limit of Quantitation
NEMI	National Environmental Method Index
PGB	Pregabalin
PDE	Permitted Daily Exposure
RSM	Response Surface Modeling
RGB	Red, Green and Blue
WAC	White Analytical Chemistry

Supplementary Information

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Additional file 1. UV spectrum of PGB and fluorescence spectrum of blank.

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The data will be availed by corresponding author of manuscript on reasonable request.

Declarations

Ethics approval and consent to participate
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Consent for publication
Our research work does not include any human study. The manuscript entitled 'Design of Experiments and White Analytical Chemistry-driven Green and Sensitive Spectrofluorimetric Estimation of Pregabalin in its Pharmaceutical Dosage Forms and Spiked Human Plasma' has been solely submitted to Future Journal of Pharmaceutical Sciences.

Competing interests
The authors of the manuscript already declared that they do not have any conflicts of interest for the publication of the manuscript.

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