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Simultaneously quantifying a novel five-component anti- migraine formulation containing ergotamine, propyphenazone, caffeine, camylofin, and mecloxamine using UV spectrophotometry and chemometric models

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Abstract

This study presents a new method for simultaneously quantifying a complex anti-migraine formulation containing five components (ergotamine, propyphenazone, caffeine, camylofin, and mecloxamine) using UV spectrophotometry and chemometric models. The formulation presents analytical challenges due to the wide variation in component concentrations (ERG: PRO: CAF: CAM: MEC ratio of 0.075:20:8:5:4) and highly overlapping UV spectra. To create a comprehensive validation dataset, the Kennard-Stone Clustering Algorithm was used to address the limitations of arbitrary data partitioning in chemometric methods. Three different chemometric models were evaluated: Classical Least Squares (CLS), Partial Least Squares (PLS), and Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS). Among these, MCR-ALS demonstrated excellent performance, achieving recovery values of 98–102% for all components, accompanied by minimal root mean square errors of calibration (0.072–0.378) and prediction (0.077–0.404). Moreover, the model exhibited high accuracy, with relative errors ranging from 1.936 to 3.121%, bias-corrected mean square errors between 0.074 and 0.389, and a good sensitivity (0.2097–1.2898 µg mL^{-1}) for all components. The Elliptical Joint Confidence Region analysis further confirmed the predictive performance of the models, with MCR-ALS consistently showing the smallest ellipses closest to the ideal point (slope = 1, intercept = 0) for most analytes, indicating superior accuracy and precision. The approach's sustainability was rigorously assessed using six advanced metrics, validating its environmental friendliness, economic viability, and practical application. This approach effectively resolves complex pharmaceutical formulations, contributing to sustainable development objectives in quality control processes.

Keywords UV Spectrophotometry, Chemometric models, Kennard stone clustering algorithm, Multi-component pharmaceutical analysis, Sustainability assessment

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Introduction

The pursuit of sustainable, cost-effective, and environmentally conscious practices in pharmaceutical analysis has become a paramount imperative. The recent market launch of a novel fixed-dose anti-migraine formulation of ergotamine (ERG), propyphenazone (PRO), caffeine (CAF), camylofin (CAM), and mecloxamine (MEC) has posed a considerable analytical problem. To date, only a single analytical method has been reported for this complex mixture [1], and it falls short of aligning with the progressive principles of green analytical chemistry (GAC) and white analytical chemistry (WAC). The existing method's reliance on harmful solvents, intricate multi-stage procedures, expensive equipment, and extensive laboratory infrastructure not only contradicts sustainable practices but also poses significant risks to human health, the environment, and practical economic considerations, necessitating a paradigm shift towards the development of innovative analytical approaches that embrace these emerging disciplines [2–5].

The quantitative determination of this fixed-dose combination presents significant analytical challenges, primarily due to the vastly different concentrations of its components. The challenging ratio of ERG (0.075), PRO (20), CAF (8), CAM (5), and MEC (4) requires a method with high sensitivity and a wide linear range. ERG, present in the lowest concentration, demands exceptional sensitivity for accurate quantification. Its instability and light sensitivity further complicate analysis. PRO, the highest concentrated component, necessitates careful sample preparation to avoid detector saturation while maintaining the working range. CAF, a common ingredient in many formulations, may introduce potential interferences. CAM and MEC, present in intermediate concentrations, add to the complexity of the mixture due to potential spectral overlaps, as shown in (Fig. 1). Moreover, the structural similarities and potential interactions between these compounds can lead to matrix effects, further complicating their simultaneous determination. These factors collectively underscore the need for a robust, sensitive, and selective analytical method capable of accurately quantifying all five components in a single analysis.

In this framework, UV–visible (UV–Vis) spectrophotometry presents itself as an effective approach, aligning with the sustainability goals of modern analytical chemistry. It offers a cost-efficient solution by utilizing affordable reagents and simple apparatus, including cuvettes and light sources, while producing minimal hazardous waste. This technique adheres to the tenets of both GAC and WAC [6, 7]. However, significant spectral overlaps frequently make it difficult to directly quantify pharmaceuticals [8], a challenge that can be surmounted through the strategic implementation of chemometrics solutions. By harnessing the power of chemometrics, UV–visible spectroscopy is transformed into a practical green analytical tool, ideally suited for efficient and sustainable pharmaceutical quality control workflows [9–12].

A common pitfall in numerous recent chemometrics research studies is the reliance on random partitioning to segregate the sample data into training and validation subsets [13, 14]. Even though random splitting is simple, it can result in validation sets that do not adequately represent the full sample space, potentially leading to biased outcomes or an overestimation of the model's accuracy. This outcome directly contradicts the sustainable objectives of resource efficiency and reliability. To surmount this formidable challenge, our research harnesses the Kennard Stone Clustering Algorithm (KSC), an advanced statistical approach, to construct truly representative validation sets [15]. This method partitions the modeled variable space into distinct clusters, ensuring that validation samples encompass the full spectrum and distribution of each variable. This meticulous clustering and sample selection strategy comprehensively covers the sample space, enabling an unbiased assessment of the chemometric model's predictive capabilities across all data domains. KSC enhances analytical sustainability by minimizing material consumption and waste generation while bolstering reliability through fewer, strategically distributed validation samples.

In this context, our study addresses five crucial objectives: (1) Developing an environmentally benign, highly sensitive, and cost-effective chemometric quantification method for ERG, PRO, CAF, CAM, and MEC without prior separation, marking a significant improvement over the existing method; (2) Positioning chemometrics as a sustainable analytical alternative to traditional methods, facilitating economical and routine quality control processes; (3) Systematically leveraging KSC to construct validation sets that rigorously assess predictive reliability across all concentration ranges, minimizing bias and overfitting; (4) Conducting a comprehensive greenness evaluation using state-of-the-art tools including the Carbon Footprint Analysis (CFA), National Environmental Method Index (NEMI), Complementary Green Analytical Procedure Index (Complex GAPI), and Analytical Greenness Metric (AGREE) analysis, providing a multifaceted assessment of environmental impact; and (5) Pioneering "whiteness" and "blueness" assessments through the novel application of the Blue Applicability Grade Index (BAGI) and Red–Green–Blue 12 (RGB12) metrics, quantifying the practicality, sustainability, analytical performance, and cost-effectiveness advantages over existing methods. This innovative approach heralds a paradigm shift towards eco-friendly, cost-effective, and practical

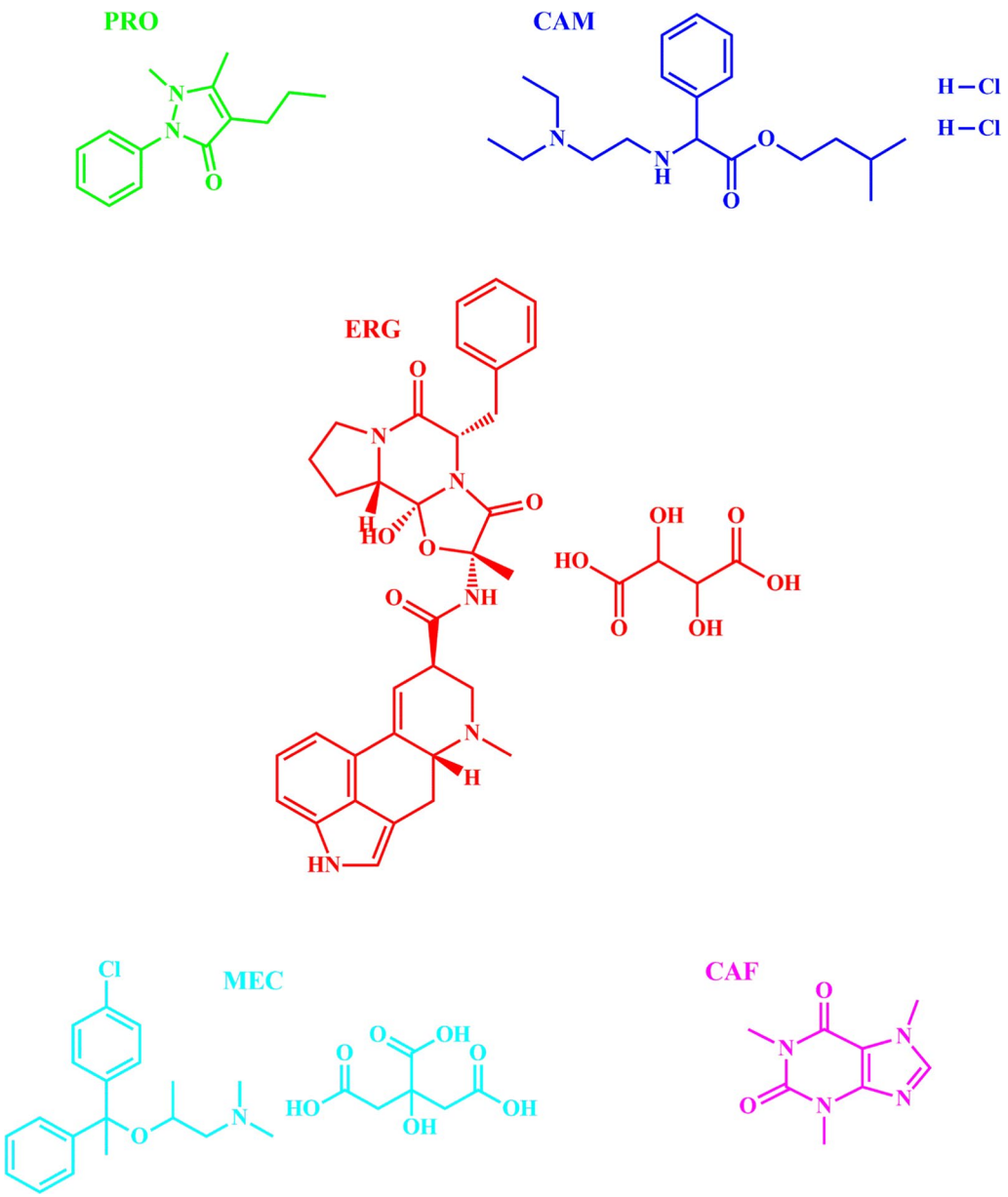


Fig. 1 The chemical structure of ERG, PRO, CAF, CAM, and MEC

analytical strategies, aligning with sustainable development principles and quality control excellence in pharmaceutical analysis.

Experimental

Analytical instruments and apparatus

Gathering spectral information was accomplished utilizing a Shimadzu UV-1800 double-beam spectrophotometer equipped with 1 cm quartz cells. Coupled with quartz cuvettes of 1 cm path length. The instrument's operation was managed through UV-Probe software (version 2.42). To optimize spectral resolution and data density, we configured the spectrophotometer with a slit width of 1.0 nm and implemented a fine-grained 0.1 nm sampling interval. Measurements were conducted in fast scan mode with a single sweep to balance efficiency and data quality. In addition, a high-precision Shimadzu analytical balance (AGE-220) was utilized for accurate sample preparation, and an ultrasonic bath for extraction was provided by Julabo Labortechnik of Germany. We processed the data and performed chemometrics analysis using Matlab R2013a with PLS Toolbox v2.0 and MCR-ALS Toolbox

(an allowed program accessible at http://www.mcrals.info). To implement KSC, we used the built-in scripts of MATLAB R2013a (version 8.2.0.701), which make use of advanced statistical algorithms.

Chemicals and materials

All chemicals were of analytical grade. We got ERG, PRO, CAF, CAM and MEC as reference standard compounds from the National Organization for Drug Control and Research of Egypt. They were certified to be 99.58%, 99.39%, 99.75%, 99.63% and 99.72% pure, respectively. Ethanol was bought from Sigma Aldrich Co., St.Louis, USA. The SPASMOMIGRAINE® tablets (batch no: S2456) made by Kahira Pharmaceuticals & Chemical Industries Company (Cairo, Egypt) and containing 0.75 mg ERG, 200 mg PRO, 80 mg CAF, 50 mg CAM and 40 mg MEC in each tablet, was bought from a local pharmacy.

Standard solutions

We prepared primary stock solutions for ERG, PRO, CAF, CAM, and MEC at a concentration of 1000 μg mL^{-1}. This process involved precise weighing of 100 mg of each reference standard using an analytical balance, followed by dissolution in ethanol within 100 mL volumetric flasks. The solutions were then brought to volume with meticulous attention to the meniscus. The stock solutions exhibited stability for one month when refrigerated at 4 °C. To attain the requisite concentration ranges for analysis, we performed daily dilutions of the stock solutions using ethanol to create fresh working standard solutions, ensuring optimal sample integrity.

Working range and spectral properties

To elucidate the spectral characteristics of each analyte, we acquired individual UV absorption spectra for ERG, PRO, CAF, CAM, and MEC across the 200–400 nm wavelength range. For this spectral profiling, we utilized standard solutions with concentrations of 1 μg mL^{-1} ERG, 20 μg mL^{-1} PRO, 8 μg mL^{-1} CAF, 5 μg mL^{-1} CAM, and 4 μg mL^{-1} MEC. These specific concentrations were strategically selected to bridge the gap between pharmaceutical formulation ratios and the linear dynamic range of our analytical method. To establish and validate the method's working range, we conducted a comprehensive analysis of ERG, PRO, CAF, CAM, and MEC mixtures, scanning from 200 to 400 nm. The concentration working ranges were carefully optimized for each analyte: 1–5 μg mL^{-1} for ERG, 10–30 μg mL^{-1} for PRO, 4–12 μg mL^{-1} for CAF, 1–9 μg mL^{-1} for CAM, and 2–6 μg mL^{-1} for MEC. These meticulously chosen intervals served a dual purpose: they aligned with the linear response capabilities of our spectrophotometric instrumentation while accurately reflecting the anticipated concentration levels in pharmaceutical formulations, thereby ensuring robust analytical performance across the relevant concentration domain.

Methodology and design of experiment

A methodical experimental outline is essential for attaining spectral data that is both representative and abundant in information. A multiple-level, and multiple-factor calibration set consisting of twenty-five mixtures was developed, following the strategy presented by Brereton et al. [16]. As a result, a set of 25 mixes was created for calibration with different ERG, PRO, CAF, CAM and MEC concentrations ranging from 1 to 5, 10–30, 4–12, 1–9, and 2–6 μg mL^{-1} for ERG, PRO, CAF, CAM and MEC, respectively. KSC was used to build a validation set that reliably validates the model and provides an illustrative sample of the concentration space. To construct a robust validation set, we implemented a stratified sampling approach, splitting the concentration range into thirteen equally probable strata. From each stratum, we selected one unique mixture, resulting in a comprehensive set of 13 distinct validation samples. This sampling strategy ensures thorough coverage of the entire concentration domain, enhancing the reliability of our method validation. Sample preparation was executed with precision using calibrated micropipettes for accurate volume transfers. Ethanol served as our solvent of choice, balancing effective analyte dissolution with environmental considerations. All mixes were formulated in 25 mL volumetric flasks, ensuring consistent and precise final volumes. Spectral acquisition was performed using 1 cm quartz cuvettes, chosen for their superior optical properties and chemical inertness. We recorded absorption spectra over 200–400 nm wavelength range, capturing the full UV spectral fingerprint of our analytes. To account for background absorbance and ensure measurement accuracy, we employed ethanol as a blank reference. This carefully designed experimental approach not only meets the rigorous standards of analytical method development but also demonstrates our commitment to sustainable and responsible scientific practices in pharmaceutical analysis. Lower and higher spectral ranges were thrown out because they had too much noise or no signals, making the working spectral data matrix 220–350 nm with a resolution of 1 nm (131 data points), as shown in (Fig. 2). This spectrum information was used to create and verify the chemometric models.

Models' development and refinement

Our study employed three distinct chemometric regression approaches: classical least squares (CLS), partial least squares (PLS), and multivariate curve

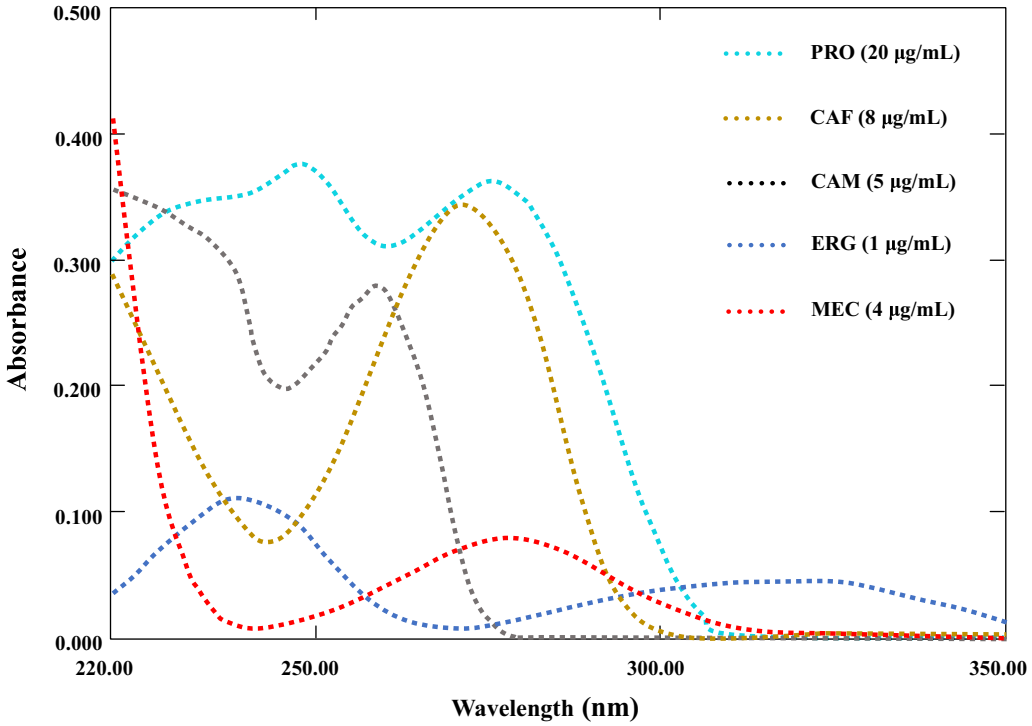


Fig. 2 The zero-order absorption spectrum of ERG, PRO, CAF, CAM, and MEC

resolution-alternating least squares (MCR-ALS). To ensure model robustness and mitigate overfitting, we meticulously optimized each calibration model using a comprehensive 25-mixture design calibration set. For the CLS model, we implemented a wavelength-specific regression strategy, eschewing the integration of results into latent variables (LVs). To enhance model performance, we utilized a moving window wavelength selection technique. We systematically evaluated window widths from 5 to 30 nm via cross-validation to determine the ideal spectral smoothing level that effectively reduces noise while preserving crucial quantitative information. In developing the PLS model, we methodically varied the number of LVs from 1 to 10. The optimal LV count was determined using Venetian blinds cross-validation, with the root mean squared error of cross-validation (RMSECV) serving as our performance metric. This approach allowed us to strike a balance between model fit and complexity. For the MCR-ALS model, constraint optimization played a pivotal role in calibration refinement. We applied non-negative least squares (nnl) or non-negativity constraints to both spectral profiles and concentration. This strategy facilitated the attainment of satisfactory parameters with minimal iterative cycles, enhancing computational efficiency. Through this rigorous optimization process, we aimed to develop highly reliable and accurate chemometric models capable of addressing the complex analytical challenges presented by our multi-component pharmaceutical formulation.

Analytical performance metrics

The predictive power, robustness, precision, accuracy, and sensitivity of the optimized models were assessed by computing a number of critical parameters [17]. Using the calibration set spectra, root mean square error of calibration (RMSEC), standard error of calibration (SEC), and RMSECV were calculated as pointers of the model's fitting and predictive power on the calibration set.

Relative root mean square error of prediction (RRMSEP) was used to quantify predictive accuracy for validation set performance. Using the root mean square error of prediction (RMSEP), the generalization ability of the model was evaluated overall. Additionally, bias-corrected mean square error of prediction (BCRMSEP) was used to assess the precision and predictability of fresh samples. To compute RMSEP, RMSECV, and RMSEC, we use the following equations [17]:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(yi - \widehat{yi})^2}{n}}$$

The subsequent equations were employed to calculate the further metrics of merit:

$$Bias = \frac{\sum_{i=1}^{n}(yi - \widehat{yi})}{n}$$

$$SEC = \sqrt{\frac{\sum_{i=1}^{n}(yi - \widehat{yi} - bias)^2}{n-1}}$$

$$RRMSEP\% = \frac{\frac{1}{n}\sqrt{\sum_{i=1}^{n}(yi - \widehat{yi})^2}}{\bar{y}i} X100$$

$$BCRMSEP = \frac{\sum_{i=1}^{n}(yi - \widehat{yi})^2}{n} - (bias)^2$$

The outcome of the cross-validation (RMSECV), validation (RMSEP), and calibration (RMSEC) procedures is denoted by the value yi. The variables n, and yi represent the overall number of samples, and the experimental outcome for sample i, respectively.

To assess the accuracy, (2, 3, and 4 µg mL^{-1}) for ERG, (15, 20, and 25 µg mL^{-1}) for PRO, (6, 8, and 10 µg mL^{-1}) for CAF, (3, 5, and 7 µg mL^{-1}) for CAM and (3, 4, and 5 µg mL^{-1}) for MEC were determined in triplicate at three different concentrations inside the linear range, and the percent recoveries (%R) were calculated. The percent relative standard deviation (%RSD) quantified precision for repeatability (intra-day) and intermediate (inter-day) precision. Three different samples were tested three times, once on the same day and three times on three different days, (2, 3, and 4 µg mL^{-1}) for ERG, (15, 20, and 25 µg mL^{-1}) for PRO, (6, 8, and 10 µg mL^{-1}) for CAF, (3, 5, and 7 µg mL^{-1}) for CAM and (3, 4, and 5 µg mL^{-1}) for MEC. Robustness was evaluated through experiments employing marginally different wavelength intervals (0.9 nm as opposed to 1 nm), scan speeds (medium as opposed to fast scan), and spectral bandwidths (0.8 nm as opposed to 1 nm slit width). The method's robustness was shown by its capacity to endure these alterations. To evaluate sensitivity, net analyte signals were utilized to compute the limits of quantification (LOQ) and detection (LOD). This inclusive validation offers a detailed insight into how well, accurately, precisely, sensitively, and robustly the models we made can predict the analysis of pharmaceutical mixtures.

Pharmaceutical dosage forms analysis

The content of 10 tablets of SPASMOMIGRAINE® tablets was accurately weighed, finely powdered, and thoroughly mixed. A quantity of powder equivalent to 0.75 mg ERG, 200 mg PRO, 80 mg CAF, 50 mg CAM, and 40 mg MEC was transferred into a volumetric flask with a capacity of 100 mL. To extract the drugs into the solution, about 80 mL of ethanol was added, and it was sonicated for 15 min. The volume was filled with ethanol, thoroughly mixed, and then diluted with ethanol to obtain concentrations of 7.5 µg mL^{-1} ERG, 2000 µg mL^{-1} PRO, 800 µg mL^{-1} CAF, 500 µg mL^{-1} CAM, and 400 µg mL^{-1} MEC. Subsequently, the mixture underwent filtration utilizing a 0.45 µm membrane filter. Appropriate aliquots of the clear filtrate were diluted with ethanol in volumetric flasks (10 mL). At this stage, while the concentrations of PRO, CAF, CAM, and MEC fell within their respective linear ranges, the ERG concentration was below the limit of quantification. To address this, a standard addition approach was employed. A 1.5 µg mL^{-1} ERG standard solution was added to each determination, bringing the total ERG concentration within the working range. These diluted sample solutions' absorption spectra were obtained between 200 and 400 nm in relation to an ethanol blank. The spectra were examined using the proposed chemometric models to calculate the concentrations of ERG, PRO, CAF, CAM, and MEC in the pharmaceutical mixture. By subtracting the additional standard concentration from the total measured concentration, the true ERG concentration in the samples was determined. The accuracy was measured by the addition of spiked standards in triplicate at 4 distinct levels of concentration. We calculated RSD% and R%.

Results and discussion

Green solvent selection

Evaluation using GSST tool

An essential component of achieving sustainability in analytical procedures is choosing the right green solvents. A number of pharmaceutical companies have published solvent sustainability recommendations that use data from safety data sheets (SDS) to analyze the advantages and disadvantages of recommended solvents. These companies include GlaxoSmithKline, Sanofi, and Pfizer, among many others. We used a solvent selection tool that was recently introduced by Larsen et al. [18] to help us with this process. Using this chemometric tool, we can compare and evaluate solvents quantitatively according to important eco-friendliness criteria like health, safety, environmental impact, and waste management. For every solvent, it computes a composite greenness score (G), where higher scores correspond to more sustainability. We used this tool with seven different polar solvents, including acetonitrile, ethyl acetate, ethanol, hexane, chloroform, and water. Water, acetonitrile, ethyl acetate, ethanol, and methanol had much higher G scores than harmful solvents like hexane and chloroform, according to the software analysis (Fig. 3**).** To illustrate, acetonitrile, water, ethyl acetate, ethanol, and methanol all achieved high G scores —7.3, 6.7, 6.6, 5.8, and 5.8, respectively, according to positive evaluations in the areas of health

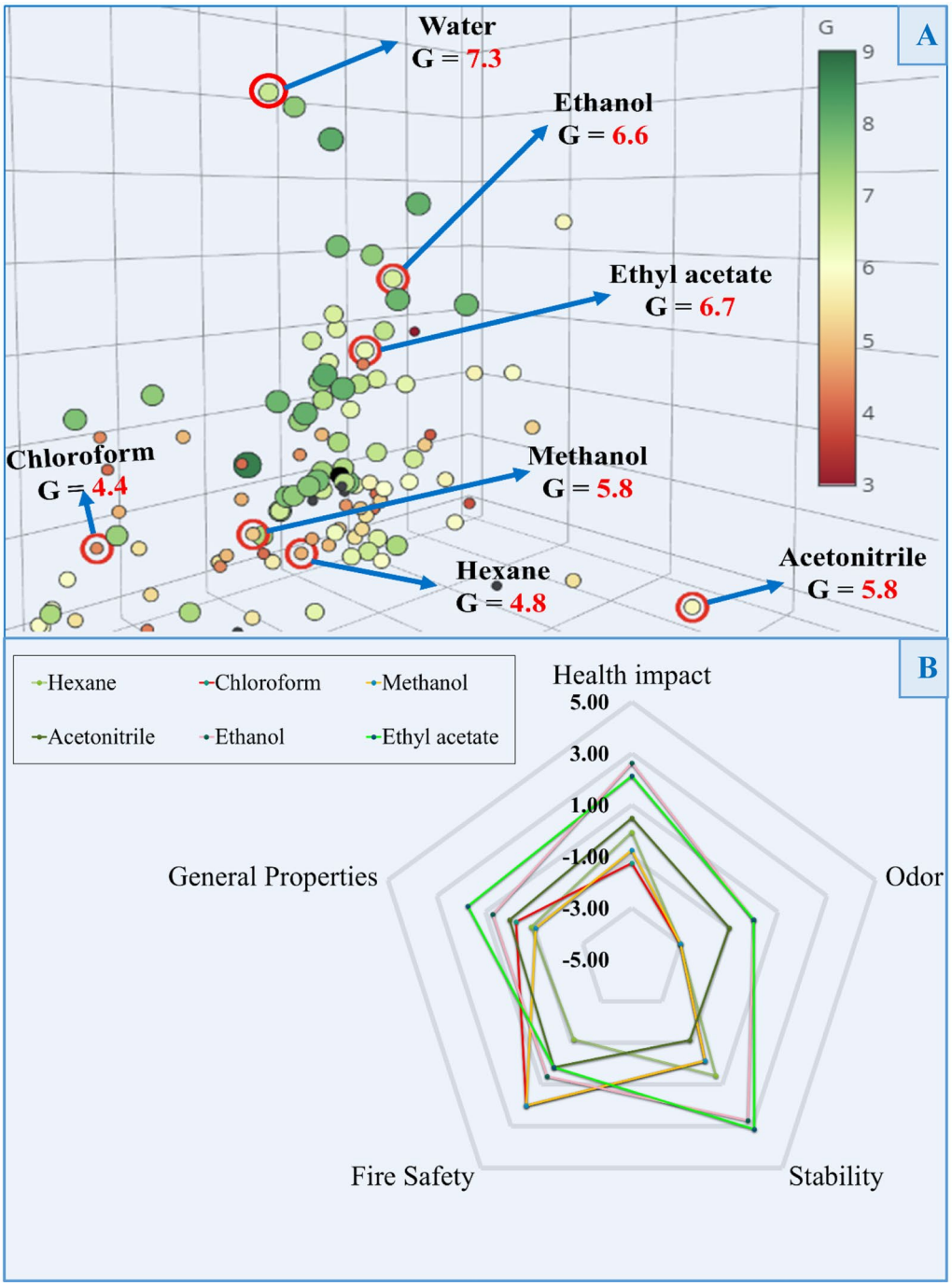


Fig. 3 **a** G scores by GSST attained by the online tool. **b** Spider diagram attained by SDAGI

effects, safety, environmental effects, and waste disposal. As illustrated in (Fig. 3). We chose acetonitrile, water, ethyl acetate, ethanol, and methanol as more environmentally friendly solvents for additional evaluation based on these greenness evaluations. This fits in with our plan to create an analytical technique that follows green chemistry philosophies by using safer, more environmentally friendly chemicals.

SDAGI assessment

Even though tools such as GSST facilitate initial solvent selection, a more thorough evaluation of reagent

greenness utilizing comprehensive investigational data is required. When assessing solvent and reagent greenness, the SDAGI tool offers a useful qualitative technique that is dependent on SDS data [19, 20]. This approach uses Safety, Health, and Environmental (SHE) data on the properties and effects of reagents. Greenness ratings based on important criteria can be computed and visual spider diagrams can be made to evaluate a solvent's greenness. Marks from -5 to + 5, derived from the five assessment subcategories of fire safety, general properties, health impact, stability, and odor are displayed in the hierarchical spider plot. Secondary spider charts show subgroup specifics, as (Fig. 3) demonstrates. Using a spider diagram to evaluate and compare chemicals visually is a straightforward way. The percentage of the available data that was used to generate the Greenness Index outputs is shown in the "Greenness Index Table". Using SDAGI, we examined SDS data and discovered that, as indicated in (Table S1), methanol and acetonitrile had scores of -0.12 and -0.30, while ethanol and ethyl acetate showed larger safety zones and elevated average greenness scores (1.33 and 1.44 for ethanol and ethyl acetate, respectively). Water, ethyl acetate, and ethanol were therefore chosen as the more environmentally friendly solvents for more testing in order to determine which green solvent would be best. However, after evaluating the UV spectra of ERG, PRO, CAF, CAM and MEC in the 3 solvents, we found that ethanol provided a higher UV response and better spectral shape compared to water and ethyl acetate. Considering the superior UV analytical performance of ethanol along with its environmentally friendly nature, we concluded that ethanol is the most suitable green solvent for further studies involving ERG, PRO, CAF, CAM and MEC.

Development of CLS, PLS, and MCR-ALS chemometric models

The complex spectral profiles of ERG, PRO, CAF, CAM and MEC exhibit significant overlap in their UV absorption spectra, as evidenced in (Fig. 2). This spectral congestion precludes direct quantification through conventional univariate methods, necessitating the application of advanced chemometric models [21–23]. To tackle this analytical challenge, we developed and optimized three multivariate chemometric models using the complete spectrum data of the five-component mixture.

Spectral data acquisition was performed over the range of 200–400 nm, capturing the characteristic UV–vis fingerprints of the analytes. While these spectral signatures encode the requisite concentration information, the substantial overlap impedes straightforward correlation between absorbance and analyte levels. Chemometric modeling enables the extraction of latent concentration data from the intricate spectral matrix.

An essential phase in model building entailed the careful selection of spectral areas to enhance valuable signals while reducing the impact of noise and spectral artifacts. Using an iterative optimization procedure, including signal-to-noise ratios, probable interferents, and preliminary model performance measurements, we established an ideal spectral window of 220–350 nm, yielding 131 data points per spectrum. The resultant data matrix was utilized as input for the development and optimization of CLS, PLS, and MCR-ALS models employing MATLAB software.

To validate the fundamental assumption of spectral additivity underpinning CLS and MCR-ALS models, we conducted a rigorous additivity assessment. This evaluation involved the preparation of a synthetic standard mixture mirroring the concentrations found in the pharmaceutical formulation. We then acquired and compared three distinct spectral profiles: (1) The UV spectrum of the synthetic standard mixture, (2) the spectrum of the actual pharmaceutical formulation, and (3) a composite spectrum generated by the mathematical summation of individual component spectra at their respective concentrations.

Comparative analysis revealed remarkable concordance among these spectra, with deviations not exceeding 2% at any wavelength across the analytical range. Spectral features, including peak positions and relative intensities, demonstrated high consistency. Notably, we observed no emergent peaks or spectral shifts in the mixture spectra relative to the calculated composite, indicating the absence of significant inter-analyte interactions (Fig. S1).

Statistical evaluation using a paired t-test to compare absorbance values between the synthetic mixture spectrum and the mathematically derived composite showed no statistically significant differences ($p > 0.05$) across all wavelengths. Furthermore, residual analysis of the difference spectrum between these two profiles revealed only stochastic noise, devoid of systematic deviations. These findings provide robust support for the additivity assumption, confirming the absence of spectral-altering interactions among the analytes in the mixture state. This validation substantiates the applicability of CLS and MCR-ALS models alongside PLS for this complex analytical system.

Calibration set design

To find the best combination of the three components for the calibration set, we used an experimental design with multiple levels and factors that Brereton et al. [16] suggested to create 25 different mixtures. Improved model accuracy is achieved by using this approach to create

analyte concentration profiles that are not correlated. Different concentrations were tested at five levels, with 0 being the middle point and the others being −2, −1, 0, +1, and +2 As shown in (Table 1). The analyte profiles' lack of correlation and the structured variation in concentrations help the model more successfully separate each component's spectrum impact. As a result, the quantification accuracy is improved, and overfitting is prevented. This saved time and money in the lab by reducing the quantity of materials used, chemical waste, and calibration sample requirements, consequently, making the method more environmentally compatible. In general, the calibrations design of multilevel and multifactor enhanced reliability and accuracy through careful modeling and selection of the complete analyte concentration spectrum. This aligns with the fundamental ideas that guide the creation of ecologically friendly analytical procedures.

Design and optimization of validation set

In order to provide a thorough assessment of the predictive abilities, the validation set must include all concentration ranges predicted in the calibration procedure. Simple random sampling carries the risk of incomplete coverage, which could result in prejudiced accuracy estimates. To address this crucial constraint, the validation set was carefully created using the KSC. To create a solid and representative validation set that covers the whole multivariate concentration space, the KSC algorithm is carefully used. MATLAB was used to run the KSC algorithm with the aid of pre-written scripts. The multicomponent concentration data was methodically stratified into discrete clusters along several dimensions by these scripts. One validation sample was chosen from each stratum, which corresponds to a concentration regime that is equally likely. This approach ensured comprehensive and equitable coverage of the full analyte concentration ranges and their combinatorial distributions. By optimally distributing validation samples across clustered data strata, KSC mitigated potential biases arising from incomplete coverage or uneven sample densities within the multivariate space. This meticulous, statistically grounded sampling technique enabled a relatively small, but very informative, validation set of 13 mixtures that could rigorously test the predictive power of the chemometric models over the whole range of expected sample compositions, as presented in (Table 1). The scatter plots in (Figs. 4, 5) show how a consistent scattering pattern was formed by the thirteen validation samples over the whole analyte range. The KSC algorithm acquired broad coverage with markedly fewer samples than standard random sampling. Through improved concentration space sampling efficiency, the KSC algorithm allowed a smaller but more informative validation set to be applied. This tactic decreased the number of materials used, the amount of waste produced, and the related expenses, which enhanced the method's greenness. Furthermore, the model's resilience in managing diverse combinations

Table 1 The five-level five-factor experimental design of 25 calibrations mixtures together with the 13 validation set mixtures used in the chemometric methods

Mix no	Calibration set (µg/mL)				
	ERG	PRO	CAF	CAM	MEC
1	3	20	8	5	4
2	3	10	4	9	3
3	1	10	12	3	6
4	1	30	6	9	4
5	5	15	12	5	3
6	2	30	8	3	3
7	5	20	6	3	5
8	3	15	6	7	6
9	2	15	10	9	5
10	2	25	12	7	4
11	4	30	10	5	6
12	5	25	8	9	6
13	4	20	12	9	2
14	3	30	12	1	5
15	5	30	4	7	2
16	5	10	10	1	4
17	1	25	4	5	5
18	4	10	8	7	5
19	1	20	10	7	3
20	3	25	10	3	2
21	4	25	6	1	3
22	4	15	4	3	4
23	2	10	6	5	2
24	1	15	8	1	2
25	2	20	4	1	6
Validation set (µg/mL)					
1	3	15	11	2	4
2	3	23	8	4	3
3	2	13	8	3	2
4	4	13	6	8	4
5	3	26	10	9	6
6	1	21	4	2	4
7	2	29	7	5	4
8	2	17	5	5	3
9	4	10	6	6	5
10	4	24	11	7	3
11	2	28	9	8	4
12	5	18	8	2	5
13	4	21	12	6	5

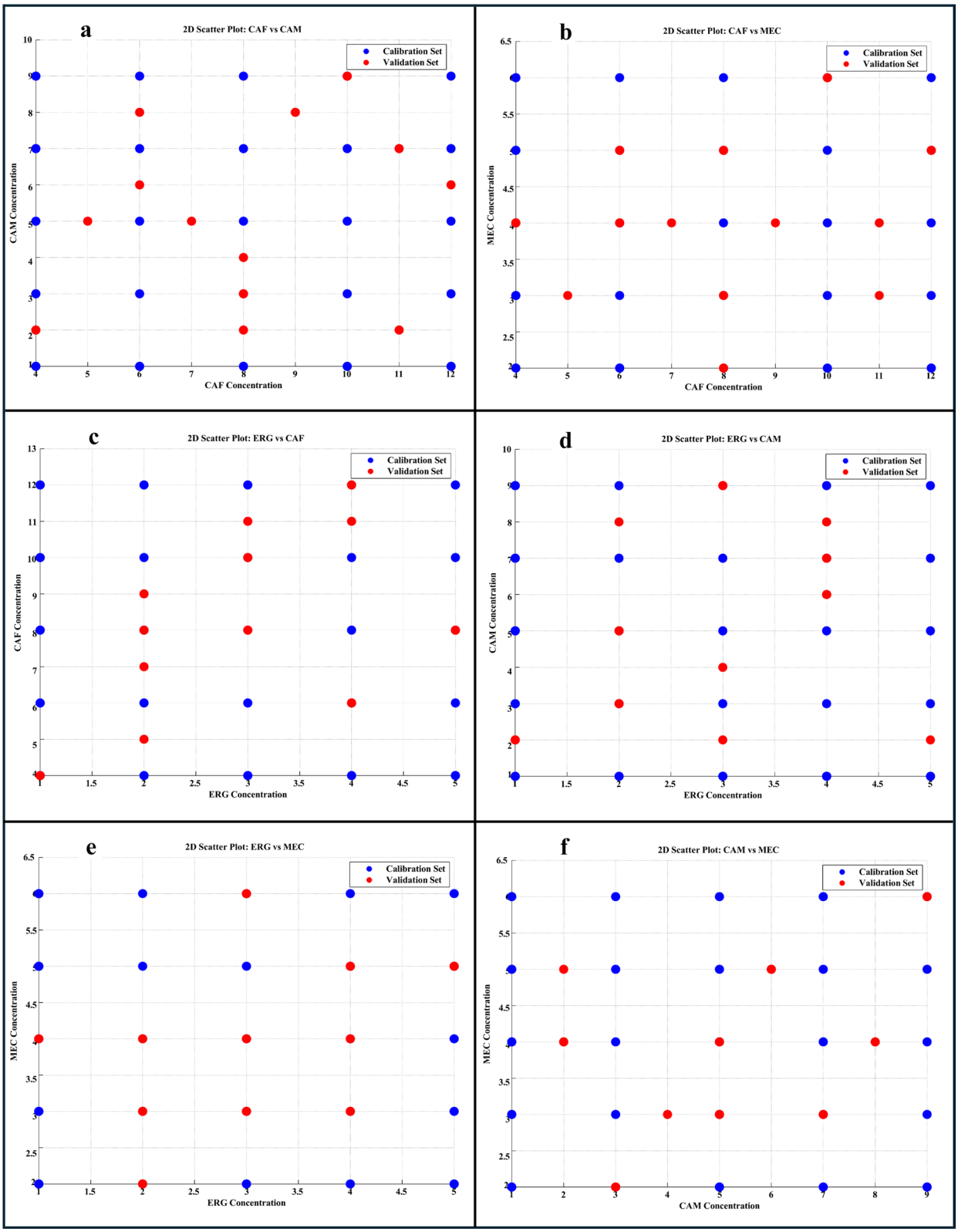


Fig. 4 2D scatter plot of the validation set of (**a**) CAF versus CAM, **b** CAF versus MEC, **c** ERG versus CAF, **d** ERG versus CAM, **e** ERG versus MEC, and **f** CAM versus MEC designed by KSC design as optimal-space filling design

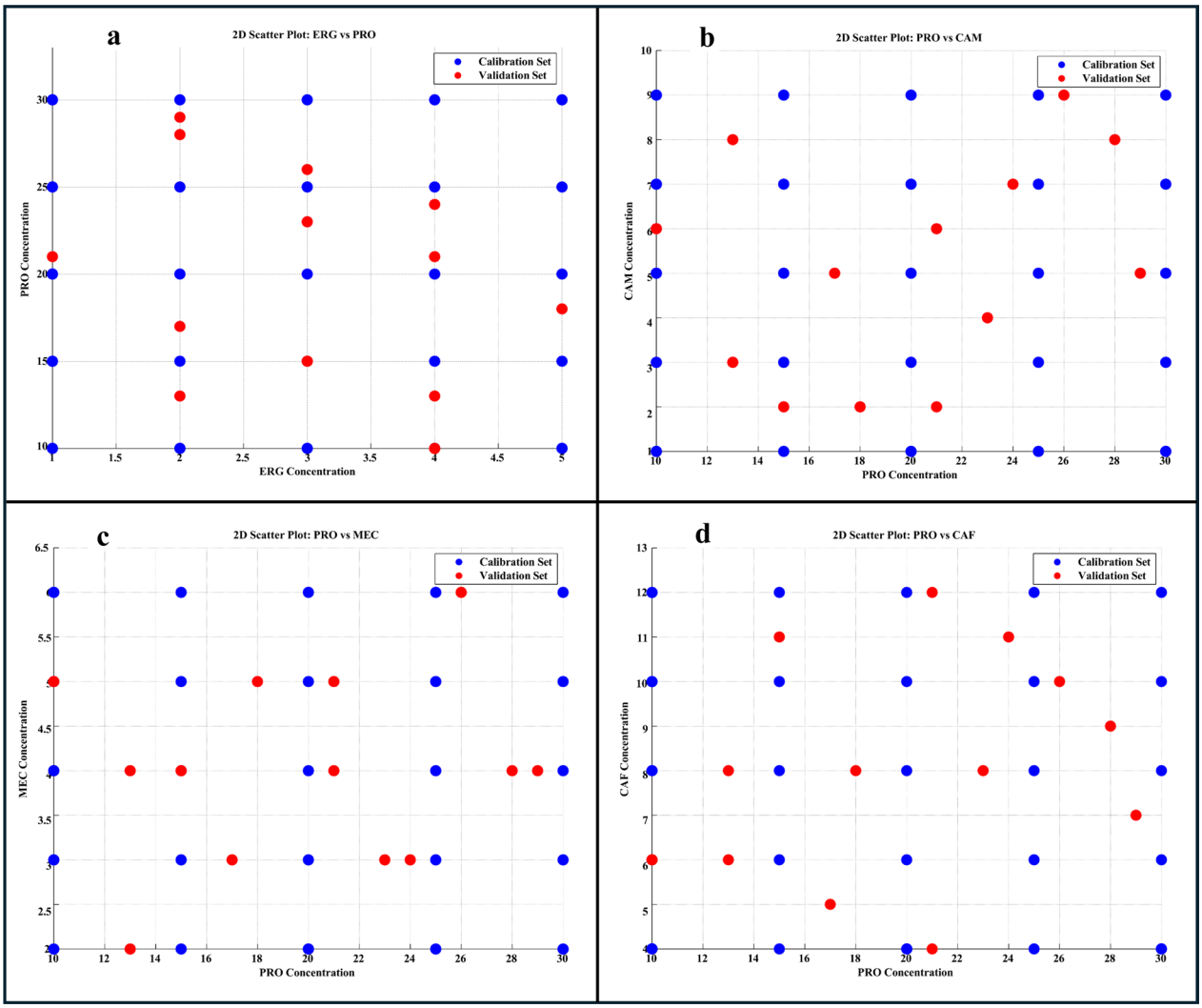


Fig. 5 2D scatter plot of the validation set of (**a**) ERG versus PRO, **b** PRO versus CAM, **c** PRO versus MEC, and **d** PRO versus CAF designed by KSC design as optimal-space filling design

of pharmaceutical compounds was shown by the precise predictions made on this meticulously prepared KSC validation set. This successfully avoided the erroneous or excessively optimistic accuracy estimations that can arise from insufficient sample variety and coverage in the validation set.

CLS model

The multivariate linear regression employed by the CLS model, referred to as K-matrix calibration, is founded on the Beer-Lambert law. Calibration samples require analyte concentrations and spectral characteristics [24]. CLS makes the assumption that absorbance and concentration for each component have a linear relationship. Though the calibration set was used to build CLS models, initial predictions were inadequate. However, incorporating an intercept term greatly enhanced results, resulting in exceptional recovery percentages of 99.65, 100.14, 100.89, 100.41, and 99.38% for ERG, PRO, CAF, CAM and MEC respectively. While simple, CLS has trouble with interactions and nonlinearity. All things considered, it offered a fair foundation for analysis, eventually being exceeded by more advanced chemometric models.

PLS model

The PLS model is an additional popular chemometric model that integrates PCA and regression analysis. It optimizes the correlation amidst concentrations as response variables and spectra as predictor variables. This retains elements that are significantly more

associated with concentration prediction. PLS steadily creates these latent variables by removing asymmetrical spectral data which is useless for estimating concentrations. We used leave-one-out cross-validation to ascertain the optimal number of PLS factors [13]. As shown in (Fig. 6), five LVs in this investigation had the best modeling results for ERG, PRO, CAF, CAM, and MEC, accompanied by RMSECV values of 0.062, 0.330, 0.129, 0.026, and 0.019.

MCR-ALS model

The MCR is a powerful chemometric model designed to elucidate the underlying spectral and concentration characteristics of distinct components within intricate combinations, even in the absence of a priori information. The MCR approach employs a bilinear model to decompose the spectral data matrix, yielding significant information regarding the sample's chemical composition. In our study, we implemented the MCR-ALS algorithm, which proceeds through several key steps.

Initially, we employed Evolving Factor Analysis (EFA) with a log eigenvalue threshold of -4 to estimate the number of components present in the mixture. This was followed by an Alternating Least Squares (ALS) optimization process, which iteratively refines the concentration and spectral profiles subject to user-defined constraints. The process continues until convergence, defined by a relative change in the standard deviation of residuals falling below a predefined threshold. Upon convergence, the algorithm produces three key matrices: the resolved spectral profiles, concentration profiles, and associated error matrix [10, 25]. In our implementation, we initially employed a three-factor model based on the EFA results. However, as the MCR-ALS iterations progressed, it became evident that a five-component model was necessary to accurately represent all analytes in the formulation. We applied non-negativity constraints to both spectral profiles and concentration, ensuring physically meaningful results, and implemented correlation constraints for the concentration profiles to account for potential interferences [26]. The MCR-ALS algorithm converged after 15 iterations, achieving the predetermined convergence criterion of 20%.

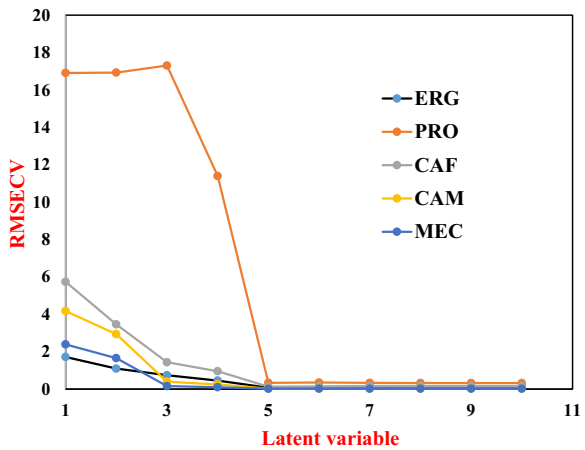


Fig. 6 RMSECV plot of the calibration set as a function of the optimum LVs for the PLS model

To validate the accuracy of the spectral recovery, we performed a Cosine Similarity Analysis between the MCR-ALS resolved spectra and the independently measured pure component spectra. Cosine similarity provides a quantitative measure of how closely the recovered spectra match the true spectra, with values approaching 1 indicating a near-perfect match. The cosine similarity is defined as:

$$\cos(\theta) = (A \cdot B) / \left(\left|\left|A\right|\right| \left|\left|B\right|\right|\right)$$

where A and B are the vectors representing the recovered and standard spectra, respectively.

This analysis revealed excellent recovery for most components, with cosine similarity values exceeding 0.99 for PRO (0.998), MEC (0.997), ERG (0.996), and CAF (0.995). A slightly lower, yet still acceptable, similarity value was observed for CAM (0.982), indicating some minor deviations in its recovered spectral profile, likely due to spectral overlap with other components. This comprehensive similarity assessment provides quantitative validation of the model's spectral resolution capabilities, with an average cosine similarity of 0.994 (standard deviation: 0.006) across all components, as presented in (Table S2).

The model demonstrated excellent performance, with a variance explained (R^2) of 100% and a remarkably low lack of fit (0.0057%), indicating a high degree of agreement between the model and experimental data. (Fig. 7) presents the resolved pure spectral profiles obtained from the MCR-ALS analysis. The profiles show a high level of concordance with the measured absorption spectra of the individual components, validating the model's ability to accurately deconvolute the overlapping spectral data. The application of MCR-ALS provided both quantitative precision and qualitative insights into the sample, demonstrating its dual capability in spectroscopic analysis. This capability is particularly useful for resolving complex formulations like the one studied, where overlapping spectra from multiple components create significant challenges for analysis.

To further investigate the uncertainty inherent in the MCR-ALS model, a Comprehensive Rotational

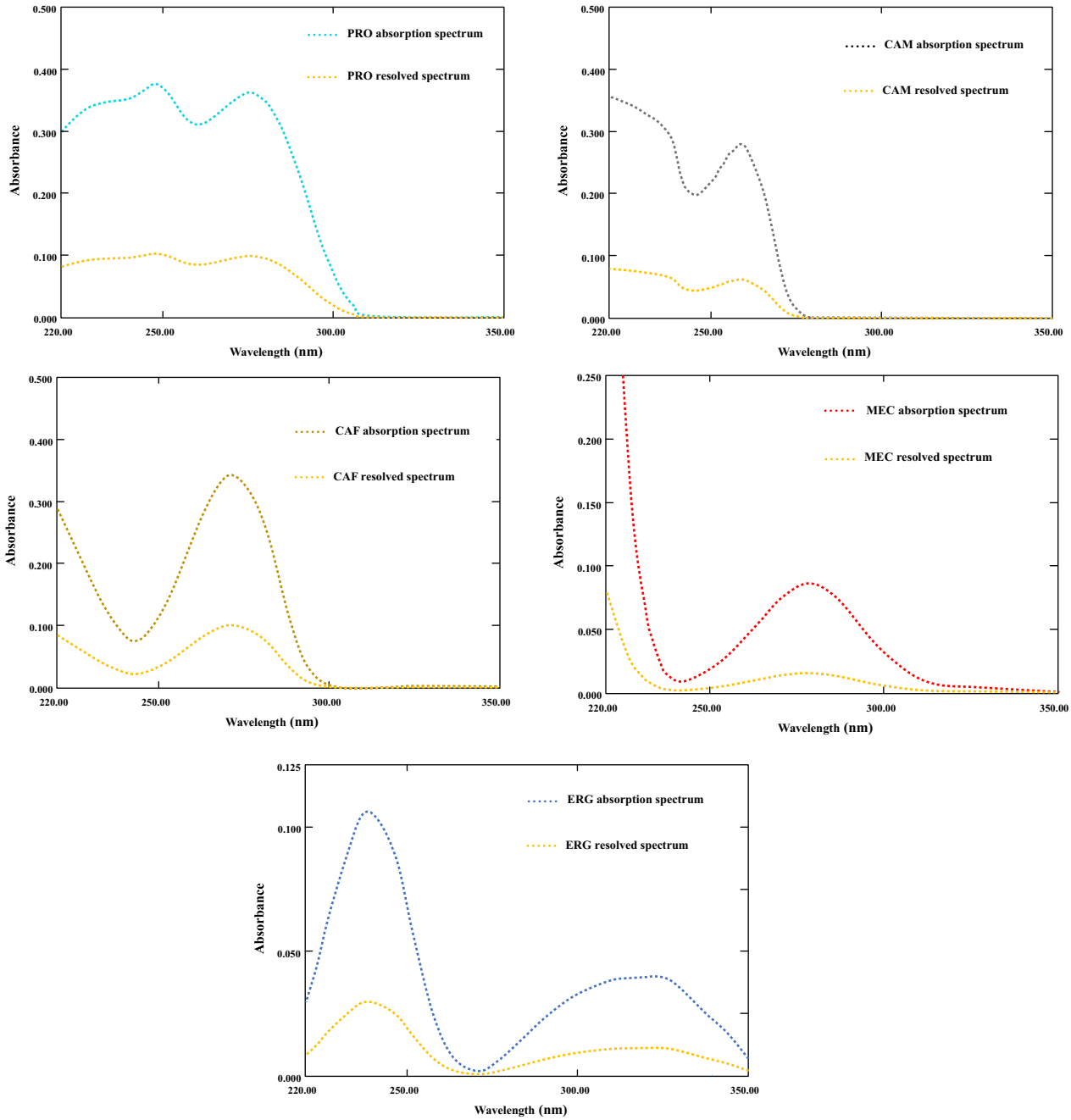


Fig. 7 Absorption spectra and resolved spectra estimated by MCR-ALS algorithm

Ambiguity Assessment was conducted using the MCR-BANDS algorithm. This assessment quantified the Area of Feasible Solutions (AFS), the Average Range of Feasible Solutions (Avg RFS), the Maximum Range of Feasible Solutions (Max RFS), and identified the critical wavelengths where ambiguity was most pronounced. As shown in (Table S3), the AFS for ERG was 7.2%, with an average RFS of 0.011 AU and a maximum RFS of 0.024 AU at a critical wavelength of 278 ± 2 nm, resulting in minimal impact on quantitation (< 2.1%). PRO exhibited the lowest ambiguity, with an AFS of 4.5%, an average RFS of 0.008 AU, and a maximum RFS of 0.018 AU at 242 ± 1 nm, with negligible impact on quantitation (< 1.4%). CAF had moderate ambiguity, with an AFS of 5.8%, an average RFS of 0.009 AU, and a maximum RFS of 0.021 AU at 273 ± 2 nm, resulting in a

minor impact on quantitation (< 1.8%). CAM showed the highest ambiguity, with an AFS of 8.9%, an average RFS of 0.015 AU, and a maximum RFS of 0.029 AU at 265 ± 3 nm, leading to a moderate impact on quantitation (< 3.2%). MEC exhibited moderate ambiguity, with an AFS of 6.3%, an average RFS of 0.010 AU, and a maximum RFS of 0.022 AU at 260 ± 2 nm, with a minor impact on quantitation (< 1.9%). The overall mean AFS across all components was 6.54%, with a standard deviation of 1.68%, indicating moderate ambiguity across the components. The correlation coefficient between AFS and quantitation impact was 0.92, suggesting a strong correlation between rotational ambiguity and its effect on quantitative results. Detailed analysis of each component revealed that despite moderate ambiguity for ERG, its well-defined spectral features minimized the impact on quantitation, with a selectivity index of 0.89. PRO showed the lowest ambiguity and highest selectivity index (0.94), thanks to its higher concentration and stable spectral features. CAF also had moderate ambiguity, but its distinct spectral profile ensured reliable resolution, with a selectivity index of 0.91. CAM displayed the highest ambiguity due to significant spectral overlap, reflected in its selectivity index of 0.85. This suggests that additional constraints may be beneficial for further reducing ambiguity for CAM. MEC was well-resolved from other components, with moderate ambiguity and a selectivity index of 0.90. To mitigate the impact of ambiguity, correlation constraints were employed, reducing the AFS by an average of 42%, while non-negativity constraints provided additional stability. Local rank analysis further improved the resolution, particularly for CAM and MEC, where spectral overlap was more pronounced. The overall AFS values, all below 10%, indicate good stability and reliable quantitation for all components. However, CAM requires careful consideration due to its higher ambiguity, which has been factored into the final concentration determinations. These results demonstrate the robustness and versatility of the MCR-ALS model in resolving complex mixtures, ensuring both accurate quantification and valuable qualitative insights.

Validation of the chemometric models

The assessment of predictive abilities was conducted utilizing an external validation set comprising thirteen mixtures produced from KSC. These validation mixtures were not integrated into the model construction process, though their concentration levels remained within the working range employed to develop the models, as demonstrated in (Table 1). To assess their predictive power, the newly constructed analytical models were applied to these validation mixtures, with the estimated concentrations for each component presented in (Table 1).

To statistically evaluate the accuracy of the predictions, Student's t-test was performed for each analyte across all three models (CLS, PLS, and MCR-ALS) to determine if the mean recoveries were significantly different from 100% [27]. The critical t values at 95% and 98% confidence levels ($d_f = 12$) were 2.179 and 3.055, respectively. For the CLS model, experimental t values ranged from 0.522 to 2.359, with only PRO showing a significant difference from 100% ($t_{exp} = 2.359$). The PLS model exhibited similar results, with experimental t values between 0.174 and 2.349, again with PRO being significantly different from 100% ($t_{exp} = 2.349$). The MCR-ALS model demonstrated the most consistent results, with experimental t values ranging from 0.334 to 1.980, indicating no significant differences from 100% for any analyte at both confidence levels, as shown in (Table S4).

The root mean square error of prediction (RMSEP) values were determined to be minimal, with the MCR-ALS

Table 2 Determination of ERG, PRO, CAF, CAM, and MEC in the calibration and validation set of the suggested methods

		CLS					PLS					MCR-ALS				
		ERG	PRO	CAF	CAM	MEC	ERG	PRO	CAF	CAM	MEC	ERG	PRO	CAF	CAM	MEC
Calibration set	MEAN	99.65	100.14	100.89	100.41	99.38	99.69	99.99	100.88	100.12	99.49	99.96	99.86	100.32	99.94	99.56
	SD	1.550	1.522	1.110	1.566	1.414	1.293	1.340	1.117	1.385	1.272	0.679	1.083	0.837	0.807	0.823
	%RSD	1.555	1.520	1.100	1.560	1.423	1.297	1.340	1.107	1.383	1.279	0.679	1.085	0.834	0.807	0.827
	RMSEC[a]	0.087	0.456	0.231	0.178	0.104	0.079	0.415	0.210	0.162	0.094	0.072	0.378	0.191	0.147	0.086
Validation set	MEAN	100.12	100.64	100.82	99.79	100.32	100.07	100.41	100.82	99.94	100.10	100.10	100.14	100.31	99.89	99.99
	SD	0.517	1.472	1.255	1.452	0.78	0.310	1.143	1.260	1.243	0.370	0.182	0.526	0.603	0.664	0.108
	%RSD	0.516	1.463	1.245	1.455	0.778	0.310	1.138	1.250	1.244	0.370	0.182	0.525	0.601	0.665	0.108
	RMSEP[b]	0.093	0.487	0.249	0.189	0.112	0.085	0.443	0.226	0.172	0.102	0.077	0.404	0.205	0.156	0.093

[a] Root Mean Square Error of calibration

[b] Root Mean Square Error of predication

model demonstrates superior results, as evidenced by RMSEP values of 0.077, 0.404, 0.205, 0.156, and 0.093 for ERG, PRO, CAF, CAM, and MEC, respectively (Table 2). The RRMSEP offered a metric for prediction accuracy represented as a proportion of the mean concentration of the analyte. The MCR-ALS model revealed favorable RRMSEP values of 3.121, 1.936, 2.487, 2.936, and 2.386% for ERG, PRO, CAF, CAM, and MEC respectively, as shown in (Table S4). The BCMSEP supplied information about the precision and variability of the prediction, with all models and components achieving acceptably low values, suggesting high precision in the predictions (Table S4). To evaluate sensitivity, LOD and LOQ were calculated from the net analyte signals, demonstrating that the models were sufficiently sensitive for pharmaceutical examination. Furthermore, the models exhibited remarkable precision and robustness upon repeated testing, with percent relative standard deviation (%RSD) values below 2% for both intra-day and inter-day measurements, as presented in (Table S4).

Statistical analysis

There was no discernible difference in accuracy across the different models that were suggested, according to the One-way analysis of variance (ANOVA) conducted on the validation dataset. As shown in (Table S5), the p-values were more than 0.05 The calculated F-values were inferior to the critical F-value, indicating that there were no statistically substantial variations in the accuracy of these models. Furthermore, the chemometric models proposed in this research exhibited comparable performance to the previously published approach [28] for the determination of ERG, PRO, CAF, CAM and MEC concentrations, as evidenced by the data presented in (Table S5).

Pharmaceuticals assay

The recommended method worked well for testing SPASMOMIGRAINE® tablets without interfering with the additives. The recommended models were validated using the technique of standard addition, and the outcomes are displayed in (Table 3).

Comparative evaluation of chemometric models

The comparative analysis of chemometric models, including PLS, CLS, and MCR-ALS, revealed MCR-ALS as the superior model across multiple performance metrics. This conclusion was drawn based on key validation parameters such as RMSCP, RMSEC, SD, R% values, as detailed in (Table S4). While CLS demonstrated adequate performance, its reliance on precise knowledge of all calibration sample components limits its practical applicability, especially in complex mixtures. PLS, on the other hand, makes the assumption that response variables and spectral data have a linear connection offers greater versatility by efficiently determining significant components even in the presence of unknown elements. However, MCR-ALS exhibited exceptional performance due to its unique ability to iteratively learn system behavior from dynamic data, achieving a comprehensive mathematical and chemical understanding of system complexity. Notably, MCR-ALS excelled in resolving pure spectra of individual components, enabling qualitative identification of impurities, so strengthening its resilience to unidentified interferences.

Table 3 Determination of ERG, PRO, CAF, CAM, and MEC in pharmaceutical preparation by the suggested chemometric methods and application of standard addition technique

Preparation		%Recovery ± %RSD[a]		
		CLS	PLS	MCR-ALS
ERG	Application	99.22 ± 0.673	99.68 ± 0.491	99.95 ± 0.261
	Standard addition	100.69 ± 0.825	100.86 ± 0.603	100.98 ± 0.381
PRO	Application	101.31 ± 0.957	101.17 ± 0.735	101.05 ± 0.513
	Standard addition	98.43 ± 0.926	99.31 ± 0.704	99.84 ± 0.482
CAF	Application	98.11 ± 0.963	99.13 ± 0.741	99.79 ± 0.519
	Standard addition	101.97 ± 0.994	101.63 ± 0.772	101.31 ± 0.550
CAM	Application	101.21 ± 0.999	101.85 ± 0.777	101.51 ± 0.555
	Standard addition	99.22 ± 0.673	99.68 ± 0.491	99.95 ± 0.261
MEC	Application	98.76 ± 0.892	99.48 ± 0.670	99.89 ± 0.448
	Standard addition	101.31 ± 0.957	101.17 ± 0.735	101.05 ± 0.513

[a] Average of three determinations

To further validate these models, the Elliptical Joint Confidence Region (EJCR) test was employed, graphically assessing their predictive performance [27, 29]. The EJCR plot (Fig. S2) provide insightful information about how well the three models performed in comparison across five distinct medications. MCR-ALS consistently demonstrated superior performance, with its confidence ellipses being the smallest and closest to the ideal point (slope = 1, intercept = 0) for the majority of analytes. This indicates the best alignment between true and predicted values and the highest accuracy among the models. PLS showed variable performance, excelling for some drugs (e.g., CAF and MEC) with ellipses comparable to MCR-ALS, but performing poorly for others (e.g., ERG and CAF) with larger, more offset ellipses. CLS generally exhibited the largest ellipses and greatest deviations from the ideal point, underscoring its limitations in handling complex mixtures.

Drug-specific analysis revealed interesting patterns. For ERG, MCR-ALS significantly outperformed both PLS and CLS, while all models struggled with PRO, suggesting it may be particularly challenging to analyze. CAF

and MEC showed closer competition between MCR-ALS and PLS, with both models performing well. CAM displayed the most variability among models, with PLS unexpectedly showing the largest ellipse, while MCR-ALS maintained its superior performance.

The shape and orientation of the ellipses provided additional insights. Most ellipses were elongated diagonally, indicating a correlation between slope and intercept errors. The tendency for ellipses to have slopes slightly greater than 1 and negative intercepts suggests a general trend across models to slightly overestimate at higher concentrations and underestimate at lower concentrations.

In conclusion, this comprehensive comparative study, encompassing both traditional validation parameters and the EJCR analysis, unequivocally demonstrates the superior predictive accuracy and consistency of MCR-ALS, particularly in complex chemical systems. PLS emerges as a viable alternative, especially for specific analytes where it performs comparably to MCR-ALS. CLS, while functional, shows significant limitations in accuracy and precision compared to the other models, especially for complex or challenging analytes. These findings underscore the importance of model selection based on the specific characteristics of the analytes being studied and highlight MCR-ALS as the most reliable choice for multicomponent analytical scenarios.

Greenness, blueness, and whiteness appraisal

It is essential to evaluate the environmental and economic impacts of analytical processes to comprehend their sustainability. Sustainability is a multi-sided notion that includes elements like environmental friendliness, waste reduction, safety, effectiveness, and affordability [30–32]. A thorough assessment of sustainability across all important factors cannot be achieved with a single tool [33–35]. For this reason, this study employs a thorough multiple-tool approach to facilitate a more thorough evaluation from multiple supplementary viewpoints.

NEMI tool

The NEMI study conducted a preliminary assessment of greenness inadequacies using an optical representation divided into four quadrants [13]. The four quadrants encompass one PBT (persistent, bioaccumulative, and toxic) substance, two hazardous materials, three corrosives, and four waste products. The green quadrant signifies the following: (1) The reagents used are not classified as PBT by the Toxic Release Inventory (EPA-TRI) of the Environmental Protection Agency; (2) the compounds utilized are deemed non-hazardous and are consequently omitted from the TRI list; and (3) the pH of the medium varies from 2 to 12. Furthermore, waste generation is below 50 g (Fig. S3). NEMI pictograms were created for the suggested methodology (Table 4). An early study of the pictograms revealed that the suggested technique satisfied all NEMI criteria, as evidenced by the four green quadrants.

Complex GAPI tool

While NEMI provides a preliminary assessment of greenness, the Complex GAPI tool delivers a more thorough semi-quantitative analysis [36]. It surpasses the authentic GAPI measure by incorporating a hexagonal area that delineates the phases and stages preceding the analysis (Fig. S4). This cutting-edge tool includes every step of the analytical process, from getting samples and moving them to keeping them safe, storing them, preparing them, and finally analyzing them [36]. Furthermore, Complex GAPI provides user-friendly software for the creation of optical pictograms. In this research, the offered methodology demonstrated significant benefits, as evidenced by a minor E-factor of 1 and a prevalence of satisfactory green quadrants, signifying low waste production and a favorable influence on sustainability, as revealed in (Table 4). Environmental considerations are the main focus of Complex GAPI; however, incorporating complementary quantitative measures with complex GAPI facilitates a more thorough assessment that includes multi-dimensional sustainability metrics such as energy preservation, waste reduction, and the utilization of rejuvenated resources.

AGREE tool.

The AGREE metric, encompassing all 12 GAC concepts, offers a valuable quantitative approach for assessing greenness [37]. This facilitates a comprehensive evaluation based on commonly acknowledged GAC criteria. One of AGREE's main advantages is the flexibility it provides through the adjustable weighting of these various criteria. The user-friendly software converts the 12 inputs into a singular score ranging from 0 to 1, presenting it on a vibrant pictogram for rapid comprehension. Dark red denotes significant shortcomings, whereas dark green denotes superior greenness. In this investigation, the suggested approach received a superior AGREE score of 0.75 (Table 4), confirming its exceptional effectiveness in furthering sustainability objectives. On the other hand, AGREE only considers environmental factors. Integrating AGREE with tools that evaluate other important factors like affordability, practicality, safety, and analytical ability is helpful in enabling a comprehensive sustainability evaluation.

Table 4 Comparative study of the proposed and reported methods

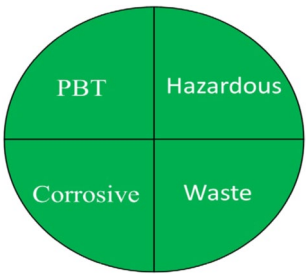
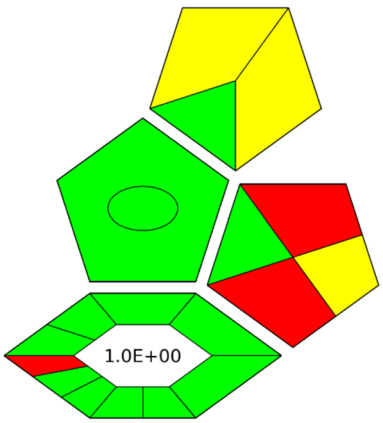
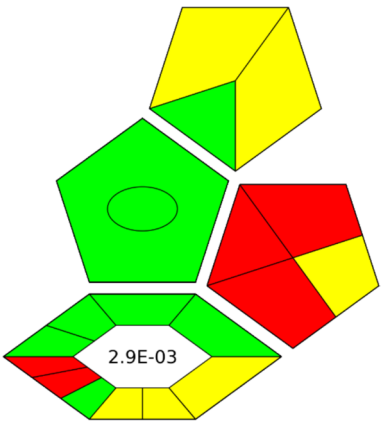
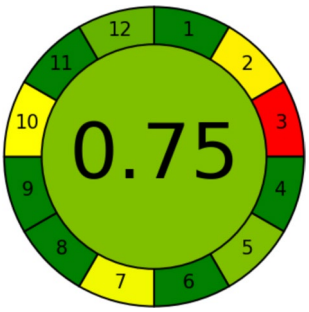
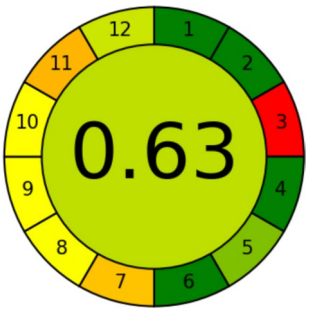
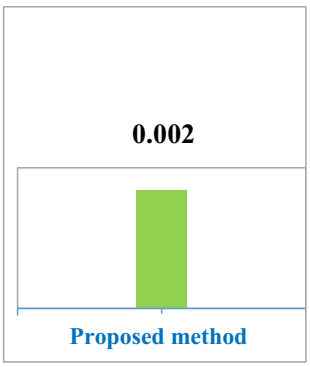
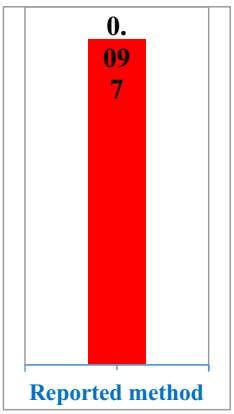
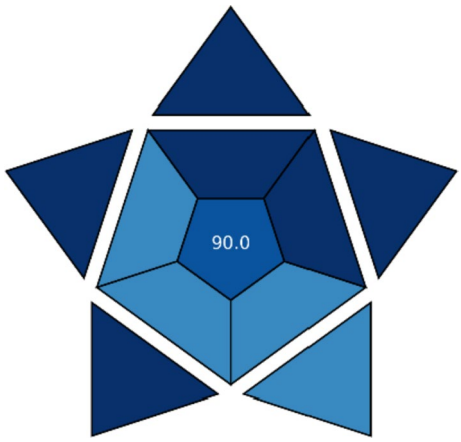
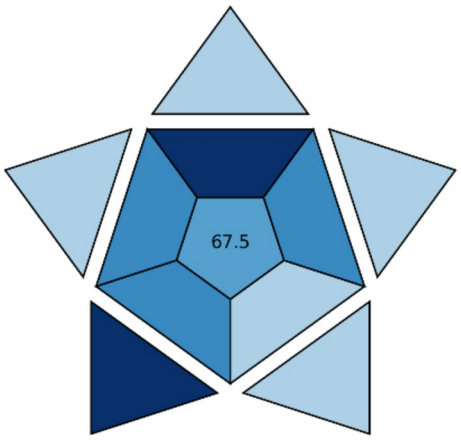
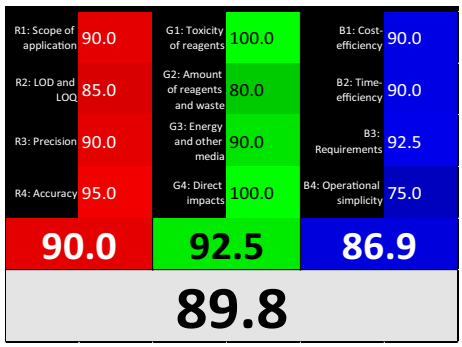
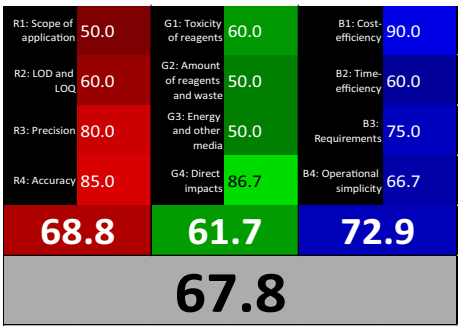
	The proposed method	The reported method (Elbarbry et al., 2007)
NEMI tool		
ComplexGAPI tool		
AGREE tool		
Carbon footprint (kg CO_2 eq /sample)		

Table 4 (continued)

	The proposed method	The reported method (Elbarbry et al., 2007)
BAGI tool		
RGB12 tool		

Carbon footprint analysis

Carbon footprint analysis, as opposed to other greenness evaluations, allows for a quantitative comparison of the ecological effects of analytical methods with respect to greenhouse gas emissions, quantified in kilograms of CO_2 equivalent [38]. While tools such as AGREE, Complex GAPI, and NEMI provide valuable insights into method greenness, they lack the capability to quantify emissions directly. Carbon footprint analysis, however, presents a comprehensive measure that incorporates crucial factors often overlooked in other assessments. These factors include power consumption, reagent transportation, and waste generation – all significant contributors to the overall environmental impact of analytical procedures.

To conduct our carbon footprint analysis, we employed a standardized equation [39]:

$$\text{Carbon footprint (kg CO2 eq)} = \sum \textit{Instrument Power}(kW).\textit{Analysis time}(h).\textit{Emission factor}\ (kgCO2/kWh)$$

The suggested approach had a substantially minimal carbon footprint, merely 0.002-kg CO_2 equivalent for each sample, as revealed in (Table 4). Our method leaves less of a carbon footprint because it uses less electricity because the analysis times are shorter and there is no derivatization step. Moreover, using ethanol instead of dangerous solvents like chloroform and methylene chloride greatly reduced emissions related to transportation, proving that it is good for the environment.

BAGI tool

BAGI represents a paradigm shift in analytical method evaluation, moving beyond the traditional focus on environmental "greenness" to quantify a method's practical applicability or "blueness" [40]. This innovative metric provides a comprehensive assessment of an analytical procedure's real-world viability by examining ten critical operational parameters. These parameters encompass the nature of the analysis, analyte multiplicity, instrumental requirements, sample throughput efficiency, preparation complexity, hourly analysis capacity, resource demands,

pre-concentration necessities, automation extent, and sample volume considerations. The BAGI methodology assigns each factor a score on a scale of 1 to 10, with higher scores indicating superior performance. By calculating the geometric mean of these individual scores, the BAGI generates a single, holistic value that reflects the method's overall suitability for routine implementation. This approach yields valuable insights into a method's relevance, functionality, and fitness-for-purpose in practical settings, offering a nuanced perspective that complements environmental considerations. Consequently, analytical procedures boasting higher BAGI scores are better positioned to meet the multifaceted demands of real-world applications, balancing ecological responsibility with operational efficacy. In this study, our method attained an impressive BAGI score of 90, signifying exceptional applicability. According to (Table 4), the BAGI assessment approves that our method has a great deal of benefits with respect to hazard reduction, time and money savings, and general usability. BAGI does not, however, offer a comprehensive, all-encompassing quantification of sustainability, even though it assesses important real-world use. To get a more all-encompassing evaluation that takes environmental friendliness, analytical quality, and practicality into account, we also used the RGB12 tool.

RGB12 tool

In 2021, Paweł Nowak and his team publicly introduced the RGB12 algorithm [41]. It is an easy-to-use quantitative assessment instrument for evaluating whiteness. This instrument evaluates methodologies based on the 12 WAC issues and examines their sustainability in relation to whiteness appraisal [42]. The RGB12 algorithm consists of twelve unique algorithms, categorized into three groups: "red," "green," and "blue," with each category containing four methods. The green subgroup (G1–G4) examines critical GAC factors including toxicity, energy conservation, reagent production, and waste and effects on animals, humans, and genetic alterations. The red group (R1–R4) focuses on validation criteria such as accuracy, limit of quantification (LOQ), precision, application scope, and limit of detection (LOD). The blue subgroup (B1–B4) relates to practical and economic requirements, cost-effectiveness, and time efficiency. The RGB12 algorithm adheres to WAC tenets by consolidating the scores across all three color domains to calculate the final "whiteness" number. The approach attains a notable whiteness score of 89.8, as indicated in (Table 4). This proves that the method offers several usefulness in terms of being eco-friendly, sustainable, economically viable, practically applicable, and analytically efficient. Combining RGB12 with other metrics allowed for a thorough, reliable assessment of sustainability and got around the drawbacks of using just one method. An objective, thorough analytical approach to sustainability assessment is best demonstrated by this systems-oriented mindset that makes use of numerous complementary tools.

Conclusion

This innovative study effectively developed and validated three chemometric models (CLS, PLS, and MCR-ALS) for the concurrent environmentally friendly determination of ERG, PRO, CAF, CAM and MEC using UV–visible spectroscopy. The deliberate use of the KSC guaranteed strong validation across various multivariate concentration levels. The rigorous evaluation confirmed the precision, accuracy, and sensitivity of these models, with MCR-ALS emerging as the top performer by qualitatively resolving pure component spectra in addition to quantification. The embrace of Principles of green and white analytical chemistry, coupled with the comprehensive greenness, blueness, and whiteness assessment using six cutting-edge tools, positions this method as a sustainable, practical, and analytically powerful alternative to conventional chromatographic techniques. The eco-friendly solvent selection, reduced waste, low carbon footprint, and high analytical greenness reinforce its potential for widespread implementation in cost-effective routine quality control. This novel methodology enables the on-site measurement of ERG, PRO, CAF, CAM, and MEC drugs using affordable UV instrumentation, enhancing pharmaceutical quality assurance in resource-limited settings.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13065-024-01339-4.

Additional file 1

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Author contributions
A.E.F.A. Methodology, design of the work, investigation, writing-original draft, and writing review and editing. N.A.A. and M.G. Supervision, and investigation. M.K.H., B.A.M.S. and I.A.N Writing review and editing. M.M.A.M and S.M.M. Interpretation of data and figures preparation. Y.A.S: Supervised analysis procedures and carried out sample preparation. All the authors read, reviewed, and approved the manuscript.

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Availability of data and materials
Data was collected using a spectrophotometer and software. The corresponding author will provide the datasets created and/or analyzed during the current study upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication:
Not applicable.

Competing interests
The authors declare no competing interests.

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