



Evaluating the greenness of MP-AES spectroscopy through AGREE metrics

Luca Vignali^a, Elena Bernardi^{a,b,*}, Enrico Sassoni^c, Martina Cappelletti^d,
Elena Cofini^e, Daniele Cespi^{a,b,**}

^a Department of Industrial Chemistry "Toso Montanari", University of Bologna, via Piero Gobetti 85, 40129, Bologna, Italy

^b Interdepartmental Centre of Industrial Research "Renewable Resources, Environment, Sea and Energy", University of Bologna, via Angherà 22, 47922, Rimini, Italy

^c Dept. Civil, Chemical, Environmental & Materials Engineering (DICAM), University of Bologna, Via Terracini 28, 40131, Bologna, Italy

^d Department of Pharmacy and Biotechnology, University of Bologna, Via Irnerio 42, 40126, Bologna, Italy

^e Department of Cultural Heritage, University of Bologna, via degli Ariani 1, 48121, Ravenna, Italy

ARTICLE INFO

Keywords:

Green analytical chemistry
Green sample preparation
AGREE
AGREEprep
MP-AES
Cultural heritage

ABSTRACT

The principles of green chemistry have progressively expanded into green analytical chemistry (GAC) and green sample preparation (GSP), fostering the development of quantitative tools such as AGREE and AGREEprep to assess the environmental sustainability of analytical workflows. Although these tools are increasingly adopted, their application is often limited to numerical scoring, with scarce discussion of the underlying assumptions and decision-making processes.

This study critically evaluates the applicability and effectiveness of AGREE and AGREEprep in the context of Microwave-induced Plasma Atomic Emission Spectroscopy (MP-AES) analyses, using a real analytical workflow adopted within the Italian PRIN2022 project SECURE-COATS focused on developing functionalized and sustainable coatings for the conservation of stone materials in cultural heritage. The case study involves the determination of Ag, Zn, Ca and P in aqueous solutions obtained from immersion tests in bacterial growth media and from artificial ageing tests, aimed at assessing, respectively, the release of potentially antibacterial metals and the durability of the new coatings.

A systematic and transparent data collection was performed to populate both AGREE tools, and the rationale behind each input parameter is explicitly discussed. This approach allows a critical assessment of how methodological choices, instrumental features and sample preparation steps influence the final greenness scores.

This work demonstrates their value as decision-support instruments for improving analytical processes also in the field of chemistry for cultural heritage, by reducing both environmental impact and operational costs. The provided data and proposed methodology could serve as a useful reference framework for future green assessments.

1. Introduction

Over the years, chemistry has played a key role in societal development, but its advancements have often negatively impacted the environment [1,2]. Awareness of these issues emerged in the 1960s with Rachel Carson's book *Silent Spring*, fostering a growing focus on more sustainable chemistry practices [1]. In 1991, Paul Anastas introduced the concept of green chemistry, which was further consolidated in 1998 with the formulation of twelve guiding principles co-authored with John Warner [3]. These principles provide a framework for the sustainable design of chemical products and processes [1,4,5]. Later, the concept of

Green Analytical Chemistry (GAC) emerged, focusing on making laboratory analytical methodologies more environmentally sustainable [2,6,7]. In 2013, Galuska et al. proposed the twelve principles of GAC, adopting a similar approach to that of Anastas and Warner [1,6,8–10], with the aim of protecting the environment and ensuring human safety during the development and implementation of analytical procedures [6,11]. The relevance of GAC lies in the fact that analytical methods can significantly contribute to environmental issues. This is because various stages of the procedures require hazardous chemicals, generating toxic waste in quantities far greater than the initial sample [7,12–14].

A crucial step in analytical methodologies is sample preparation,

* Corresponding author. Department of Industrial Chemistry "Toso Montanari", University of Bologna, via Piero Gobetti 85, 40129 Bologna, Italy.

** Corresponding author. Department of Industrial Chemistry "Toso Montanari", University of Bologna, via Piero Gobetti 85, 40129 Bologna, Italy.

E-mail addresses: elena.bernardi@unibo.it (E. Bernardi), daniele.cespi2@unibo.it (D. Cespi).

often essential for isolating and concentrating the analyte, but also highly impactful due to its significant consumption of solvents, reagents, and materials [2,12,13,15]. The first principle of GAC advocates the use of direct analytical techniques to avoid sample preparation [6]. However, this principle does not adequately address the complexity of this stage, which is critical to ensuring compatibility with the chosen analytical technique [16]. To bridge this gap, the concept of Green Sample Preparation (GSP) was then introduced, extending the first principle of GAC through ten guidelines aimed at making this stage more sustainable and improving overall analytical methodologies [13,16].

In recent years, considerable efforts have been dedicated to designing analytical processes in line with GAC principles [10,17,18]. However, many claims regarding the environmental sustainability of analytical procedures lack quantitative assessments and are often based on subjective impressions or uncertain assumptions, primarily due to the absence of standardized evaluation criteria [7,12,19–21]. This underscores the need for robust tools capable of assessing the environmental impact of analytical processes through objective indicators [10].

To address this challenge, several tools based on GAC have been developed, all sharing the common goal of assessing the environmental impact of analytical procedures. These include, for instance, NEMI [22], Analytical Eco-scale [23], GAPI [21], BAGI [24], AMGS [25], CALIFICAMENT [10,20,26–35]. However, the application of this greenness metric tools is sometimes inconsistent, which has raised concerns about the reproducibility and reliability of their results. Recent guidelines by Yahya et al. provide a systematic framework for selecting and applying these tools properly, demonstrating that, while they produce correlated outcomes, standardized protocols are essential to obtain meaningful and comparable assessments [28], which is precisely the focus of the present work.

In this study, AGREE and AGREEprep were selected as they are among the most recent and complementary tools, as also emphasized by Yahya et al. [28], allowing for a comprehensive assessment of the analytical method. Introduced in 2020, AGREE was the first software designed to evaluate analytical procedures based on the twelve principles of GAC [36]. In 2022, the framework was expanded with AGREEprep, a tool specifically designed to assess sample preparation techniques according to the ten principles of GSP [13].

Several studies investigate the application of these two approaches in the GAC and GSP sectors, focusing on their ability to evaluate the environmental impact of analytical procedures and identify opportunities for improvement [17,18,26,37]. These tools have been applied to various analytical techniques, such as chromatography, spectroscopy and microextraction, to assess their alignment with sustainability principles, highlighting critical points in workflows [38–41]. However, some studies emphasize a lack of comprehensive information regarding their objective and systematic application [9,24,27]. This is mainly because users, when modifying the relative weight of the criteria, do not justify their choices, as noted by the developers of the AGREE tools [13,15]. This lack of justification results in findings that cannot be compared with similar studies and are difficult for readers to interpret.

To our knowledge, only two studies have applied AGREE and AGREEprep to analytical procedures involving MP-AES [40,42]. These studies primarily focused on using the tools to optimize analytical methods and compare them with conventional procedures. They suggest that MP-AES represents a more environmentally friendly and versatile analytical technique compared to traditional ones such as flame atomic absorption spectrometry (FAAS) [40,42,43]. Furthermore, MP-AES allows for the simultaneous determination of multiple analytes, thereby reducing energy consumption, analysis time, and sample volume. However, as previously mentioned, even in these cases, no justification is provided for the choices made to obtain the results.

In contrast, the present work evaluates metal analysis in aqueous solutions by providing a detailed examination of the responses generated by the two tools, offering a comprehensive analysis that can serve as a reference for future research employing MP-AES.

Furthermore, to our knowledge, no application of AGREE and AGREEprep has been reported in the field of cultural heritage chemistry. This work addresses this gap by analysing samples obtained in the context of the Italian project PRIN2022 SECURE-COATS (Safe, Eco-friendly, and Durable Coatings to Prevent Deterioration of Heritage Stones) as a case study. The main aim is to provide clear and structured guidelines for the application of AGREE and AGREEprep in this chemical sector, ensuring an objective evaluation of results and offering a reference framework for future research employing MP-AES in both analytical and cultural heritage chemistry. This work seeks to standardize the use of these tools as much as possible, making their application simpler, clearer, and faster for future researchers. By doing so, it aims to facilitate consistent and efficient evaluations, promoting wider adoption and improving comparability across studies. To achieve this, the study provides practical elements such as energy data, examples of justification for the choices and weights used in the integrated application of AGREE and AGREEprep, the application to a tool that had not previously been assessed (MP-AES), and a step-by-step description of the entire process.

In summary, this work presents four key innovations: (i) it provides a clear justification for a more comprehensive application of the AGREE and AGREEprep tools in the evaluation of MP-AES procedures; (ii) it demonstrates their application in the field of cultural heritage chemistry through the analysis of PRIN2022 SECURE-COATS samples; (iii) it establishes a reference framework to support reproducible and comparable sustainability assessments across analytical chemistry and cultural heritage contexts; and (iv) it offers practical, implementation-oriented guidelines, including energy data, weighting justifications, and step-by-step protocols.

In the following sections, the methodologies used to evaluate the "greenness" of the MP-AES analysis are described in detail, with particular emphasis on the application of the AGREE and AGREEprep criteria. For a better understanding of the experimental data and procedures, reference is also made to the supplementary material: [Tables S1 and S3](#) explain the motivation behind each response and the weighting of the criteria, while [Table S2](#) and [Fig. S1](#) complete the description of how the data collection for the experimentally calculated criteria was conducted. In addition, [Fig. S2](#) represents the complementary scores with equal weights.

2. Methodology

In this study, MP-AES was applied to determine the concentration of target analytes (i.e., Ag, Zn, Ca, and P) in different aqueous samples obtained within the SECURE-COATS project. This section outlines the context in which the analytical criteria arise. It then describes the application of AGREEprep and AGREE, offering a thorough evaluation of both the sample preparation process and the analytical methods.

SECURE-COATS project, funded by the national program PRIN (*in Italian*, Progetti di Rilevante Interesse Nazionale) 2022, was selected as reference case study. It aims to conserve stone materials in cultural heritage through innovative and sustainable treatments based on functionalized hydroxyapatite (HAP) coatings. The treatments are designed to combine three functions: antifouling, anti-staining and self-diagnostics. One route to obtain these abilities is to functionalise the HAP coatings by addition of silver or zinc. As environmental sustainability of the treatments is one of the key points of the project, it may also be useful to understand and assess the alignment of the analytical methodologies applied during the coating development phase with the principles of green analytical chemistry.

To investigate the antifouling properties of the treatments when applied to marble samples, the release of functionalizing metals with possible antibacterial activity, such as Ag and Zn, in a commonly used bacterial growth medium was evaluated. Functionalized marble samples (1 × 1 cm), along with non-functionalized control samples, were placed in 24-well plates (one sample per well), each containing 2 mL of tryptic soy broth (TSB) (Conda). The plates were incubated at 37 °C under

shaking (100 rpm) for 24 h, after which the solutions were collected, filtered, and analysed using MP-AES.

To assess the durability of treatments, *run-off* tests were conducted to simulate the impact of heavy rain. This was achieved by using a peristaltic pump that periodically distributed droplets of synthetic rainwater onto 2.5×2.5 cm marble samples [44]. The solutions are then collected and analysed to monitor the release of the functionalizing metals Ag and Zn, as well as markers of the substrate/coating degradation, such as Ca and P. Marble samples were prepared in a different location from where *run-off* tests and subsequent MP-AES analyses were conducted.

2.1. Description of sample preparation and analytical procedure

To evaluate the analytical methodologies, it was necessary to identify all steps involved in sample preparation and in MP-AES working session.

For the samples collected from the *run-off* test, the first step involved reducing the sample volume to approximately 50 mL. This miniaturization process was achieved by transferring the sampled solutions from storage tanks into HDPE bottles. The samples were then acidified with 0.1 mL of 65% HNO₃ (Suprapur®) using a Pasteur pipette and stored at 4 °C until MP-AES working session. For calcium determination, an additional step was required: 0.05 mL of CsCl 0.83 M (Carlo Erba) solution was added, with a micropipette, to the sample before MP-AES working session as an ionisation suppressor.

For the metal release test in the bacterial growth medium, sample preparation required a different approach. The samples, which arrived from an external location, had a volume of 1.5 mL and were contained in Eppendorf tubes®. Before MP-AES working session, approximately 0.1 mL of nitric acid was added to each sample.

The operation of MP-AES is based on atomic emission spectroscopy, in which the sample is nebulised, and its elements are subsequently transformed into atomic or elementary ionic species in the gas phase through a process called atomisation. During atomisation, a fraction of these species becomes excited, and upon returning to the ground state or a lower energy level, they emit radiation in the UV-Visible range. Atomic emission spectroscopy exploits this phenomenon to obtain qualitative and quantitative information about the elements present in the sample. The atomisation source is a nitrogen plasma generated by a microwave-induced electromagnetic field, reaching a temperature of approximately 5000 K. During the ignition phase, which lasts about 10 s and involves an electrical discharge, argon is used to facilitate the initial formation of the plasma. Nitrogen is then used to support the plasma, which is maintained and stabilised by the electromagnetic field generated by microwaves. Compressed air is supplied to protect the pre-optics by passing through a filter. Additionally, the computer manages the entire examination process, collecting data and controlling the instrument's operation.

The MP-AES constantly requires an aqueous solution, as the torch cannot operate dry. When a sample is not being analysed, the peristaltic pump draws a washing solution. This solution is nebulised before reaching the torch, but only about 15% of the generated droplets reach the torch. The material that reaches the torch is converted into gaseous waste, which is released into the atmosphere after being conveyed into an exhaust hood, while the remaining liquid is collected as waste and sent to a treatment plant for disposal.

Before starting the MP-AES analyses, single or multi element standards are prepared depending on the number and type of analytes to be measured. Their concentrations are selected to cover the expected

Table 1

– Summary of reagents, materials and apparatus used in sample preparation and analytical procedure.

Reagents	Materials	Apparatus
Nitric acid 65% (HNO ₃) (Merck, Suprapur®)	High-density polyethylene (HDPE) bottles	MP-AES
Cesium chloride (CsCl) (Carlo Erba)	Pasteur pipettes	Fume-hood
Milli-Q water ($\rho = 18.2$ M Ω cm at 25 °C)	Micropipette tips	-
-	Eppendorf tubes®	-

concentration range of metals present in the sample, through the construction of a calibration line. Once the calibration is established, the samples are analysed.

During the working session, the capillaries used to draw up the sample solutions are cleaned with a washing solution composed of Milli-Q water ($\rho = 18.2$ M Ω cm at 25 °C) and 2% Suprapur® nitric acid. Despite the availability of the autosampler, the sample change and washing steps were carried out manually. This choice avoids an additional source of energy consumption and was made to reduce the analysis time and sample demand associated with longer capillaries in the autosampler.

The laboratory reagents, materials and apparatus for sample preparation and analytical procedure are summarized in Table 1.

Specific operating times, adapted to the functioning of the instrument used, were identified. The first times to consider when filling out the worksheet are related to sample withdrawal. Indeed, the sample withdrawal time corresponds to the total time required by the MP-AES to analyse a sample. This time is divided into three phases.

1. Uptake time: this is the period during which the pump transfers the sample to the torch. During this phase, the pump usually operates at maximum speed (80 rpm) for 15 s, as is the case in this study. These two parameters are set in the work schedule before the analyte determination begins and can vary according to the length of the capillaries carrying the sample to the nebulizer.
2. Signal stabilization time: during this phase, the sample signal stabilizes within the plasma. The pump runs at the set speed (here, 15 rpm). This time is also set in the work schedule and, as in this case, usually lasts 15 s.
3. Analyte determination time: this is the period the instrument takes to analyse the various analytes of interest. The pump runs at the set speed (here, 15 rpm) and the duration of this phase depends on the number and type of analytes being measured.

The other type of times identified are related to the general functioning of the instrument. Consequently, the total working session time was divided into the following main phases.

1. Ignition and plasma stabilization: includes the time needed to ignite the torch, which ranges from 10 to 15 s, and the time to stabilize the plasma (ca. 15 min).
2. Running: the period during which the analyte determination takes place, including the construction of the calibration curve (varies based on parameters like number of samples, number of standard and analyte type).
3. Washing: a phase of approximately 10 min during which the torch is cleaned with a washing solution to remove any sample residues.

4. Cooling: the phase in which the torch is cooled for about 10 min after being turned off, while keeping the flow of compressed air and nitrogen active.
5. Standby: the time during which the instrument and the torch remain switched on between two analyses.

2.2. AGREE and AGREEprep tools and data collection

AGREE and AGREEprep use a consistent approach for their evaluations, involving a questionnaire based on the principles of GAC and GSP. Each answer is assigned a score ranging from 0 (minimum, indicating no alignment with the principle) to 1 (maximum, indicating full alignment) [15]. The responses are converted into scores using equations provided in literature [13,15]. The final result is obtained by calculating a weighted average of all answers, as users can assign different weight (from 1 to 4) to each principle [15,36]. This score is represented by a pictogram that visualises the performance of each criterion on a color-coded scale, reflecting both the user's assigned weight and the alignment with the principles [15].

The evaluations conducted using AGREE and AGREEprep involved fourteen MP-AES working sessions. As highlighted by the developers [15], one of the advantages of the AGREE tools is their flexibility in assigning different weights to each criterion based on the importance given by the user. Although this approach may involve some subjectivity, it was adopted in this case study by presenting the results in two distinct scenarios.

1. Baseline scenario: application of the original default weights assigned to each criterion, both for AGREE (where all criteria have the same weight) and AGREEprep.
2. Alternative scenario: adjustment of weights to better reflect requirements and evaluations often needed for chemical analysis in the field of cultural heritage. This scenario was not applied to AGREEprep, as its weights are already representative of this context.

The weights assigned by the developers of AGREEprep, for each of the 10 AGREEprep criteria, are represented in the supplementary file with the corresponding motivation (Table S1 and Section 2.1). These criteria are represented in Fig. 1.

Table 2 shows the weights assigned to each AGREE criterion, which are represented in Fig. 2, along with the corresponding justifications, trying to limit subjectivity.

To perform evaluations using AGREEprep and AGREE, all the relevant information was gathered to answer the ten or twelve related criteria, as shown in Figs. 1 and 2, respectively. A detailed explanation of

Table 2
Weight of AGREE for the Alternative scenario.

Principle	Weight	Motivation
1	1	Sample preparation is important for the analyte determination but is not prioritized in this specific context.
2	3	Minimizing the sample size is relevant from the perspective of resource and reagent consumption. However, there is a limit to volume reduction, as the sample must remain representative depending on the type of analyte determination.
3	2	Performing analyses directly <i>in situ</i> depends on the type of analyte determination. In certain cases, transporting equipment to the field is not realistically possible, as in this case study.
4	3	Reducing the steps in an analytical procedure saves time, energy, and materials; however, this depends on the type of analyte determination.
5	2	Automation is important for operator safety, as it reduces exposure to hazardous samples. However, in this case, the choice not to use the autosampler, as described in Section 2.1, proved advantageous. Miniaturization aligns with the concept of Principle 2, as both aim to reduce sample volume, limiting the use of reagents, solvents, and energy. For this reason, its weight was set lower, since the benefit of reduced sample volume would otherwise be effectively counted twice.
6	2	Derivatization is environmentally relevant but not common for the determination of metals and inorganic components.
7	3	Preventing waste generation is crucial both environmentally and economically. This principle is also connected to Principle 2, as the amount of waste produced depends on the sample size; therefore, the assigned weight remains the same.
8	3	The speed at which the instrument analyses the analytes is important because it affects analysis time and energy consumption, two essential factors for the overall evaluation.
9	4	Energy consumption must be minimized under all circumstances.
10	1	Reagents derived from renewable resources are not yet widely available in the cultural heritage sector.
11	3	Reducing the use of toxic reagents is important, although it is not always possible to eliminate or replace them.
12	4	Operator health and safety must always be the top priority.

all the answers is provided in the supplementary file (Tables S1 and S3 within Section 2.1 and 2.2). The following outlines how the criteria requiring specific calculation was answered.

Among the ten criteria of AGREEprep, data were collected through experiments for the fourth and sixth. During the data collection, the standard preparation was not considered in accordance with the guidelines provided by Wojnowski W. et al. [13]. The fourth principle recommends minimizing waste; therefore, AGREEprep requires the input of the waste volume generated during sample preparation. To

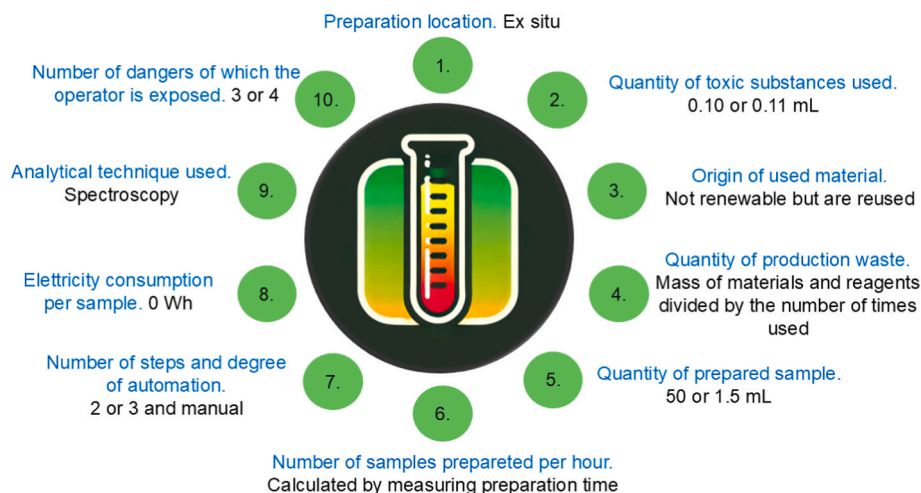


Fig. 1. AGREEprep criteria (blue) with corresponding responses (black).

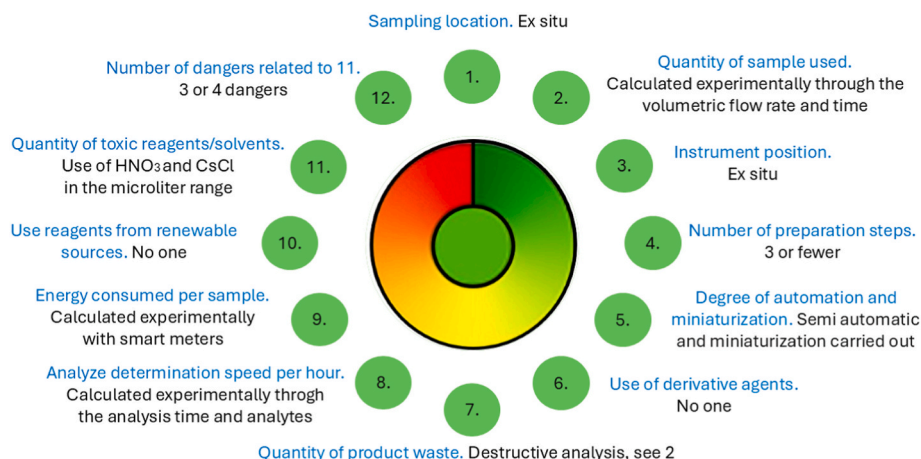


Fig. 2. AGREE criteria (blue) with corresponding response (black).

quantify this, a flowchart from Pena-Pereira et al. [45] was used. In this study, the total waste generated was calculated by summing the amounts of all the chemicals used (HNO₃ and CsCl) and the masses of all the disposable materials (high density polyethylene-HDPE bottle, Pasteur pipette, micropipette tip, and Eppendorf tube®), the latter normalized per the number of uses.

The sixth principle states that sample throughput should be maximised. The criteria required the determination of the number of samples prepared per hour. To quantify this data for the preparative phase, the time required to prepare a defined number of samples was measured with a stopwatch and then scaled to an hourly rate.

A limitation encountered during the determination of energy consumption was the omission of the fume hood's contribution during the addition of nitric acid. This exclusion could affect response 8.

Among the 12 criteria of AGREE, data were collected through experiments for the second, eighth and ninth. As mentioned before, these criteria are calculated by referring to specific operational times adapted to the instrument's functioning.

In second criterion, the analysed sample volume needs to be determined. To compute it, data on the volumetric flow rate and sample withdrawal time are needed, as this information is not included in the manual, being dependent on the instrumental parameter set (Section 2.1). The volumetric flow rate depends on the angular velocity of the peristaltic pump, which operates at either 15 or 80 rpm. The volume and time values were obtained by measuring, using a stopwatch, the time required to collect 1 mL of washing solution from a graduated cylinder. Four measurements of the time were taken for each of the two angular velocities of the peristaltic pump.

Once the two flow rates were known, the sampled volume was calculated taking into account the three phases of withdrawal (Section 2.1). The uptake and stabilization times were predefined in the work schedule, while the analyte determination time depended on the number and type of analytes. For this reason, the sample withdrawal time, Table 3 was measured three times manually using a stopwatch.

The eighth criterion required the number of analytes determined in one analyte determination and the number of samples analysed per hour. The total MP-AES working session time considered was determined by summing the ignition and plasma stabilization, analysis,

Table 3

– Data used to calculate the volumetric flow rate.

Angular velocity (rpm)	V (mL)	Average time (min)	Q (mL/min)
15	1.00 ± 0.25	4.00 ± 0.02	0.25 ± 0.06
80	1.00 ± 0.25	0.75 ± 0.02	1.33 ± 0.33

washing, and cooling phases (Section 2.1).

The ninth criterion required the instrument used or the energy consumed per sample. As the MP-AES was not among the predefined instruments, a smart meter (Tapo P110) was used to measure energy consumption every 5 min during the MP-AES working session. On the day the MP-AES energy consumption data, shown in Fig. S1 in supporting information, were collected, two MP-AES working sessions were performed. The working session is comprised of creating the calibration curve and analysing the samples. As illustrated in Fig. S1 and the corresponding paragraph (1.2.1) of the supporting information, it was possible to assign the energy consumption to each operating phase. In addition, the energy consumption of the MP-AES was measured and recorded twice (in one case, at 5-min intervals; in the other, hourly) and consistent results were obtained. The energy consumption recorded every 5 min is reported in Fig. S1 of the supplementary information. The operating phases for the MP-AES working session, as defined in Section 2.1, are related to the conditions where the power consumption changes.

1. Ignition and plasma stabilization: 1976 W;
2. Running: 1963 W;
3. Washing: 1963 W;
4. Cooling: 37 W;
5. Standby: 1963 W.

To calculate the energy consumption of MP-AES working session, the following equation was used (Equation (1)) [46]:

$$E_{tot} = \sum_{i=1}^5 E_i = \sum_{i=1}^5 (P_i \times t_i) \quad \text{Equation 1}$$

Where.

- E_{tot} is the total energy consumed (kWh);
- E_i is the energy consumed during the phase i of the analysis (kWh);
- P_i is the power required during phase i (kW);
- t_i is the time for phase i (h).

The total energy consumption must then be divided by the number of analysed samples to provide the data required by AGREE.

With regard to energy consumption for these five phases (Table 4), for future surveys involving this equipment, it will be sufficient to collect only time data, thus eliminating the need for direct energy measurements.

To calculate the energy consumption per sample using Equation (1), the duration of each phase reported in Table 4 must be recorded (t_i) and

Table 4
Collected data for criterion 9.

Number of MP-AES working session	Ignition and stabilization (h)	Running (h)	Washing (h)	Cooling (h)	Standby (h)	Number of sample analysed	Energy consumed per sample (kWh)
1	0.25	0.85	0.00	0.00	0.00	10	0.22
2	0.00	0.72	0.00	0.00	1.75	11	0.44
3	0.00	0.87	0.08	0.17	0.17	14	0.16
4	0.00	0.45	0.08	0.17	0.30	9	0.18
5	0.25	0.48	0.00	0.00	0.00	7	0.21
6	0.25	1.75	0.08	0.17	0.00	44	0.09
7	0.25	1.57	0.08	0.00	0.00	24	0.16
8	0.00	1.67	0.08	0.17	1.60	38	0.17
9	0.50	1.05	0.00	0.00	0.00	22	0.14
10	0.00	0.67	0.08	0.17	0.68	19	0.15
11	0.30	0.80	0.08	0.17	0.00	17	0.14
12	0.25	0.83	0.08	0.17	0.00	23	0.10
13	0.25	0.73	0.08	0.00	0.00	24	0.09
14	0.25	0.58	0.08	0.17	0.00	5	0.36

multiplied by its associated Power requirement (P_i), the resulting values (E_i) are then summed and divided by the number of samples analysed per session. An illustrative example is provided below for MP-AES session 14 (Equation (2)):

$$E_{\text{sample}} = \frac{(1.976 \times 0.25) + (1.963 \times 0.58) + (1.963 \times 0.08) + (0.037 \times 0.17) + (1.963 \times 0.00)}{5} = 0.36 \text{ kWh} \tag{Equation 2}$$

In this study, it was not possible to quantify the energy consumption associated with the fume extraction hood used by the MP-AES, as it is part of a centralised system. This makes it difficult to obtain or estimate specific data, as it would require an allocation across the entire facility an approach that is unfeasible.

To ensure transparency and reproducibility, the system boundaries defining the scope of the AGREEp prep and AGREE assessments are explicitly outlined in Table 5.

The limitations of AGREE identified while answering the criteria include the omission of standards, which may affect some responses. This includes criterion 7 (waste amount increased), which is also biased by not considering the generation of gaseous waste (N_2 and air with sample exhaust fumes) during instrument operation. However, as stated above, it was decided to follow the AGREEp prep guidelines, which recommend excluding the preparation of standards, in order to assess the impact of this choice on the final results. As indicated, this exclusion may influence the outcomes and should therefore be taken into account when making the final decision.

3. Results and discussion

This section presents the outcomes of the AGREEp prep and AGREE evaluations applied to the analytical procedures used in the study. These

evaluations involved twelve MP-AES work session where the samples were derived from *run-off* test and two from the metal release test in bacterial growth medium.

The AGREEp prep results are shown in Fig. 3. The numbers represent the different MP-AES work session (number 9 and 10 refer to samples coming from metal release tests).

Some analyses were grouped together in the same evaluation due to shared input data. The results are generally consistent, with an average score of 0.53. This score classifies the method as “green”, although it remains significantly below the ideal benchmark of 1. The analyses that deviate most positively are 9 and 10, as they derive from metal release tests using 24-well plates that require a smaller sample volume. Furthermore, Fig. 3 highlights the most critical criteria (marked in orange and red) that are described below per number of criterion.

- 1. Place of preparation: for all analyses, the samples are transferred from a location different from where the determination is conducted, needing a transportation step;
- 4. Waste: the amount of waste generated is high, as it includes all disposable and chemicals used, divided by their number of uses;

Table 5
– System boundaries for AGREEp prep and AGREE assessments: elements included (green) vs. excluded (red).

<i>AGREE</i>	<i>AGREEp prep</i>
<i>Transport</i>	<i>Transport</i>
<i>Energy consumption of MP-AES</i>	<i>Samples solutions (also reagents)</i>
<i>Samples solutions (also reagents)</i>	<i>Waste from laboratory glassware</i>
<i>Number of sample analysed</i>	<i>Number of sample prepared</i>
<i>Standards consider indirectly in the analysis time</i>	<i>Sample preparation time</i>
<i>Calibration line and re-runs</i>	<i>Standard preparation</i>
<i>Energy consumption of MP-AES hood</i>	<i>Energy consumption of fume hood</i>
<i>Gaseous waste from MP-AES</i>	<i>Energy consumed by storing samples (fridge consumption)</i>
<i>Standards not consider as samples</i>	

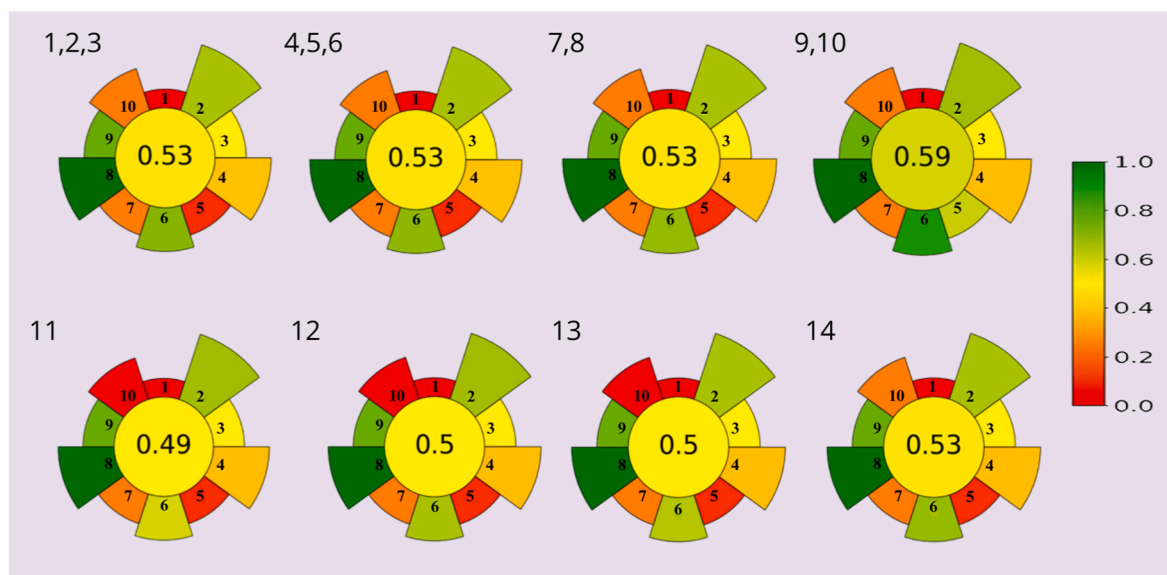


Fig. 3. AGREEprep results. Each diagram summarizes one or more MP-AES work sessions: the numbering (e.g., 1,2,3; 4,5,6; etc.) groups individual sessions with the same operating conditions.

- 5. Sample size: for the run-off samples the volume is 50 mL to ensure there is an ample amount available for any eventuality. However, given the volume requirements of the instrument, the sample volume preserved for this type of determination could be reduced to 10 mL or less, depending on the number of metals to be analysed. This reduction could also decrease the amount of acid needed for sample acidification;
- 7. Integration and automation: this aspect is partially critical because the preparation phase consists of two steps, carried out manually;
- 10. Number of hazards: the most hazardous step for the operator's health is the addition of HNO_3 (GHS03, GHS05 and GHS06). Additionally, when analysing Ca, the number of hazards increases to four due to the addition of CsCl (GHS07 and GHS08).

The results from AGREE evaluations are shown in Fig. 4 and are ordered according to the studied analyte, as the MP-AES has different sensitivities for each element. Consequently, any difficulties encountered during the MP-AES working session, such as increased analysis time and energy consumption, depend on this factor. The numbers represent the same analyses described previously.

The results of the AGREE analyses are generally consistent, with an average score of 0.60 for the baseline scenario and 0.67 for the alternative scenario. These scores suggest that the analytical method can be classified as “green”, indicating low environmental impact, as they exceed the threshold of 0.5. However, there remains room for improvement. However, the P determination shows the lowest score, as the MP-AES instrument struggles to detect phosphorus at concentrations near single ppm levels, as in the case for the analysed sample. As evident from Fig. 4, the analysis of phosphorus specifically impacts criteria 8 (samples analysed per hour) and 9 (energy consumption per sample), due to the increased per-sample analysis time stemming from the limited sensitivity of MP-AES towards this analyte. Among the operating phases, only the running time depends directly on the analyte type, whereas variations in other phases are minimal and primarily operator-controlled rather than analyte-specific.

Accordingly, the average running time per sample was calculated for each analyte (Ag: 0.06 h; Zn: 0.05 h; Ca: 0.04 h; P: 0.12 h). These results confirm that phosphorus exhibits the longest running time per sample, at least double that of the other analytes, explaining its lower AGREE scores.

The most critical criteria (scores <0.5) are.

- 1. Use of direct analytical techniques: in this case not applicable, since sample preparation and transportation (for metal release in bacterial growth medium samples and marble specimens) were necessary;
- 3. Position of the analytical device: as previously mentioned, the metal release samples, and marble specimens underwent transportation;
- 10. Source of the reagents: the reagents used do not come from renewable sources;
- 12. Safety: HNO_3 was used.

To improve criteria 1 and 3, practical strategies relevant to cultural heritage fieldworks could be implemented: (i) portable sampling and preservation kits, (ii) on-site analytical tools, such as portable electrochemical sensors [47,48]. While these approaches eliminate sample transport, they may present limitations depending on the analysis type, including reduced sensitivity and greater challenges in results interpretation [49].

Conversely, criteria 4, 5, and 6 consistently achieve high scores. Criteria 8 and 9, while varying across analyses, maintain high scores, indicating that analytes are determined quickly and energy consumption is low.

The results obtained from the two tools are not presented with an error, which would be useful for more accurate comparisons. However, this is challenging as the error could only be associated with quantitative rather than qualitative responses.

In the alternative scenario, the average results increased by 7% compared to the baseline scenario, as criteria with better scores received greater weighting. Moreover, to obtain a more representative assessment of both sample preparation and the analytical method, a weighted average of the scores from AGREE and AGREEprep was calculated using a weight ratio of 3:1. This weighting was chosen to emphasize the analytical procedure over sample preparation, reflecting its higher resource demands. Indeed, sample preparation entails negligible energy consumption and requires a markedly shorter average time per sample (0.048 h prepared vs. 0.083 h analysed). However, as a certain degree of subjectivity is inherent to all weighting procedures, the 1:1 scenario was also included in the ESI for comparison (section 3). This approach simplifies the final score, shown in Fig. 5, into a single, comprehensive metric. Although subjective, this approach provides a practical way for combining the two phases in method evaluations. To assess the impact of

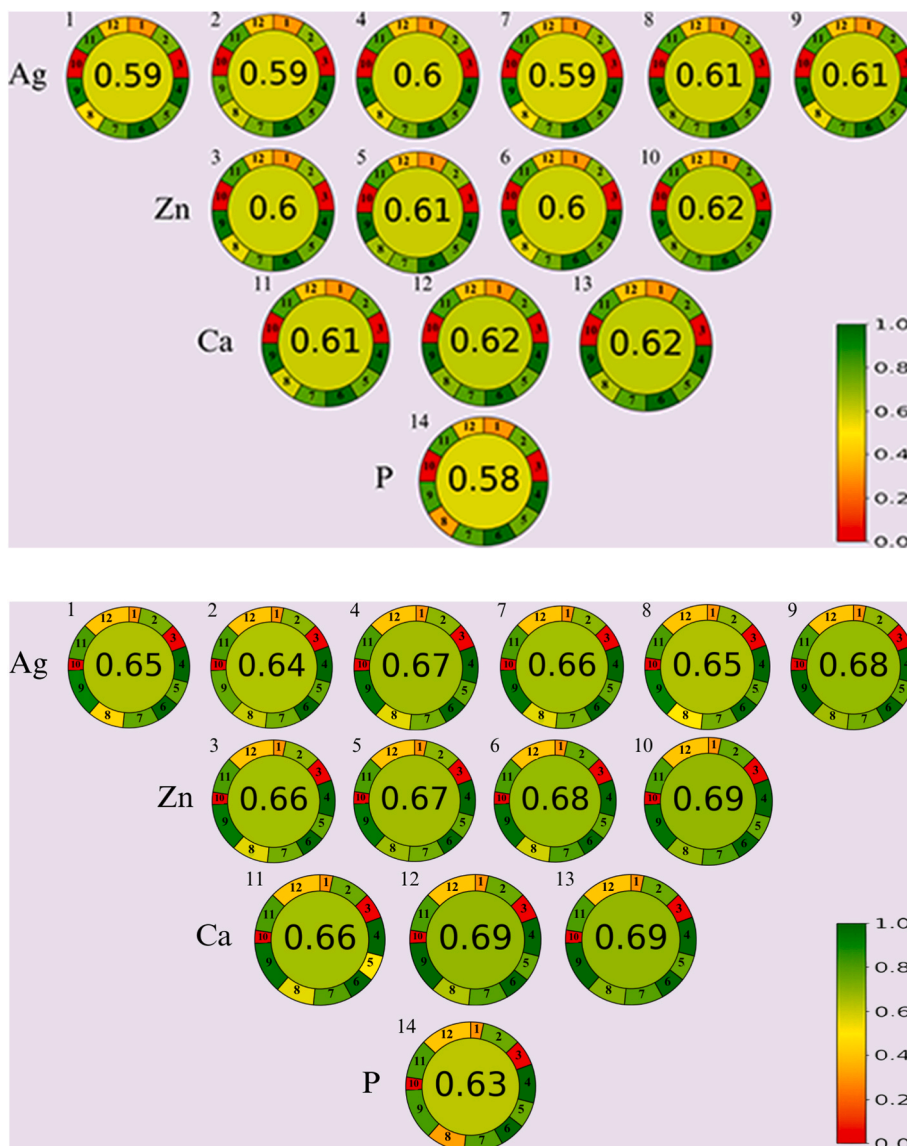


Fig. 4. AGREE results: baseline scenario (I) and alternative scenario (II). The numbers correspond to the same MP-AES work sessions as in Fig. 3. Sessions are grouped according to the element analysed in them and reported on the left.

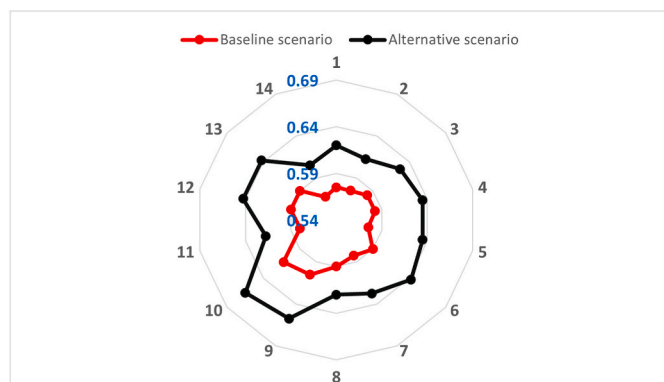


Fig. 5. Comparison of complementary scores in the two scenarios; the external numbers represent the MP-AES working session (black) and the internal numbers represent the complementary score (blue).

these choices, practitioners may consider performing a sensitivity analysis by varying the weighting ratio.

The average complementary score is 0.59 in the baseline scenario and 0.63 in the alternative scenario. Comparing the average scores of the two scenarios obtained by AGREE (0.60, 0.67) and by the complementary scores' calculation reveals that the difference between the two scenarios is more consistent in the latter case. This is likely because AGREEprep results tend to smooth the scores, making them more uniform. Moreover, the difference in the baseline scenario is 0.01 between the AGREE results and the complementary ones, while in the alternative scenario it is 0.04. This larger difference could be attributed to the fact that it tends to limit the weight changes that occurred in the alternative scenario, which, however objective they may seem, will always remain subjective.

In addition, the average complementary score of the equal weights (1:1), represented in Fig. S2 of the supplementary materials (section 3), was 0.57 in the baseline scenario and 0.60 in the alternative scenario. Comparison of the two weightings reveals that the difference with the 3:1 ratio ($\Delta = 0.04$ between scenario averages) compared to 1:1 ($\Delta = 0.03$) is marginal. In this case study, these findings indicate a limited sensitivity of the results to the selected weighting approach, confirming

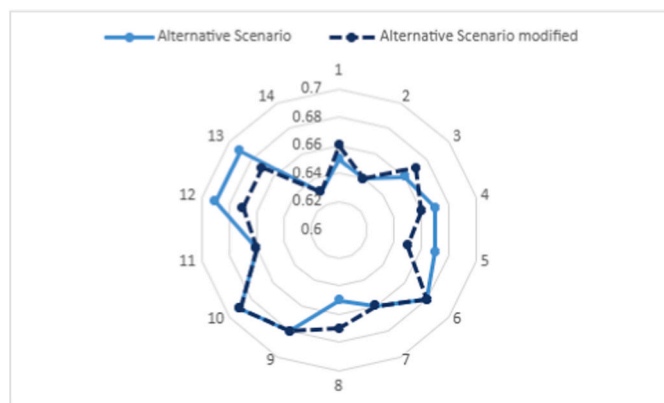


Fig. 6. – Sensitivity analysis: comparison between the Alternative scenario and the modified Alternative scenario.

result stability across reasonable assumptions.

Considering the results obtained, the suggestions to improve the scores and, consequently, reduce environmental impacts are.

- Criterion 4 of AGREEprep (waste production): reuse HDPE bottles as many times as possible by washing them after use, as they are the primary contributors to the final waste (Table S2 within Section 2.1.1 of the supplementary file).
- Criterion 5 of AGREEprep (sample size): reduce the volume of sample stored as much as possible to decrease energy consumption during the storage phase and to minimise the use of nitric acid.
- Criterion 9 of AGREE (energy consumption): limit standby times exceeding 15 min, switching off the instrument, as much as possible to reduce energy consumption. The 15 min threshold is related to the ignition and plasma stabilization time that occurs when the MP-AES is turned on.

A sensitivity analysis was performed on the AGREE alternative scenario by selectively modifying the weights assigned to criteria 9 and 12, which had received the maximum weight of 4 and were reduced to 3. These two criteria were specifically chosen due to their highest weighting in the original assessment.

Fig. 6 presents the results of this sensitivity analysis, comparing the original alternative scenario with the modified version. As shown, the final AGREE scores varied by a maximum of ± 0.02 , while remaining unchanged in some cases. This analysis demonstrates the low sensitivity of the AGREE score to weight adjustments, considering that only 2 out of 12 criteria were modified by 1 point each, confirming the robustness of the results and reinforcing the value of the justified weighting approach adopted in this study.

This study's approach can be adopted by those applying the two tools to MP-AES metal analyses required for material testing in cultural heritage applications, or in cases with similar specific constraints (e.g. variables -often low-sample volumes, low analyte concentrations, complex matrix, interferences). These tools can be adapted to different fields by adjusting the criteria weights based on the study's objectives, as demonstrated in this research, and can also be combined with other metrics. For example, AGREE, complex GAPI, BAGI, and RGB12 have been jointly applied in pharmaceutical studies to evaluate the greenness, blueness, and whiteness of analytical methods, supporting the environmental sustainability of the proposed approaches [50,51].

To assist practitioners in the usage, the following actions are recommended.

1. Flowsheet: create a diagram describing for the preparatory and analytical phases to visualise the entire analytical method. The

flowsheet should include all available data, such as the volume of reagent added and the instrumentation used.

2. Criteria: understand the tool's requirements by carefully reviewing each request per criteria.
3. Data collection: set up a checklist (spreadsheet file) to collect all the required information to fill each criterion.
4. Data gap: literature review, find analytical/theoretical procedure to fill the data gap.
5. Weighting: evaluate if the criteria weights adequately represent the study's objectives. If not, adjust them accordingly and justify the choices made.
6. Filling: complete the questionnaire based on the gathered information.
7. Interpretation: does the results satisfy the expectations? Yes/no and why. Draw conclusions from the results, comparing them, if possible, with similar studies in the literature.

4. Conclusion

This work aims to address the lack of guidelines in the literature regarding the application and interpretation of AGREE tools by outlining the steps taken to perform evaluations with both methodologies. Additionally, an effort was made to simplify data collection by developing a procedure that reflects metal analyses in aqueous solutions conducted with MP-AES. The data considered are those required to answer the criteria involving experimental calculations: principles 2 and 6 for AGREEprep, and 2, 8, and 9 for AGREE. Especially, as regards the instrument's energy consumption (criterion 9), average power consumption values were identified for each phase of the working session, allowing future AGREE analyses applied to MP-AES to require only the measurement of specific time intervals.

These two tools were applied for the first time to laboratory analyses conducted in a research context dedicated to developing new protective coatings for cultural heritage. Certain aspects of this sector influenced the operating conditions, as it required working with low sample volumes. This can impact the sampling time or the repetition frequency of analyses. While this need can reduce environmental impacts, it means that if an error occurs during a MP-AES session, you cannot repeat it.

From this study, the advantages of using AGREEprep and AGREE include accessibility (open access), ease of use without requiring a supercomputer, rapid evaluation capabilities, and adaptability to various analytical methods. However, the only limitations have been identified are the lack of error estimation and in AGREE the gaseous waste, such as those produced during MP-AES operation, is not considered. Instead, common errors that users could make include introducing subjectivity into the weighting of criteria without providing a proper explanation, since some degree of intrinsic subjectivity cannot be entirely avoided, and making incorrect comparisons by including metrological parameters outside the scope of the tool, such as LoD (limit of detection) and LoQ (limit of quantification).

For the evaluated case study, a more representative assessment was proposed by combining the results of AGREE and AGREEprep under both baseline conditions (default weights assigned to each criterion) and alternative scenarios, in which the criteria weights were adjusted to better reflect the specific requirements of the case study.

In this context, a weight ratio of 3:1 was applied between AGREE and AGREEprep, assigning greater importance to AGREE, as it provides a more representative evaluation. Although the findings in this case show a certain stability across reasonable assumptions, this approach can facilitate the overall interpretation of the data.

Future developments in the assessment of analytical methods could focus on integrating the AGREE and AGREEprep tools with comprehensive models such as the RGB (red, green, and blue) [52]. The RGB model adopted within the framework of White Analytical Chemistry (WAC) exemplifies this comprehensive approach by combining these three aspects: the red component assesses analytical efficiency through

criteria such as accuracy, precision, and sensitivity; the green component quantifies environmental impact and safety; and the blue component focuses on productivity, economic efficiency, operational simplicity, and practical applicability [52–55]. These combined approaches enable a holistic evaluation by addressing different facets of analytical performance. Additionally, if limited information or time is available for the evaluation of an analytical method, or if one wishes to focus on only one of the three aspects, the use of complementary tools becomes essential. The Blue Applicability Grade Index (BAGI), developed in 2023, which shares the user-friendly features of AGREE, including graphical result representation through color-coded pictograms and rapid assessment capabilities, and is inspired by the WAC philosophy [24,56]. BAGI integrates evaluations of practical applicability and economic considerations, thus extending the assessment beyond purely green metrics [24]. Compared to other green analytical chemistry tools like GAPI [57] or the Analytical Eco-Scale [23], BAGI [24] and RGB model [52] offer more holistic or user-friendly perspectives, while each tool maintains distinct strengths and ideal applications. Incorporating these models alongside existing metrics could enrich future sustainability evaluations and support the optimization of analytical methods that balance environmental, economic, and performance criteria.

Moreover, there is another approach, particularly used in the pharmaceutical sector, which involves the combined adoption of QbD (Quality by Design) and GAC to enhance the efficiency and sustainability of analytical processes. This integration enables the development of methods that are both more robust and environmentally friendly [14, 58–60].

Another future development can be the integration of ChlorTox scale with criteria 11 and 12 of the AGREE. ChlorTox scale is already an internationally recognized parameter in the scientific community. It was also proposed by Nowak et al. in the RGBsynt model to quantify chemical risk [53]. This chemical risk indicator allows for the independent evaluation of hazards associated with each reagent, providing a quantitative comparison between different synthesis or analytical methods [61].

Finally, a further integration with life cycle assessment (LCA) methodology is also recommended to create a holistic evaluation framework that balances environmental, economic, and analytical efficiency [62–64]. However, it is essential to adapt the type of evaluation to the time and resources available, as more time and comprehensive data allow for a more accurate and robust assessment. The integration of models such as LCA increases the time and effort required for evaluation due to the extensive data collection involved. Expanding these tools to other branches of chemistry, as demonstrated by the adaptation of the RGB model to organic synthesis (RGBsynt), opens exciting opportunities for cross disciplinary applications.

CRediT authorship contribution statement

Luca Vignali: Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Elena Bernardi:** Writing – review & editing, Validation, Supervision, Resources, Conceptualization. **Enrico Sassoni:** Writing – review & editing, Validation, Funding acquisition. **Martina Cappelletti:** Writing – review & editing, Validation. **Elena Cofini:** Supervision, Formal analysis. **Daniele Cespi:** Writing – review & editing, Validation, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was partially funded by the project “SECURE COATS” (Safe, eco-friendly, and durable coatings to prevent deterioration of heritage stones, MUR code: “202288LY27”), funded by EU – Next Generation EU, Mission 4, Component 1, CUP: J53D23003410006.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.talanta.2026.129536>.

Data availability

All data used are within the manuscript and electronic supplementary information

References

- [1] B.A. de Marco, B.S. Rechelo, E.G. Tófoli, A.C. Kogawa, H.R.N. Salgado, Evolution of green chemistry and its multidimensional impacts: a review, *Saudi Pharm. J.* 27 (2019) 1–8, <https://doi.org/10.1016/j.jsps.2018.07.011>.
- [2] M. Tobiszewski, A. Mechlińska, B. Zygmunt, J. Namieśnik, Green analytical chemistry in sample preparation for determination of trace organic pollutants, *TrAC, Trends Anal. Chem.* 28 (2009) 943–951, <https://doi.org/10.1016/j.trac.2009.06.001>.
- [3] P.T. Anastas, J.C. Warner, *Green Chemistry: Theory and Practice*, Oxford University Press Inc., New York, United States, 1998 n.d.
- [4] J.H. Clark, D.J. Macquarrie, *Handbook of Green Chemistry and Technology*, Blackwell Science, 2002.
- [5] P. Anastas, N. Eghbali, *Green chemistry: principles and practice*, *Chem. Soc. Rev.* 39 (2010) 301–312, <https://doi.org/10.1039/b918763b>.
- [6] A. Gatuszka, Z. Migaszewski, J. Namieśnik, The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices, *TrAC, Trends Anal. Chem.* 50 (2013) 78–84, <https://doi.org/10.1016/j.trac.2013.04.010>.
- [7] M. Tobiszewski, Metrics for green analytical chemistry, *Anal. Methods* 8 (2016) 2993–2999, <https://doi.org/10.1039/c6ay00478d>.
- [8] S. Armenta, S. Garrigues, M. de la Guardia, Green analytical chemistry, *TrAC, Trends Anal. Chem.* 27 (2008) 497–511, <https://doi.org/10.1016/j.trac.2008.05.003>.
- [9] M. Koel, M. Kaljurand, Application of the principles of green chemistry in analytical chemistry, *Pure Appl. Chem.* 78 (2006) 1993–2002, <https://doi.org/10.1351/pac200678111993>.
- [10] B. Raccary, P. Loubet, C. Peres, G. Sonnemann, Evaluating the environmental impacts of analytical chemistry methods: from a critical review towards a proposal using a life cycle approach, *TrAC, Trends Anal. Chem.* 147 (2022), <https://doi.org/10.1016/j.trac.2022.116525>.
- [11] P.M. Nowak, R. Wietecha-Postuszny, J. Pawliszyn, White analytical chemistry: an approach to reconcile the principles of green analytical chemistry and functionality, *TrAC, Trends Anal. Chem.* 138 (2021), <https://doi.org/10.1016/j.trac.2021.116223>.
- [12] L.H. Keith, L.U. Gron, J.L. Young, Green analytical methodologies, *Chem Rev* 107 (2007) 2695–2708, <https://doi.org/10.1021/cr068359e>.
- [13] W. Wojnowski, M. Tobiszewski, F. Pena-Pereira, E. Psillakis, AGREeprep – analytical greenness metric for sample preparation, *TrAC, Trends Anal. Chem.* 149 (2022), <https://doi.org/10.1016/j.trac.2022.116553>.
- [14] C. Boussès, L. Ferey, E. Vedrines, K. Gaudin, Using an innovative combination of quality-by-design and green analytical chemistry approaches for the development of a stability indicating UHPLC method in pharmaceutical products, *J. Pharm. Biomed. Anal.* 115 (2015) 114–122, <https://doi.org/10.1016/j.jpba.2015.07.003>.
- [15] F. Pena-Pereira, W. Wojnowski, M. Tobiszewski, AGREE - Analytical GREENness metric approach and software, *Anal. Chem.* 92 (2020) 10076–10082, <https://doi.org/10.1021/acs.analchem.0c01887>.
- [16] Á.I. López-Lorente, F. Pena-Pereira, S. Pedersen-Bjerggaard, V.G. Zuin, S.A. Ozkan, E. Psillakis, The ten principles of green sample preparation, *TrAC, Trends Anal. Chem.* 148 (2022), <https://doi.org/10.1016/j.trac.2022.116530>.
- [17] M.S. Imam, M.M. Abdelrahman, How environmentally friendly is the analytical process? A paradigm overview of ten greenness assessment metric approaches for analytical methods, *Trends Environ. Anal. Chem.* 38 (2023), <https://doi.org/10.1016/j.teac.2023.e00202>.
- [18] A. Cetinkaya, S.I. Kaya, S.A. Ozkan, An overview of the current progress in green analytical chemistry by evaluating recent studies using greenness assessment tools, *TrAC, Trends Anal. Chem.* 168 (2023), <https://doi.org/10.1016/j.trac.2023.117330>.
- [19] A. Ballester-Caudet, P. Campins-Falcó, B. Pérez, R. Sancho, M. Lorente, G. Sastre, et al., A new tool for evaluating and/or selecting analytical methods: summarizing the information in a hexagon, *TrAC, Trends Anal. Chem.* 118 (2019) 538–547, <https://doi.org/10.1016/j.trac.2019.06.015>.
- [20] J. Martínez, J.F. Cortés, R. Miranda, Green chemistry metrics, A review, *Processes* 10 (2022), <https://doi.org/10.3390/pr10071274>.

- [21] J. Plotka-Wasyłka, A new tool for evaluation of analytical procedure, *Green Analytical Procedure Index* (2017), <https://doi.org/10.1039/x0xx00000x>.
- [22] NEMI(National Environmental Methods Index. <https://www.nemi.gov/home/>, 2002.
- [23] A. Gatuszka, Z.M. Migaszewski, P. Konieczka, J. Namieśnik, Analytical eco-scale for assessing the greenness of analytical procedures, *TrAC, Trends Anal. Chem.* 37 (2012) 61–72, <https://doi.org/10.1016/j.trac.2012.03.013>.
- [24] N. Manousi, W. Wojnowski, J. Plotka-Wasyłka, V. Samanidou, Blue applicability grade index (BAGI) and software: a new tool for the evaluation of method practicality, *Green Chem.* 25 (2023) 7598–7604, <https://doi.org/10.1039/d3gc02347h>.
- [25] M.B. Hicks, W. Farrell, C. Aurigemma, L. Lehmann, L. Weisel, K. Nadeau, et al., Making the move towards modernized greener separations: introduction of the analytical method greenness score (AMGS) calculator, *Green Chem.* 21 (2019) 1816–1826, <https://doi.org/10.1039/c8gc03875a>.
- [26] H.A. Silva-Neto, L.F. de Lima, D.S. Rocha, V.N. Ataíde, G.N. Meloni, G. Moro, et al., Recent achievements of greenness metrics on paper-based electrochemical (bio) sensors for environmental and clinical analysis, *TrAC, Trends Anal. Chem.* 174 (2024), <https://doi.org/10.1016/j.trac.2024.117675>.
- [27] L.P. Kowtharapu, N.K. Katarji, S.K. Muchakayala, V.M. Marisetti, Green metric tools for analytical methods assessment critical review, case studies and crucify, *TrAC, Trends Anal. Chem.* 166 (2023), <https://doi.org/10.1016/j.trac.2023.117196>.
- [28] L.A. Yahya, C. Vakh, O. Dushna, O. Kalisz, S. Bocian, M. Tobiszewski, Guidelines on the proper selection of greenness and related metric tools in analytical chemistry – a tutorial, *Anal. Chim. Acta* 1357 (2025), <https://doi.org/10.1016/j.aca.2025.344052>.
- [29] L. Durand, L. Wiest, E. Vulliet, Analytical chemistry in the era of sustainability: evaluating tools and challenges for a greener future, *Trends Environ. Anal. Chem.* 47 (2025), <https://doi.org/10.1016/j.teac.2025.e00275>.
- [30] N. Yahaya, A.H. Mohamed, M. Miskam, A.S. Abdul Keyon, S.H. Loh, N. Mohamad Zain, et al., Green analytical chemistry metrics for evaluation of microextraction methods: fascinating or essential tools in real-world applications? *TrAC, Trends Anal. Chem.* 172 (2024) 117587 <https://doi.org/10.1016/j.trac.2024.117587>.
- [31] S.F. Hammad, M.A.A. Hamid, L. Adly, S.H. Elagamy, Comprehensive review of greenness, whiteness, and blueness assessments of analytical methods, *Green Anal Chem* 12 (2025) 100209, <https://doi.org/10.1016/j.greac.2025.100209>.
- [32] Fuente-Ballesteros A, Jano A, Haque SM. Emerging Tools for Holistic Evaluation of Analytical Methods n.d.
- [33] Mansour FR, Bedair A, Hamed M. The Green Component: Assessing the Environmental Impact of Analytical Methods n.d.
- [34] S.I. Kaya, G. Ozelikay-Akyildiz, S.A. Ozkan, Green metrics and green analytical applications: a comprehensive outlook from developing countries to advanced applications, *Green Anal Chem* 11 (2024) 100159, <https://doi.org/10.1016/j.greac.2024.100159>.
- [35] H.M. Lotfy, G. Tiris, A.A. Genc, R.H. Obaydo, N. Erk, Sustainable signal processing spectrophotometric analysis of candesartan, hydrochlorothiazide, and amlodipine: green methods aligned with global sustainability goals, *Spectrochim. Acta Mol. Biomol. Spectrosc.* 338 (2025) 126157, <https://doi.org/10.1016/j.saa.2025.126157>.
- [36] Pena-Pereira F, Wojnowski W, Tobiszewski M. AGREE-Analytical Greenness Metric Approach and Software Supporting Information: Software Documentation for the Analytical Greenness Calculator. n.d.
- [37] P.H. Pour, F.M. Suzaei, S.M. Daryanavard, Greenness assessment of microextraction techniques in therapeutic drug monitoring, *Bioanalysis* 16 (2024) 249–278, <https://doi.org/10.4155/bio-2023-0266>.
- [38] G. Wejnerowska, I. Narloch, Comparison of the greenness assessment of chromatographic methods used for analysis of UV filters in cosmetic samples, *Analytica* 4 (2023) 447–455, <https://doi.org/10.3390/analytica4040032>.
- [39] D. Moema, T.A. Makwakwa, B.E. Gebreyohannes, S. Dube, M.M. Nindi, Hollow fiber liquid phase microextraction of fluoroquinolones in chicken livers followed by high pressure liquid chromatography: greenness assessment using National Environmental Methods Index Label (NEMI), green analytical procedure index (GAPI), Analytical GREEnness metric (AGREE), and Eco Scale, *J. Food Compos. Anal.* 117 (2023), <https://doi.org/10.1016/j.jfca.2023.105131>.
- [40] J. Silva, V. Bühl, F. Iaquinta, M. Pistón, Should we think about green or white analytical chemistry? Case study: accelerated sample preparation using an ultrasonic bath for the simultaneous determination of Mn and Fe in beef, *Heliyon* 9 (2023), <https://doi.org/10.1016/j.heliyon.2023.e20967>.
- [41] K. Marakova, R. Tomasovsky, M. Opetova, K.A. Schug, Greenness of proteomic sample preparation and analysis techniques for biopharmaceuticals, *TrAC, Trends Anal. Chem.* 171 (2024), <https://doi.org/10.1016/j.trac.2023.117490>.
- [42] J. Silva, M. Pistón, Evaluation of sisal fiber as biosorbent for online preconcentration and determination of Cu and Mn coupled to MP AES using the analytical greenness metric approach, *Braz. J. Anal. Chem* 10 (2023) 73–79, <https://doi.org/10.30744/brjac.2179-3425.TN-11-2023>.
- [43] V. Balaram, Microwave plasma atomic emission spectrometry (MP-AES) and its applications – a critical review, *Microchem. J.* 159 (2020), <https://doi.org/10.1016/j.micro.2020.105483>.
- [44] S. Spadavecchia, C. Chiavari, F. Ospitali, S. Gualtieri, A.C. Hillar, E. Bernardi, Evaluation of the effectiveness of coatings for the protection of outdoor terracotta artworks through artificial ageing tests, *J. Cult. Herit.* 70 (2024) 213–222, <https://doi.org/10.1016/j.culher.2024.09.008>.
- [45] F. Pena-Pereira, M. Tobiszewski, W. Wojnowski, E. Psillakis, A tutorial on AGREEprep an analytical greenness metric for sample preparation, *Adv. Sample Prep* 3 (2022), <https://doi.org/10.1016/j.sampre.2022.100025>.
- [46] J. Giménez, B. Bayarri, Ó. González, S. Malato, J. Peral, S. Esplugas, Advanced oxidation processes at laboratory Scale: environmental and economic impacts, *ACS Sustain Chem Eng* 3 (2015) 3188–3196, <https://doi.org/10.1021/acssuschemeng.5b00778>.
- [47] E. Valentini, Smart electrochemical portable tools for cultural heritage analysis: a review, *Sensors (Switzerland)* 19 (2019), <https://doi.org/10.3390/s19194303>.
- [48] J. Jehlička, A. Culka, Critical evaluation of portable Raman spectrometers: from rock outcrops and planetary analogs to cultural heritage – a review, *Anal. Chim. Acta* 1209 (2022) 339027, <https://doi.org/10.1016/j.aca.2021.339027>.
- [49] L. Dayet, F. d'Errico, M.G. Diez, J. Zilhão, Critical evaluation of in situ analyses for the characterisation of red pigments in rock paintings: a case study from El Castillo, Spain, *PLoS One* 17 (2022), <https://doi.org/10.1371/journal.pone.0262143>.
- [50] W.N. Abd-ALGhafar, R. Abo Shabana, R. El-Shaheny, M.M. Tolba, A fluorescence switch-off nanosensor for sensitive determination of the antiandrogen drug flutamide in pharmaceutical and environmental samples. Analytical method greenness, blueness, and whiteness assessment, *Microchem. J.* 204 (2024) 111078, <https://doi.org/10.1016/j.micro.2024.111078>.
- [51] W.N. Abd-ALGhafar, R.A. Shabana, R. El-Shaheny, M.M. Tolba, Integrating bio-based luminescent QDs nanosensors and factorial-design-optimized sugaring-out homogenous liquid–liquid microextraction for sensitive determination of two neurologic drugs, *Microchem. J.* 210 (2025) 113034, <https://doi.org/10.1016/j.micro.2025.113034>.
- [52] P.M. Nowak, P. Kościelniak, What color is your method? Adaptation of the rgb additive color model to analytical method evaluation, *Anal. Chem.* 91 (2019) 10343–10352, <https://doi.org/10.1021/acs.analchem.9b01872>.
- [53] P.M. Nowak, M. Kamiński, W. Trybała, V. Canale, P. Zajdel, Comparison of greenness and whiteness of selected mechanochemical and solution-based reactions using a new RGBsynt model, *Green Chem.* (2024), <https://doi.org/10.1039/d4gc05097e>.
- [54] P.M. Nowak, P. Kościelniak, M. Tobiszewski, A. Ballester-Caudet, P. Campins-Falcó, Overview of the three multicriteria approaches applied to a global assessment of analytical methods, *TrAC, Trends Anal. Chem.* 133 (2020), <https://doi.org/10.1016/j.trac.2020.116065>.
- [55] Locatelli M, Perrucci M, Fuente-Ballesteros A. White Analytical Chemistry: Balancing Performance, Sustainability, and Practicality in Modern Methods n.d.
- [56] Manousi N, Fuente-Ballesteros A, Bernal J. The Blue Component of Analytical Chemistry: Assessing the Practicality of Analytical Methods n.d.
- [57] J. Plotka-Wasyłka, A new tool for the evaluation of the analytical procedure: Green Analytical Procedure Index, *Talanta* 181 (2018) 204–209, <https://doi.org/10.1016/j.talanta.2018.01.013>.
- [58] A. Balekundri, E.D. Ahire, R.U. Shelke, D.D. Rishipathak, S.J. Kshirsagar, Eco-friendly HPTLC method for Trifluridine and Tipiracil determination: Quality-by-design meets green analytical chemistry, *Green Anal Chem* 13 (2025) 100234, <https://doi.org/10.1016/j.greac.2025.100234>.
- [59] A.M. Valvi, R.U. Shelke, S.S. Ghodke, D.D. Rishipathak, Quality by design and green analytical chemistry: a review of novel approaches to chromatographic method development, *Biosci. Biotechnol. Res. Asia* 22 (2025) 497–520, <https://doi.org/10.13005/bbra/3379>.
- [60] K. Chagarlamudi, V.K.S. Maddala, K. Gandla, A stability-indicating and environmentally sustainable UPLC method for simultaneous determination of rizatriptan, meloxicam, and related N-nitrosamine impurities, *J. Pharm. Biomed. Anal. Open* 6 (2025) 100087, <https://doi.org/10.1016/j.jpba.2025.100087>.
- [61] P.M. Nowak, R. Więtecha-Postuszny, J. Plotka-Wasyłka, M. Tobiszewski, How to evaluate methods used in chemical laboratories in terms of the total chemical risk? – a ChlorTox Scale, *Green Anal Chem* 5 (2023), <https://doi.org/10.1016/j.greac.2023.100056>.
- [62] International Organization for Standardization (ISO), 14040:2006/Amd 1:2020, Environmental Management-Life Cycle Assessment- Principles and Framework, ISO, Geneva, Switzerland, 2006 n.d.
- [63] International Organization for Standardization (ISO), 14044:2006/Amd 1:2017, Environmental Management Life Cycle Assessment-Requirements and Guidelines-Amendment 1, ISO, Geneva, Switzerland, 2006 n.d.
- [64] International Organization for Standardization (ISO), 14044:2006/Amd 2:2020, Environmental Management-Life Cycle Assessment- Requirements and Guidelines-Amendment 2, ISO, Geneva, Switzerland, 2020 n.d.