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Review paper

Green analytical chemistry metrics for evaluating the greenness of analytical procedures

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A B S T R A C T

Green analytical chemistry (GAC) focuses on mitigating the adverse effects of analytical activities on human safety, human health, and environment. In addition to the 12 principles of GAC, proper GAC tools should be developed and employed to assess the greenness of different analytical assays. The 15 widely used GAC metrics, i.e., national environmental methods index (NEMI), advanced NEMI, assessment of green profile (AGP), chloroform-oriented toxicity estimation scale (ChlorTox Scale), Analytical Eco-Scale, Green Certificate Modified Eco-Scale, analytical method greenness score (AMGS), green analytical procedure index (GAPI), ComplexGAPI, red-green-blue (RGB) additive color model, RGB 12 algorithm, analytical greenness calculator (AGREE), AGREE preparation (AGREEprep), HEXAGON, and blue applicability grade index (BAGI), are selected as the typical tools. This article comprehensively presents and elucidates the principles, characteristics, merits, and demerits of 15 widely used GAC tools. This review is helpful for researchers to use the current GAC metrics to assess the environmental sustainability of analytical assays.

1. Introduction

Green chemistry (GC) has received global attention in recent decades owing to its role in promoting sustainable practices in laboratories and industries [1–5]. Anastas and Warner [6] articulated 12 principles of GC, which provide guidance and a framework for effectively implementing sustainable practices in related fields [6,7] (Fig. S1A). Green analytical chemistry (GAC) has emerged as a new approach to mitigate the adverse effects of analytical activities on the environment, human safety, and human health [8–10]. To effectively implement GAC, it is crucial to adopt key measures. These measures include the use of less toxic solventless extraction techniques, the miniaturization of sample processing technologies, and the application of environmentally friendly detection instruments [11,12].

Generally, GAC serves as a catalyst for advancing analytical chemistry [13]. The primary challenge of GAC is to balance the reduction of the adverse effects of analytical procedures on the environment with the improvement of the quality of analysis results [14]. Guidelines and principles of GAC are crucial for effectively addressing these challenges. Gałuszka et al. [15] revised the 12 principles of GC by selecting four from the original set and incorporating eight additional principles to formulate the 12 principles of GAC (Fig. S1B). The 12 principles of GAC include various aspects of analytical methods. These principles serve as crucial guidelines for implementing greener practices in corresponding analytical procedures [16]. Notably, these principles can be represented by the mnemonic significance. Additionally, López-Lorente et al. [17] proposed 10 principles of green sample preparation (GSP) (Fig. S1C). These GSP principles serve as a road map for evaluating the greenness of different sample preparation methods.

From the perspective of GAC, assessing the greenness of different analytical procedures is vital. However, owing to the diverse nature of analytical procedures and their specific requirements, the universal applicability of the 12 GAC principles to these procedures may be limited [18]. Additionally, certain undesirable analytical procedures may be unavoidable in various conditions. Therefore, in the GAC field, it is crucial to evaluate the greenness of different analytical procedures and significantly minimize the negative effects of analytical assays on the environment and human safety [19].

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Traditional GAC metrics are often unsuitable for evaluating the greenness of analytical assays [19,20]. To address this limitation, numerous greenness metrics have been developed and applied in the GAC field. The number of published papers related to these GAC tools is shown in Fig. 1. The information about the related GAC metrics is summarized in Table S1 [21–39].

Some GAC metrics are developed for specific analytical assays, while other metrics are universally applicable across most analytical assays [40]. National environmental methods index (NEMI), advanced NEMI, assessment of green profile (AGP), Analytical Eco-Scale, Green Certificate Modified Eco-scale, analytical method greenness score (AMGS), red-green-blue (RGB) additive color model, RGB 12 algorithm, green analytical procedure index (GAPI), ComplexGAPI, blue applicability grade index (BAGI), analytical greenness calculator (AGREE), HEXAGON, AGREE preparation (AGREEprep), and chloroform-oriented toxicity estimation scale (ChlorTox Scale) are widely used in the GAC field (Fig. S2). These metrics apply to a wide range of analytical methods [41–44]. Therefore, in this article, 15 developed metrics have been selected as representative tools in the GAC field.

This article presents and elucidates the principles, merits, limitations, and practical examples of using GAC metrics to assess the greenness of different analytical assays. Additionally, it highlights the current needs and future perspectives of GAC tools and provides valuable insights into the current GAC metrics.

2. NEMI, advanced NEMI, and AGP

In 2002, NEMI was developed by the Methods and Data Comparability Board (MDCB) [45]. It is one of the oldest GAC metrics. The website of NEMI is http://www.nemi.gov [22]. NEMI can be used as a searchable database. Its pictogram is a circle which has four parts and each part represents a different criterion (Fig. 2A) [19]. The respective field of NEMI pictogram is labeled with green color when the criterion value is met. If not, the corresponding part is uncolored. The first part of NEMI label can be colored green with the requirement that the chemicals used in the corresponding analytical procedure are not present on the persistent, bioaccumulative, and toxic chemicals (PBT) list [46]. The second part of NEMI label can be marked with green if none of the solvents employed in the corresponding analytical procedures is hazardous and present on D, F, P, or U hazardous wastes lists. The third part of NEMI label can be labeled with green color with the requirement that the pH of the sample is between 2 and 12. In this case, corrosive effect to the environment can be avoided. The fourth part of NEMI label can be marked with green color with the requirement that the amount of waste produced is no more than 50 g.

NEMI has numerous merits as one of the oldest greenness metric systems [45]. NEMI is a simple GAC metric. Moreover, immediate and general information concerning the impacts of corresponding analytical procedures on environment could be obtained just by a glance at the NEMI symbol. However, there are also some limitations for application of NEMI in GAC field. For instance, the information provided by NEMI is general. Its searching process and operation process are relative time consuming [47]. Furthermore, NEMI symbol can only provide qualitative information. It is not a semi-quantitative or quantitative tool. The greenness of three different analytical assays is evaluated by NEMI. The first analytical assay is an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method coupled with liquid-liquid extraction to determine guaifenesin and bromhexine in human plasma [48]. The second method is a high performance

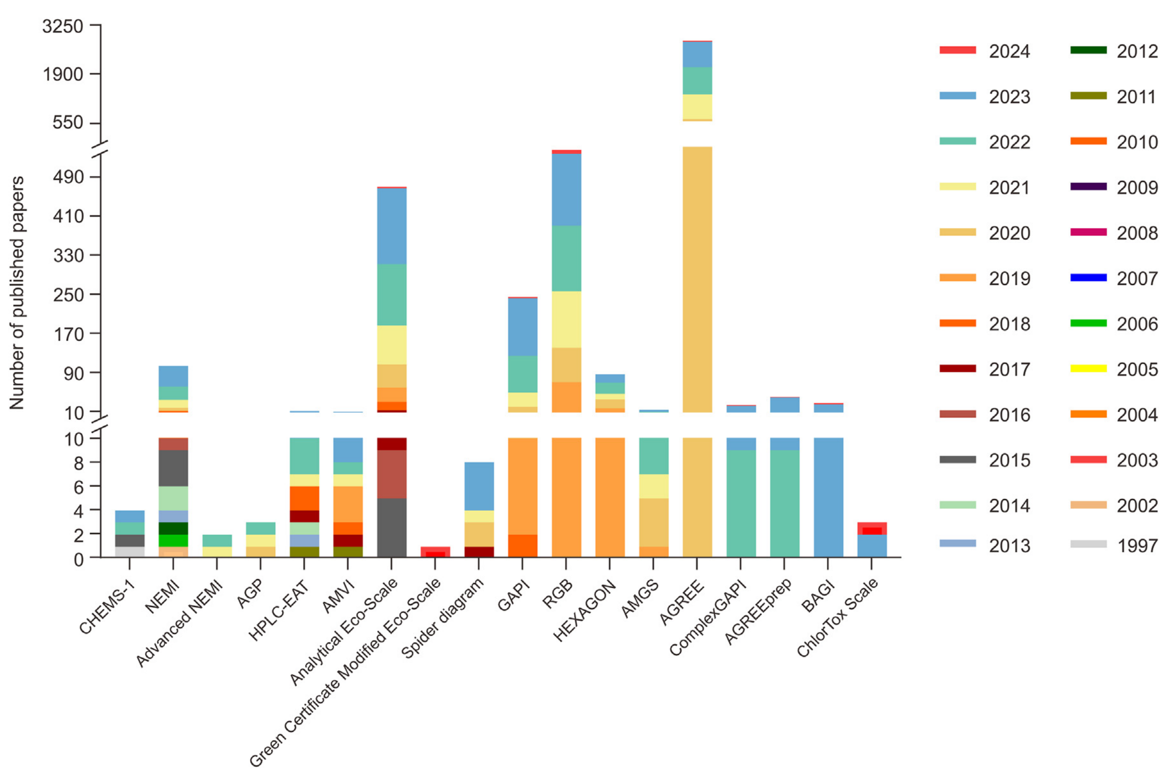

Fig. 1. The number of published papers that related to these green analytical chemistry (GAC) tools up till the present moment. The keywords searched in the database of Web of Science are the corresponding metrics and analytical methods. CHEMS-1: chemical hazard evaluation for management strategies; NEMI: national environmental methods index; AGP: assessment of green profile; HPLC-EAT: high performance liquid chromatography-environmental assessment tool; AMVI: analytical method volume intensity; GAPI: green analytical procedure index; RGB: red-green-blue; AMGS: analytical method greenness score; AGREE: analytical greenness calculator; AGREEprep: AGREE preparation; BAGI: blue applicability grade index; ChlorTox Scale: chloroform-oriented toxicity estimation scale.

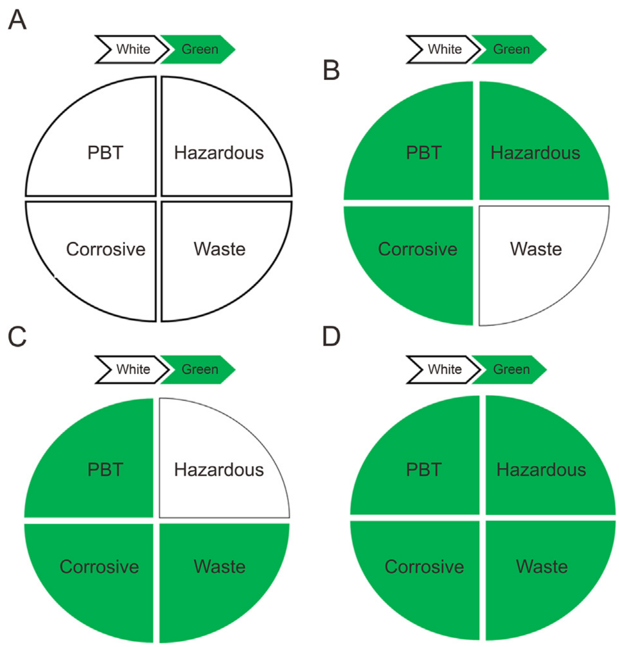

Fig. 2. The typical output pictogram of national environmental methods index (NEMI) and the examples of using NEMI for assessing the greenness of some selected analytical procedures. (A) The typical output pictogram of NEMI [19]. (B–D) Examples of using NEMI for assessing the greenness of some selected analytical procedures: determination of guaifenesin and bromhexine in human plasma by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) coupled with liquid-liquid extraction (B), determination of oxytetracycline and bromhexine in spiked milk samples by high performance liquid chromatography-ultraviolet (HPLC-UV) (C), and simultaneous quantitative analysis of carbinoxamine maleate, paracetamol, and pseudoephedrine hydrochloride in their pure form and marketed combination by ultraviolet (D). PBT: persistent, bioaccumulative, and toxic chemicals. Reprint from Ref. [19] with permission.

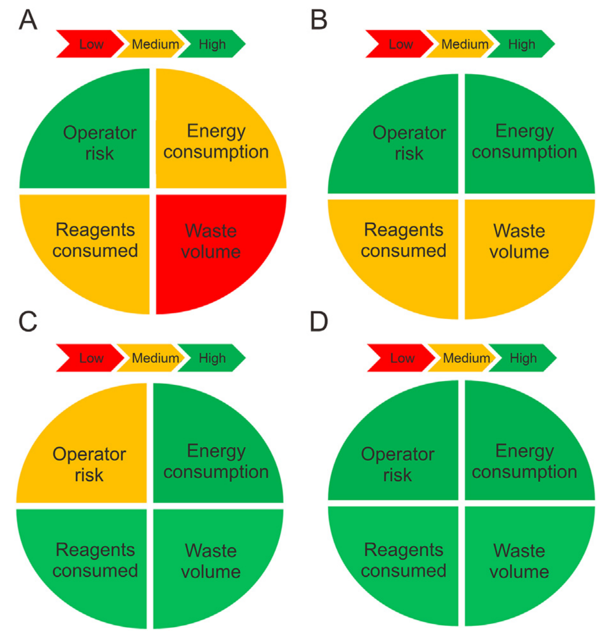

Fig. 3. The typical output pictogram of advanced national environmental methods index (advanced NEMI) and the examples of using advanced NEMI for assessing the greenness of some selected analytical procedures. (A) The typical output pictogram of advanced NEMI [16]. (B–D) Examples of using advanced NEMI for assessing the greenness of some selected analytical procedures: determination of guaifenesin and bromhexine in human plasma by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) coupled with liquid-liquid extraction (B), determination of oxytetracycline and bromhexine in spiked milk samples by high performance liquid chromatography-ultraviolet (HPLC-UV) (C), and simultaneous quantitative analysis of carbinoxamine maleate, paracetamol, and pseudoephedrine hydrochloride in their pure form and marketed combination by UV (D). Reprint from Ref. [16] with permission.

liquid chromatography-ultraviolet (HPLC-UV) assay to determine oxytetracycline and bromhexine in spiked milk samples [49]. The third assay is an UV method without chromatographic separation for simultaneous quantification of carbinoxamine maleate, paracetamol, and pseudoephedrine hydrochloride in their pure form and marketed combination [50]. These tested assays used different sample preparation procedures and different detection instruments. The results evaluated by NEMI are shown in Figs. 2B–D and Table S2 [48–50]. The results indicated that the greenness of the third assay with more detected analytes, higher sample throughput, and less run time is better.

Researchers improved NEMI with the purpose to increase the quantitative capability of this GAC metric. This metric is called advanced NEMI [23]. A color scale of green, yellow, and red is employed in advanced NEMI to reveal the greenness of related analytical procedure (Fig. 3A) [16]. This enhancement provides the advanced NEMI with quantitative capabilities and expands its perspective on evaluating analytical methods. The greenness of the above three different analytical assays is also evaluated by advanced NEMI. The results are shown in Figs. 3B–D and Table S2 [48–50].

NEMI was further improved as a new metric called AGP [51]. Compare to the original NEMI, the AGP is divided into five sections to assess the greenness of the analytical process with regard to safety, health, energy, waste, and the environment [24]. The pictogram of AGP is shown in Fig. 4A [30]. Each section's green rating is determined by reference to National Fire Protection Association (NFPA) scores and specified dosage ranges, which are visually represented on the pictogram using three different colors [52]. The greenness of the above three different analytical assays is also evaluated by AGP. The results are shown in Figs. 4B–D and Table S2 [48–50].

3. Analytical Eco-Scale and Green Certificate Modified Eco-Scale

Analytical Eco-Scale, proposed in 2012, is a widely used GAC tool [27]. The GAC metric operates on the principle of assigning a total score of 100 points for an ideal green analysis. Penalty points are subtracted based on the amounts of solvents or reagents, energy consumption, hazards, and the quantity of waste produced during the analysis [53]. To be considered an "ideal green analysis", an analytical method must meet three conditions. First, the solvents or reagents used in the analytical procedures should not pose any health, environmental, or physical hazards. Second, the energy consumed for each sample should be less than 0.1 kWh. Third, no waste should be produced during analytical procedures. However, only a limited number of analytical methods fully meet the criteria of "ideal green analysis" [54]. Only a few direct analytical assays without sample processing are qualified as ideal green analyses [55].

The negative effects of hazardous substances depend on their quantity. Therefore, the total penalty points for an analytical procedure can be calculated by multiplying the sub-total penalty points corresponding to the specified hazard and amount. The cumulative penalty points for the entire analytical assay are included in the Eco-Scale calculation. The Analytical Eco-Scale score is calculated as 100 minus total points. Conversely, under the Globally Harmonized

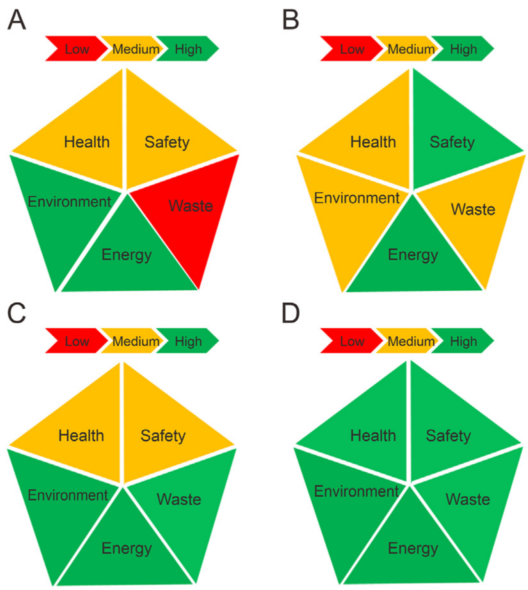

Fig. 4. The typical output pictogram of assessment of green profile (AGP) and the examples of using AGP for assessing the greenness of some selected analytical procedures. (A) The typical output pictogram of AGP [30]. (B–D) Examples of using AGP for assessing the greenness of some selected analytical procedures: determination of guaifenesin and bromhexine in human plasma by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) coupled with liquid-liquid extraction (B), determination of oxytetracycline and bromhexine in spiked milk samples by high performance liquid chromatography-ultraviolet (HPLC-UV) (C), and simultaneous quantitative analysis of carbinoxamine maleate, paracetamol, and pseudoephedrine hydrochloride in their pure form and marketed combination by UV (D). Reprint from Ref. [30] with permission.

System of Classification and Labeling of Chemicals (GHS), penalty points may be assigned based on the solvents used in analytical procedures [56]. Different solvents or reagents may have different numbers of pictograms and signal words. GHS uses nine pictograms (such as corrosion, flame, skull, crossbones, flame over a circle, exclamation mark, gas cylinder, environment hazard, and health hazard) and two signal words (danger and warning) to indicate the hazards associated with chemicals [56]. According to the principles of this metric, the number of the pictograms is related to the penalty points. Each pictogram earns one penalty point. For example, acetonitrile has two penalty points owing to its two pictograms, while benzoic acid has one penalty point owing to its one pictogram. In GHS, “danger” and “warning” serve as the two signal words. For danger, the hazard penalty points are calculated by multiplying the number of the pictograms by two. For warning, the hazard penalty points are determined by multiplying the number of pictograms by one. The penalty points calculated from the signal word and the number of pictograms is then multiplied by the penalty points associated with the quantity of the solvent or reagent. The formula for calculating the total penalty points of the solvent is shown as follows: total penalty points of solvent = number of pictograms × signal word × penalty points of the quantity of solvent or reagent. In this formula, the penalty point of the quantity of solvent or reagent is determined as follows. If the amount of solvent or reagent is less than 10 mL or 10 g, the penalty point of the quantity of solvent or reagent is 1. If the amount of solvent or reagent is more than 10 mL or 10 g and less than 100 mL or 100 g, the penalty point of the quantity of solvent or reagent is 2. If the amount of solvent or reagent is more than 100 mL or 100 g, the penalty point of the quantity of solvent or reagent is 3. The penalty point for energy consumption during analytical procedures is assigned based on the following criteria. If the energy consumption is below 0.1 kWh for each sample, the penalty point is 0. If the energy consumption exceeds 0.1 kWh and is below or equal to 1.5 kWh for each sample, the penalty point is 1. If the energy consumption exceeds 1.5 kWh for each sample, the penalty point is 2 [57]. The penalty point for the occupational hazard during analytical procedures is assigned based on the following criteria. If any vapors or gases are released into the environment during analysis, three penalty points should be assigned. If no vapors or gases is released into the environment during analysis, no penalty point should be subtracted. The penalty point for generated waste during analytical procedures is assigned based on the following criteria. If the amount of waste generated is below 1 mL or 1 g, the penalty point is 1. If the amount of waste generated exceeds 1 mL or 1 g but is below 10 mL or 10 g, the penalty point is 3. If the amount of waste generated exceeds 10 mL or 10 g, the penalty point is 5. The penalty point for treating the waste generated during analytical procedures is assigned based on the following criteria. If the generated waste is recycled, the penalty point is 0. If the generated waste is degraded, the penalty point is 1. If the generated waste is passivated, the penalty point is 2. If the generated waste is not processed, the penalty point is 3. The final total calculated score of this metric can be ranked on a scale. Each score value has a distinct meaning. An analytical method with a score above 75 indicates an excellent level of greenness in the assay. An analytical method with a score ranging from 50 to 75 indicates an acceptable level of greenness in the assay. A score below 50 suggests inadequate greenness in the assay [58].

Generally, the Analytical Eco-Scale, a widely used GAC metric, exhibits several advantages. First, this metric enables the quantitative assessment of the impacts of the corresponding analytical assay on the environment and human health. Second, the metric is simple. Moreover, the metric provides easily obtainable calculated scores and facilitates result comparisons between different analytical assays. However, Analytical Eco-Scale also has some disadvantages. For example, the total calculated score of the GAC metric cannot provide detailed information about the structure of the hazards [59]. Additionally, the final score of the metric does not provide insights into the causes of the impacts of analytical assays on the environment. Gallart-Mateu et al. [28] improved this GAC metric and proposed Green Certificate Modified Eco-Scale (Fig. 5A) [28]. Eco-scale values were categorized into seven levels (i.e., A–G) and visually represented by different colors [60]. The examples of using the two GAC metrics to evaluate the greenness of some selected analytical methods are summarized and shown in Figs. 5B–D and Tables S3 [61], S4 [62], and S5 [63].

4. GAPI and ComplexGAPI

GAPI was proposed by Płotka-Wasylka [30]. The greenness of different stages of entire analytical assay can be evaluated and revealed by the five pentagrams of GAPI pictogram [64]. A color scale with three different levels (green, yellow, and red colors indicate low, medium, and high impact of the analytical method on the environment and human healthy, respectively) is applied in GAPI pictogram to reveal the greenness of different stages of the entire analytical method [65]. Just like NEMI metric, each pentagram of GAPI symbol represents a different part of the corresponding analytical assay. The related part should be marked with green color if the specific conditions are fulfilled. Otherwise, the related part of the pentagram is marked with yellow or red color which means moderate or high impact of the related analytical assay on human health or environment [66]. Generally, this GAC metric is an excellent semi-quantitative tool.

GAPI symbol is composed of five pentagrams (Fig. 6A) [30]. The five pentagrams are associated with different steps of the analytical assay [67]. The detailed description of the parameters used in GAPI is

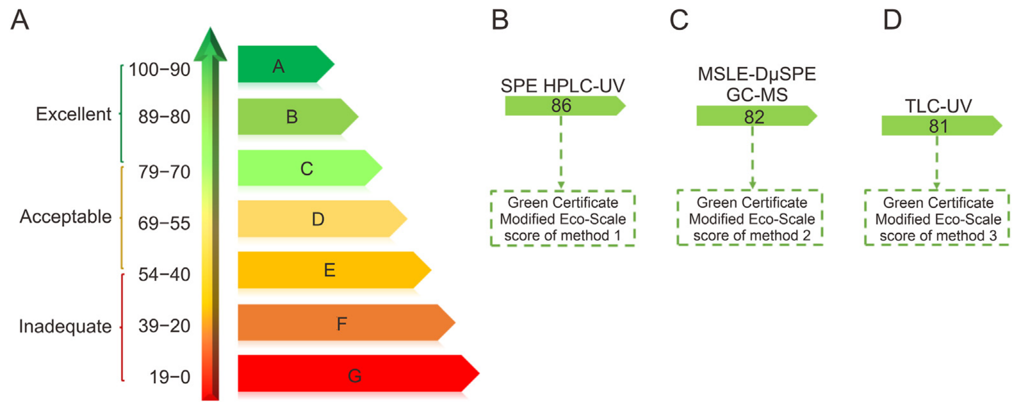

Fig. 5. The typical output pictogram of using both Analytical Eco-Scale and Green Certificate Modified Eco-Scale to assess the greenness of three selected analytical methods. (A) The typical output pictogram of Green Certificate Modified Eco-Scale [28]. (B–D) Examples of using both Analytical Eco-Scale and Green Certificate Modified Eco-Scale for assessing the greenness of some selected analytical procedures: a green high performance liquid chromatography-ultraviolet (HPLC-UV) method coupled with solid phase extraction (SPE) as sample processing procedure for the detection of sulfonamides residues in different animal-origin foods (B), a microscalec solid-liquid extraction (MSLE) assay using a miniaturized device combined with cleanup via dispersive micro-solid-phase extraction (DμSPE) for determination of *n*-alkanes in marine sediments by gas chromatography-mass spectrometry (GC-MS) (C), and a thin-layer chromatography-ultraviolet (TLC-UV) assay for simultaneous determination of norfloxacin and tinidazole (D). Reprint from Ref. [28] with permission.

illustrated in Table S6. The first pentagram of GAPI pictogram has four parts and it is associated with sampling. The first part marked with number 1 is associated with sample collection. This part is marked with green, yellow, and red colors corresponding with the sample collection is in-line, on-line or at-line, and off-line, respectively. The second part marked with number 2 is associated with sample preservation. This part is marked with green, yellow, and red colors corresponding with the sample preservation is not needed, chemical or physical preservation is required, and both chemical and physical preservation are needed, respectively [68]. The third part marked with number 3 is associated with sample transportation. This part is marked with green and yellow colors corresponding with the sample transportation is not needed and sample transportation is needed, respectively. The fourth part marked with number 4 is

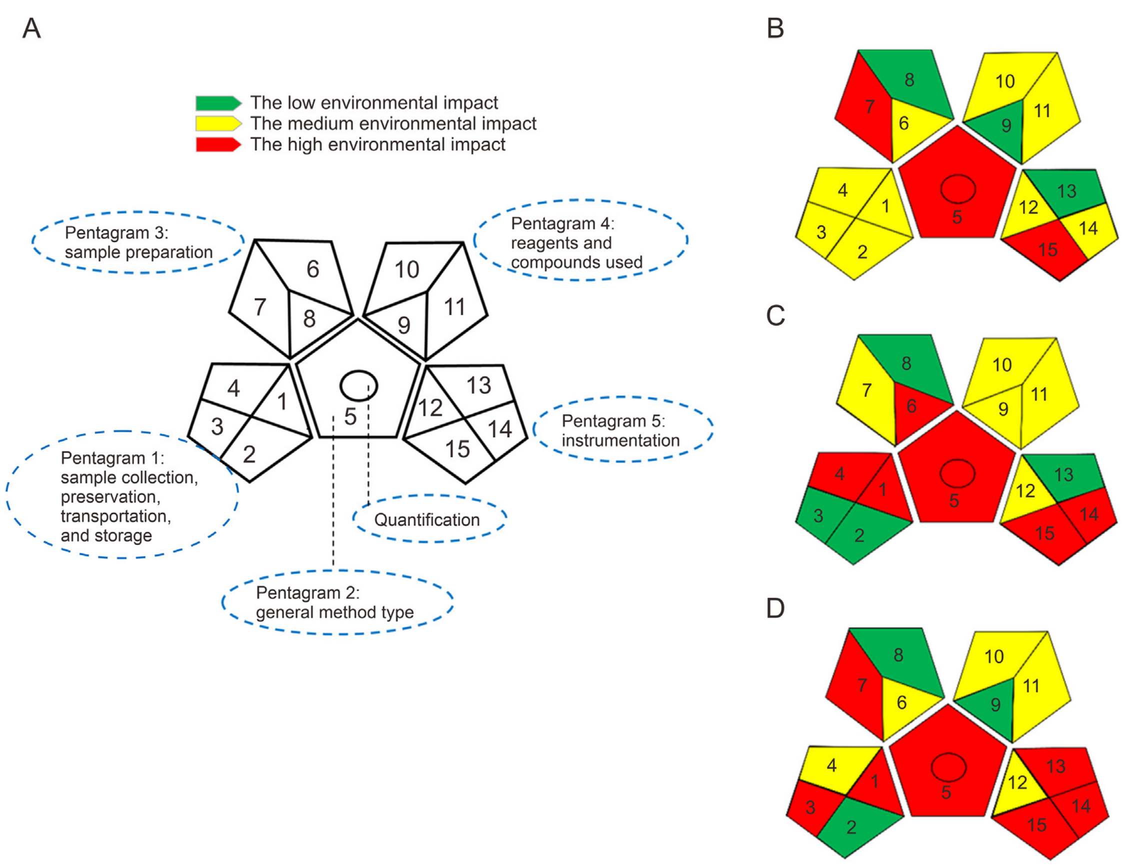

Fig. 6. The typical output pictogram of green analytical procedure index (GAPI) and the examples of using GAPI for assessing the greenness of some selected analytical procedures. (A) The typical output pictogram of GAPI [30]. (B–D) Examples of using GAPI for assessing the greenness of some selected analytical procedures: monitoring of guaifenesin and bromohexine hydrochloride in human plasma by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) (B), determination of bromhexine and oxytetracycline residues in milk by high performance liquid chromatography-ultraviolet (HPLC-UV) (C), and determination of polycyclic aromatic hydrocarbons (PAHs) in real water samples using gas chromatography (GC) with flame ionization detection (FID) (D). Reprint from Ref. [30] with permission.

associated with sample storage. This part is marked with green, yellow, and red colors corresponding with the sample storage is not needed, sample storage is under normal conditions, and sample storage is under special conditions, respectively. The second pentagram of GAPI pictogram has only one part. This part marked with number 5 is related to the type of the analytical method. It is marked with green, yellow, and red colors corresponding with the sample processing is not needed, simple sample processing is needed, and extraction procedure is further needed for sample processing, respectively. Furthermore, a circle inside the GAPI pentagram could be used to demonstrate the performance of the analytical assay. If the circle is placed inside the pentagram, which means the analytical assay could be used to both qualitative and quantitative analyses of target compounds. Otherwise, if the method can be only used to qualitative analysis of chemicals, the circle will not be placed inside the pentagram [69]. The third pentagram of GAPI pictogram has 3 parts and it is associated with different stages of sample processing. The first part marked with number 6 is associated with the scale of extraction. This part is marked with green, yellow, and red colors corresponding with the extraction procedure is performed at nano-scale, micro-scale, and macro-scale, respectively. Meanwhile, the second part marked with number 7 is associated with the nature of the reagent or solvent used in the extraction stage. This part is marked with green, yellow, and red colors corresponding with the assay is solventless or solvent-free, green solvents are used, and non-green solvents are used in the extraction step, respectively. The third part marked with number 8 is associated with the additional sample processing. This part is marked with green, yellow, and red colors corresponding with none of additional sample preparation procedure is needed, simple additional sample preparation procedure is needed, and advanced or complicated additional sample preparation procedure is needed, respectively. The fourth pentagram of GAPI pictogram has 3 parts and it is associated with the volume or quantity of solvents and the safety and health hazards of the employed reagents. The first part marked with number 9 is associated with the volume or quantity of the solvents used in the analytical assay. This part is marked with green, yellow, and red colors corresponding with the amount or volume of the solvents is less than 10 g or 10 mL, larger than 10 g or 10 mL and less than 100 g or 100 mL, and larger than 100 g or 100 mL, respectively. The second part marked with number 10 is related to the health hazard of the reagents employed in analytical procedure. This part is marked with green color corresponding with the toxicity of the solvents or reagents is low and the NFPA health hazard related score of these solvents is 0 or 1. This part is marked with yellow color corresponding with the toxicity of the solvents or reagents is moderate and the NFPA health hazard score of these solvents is 2 or 3. This part is marked with red color corresponding with the toxicity of the solvents or reagents is high and the NFPA health hazard score of these solvents is 4. The third part marked with number 11 is related to the safety hazard of the reagents employed in the analytical procedure. This part is marked with green color corresponding with the value of NFPA instability or flammability score of these solvents is 0 or 1. This part is marked with yellow color corresponding with the value of NFPA instability or flammability score of these solvents is 2 or 3. This part is marked with red color corresponding with the value of NFPA instability or flammability score of these solvents is 4. The fifth pentagram of GAPI pictogram has 4 parts and it is associated with energy consumption by the instruments, occupational hazards, wastes produced, and their treatment. The first part marked with number 12 is associated with the energy consumed by the instrument for each sample. This part is marked with green, yellow, and red colors corresponding with the energy consumed by the instruments for each sample is < 0.1 kWh, 0.1–1.5 kWh, and >1.5 kWh, respectively. The second part marked with number 13 is related to the occupational hazards. This part is marked with green color if the analytical procedures are hermetic sealed and there is no vapor or gas is emitted into the air. Otherwise, this part is marked with red color if any vapors or gases are emitted into the air. The third part marked with number 14 is associated with the quantity of waste produced during analytical procedure. This part is marked with green, yellow, and red colors corresponding with the quantity or volume of the waste generated is < 1 g or 1 mL, 1–10 g or 1–10 mL, and >10 g or 10 mL, respectively. The fourth part marked with number 15 is associated with the way to treat the waste. This part is marked with green color if the waste is recycled. This part is labeled with yellow color if the waste is processed with passivation or degradation. This part is labeled with red color if the waste is not processed at all.

Generally, as an excellent semi-quantitative tool, GAPI has many advantages [70]. The greenness of numerous aspects of a corresponding analytical method can be evaluated by GAPI. GAPI is a simple GAC metric and it could provide both qualitative and quantitative information about the greenness of the corresponding assay. However, this GAC metric is not perfect. It also has some limitations. The label used for the quantity or volume of solvents and waste is not precise. For example, the amount of generated waste during analytical procedure A is 10.1 g; Meanwhile, the amount of generated waste during analytical procedure B is 1000 g; Obviously, the impacts of the assay A and assay B on human health and environment are really different. However, assay A and assay B have the same GAPI label. The examples of using GAPI to evaluate the greenness of some selected assays are summarized in Figs. 6B–D and Table S7 [48,49,71].

Płotka-Wasylka and Wojnowski improved GAPI and proposed ComplexGAPI (Fig. 7A) [35]. An extra hexagonal pictogram was added to the original output graph to indicate the greenness of corresponding analytical procedures carried out before sample preparation and final detection. Numerous aspects are included in the hexagonal pictogram. The detailed information about its hexagonal part is summarized and illustrated in Table S6. ComplexGAPI is a semi-quantitative tool. The examples of using ComplexGAPI to evaluate the greenness of some selected assays are illustrated in Figs. 7B–D and Table S8 [72–74].

5. AGREE and AGREEprep

Pena-Pereira et al. [34] proposed AGREE, a relatively new GAC tool. The software can be obtained at https://mostwiedzy.pl/wojciech-wojnowski,174235-1/AGREE. AGREE is a simple and comprehensive tool for assessing the greenness of analytical assays, providing informative and easily interpretable results. The calculation of AGREE metric is derived from the 12 principles of GAC, which are transformed into a 0–1 scale. Moreover, the final score of this GAC metric is calculated through the assessment of these 12 GAC principles [75]. Fig. 8A [34] shows the pictogram of AGREE. Table S9 [34] outlines the procedures for assigning scores in this GAC metric. Additionally, the center of the AGREE pictogram reveals both the color and the final score of the greenness evaluation results [76]. The 12 principles of GAC are represented as 12 segments around the central field of the pictogram. The width of each segment in the AGREE pictogram indicates the weightage assigned to each principle. Furthermore, the color of each segment in the AGREE pictogram can be changed from dark green to red. Therefore, AGREE pictogram provides a simple assessment of the greenness of the analytical assay based on the 12 GAC principles [77]. The application examples of using AGREE metric to evaluate the greenness of some selected assays are illustrated in Figs. 8B–D and Table S10 [78–80].

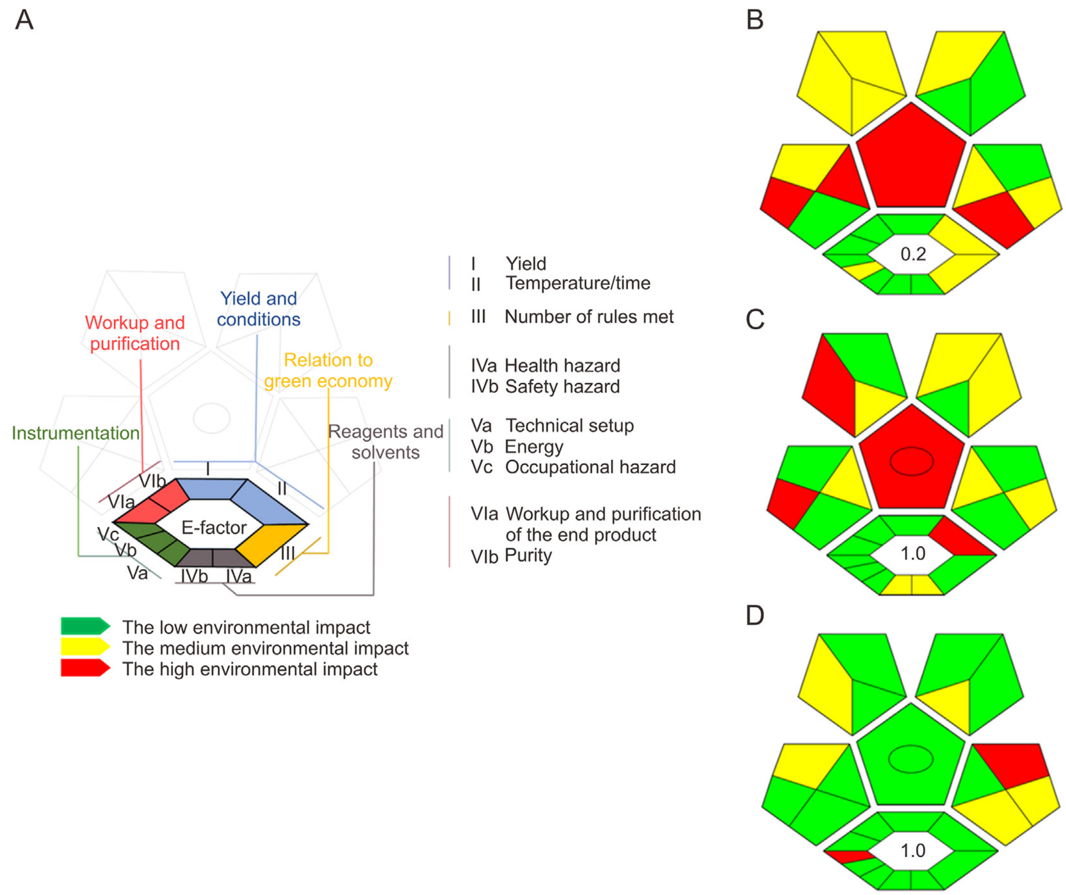

Fig. 7. The typical output pictogram of complex green analytical procedure index (ComplexGAPI) and the examples of using ComplexGAPI for assessing the greenness of some selected analytical procedures. (A) The typical output pictogram of ComplexGAPI [35]. (B–D) Examples of using ComplexGAPI for assessing the greenness of some selected analytical procedures: determination of dimethyl phthalate in beverage samples by a deep eutectic solvent-based ferrofluid assisted liquid-liquid microextraction method and monitoring by high performance liquid chromatography-ultraviolet (HPLC-UV) (B), a monolithic capsule phase microextraction method combined with HPLC-diode array detector (DAD) for monitoring of benzoyl urea insecticides in apple juice samples (C), and nanoparticle-modified carbon paste sensor for ultrasensitive detection of lignocaine and its metabolite residues in bovine food samples (D). Reprint from Ref. [35] with permission.

Overall, AGREE, a comprehensive greenness metric system, exhibits various advantages. Notably, the AGREE metric includes all 12 GAC principles [81], providing both color- and numeric-based results to assess the greenness of different analytical assays. The greenness evaluation process of the AGREE metric is flexible and simple to perform. However, similar to GAPI, AGREE also has some limitations. The AGREE calculation excludes compounds, solvents, energy consumption, and waste generated in pre-extraction procedures. Additionally, the AGREE evaluation does not account for the greenness of sample preparation [37].

AGREEprep is developed to fill this gap and serves as a comprehensive GAC metric based on the 10 principles of GSP [17]. According to the GSP principles, AGREEprep thoroughly evaluates the greenness of sample preparation procedures [82]. The aspects of sample preparation include the sample preparation site, safety of solvents, sustainability of materials, waste minimization, integration and automation of steps, reduction of sample volume, maximization of sample throughput, reduction of energy consumption, selection of environmentally friendly configurations for sample post-preparation, and operator safety. The 10 principles of GSP are converted into a 0–1 scale. Moreover, the final score of this GAC metric is calculated based on the evaluation of all 10 GSP principles [83]. Fig. 9A [37] shows the pictogram of AGREEprep. Table S11 [37] outlines the procedures for assigning scores in this GAC tool. The center of the AGREEprep pictogram reveals both the color and the final score of the greenness evaluation results. The 10 principles of GSP are represented as 10 segments around the central field in the pictogram. The color of each segment in the AGREEprep pictogram can transform from dark green (score, 1) to red (score, 0). The AGREEprep pictogram provides an easy assessment of the greenness of sample preparation procedures based on the 10 GSP principles. The examples of using AGREEprep to evaluate the greenness of some selected assays are illustrated in Figs. 9B–D and Table S12 [84–86]. However, AGREEprep also has certain limitations. In particular, AGREEprep does not account for sample storage, sample transportation, and economic costs in its evaluation.

6. RGB additive color model and RGB 12 algorithm

The RGB model is a comprehensive GAC metric [31]. It is also defined as “white” analytical chemistry [87]. Three primary colors (red, green, and blue) are employed in RGB metric to represent the greenness related parameters of analytical assay [31]. Red color is related to analytical performance. Green color is related to safety/eco-friendliness and blue color is related to productivity/practical effectiveness (Fig. 10A) [31]. The degree of compliance of the analytical assay with the RGB criteria is assessed quantitatively by a color score (CS). Furthermore, the range of CS is from 0% to 100%, and the greenness of analytical assay can be determined by the CS value. The satisfaction range of CS is 66.6%–100%, the tolerance

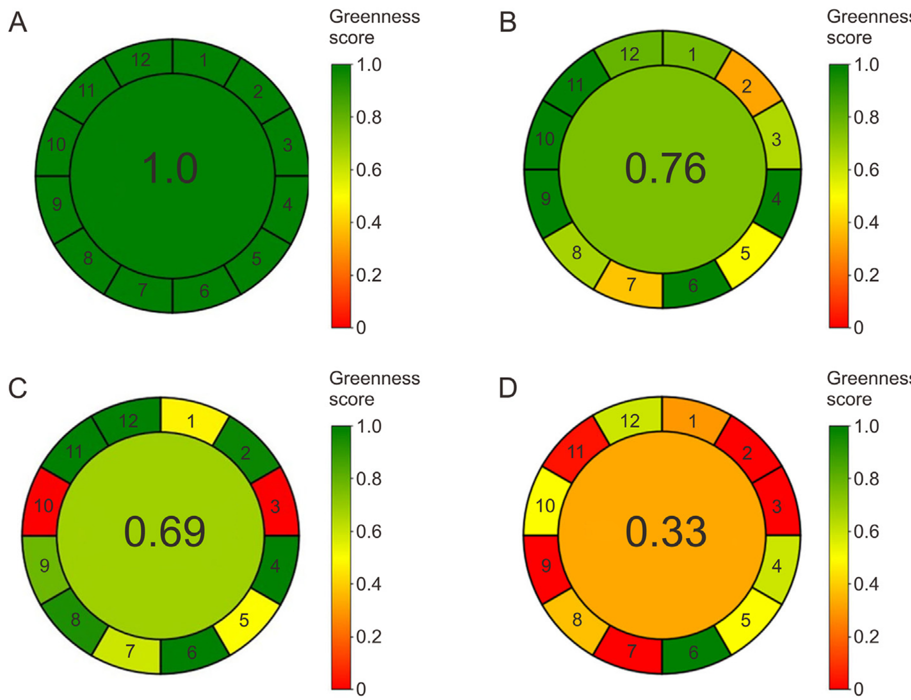

Fig. 8. The typical output pictogram of analytical greenness calculator (AGREE) and the examples of using AGREE for assessing the greenness of some selected analytical procedures. (A) The typical output pictogram of AGREE [34]. (B–D) Examples of using AGREE for assessing the greenness of some selected analytical procedures: simultaneous quantification of carvedilol and ivabradine in tablets by derivative synchronous spectrofluorimetric method (B), determination of paracetamol (PAR), aspirin (ASP), and diphenhydramine (DIPH) by high-performance thin layer chromatographic (HPTLC) (C), and determination of 16 polycyclic aromatic hydrocarbons in environmental water samples by gas chromatography-mass spectrometry (GC-MS) coupled with solid-phase extraction (D). Reprint from Ref. [34] with permission.

range of CS is 33.3%–66.6%, and the unsatisfactory range of CS is 0%–33.3%. Therefore, analysts can use CS to evaluate whether the analytical assay is aligned with the concepts of white analytical chemistry. Moreover, the final color by combining the CS of analytical assay can also be obtained. The entire evaluation process of RGB model is carried out by using Excel (Table S13).

RGB is a comprehensive GAC metric. Safety, performance, and reproducibility of analytical method are all considered in RGB. However, the greenness evaluation of analytical assays by RGB is relatively complex and time consuming. The examples of using RGB metric to evaluate the greenness of some selected assays are summarized in Tables S14 [88], S15 [89], and S16 [90].

RGB 12 algorithm was proposed in 2021 as a new edition of RGB metric [36]. The greenness evaluation of analytical assays by RGB 12 algorithm is based on 12 criteria (Fig. 10B). These criteria include red principles (analytical performance), green principles (GC), and blue principles (practical side) [36]. The entire evaluation process of RGB 12 algorithm is also performed by using Excel. The detailed information about the red, green, and blue principles of RGB 12 algorithm is shown in Table S17. The examples of using RGB 12 algorithm to evaluate the greenness of some selected assays are summarized in Tables S18 [91], S19 [92], and S20 [93].

7. AMGS

Hicks et al. [33] proposed AMGS as a new GAC metric. The AMGS score indicates the greenness of an analytical assay. A smaller score indicates better greenness in the tested assay. The greenness score calculated by this metric is based on safety, health, environmental assessment of the reagents, waste generation, instrument energy consumption, and solvent energy required during analytical procedures. The greenness score of analytical assays can be calculated using AMGS, and additional information can be found on its website https://www.acsgcipr.org/amgs. The AMGS is calculated from a weighted combination of three key parameters: the solvent consumption, the safety, health, and environmental (SHE) attributes of the solvent, and the cumulative energy demand associated with the solvent. The AMGS calculation is shown in Eq. 1. m_{Sn} is the mass of solvent n consumed for each sample during sample preparation. m_{In} is the mass of mobile phase which is composed of solvent n consumed by the instrument. S_n is a parameter related to the safety of solvent n. H_n is a parameter related to the health of solvent n. E_n is a parameter related to environmental friendliness of the solvent n. CED_n is the cumulative energy required to produce and dispose of solvent n. E_i is the instrumental energy consumption. R is the injection times for each analytical batch.

$$\text{AMGS} = \frac{(m_{Sn} + m_{In}) \times \left(\left(3\sqrt{S_n \times H_n \times E_n + CED_n} + (E_i \times R)\right)\right)}{\text{Number of analytes of interest}} \quad \text{Eq. 1}$$

The calculation principles and corresponding parameters of AMGS are summarized and shown in Table S21. Greenness score, instrument energy score, solvent energy score, and solvent SHE score can be calculated according to the AMGS rules [94]. AMGS is a single, concise GAC tool used to quantitatively assess the overall greenness of different assays. However, AMGS does not utilize a pictogram or color scale but only uses a score to indicate the greenness of different assays. Additionally, the calculation process of AGMS is relatively complex and time-consuming. The application examples of using AMGS to evaluate the greenness of three selected assays are summarized in Table 1 [94–96].

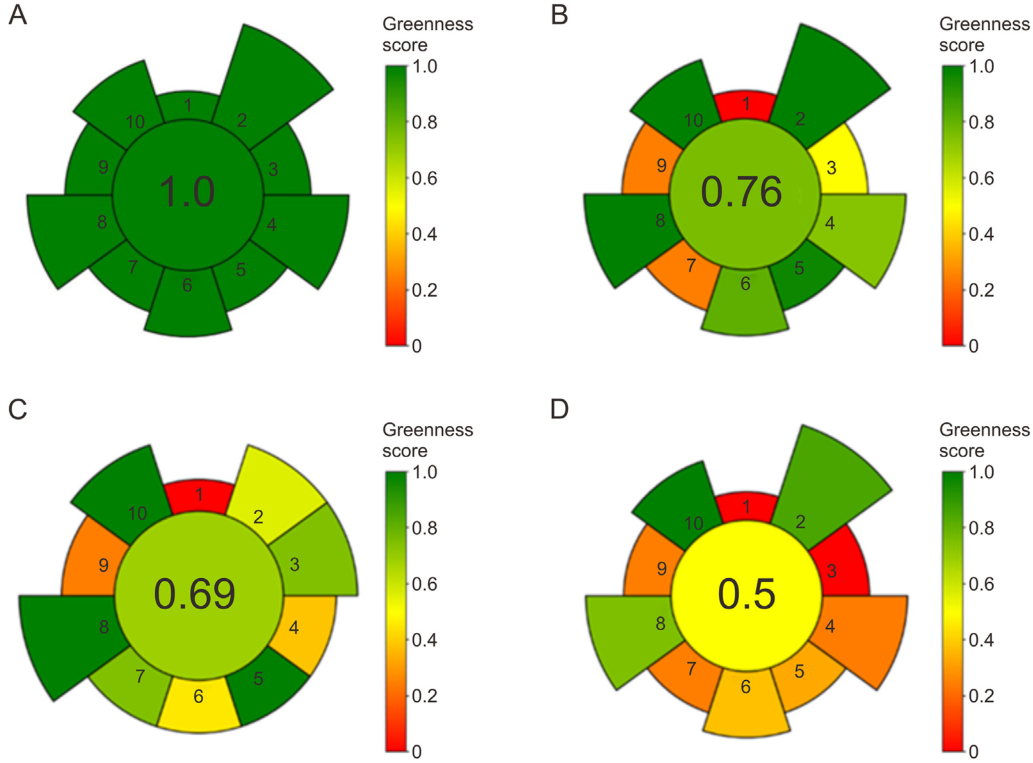

Fig. 9. The typical output pictogram of analytical greenness calculator preparation (AGREEprep) and the examples of using AGREEprep for assessing the greenness of some selected analytical procedures. (A) The typical output pictogram of AGREEprep [37]. (B–D) Examples of using AGREEprep for assessing the greenness of some selected analytical procedures: determination of tryptamine analogs in whole blood by 96-well electromembrane extraction and ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) (B), determination of beta-blockers in serum by on-line liquid-liquid extraction coupled with UPLC-MS/MS (C), and simultaneous determination of paclitaxel and vincristine in environmental water and urine samples by dispersive micro solid phase extraction coupled with high performance liquid chromatography (HPLC) (D). Reprint from Ref. [37] with permission.

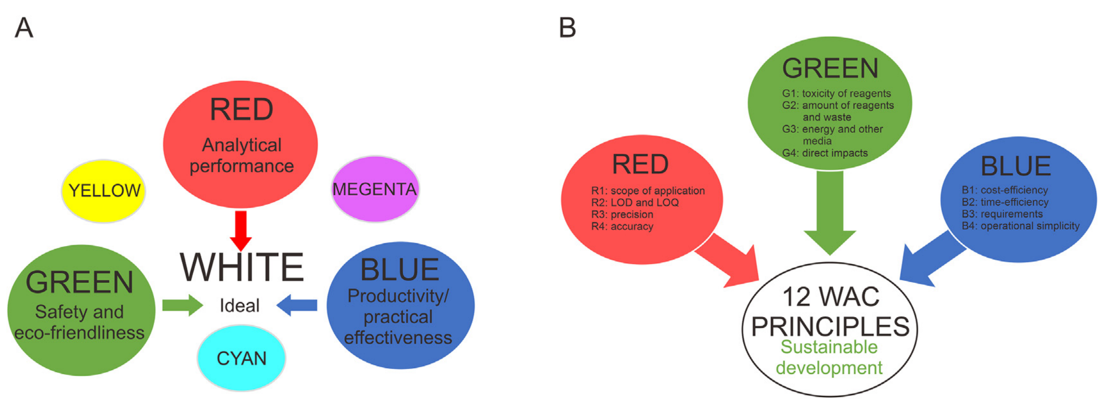

Fig. 10. The pictogram of red-green-blue (RGB) additive color model and RGB 12 algorithm. (A) The pictogram of RGB additive color model [31]. (B) The pictogram of RGB 12 algorithm. LOD: limit of detection; LOQ: limit of quantification; WAC: white analytical chemistry. Reprint from Ref. [31] with permission.

8. BAGI

Manousi et al. [38] proposed BAGI, a new GAC metric, to evaluate the practicability and greenness of analytical methods. The greenness evaluation of the BAGI-related analytical assay is based on the principles of white analytical chemistry. BAGI uses a score, a color scale, and a pictogram to indicate the greenness of tested assays. The score range of this GAC metric is from 25 to 100. A higher score on the BAGI metric indicates better greenness of the assay. Fig. 11A [38] shows the pictogram of BAGI. The greenness score of the tested analytical assay can be calculated using BAGI, and its official website is at https://bagi-index.anvil.app. The BAGI metric incorporates 10 criteria to assess the practicability and greenness of different assays. The attributes, score values, and related hues of this GAC metric are shown in Table S22. The greenness of analytical assays can be easily and rapidly determined using BAGI. However, the comprehensiveness of BAGI still requires improvements. In particular, BAGI does not include the SHE assessment of reagents and waste generation in its evaluation process. Overall, BAGI is a simple and efficient GAC metric and

Table 1
The examples of using analytical method greenness score (AMGS) for assessing the greenness of selected analytical methods.

Selected analytical assays for greenness evaluation	Category	Category score	Percent of greenness score (%)	Greenness score	Refs.
A HPLC-DAD assay for determination of hederacoside C, thymol, and potassium sorbate	1	85.87	22.35	384.15	[94]
	2	49.16	49.16		
	3	28.46	28.48		
An UPLC-PDA assay for determination of isosorbide dinitrate and hydralazine hydrochloride	1	5.72	12.82	44.61	[95]
	2	3.81	8.55		
	3	35.08	78.64		
An UPLC-PDA assay for determination of pitavastatin and ezetimibe	1	5.72	11.58	49.38	[96]
	2	3.80	7.70		
	3	39.85	80.72		

Category 1: instrument energy score; category 2: solvent energy score; and category 3: solvent safety, health, and environmental (SHE) score. HPLC-DAD: high performance liquid chromatography-diode array detection: UPLC-PDA: ultra-performance liquid chromatography-photodiode array detection.

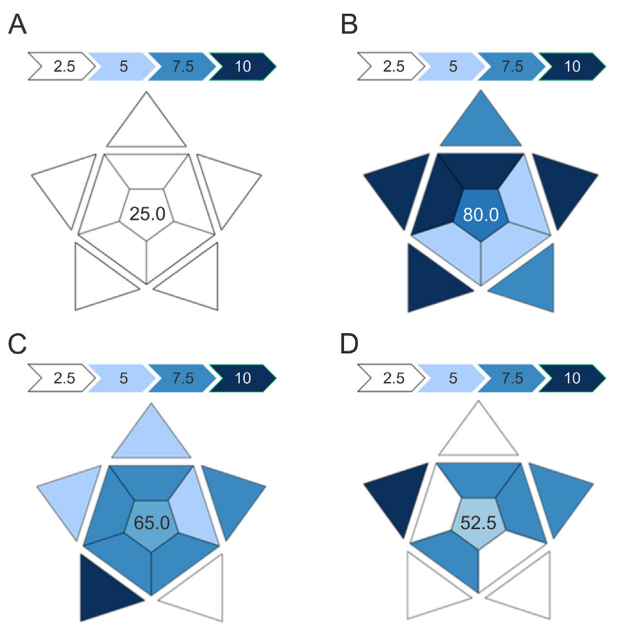

Fig. 11. The typical output pictogram of blue applicability grade index (BAGI) and the examples of using BAGI for assessing the greenness of some selected analytical procedures. (A) The typical output pictogram of BAGI [38]. (B–D) Examples of using BAGI for assessing the greenness of some selected analytical procedures: analysis of phthalates, polycyclic aromatic hydrocarbons, and pesticide residues in infant formula by single-run gas chromatography-mass spectrometry (GC-MS) method (B), analysis of endocrine disrupting chemicals in milk and environmental water samples by high performance liquid chromatography-ultraviolet (HPLC-UV) (C), and analysis of morpholine (MOR) in the pericarp of orange and apple fruit specimens by an innovative spectrofluorometric assay (D). Reprint from Ref. [38] with permission.

serves as a vital complement to the current GAC tools, such as AGREEprep and ComplexGAPI. The examples of using BAGI to evaluate the greenness of three selected assays are illustrated in Figs. 11B–D and Table S23 [97–99].

9. ChlorTox scale

Nowak et al. [39] proposed the ChlorTox Scale, a new GAC metric, for evaluating the health and environmental risks related to chemicals. The health and environmental risk posed by a specific chemical can be determined using the ChlorTox value. Additionally, the cumulative ChlorTox values obtained for all solvents used in analytical procedures represent the overall risk associated with the corresponding analytical assay. The ChlorTox value is determined by comparing the hazards of the chemical being evaluated with the standard chemical. In particular, chloroform serves as the standard chemical in the ChlorTox Scale. The authors use the weighted hazards number (WHN) model to calculate the overall hazard value of chloroform, which is 5.75. This score is then used as a reference point for assessing the hazard of other chemicals. The equivalent mass of chloroform is the unit of ChlorTox, reflecting the degree of evaluated risks. The calculation formulas for this GAC metric are shown in Eqs. 2 and 3. CH_{sub} is the overall chemical hazard level of the substance concerned. The corresponding CH_{sub} values can be found in a specially developed database [100]. m_{sub} is the mass of the substance consumed in a single analysis. m_N is the total mass of the substances directly consumed in N analyses. m' is the mass of substance consumed by additional mandatory procedures. N is the longest possible series in the analysis.

$$\text{ChlorTox} = \frac{CH_{sub}}{CH_{CHCl_3}} \times m_{sub} \qquad \text{Eq. 2}$$

$$m_{sub} = \frac{m_N + m'}{N} \qquad \text{Eq. 3}$$

The overall risk assessed by the ChlorTox Scale for an analytical method is based on the hazardous properties and amounts of the chemical. The overall chemical risk of an analytical assay can be easily evaluated using the ChlorTox value. However, the result obtained from the ChlorTox assessment represents a semi-quantitative measure. Hence, the comprehensiveness of ChlorTox still requires improvement. ChlorTox only uses values to indicate the chemical risk of analytical assays without the use of a pictogram or color scale. Waste pretreatment and method performance are not included in the greenness evaluation. Moreover, the calculation process of ChlorTox is relatively complex. The examples of using ChlorTox to assess the greenness of three selected assays are illustrated in Table 2 [101–103].

10. HEXAGON

HEXAGON was proposed as a new GAC metric for quantitative evaluation of the greenness of analytical assays [32]. Six blocks just like residues, carbon footprint, economic cost, toxicity/safety, figures of merit 1, and figures of merit 2 are included in the pictogram of HEXAGON to reveal the greenness of analytical assay in terms of different aspects [32]. The penalty score for each aspect is converted to 0–4. The lower the value, the better the greenness. The corresponding parameters, implication, and interpretation of different blocks of HEXAGON are summarized and shown in Fig. 12A [32]. The detailed calculation rules of HEXAGON are summarized and shown in Tables S24–S30.

HEXAGON is a new GAC metric for greenness evaluation of analytical methods with more comprehensive scope. Numerous factors just like environmental aspect, safety aspect, economic and social impacts are all included in HEXAGON for greenness

Table 2
The examples of using chloroform-oriented toxicity estimation scale (ChlorTox Scale) for assessing the greenness of selected analytical methods.

Method	Chemical	Relative hazard (WHN)	m_{sub} (mg)	ChlorTox in WHN model (g)	Refs.
HPLC-UV	Methanol	0.57	3531.43	2.010	[101]
	Acetonitrile	0.39	5234.76	2.040	
	Phosphoric acid	0.57	337.00	0.190	
LLME and HPLC-UV	Menthol:thymol (1:1, *V*/*V*)	0.78	401.50	0.313	[102]
	Chloroform	1.00	749.00	0.749	
	Acetonitrile	0.39	1256.00	0.490	
	Formic acid	0.57	15.86	0.009	
HPTLC	Trichloromethane	1.00	3600.00	1.800	[103]
	Methanol	0.56	400.00	0.110	

WHN: weighted hazards number; m_{sub}: the mass of the substance consumed in a single analysis; HPLC-UV: high performance liquid chromatography-ultraviolet; LLME: liquid-liquid microextraction; HPTLC: high-performance thin-layer chromatography.

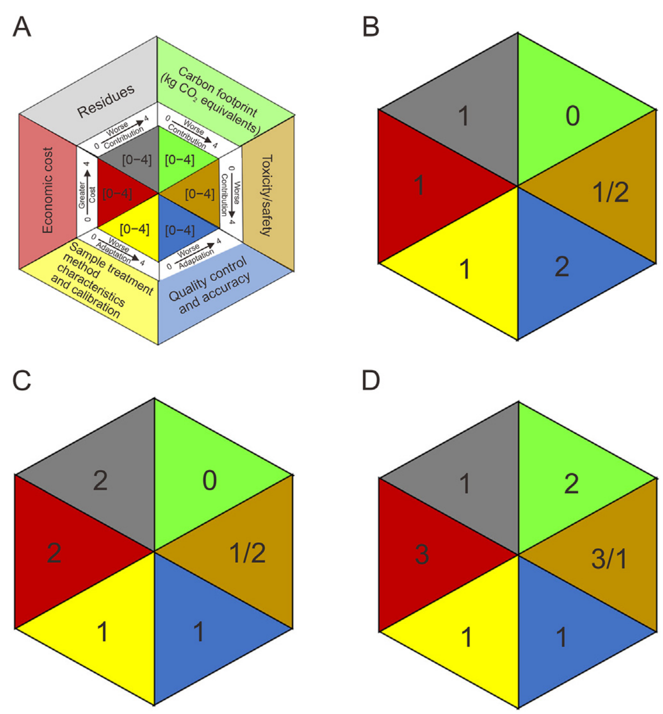

Fig. 12. The typical output pictogram of HEXAGON and the examples of using HEXAGON for assessing the greenness of some selected analytical procedures. (A) The typical output pictogram of HEXAGON [32]. (B–D) Examples of using HEXAGON for assessing the greenness of some selected analytical procedures: smart-chemometric spectrophotometric assay for determination of six gastric proton-pump inhibitors (B), liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay for determination of hydrochlorothiazide (HCT) and five antihypertensive drugs (C), and liquid chromatography assay with diode array detector (HPLC-DAD) for determination of sulfonate-based dyes in meat samples (D). Reprint from Ref. [32] with permission.

evaluation of analytical assays. Moreover, the greenness evaluation process of HEXAGON is more standardized. Therefore, the output greenness evaluation results of HEXAGON are more accurate. However, HEXAGON also has some limitations. For example, more information should be collected in HEXAGON for greenness evaluation. The calculation process of HEXAGON is complex and laborious. The examples of using HEXAGON to assess the greenness of three selected assays are illustrated in Figs. 12B–D and Table S31 [104–106].

11. Conclusion and future perspectives

This article presents various GAC metrics with different principles, strengths, and weaknesses. The evaluation results for the greenness of GAC metrics, such as NEMI and advanced NEMI, are based on qualitative measures. The results generated by advanced pictograms, ChlorTox, GAPI, ComplexGAPI, Analytical Eco-Scale, and Green Certificate Modified Eco-Scale include both qualitative and semi-quantitative aspects. The results produced by BAGI, AGREE, AGREEprep, AMGS, RGB additive color model, RGB 12 algorithm, and HEXAGON include both quantitative and qualitative aspects. NEMI, advanced NEMI, and advanced pictograms use pictograms to visually represent the greenness of analytical assays. AMGS, ChlorTox, and Analytical Eco-Scale only use scores to indicate the greenness of analytical methods. GAPI and ComplexGAPI use both a color scale and a pictogram to represent the greenness of analytical methods. The RGB additive color model and RGB 12 algorithm use both a score and a color scale to indicate the greenness of analytical assays. AGREE, AGREEprep, BAGI, Green Certificate Modified Eco-Scale, and HEXAGON use a score, a pictogram, and a color scale to indicate the greenness of analytical methods. To obtain more comprehensive and reliable greenness assessment results, it is recommended to use multiple different GAC metrics for evaluating the greenness of different assays. The increasing interest in the GAC field indicates the need for new perspectives on GAC metrics. Newly developed GAC metrics should be simple, flexible, and easy to operate. The principles considered in the metrics should be comprehensive, and the result should be simple, clear, and easy to interpret. Moreover, the development of the greenness model requires large input datasets. New software should be developed and used to assess the greenness of various assays on different scales and improve the calculation of the greenness metrics. Generally, this article comprehensively presents and elucidates the principles, characteristics, advantages, and disadvantages of 15 widely used GAC tools. Additionally, the article provides valuable insights into the current GAC tools.

CRediT authorship contribution statement

Lei Yin: Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition. **Luyao Yu:** Writing – review & editing, Investigation. **Yingxia Guo:** Writing – review & editing. **Chuya Wang:** Writing – review & editing. **Yuncheng Ge:** Writing – review & editing. **Xinyue Zheng:** Writing – review & editing. **Ning Zhang:** Writing – review & editing. **Jiansong You:** Investigation. **Yong Zhang:** Project administration, Funding acquisition. **Meiyun Shi:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpha.2024.101013.

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