



Green Analytical Chemistry (GAC): Metrics, Method Development, and Regulatory Acceptance

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ABSTRACT:

In order to reduce environmental effect while preserving analytical efficiency, precision, and dependability, Green Analytical Chemistry (GAC) has evolved as a novel and sustainable technique that incorporates the concepts of green chemistry with analytical sciences. The development of greener analytical techniques has quickened due to growing concerns about the excessive use of hazardous chemicals, high energy requirements, and production of toxic waste from current analytical methods. The basic ideas and twelve principles of GAC are thoroughly covered in this review, with a focus on waste reduction, cleaner solvents and reagents, energy efficiency, miniaturization, automation, and real-time analysis. The principles, applications, strengths, and limitations of several greenness assessment tools, such as the National Environmental Methods Index (NEMI), Analytical Eco-Scale, Green Analytical Procedure Index (GAPI), Modified GAPI (MoGAPI), Analytical GREENness Metric (AGREE), and Greenness Evaluation Metric for Analytical Methods (GEMAM), are critically examined. With an emphasis on the incorporation of Analytical Quality by Design (AQbD), the use of environmentally safe solvents, miniaturized analytical platforms, and cutting-edge sample preparation methods like solid-phase microextraction and microwave-assisted extraction, the review also highlights recent developments in the development of green analytical methods. To illustrate the usefulness of GAC, applications in the food, pharmaceutical, environmental, and bioanalytical domains are examined. Additionally, the present state of regulatory adoption of green analytical methods by international organizations, such as the European Medicines Agency (EMA), the United States Food and Drug Administration (FDA), and the International Council for Harmonization (ICH), is reviewed. Future prospects on artificial intelligence-assisted optimization and sustainability-oriented analytical workflows are also discussed, along with challenges related to greenness evaluation, method sensitivity, and universal standardization.

Keywords: Green Analytical Chemistry (GAC); Green Analytical Procedure Index (GAPI); AGREE; Analytical Eco-Scale; Analytical Quality by Design (AQbD); Sustainable Analytical Methods; Green Solvents; Regulatory Acceptance

Introduction:

Green Analytical Chemistry (GAC) aims to reduce the environmental and health impact of analytical methods while maintaining or improving analytical performance and data quality. It extends the 12

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principles of green chemistry to sample handling, preparation, separation, detection, waste management, and occupational safety.

Over the last two decades, GAC has evolved from a conceptual framework into a practical paradigm, driven by pressure to lower solvent use, energy consumption, and hazardous-waste generation in quality-control and research laboratories. Pharmaceutical, environmental, food, and clinical laboratories now apply GAC-driven metrics, miniaturization, automation, and alternative reagents to redesign classical methods.[1]

This review covers:

1. Major GAC metrics (NEMI, Eco-Scale, GAPI/MoGAPI, AGREE, GEMAM).
2. Practical strategies for GAC-driven method development, especially in HPLC and sample preparation.
3. Current status and perspectives on regulatory acceptance of green analytical methods by ICH-aligned agencies (FDA, EMA).

2. Core Principles of Green Analytical Chemistry

The 12 principles of GAC are summarized under the SIGNIFICANCE mnemonic and form the basis of most modern greenness metrics. PROCESSES such as miniaturization, automation, in-situ or on-line measurements, and elimination or replacement of toxic reagents are central to GAC.[2-8]

Relevant GAC principles include:

1. Minimal sample size and remote or in-situ sensing to cut sample treatment and waste.
2. Energy efficiency and renewable-source reagents (e.g., bio-based solvents).
3. Avoidance of derivatization and hazardous reagents (e.g., hexane, chloroform).

These principles are operationalized through quantitative metrics discussed in the next section.

3. Greenness Metrics in Analytical Chemistry

3.1. National Environmental Method Index (NEMI)

NEMI is a qualitative pictogram comprising four quadrants representing:

1. No PBT (persistent, bioaccumulative, toxic) reagents.
2. No EPA-listed hazardous waste.
3. pH between 2 and 12.
4. Waste ≤ 50 g.[6][4]

If all are met, the NEMI label is “green” in all four sectors; otherwise, the unsatisfied sectors remain white. Although simple, NEMI lacks quantitative scoring and is often combined with other tools.[4][6]

3.2. Analytical Eco-Scale

The Analytical Eco-Scale starts from a baseline score of 100 points and subtracts penalty points (PPs) for:

1. Chemical hazard and amount.
2. Energy consumption.
3. Waste volume and waste-treatment adequacy.
4. Occupational hazard (e.g., vapors released).

Scoring levels:

5. ≥ 75 points: excellent green method.
6. 50–74 points: acceptable.
7. < 50 points: not green.

Eco-Scale is widely used because criteria are explicit, but it does not visually indicate which step is responsible for penalties.[10]

3.3. Green Analytical Procedure Index (GAPI / MoGAPI)

GAPI uses a pictogram of five pentagrams (sample handling, method type, sample preparation, reagents/solvents, instrumentation) scored as green/yellow/red. Each sector is subdivided into 15 sub-categories, for example:[12]

1. Sampling: in-line, on-line, at-line, off-line.
2. Sample preparation scale: nano, micro, macro.
3. Solvent health hazard (NFPA-based), energy < 0.1 – 1.5 -kW h per sample, and waste < 1 , 1 – 10 , > 10 mL.[6]

Modified GAPI (MoGAPI) extends this by adding a total score (e.g., 0–100) and dedicated software, improving comparability between methods.[11]

3.4. AGREE (Analytical GREENness Metric)

AGREE is currently the most comprehensive metric, mapping all 12 GAC principles into a single circular pictogram and an overall score on a 0–1 scale. Example criteria include:[5][1][6]

1. Sample pretreatment: remote sensing and non-invasive methods score highest; external batch treatment scores lowest.[5][6]
2. Automation and miniaturization: fully automatic miniaturized methods score 1.0; manual non-miniaturized methods = 0.0.[5][6]

3. Waste volume, energy/sample, number of analytes per hour, use of bio-based reagents, and toxic-reagent use.[6][5]

AGREE software applies user-selectable weights and outputs a color-coded “clock-like” diagram along with a global score (e.g., 0.72 for a greener UFLC method).[5][6]

3.5. Emerging tools (GEMAM, AMGS, BAGI)

Recent systems include:

1. GEMAM (Greenness Evaluation Metric for Analytical Methods): spans 12 GAC principles and 10 sample-preparation factors, with a 0–10 numerical score and a color-coded pictogram.[3]
2. AMGS (Analytical Method Greenness Score): developed for pharmaceutical separations, focusing on column length, mobile-phase composition, and run-time reductions.[8]
3. BAGI (Blue Applicability Grade Index): evaluates method practicality rather than greenness, complementing GAC metrics.[6]

These tools highlight the trend toward multi-criteria, software-based greenness evaluation.

4. Comparative Table of GAC Metrics

Table 1. Overview of major GAC metrics

Metric	Score type	Representation	Strengths	Main limitations
NEMI	Qualitative	4-quadrant pictogram [4][6]	Simple, easy to interpret; widely recognized.	No numerical score; limited hazard coverage; manual look-up.
Eco-Scale	Numerical (100→0)	Single value + criteria table [10][6]	Considers multiple impact factors; clear scoring rules.	Does not show which step is problematic; manual scoring.
GAPI/MoGAPI	Semi-quantitative + pictogram	5 pentagrams, 15 sub-categories [12][6]	Visual identification of weak steps; MoGAPI adds total score and software.	Complex to fill; some categories are ambiguous.
AGREE	0–1 scale + pictogram	12-sector wheel [11][5][6]	Covers all 12 principles; software-based; flexible weights.	Confusing weight allocation; steep learning curve.
GEMAM	0–10 numerical + pictogram [3]	Color-coded sectors	Simple interface; includes sample-preparation factors.	Newer, fewer published validations.

5. Example: Greenness Evaluation of a UFLC Method

Consider a UFLC method for quantification of Omarigliptin (pharmaceutical tablets) assessed with NEMI, Eco-Scale, MoGAPI, and AGREE.[6]

5.1. Data (hypothetical illustration)

1. Mobile phase: methanol–phosphoric acid buffer.

2. Waste per analysis: 1.32 mL.
3. Toxic solvents used: ~0.43 mL methanol.
4. Waste treated via passivation.
5. No vapors released during analysis.[6]

5.2. Metric results (Figure 1 concept)

Create a combined figure in Word with four subpanels:

- **Panel A (NEMI):** 3 green quadrants, 1 white (methanol and phosphoric acid are hazardous EPA wastes).[6]
- **Panel B (Eco-Scale):** Total score 85 (excellent greenness).[6]
- **Panel C (MoGAPI):** Mainly green sectors; two red sectors (offline sample preparation, non-green solvents); total score 80.[6]
- **Panel D (AGREE):** Overall score 0.72, with weak green/yellow sectors for off-line pretreatment, moderate waste volume, single-analyte throughput, and conventional solvents.[6]

These four pictograms can be plotted in Word as four small circles/boxes (NEMI and AGREE) and pentagrams (GAPI/MoGAPI) placed side-by-side.

6. Method Development under GAC Principles

6.1. Miniaturization and Automation

Key strategies include:

1. Replacement of HPLC with U/HPLC or SFC to reduce column length, flow rate, and run time.[8]
2. On-line or at-line sample preparation instead of off-line extraction.[9][6]

Example: A miniaturized UFLC method for Omarigliptin can cut methanol consumption by 50–70% compared with classical HPLC, improving both Eco-Scale and AGREE scores.[6]

6.2. Green Solvents and Derivatization

1. Replacement of hexane, chloroform, and dichloromethane with ethanol, acetone, ethyl acetate, or bio-based solvents improves hazard and waste metrics.[8]
2. Avoiding derivatization or using greener derivatization agents (water-based, enzymatic, low-toxicity reagents) reduces Eco-Scale penalty points.

6.3. Sample-Preparation Innovations

1. Microwave-assisted, ultrasound-assisted, and stir-bar sorptive extraction reduce solvent volume and energy vs. Soxhlet extraction.[3]

2. Solid-phase microextraction (SPME) and headspace-SPME eliminate or cut organic-solvent use, improving NEMI and AGREE waste sectors.[9]

A comparative table can be added:

Table 2. Sample-preparation techniques and their impact on key GAC metrics

Technique	Solvent volume (mL/sample)	Typical energy use (kW h/sample)	Approx. Eco-Scale waste PP	Notes
Soxhlet extraction	100–200	High (continuous heating)	5–10	Highest PPs; poor GAPI/MoGAPI.
Accelerated solvent extraction	20–50	Medium	3-5	Moderate improvement.
Microwave-assisted	10–30	Medium–low	2-4	Faster; lower solvent.
SPME/HS-SPME	0–5	Low	0-1	Best greenness scores.

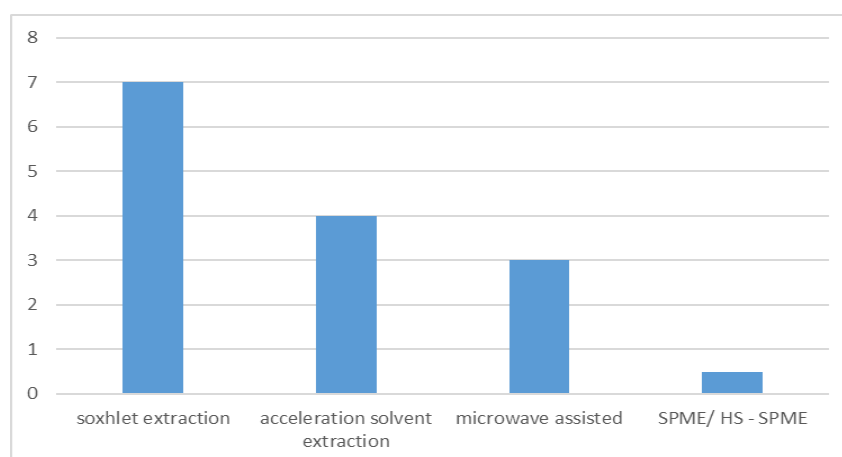


Figure 1: Solvent volume” Vs “Eco-Scale PPs”

7. Regulatory Acceptance of Green Analytical Methods

7.1. ICH-aligned guidelines and GAC

ICH Q2(R1) on analytical validation and Q3A–Q3D on impurities do not explicitly mandate greenness but implicitly favor specific, robust, and robust methods that can be aligned with GAC principles. Modern Quality by Design (QbD) and lifecycle-management approaches allow method optimization toward lower solvent use and shorter run times without compromising selectivity or robustness.[13][7][9][10]

Regulatory agencies increasingly recognize that:

1. Reduced solvent use lowers environmental and occupational risk.
2. Automated and miniaturized methods improve reproducibility and reduce human error.[7][11]

7.2. FDA, EMA, and EPA-type considerations

1. The U.S. FDA and EMA encourage sustainable pharmaceutical development and analytical practices, including solvent substitution and green separation methods.[7][10]
2. Process Mass Intensity (PMI) and E-factor concepts from green chemistry are being adapted to assess analytical method impact, even though dedicated GAC metrics like AGREE are not yet formalized in guidance.[11][8]

Green methods are typically accepted if they meet:

1. ICH-compliant selectivity, robustness, accuracy, and precision.
2. Controlled variance in solvent composition and run time (e.g., via AQbD and design of experiments).[13-9]

7.3. Case-based trend: Green methods in submissions

Recent pharmaceutical-analytical case studies show that:

1. Eco-Scale- and AGREE-evaluated UFLC/HPLC methods are increasingly included in regulatory dossiers as part of sustainability-oriented documentation.[7]
2. Greenness profiles are reported as supplementary information, demonstrating commitment to environmental stewardship without changing core validation data.[11-10]

8. Practical Integration into Analytical Workflows

8.1. When to apply GAC metrics

GAC metrics should be integrated at:

1. Early development (screening alternative solvents and techniques).
2. Final optimization (comparing candidate methods with AGREE or MoGAPI).[1-6]

8.2. Workflow proposal

1. Define analytical requirements (LOQ, precision, selectivity).
2. Screen miniaturized, on-line, or automated methods (UPLC, SFC, SPME).
3. Evaluate a short-list of methods using:
 - a. Eco-Scale (quick numerical greenness).
 - b. MoGAPI (visual sector-wise weaknesses).
 - c. AGREE/GEMAM (comprehensive GAC-principle coverage).
4. Select the greenest method that still meets validation criteria and regulatory expectations.

8.3. Example: AGREE-driven HPLC optimization

In a typical HPLC optimization, one can vary:

1. Column length (e.g., 150 mm vs. 50 mm).
2. Flow rate and gradient program.
3. Mobile-phase composition (acetonitrile vs. methanol vs. ethanol).

For each variant, compute:

1. Eco-Scale score (waste and hazard).
2. AGREE score (automation, energy, waste, toxic reagents).

Plot a scatter plot in Word: x-axis = flow rate (mL/min), y-axis = AGREE score (0–1), with markers for each method. This shows how lower flow/energy improves the greenness score while retaining adequate resolution.

9. Challenges and Limitations

1. Subjectivity in weighting (AGREE, MoGAPI) can lead to different greenness rankings for the same method.[6]
2. Manual data entry and CAS-lookup for every reagent are time-consuming, especially for Eco-Scale and complex GAPI extensions.[6]
3. Analytical performance vs. greenness trade-offs: a method with lower solvent use might have higher detection

Conclusion:

In this review article, nanobiomaterials, which are used in the field of medical science, have been discussed and their toxicity effects investigated. It is obvious that most nondegradable nanoparticles considered in this review are toxic and can influence the body's cells.

The swift evolution of nanotechnology has significantly impacted sectors such as healthcare, environmental management, and agriculture, offering unprecedented opportunities for precision medicine and reduced dependency on environmentally detrimental agrochemicals. However, the expanding integration of nanoparticles into biological systems has raised valid concerns regarding their potential toxicity and long-term biocompatibility. This review comprehensively analyses the current approaches to assessing nanotoxicity, highlighting recent advancements and persisting challenges. It delves into the underlying physiological and molecular mechanisms associated with nanoparticle-induced toxicity, including the complex interplay of oxidative stress, inflammation, DNA damage, and neurotoxic effects. Developing advanced *In vitro*, *in vivo* and *in silico* testing platforms, high-throughput screening technologies, and alternative model organisms has enhanced nanoparticle toxicity evaluations' precision and ethical acceptability. Despite these improvements, the lack of standardized testing protocols and the need for more predictive, biologically relevant models

remain significant barriers. The regulatory framework surrounding nano toxicology continues to evolve, with ongoing efforts to establish harmonized guidelines and robust risk assessment strategies.

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