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Guidelines on the proper selection of greenness and related metric tools in analytical chemistry

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ABSTRACT

Background: The metric tools that aim to assess greenness, whiteness or related aspects of analytical procedures are gaining in popularity. At the same time, their application is not standardized, leading to unintentional abuses.

Results: Within this study, two datasets are created, consisting of the greenness assessment results for 27 (for general assessment) and 3 (for reproducibility study) analytical procedures, obtained with available greenness and related metric tools. The first dataset was assessed with multivariate statistical tools, and the analysis shows that metric tools give correlated results. The second dataset was used to calculate the reproducibility of metric tools results.

Significance and Novelty: Based on the results of multivariate statistics and reproducibility analyses, we propose guidelines for the application of metric tools. Obeying these guidelines will result in a more standardized approach to assessment, consistency of results, and drawing more meaningful conclusions.

Keywords: *green analytical chemistry; greenness metrics; white analytical chemistry; whiteness metrics*

Graphical Abstract

1. Introduction

The need to move towards more sustainable practices has become critical in today's world due to the escalating environmental degradation and its direct impact on human health and ecosystems. Rising levels of air, water, and soil pollution contribute to climate change, biodiversity loss, and an increase in diseases, all of which threaten global well-being [1]. The sustainable development principle "think globally, act locally" means that all professions should make efforts to make their activities more sustainable.

Therefore, in recent years, analytical chemistry is increasingly adapting sustainable and environmentally friendly practices, leading to the development of various metric tools to assess and minimize laboratory procedures' environmental impact [2]. While these metrics share the common goal of accounting of environmental impact and enhancing sustainability, their specific implementation and focus can vary significantly depending on the context [3].

In analytical procedures, "greenness" metrics are typically designed to evaluate the environmental impact of methods used for detecting, quantifying, or characterizing chemical substances. The term greenness refers to the impacts within environmental, health and safety criteria. These metrics focus on factors such as solvent selection, reagent minimization, waste reduction, and energy efficiency, in alignment with the 12 principles of green analytical chemistry (GAC) [4]. On the other hand, in the context of chemical processes, greenness metrics have a broader focus, addressing aspects such as reaction efficiency, atom economy, renewable feedstock utilization, and life cycle assessment, in accordance with the 12 principles of green chemistry [5]. To avoid confusion, it is recommended that metrics related to GAC be referred to as GAC metric tools to distinguish them from other greenness metrics. Below is a brief overview of some of the tools used in GAC and sustainability assessments. Moreover, summary of review articles discussing metric tools is presented in table 1.

Table 1. Summary of review articles discussing GAC and related metric tools

Review article	Metric tools reviewed	Ref
Green chemical analysis: main principles and current efforts towards greener analytical methodologies	NEMI, Eco-Scale, GAPI, HEXAGON, AGREE, ComplexGAPI, AGREEprep	[3]
Green metric tools for analytical methods assessment critical review, case studies and crucify	NEMI, Eco-Scale, GAPI, AGREE, Complex GAPI, HPLC-EAT, AMVI, AMGS, Modified NEMI, AGREEprep, Spider diagram, GSST and iGAL.	[6]
Overview of sixteen green analytical chemistry metrics for evaluation of the greenness of analytical methods	CHEMS-1, NEMI, Modified NEMI, AGP, HPLC-EAT, AMVI, Eco-scale, Green Certificate Modified Eco-scale, GAPI, RGB, HEXAGON, AMGS, AGREE, Complex GAPI,	[7]

	AGREEprep and Spider diagram	
How environmentally friendly is the analytical process? A paradigm overview of ten greenness assessment metric approaches for analytical methods	NEMI, Modified NEMI, analytical Eco-Scale, HPLC-EAT, AMVI, GAPI, ComplexGAPI, AMGS, AGREE and AGREEprep.	[8]
Assessing the Greenness and Environmental Friendliness of Analytical Methods: Modern Approaches and Recent Computational Programs	AGREE, AGREEprep, ComplexGAPI, RGB12, and ChlorTox and BAGI.	[9]
Green analytical chemistry metrics for evaluating the greenness of analytical procedures.	NEMI, advanced NEMI, AGP, AES, Green Certificate Modified Eco-Scale, GAPI,ComplexGAPI, AGREE, AGREEprep RGB, RGB12, AMGS, BAGI, ChlorTox and HEXAGON	[10]
Green metrics and green analytical applications: A comprehensive outlook from developing countries to advanced applications.	NEMI, Modified NEMI, Eco-Scale, GAPI, ComplexGAPI, Modified GAPI (MoGAPI), ComplexMoGAPI, AGREE, AGREEprep, AMGS, BAGI, ChlorTox, AMVI, SPMS and RGB	[11]
An overview of the current progress in green analytical chemistry by evaluating recent studies using greenness assessment tools	NEMI, Eco-Scale, GAPI, AGREE, AMGS, ChlorTox, RGB and RGB12.	[12]
Exploring sustainable analytical techniques using G score and future innovations in green analytical chemistry	NEMI, Modified NEMI, Eco-Scale, GAPI, AGREE, AMGS, Complex GAPI and AGREEprep and G score.	[13]
Green Chemistry Metrics in Analytical Chemistry	CHEMS-1, NEMI, AMVI, HPLCEAT, Eco-Scale, GAPI, Hexagon, RGB, AGREE, ComplexGAPI and ChlorTox.	[14]
Green profile tools: Current status and future perspectives	NEMI, Eco-scale, GAPI, ComplexGAPI, RGB, HEXAGON, AGREE and AGREEprep.	[15]

*) **NEMI** - National Environmental Methods Index; **Eco-Scale** - Ecological Scale for Analytical Chemistry; **GAPI** - Green Analytical Procedure Index; **AGREE** - Analytical Greenness Calculator; BAGI: Blue Analytical Greenness Index; **ChlorTox** - Chloroform-oriented Toxicity Estimation Scale; Complex **GAPI** - Complex Green Analytical Procedure Index; **HPLC-EAT** - High-Performance Liquid Chromatography - Environmental Assessment Tool; **AMVI** - Analytical Method Volatility Index; **AMGS** - Analytical Method Greenness Score; **Modified NEMI** - Modified National Environmental Methods Index; **AGREEprep** - Analytical Greenness Calculator for Sample Preparation; **Spider Diagram** - A graphical representation of analytical procedure attributes in a circular diagram; **GSST** - Green Sample Treatment Score; **iGAL** - Integrated Green Analytical Lab; **SPMS** – Sample Preparation Metric of Sustainability; **RGB**: Red, Green, and Blue model assessment

One of the oldest GAC tools introduced by Keith et al. in 2007 [16] is the National Environmental Methods Index (NEMI). It is represented by a circle divided into four parts that relate to the use of persistent, bioaccumulative, and toxic reagents, their hazardous nature, corrosivity, and waste. Each section can be green, indicating a greener method, or left blank, signaling a lack of greenness. It is a simple, qualitative tool that provides fundamental information on the harmfulness of the procedure. Five years later, Gałuszka et al. proposed another metric called the Analytical Eco-Scale (AES) [17]. This tool is based on penalty points assigned for using toxic reagents, waste generation, and high energy consumption. Similar to NEMI, AES is characterized by its simplicity; however, it provides semi-quantitative information about the harmful effects of the analytical method. To focus more on the sustainability of reagents and solvents applied in analytical procedures Tobiszewski and Namieśnik proposed in 2015 the CHEMS-1 tool [18]. This model uses toxicological and exposure data found in safety data sheets to calculate hazard values associated with using solvents. CHEMS-1 can be particularly useful when solvents are selected in the early stages of procedure development.

To perform a more comprehensive assessment, considering the steps from sampling to determination, Płotka-Wasylka presented the Green Analytical Procedure Index tool (GAPI) in 2018 [19]. To obtain a result about the environmental impact of a method, it is necessary to use software that generates a pictogram of five pentagrams and a scale based on the colours green, yellow, and red. Thus, GAPI allows for a quick semi-quantitative comparison and selection of the most environmentally friendly method.

A year later, two more complex greenness assessment tools were developed. One of them, the RGB model, introduced by Nowak and Kościelniak [20], is designed to evaluate analytical methods using colors: red for analytical performance, green for compliance with green chemistry principles, and blue for practical effectiveness. Therefore, the final result considers both environmental, ecological, and qualitative aspects. The second evaluation tool, called Hexagon, was proposed by Ballester-Caudet et al. [21] to assess analytical methods based on criteria such as analytical performance, sustainability, environmental impact, and economic cost, using penalty points, which are similar in assumptions to Analytical Eco-Scale. However, the results are visualized on a hexagonal pictogram for easy and quick comparison, offering a guideline for selecting methods that align with GAC while balancing performance, safety, and cost-effectiveness.

In 2020, the Analytical GREEnness Calculator (AGREE) was developed by Pena-Pereira et al. [22]. This tool is directly based on 12 principles of GAC, with each principle assigned a specific weight. AGREE uses a color-coded scale – red, yellow, and green – to reflect the level of greenness in a given method. Employing software generates a clock-like pictogram displaying the final score of the analytical method from 0 to 1. This approach is comprehensive, flexible, easy-to-interpret, offering clear and informative results. To pay particular attention to the sample preparation stage, which is often the most resource-intensive part of the analytical process, AGREEprep software was developed two years later [23]. This tool assesses the environmental impact of sample preparation techniques and promotes more sustainable approaches.

As interest in tools for evaluating the greenness of analytical methods has grown, three notable tools were introduced in 2023. One of them, the Sample Preparation Metric of Sustainability tool (SPMS) presented by Gonzalez-Martín et al. [24] is designed to assess the greenness of the extraction technique. This metric evaluates nine parameters across four categories – sample, extractant, procedure information, and energy consumption with waste generation. Based on a graph, SPMS displays the greenness results for key preparation parameters and a total evaluation of this stage within the analytical procedure. Another one – need, quality, and sustainability (NQS) index [25] has been proposed by Grudpan et al. The application of the NQS index indicates that natural reagents enhance analytical procedures, particularly in terms of need and sustainability. As an evaluation tool, the NQS index can aid in developing analytical methods that address social needs, improve analytical performance, and promote global sustainability.

The third tool discussed is the chloroform-equivalency toxicity assessment scale (ChlorTox Scale) developed by Nowak et al. [26]. It provides a straightforward way to evaluate risk by measuring the toxicity of reagents used in an analytical method. This is performed by comparing the substance in question with chloroform as a reference substance. Like the CHEMS-1 tool, ChlorTox focuses exclusively on the harmfulness of the reagents and solvents. The last one, proposed by Manousi et al. is called Blue Applicability Grade Index (BAGI) [27]. BAGI tool is used to assess the practicality of analytical methods, focusing on one of the pillars of White Analytical Chemistry (WAC). By evaluating ten key attributes – such as analysis type, sample throughput, reagent use, and automation – BAGI software generates a blue-toned pictogram along with a score within 25-100 points scale, allowing users to compare different methods and identify their practical strengths and weaknesses.

Green sample preparation (GSP) is a crucial component of green analytical chemistry, guided by the 10 principles of GSP, which emphasize reducing solvent use, minimizing waste, enhancing energy efficiency, and prioritizing safer, sustainable alternatives. The relationship between green chemistry, GAC, and GSP is well-documented in the literature [3]. However, ambiguity often arises regarding the role of material synthesis in assessments using GAC metric tools. Material synthesis generally falls under the broader domain of green chemistry, as it focuses on designing and producing materials with minimal environmental impact, aligning with principles such as atom economy, waste prevention, and the use of renewable feedstocks.

White chemistry is the latest concept that encompasses all aspects of green analytical chemistry. It integrates various dimensions of sustainability, using a color-coded, Red (R) represents analytical performance, measured through validation criteria that assess the quality of results; Green (G) focuses on safety and environmental friendliness; and Blue (B) reflects practical efficiency and productivity [20]. On the other hand, when viewed from the perspective of stages, the analytical procedure can be divided into steps such as sampling (including handling and transport), sample preparation, and determination (including data processing) [28]. Hence, based on their applicability, GAC and related metric tools can be mapped to these stages, as shown in Figure 1.

Completing the advancement of GAC, the Circular Analytical Chemistry (CAC) builds upon the principles of GAC by incorporating circular economy strategies to minimize resource depletion and waste generation. It emphasizes the reuse, recycling, and regeneration of materials throughout the entire analytical process, ensuring a sustainable and closed-loop system. Successful implementation requires collaboration among researchers, industries, and policymakers to drive systemic change and support global sustainability efforts [65]. In detail the milestones of concept and GAC metric tools are presented in Table S2.

Metric tools primarily evaluate the environmental friendliness of materials used in analytical procedures, rather than the complexity or sustainability of its material synthesis process. For example, in the BAGI metric tool, material synthesis is addressed in Section 7: Reagents and Materials, which distinguishes whether the material is synthesized or commercially available [27]. Similarly, the AGREE metric tool considers material synthesis in Section 10: Preference for reagents obtained from renewable sources, categorizing whether they are derived from renewable sources or not [22]. However, when applying metrics to assess analytical procedures, the volume of materials used and the waste generated during the synthesis process are typically excluded from the calculation. The primary focus of GAC metrics is the greenness of the analytical process itself, while the environmental impact of material synthesis is addressed within the broader scope of green chemistry. In this paper, the focus is on the application and evaluation of GAC and related metric tools that deal with analytical methods.

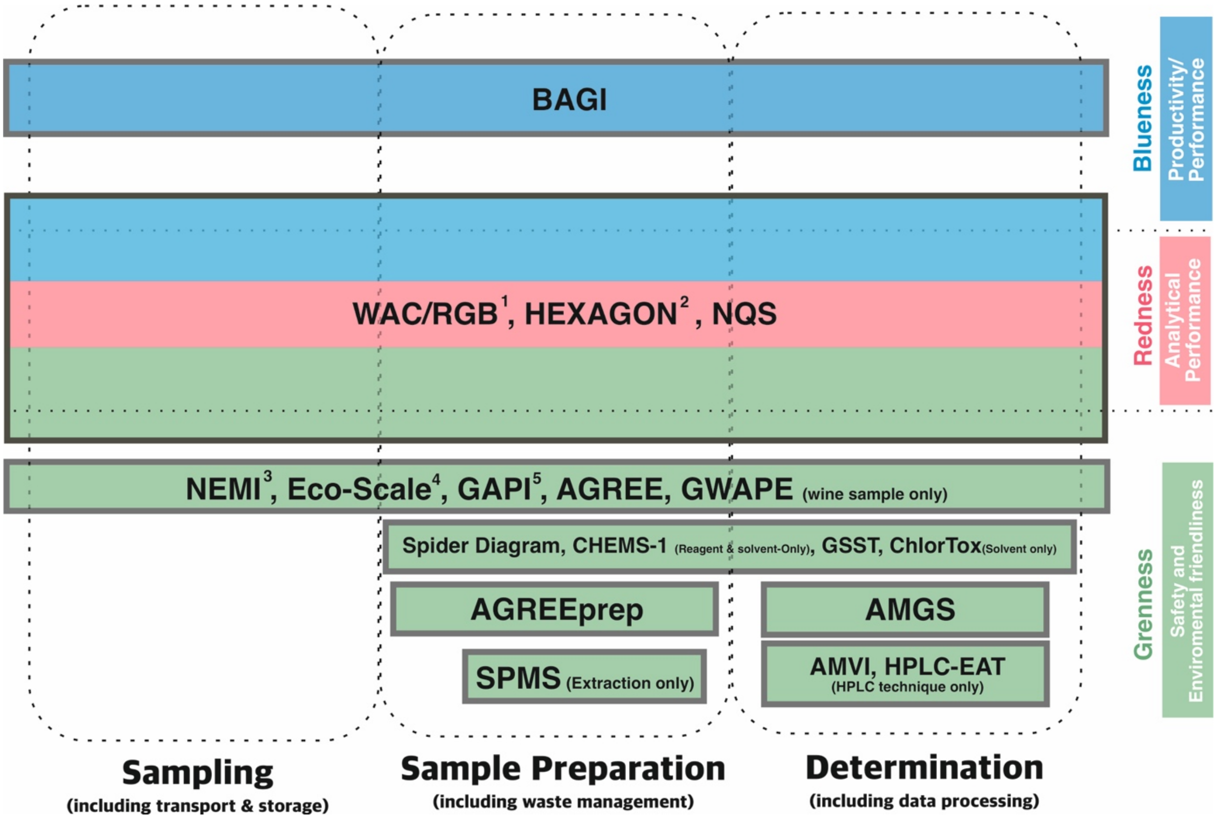

Figure 1. Positioning mapping GAC and related metric tools across Redness, Greenness, Blueness division and Sampling, sample preparation, determination. 1)**WAC/RGB** including its development such as RGB12 and RGBFast; 2)**HEXAGON** including its previous name called CALIFICAMET; 3)**NEMI** including its development Modified NEMI and Assessment of Green Profile (AGP); 4)**Eco-Scale** also known as Analytical Eco-Scale (AES) including its development called Green Certificate Modified Eco-Scale; 5)**GAPI** including its development ComplexGAPI, ModifiedGAPI (MoGAPI) and ComplexMoGAPI

Researchers have increasingly embraced the implementation of metric tools, demonstrating growing enthusiasm for their application in comparative studies. These tools have been successfully utilized to compare the determination of various analytes, contributing to the assessment of greener, more sustainable analytical practices. A growing number of publications have leveraged GAC and related metric tools to evaluate the sustainability and efficiency of analytical procedures, as presented in Supplementary Table S1.

Despite the development of many metric tools, the most used ones in comparative studies are Eco-Scale, GAPI, AGREE, and BAGI. This raises the question of whether further development of new GAC and related metric tools is necessary. Comparative studies have shown that re-evaluations conducted by both original authors and reviewers often yield different results, highlighting concerns about the reproducibility of assessments. Additionally, some studies have applied these tools incorrectly or in ways that are impractical, raising questions about their reliability. Therefore, the aim of the paper is to give evidence-supported guidelines on the proper selection of GAC and related metric tools used to fit with the goals of assessment of the user and to investigate the reproducibility of the results assessment obtained with metric tools to get more insights into their applicability.

2. Dataset

To get the information on the quality of results obtained with the assessment tools we created the test dataset consisting of assessment results for analytical procedures. The first group was used for the purpose of multivariate analysis, while the second group was utilized to assess reproducibility. The first group comprises 27 analytical procedures selected from scientific literature based on their relevance to analytical chemistry (Table 2). These procedures span various techniques, varying in complexity, sample preparation methods, and instrumentation. The selection ensures a comprehensive representation of recent trends in research and industry, covering diverse analytes and sample matrices. The second group consists of three additional analytical procedures, chosen with the same criteria as the first group and used specifically for reproducibility assessment (Table 3).

The analytical procedures in this study were assessed using 11 commonly employed metric tools for comparison. These tools are frequently referenced in the literature to evaluate analytical methods' environmental and sustainability aspects. The 11 metric tools used in this study include NEMI, GAPI, EcoScale, AGREE, AGREEprep, SPMS, ChlorTox, CHEMS-1, RGB, HEXAGON, BAGI. The assessment was performed using the original tools and guidelines provided by the publications that developed these metric tools. To minimize the bias potentially introduced by the user, all 27 protocols were assessed with a given tool by the same assessor. The results obtained with some of the tools are numbers; they were applied directly. Some loss of information was due to applying the final result without considering the scores within categories or criteria. NEMI and GAPI results are pictograms, so to obtain numerical input certain assumptions had to be made. In the case of NEMI, the green fields were counted; for GAPI, every green field got two points, while yellow got one point. Again, the information on performance within the criteria was lost.

There are also other assessment tools applied in analytical chemistry. They are not included in the assessment as their areas of application are different from the scope of the assessment. ComplexMoGAPI [29] introduces the preparation or synthesis of nonconventional materials step into the assessment, while AGREEMIP [30] is dedicated to the assessment of molecularly imprinted polymers synthesis, that may be further applied in analytical protocols. Analytical Method Greenness Score (AMGS) [31] is focused on the assessment of greenness assessment of separation, while HPLC – Environmental Assessment Tool (HPLC-EAT) [32] and Analytical Method Volume Intensity (AMVI) [33] deal with liquid chromatography (LC) separation processes. Additionally, specialized tools like GWAPE (Green Wine Analytical Procedure Evaluation) [34] have been introduced for specific applications, such as wine analysis, where the reduced use of hazardous reagents necessitates the adaptation or omission of certain green analytical chemistry criteria.

For the multivariate analysis, three assessors with prior knowledge and experience using the metric tools were selected to evaluate the 27 analytical procedures. Each procedure was assessed using all 11 metric tools to ensure a comprehensive evaluation. The resulting data were then processed using statistical software to perform Cluster Analysis, Principal Component Analysis, and Correlation Analysis. The assessment results are presented in detail in Supplementary Table S2

For the reproducibility study, five assessors, all working in analytical chemistry, were asked to use all metric tools to assess the three selected procedures. The assessors conducted the evaluations independently to ensure consistency in the reproducibility analysis. The assessment results are presented in detail in Supplementary Table S3 - S13.

Table 2. Data set of an analytical procedure for multivariate analysis

No	Analytical procedure	Analytical techniques	Analytes	Ref
1	Quantification of Cu and Zn in antifouling paint films by XRF	XRF	Copper (Cu), Zinc (Zn)	[35]
2	A novel silica supported chitosan/glutaraldehyde as an efficient sorbent in solid phase extraction coupling with HPLC for the determination of Penicillin G in water and wastewater samples	SPE + HPLC-UV	Penicillin G	[36]
3	Deep eutectic mixture membrane-based microextraction: HPLC-FLD determination of phenols in smoked food samples	Membrane Microextraction + HPLC-FLD	Phenols	[37]
4	Sensitive determination of phenylurea herbicides in soybean milk and tomato samples by a novel hypercrosslinked polymer based solid-phase extraction coupled with high performance liquid chromatography	SPE + HPLC-DAD	Phenylurea herbicides	[38]
5	Evaluation of craft beers through the direct determination of amino acids by capillary	CE UV/Vis DAD	Amino Acids	[39]

	electrophoresis and principal component analysis			
6	Optimization and validation of a SPME-GC/MS method for the determination of volatile compounds, including enantiomeric analysis, in northern highbush blueberries (Vaccinium corymbosum L.)	SPME + GC-MS	Volatile Compounds	[40]
7	Simultaneous determination of aflatoxins B1, B2, G1 and G2 in commercial rices using immunoaffinity column clean-up and HPLC-MS/MS	Immunoaffinity Column + HPLC-MS/MS	Aflatoxins (B1, B2, G1, G2)	[41]
8	Imidazolium-based task-specific ionic liquid for selective Ag, Cu, Pd and Pt determination by means of dispersive liquid-liquid microextraction and inductively coupled plasma optical emission spectrometry	DLLME + ICP-OES	Silver (Ag), Copper (Cu), Palladium (Pd), Platinum (Pt)	[42]
9	Evaluation of microwave-assisted ultraviolet digestion method for rice and wheat for subsequent spectrometric determination of As, Cd, Hg and Pb	MW-UV + (SF-ICP-MS and CVG-AAS)	Arsenic (As), Cadmium (Cd), Mercury (Hg), Lead (Pb)	[43]
10	Novel highly sensitive conductometric biosensor based on arginine deiminase from Mycoplasma hominis for determination of arginine	Conductometric Biosensor	Arginine	[44]
11	Optimization and application of ultrasonic extraction and Soxhlet extraction T followed by solid phase extraction for the determination of triazine pesticides in soil and sediment	Ultrasonic Extraction + Soxhlet + SPE	Triazine Pesticides	[45]
12	Determination of metals and trace elements in water and wastes by inductively coupled plasma-atomic emission spectrometry	ICP-AES	Metals and Trace Elements	[46]
13	Determination of total cyanide by semi-automated colorimetry	Colorimetry	Total Cyanide	[47]
14	Optimization of extraction of total trans-resveratrol from peanut seeds and its determination by HPLC	SPE + HPLC-UV	Trans-resveratrol	[48]
15	Determination of organic compounds in drinking water by liquid-solid extraction and capillary column gas chromatography/mass spectrometry	Liquid-Solid Extraction + GC-MS	Organic Compounds	[49]
16	Determination of purgeable organic pollutants in industrial discharges and other environmental samples by gas	GC/MS	Purgeable Organic Pollutants	[50]

	chromatography combined with mass spectrometry (GC/MS)			
17	Determination of 117 endocrine disruptors (EDCs) in water using SBSE TD–GC-MS/MS under the European Water Framework Directive	SBSE + TD-GC-MS/MS	Endocrine Disruptors	[51]
18	Determination of water content of crude oil by azeotropic distillation Karl Fischer coulometric titration	Azeotropic Distillation + Karl Fischer Titration	Water Content	[52]
19	Colorimetric and smartphone-integrated paper device for on-site determination of arsenic (III) using sucrose modified gold nanoparticles as a nanoprobe	µSPE-DIC	Arsenic (III)	[53]
20	A validated method for the quantitative determination of sugars in honey using high-performance thin-layer chromatography	HPTLC	Sugars in Honey	[54]
21	Determination of Cr(III) and Cr(VI) in water by dual-gel electromembrane extraction and a microfluidic paper-based device	EME + Microfluidic paper Device	Chromium (III), Chromium (VI)	[55]
22	Determination of imidacloprid and acetamiprid in bottled juice by a new DLLME-HPLC	DLLME + HPLC-DAD	Imidacloprid, Acetamiprid	[56]
23	Determination of trace elements in meat and fish samples by MIP OES using solid-phase extraction	SPE + MIP OES	Trace Elements	[57]
24	A simple strategy based on deep eutectic solvent for determination of aflatoxins in rice samples	LPME + HPLC-FLD	Aflatoxins	[58]
25	Electrochemical immunoassay for determination of glycated albumin using nanozymes	Electrochemical Immunoassay	Glycated Albumin	[59]
26	Determination of polybrominated diphenyl ethers and metabolites by single-drop microextraction and GC–MS/MS	SDME + GC-MS/MS	Polybrominated Diphenyl Ethers	[60]
27	Simultaneous determination of fuel oxygenates and BTEX using direct aqueous injection gas chromatography mass spectrometry (DAI-GC/MS)	DAI-GC/MS	Fuel Oxygenates, BTEX	[61]

*) **XRF** - X-ray fluorescence; **SPE + HPLC** - Solid-phase extraction coupled with high-performance liquid chromatography; **HPLC-FLD** - High-performance liquid chromatography and fluorescence detection; **CE UV/Vis** diode array detector - Capillary electrophoresis with ultraviolet/visible diode array detection; **SPME + GC-MS** - Solid-phase microextraction coupled with gas chromatography-mass spectrometry; **HPLC-MS/MS** - High-performance liquid chromatography-tandem mass spectrometry; **DLLME + ICP-OES** - Dispersive liquid-liquid microextraction coupled with inductively coupled plasma optical emission spectrometry; **MW-UV** - Microwave-assisted ultraviolet digestion; **SF-ICP-MS**- sector-field inductively coupled plasma mass spectrometry; **CVG-AAS -** Chemical vapor generation coupled to atomic absorption spectrometry; **ICP-AES** - Inductively Coupled Plasma Atomic Emission Spectrometry; **GC-MS** - Gas

chromatography-mass spectrometry; **SBSE + TD-GC-MS/MS** - Stir Bar Sorptive Extraction coupled with Thermal Desorption-Gas Chromatography-Mass Spectrometry; **µSPE-DIC** - Micro-solid-phase extraction coupled with digital image colorimetry; **TLC** - Thin-layer chromatography; **EME** - Electromembrane Extraction; **SPE MIP OES** - Solid-phase extraction coupled with Microwave-Induced Plasma Optical Emission Spectrometry; **LPME** – Liquid-Phase Microextraction; **SDME GC-MS/MS** - Single-drop microextraction coupled with gas chromatography-tandem mass spectrometry; **DAI-GC/MS** - Direct Aqueous Injection Gas Chromatography-Mass Spectrometry.

Table 3. Data set of analytical procedure for reproducibility analysis

No	Analytical procedure	Analytical techniques	Analytes	Ref
1	Determination of toxic heavy metals in rice samples using ultrasound-assisted emulsification microextraction combined with inductively coupled plasma optical emission spectroscopy	UAE microextraction + ICP-OES	Toxic heavy metals	[62]
2	Construction of a magnetic solid-phase extraction method for the analysis of azole pesticides residue in medicinal plants	Magnetic SPE + HPLC-UV	Azole pesticides	[63]
3	A novel magnetic molecularly imprinted polymer for selective extraction and determination of quercetin in plant samples	SPE MMIP + PAD	Quercetin	[64]

***) UAE microextraction + ICP-OES** - Ultrasound-Assisted Extraction microextraction coupled with Inductively Coupled Plasma Optical Emission Spectrometry; **Magnetic SPE + HPLC** - Magnetic Solid-Phase Extraction coupled with High-Performance Liquid Chromatography; **SPE MMIP + (PAD)** - Solid-Phase Extraction with Molecularly Imprinted Polymer coupled with paper-based analytical device

3. Cluster analysis

To understand the results obtained with all assessment tools, cluster analysis was performed to group the results according to similarity. The dataset consists of the results for n = 27 analytical procedures that are assessed with m = 11 assessment tools. The data was standardized, and variables ChlorTox, CHEMS-1, and Hexagon were transformed by multiplication with -1 to obtain the same preference function – the higher the score, the better. Then, cluster analysis was performed using the Ward method and Euclidean distance for cluster formation. The results are presented in Figure 2.

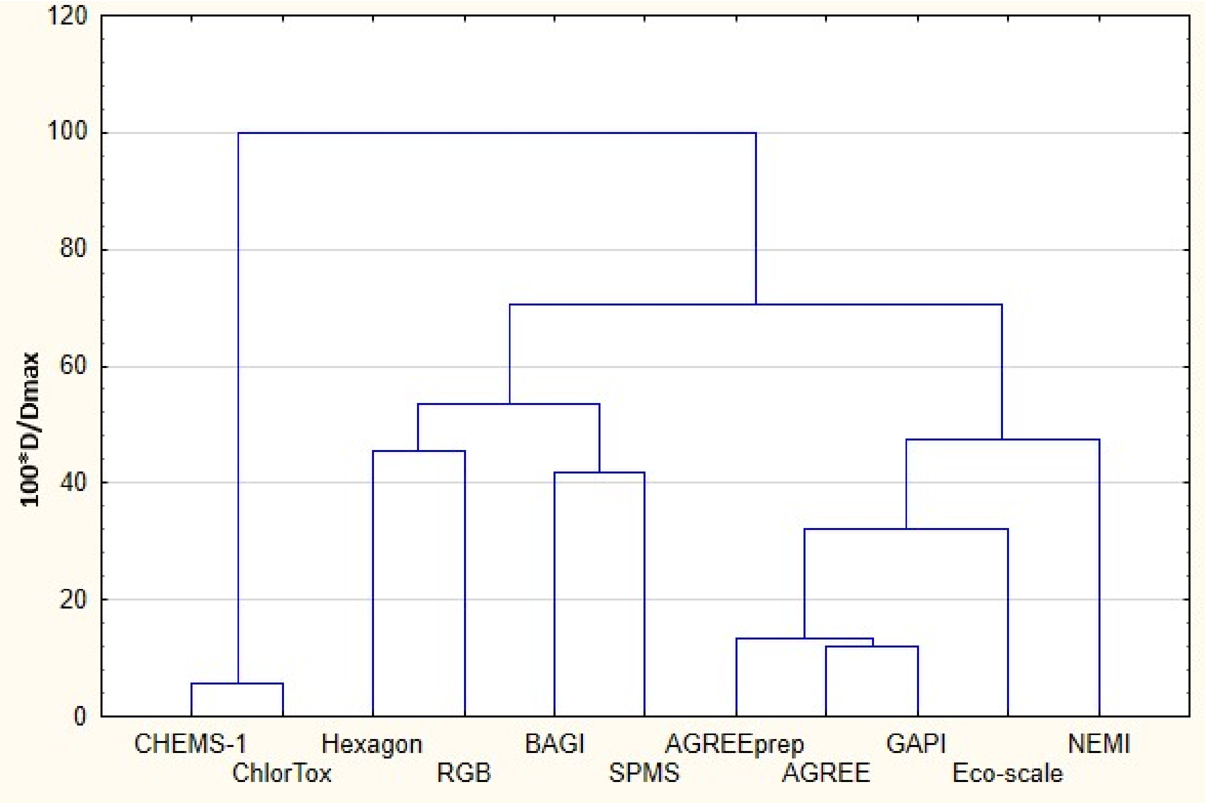

Figure 2. The results of cluster analysis of the result obtained with different assessment tools for the dataset of 27 analytical procedures.

Cluster analysis can give substantial information on similarities and dissimilarities of the results obtained with different tools. The first cluster is formed by CHEMS-1 and ChlorTox tools as they give the most similar results. The first one considers the volumes and hazards of solvents, while the second one considers the amounts and hazards of reagents applied in the analytical procedure. In contrast to other tools, they are characterized by no upper scale limit for their scores. Together with the consideration of only reagent aspects of procedures, this is why the cluster is dissimilar from all others.

The second cluster is formed by AGREE and GAPI, which are slightly more similar than AGREEprep. AGREE and GAPI are characterized by similar assessment criteria (though treated in different ways). AGREEprep is similar to them, as the assessment criteria are not much different. In contrast to the other two, it deals only with sample preparation. Since almost all of the considered procedures include sample preparation steps, and this step is considered as the most problematic in terms of greenness, AGREEprep is clustered together with AGREE and GAPI. More loosely similar to AGREE, GAPI and AGREEprep are Eco-scale and NEMI results. Both tools consider only some of the criteria included in the formerly described three; in the case of NEMI only four criteria are included in the yes-no threshold. Therefore, the results follow the general trend of other tools but with serious deviations. All five tools form the general cluster that can group the tools for greenness assessment.

The remaining four assessment procedures form a loose cluster. Hexagon and RGB form a cluster as they deal with the global assessment of procedures, so with metrological, economic, and greenness characteristics of the procedures. The second subcluster is formed by BAGI and SPMS tools; the first assesses the method's applicability, while the second only assesses the extraction step, if present in the procedure. They have some common assessment criteria, such as sample amount, time of the process, the scale of the extraction process, and parameters referring to automation degree. The loose cluster is formed by global assessment tools and SPMS.

The results of cluster analysis give hints on the application of analytical procedures assessment tools together. The tools that give similar results should not be applied together as they repeat information, it is rather better to find complementary metric tools if more than one has to be applied.

4. Principal component analysis

The principal component analysis (PCA) application on standardized dataset showed that the first three principals explain 74.73% of the initial variance of the dataset (Figure 3). The results are generally in accordance with CA results. Within PC1 and PC2, two of the most significant PCs, all assessment tools carry similar information, except ChlorTox and CHEMS-1. The assessment tools, forming a cluster referring to greenness assessment in CA, are similar according to all three PCs.

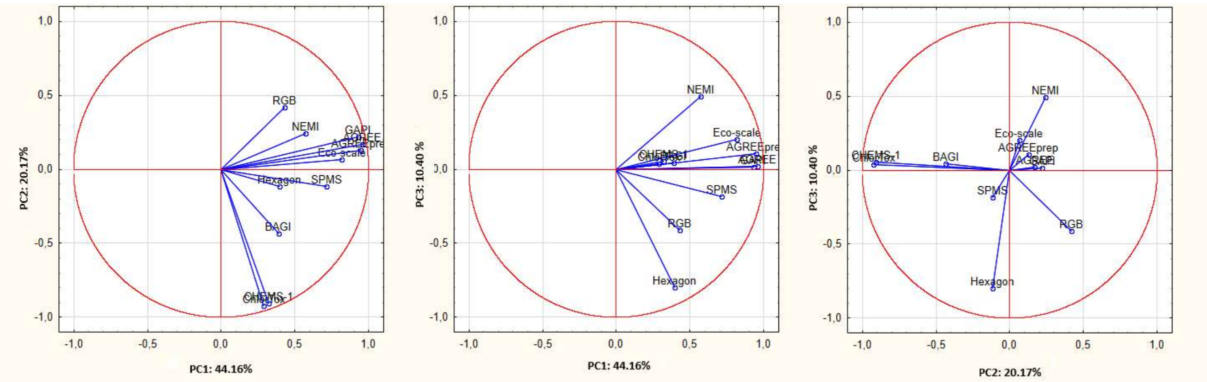

Figure 3. Variables presented in principal components planes.

5. Correlation analysis

Correlation analysis was performed to obtain further information on the dataset (Figure 4). The correlations are visualized for the raw data, so for ChlorTox, CHEMS-1, and Hexagon tools, negative correlations are expected with other tools (since the preference function is different for these three - the lower, the better). The first result is that the correlations for GAPI, AGREE, and AGREEprep are good. Eco-scale correlates with them, but to a lesser extent, which can be explained by taking into assessment a relatively limited number of factors. NEMI, on the other hand, shows a similar trend, but the correlation is low due to the limited "resolution" of the tool. By stating "resolution," it means that only five values of the final result can be obtained – 0, 1, 2, 3, or 4, which is probably not enough, in comparison to other tools, to differentiate greenness. The results obtained with RGB, Hexagon, SPMS, and BAGI do not show clear trends. It is because they have slightly different assessment criteria or the assessment results are subjected to bias.

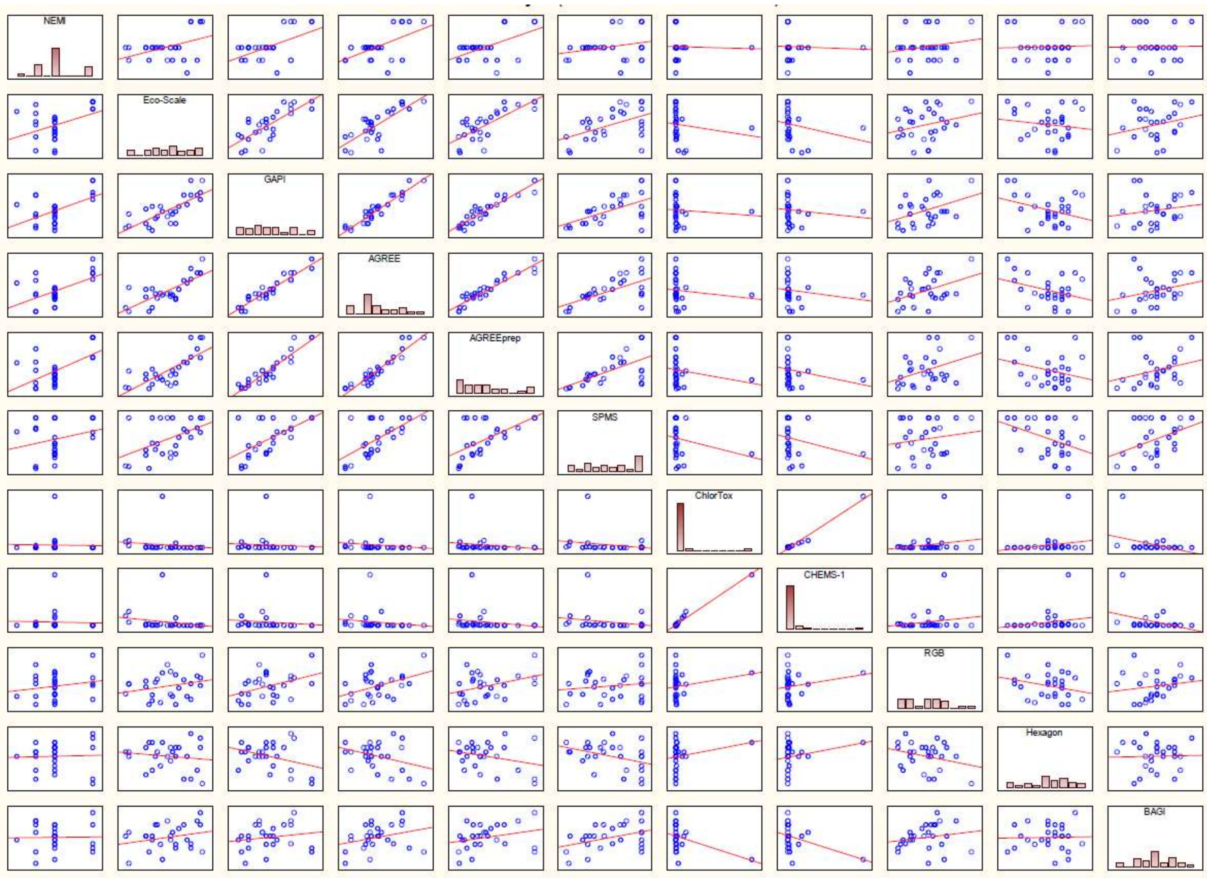

Figure 4. The correlations of the raw results obtained with all assessment tools.

6. Reproducibility assessment

Reproducibility is a fundamental aspect of scientific validity, especially in the context of analytical procedures. It refers to the ability of a method or experiment to produce consistent results when repeated under different conditions or by different individuals. The assessors, selected based on their focus on analytical chemistry, represented a diverse range of perspectives, as their analytical backgrounds and experience vary. Although all assessors were familiar with the metric tools, their level of expertise with each tool varied. To assess the reproducibility of analytical procedures the initial step involves conducting a descriptive statistical analysis. Descriptive statistics provide a general overview of variability in the scores assigned by different assessors, offering insight into how consistently the analytical methods are evaluated. Key metrics used in this context are the Mean, Standard Deviation (StD), and Coefficient of Variation (CV). These statistics help quantify the agreement or discrepancy among the assessors' evaluations. The results of assessments used in the reproducibility study are presented in Table 4.

Table 4. Descriptive Statistics for Scores Assigned by Different Assessors to Various Metric Tools (Mean, Standard Deviation, and Coefficient of Variation). For raw results see table S1.

Metric tools	Analytical procedure	Mean ± StD (n=5)	CV (%)
	Procedure 1	68.6 ± 7.2	10.44%
	Procedure 2	71 ± 12	16.72%
Eco-Scale	Procedure 3	82.4 ± 3.6	4.34%
	Procedure 1	52 ± 8.2	15.73%
	Procedure 2	55.5 ± 6.5	11.66%
BAGI	Procedure 3	53 ± 11	21.46%
	Procedure 1	4.57 ± 0.85	18.66%
	Procedure 2	5.3 ± 1.1	20.50%
SPMS	Procedure 3	6.5 ± 1.1	17.01%
	Procedure 1	0.37 ± 0.10	28.03%
	Procedure 2	0.428 ± 0.094	22.00%
AGREE	Procedure 3	0.576 ± 0.062	10.81%
	Procedure 1	16.2 ± 2.9	18.21%
	Procedure 2	14.8 ± 3.3	22.10%
HEXAGON	Procedure 3	11.4 ± 5.7	49.85%
	Procedure 1	0.15 ± 0.11	69.96%
	Procedure 2	0.28 ± 0.12	34.64%
AGREEprep	Procedure 3	0.428 ± 0.061	14.15%
	Procedure 1	51 ± 30	57.69%
	Procedure 2	46 ± 26	56.77%
RGB	Procedure 3	63.3 ± 6.8	10.75%
	Procedure 1	0.30 ± 0.21	69.72%
	Procedure 2	0.60 ± 0.38	63.19%
NEMI	Procedure 3	0.60 ± 0.45	75.69%
	Procedure 1	63 ± 114	179.58%
	Procedure 2	302 ± 220	72.94%
CHEMS-1	Procedure 3	81 ± 122	149.71%
	Procedure 1	9 ± 18	197.80%
	Procedure 2	22 ± 42	192.47%
ChlorTox	Procedure 3	2.7 ± 4.0	149.61%

As shown in Table 4, the metrics CHEMS-1 and ChlorTox exhibit the highest CVs, ranging from 149.61% to 197.80% for CHEMS-1 and 72.94% to 179.58% for ChlorTox. These high CV values indicate substantial variability in the scoring by different assessors, suggesting poor agreement and significant inconsistency in their evaluations. This might point to a lack of standardization in how assessors interpret or apply these specific metric tools, or it could reflect inherent challenges in the metrics themselves, such as complexity or subjective interpretation of certain criteria. It is also related to the scoring system that is open, no score limit is possible. Among the metric tools evaluated, Eco-Scale and BAGI stand out as having the best agreement between assessors, with Coefficient of Variation (CV) values of 10.50%, 9.81%, and 16.28%, respectively. These relatively low CV values indicate strong consistency in the assessment, suggesting that these tools are more standardized or more accessible to apply uniformly. A closer look at GAPI results presented in table S3 shows that the pictograms are not reproducible, and the transformation procedure to numbers resulted in relatively good CV values. CV cannot be calculated for GAPI as it does not have numerical final result. The pictograms presented in table S3 indicate significant variability in how assessors apply these tools, meaning their scores differ widely. This inconsistency raises concerns about the reliability and reproducibility of results when using all the tools, as the high degree of variability may indicate that the tools lack clarity, are open to interpretation, or are difficult to apply uniformly across different assessors. For instance, tools with poorer CVs may involve more subjective elements, where assessors must make judgment calls that can vary significantly based on their individual expertise, experience, or interpretation of the tool's criteria. This degree of variability may originate from a lack of precise data that is presented in the manuscripts while needed to be included as inputs into metric tools. Some aspects of analytical protocols are poorly described, like the volumes of solvents, reagents, times of different processes, and energy demands. This results in the need to make assumptions and inconsistencies in input data. Another explanation lies in the source data itself, here scientific articles. For some reported analytical protocols, not every piece of information is directly stated, and some assumptions need to be made. A closer look at the CVs obtained shows that procedure 1 generally has the highest values of CVs. This supports the statement that deviations in results at least partially originate from the source data.

Heatmap and cluster analysis were conducted to explore the similarities and tendencies in how the assessors evaluated the analytical procedures. These methods help identify patterns of agreement and divergence among the assessors' scoring behaviors (Figure 5). For Analytical Procedure 1, the analysis revealed three distinct groups of assessors with similar evaluation patterns - Group 1: Assessor 1 and Assessor 2; Group 2: Assessor 4 and Assessor 5; Group 3: Assessor 3 (who formed a separate group, indicating a more unique assessment pattern). For Analytical Procedure 2, the assessors also formed three groups, but with a slightly different clustering - Group 1: Assessor 1 and Assessor 2 (consistent with their behavior in Procedure 1); Group 2: Assessor 3 and Assessor 4; Group 3: Assessor 5 (who now stands alone in this assessment). Similarly, for Analytical Procedure 3, the same grouping as Procedure 2 was observed - Group 1: Assessor 1 and Assessor 2; Group 2: Assessor 3 and Assessor 4; Group 3: Assessor 5 (remaining separate again). The consistent pairing of Assessor 1 and Assessor 2 across all three procedures suggests that these two assessors exhibit similar rating tendencies, potentially due to shared perspectives, similar interpretations of the metric tools, or aligned evaluative criteria. On the other hand, other assessors display varying degrees of alignment depending on the procedure being evaluated, which may indicate more individualized approaches to assessment. These results indicate that uncertainty in scores is also introduced by the assessors. To improve the quality of the assessment results, it is advised to compare the newly developed procedure with previous procedures to show improvement. What is more, the analyses should be performed by the same assessor.

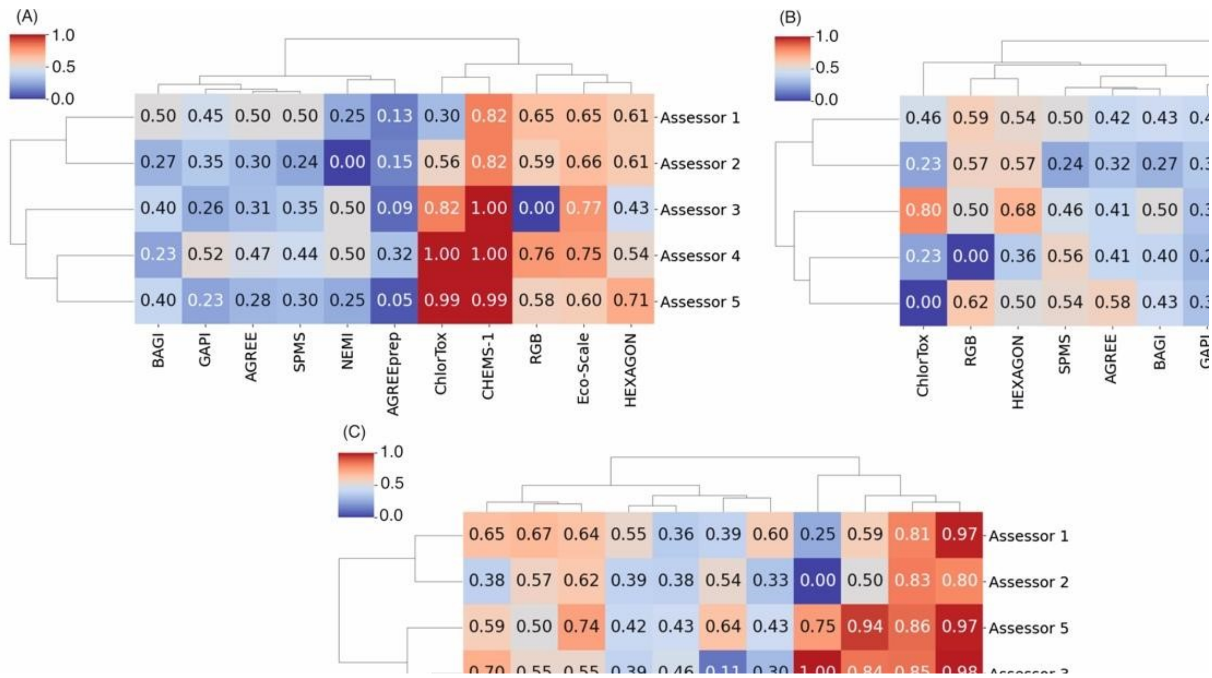

Figure 5. Dendrogram heatmaps and cluster analysis of assessments towards analytical procedure 1 (A), procedure 2 (B) and procedure 3 (C).

7. Recommendations and Guidelines

Based on the analysis and discussion, valuable insights have been generated for the further improvement of metric tools. First, the assessment process often encounters missing data or insufficient information due to incomplete descriptions in the publications. Therefore, this section presents suggestions for developing and describing novel analytical procedure protocols. Second, the results indicate that some metric tools provide similar outcomes. As a result, it is recommended that users select a combination of tools to achieve a more comprehensive assessment. Lastly, recommendations are provided for the potential development of new metric tools.

Guidelines for analytical procedure developers:

It is well-known that the development of new analytical procedures often focuses on enhancing analytical performance through method validation. Authors frequently mention replacing reagents and solvents with "greener" alternatives. However, detailed information regarding the waste generated, the volume of reagents used is frequently missing. Therefore, to better align the development with GAC principles, the CLEAN-S Protocol for developing analytical procedures is suggested.

C Clarity in procedure description

Apart from performing validation methods, a novel analytical procedure should be described with clear, detailed information, ensuring that not only it can be replicated by the other researcher also includes explaining the specific improvements made reflecting 12 GAC principles.

L Lifecycle Waste Reduction

The generation of minimal waste should be a key consideration and described in detail for both single analyses and daily routines. This aspect is often overlooked. It would be helpful if authors provided specific information on the waste generated at each stage of the analysis, including the information if the materials and auxiliaries can be reused or are single use.

E Energy Efficiency

The *Never Waste Energy* principle should be emphasized. Provide details on energy consumption at each stage of analysis and demonstrate how energy usage has been minimized throughout the process.

A Analysis Time

The time required for each stage of analysis, as well as how many samples can be analyzed in each time frame, should be included to assess the efficiency and practicality of the procedure for routine use.

N Non-Toxic Reagents and Solvents

The toxicity of reagents and solvents should be minimized. Authors should clearly describe the toxic characteristics of the chemicals used and detail efforts made to eliminate or replace hazardous substances with safer, non-toxic alternatives.

S Safety Considerations

The safety of the operator and others involved in the analysis should be addressed. Improving safety conditions for analysts is an important part of advancing a new analytical procedure. Including the exposure of chemical and possible contaminants toward the operator.

Simple GUIDELINES for metric tools users

In this section, the simple **GUIDELINES** provide a practical framework for effectively selecting, applying GAC and related metric tools is suggested presented below:

G Gather Information

Before applying any metric tool, take time to understand the underlying logic, algorithms, and principles that drive the assessment. Reviewing the documentation thoroughly helps ensure accurate application and meaningful interpretation of results.

U Use the right tools

Pick the appropriate metric tool for the specific task at hand, depending on the goal of the analysis (see Figure 1).

I Implement Comparisons

A single analysis is not sufficient. Always compare the developed procedure with previously published and/or standard methods. This comparison should evidence progress in terms of greenness and related factors.

D Do not duplicate

Many metric tools provide similar information. Avoid using multiple tools that serve similar purposes. Instead, choose tools designed for different analysis goals. Grouping of similar tools based on the data:

Greenness: Eco-scale, GAPI, and AGREE

Sample preparation : AGREEprep and SPMS

Reagent/solvent : CHEMS-1 and ChlorTox

Global assessment : RGB model, Hexagon, and BAGI

Assessment using multiple metric tools which have the same parameter would not bring new perspective.

E **Ensure consistency**

Maintain reliability by having a single assessor handle evaluations to reduce variability and enhance credibility.

L **Learn by testing**

Conduct test runs with sample data to get comfortable with the metric tool's operation before full application.

I **Improve with training**

Participate in any available training sessions, webinars, or workshops provided by the tool developers to deepen your understanding

N **Navigate Available Options**

Do not rely on similar tools repeatedly. Always assess whether different tools offer distinct insights to avoid unnecessary overlap.

E **Examine Reports Critically**

Reviews should go beyond superficial comparisons; they must include clear, focused recommendations for the future development of analytical techniques.

S **Summarize Key Findings**

Many recent review articles in the literature focus on applying metric tools to compare analytical protocols. However, many of these reviews lack clear, actionable conclusions. Reviews should provide detailed guidelines for improving analytical protocols and highlight which techniques show promise for further development. Without such insights, these reviews risk being perceived as repetitive and offering limited value to the analytical community

Recommendation for possibly new development of GAC and related metric tools

Researchers are continually creating new metric tools, often marketing them as innovative advancements. However, these developments are unlikely to offer significant breakthroughs. New tools typically introduce only minor changes in visual design or slight adjustments in their algorithms. Rather than focusing on creating new tools, the real progress lies in the practical application of existing tools. This approach is more likely to enhance the understanding and highlight key improvements in the development of novel analytical procedures.

A more promising development would be the creation of an assessment system based on life-cycle analysis. There is also a space for the development of tools for assessing the techniques or materials that do not fall into the analytical mainstream and consequently require special approach.

8. Conclusions

The article gives evidence-supported guidelines on applying greenness metrics and related tools. The metric tool has to be properly selected to meet the needs of the analysis. The results of the application of metric tools are generally in agreement, so the application of more than one tool has to be carefully considered. Their application to evaluate newly developed procedures has to be reasonably conducted; comparison with previous protocols is especially significant. For the first time, reproducibility of the results is discussed. Inconsistencies in the results obtained by different assessors appear, and they may be related to misunderstanding of tool input data or lack of proper data in the manuscript describing analytical protocol and resulting in wrong assumptions. The reproducibility study gives insights into the quality of results, but it should be further studied.

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Conflict of Interest

No conflict of interest to declare

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