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Green metric tools and white assessment in bioanalytical method development and validation: A comprehensive review

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Abstract

The increasing demand for sustainable and eco-friendly practices in pharmaceutical and bioanalytical research has prompted the integration of green chemistry principles into analytical method development. This review discusses the implementation of Green Analytical Chemistry (GAC) metrics, such as Analytical Eco-Scale, Green Analytical Procedure Index (GAPI), and Analytical Greenness (AGREE), as well as the emerging White Analytical Chemistry (WAC) concept, in the development and validation of bioanalytical methods. We highlight the significance, methodology, and applications of these tools in assessing the environmental impact, efficiency, and overall sustainability of bioanalytical procedures. Case studies from recent literature are examined to illustrate practical applications and future directions.

Keywords: Green Analytical Chemistry; White Analytical Chemistry; Bioanalytical Methods; Method

1. Introduction

The field of bioanalytical method development and validation plays a crucial role in pharmaceutical analysis, particularly in pharmacokinetics, drug metabolism, and therapeutic monitoring. Traditionally, analytical methods were optimized primarily for sensitivity and specificity, often overlooking environmental and safety considerations. In recent years, the emphasis on sustainable development and green chemistry has led to a paradigm shift toward environmentally benign analytical practices. These include reducing solvent and reagent use, utilizing safer alternatives, and developing miniaturized, automation-friendly systems.

2. Green Analytical Chemistry (GAC)

Green Analytical Chemistry aims to reduce or eliminate hazardous substances in analytical processes, minimize waste generation, and promote safer, energy efficient techniques. Several metric tools have been introduced to quantify the greenness of analytical procedures:

2.1. Analytical Eco-Scale

This semi-quantitative tool starts with a base score of 100 and deducts penalty points for non-green aspects like hazardous reagents, excessive energy consumption, and waste production. A final score above 75 is considered excellent, 50–75 acceptable, and below 50 inadequate.

- **Strengths**: Simple and widely used.
- **Weaknesses**: Subjective penalty assignment, limited scope for emerging technologies.

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2.2. Green Analytical Procedure Index (GAPI)

GAPI uses a 5-point pentagram diagram to assess greenness across sample collection, preservation, preparation, analysis, and waste. Each step is color-coded (green, yellow, red) for quick visualization. It is especially useful in highlighting areas for improvement in the overall method lifecycle.

- **Strengths**: Comprehensive (covers sample collection to waste disposal); user-friendly.
- **Weaknesses**: Lacks quantitative scoring; visual interpretation can be ambiguous.

2.3. Analytical GREEnness (AGREE)

AGREE software evaluates methods based on the 12 principles of GAC, generating a circular, color-coded graph with a unified score (0–1). This allows for a nuanced and comprehensive assessment that is easy to interpret.

- **Strengths**: Quantitative, automated, based on 12 principles.
- **Weaknesses**: Requires complete information on method parameters; can oversimplify complex trade-offs.

2.4. NEMI (National Environmental Methods Index)

A qualitative tool that assesses method greenness using four quadrants—persistent, bio accumulative, toxic, hazardous waste, and corrosivity.

- **Strengths**: Quick and simple.
- **Weaknesses**: Too simplistic for complex bioanalytical workflows.

Table 1 Comparison of Green and White Analytical Chemistry Assessment Tools

Parameter	**GAPI (Green Analytical Procedure Index)**	**AGREE (Analytical GREEnness)**	**Eco-Scale**	**WAC (White Analytical Chemistry)**
Purpose	Visual assessment of greenness across analytical workflow	Comprehensive scoring based on 12 GAC principles	Semi-quantitative score based on penalties for hazards/waste	Integrated evaluation balancing analytical, environmental, and economic factors
Assessment Format	Pentagram with color-coded zones (green, yellow, red)	Circular wheel with 12 segments and unified score (0–1)	Numeric score from 100, penalty points deducted	RGB color model representing balance of 3 pillars
Focus Areas	Sample collection, preservation, preparation, analysis, waste	12 GAC principles including reagent, energy, waste, safety	Reagent hazards, energy use, waste generation	Analytical performance, environmental impact, and economic/social sustainability
Output Interpretation	Visual identification of strengths and weaknesses	Holistic score for easy comparison	>75 excellent, 50–75 acceptable, <50 poor	White color indicates balanced method quality
Ease of Use	Moderate; requires detailed workflow data	User-friendly software	Simple calculation	Requires comprehensive data on all three pillars
Regulatory Use	Increasing acceptance for method optimization	Growing use in research and regulatory submissions	Widely used for greenness reporting	Emerging tool aligned with sustainable goals
Strengths	Highlights specific steps for improvement	Standardized, holistic, visually intuitive	Quick, easy to calculate	Balances all key sustainability aspects
Limitations	Subjective color coding, limited economic considerations	Learning curve; needs software	Does not include economic/social factors	New concept; needs broader adoption and validation
Typical Applications	Method development and green screening	Green method validation and comparison	Greenness scoring in publications	Sustainability evaluation in bioanalysis and pharma

3. White Analytical Chemistry (WAC)

While GAC focuses on environmental aspects, WAC introduces a balanced approach integrating analytical performance (red), environmental impact (green), and economic/social sustainability (blue). The WAC framework emphasizes the "whiteness" or overall quality and applicability of the method, aiming for equilibrium among all pillars of sustainability. WAC scoring typically uses the RGB model, with each axis representing one of the three dimensions. Optimal methods are those that balance analytical efficiency, low environmental impact, and practical utility, resulting in a neutral white hue. This makes WAC particularly relevant for regulatory environments and industries focused on cost-effectiveness and safety.

4. Principles of Green and White Analytical Chemistry

GAC is guided by 12 principles, emphasizing:

- Use of non-toxic and renewable solvents.
- Miniaturization and automation.
- Reduction of energy and reagent consumption.
- On-site and real-time monitoring.

WAC builds upon GAC by introducing a three-dimensional (3D) concept: - Red (Analytical performance): accuracy, precision, selectivity, sensitivity - Green (Environmental impact): toxicity, waste, resource consumption - Blue (Practicality): cost-effectiveness, simplicity, speed, robustness.

The ideal analytical method is located at the white core of the 3D evaluation sphere, ensuring balance across these dimensions.

Figure 1 Twelve Principles of Green Analytical Chemistry

5. Historical Background

The drive toward sustainability in analytical sciences has its roots in the principles of Green Chemistry, first articulated by Anastas and Warner in the 1990s. This movement led to the formalization of Green Analytical Chemistry (GAC) in the early 2000s, focusing on minimizing the environmental footprint of analytical procedures. In response, several metric-based tools were developed to quantitatively and qualitatively evaluate method greenness: the Analytical Eco-Scale (Gałuszka et al., 2012), Green Analytical Procedure Index (GAPI) (Nowak and Wietecha-Posłuszny, 2016), and AGREE (Nowak et al., 2020), each offering unique scoring systems and visual outputs to assess adherence to green chemistry principles.

However, as these tools matured, it became evident that evaluating only the environmental impact of a method overlooked other crucial dimensions such as analytical performance and operational feasibility. This gap led to the conceptualization of White Analytical Chemistry (WAC) by Nowak et al. in 2021. WAC uses the RGB (Red-Green-Blue) model to represent the three essential pillars of sustainable analytical methods:

- Red for analytical performance (e.g., accuracy, precision, selectivity),
- Green for environmental impact (as per GAC),
- Blue for economic and practical aspects (e.g., cost-effectiveness, user-friendliness, societal value).

The convergence of these three dimensions into a "white" output symbolizes a method that is not only eco-friendly but also analytically robust and socially responsible.

In the field of bioanalytical method development and validation, which is heavily regulated and involves complex biological matrices, these tools have found increasing relevance. While GAC tools help in reducing hazardous waste and optimizing reagent use, WAC ensures that these greener methods do not compromise on analytical integrity or usability. This evolution from "green-only" to "white-inclusive" assessment marks a significant shift toward holistic method design, aligning with modern demands for sustainability, compliance, and performance.

6. Application in Bioanalytical Method Development

The bioanalytical domain presents unique challenges, such as complex biological matrices and stringent regulatory requirements. The integration of GAC and WAC tools provides a structured approach for:

- Selecting greener solvents and reagents (e.g., replacing acetonitrile with ethanol or propylene carbonate)
- Minimizing sample preparation through techniques like solid-phase microextraction (SPME), dispersive liquid–liquid microextraction (DLLME), and protein precipitation using minimal solvent volumes
- Enhancing method robustness and reproducibility using automation and miniaturized systems
- Facilitating regulatory compliance through validated sustainable practices in line with FDA and EMA bioanalytical guidelines.

6.1. Regulatory Perspectives on Green Bioanalytical Methods

Regulatory agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), while not yet issuing explicit standalone guidelines solely focused on green analytical chemistry in bioanalysis, are increasingly promoting sustainability within their broader regulatory frameworks. These agencies emphasize the development of robust, reliable, and reproducible bioanalytical methods that inherently align with green chemistry principles by minimizing resource consumption and environmental impact.

The FDA’s Bioanalytical Method Validation Guidance encourages method development and validation approaches consistent with Quality by Design (QbD) principles, fostering efficient and risk-based methodologies that reduce waste and energy use. Although specific mandates on greenness are evolving, the FDA supports initiatives promoting sustainable laboratory practices and environmental stewardship within pharmaceutical research.

Similarly, the EMA advocates for sustainability through its adherence to Good Laboratory Practice (GLP) and bioanalytical method validation guidelines, while aligning with the broader European Green Deal objectives. EMA’s scientific advice and regulatory framework increasingly integrate environmental considerations, encouraging the adoption of green chemistry approaches in pharmaceutical development, including bioanalysis.

Globally, there is a trend toward incorporating environmental sustainability as a key factor in pharmaceutical quality assessments. Emerging harmonization efforts, such as those by the International Council for Harmonisation (ICH), are beginning to include sustainability considerations, which may shape future regulatory expectations. The integration of green and white assessment tools within bioanalytical workflows aligns with these evolving trends and offers a proactive strategy to meet upcoming regulatory and societal demands.

Incorporating these regulatory perspectives underscores the importance of greenmetrics and white assessment tools, not only for environmental benefits but also for ensuring compliance and competitiveness in an increasingly sustainability-focused pharmaceutical landscape.

7. Case Studies and Literature Insights

Several studies have successfully employed green and white assessment tools in bioanalytical method development. Examples include:

- Naguib et al. developed HPLC methods for paracetamol analysis in pharmaceuticals and biological fluids and assessed their greenness using Eco-Scale and GAPI. Their results showed that thoughtful method design significantly minimized hazardous solvent usage and improved sustainability (Naguib et al., 2023).
- Elsheikh et al. integrated experimental design with green chemistry to optimize a stabilityindicating HPLC method for zonisamide. The method was validated using Eco-Scale, GAPI, and AGREE, emphasizing its eco-friendliness (Elsheikh et al., 2023).
- A recent BMC Chemistry study designed and validated an HPLC method for β-sitosterol, using color-coded tools like AGREE, GAPI, and Eco-Scale for a holistic green assessment. The method demonstrated how multiple greenness metrics can complement each other (BMC Chemistry, 2024).
- Salman et al. developed a fast and sensitive HPLC-MS/MS method for deucravacitinib quantification in plasma and used AGREE to evaluate its greenness. Their approach highlighted the balance between sensitivity and environmental responsibility (Salman et al., 2023).
- Saroj et al. proposed a synergistic model combining Green Analytical Chemistry and Quality by Design (QbD), arguing that analytical methods should be not only green but also robust, efficient, and regulatory compliant (Saroj et al., 2018).

These examples underscore the value of green and white assessments in identifying greener alternatives and optimizing existing methods for higher sustainability.

8. Critical Comparison and Discussion:

Table 2 Comparison of Green-metric Tools

Metric Tool	Quantitative	Visual	Suited for Bioanalysis	Limitations
Eco-Scale	Yes	No	Moderate	Not bio-specific
GAPI	No	Yes	Good	Visual ambiguity
AGREE	Yes	Yes	Excellent	Needs full data
NEMI	No	Yes	Poor	Too general

- **Integration**: No single tool is ideal. Combining AGREE with GAPI offers both quantitative insight and holistic visualization.
- **Regulatory Acceptance**: Still lacking; these tools are mainly academic.
- **Automation Potential**: AGREE's software could be extended to support automated method development in pharma.

9. Challenges and Future Perspectives

Despite the growing adoption of GAC and WAC tools, several challenges remain:

- Lack of standardization in metric application across laboratories and journals
- Limited awareness and training among analysts, especially in low-resource settings
- Difficulty in balancing sensitivity and selectivity with environmental friendliness
- Need for integration with quality-by-design (QbD) frameworks, which focus on robustness and lifecycle management

9.1. Future directions include

- Development of hybrid assessment software integrating AGREE, GAPI, and WAC scoring systems
- Encouraging inclusion of green and white assessments in regulatory submissions
- Creating open-access databases of green-validated bioanalytical methods

- Promoting academic-industrial collaborations for greener analytical platforms

10. Conclusion

Greenmetric tools such as GAPI, AGREE, and Analytical Eco-Scale have revolutionized the evaluation of environmental impact in bioanalytical method development. These tools facilitate the design of greener, safer, and more sustainable analytical protocols. The emerging concept of White Analytical Chemistry adds a valuable dimension by balancing analytical performance, environmental sustainability, and economic feasibility—offering a holistic framework for method assessment.

Incorporating green and white assessment tools early in method development can significantly reduce hazardous waste, minimize resource consumption, and improve compliance with regulatory guidelines. Although challenges remain, including standardization and training, the integration of these tools represents a forward-thinking approach that aligns with global sustainability goals.

Future efforts should focus on harmonizing assessment methodologies, developing hybrid software tools, and fostering interdisciplinary collaboration to mainstream green and white analytical practices in bioanalysis.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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