

Supporting Information

Evaluating the greenness of MP-AES spectroscopy through AGREE metrics

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28 **1 Brief overview of Green tools**

29 In recent years, significant progress has been made in developing analytical processes aligned with GAC principles [1–
30 3]. Nevertheless, many statements about the environmental sustainability of analytical procedures lack quantitative
31 validation and are often rooted in subjective judgments or uncertain assumptions, largely because standardized assessment
32 criteria are missing [4–8]. This points to a distinct need for reliable tools that can objectively measure the environmental
33 impact of analytical methods [1].

34 To meet this need, several GAC-based tools have been introduced, all designed to evaluate the environmental impact of
35 analytical procedures [9,10]. These include:

- 36 i. NEMI (National Environment Methods Index), developed in 2002, collects environmental techniques and
37 analytical methods. The evaluation is represented by a pictogram divided into four parts, which turns green
38 if the criteria are met. The criteria are: absence of toxic substances, avoidance of hazardous waste
39 production, sample's pH, and amount of waste generated [2,3,11];
- 40 ii. Analytical Eco-Scale, derived from Eco-Scale [12], originally designed for organic synthesis, this method
41 assumes an "ideal" process that does not use hazardous reagents for health and the environment, assigning
42 it a score of 100. Penalty points are subtracted based on the deviation of the procedure from this ideal
43 situation [13];
- 44 iii. GAPI (Green Analytical Procedure Index) and complexGAPI represent each phase of the analytical method
45 results through a pictogram composed of five pentagrams in GAPI, while complexGAPI adds a hexagon for
46 a more detailed analysis, particularly focusing on sample preparation aspects [6,14];
- 47 iv. BAGI (Blue Applicability Grade Index), developed in 2023 and inspired by GAPI for its graphical
48 representation of results through a colour-coded pictogram and its rapid evaluation capabilities [6,15].
49 Additionally, it draws inspiration from the WAC (white analytical chemistry) approach, which, for the first
50 time, integrates analytical performance and economic aspects into the evaluation of an analytical method
51 [16];
- 52 v. AMGS (Analytical Methods Greenness Score), developed in collaboration with eight pharmaceutical
53 companies, it evaluates methods based on three main variables: choice of instrument that influences the
54 energetic consumed, choice of solvent which affects the energy demand, and waste generation that refers to
55 health and environmental safety [17];
- 56 vi. CALIFICAMENT (CALculating the Impact of a FInding by Combined Assays of Multiple Environmental
57 Norms and Thresholds). This latest tool was designed to identify weaknesses in analytical methods, by
58 extending the Eco-Scale concept. Its hexagonal shape visually represents different aspects, such as process
59 carbon footprint, waste production, hazardous chemicals, analytical performance, and cost [4].

60

61 **2 Inventory analysis**

62 To obtain an evaluation using AGREE and AGREEprep, it was necessary to gather all the relevant information required
63 to answer the evaluation criteria. Comprehensive responses and explanations are presented in the following sections.

64 **2.1 AGREEprep**

65 The responses assigned to AGREEprep, along with their explanations, are summarized in Table S1.

66 *Table S1 - AGREEprep's responses with their motivation.*

Criterion	Response	Motivation	Weight
1. Preparation location	Ex situ	Both the run-off and antibacterial analyses required material to be transferred between different locations (i.e. marble specimens for the run-off test or the samples themselves, respectively).	1
2. Quantity of toxic substances used	0.10 or 0.11mL	In the procedure is added 0.1 mL of HNO ₃ and when requested 0.05 mL of CsCl	5
3. Origin of used material	Not renewable, but reused.	Some materials are properly washed and then reused	2
4. Quantity of production waste	Calculated experimentally	Explanation is provided in paragraph 2.1.1	4
5. Quantity of prepared sample	1.5 or 50 mL	1.5 mL is the volume of the sample of antibacterial test. 50 mL is the volume of sample derived from <i>run-off</i> test	2
6. Number of samples prepared per hour	Calculated experimentally	Explanation is provided in the paragraph 2.1.1	3
7. Number of steps and degree of automation	2 or 3 and manual	The preparation phases involve miniaturization, acidification and sometimes the addition of CsCl	2
8. Electricity consumption per sample	0 Wh	No electrical instruments are used for sample preparation	4
9. Analytical technique used	spectrophotometry, etc.	The response closest to spectroscopy was selected, as no exact match was available.	2
10. Number of dangers of which the operator is exposed	3 or 4	In the safety data sheet of HNO ₃ , three hazards are reported, while only one is listed for CsCl	3

67

68 Reasons why the weights, proposed by AGREEprep, were already representative and were not modified in the alternative
69 scenario:

- 70 1. Performing analyses directly *in situ* depends on the type of analyte determination. In certain cases, transporting
71 equipment to the field is not realistically possible, as in this case study.
- 72 2. Operator health and safety must always be the top priority.
- 73 3. Glassware derived from renewable resources are not yet widely available in the cultural heritage sector.
- 74 4. Preventing waste generation is crucial both environmentally and economically.
- 75 5. Minimizing the sample size is relevant from the perspective of resource and reagent consumption. However,
76 there is a limit to volume reduction, as a certain quantity must be stored for repeat the analysis, if necessary.
- 77 6. The speed of preparation is important for both working time and energy consumption.
- 78 7. Automation is important for operator safety, as it reduces exposure to hazardous samples. The limited number
79 of steps aims to reduce the use of reagents, solvents and energy.
- 80 8. Energy consumption must be minimized under all circumstances.

- 81 9. The selection of the instrument can help estimate the future energy consumption in the analysis phase.
 82 10. Reducing the use of toxic reagents is important, although it is not always possible to eliminate or replace them.

83

84 2.1.1 Response calculated experimentally

85 The following section describes the data collected and the calculations performed to determine the responses for principles
 86 4 and 6 of AGREEprep. The fourth principle requires the calculation of the waste volume generated during sample
 87 preparation. To quantify this, the article by Pena-Pereira *et al.* [18] was consulted, which provides both a flow diagram
 88 to identify the types of waste produced and an equation to quantify the waste based on their type. This equation takes into
 89 account several factors: the mass of single use materials, the contribution of reusable materials, non-recoverable
 90 chemicals, recoverable chemicals, and the mass of contaminated or diluted sample.

91 In the present case, the waste volume was calculated by summing their total mass and dividing it by the number of uses,
 92 present in the Table S2. The generated waste mainly came from materials such as HDPE bottles, Pasteur pipettes,
 93 micropipette tips, Eppendorf tubes[®], and reagents like HNO₃ e CsCl.

94 *Table S2 – mass and number of uses of disposable glassware and plastics used in the preparation phase.*

	Average mass (g)	Number of uses
HDPE bottles	15.86	50
Pasteur pipettes	3.08	1000
Micropipette tips	0.32	1
Eppendorf tubes[®]	1.20	1

95

96 The sixth principle requires determining the number of samples prepared per hour. To obtain this data from the preparation
 97 phase, the time needed to prepare a known quantity of samples was measured using a stopwatch. Subsequently, the
 98 following formula was applied to calculate the number of samples prepared per hour (Equation 1):

$$99 \quad n_{\text{samples/hour}} = \frac{n_{\text{samples,1h}}}{t_{\text{measured}}}$$

100

Equation 1

101 **Where:**

- 102 • $n_{\text{samples/hour}}$ represents the number of samples prepared per hour;
- 103 • $n_{\text{samples, 1h}}$ is the number of samples prepared in one hour;
- 104 • t_{measured} is the time measured to prepare the batch of sample (h).

105

106 2.2 AGREE

107 The responses provided, along with their explanations, are summarised in Table S3.

Table S3 – AGREE's responses with their motivation.

Criterion	Response	Motivation
1. Sampling location	Ex situ	The evaluation focuses on the analysis performed with MP-AES, excluding the aging and antibacterial testing phases from the boundaries of the analytical system
2. Quantity of sample used	Calculated experimentally	Explanation is provided in the paragraph 2.2 of the manuscript
3. Instrument position	Ex situ	MP-AES is located in a different place than where the coating was applied to the marble samples or antibacterial tests were carried out
4. Number of preparation steps	3 or fewer	The preparation phases involve miniaturization, acidification and sometimes the addition of CsCl
5. Automated and miniaturized	Semiautomatic and miniaturization carried out	The analyses were conducted with manual sample replacement. Miniaturization is part of the procedure
6. Use of derivate agents	None	Not present
7. Quantity of product waste	Calculated experimentally	Explanation is provided in the paragraph 2.2 of the manuscript
8. Determination speed per hour	Calculated experimentally	Explanation is provided in the paragraph 2.2 of the manuscript
9. Energy consumed per sample	Calculated experimentally	Explanation is provided in the paragraph 2.2 of the manuscript
10. Use of renewable reagents	None	The reagents used are not derived from renewable sources
11. Quantity of toxic reagents/solvents	Use of HNO ₃ and CsCl in the microliter range per sample	0.1 mL of HNO ₃ is added for 1.5 or 50 mL of sample and this number is divided for the volume of analysed sample. The score is 0.8 if the volume added is < 0.1 mL[19]. Additionally, with the addition of CsCl, the volume is less than 0.1 mL.
12. Number of dangers related to 11.	3 or 4	In the safety data sheet of HNO ₃ , three hazards are reported, while only one is listed for CsCl

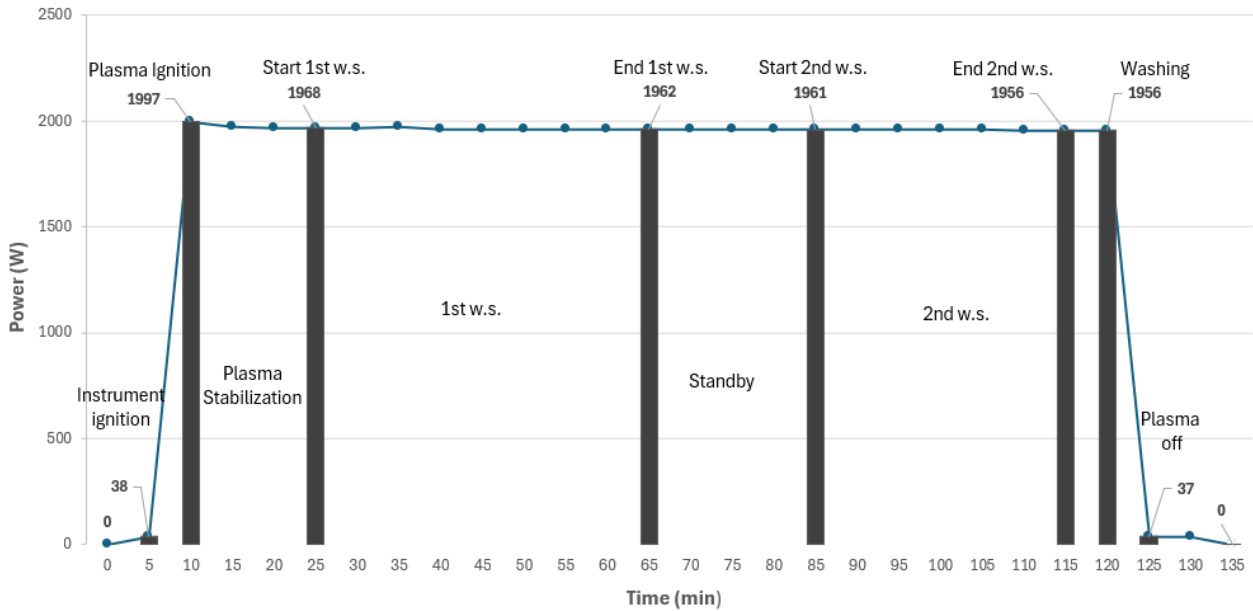
109

110 2.2.1 Response calculated experimentally

111 The following section describes how the electrical energy consumption of the MP-AES was quantified, serving to
 112 complement the information presented in Section 2.2 of the manuscript. Quantifying the energy consumed per sample is
 113 necessary to satisfy the ninth criterion. For this quantification, it was determined that the power required does not depend
 114 on the type of analyte of interest or the complexity of the matrix. Figure S1 illustrates how the required power varies
 115 according to the instrument's different operating phases (section **Errore. L'origine riferimento non è stata trovata.**, in
 116 the manuscript):

- 117 • 5 minutes: instrument ignition;
- 118 • 10 minutes: torch ignition;
- 119 • 25 minutes: start of the first working session (w.s.) after torch stabilization;
- 120 • 65 minutes: end of the first w.s.;
- 121 • 85 minutes: start of the second w.s.;

- 122 • 115 minutes: end of the second w.s.;
- 123 • 120 minutes: washing phase;
- 124 • 125 minutes: instrument on, torch off;
- 125 • 135 minutes: instrument shutdown.



126

127 *Figure S1 - Power consumption over time and operation phases.*

128 From Figure S1, it is evident that power consumption varies across three main conditions, for which average reference
 129 power values were calculated. These are useful for determining the power consumption of an MP-AES working session:

- 130 1. Instrument on with torch off: 37 W;
- 131 2. Torch ignition and stabilization: 1976 W;
- 132 3. Instrument on with torch on: 1963 W.

133 The average power during torch ignition was calculated over the first 15 minutes, the period during which the instrument
 134 stabilizes. This approach was chosen because the actual ignition duration (~10 seconds) is too brief to be captured
 135 accurately by the smart meter, which records average power every 5 minutes.

136 The operating phases, defined in paragraph **Errore. L'origine riferimento non è stata trovata.** in the manuscript, for
 137 the MP-AES working session are related to the three conditions, where the power consumption changes:

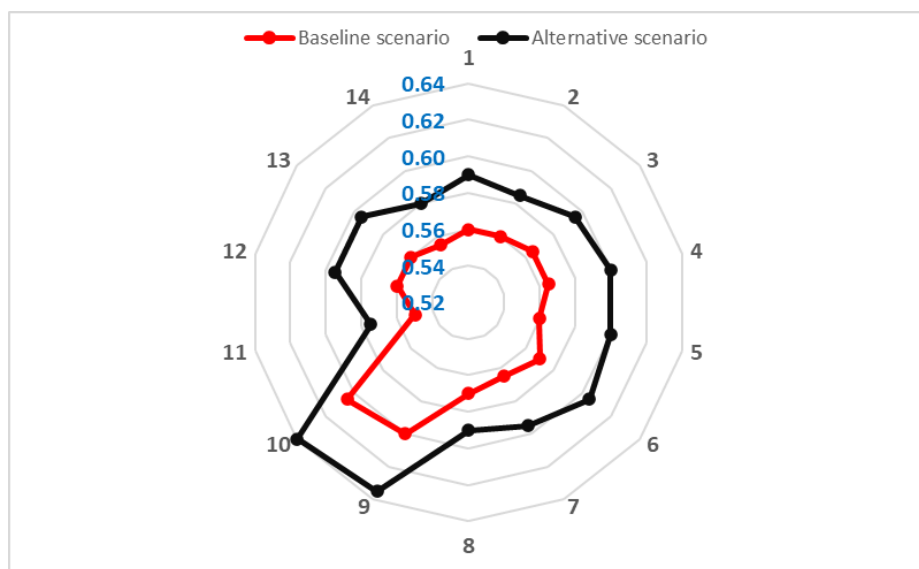
- 138 1. Ignition and plasma stabilization: 1976 W;
- 139 2. Running: 1963 W;
- 140 3. Washing: 1963 W;
- 141 4. Cooling: 37 W;
- 142 5. Standby: 1963 W.

143 Assigning these specific energy consumptions to the operating phases was crucial for simplifying the quantification of
 144 energy use in future analyses. Consequently, subsequent measurements only require the time of each operating phase to
 145 be recorded.

146

147 3 Results

148 To enable direct comparison with the 3:1 weighting presented in Figure 5, the complementary score was also calculated
149 using equal weights (1:1) for AGREE and AGREEprep. These results are shown in Figure S2.



150

151 *Figure S2 - Comparison of complementary scores (1:1) between the baseline and alternative scenarios. External numbers represent*
152 *MP-AES working sessions (black); internal numbers indicate complementary scores (blue).*

153 The average complementary score was 0.57 in the baseline scenario and 0.60 in the alternative scenario. To facilitate
154 comparison with manuscript Figure 5 (3:1 weighting), equal weights were applied here. The inter-scenario difference
155 remains modest ($\Delta = 0.03$), with no outliers exceeding ± 0.02 . No significant sensitivity to equal weighting was observed,
156 confirming result stability across reasonable assumptions.

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