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Green and White Analytical Chemistry in Spectrophotometric Pharmaceutical Analysis: Assessment Tools, Chemometrics, and Recent Developments: A Review

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Abstract

Shifting sustainable practices in consumer demand parallel the introduction of eco-friendly and performance-based practices in analytical chemistry with White (WAC) and Green Analytical Chemistry (GAC). The purpose of this paper is to detail the advancements in pharmaceuticals quality control using UV-Vis spectrophotometry from GAC to WAC. The metrics of NEMI, Analytical Eco-Scale, GAPI/MoGAPI, AGREE, RGB/RGB12, and BAGI will be reviewed regarding their concepts, advantages, and disadvantages. PLS, PCR, MCR-ALS, and derivative spectrophotometry will be discussed as chemometric methods for multicomponent pharmaceutical analysis, as well as recent advancements in chemometric processes incorporating AI and ML. A review of the literature from 2019 to 2025 in Scopus, Web of Science, and PubMed yielded 777 publications, of which 37 met all criteria for inclusion. PLS-based chemometric models were the most prominent (~42%), followed by PCR (~21%), AI-based techniques (SVR, ANN, MCR-ALS) (~15%), indicative of promising growth. The mean RGB12 whiteness score was 84/100, with 52% of studies scored between 80-90. AGREE scores of >0.70 were consistently met with the use of aqueous and/or low-toxicity solvent methods. It is clear from the review that GAC/WAC metrics related to chemometric advances can provide robust, cost-effective, and reliable eco-analytical methods for routine pharmaceutical quality control.

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1. Introduction

Concerns about pollution and chemicals in our environment have impacted the field of analytical chemistry over the last 30 years. Traditional methods of analysis in pharmaceutical chemistry often take advantage of the use of large quantities of toxic organic solvents, and generate substantial chemical waste, which poses health risks to laboratory staff. In response, Green Analytical Chemistry (GAC) was proposed in 1999 and offers a framework to devise analytical procedures that provide analytical performance while reducing environmental and health concerns. ^[1-3]

The twelve principles of GAC, introduced by Gałuszka *et al.* in 2013, are the basis of the development of greener analytical procedures. This set of principles, represented by the mnemonic SIGNIFICANCE, incorporates waste reduction, reduction of reagent toxicity, energy saving, simplification of sample preparation, and safety of the analyst. They have been quickly adopted in the pharmaceutical analysis as the standards for method development in sustainability. ^[4]

Due to its simplicity, low cost, low energy consumption, and low solvent consumption, UV-Vis spectrophotometry is one of the most commonly used techniques in pharmaceutical quality control. It is inherently compatible with the principles of GAC, making it appropriate for application in green pharmaceutical analysis. Coupled with chemometric approaches,

spectrophotometric methods can overcome the need for chromatographic separation by effectively determining multiple components of complex pharmaceutical formulations.^[5, 6]

While GAC aims at environmental sustainability only, a more comprehensive evaluative framework, White Analytical Chemistry (WAC), aims at a single system incorporating analytical performance (red), eco-friendliness (green), and operational feasibility (blue), which is often represented through the RGB color model.

This evolution signifies a fundamental shift to a more holistic approach to analytical method development where sustainability, practicality, and scientific rigor are in equilibrium.^[7]

This review seeks to: (1) describe the conceptual development from GAC to WAC; (2) critique the existing tools for assessing greenness and whiteness; (3) provide an insight into the significance of chemometrics in the spectrophotometric analysis of pharmaceuticals; and (4) provide an overview of recent developments and the prospects for the future in this field.

2. Green Analytical Chemistry: Foundations and Principles

2.1. Historical Background

Anastas first articulated the concept of Green Chemistry in 1990 as the design of chemical processes aimed at minimizing or completely removing dangerous substances. The idea of the Green Analytical Chemistry (GAC) branch was introduced in 1999. Its purpose is to minimize the environmental impact of analytical methods. The 12 GAC principles were introduced in 2013 as the first detailed principles framework for any analytical domain.^[1,3,4]

Direct analysis of samples without any preparation, miniaturization of the methods and procedures, automation, complete elimination of solvents (or replacement of solvents with green solvents), waste reduction, in-field analysis, avoidance of derivatization, and use of reagents from renewable sources are the principles of GAC. GAC principles have been adapted in the field of pharmaceutical analysis, and other eco-friendly spectrophotometric methods have been developed to preserve or improve the performance of existing valid methods.^[4, 5]

2.2. Spectrophotometry as a Green Analytical Platform

UV-Vis spectrophotometry has unique advantages which give it a good fit within the GAC principles. Its moderate energy needs (usually less than 0.1 kWh/sample), low solvent needs, and minimal waste with little to no derivatization all make it a good choice for the analysis of pharmaceuticals while being mindful of green chemistry practices, especially in places with limited resources.^[5, 8]

Derivative spectrophotometry, as well as dual-wavelength and ratio spectra methods, expand the versatility of the technique. These methods allow for the analysis of drug mixtures – both binary and ternary – without the need for separation. As an example, the first and second derivative methods have been used to analyze mixtures of cefixime and cephalexin as well as furosemide and carbamazepine,

diazepam, and carvedilol in various pharmaceutical formulations.^[9]

3. Greenness Assessment Tools

3.1. NEMI

The National Environmental Method Index (NEMI) began in 2002 and was recognized as an early GAC metric. Its signature graphic is a circle divided into four quadrants. Each quadrant is colored green if certain environmental criteria are satisfied. These are: the absence of PBTs; absence of hazardous waste solvents; a pH of the sample that is between 2 and 12; and waste less than 50 g.^[10]

The simplicity of NEMI is what sells it, and a quick look at the pictogram offers an immediate overview of that. While great for a quick look, this is somewhat limited, as it does not provide a quantitative assessment, in which case the software does not help as one would still need to manually search compound names in the EPA-TRI. Therefore, it is more appropriate for a qualitative metric, where it is often used to supplement or complement more quantitative metrics.^[11,12]

3.2. Analytical Eco-Scale

The Analytical Eco-Scale is a system developed by Gałuszka *et al.* in 2012 based on a negative point system that starts from the perfect score of 100 and subtracts points based on how a method deviates from the green criteria. Some of the elements taken into consideration are classified based on their chemical hazard, the amount of the dispersing agent, energy consumption, occupational dangers, and the waste generated and how it is disposed of. Points are deducted based on the amount of the dispersing agent present (amounts less than 10 ml lead to a single point penalty, 10 to 100 ml lead to a 2 point penalty, and over 100 ml lead to a 3 point penalty). An occupational danger is based on the release of (potentially) harmful vapors, and the generated waste.^[12]

Methods that score above 75 are considered to be excellent green methods, those that score between 50 and 75 are considered to be acceptable green methods, and those methods that score below 50 are considered to be non-green. The Eco-Scale is a tool that offers a semi-quantitative evaluation of a method, and offers methods with clear evaluation criteria and easy calculation. Nevertheless, this system has a substantial shortcoming in that it is unable to reveal steps and reagents that are the sources of method non-optimization. Recently, the Eco-Scale has been used alongside GAPI and AGREE

3.3. GAPI and MoGAPI

The Green Analytical Procedure Index (GAPI) describes the environmental risk of the entire analytical process in five steps or stages: sampling, method, sample prep, reagents/solvents, and instruments. Each step of the five pentagrams is assessed on low (green), moderate (yellow), and high (red) impact levels. This framework was introduced in 2018 by Płotka-Wasyłka.^[15]

The Modified Green Analytical Procedure Index (MoGAPI) framework was designed to meet the analytical process assessment needs in 2024. This was made possible through

an overall score derived from 15 components of the complete analytical process. As such, it allows comparison between methods. MoGAPI also has user-friendly online software that creates the assessment pictogram, therefore, saving time. Both systems provide an assessment of the analytical process with a pictogram that assists in the identification of the most critical components of the entire analytical process. However, the categories for reagents and waste are not defined, resulting in vague assessments. [12,16]

3.4. AGREE

Developed in 2020 by Pena-Pereira *et al.*, the Analytical GREENess (AGREE) metric is currently the most widely used and comprehensive GAC tool. AGREE maps all twelve principles of GAC onto a circular pictogram divided into twelve sectors, each individually scored on a 0–1 scale; the final score is the weighted average of all twelve sectors. The color of each sector transitions from dark green (score = 1.0) to red (score = 0), providing simultaneous qualitative and quantitative information. [17]

An AGREE score ≥ 0.75 is generally considered indicative of excellent greenness; scores around 0.60–0.75 suggest acceptable greenness. The metric is operationalized through a user-friendly, freely available software that automatically generates the pictogram upon data entry. Its major advantage over earlier tools is the inclusion of all twelve GAC principles, including sample throughput, derivatization, and renewable reagent sourcing, which are omitted in simpler metrics. In 2022, AGREEprep was introduced as a complementary tool specifically designed to assess the greenness of sample preparation procedures based on ten principles of Green Sample Preparation (GSP). [11,17,18]

4. White Analytical Chemistry: The Expanded Framework

4.1. Concept and Principles

White Analytical Chemistry (WAC) represents an evolution beyond environmental-only metrics by integrating three equally weighted dimensions of analytical method quality. These dimensions are represented by the RGB color model: [7]

- **Red (R):** Analytical performance — including accuracy, precision, selectivity, sensitivity, and limits of detection/quantification
- **Green (G):** Eco-friendliness and safety — including solvent toxicity, waste generation, and environmental impact
- **Blue (B):** Productivity and practical effectiveness — including throughput, cost, simplicity, and automation potential [19][7]

Ten guiding principles of WAC have been proposed to standardize the development and evaluation of analytical tools within this framework. These principles address the need for consistent, standardized guidelines for metric development, replacing ad hoc approaches that have led to inconsistencies in the current landscape of assessment tools [20].

4.2. RGB and RGB12 Algorithm

The RGB additive color model, the first operational WAC metric, quantifies method "whiteness" through a composite Color Score (CS) ranging from 0% to 100%. A CS of 66.6%–100% is considered satisfactory, 33.3%–66.6% is tolerable, and below 33.3% is unsatisfactory. The RGB12 algorithm, introduced in 2021 as an updated edition, applies 12 explicit criteria across the red, green, and blue dimensions and is executed using an Excel-based spreadsheet, making it widely accessible [19].

The RGB12 model has been incorporated into pharmaceutical spectrophotometric analyses, providing a more realistic, multi-dimensional evaluation over the single-dimensional green metrics. By integrating performance analytical data with ambient and operational data, RGB12 offers researchers opportunity to determine eco-friendly, analytically sound, and operationally feasible methods for everyday applications in laboratory environments. [21]

4.3. BAGI

The Blue Applicability Grade Index (BAGI), introduced by Manousi *et al.* in 2023, evaluates method practicality within the WAC framework by examining ten factors that include analysis type, analysis cost, the number of analytes run, automation, throughput, and laboratory equipment. BAGI generates a score between 25 and 100. A higher score indicates a higher level of practicality. [22]

The BAGI is a free online tool that can be accessed at the site <https://bagi-index.anvil.app>. It is proposed as an additional tool in combination with the AGREE, MoGAPI, and ComplexGAPI tools to evaluate method practicality. When combined with AGREE and RGB12, BAGI provides full coverage of all three WAC dimensions. It is important to mention that, at this time, BAGI does not evaluate the safety, health, and environmental (SHE) of the reagents, which is a potential area for future development. [11,12,22]

5. Chemometrics in Spectrophotometric Pharmaceutical Analysis

5.1. Overview and Significance

Chemometrics involves using mathematical and statistical methods to analyze relationships in complex chemical data. In a spectrophotometric analysis of pharmaceuticals, chemometrics is useful for addressing overlapping absorption spectra of pharmaceutical products. Therefore, chemometrics methods allow for the concurrent quantification of agents without physical separation. This method primarily addresses GAC and WAC because it reduces the number of sample preparation steps, reduces the use of derivatizing agents and decreases the overall waste produced. [6, 23]

The most common chemometric methods in spectrophotometric UV-Vis pharmaceutical analyses include: artificial neural networks (ANN), multivariate curve resolution–alternating least squares (MCR-ALS), partial least squares (PLS-1 and PLS-2), principal component regression (PCR), inverse least squares (ILS), and classical least squares (CLS). The different methods all vary in their data requirements and their appropriateness for a given analysis. [6, 23]

5.2. Derivative Spectrophotometry

Using the first, second, third, or fourth derivatives of absorbance spectra, derivative spectrophotometry alters the original spectra to enhance the differences between the spectra of the analytes, allowing for the resolving of overlapping spectra. Some of the methods that are employed at chosen derivative wavelengths for accurate component quantification in mixtures include zero-crossing, peak-to-baseline, and peak-to-peak amplitude analyses. For instance, the first and second derivative spectra have been used to simultaneously analyze cefixime and cephalixin, while the fourth-order derivative spectra have separated a quaternary mixture of furosemide, carbamazepine, diazepam, and carvedilol, among others.

The advantages of derivative spectrophotometry include its simplicity, low cost, rapid execution, and freedom from complex data preprocessing requirements. It is particularly suited for binary and ternary pharmaceutical mixtures where the spectral differences are moderate and can be enhanced through differentiation. Integration with green assessment tools has confirmed that derivative spectrophotometric methods consistently achieve high Eco-Scale scores and favorable AGREE values^[9, 24].

5.3. Partial Least Squares (PLS)

Partial Least Squares (PLS) regression is the most widely used multivariate chemometric technique in spectrophotometric pharmaceutical analysis. PLS constructs latent variables (components) that maximize the covariance between spectral data (X matrix) and concentration data (Y matrix), effectively handling strongly overlapping spectra and collinear data that would challenge univariate methods. Both PLS-1 (single response variable) and PLS-2 (multiple response variables) have been successfully applied to the simultaneous determination of multi-component drug formulations^[6, 23, 25].

A recent study showed how PLS can be used to identify metronidazole (MNZ) and diloxanide furoate (DLF) and how, with the aid of chemometrics, UV spectrophotometry can avoid chemical separations. The method is rapid and, being environmental, is considered to be excellent in terms of accuracy and precision. Similarly, the use of PLS for the parallel determination of montelukast sodium, rupatadine fumarate, and desloratadine in pharmaceuticals is a low-cost alternative to HPLC.

5.4. Principal Component Regression (PCR)

Principal Component Regression (PCR) performs principal component analysis (PCA) on the spectral data matrix to reduce its dimensionality into orthogonal principal components, which are then used as predictors in a regression model. During decomposition, PCR doesn't utilize concentration data, potentially harming its predictive capacity. Nevertheless, PCR is very helpful when the main objective is the extraction of spectral noise and the management of collinearity before regression.^[6, 23]

In the comparative studies of pharmaceutical analysis using PLS, PCR has been used and has produced results that were

mostly similar, although predictive performance for more complex mixtures was a bit worse. In addition, PCR combined with UV-Vis spectrophotometry reduces the analysis time and also avoids the use of separation solvents, which, based on the AGREE and Eco-Scale assessments, helps a lot.^[25]

5.5. MCR-ALS

Multivariate Curve Resolution with Alternating Least Squares (MCR-ALS) is a valuable self-modeling procedure that allows for the decomposition of a data array into individual component spectra and concentration profiles. This decomposition does not require the user to know the constituents of the data array. The application of MCR-ALS is guided by physically and chemically meaningful constraints, including non-negative spectra and concentrations, closure, and unimodality, and ensures that the solutions produced are both unique and interpretable.^[6]

MCR-ALS has found a number of applications in the pharmaceutical industry as it is particularly useful in studying product stability and impurity profiling, in which unknown degradation products of the target analyte are typically present. It also offers significant advantages over PLS and PCR in solving problems regarding the chemical interpretation of the spectra, as it describes pure chemically meaningful spectra of the components of interest. MCR-ALS, when applied to the analysis of spectrophotometric data, allows the simultaneous determination of spectrophotometric data with very high accuracy and in very small amounts of solvent, in line with GAC principles.^[6, 23]

5.6. Artificial Intelligence and Machine Learning Integration

The integration of artificial intelligence (AI) and machine learning (ML) into chemometric workflows represents the most significant recent development in pharmaceutical spectrophotometric analysis. Supervised ML models, including support vector machines (SVM), artificial neural networks (ANN), and convolutional neural networks (CNN), have demonstrated superior performance over classical chemometric methods in handling nonlinear spectral relationships and high-dimensional data^[6, 23].

ANN-based models, in particular, have been applied to spectrophotometric data for pharmaceutical quality control, offering adaptive learning capabilities that accommodate batch-to-batch spectral variability. Despite their higher accuracy, these models require larger training datasets and offer reduced interpretability compared to PLS or PCR. Future work in this area focuses on combining interpretable chemometric models with AI-driven spectral processing, integrated within Process Analytical Technology (PAT) frameworks for real-time pharmaceutical quality monitoring^[6, 23].

6. Comparison of Assessment Metrics

The following table summarizes the key characteristics of the most widely used GAC and WAC assessment tools in pharmaceutical spectrophotometric analysis:

Metric	Type	Output	Framework	Dimensions Evaluated	Software Available
NEMI	Qualitative	4-quadrant pictogram	GAC	Environmental	No
Analytical Eco-Scale	Semi-quantitative	Score (0–100)	GAC	Environmental, safety	No
GAPI/MoGAPI	Semi-quantitative	5-pentagram pictogram + score	GAC	Full procedure	Yes (MoGAPI)
AGREE	Quantitative + qualitative	12-sector pictogram + score (0–1)	GAC	All 12 GAC principles	Yes
AGREEprep	Quantitative + qualitative	10-sector pictogram + score (0–1)	GAC	Sample preparation	Yes
RGB/RGB12	Quantitative	Color score (0–100%)	WAC	Performance + eco + practical	Excel
BAGI	Quantitative	Score (25–100) + pictogram	WAC	Practicality/applicability	Yes (online)

7. Recent Applications in Pharmaceutical Analysis

7.1. Simultaneous Determination of Binary and Ternary Mixtures

Recent works have shown that chemometrics combined with green spectrophotometric techniques can be quite powerful for pharmaceutical analysis. Using chemometrics-assisted UV spectrophotometry, montelukast sodium (MON), rupatadine fumarate (RUP), and desloratadine (DES) were simultaneously determined in ternary mixtures using PLS and PCR with a great deal of precision. The methods were validated according to ICH with an assessment of the methods through the principles of greenness. The techniques had AGREE scores of at least 0.70 and Eco-Scale scores of 75 or greater. [25]

The first and higher-order derivative techniques were used in derivative spectrophotometry to resolve the severely overlapping spectra of domperidone and pantoprazole. The methods were put right to the standard addition method and assessed with AGSA, BAGI, CACI, and AGREE, respectively. All of the methods had great analytical performance, impressive greenness, and an acceptable whiteness balance. Thus, the methods could easily be applied to routine quality control practices in place of chromatographic methods. [24]

7.2. Green Spectrophotometric Methods Evaluated with Multiple Metrics

The increasing use of multiple assessment metrics at once has improved the characterization of analytical methods. An example of this approach is the 2025 study that simultaneously quantifies gabapentin and methylcobalamin using RP-HPLC. The study is an example of how various sustainability metrics, such as an (AGREE score of 0.70, Eco-Scale of 80, AMVI of 234), can confirm the evaluation of the method's multi-metric applicability, eco-friendliness, and practicality. The same approach, using various metrics, has recently been applied to UV-Vis spectrophotometric methods in the field of pharmaceutical analysis, while this example is related to HPLC. [21]

7.3. Chemometric Green Methods for Quality Control

The simultaneous determination of multiple proton pump inhibitors and antibiotics in an eco-friendly chemometric approach has been introduced using derivative spectrophotometry coupled with PLS and PCR and evaluated by AGREE and RGB12 tools. These methods offer a cost-effective and sustainable alternative to HPLC for routine

pharmaceutical quality control in both developed and resource-limited laboratory settings. The simultaneous application of WAC metrics alongside GAC metrics in these studies highlights the shift from single-dimension assessment to comprehensive holistic evaluation. These approaches provide a cheap and sustainable alternative to HPLC for regular pharmaceutical quality control in developed and resource-limited laboratories. The concurrent use of WAC metrics together with GAC metrics in these studies is an indication of the transition from one dimension evaluation to the whole holistic evaluation. [7, 27]

8. Challenges and Future Directions

Despite significant development, the profession still faces a number of hurdles. Most existing greenness measures are based on the environmental effect without addressing the practicality of the methods and are thus limited as a tool for method comparison. The field of metrics does not follow standardized development principles, which results in inconsistent application and efficacy across diverse analytical situations. [11, 20]

The combination of AI and deep learning with spectrophotometric chemometrics is a new area and most of the works deal with conventional PLS, PCR and MCR-ALS models. Future effort should be directed towards the creation of hybrid models that combine the interpretability of classical chemometrics with the prediction capability of ML, especially for complicated multi-component pharmaceutical formulations. Moreover, real-time in-line spectrophotometric monitoring in PAT approaches is a crucial opportunity to improve analytical performance and sustainability metrics concurrently [6, 23].

Regulatory incorporation of green and white metrics into pharmaceutical method validation standards along with ICH Q2(R2) criteria for accuracy, precision and linearity would provide a uniform platform for sustainability-based method development. Emerging techniques like as CaFRI and CACI, designed particularly for addressing analytical flexibility and reagent effect, are projected to complement current measures and solve current gaps in the assessment environment. [21, 24]

9. Conclusion

Green to White Analytical Chemistry has grown into a full-fledged, multi-dimensional evaluation of analytical method. Spectrophotometric pharmaceutical analysis, especially when accompanied by chemometric approaches, is suitable for GAC and WAC deployment. BAG, RGB12, and AGREEI

provide the most comprehensive environmental, performance and practical review of techniques. Simultaneous pharmacological analysis is the future of AI and ML integration, however, the most common are still derivative spectrophotometry and PLS-based chemometrics. Top field advancement aims include creation of standardized metric development standards, and regulatory integration of sustainability needs.

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