analytica

MDPI

Article

TriCA as a Triple-Color Assessment Tool for Analytical Methods

Fotouh R. Mansour [1,2,*], Khalid M. Omer [3], Sameera Sh. Mohammed Ameen [4], Marcello Locatelli [5,*], Imran Ali [6] and Alaa Bedair [7]

1 Department of Medicinal Chemistry, Faculty of Pharmacy, King Salman International University (KSIU), El-Tor 11371, Egypt
2 Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Tanta University, Tanta 31111, Egypt
3 Department of Chemistry, College of Science, University of Sulaimani, Sulaymaniyah 46002, Kurdistan Region, Iraq; khalid.omer@univsul.edu.iq
4 Department of Chemistry, College of Science, University of Zakho, Zakho 42002, Kurdistan Region, Iraq; sameera.m.ameen@uoz.edu.krd
5 Department of Science, University "G. d'Annunzio" of Chieti-Pescara, Via dei Vestini 31, 66100 Chieti, Italy
6 Department of Chemistry, Jamia Millia Islamia (Central University), Jamia Nagar, New Delhi 110025, India; drimran.chiral@gmail.com or drimran_ali@yahoo.com
7 Department of Analytical Chemistry, Faculty of Pharmacy, University of Sadat City, Sadat City 32958, Egypt; alaa.m.bedair@gmail.com
* Correspondence: fotouhrashed@pharm.tanta.edu.eg (F.R.M.); marcello.locatelli@unich.it (M.L.)

Abstract

The evaluation of analytical methods has evolved beyond traditional validation parameters to encompass environmental sustainability, analytical reliability, and practical applicability. While numerous assessment tools exist for individual dimensions, integrated multicolor frameworks often impose rigid parameter selection, assign equal weights to all dimensions regardless of analytical context, or lack the granular diagnostic feedback necessary for method improvement. To address these limitations, we present the Triple-Color Assessment (TriCA) tool, a web-based platform that integrates three validated metrics into a unified scoring system with a hierarchical weighting scheme. TriCA combines the Analytical Green Star Area (AGSA) for greenness assessment, the Click Analytical Chemistry Index (CACI) for blueness evaluation, and the Analytical Method Reliability Index (AMRI) for redness scoring. While these metrics are provided as defaults, users may select alternative assessment tools within each dimension according to their specific preferences or requirements. TriCA features a user-customizable weighting system with recommended defaults (red = 3, blue = 2, green = 1), reflecting the logical priority that a method must first be valid, then practical, and finally green. Users can adjust these weights for specific contexts, while the underlying metric scoring protocols (AGSA, CACI, and AMRI) remain fixed to ensure reproducibility. Beyond composite scoring, TriCA generates three detailed pictograms, 12 segments for AGSA, eight segments for CACI, and 10 segments for AMRI, each color-coded to show performance at the individual criterion level. This diagnostic capability enables analysts to identify exactly which green chemistry principles, practicality criteria, or validation parameters are deficient, transforming assessment from an opaque scoring exercise into actionable guidance for method improvement. Seven case studies encompassing diverse analytical techniques demonstrate TriCA's discriminative power and diagnostic utility. The tool is freely accessible at bit.ly/TriCA, offering the analytical community a transparent, reproducible, and diagnostically rich framework for method evaluation, development, and optimization.

Academic Editor: Simone Morais

Received: 4 June 2026
Revised: 21 July 2026
Accepted: 24 July 2026
Published: 4 August 2026

 https://doi.org/10.3390/analytica7030052

Keywords: white analytical chemistry; green analytical chemistry; method validation; sustainability metrics; analytical performance assessment; TriCA tool

1. Introduction

The evaluation of analytical methods has evolved far beyond traditional validation parameters, driven by an increasing recognition that method quality cannot be defined solely by accuracy and precision [1,2]. Modern analytical chemistry demands a multidimensional perspective that balances environmental responsibility, analytical reliability, and practical applicability, three pillars that collectively determine whether a method is truly fit for its intended purpose [3,4]. This paradigm shift has given rise to a diverse spectrum of assessment metrics, each represented by a distinct color: green for environmental sustainability, red for analytical performance and validation, blue for practicality and operational efficiency, white for holistic integration, yellow for lifecycle sustainability, and violet for innovation [5–7]. Among these, the three primary dimensions, green, red, and blue, form the foundational RGB model of White Analytical Chemistry (WAC), where their balanced combination yields a "white" method representing optimal compromise between ecological friendliness, analytical excellence, and practical utility [8].

The proliferation of assessment tools over the past decade reflects the growing maturity of this field [9,10]. Greenness metrics such as the Analytical Green Star Area (AGSA) [11], AGREE [12], and GAPI [13] provide structured frameworks for evaluating environmental impact based on the 12 principles of Green Analytical Chemistry. Redness metrics, including the recently introduced Analytical Method Reliability Index (AMRI) [14] and the Red Analytical Performance Index (RAPI) [15], offer quantitative scoring of validation parameters aligned with regulatory guidelines. Blueness metrics such as the Click Analytical Chemistry Index (CACI) [16] and the Blue Applicability Grade Index (BAGI) [17] assess practicality, cost-effectiveness, and operational simplicity. While each tool serves its specific purpose admirably, the analytical community increasingly recognizes that applying multiple metrics in isolation is time-consuming, potentially redundant, and fails to provide an integrated picture of overall method quality [18,19].

Recent efforts have sought to harmonize these disparate assessment approaches into unified frameworks. The RGB12 model [7] and its streamlined version RGBfast [20] integrate red, green, and blue criteria into a single evaluation, while the Whiteness Evaluation for Chemical Analysis (WECA) tool [21] offers customizable weighting of the three pillars, but is limited to a maximum of 12 criteria. The Multicolor Assessment Platform (MA-Tool) [22] further combines greenness, blueness, redness, and innovation metrics. However, a critical examination reveals significant gaps in existing multicolor models. Many frameworks either impose rigid parameter selection (e.g., RGB12 requires evaluation of exactly 12 criteria regardless of method specificity) or limit the number of parameters per color, potentially omitting contextually relevant attributes. More importantly, most multicolor tools typically assign default equal weights to all three dimensions, implicitly assuming that analytical performance, environmental sustainability, and practicality are equally important in every analytical context—an assumption that rarely holds true in practice [23]. For instance, a method intended for emergency toxicology screening prioritizes speed and simplicity (blue) over environmental impact (green), whereas a routine environmental monitoring method for regulatory compliance may require exceptional analytical rigor (red) with moderate practicality requirements. Furthermore, several existing multicolor tools focus primarily on greenness and blueness while giving insufficient attention to redness, the fundamental pillar of analytical credibility [24]. This imbalance is particularly

https://doi.org/10.3390/analytica7030052

concerning given that a method cannot be considered truly "white" if it fails to demonstrate robust analytical performance, regardless of how environmentally friendly or practical it may be.

To address these limitations, we present the Triple-Color Assessment (TriCA) tool, a novel, web-based platform that integrates three validated metrics into a unified scoring system with customizable weights and recommended default values. TriCA combines the AGSA for greenness assessment, the CACI for blueness evaluation, and the AMRI for redness scoring, each rigorously developed and grounded in established analytical principles. Unlike existing multicolor frameworks, TriCA allows users to assign differential importance to each dimension based on the specific analytical context, thereby providing a truly flexible and purpose-dependent evaluation. The tool generates an overall whiteness score together with color-coded visual indicators for each dimension, enabling rapid interpretation of method strengths and weaknesses. By harmonizing three validated metrics into a single, user-friendly interface with customizable weighting, TriCA addresses the urgent need for practical, transparent, and context-aware assessment of analytical methods, supporting informed decision-making in method development, selection, and optimization across diverse application domains.

2. Methodology

2.1. Overview of the TriCA Framework

The Triple-Color Assessment (TriCA) tool integrates three established metrics, AGSA (greenness), CACI (blueness), and AMRI (redness), into a unified web-based platform for comprehensive analytical method evaluation. The framework is grounded in the RGB additive color model of White Analytical Chemistry (WAC), where the balanced combination of green, blue, and red dimensions produces a "white" method representing optimal harmony between environmental sustainability, practical applicability, and analytical reliability [7,8]. Unlike existing multicolor tools that impose equal weighting across dimensions, TriCA introduces user-configurable importance factors, enabling context-aware assessment that reflects the specific priorities of each analytical application.

The TriCA tool is implemented as an interactive web application accessible at bit.ly/TriCA, featuring a structured form-based interface for data input and dynamic visual output. Users complete three distinct sections corresponding to the green, blue, and red assessment domains. Within each section, responses are selected from predefined options aligned with the original metric criteria, ensuring consistency and reproducibility across evaluations. The tool automatically computes domain-specific percentage scores, applies user-defined weights, generates a composite whiteness score, and produces color-coded visual indicators for immediate interpretation.

2.2. Greenness Assessment: Analytical Green Star Area (AGSA)

The greenness dimension of TriCA employs the AGSA metric, a comprehensive tool specifically designed to evaluate analytical methods against the 12 Principles of Green Analytical Chemistry (GAC) [11]. AGSA was selected over alternative greenness metrics due to its explicit alignment with GAC principles, its built-in quantitative scoring (0–100 scale), its visual star-shaped output, and its resistance to user bias through equal weighting of all 12 principles.

The AGSA framework evaluates each of the 12 GAC principles through specific questions with three-tiered response options. The principles assessed include: direct analysis (minimizing sample treatment), minimum sample size (reducing resource consumption), in situ measurements (performing analysis at the sampling site), integration of analytical processes (combining steps into unified workflows), automation and miniaturization (reducing

https://doi.org/10.3390/analytica7030052

manual intervention and physical footprint), avoidance of derivatization (eliminating additional reaction steps), waste minimization and management (reducing and responsibly handling waste), high throughput (analyzing multiple analytes simultaneously), low energy consumption (minimizing power requirements), use of renewable reagents (prioritizing sustainable sources), low toxicity (selecting safer chemicals), and operator safety (minimizing personal protective equipment requirements).

Each response option carries a point value ranging from 0.5 to 3 points, with higher scores indicating superior greenness. Principles 5 and 7 each contain two sub-questions (automation/miniaturization and waste volume/management, respectively), with points summed within each principle. The maximum possible total score is 36 points (12 principles × 3 points maximum per principle). The AGSA percentage score is calculated as:

$$\text{AGSA Score}(\%) = \left(\frac{\sum_{i=1}^{12} S_i}{36 - P_{\text{NA}}} \right) \times 100$$

where S_i is the score obtained for principle i, and P_{NA} represents points deducted for principles marked as "not applicable" (each non-applicable principle reduces the denominator by 3 points). Methods scoring $\geq$75% are classified as optimal greenness, 50–74% as acceptable, and $<$50% as inadequate. For the integration into TriCA, the tool automatically computes the AGSA percentage based on user selections, with "not applicable" options properly excluded from denominator calculations.

2.3. Blueness Assessment: Click Analytical Chemistry Index (CACI)

The blueness dimension of TriCA employs the CACI, a metric designed to assess the practicality, simplicity, and operational efficiency of analytical methods [16]. CACI was selected because it uniquely addresses the "clickability" of analytical procedures emphasizing rapid, modular, user-friendly processes that enable on-site and time-sensitive applications while incorporating parameters overlooked by other blueness metrics such as BAGI [17], including cost analysis and matrix applicability.

The CACI framework evaluates analytical methods across eight core criteria. Sample size assesses the volume or mass required for a single analysis, with smaller samples ($\leq$1 mL or g) receiving higher scores. Sample preparation complexity distinguishes between no preparation (direct analysis), minimal preparation (dilution, filtration), and conventional preparation (extraction, derivatization). Feasibility encompasses three sub-criteria: availability of chemicals and reagents (commercially available, easily synthesized, or laborious synthesis), instrument accessibility (standard laboratory equipment versus specialized instruments), and cost per sample (categorized as <\$10, \$10–\$100, or >\$100 per sample). Application scope evaluates the method type (quantitative, semi-quantitative, or qualitative), number of analytes determinable simultaneously (single, 2–3, or >3 analytes), and number of applicable matrices (single, 2–3, or >3 matrices). Portability classifies instrumentation as portable, miniaturized but not portable, or neither. Automation level distinguishes automatic, semi-automatic, and manual methods. Sensitivity assesses the lower limit of quantitation relative to the target concentration ($\leq$1%, $\leq$10%, or $>$10% of target). Finally, total analysis time (sample preparation plus instrument run time) is scored on a graduated scale from 6 points ($\leq$5 min) to 1 point ($>$300 min).

The maximum possible CACI score is 27 points. The CACI percentage is calculated as:

$$\text{CACI Score}(\%) = \left(\frac{\sum_{i=1}^{8} S_i}{27}\right) \times 100$$

where S_i represents the score for each criterion. For the feasibility and application domains (each containing three sub-criteria), the sub-criterion scores are summed and divided by 3

https://doi.org/10.3390/analytica7030052

to ensure each domain contributes equally to the total score. The tool computes the final CACI percentage automatically upon user input.

2.4. Redness Assessment: Analytical Method Reliability Index (AMRI)

The redness dimension of TriCA employs the AMRI, a metric specifically designed to provide standardized, quantitative assessment of analytical method reliability beyond binary validation outcomes. AMRI [14] was selected as a default tool over the alternative RAPI [15] because it anchors scoring directly to application-specific acceptance criteria prescribed in international regulatory guidelines (ICH Q2(R2), FDA, EMA), making it particularly suitable for regulated environments where compliance with specific validation standards is critical. Accordingly, the same analytical method may yield different performance scores depending on the analyst's predefined performance requirements and acceptance criteria.

The AMRI framework evaluates analytical methods across 10 validation attributes derived from regulatory guidelines. Each attribute contains one or more specific questions scored on a 0–3 scale, with assigned weights reflecting relative importance based on expert consensus. The attributes and their weights are: linearity (weight 4), range (weight 2), accuracy (weight 4), precision (weight 4), limit of detection (LOD; weight 1), limit of quantitation (LOQ; weight 2), selectivity (weight 3), robustness (weight 1), stability (weight 3), and application to real samples (weight 4).

Linearity assessment considers the coefficient of determination (r^2) and the lack-of-fit test. For r^2, scores range from 1 ($r^2 = 0.990$) to 3 ($r^2 > 0.999$), with the final score conditional on the lack-of-fit test (significant lack of fit reduces the score to 1). Range evaluation examines the ULOQ/LLOQ ratio (1 point for $\leq$10, 2 points for >10–100, 3 points for >100–1000, 4 points for >1000), the number of concentration levels tested (1 point for 5 points, 2 points for 6–9 points, 3 points for $\geq$10 points), and quartile coverage (1 point for no, 3 points for yes). Accuracy and precision are scored based on the ratio of observed %bias or %RSD to the application-specific acceptance limit (1 point for ratio ≥ 1, 2 points for $0.5 \leq$ ratio < 1, 3 points for ratio < 0.5). LOD and LOQ evaluation includes verification of practical measurement (in matrix versus solution) and replicate analysis. Selectivity considers the number of interfering substances tested and blank samples analyzed. Robustness assesses the number of variables tested and maximum %RSD observed. Stability evaluation includes whether the method is stability-indicating and the number of stability levels tested. Real sample application distinguishes between simulated/spiked samples (1 point) and authentic real samples (3 points).

The AMRI percentage score is calculated using the weighted average formula:

$$\text{AMRI Score}(\%) = \left(\frac{\sum(S_i \times W_i)}{3 \times \sum W_i} \right) \times 100$$

where S_i is the average score (0–3) for parameter i after excluding non-applicable items, and W_i is the assigned weight. Parameters marked as "not applicable" are excluded from both numerator and denominator, ensuring fair comparison across different method types. Parameters marked as "not tested/not reported" incur a penalty (effectively scoring 0.5 out of 3) without adjusting the denominator, reflecting the incompleteness of validation documentation. TriCA automatically calculates the weighted average, handles not-applicable exclusions, applies penalties for not-tested responses, and computes the final AMRI percentage.

https://doi.org/10.3390/analytica7030052

2.5. Whiteness Score Calculation with Customizable Weights

The overall whiteness score in TriCA is calculated as a weighted average of the three domain scores, allowing users to assign differential importance to greenness, blueness, and redness based on the specific analytical context. This weighting mechanism addresses a fundamental limitation of existing multicolor tools that assume equal importance across all dimensions regardless of application.

Users assign weights to each domain using a three-level ordinal scale: 1 (low importance), 2 (moderate importance), or 3 (high importance). These weights function as importance multipliers reflecting relative prioritization, not as ratio-scale quantities. The weighted whiteness score is calculated as:

$$\text{TriCA Score}(\%) = \frac{(G \times w_G) + (B \times w_B) + (R \times w_R)}{w_G + w_B + w_R}$$

where G, B, and R represent the AGSA, CACI, and AMRI percentage scores, respectively, and w_G, w_B, and w_R represent their assigned weights (each ranging from 1 to 3). The resulting score is normalized to a 0–100 scale and rounded to the nearest integer. If any domain score is unavailable, that domain is excluded from both numerator and denominator, and the weights are renormalized accordingly. The tool provides default weights for general-purpose evaluation. The weighting system gives high weight on redness ($w_R = 3$) to prioritize analytical reliability and validation compliance, moderate weight on blueness ($w_B = 2$), and low weight on greenness ($w_G = 1$), based on the authors′ expert judgment. This recommendation reflects the principle that a method must first be analytically valid (redness), then practical (blueness), and finally green (greenness). Users are free to adjust these weights according to their specific priorities. For example, user can assign higher weight to blueness for point-of-care applications or higher weight to greenness for routine environmental monitoring, while the default values serve as a scientifically grounded starting point.

2.6. Visual Encoding and User Interface

The TriCA interface is organized into two main panels: a left-side form panel containing all assessment questions organized by color-coded sections (green, blue, red), and a right-side visualization panel displaying dynamic graphical output. The visualization panel contains four circular indicators: the green circle displays the AGSA percentage score (rounded to the nearest integer). The circle background changes dynamically based on score thresholds: green (≥75%), gray (50–74%), or black (<50%). The blue circle shows the CACI percentage score with analogous color coding (blue for ≥75%, gray for 50–74%, black for <50%). The red circle displays the AMRI percentage score with analogous color-coding (red for ≥75%, gray for 50–74%, black for <50%). The central whiteness circle demonstrates the weighted TriCA score with white background for ≥75% (indicating high whiteness), gray for 50–74%, and black for <50%.

In addition to the circular indicators, TriCA includes dynamic pictogram layers for each domain that visually highlight method performance across individual criteria. For the green domain, the AGSA pictogram updates each of the 12 arms based on principle-specific scores (green for optimal compliance, gray for moderate, black for poor). For the blue domain, the CACI pictogram updates eight segments corresponding to the eight evaluation criteria. For the red domain, the AMRI pictogram updates 10 segments representing the 10 validation attributes. These visual elements enable rapid identification of specific strengths and weaknesses within each domain.

2.7. Case Study Selection

To demonstrate the utility and discriminative power of TriCA, seven case studies encompassing diverse analytical techniques and application domains were selected from the peer-reviewed literature. Each method was independently assessed using AGSA, CACI, and AMRI according to their respective scoring protocols. The domain scores were then integrated into a TriCA score.

3. Results and Discussion

Abdallah et al. [25] developed a menthol-assisted homogeneous liquid–liquid microextraction method coupled with HPLC-UV for the quantification of favipiravir, an antiviral drug used against COVID-19, in human plasma (Figure 1). The analytical workflow requires extensive sample preparation (26 min) and a relatively large sample volume (1–10 mL), with a total analysis time of 31 min. The method is fully miniaturized and semi-automated, avoids derivatization, and operates with a mixture of renewable and non-renewable reagents at an energy consumption of 1–1.5 kW per sample. Reagent toxicity is high, indicated by six or more hazard pictograms. Validation demonstrates excellent linearity with a coefficient of determination (R^2) exceeding 0.999, a range ratio (ULOQ/LLOQ) between 100 and 1000 assessed using ≥10 concentration levels, and good accuracy and precision (observed %bias and %RSD each less than 0.5 times the respective acceptance limits). However, the method exhibits critical deficiencies: the limit of detection (LOD) was neither properly calculated nor measured practically or in replicates; the limit of quantitation (LOQ) was measured practically and its associated %RSD and %bias ratios were <0.5; selectivity was not tested for interfering substances (though ≥6 blanks showed no interference); robustness was not evaluated; and the method is not stability-indicating, although two stability levels were tested. The method was successfully applied to real plasma samples and is commercially feasible (total cost < $10 per sample). Instrumentation is miniaturized but not portable, and the method is quantitative for a single analyte in a single matrix. The procedures and the TriCA score are shown in Figure 1.

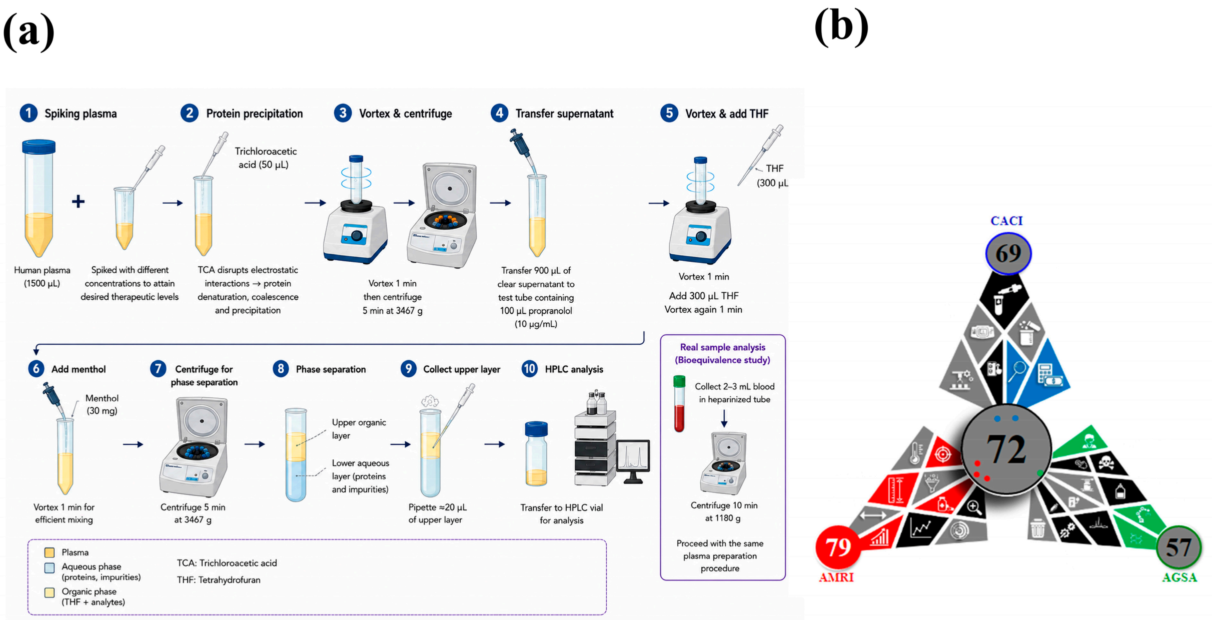

Figure 1. (**a**) A menthol-assisted homogeneous liquid–liquid microextraction for the quantification of favipiravir, (**b**) TriCA score of the proposed method.

Abdallah et al. [26] employed a sugaring-out induced homogeneous liquid–liquid microextraction as an alternative sample preparation mode for biological fluids, followed by HPLC analysis. Sample preparation is extensive, requiring 7 min, with a sample volume of 1–10 mL and total analysis time of 27 min. The method is fully miniaturized, semi-automated, and avoids derivatization, using a mixture of renewable and non-renewable reagents and consuming 1–1.5 kW per sample. Reagent toxicity is high (≥6 pictograms). Linearity is acceptable (R^2 = 0.991–0.999), with a range ratio of 100–1000 based on 6–9 concentration levels that cover all four quartiles. Accuracy and precision are good (observed %bias and %RSD ratios < 0.5 relative to acceptance limits). Nevertheless, the validation is incomplete: LOD was not properly reported, calculated, or measured practically or in replicates; LOQ was not measured practically; selectivity is poor, with fewer than three blanks showing no interference and no interfering substances tested; robustness was not tested; stability was not tested at any level; and the method is not stability-indicating. Importantly, the method was tested only on simulated (not real) samples. Feasibility is high (reagents commercially available, instruments standard, cost < $10 per sample), and the method is quantitative for 2–3 analytes but only one matrix. Instrumentation is miniaturized but not portable. Figure 2 shows the microextraction modes compared and the TriCA score of the sugaring-out induced homogeneous liquid–liquid microextraction (SULLME).

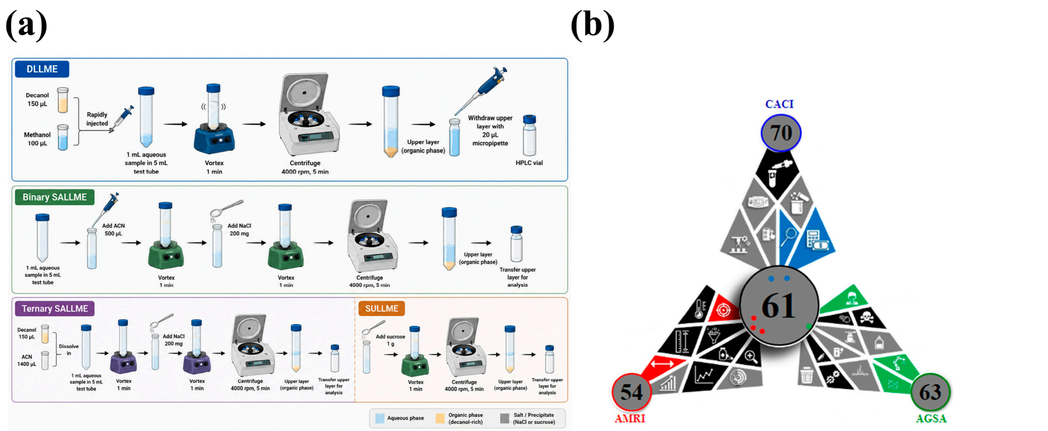

Figure 2. (**a**) Comparison of different microextraction modes, (**b**) TriCA Score of sugaring-out assisted microextraction mode for determination of antivirals in human plasma.

Priya et al. [27] report an electrochemical sensor based on a perovskite-type potassium niobate synthesized using deep eutectic solvents for the detection of bisphenol A. The method requires only moderate sample preparation (1 min) and an exceptionally small sample volume (<0.1 mL), with a total analysis time of 2 min. It is not miniaturized, is semi-automated, and avoids derivatization. Green chemistry features are outstanding, with fully renewable reagents, very low energy consumption (<0.1 kW per sample), and moderate reagent toxicity (3–5 pictograms). Linearity is good (R^2 = 0.991–0.999), and the range is extremely wide (ULOQ/LLOQ > 1000) based on 6–9 concentration levels covering all quartiles. The LOD was properly reported and calculated, though practical measurement and replicate testing were not reported; the LOQ was measured practically. However, accuracy and precision were not tested at all. Selectivity was evaluated with more than five interfering substances, but the number of blanks with no interference was not reported. Robustness and stability were not tested, and the method is not stability-indicating. The method was applied to real samples, with a sample size < 1 mL. Feasibility is high (commercially available reagents, standard lab equipment, cost < $10 per sample). The method is quantitative for a single analyte in a single matrix, with a sensitivity level > 10%

(relatively low). Instrumentation is miniaturized but not portable. The steps and the TriCA pictogram are shown in Figure 3.

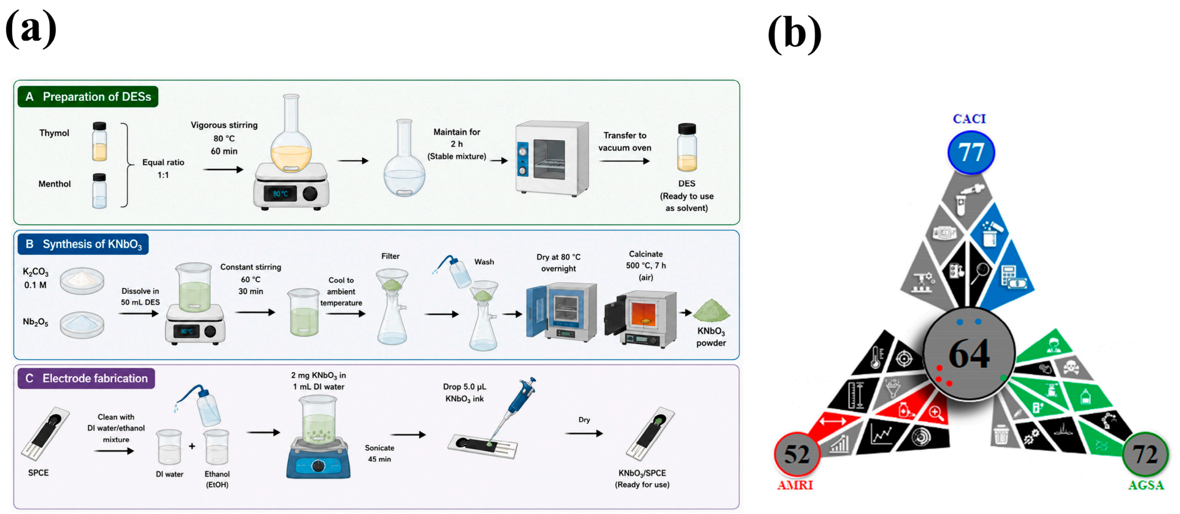

Figure 3. (**a**) Schematic of the procedure of perovskite-type potassium niobate electrochemical sensor for bisphenol A detection using deep eutectic solvents; (**b**) TriCA output showing outstanding greenness, good blueness, and poor redness due to untested accuracy, precision, robustness, and stability parameters.

Yan et al. [28] described a turn-on fluorescent method using water-soluble WS_2 quantum dots for the measurement of lipoic acid. Sample preparation is minimal (1 min) with a sample volume < 1 mL and analysis time of 2 min. The method is not miniaturized, is semi-automated, and avoids derivatization. It excels in greenness, with fully renewable reagents, very low energy (<0.1 kW per sample), and very low toxicity (only 2 pictograms). Linearity is good (R^2 = 0.991–0.999), with a moderate range ratio (10–100) assessed using more than 10 concentration levels that cover all four quartiles. Selectivity is adequate (more than five interfering substances tested), but the number of blanks without interference is low (<3). The method suffers from poor accuracy and precision: observed %bias and %RSD ratios are $\geq$1 relative to acceptance limits, indicating unacceptable performance. Although the LOD was properly reported and calculated, it was not measured practically or in replicates; the LOQ was not measured practically. Robustness was not tested, and the method is not stability-indicating (stability levels not applicable). The method was applied to real samples with a sample size < 1 mL. Feasibility is high (commercially available reagents, standard instruments, cost < $10 per sample). It is quantitative for a single analyte in a single matrix, with high sensitivity (<1%). Instrumentation is miniaturized but not portable. A summary of the procedures and the TriCA score are illustrated in Figure 4.

Abdallah et al. [29] present a supramolecular dispersive liquid–liquid microextraction using a gadolinium-based magnetic ionic liquid, followed by HPLC-UV for favipiravir determination in human plasma. Sample preparation is extensive (19 min), requiring 1–10 mL of sample, with a total analysis time of 24 min. The method is fully miniaturized, semi-automated, and derivatization-free, using a mixture of renewable and non-renewable reagents and consuming 1–1.5 kW per sample. Reagent toxicity is very high ($\geq$6 pictograms). Validation shows excellent linearity (R^2 > 0.999), an extremely wide range (ULOQ/LLOQ > 1000) based on more than 10 concentration levels covering all quartiles, and acceptable accuracy (observed %bias ratio < 0.5). Precision is moderate: repeatability and intermediate precision ratios are 0.5–1, which still meet typical acceptance criteria. The LOQ was measured practically with observed %RSD and %bias ratios in the 0.5–1 range. However, the LOD was not properly reported, calculated, or measured practically or in

https://doi.org/10.3390/analytica7030052

replicates. Selectivity was not tested for interfering substances, though ≥6 blanks showed no interference. Robustness was not tested. Stability was assessed at two levels, but the method is not stability-indicating. The method was successfully applied to real plasma samples. Feasibility is excellent (reagents commercially available, standard lab equipment, cost < $10 per sample). It is quantitative for a single analyte in a single matrix, with high sensitivity (<1%). Instrumentation is miniaturized but not portable. The procedures and the TriCA are demonstrated in Figure 5.

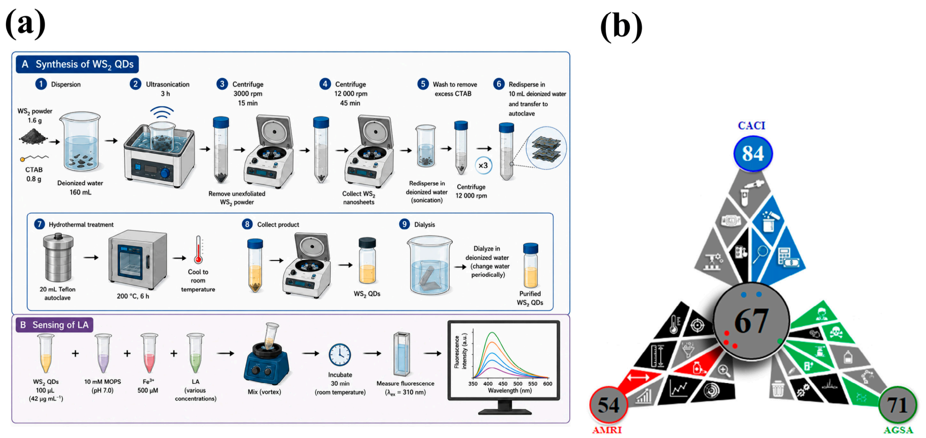

Figure 4. (**a**) Principle and (**b**) TriCA assessment of the turn-on fluorescent method using water-soluble WS_2 quantum dots for lipoic acid measurement.

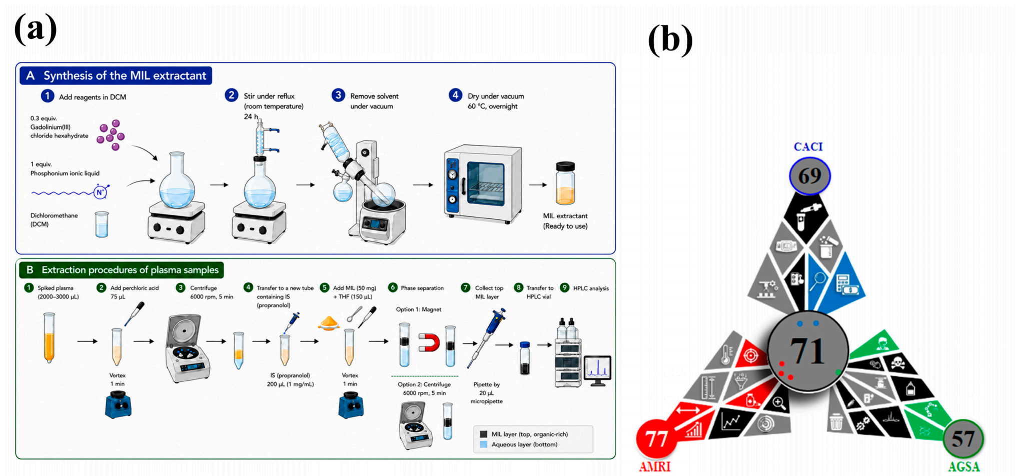

Figure 5. (**a**) Procedure and (**b**) TriCA output for the supramolecular dispersive liquid–liquid microextraction using a gadolinium-based magnetic ionic liquid for favipiravir determination.

Abdella & Elshenawy [30] described a spatial-hue smartphone-based colorimetric method for the detection and discrimination of carmine and carminic acid in food products based on differential adsorptivity. This method is exceptional for green and in situ analysis: measurements are performed directly at the sampling site; sample preparation is minimal (18 min) with a sample volume < 0.1 mL and total analysis time of 19 min; the analytical process is fully integrated; waste is <100 mL per sample; reagents are fully renewable; energy consumption is very low (<0.1 kW per sample); and toxicity is very low (2 pictograms). The instrumentation is portable. Linearity is good (R^2 = 0.991–0.999) and accuracy/precision are acceptable (observed %bias and %RSD ratios = 0.5–1). Nevertheless, the dynamic range is narrow (ULOQ/LLOQ ≤ 10) compared to other methods. Validation has several limitations: the LOD was properly reported and calculated but not measured practically or in replicates; the LOQ was not measured practically; selectivity is poor (<3 interfering substances tested and <3 blanks with no interference); robustness was not tested; stability was not tested; and the method is not stability-indicating. The method was applied to real samples, with a sample size < 1 mL. Feasibility is high (commercially available reagents, standard instruments, cost < $10 per sample). It is quantitative for 2–3 analytes and can be applied to more than three matrices, with high sensitivity (<1%). Despite its portability and green profile, the method is only semi-automated, as shown in Figure 6.

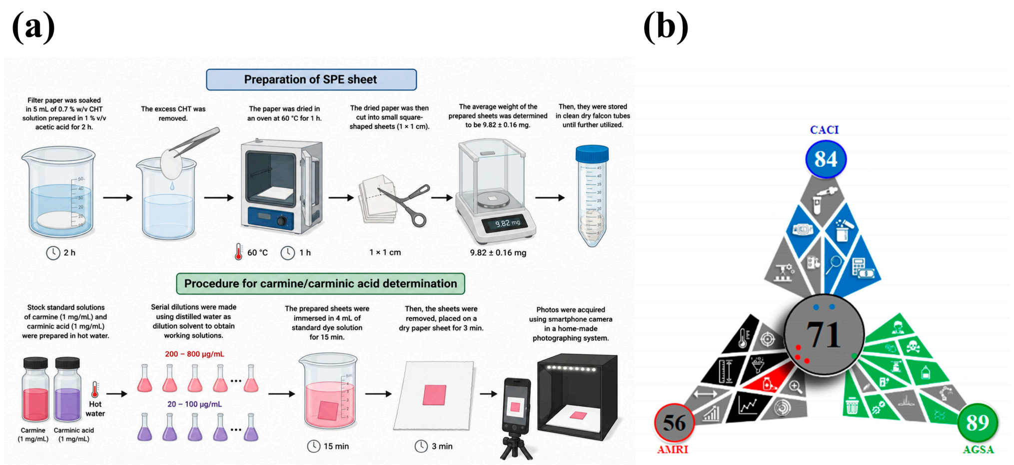

Figure 6. (**a**) Procedure and (**b**) TriCA assessment of the spatial-hue smartphone-based colorimetric method for carmine and carminic acid discrimination in food products, highlighting exceptional greenness and portability but poor selectivity, incomplete LOD/LOQ documentation, and lack of robustness testing.

Nabil et al. [31] integrated sustainable spectrophotometry with AI-driven data analysis for the simultaneous determination of carvedilol, hydrochlorothiazide, and a toxic impurity. This method represents the most comprehensive balance of greenness and validation rigor among the seven cases. It requires minimal sample treatment, performs in situ measurements at the sampling site, uses a small sample volume, and has a short analysis time of only 2 min. The analytical process is partially integrated (reducing steps and instrument use), waste is recycled (<100 mL per sample), reagents are fully renewable, energy consumption is very low (<0.1 kW per sample), and toxicity is very low (2 pictograms). Validation is outstanding: linearity is excellent ($R^2 > 0.999$); the range is wide (ULOQ/LLOQ 100–1000) based on $\geq$10concentration levels (covering all quartiles); accuracy is good (observed %bias ratio between 0.5–1); repeatability, intermediate precision, and reproducibility all show %RSD ratios < 0.5 relative to acceptance limits. The LOD was properly reported, calculated, measured practically (but only in sample matrix), and measured in more than three replicates. The LOQ was measured practically with %RSD and %bias ratios < 0.5. Selectivity is good: more than five interfering substances were tested, but <3 blanks showed no interference. Robustness was tested with more than three variables, and the maximum %RSD observed was less than 1% Stability was tested at two levels, although the method is stability-indicating. The method was applied only to simulated samples, not real ones. Feasibility is high (reagents commercially available, standard lab equipment, cost < $10 per sample). It is quantitative for 2–3 analytes and more than three matrices, with a sensitivity level $\leq$ 10%. Instrumentation is not portable nor miniaturized. The chemical structures and the TriCA output are shown in Figure 7.

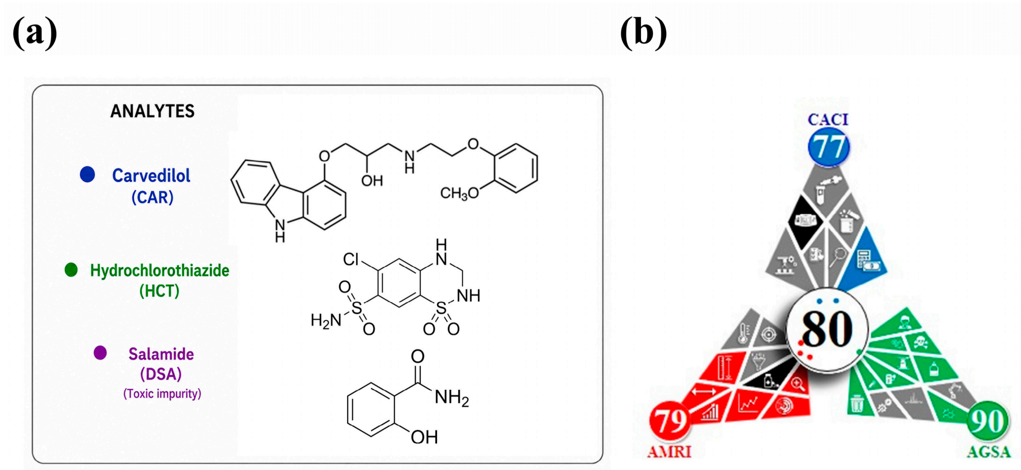

Figure 7. (**a**) Chemical structures and (**b**) TriCA output for the AI-driven sustainable spectrophotometric method for carvedilol, hydrochlorothiazide, and toxic impurity determination.

4. Comparison with Other Multicolor Assessment Tools

TriCA distinguishes itself from other multicolor assessment tools [20–22,32] through four key features: (i) a recommended validation-first weighting scheme (red = 3, blue = 2, green = 1) that reflects the logical priorities of method development; (ii) comprehensive integration of all criteria from AGSA (12 principles), CACI (8 criteria), and AMRI (10 parameters); (iii) detailed criterion-level diagnostic feedback through three integrated pictograms that guide targeted method improvement; and (iv) the ability to select the evaluation tool in each dimension.

The weighting scheme embedded in TriCA assigns the highest importance to the red dimension (analytical reliability) with a weight of 3, reflecting the non-negotiable principle that a method must be analytically valid before any other consideration becomes relevant. A method that fails to demonstrate acceptable accuracy, precision, or selectivity cannot be considered fit for purpose regardless of how environmentally friendly or operationally convenient it may be. The blue dimension (practicality) receives a weight of 2, recognizing that, among methods that are analytically valid, those that are faster, simpler, more cost-effective, and more accessible are inherently preferable. The green dimension receives a weight of 1, acknowledging that greenness is a highly desirable attribute but should not come at the expense of analytical credibility or practical usability. This hierarchical logic stands in contrast to the equal-weighting approaches of the original RGB12 model and the MA Tool, which implicitly assume that analytical performance, environmental impact, and practicality are equally important in every analytical context—an assumption that rarely holds in practice.

The comparative analysis presented in Table S1 highlights several key features that distinguish TriCA from existing multi-color assessment frameworks. Unlike RGB12 and RGBfast, which employ fixed equal weighting across all dimensions, or the MA Tool, which also applies uniform weighting, TriCA offers a user-customizable weighting system with a recommended validation-first default (red = 3, blue = 2, green = 1). This approach provides flexibility while maintaining a scientifically grounded hierarchy, allowing analysts to adapt the evaluation to their specific context without sacrificing transparency or reproducibility. Mosaic-WAC, in contrast, enforces a fixed performance-biased weighting scheme (45% red) that does not permit user adjustment, whereas WECA allows customization but is limited to a maximum of 12 criteria, whereas TriCA evaluates all 30 criteria from the three parent metrics. Perhaps the most distinctive feature of TriCA lies in its diagnostic output. While most existing tools generate a single composite pictogram and a whiteness score, TriCA produces three separate, detailed pictograms, 12 arms for AGSA, eight segments for CACI, and 10 segments for AMRI, each color-coded to reveal performance at the individual criterion level. This detailed feedback enables analysts to identify specific deficiencies in green chemistry principles, practicality criteria, or validation parameters, transforming assessment from a scoring exercise into actionable guidance for method improvement. Furthermore, with a total of 30 criteria across the three dimensions, TriCA offers substantially more comprehensive coverage than RGB12 (12 criteria) or RGBfast (six criteria), while remaining accessible through a free, open-access web platform that requires no installation and ensures reproducibility through adherence to the scoring protocols of the parent metrics. Besides, TriCA allows selecting between tools to evaluate each color dimension, which offers more flexibility.

5. Conclusions

TriCA represents a distinct contribution to the landscape of multicolor analytical method assessment tools, distinguished by its integration of three pre-validated metrics, its default validation-first weighting hierarchy, and its emphasis on criterion-level diagnostic feedback rather than composite scoring alone. By assigning the highest importance to analytical performance (red, w = 3), moderate importance to practicality (blue, w = 2), and lower importance to greenness (green, w = 1), TriCA reflects the logical priorities of analytical method development: a method must first be valid, then practical, and finally green. This hierarchical approach stands in contrast to equal-weighting frameworks that assume all dimensions are equally important in every context, as well as to flexible-weighting tools that, while valuable, may permit weighting schemes that undervalue reliability if used without careful justification. The diagnostic orientation of TriCA further differentiates it from existing tools, as it directs the analyst's attention to the three detailed pictograms that reveal performance at the individual criterion level. An analyst evaluating a method can immediately identify that robustness testing is incomplete (redness dimension), that waste management is inadequate (greenness dimension), or that sample preparation complexity is excessive (blueness dimension). This granular feedback transforms assessment from a passive scoring exercise into an active guide for targeted method improvement, making TriCA uniquely suited for method development and optimization rather than merely for final method selection or regulatory reporting. The successful application of TriCA to seven diverse case studies encompassing liquid–liquid microextraction, electrochemical sensing, fluorescent methods, smartphone-based colorimetry, and AI-driven spectrophotometry demonstrates the tool's broad applicability across analytical techniques. In each case, the AGSA, CACI, and AMRI pictograms successfully highlighted specific strengths and weaknesses that were not immediately apparent from traditional validation data alone. The hierarchical weighting scheme also proved effective in differentiating methods that excel in analytically critical dimensions from those that achieve moderate overall scores through strong greenness performance despite compromised validation. TriCA is implemented as an open-access web application with a user-friendly interface, automated score calculations, and dynamic visual outputs. The tool requires no installation, maintains transparency through published scoring equations, and ensures reproducibility by adhering strictly to the protocols of the parent metrics. While TriCA does not replace the need for comprehensive validation documentation or regulatory compliance reporting, it provides a standardized, transparent, and diagnostically powerful framework for comparative method evaluation, iterative method optimization, and educational instruction in green and white analytical chemistry principles.

https://doi.org/10.3390/analytica7030052

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/analytica7030052/s1, Table S1: Comparison of TriCA with other multi-color assessment tools for analytical method evaluation.

Author Contributions: Conceptualization, F.R.M., K.M.O., I.A., S.S.M.A. and A.B.; investigation, F.R.M., M.L. and A.B.; data curation, M.L., K.M.O. and A.B. and I.A.; writing—original draft preparation, F.R.M., M.L., K.M.O., S.S.M.A. and A.B. and I.A.; writing—review and editing, F.R.M., M.L., K.M.O., S.S.M.A. and A.B.; visualization, A.B., F.R.M. and K.M.O.; supervision, F.R.M. and M.L.; project administration, F.R.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data will be provided upon reasonable requests.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Elagamy, S.H.; Fuente-Ballesteros, A.; Chanduluru, H.K.; Obaydo, R.H. Systematic Review of Recent Metrics (2020–2025) for Greenness, Applicability, and Analytical Performance with Guidelines for Practical Use. *Results Chem.* **2026**, *20*, 103048. [CrossRef]
2. Nowak, P.M.; Kościelniak, P.; Tobiszewski, M.; Ballester-Caudet, A.; Campíns-Falcó, P. Overview of the Three Multicriteria Approaches Applied to a Global Assessment of Analytical Methods. *TrAC Trends Anal. Chem.* **2020**, *133*, 116065. [CrossRef]
3. Imam, M.S.; Abdelrahman, M.M. How Environmentally Friendly Is the Analytical Process? A Paradigm Overview of Ten Greenness Assessment Metric Approaches for Analytical Methods. *Trends Environ. Anal. Chem.* **2023**, *38*, e00202. [CrossRef]
4. Hammad, S.F.; Hamid, M.A.A.; Adly, L.; Elagamy, S.H. Comprehensive Review of Greenness, Whiteness, and Blueness Assessments of Analytical Methods. *Green Anal. Chem.* **2025**, *12*, 100209. [CrossRef]
5. Fuente-Ballesteros, A.; Martínez-Martínez, V.; Ares, A.M.; Valverde, S.; Samanidou, V.; Bernal, J. Violet Innovation Grade Index (VIGI): A New Survey-Based Metric for Evaluating Innovation in Analytical Methods. *Anal. Chem.* **2025**, *97*, 6946–6955. [CrossRef] [PubMed]
6. Sajid, M.; Płotka-Wasylka, J. Green Analytical Chemistry Metrics: A Review. *Talanta* **2022**, *238*, 123046. [CrossRef] [PubMed]
7. Nowak, P.M.; Wietecha-Posłuszny, R.; Pawliszyn, J. White Analytical Chemistry: An Approach to Reconcile the Principles of Green Analytical Chemistry and Functionality. *TrAC-Trends Anal. Chem.* **2021**, *138*, 116223. [CrossRef]
8. Nowak, P.M.; Kościelniak, P. What Color Is Your Method? Adaptation of the RGB Additive Color Model to Analytical Method Evaluation. *Anal. Chem.* **2019**, *91*, 10343–10352. [CrossRef] [PubMed]
9. Locatelli, M.; Kabir, A.; Perrucci, M.; Ulusoy, S.; Ulusoy, H.I.; Ali, I. Green Profile Tools: Current Status and Future Perspectives. *Adv. Sample Prep.* **2023**, *6*, 100068. [CrossRef]
10. Samanidou, V.F. Metric Tools in Analytical Chemistry: From Proliferation to Harmonization. *Green Anal. Chem.* **2026**, *16*, 100326. [CrossRef]
11. Mansour, F.R.; Bedair, A.; Belal, F.; Magdy, G.; Locatelli, M. Analytical Green Star Area (AGSA) as a New Tool to Assess Greenness of Analytical Methods. *Sustain. Chem. Pharm.* **2025**, *46*, 102051. [CrossRef]
12. Pena-Pereira, F.; Wojnowski, W.; Tobiszewski, M. AGREE—Analytical GREEnness Metric Approach and Software. *Anal. Chem.* **2020**, *92*, 10076–10082. [CrossRef] [PubMed]
13. Płotka-Wasylka, J. A New Tool for the Evaluation of the Analytical Procedure: Green Analytical Procedure Index. *Talanta* **2018**, *181*, 204–209. [CrossRef] [PubMed]
14. Mansour, F.R.; Locatelli, M.; Fuente-Ballesteros, A.; Bedair, A. Analytical Method Reliability Index (AMRI) as an Innovative Tool and Software for the Assessment of Analytical Procedure. *Microchem. J.* **2026**, *221*, 116826. [CrossRef]
15. Nowak, P.M.; Wojnowski, W.; Manousi, N.; Samanidou, V.; Płotka-Wasylka, J. Red Analytical Performance Index (RAPI) and Software: The Missing Tool for Assessing Methods in Terms of Analytical Performance. *Green Chem.* **2025**, *27*, 5546–5553. [CrossRef]
16. Mansour, F.R.; Bedair, A.; Locatelli, M. Click Analytical Chemistry Index as a Novel Concept and Framework, Supported with Open Source Software to Assess Analytical Methods. *Adv. Sample Prep.* **2025**, *14*, 100164. [CrossRef]
17. Manousi, N.; Wojnowski, W.; Płotka-Wasylka, J.; Samanidou, V. Blue Applicability Grade Index (BAGI) and Software: A New Tool for the Evaluation of Method Practicality. *Green Chem.* **2023**, *25*, 7598–7604. [CrossRef]
18. Nowak, P.M. How to Correctly Evaluate Greenness, Whiteness and Other "Colours"? Introducing General Rules of a Good Evaluation Practice. *Green Chem.* **2025**, *27*, 6699–6710. [CrossRef]

19. Yahya, L.A.; Vakh, C.; Dushna, O.; Kalisz, O.; Bocian, S.; Tobiszewski, M. Guidelines on the Proper Selection of Greenness and Related Metric Tools in Analytical Chemistry—A Tutorial. *Anal. Chim. Acta* **2025**, *1357*, 344052. [CrossRef] [PubMed]
20. Nowak, P.M.; Arduini, F. RGBfast—A User-Friendly Version of the Red-Green-Blue Model for Assessing Greenness and Whiteness of Analytical Methods. *Green Anal. Chem.* **2024**, *10*, 100120. [CrossRef]
21. Mansour, F.R.; Locatelli, M.; Obaydo, R.H.; Farag, A.S.; Bedair, A. Whiteness Evaluation for Chemical Analysis (WECA) as a Flexible Tool and Web-Based Software for Whiteness Assessment in Analytical Chemistry. *Analytica* **2026**, *7*, 19. [CrossRef]
22. Abbas, A.E.F.; Al kamaly, O.; Magdy, G.; Halim, M.K. MA Tool—Multi-Color Assessment Platform for White Analytical Chemistry: Unified Evaluation of Method Greenness, Practicality, Performance, and Innovation. *Microchem. J.* **2025**, *216*, 114781. [CrossRef]
23. Fuente-Ballesteros, A.; Samanidou, V.; Ares, A.M.; Bernal, J. Ten Principles for Developing and Implementing Tools in the Context of White Analytical Chemistry. *Sustain. Chem. Pharm.* **2025**, *45*, 102031. [CrossRef]
24. Saleh, S.S.; Obaydo, R.H.; El Hamd, M.A.; Rostom, Y.; Mohamed, D.; Lotfy, H.M. Guidelines for Accurate Application of Green and White Analytical Concepts: Merits Versus Demerits with Insights of Significant Milestones of Assessment Tools Applied for Antiviral Drugs. *Microchem. J.* **2024**, *199*, 109917. [CrossRef]
25. Abdallah, I.A.; Hammad, S.F.; Bedair, A.; Mansour, F.R. Menthol-Assisted Homogenous Liquid-Liquid Microextraction for HPLC/UV Determination of Favipiravir as an Antiviral for COVID-19 in Human Plasma. *J. Chromatogr. B* **2022**, *1189*, 123087. [CrossRef] [PubMed]
26. Abdallah, I.A.; Hammad, S.F.; Bedair, A.; Mansour, F.R. Sugaring-out Induced Homogeneous Liquid-liquid Microextraction as an Alternative Mode for Biological Sample Preparation: A Comparative Study. *J. Sep. Sci.* **2021**, *44*, 3117–3125. [CrossRef] [PubMed]
27. Priya, T.S.; Chen, T.-W.; Chen, S.-M.; Kokulnathan, T.; Lou, B.-S.; Al-onazi, W.A.; Al-Mohaimeed, A.M.; Elshikh, M.S.; Yu, J. Synthesis of Perovskite-Type Potassium Niobate Using Deep Eutectic Solvents: A Promising Electrode Material for Detection of Bisphenol A. *Chemosphere* **2023**, *318*, 137948. [CrossRef] [PubMed]
28. Yan, Y.; Zhang, C.; Gu, W.; Ding, C.; Li, X.; Xian, Y. Facile Synthesis of Water-Soluble WS 2 Quantum Dots for Turn-On Fluorescent Measurement of Lipoic Acid. *J. Phys. Chem. C* **2016**, *120*, 12170–12177. [CrossRef]
29. Abdallah, I.A.; Hammad, S.F.; Bedair, A.; Abdelaziz, M.A.; Danielson, N.D.; Elshafeey, A.H.; Mansour, F.R. A Gadolinium-based Magnetic Ionic Liquid for Supramolecular Dispersive Liquid–Liquid Microextraction Followed by HPLC/UV for the Determination of Favipiravir in Human Plasma. *Biomed. Chromatogr.* **2022**, *36*, e5365. [CrossRef] [PubMed]
30. Abdella, A.A.; Elshenawy, E.A. A Spatial Hue Smartphone-Based Colorimetric Detection and Discrimination of Carmine and Carminic Acid in Food Products Based on Differential Adsorptivity. *Talanta* **2025**, *282*, 127053. [CrossRef] [PubMed]
31. Nabil, M.; Nashat, N.W.; Marzouk, H.M.; Abbas, S.S.; Lotfy, H.M. Integration of Sustainable Spectrophotometric Methods and AI-Driven Data Analysis for Simultaneous Determination of Carvedilol, Hydrochlorothiazide and a Toxic Impurity. *Green Anal. Chem.* **2026**, *17*, 100354. [CrossRef]
32. Al-Khateeb, L.A.; A.M.Ali, M.; S.Batubara, A.; Gamal, M.; A.Naguib, I.; Chaudhary, A.A.; M.Ibrahim, A. The Mosaic-WAC Tool: Automating the Holistic Assessment of Green, Red, Blue, and Violet Principles. *Microchem. J.* **2026**, *226*, 118329. [CrossRef]

https://doi.org/10.3390/analytica7030052