



A Tutorial on AGREEprep an Analytical Greenness Metric for Sample Preparation[☆]

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ARTICLE INFO

Keywords:

Green analytical chemistry
Green sample preparation
Green metrics
Sample preparation
Sustainability assessment

ABSTRACT

Sample preparation is a key step in the analytical procedure and a critical component for achieving analytical greenness. AGREEprep is the first metric intended for evaluating the environmental impact of sample preparation methods. The approach consists of ten steps of assessment that correspond to the ten principles of green sample preparation and uses a user-friendly open-source software to calculate and visualize the results. Despite the simplicity of the approach, some assessment steps can be difficult to evaluate in a straightforward manner, either because essential data are not readily available or, in some cases, are poorly defined.

This walkthrough tutorial on AGREEprep serves as an in depth yet simple guide for new users that elucidates all aspects of the greenness assessment. It will prove beneficial to analysts wishing to assess the greenness of their own developed procedures or those found in the literature, which can be challenging taken that critical data is often not reported. Particular attention is given to the calculations involved in estimating the amount of waste generated and energetic requirements. The impact of weight values of criteria on the overall final score are discussed and using two hypothetical scenarios is related to the analytical goals of operators. Finally, several assessment examples are given in the supplementary information to familiarize assessors with all assessment steps.

1. Introduction

Sample preparation is a key step in analytical procedures that is essential for the separation/enrichment of analytes, the removal/minimization of matrix interferences and achieving compatibility with the determination technique. Although essential, sample preparation may increase the environmental impact of the overall analytical method, mainly because of the large requirements in solvents, reagents, chemicals, materials, and energy. On this basis, the Green Analytical Chemistry (GAC) approach excluded sample preparation from green analytical practices by suggesting in the first of its twelve principles to avoid this step [1] altogether. To address the exclusion gap created by GAC, Green Sample Preparation (GSP) was recently defined and formulated in the form of ten principles aiming to protect the environment

and human health [2]. Most of these principles are directly related to the sample preparation procedure, and two principles (1 and 9) refer to pre- and post- sample preparation analytical activities.

Although the ten principles of GSP established a road map toward the development of overall greener analytical methodologies, they did not measure the environmental impact of sample preparation methods. For this reason, a new metric tool, termed AGREEprep, was recently proposed and was the first reported metric focusing on sample preparation [3]. AGREEprep is based on ten consecutive steps of assessment that correspond to the ten principles of GSP. Compared to other published metrics, AGREEprep provided appropriate levels of accuracy and specificity for assessing the environmental impact of sample preparation methods [3], mainly because previously published metrics did not give sufficient attention to the sample preparation step. In addition to

[☆] Given her role as Editor in Chief, Eleftheria Psillakis had no involvement in the peer-review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Rafael Lucena Rodríguez.

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evaluating greenness, AGREEprep assessment can assist detecting the strengths and weaknesses of methods, thereby facilitating the greening of sample preparation procedures.

The evaluation of greenness can involve steps that are difficult to “quantify” either because values are not readily available or, in some cases, are poorly defined, e.g., energy demands, calculation of waste. The aim of this tutorial is to provide a simple and thorough guide for using the AGREEprep metric tool. All aspects on greenness evaluation are elucidated and exemplified. A special focus is given on estimating the amount of waste generated and energetic requirements. The weights of criteria and their default values are also discussed together with their impact on the final score using different hypothetical scenarios.

2. AGREEprep evaluation

The AGREEprep open access software can be obtained from mostwiedzy.pl/AGREEprep, and the code is available at git.pg.edu.pl/p174235/agreeprep. In case of difficulties with deploying the desktop version of the software, the user can access an online emulator by following the link provided at the project’s website (mostwiedzy.pl/agreeprep), where one can also find a link to a short video with instructions on how to run the emulator.

The ten individual steps of assessment in AGREEprep take scores ranging from 0 to 1, with the extremes representing the worst and ultimate performance respectively. Each criterion has a default weight to the overall score, and assessors may choose to change the default weights and adjust them to their analytical goals, provided that they justify these changes. The scores from each criterion are weighted and combined to yield the overall score that also ranges from 0 to 1, with 1 representing optimum performance.

AGREEprep software requests input data for each of the ten steps of assessment and after completion of the evaluation it produces a round pictogram with a circle in the center that shows the overall score and ten trapezoid bars corresponding to the ten criteria, each having a length equivalent to the assigned weight. The color of each element changes after evaluation, thus providing an easy way to identify the weak and strong points of the procedure, and their contribution to the final score.

A step-by-step discussion of the assessment criteria follows that exemplifies their input values and uses case studies to present the different aspects of the greenness assessment.

2.1. Input data for Criterion 1: Favor in situ sample preparation

The first criterion relates sample preparation to the sampling step and aims at the reduction of time, waste, energy, and materials to be transported to the laboratory. To assess this criterion, AGREEprep distinguishes four options. The first option, assigned with the highest score, is *in-line/in situ* sample preparation, and considers sample preparation carried out in situ directly in the investigated object with no withdrawal of samples from it. The second, *on-line/in situ* sample preparation, considers sampling and sample preparation being performed in a side-line of the object under investigation with samples being continuously withdrawn from the object and then prepared in situ and in some automated way. In most cases, such on-line systems are not commercially available and suitable interfaces need to be designed for each application [4]. It should be noted that the *on-line/in situ* option must not be mistaken with on-line/at-line analysis performed in the laboratory, consisting of systems coupling sample preparation with the consequent analysis technique [5,6]. *On site* sample preparation is the third option and involves collecting samples on site and preparing them using sample preparation device(s) brought in the sampling site. Extracts are then transferred to the laboratory for further analysis. In the last option of *ex situ* sample preparation, samples are collected from the object and then transported to the laboratory for sample preparation and analysis. This option is the worst-case scenario and is assigned with the lowest score.

2.2. Input data for Criterion 2: Use safer solvents and reagents

This criterion is focused on the application of safe, non-toxic solvents, reagents, catalysts and mineralization/derivatization agents. It favors sample preparation systems that are miniaturized or, even better, solventless and reagentless. All chemicals that are toxic via any exposure pathways are accounted here and AGREEprep assumes that any amount above 10 mL or grams is not acceptable (score 0). In practice, there are procedures that do not state the amounts of reagents used, e.g., when adjusting the pH to specific values. In such cases, estimations of these amounts can be made using personal laboratory experience after considering the type of reagent and sample size.

The chemicals that are considered safe are water, inert gases, green alternatives to hazardous chemicals, and substances that are authorized for consumption apart from ethanol. A past report reviewed several examples of natural and safe reagents [7], including enzymes or crude plant extracts. Moreover, green and bio-based solvents were recently summarized and included alcohols, esters, carboxylic acids and terpenes [8]. The same report raises an interesting point to consider after comparing for different bio-solvents, their greenness scores, toxicities towards fish, and ability for biodegradation (Table S1 in the Supplementary Information). The data showed that although most bio-based solvents are biodegradable, some of them are toxic towards aquatic organisms and should therefore be considered problematic [8].

2.3. Input data for Criterion 3: Target sustainable, reusable, and renewable materials

Criterion 3 promotes materials that are sustainable, renewable and reusable. The assigned scores are based on the percentage amount of such materials used in the sample preparation procedure.

Reusability refers to any material that can be used more than once. This typically refers to extracting materials that can be regenerated using a thermal or solvent desorption step. The terms sustainable and renewable are both related to the plant- or bio-based origin of materials. The two terms are often used interchangeably, although differences exist between them. Resources qualify as renewable if they can be replenished naturally (such as crops and biomatter), whereas sustainable resources can be maintained for the foreseeable future without compromising future generations [9]. Cellulose is one of the most important and abundant renewable material for a multitude of applications in sample preparation including paper strips and filter papers. Other examples include cork, cotton and other material that are sourced from living plants and animals powered by solar energy. It is noted that not all renewable resources qualify as sustainable and vice versa. For example, several plant-based materials cannot be replenished within a period of 10 years or less, so in the long run, they may not be renewable in a sustainable manner [9]. Moreover, biomass carbon materials can be renewable, but their generation may release greenhouse gases which can be harmful to the environment and are therefore considered unsustainable. Sustainable materials vary enormously and may include bio-based polymers or highly recyclable materials. It is noted that glass is considered highly recyclable as it can be reprocessed an indefinite number of times without requiring additional mineral resources. However, laboratory glassware when used only once generates waste, which can impact the environment [10]. In this direction, washing the glassware and reusing it, should be preferred over considering it as a single-use item.

2.4. Input data for Criterion 4: Minimize waste

The fourth criterion focuses on minimizing the waste generated during sample preparation procedures. AGREEprep gives the highest (1.0) and lowest (0.0) scores to methods that generate less than 0.1 g (or mL) and more than 50 g (or mL) of waste, respectively, and uses a logarithmic function to calculate scores when the amount of waste is between

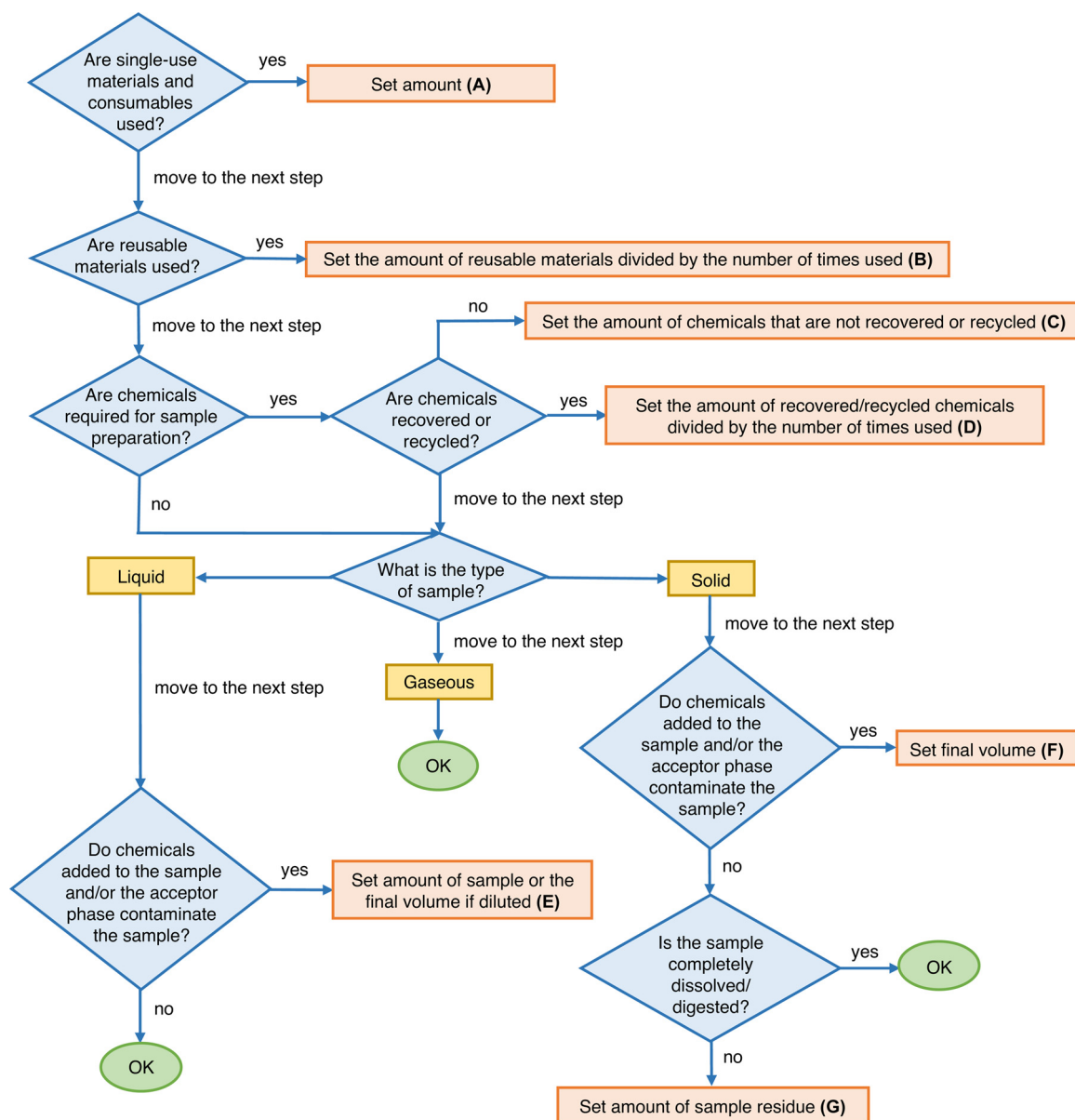


Fig. 1. Flow diagram for estimating the amount of waste generated by a sample preparation method.

these two levels. With the aim of identifying and assessing the different sources of waste generation in a systematic way, a flow diagram is provided in Fig. 1.

Initially, the amount in grams of single-use materials and consumables (A) is evaluated (e.g., micropipette tips, filters, solid-phase extraction (SPE) cartridges, extraction thimbles), followed by the amount of reusable materials (e.g., solid-phase microextraction (SPME) fibers) divided by the number of times they can be used (B). The amount of chemicals required (C) for sample preparation is then considered. Any reagents and solvents used must be considered at this step, regardless of their environmental, health, and safety issues, since these aspects are assessed in subsequent criteria. Notwithstanding, the amount (C) has to be corrected by subtracting the amount of materials divided by the number of times they can be used (D).

When the *in line/in situ* sample preparation approach is not followed, the sample itself (or part of it) can be considered as waste depending on the sample preparation method. This is the case of liquid samples treated with reagents (e.g., acids, bases or salts) or solvents, in which

case the total amount of sample is considered as waste (E). A zero contribution to waste from the sample is selected when (i) chemicals are not added to the sample, (ii) the sample does not come into contact with the acceptor phase (e.g., headspace methods) or (iii) the acceptor phase does not contaminate the sample (e.g., direct-SPME, stir bar sorption extraction (SBSE), thin film microextraction (TFME)). When solid samples are treated with clean extractants (e.g., ultrapure water or supercritical carbon dioxide without modifiers) and diluted to volume without the addition of chemicals, a zero contribution to waste is considered. Conversely, when solid samples are treated with acceptor phases that contaminate the solid matrix (e.g., Soxhlet extraction, microwave- or ultrasound-assisted extraction involving solvents other than water, supercritical fluid extraction involving modifiers), then the final volume (F) is accounted as waste (also considering the probable dilution of the extract or digestate before the analysis). Furthermore, when solid samples are not completely dissolved or digested, the resulting solid residue (G) is also considered as waste and needs to be added to the previous amount (F). In the case of gaseous samples, additional chemicals are

not considered since they are covered in the previous steps of the flow diagram. The total amount of waste is then calculated for gaseous samples as $A + B + C + D$, for liquids as $A + B + C + D + E$ and for solids as $A + B + C + D + F + G$. The only exceptions are when the chemicals considered in C and D are contained in the final volume, then the total amount of waste is calculated as $A + B + E$ for liquid samples, and $A + B + F + G$ for solid samples.

2.5. Input data for Criterion 5: Minimize sample, chemical, and material amounts

Criterion 5 is related to miniaturization and considers the sample size expressed in units of mass or volume. Although the size of the sample is in many cases not of concern from the point of view of abundance, large samples may increase energy demands (during heating, cooling, mineralizing), consumption of chemicals and waste generation, next to reducing the potential for automation and portability. For these reasons, AGREEprep considers sample sizes above 100 mL or grams not acceptable.

It should be noted that in some extreme cases the mass of sample is variable, as seen in the mass of particulate matter fallout or concentration [11] before polycyclic aromatic hydrocarbons or heavy metals determination. In such cases, it is advisable to assume the typical mass of collected particulate matter sample.

2.6. Input data for Criterion 6: Maximize sample throughput

Sample throughput does not directly affect the greenness of the sample preparation procedure but is related to energetic demands and occupational exposure risks for operators. It is defined as the number of samples prepared in one hour and is to be distinguished from the throughput of the entire analytical procedure. In general, sample throughput can be maximized in two ways. The first one is to use very short sample preparation times for rapidly processing many samples in series, one after another. Another way is to treat several samples in parallel so that even if long sample preparation times are needed, a high sample throughput can be achieved. For example, extraction of one sample by Soxhlet extraction for 24 h yields a 0.042 h^{-1} sample throughput. If the same Soxhlet extractor can accommodate up to 6 samples then extracting 6 samples for 24 h will increase sample throughput to 0.25 h^{-1} . Some more examples of sample throughput calculations are given in **Table S2** in the Supplementary Information.

2.7. Input data for Criterion 7: Integrate steps and promote automation

This criterion suggests integrating steps and automating procedures to save time, minimize human intervention and errors next to lower chemical consumption and waste generation. It is equally important that automation and to some extent integration of sample preparation steps may also reduce exposure risks for operators. To assess this criterion, two sub-criteria are considered and the resulting sub-scores are combined. Initially, the number of sample preparation steps is inserted (less or equal to 2 steps is rated with the highest score and procedures with 6 steps or more are rated with a 0 score). At this point it is important to provide the definition of a step in sample preparation: a step is as an operation or set of operations that leads to a change of sample matrix or change in sample matrix volume, properties or composition, phase separation or even change the analyte itself. Representative examples of sample preparation steps include filtration, dilution, decantation, mineralization, extraction, centrifugation, sorption, analytes derivatization, drying, lyophilization. It is advised not to consider minor operations as separate steps. For instance, the procedure of adding a mineral acid to the sample, applying microwave irradiation for 20 min and the transfer of mineralizate from cell to flask, should be treated as a single step rather than three separate steps. Moreover, if the same operation is repeated (such as a second extraction of the sample with a new portion of

solvent) and this repetition is fixed in the procedure, then it is advised to treat these series of steps as a single step. Likewise, if the sample preparation procedure states “dilute until certain parameter is reached”, “add reactant portions until change of colour” or “rinse until total transfer of sediment” and the number of repetitions is not stated, then it is advisable to treat this part of the procedure as a single step.

The criterion 7 also evaluates the degree of automation of the procedure and considers as: (i) fully automated systems those that exclude involvement of operators in the sample preparation procedure, (ii) semi-automated systems those that involve partial involvement of operators, and (iii) manual systems those that require the direct participation of analysts.

2.8. Input data for Criterion 8: Minimize energy consumption

Energy consumption is a crucial point to consider in greenness assessment. To evaluate the impact of this criterion the total energetic requirement of the electrical appliance/instrument must be estimated for the specific time needed to complete the sample preparation step, and then divided by the number of samples extracted, i.e., express energy consumption in terms of watt-hour (Wh) per sample. This normalization of energy demands is important when several samples are simultaneously prepared whilst using the same electrical appliance/instrument.

In general, watt-hour is commonly used in electrical applications and represents the unit of energy equivalent to one watt (1 W) of power expended for one hour (1 h) of time. It should be remembered that watt is a unit of power and not energy, i.e., measure of the rate at which energy flows at a moment in time and cannot quantify energetic demands of the system. The latter is typically calculated by multiplying the power of the electrical appliance (in W) with time (in h). The power value of the electrical appliance used is listed in the manufacturer's technical specifications sheet or on the back of the instrument's hardware and represent maximum values. For example, magnetic stirrers may be equipped with a hotplate having an 830 W power rating. This is the maximum value corresponding to operating the electrical appliance at the maximum temperature e.g., 300°C . For lower temperatures, the actual power value will be lower and if only magnetic stirring is applied then the power will be reduced to e.g., close to 30 W, a typical value corresponding to the maximum rotating speed. It should be mentioned here that a past report investigating the energy consumption of analytical instrumentation, measured actual power values that were about 40% of the posted maximum value given by the manufacturer [12]. In the same report, the authors applied a 40% correction factor for those systems that were unable to be physically measured.

The calculation of energy consumption can be a problematic step in assessing the greenness of analytical methods, and this includes sample preparation methods. AGREEprep takes into account the nominal wattage given by vendors to calculate scores without applying a correction factor, and the resulting score will remain valid as it is used to evaluate the comparative performance of sample preparation methods [3,13]. In AGREEprep, energy requirements below 10 Wh per sample receive the highest score equal to 1, whereas energy demands above 500 Wh per sample correspond to the worst performance (score = 0). **Table 1** lists several examples and shows the calculations for obtaining the Wh per sample values. It must be kept in mind that the nominal wattage values considered in **Table 1** are for specific models from vendors that were randomly chosen and do not correspond to average values. On the contrary, they are expected to vary even between models from the same manufacturer. **Table 1** shows that reducing the operation time and/or increasing the number of samples processed simultaneously may improve the greenness of the sample preparation method under evaluation. To further demonstrate the above, **Supplementary Table S3** shows calculated Wh per sample values for selected wattage, operation times and compares values when one sample or six samples are simultaneously processed.

Table 1

Watt-hour per sample values for different sample preparation instrumentation based on the nominal wattage (given in parentheses) and different scenarios with regards to operational time and number of samples processed.

Microwave digester (1900 W)	
0.5 h operation for 1 sample	(1900 W x 0.5 h) / 1 sample = 950 Wh/sample
0.5 h operation for 10 samples	(1900 x 0.5 h) / 10 samples = 95 Wh/sample
Manual Soxhlet extraction (2700 W)	
8 h operation for 1 sample	(2700 W x 8 h) / 1 sample = 21600 Wh/sample
8 h operation for 6 samples	(2700 W x 8 h) / 6 samples = 3600 Wh/sample
Automated Soxhlet extraction (1200 W)	
2 h operation for 6 samples	(1200 W x 2 h) / 6 samples = 400 Wh/sample
Ultrasound bath (130 W)	
0.5 h operation for 1 sample	(130 W x 0.5 h) / 1 sample = 65 Wh/sample
Shaker agitator (50 W)	
24 h operation for 1 sample	(50 W x 24 h) / 1 sample = 1200 Wh/sample
24 h operation for 10 samples	(50 W x 24 h) / 10 samples = 120 Wh/sample
Accelerated solvent extraction (500 W)	
0.2 h operation for 1 sample	(500 W x 0.2 h) / 1 sample = 100 Wh/sample

2.9. Input data for Criterion 9: Choose the greenest possible post-sample preparation configuration for analysis

Several measurement and instrumental techniques can be used after sample treatment, and the final choice may depend on the analytical performance requirements or even availability. AGREEprep distinguishes four options to assess this criterion that range from simple, readily available detection systems (such as smartphones and desktop scanners; score = 1) to advanced mass spectrometry techniques and inductively coupled plasma (score = 0). If the system used is not listed then analytical chemists performing the assessment should select, as a good approximation, the closest one or the one having similar greenness impact. Moreover, if more than one determination steps are used for the same extract, it is recommended to select the most problematic option as the final determination technique for AGREEprep assessment.

It should be noted that in some cases analysts select simple, if any, sample preparation procedures and rely on sophisticated analytical instruments with extremely high separation power to isolate analytes. This approach may be very demanding in terms of energy consumption and sometimes material inputs, shifting the environmental impact from sample preparation to the determination step. In these cases, AGREEprep will not be able to evaluate the greenness of the overall analytical procedure. Therefore, to achieve greenness in methods it is recommended to opt for simple, widely available final determination systems over sophisticated ones.

2.10. Input data for Criterion 10: Ensure safe procedures for the operator

Operators can be potentially exposed to several hazards that should be minimized and controlled. AGREEprep considers the number of the operational hazards (i.e., chemical, electrical, physical, or biological hazards) and assigns scores from 0 (involving four or more hazards) to 1 (no operational hazards). To evaluate this criterion, the number of pictograms of each of the substances used is considered to assess the exposure to chemical hazards. This information being provided in section 2 (hazards identification) of the material safety data sheet (MSDS) files. In addition, electrical, physical, and biological hazards that may result in personal injury to the operator are considered whenever available in MSDS files or the technical specifications of the apparatus used to perform sample preparation. For convenience, the pictograms that need to be counted on MSDS basis are depicted in the **Supplementary Fig. S1**. It is noted that when the same hazard pictogram appears in the MSDSs for more than one compound, then it is counted only once.

3. Weights for criteria

A closer study of the ten criteria used for the greenness assessment, shows that they are not equal in terms of their importance. For example, selecting *in-situ* sample preparation or choosing to integrate steps is presumably less significant in terms of greenness than the volume of solvents used, energy requirements or assuring safety for the analyst. For this reason, default weights are suggested and applied during assessment with the option to modify them provided that any changes in weights are clearly justified and related to the importance of the corresponding criteria.

One of the underlying reasons for changing values in weights may be to adjust the greenness assessment to the goals of the sample preparation method. To exemplify this, two hypothetical scenarios are discussed here, and the corresponding changes in weights for each criterion are summarized in **Table 2**. In the first scenario (Scenario 1), analysts seek to promote simple and automated systems with the fundamental aim to limit the operational exposure. The analysts and decide to tune the greenness assessment to their analytical goals by increasing the weights of criteria 1, 6, 7 and 9. The importance of sample size (criterion 5) is also increased, as smaller samples have a greater potential for automation. In this hypothetical scenario, the use of safe solvents/reagents/materials, safety of the operator and energy demands (criteria 2, 3, 8 and 10) are of less importance and as such, their weights are reduced. In the second scenario (Scenario 2), analysts seek to promote the application of safe reagents, materials, and the generation of benign wastes. Accordingly, the weights of criteria 3, 4, 5 and 10 are increased, pointing out the high significance of non-problematic waste, small sample size and safety of the operator. At the same time, analysts in Scenario 2 consider speed and automation (criteria 6 and 7) of less importance and as such, their weights are lowered.

The default weights and the weights assigned in the two scenarios were then applied to evaluate the greenness of two published methods. The first one considered an on-site analysis protocol based on microextraction by packed sorbent (MEPS) technique used for the determination of UV filters in water samples [14]. This fully automated procedure included the preparation of 800 μ L water sample with sorbent trapping of analytes and the extraction cycle time was 10 minutes. The procedure required 0.25 mL of MeOH for conditioning and washing and 50 μ L of ethyl acetate for analytes elution. The energy requirement for the sample preparation step was not stated and was estimated as 20 Wh per sample. The results of the AGREEprep assessments based on the three different scenarios (**Table 2**) are presented in **Fig. 2** and the evaluation reports generated by AGREEprep can be found in the **Supplementary Fig. S2A-S2C**. **Fig. 2** clearly shows the differences in weights as reflected by the size of the outer trapezoidal parts of the graphical result. Moreover, **Fig. 2** shows that the final score in Scenario 1 (promoting simple and automated systems) was the same as the one obtained with the default weights, and that the changes made in the individual scores canceled each other. Similarly, the final score of Scenario 2 (safe chemicals/materials) was almost the same as the one with default weights. Overall, the published method under evaluation being inherently simple, automated, and involving safe chemicals and materials, satisfied the analytical goals of each hypothetical scenario tested here, and for this reason adjusting the weights to the requirements of each scenario did not result in a substantial (if any) change in the final score.

The second evaluated method involved SPME combined with gas chromatography/high-resolution time-of-flight mass spectrometry for the extraction of organic micropollutants (including a UV filter) in water samples [15]. SPME was performed manually and in an *ex situ* mode. The sample size was 4 mL and SPME sampling was performed for 45 min at room temperature. Magnetic stirring was only applied during extraction and the corresponding energetic requirement was assumed to be 22.5 Wh per sample. The results of the AGREEprep assessments after applying the default weights and the two hypothetical scenarios are given in

Table 2

The default weights used in AGREEprep and the new weights based on Scenarios 1 and 2.

Criterion	Criterion description	Default weights	Scenario 1 weights	Scenario 2 weights
1	Favor <i>in situ</i> sample preparation	1	4	1
2	Use safer solvents and reagents	5	4	5
3	Target sustainable, reusable, and renewable materials	2	1	4
4	Minimize waste	4	4	5
5	Minimize sample, chemical and material amounts	2	4	4
6	Maximize sample throughput	3	5	2
7	Integrate steps and promote automation	2	5	1
8	Minimize energy consumption	4	3	4
9	Choose the greenest possible post-sample preparation configuration for analysis	2	4	2
10	Ensure safe procedures for the operator	3	2	5

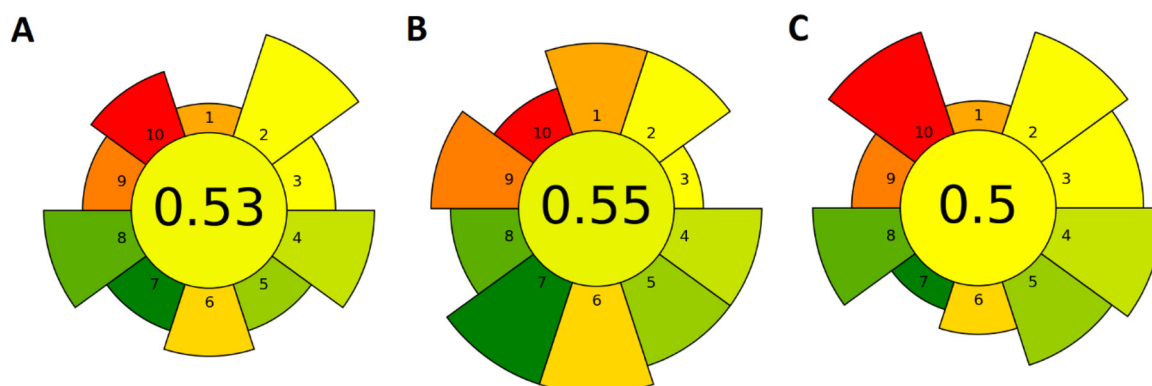
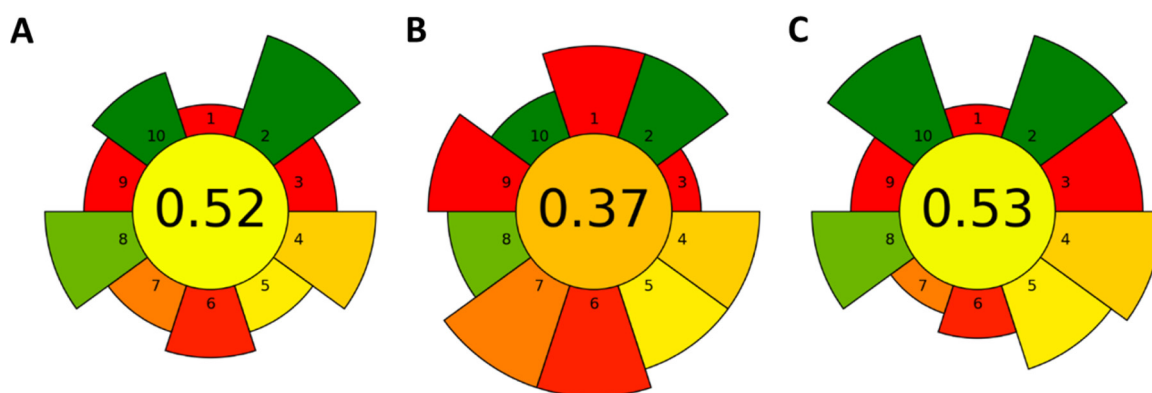
**Fig. 2.** Comparison of the assessment results on the at-line analysis protocol based on MEPS [14] after applying the (A) default weights, (B) Scenario 1 weights for a simple, automated system, (C) Scenario 2 weights for safe chemicals/materials.**Fig. 3.** Comparison of the assessment results on the SPME method used for screening organic pollutants in water [15] after applying the (A) default weights, (B) Scenario 1 weights promoting a simple, automated systems, (C) Scenario 2 weights promoting safe chemicals/materials.

Fig. 3 and the evaluation reports can be found in the Supplementary **Fig. S3A-S3C**. As seen, the final score of Scenario 1 (promoting simple and automated systems) was the lowest, reflecting the manual SPME operations required by this published procedure, but also the fact that the weak points of the method (criteria 1, 6 and 9) received more attention. On the other hand, the final score of Scenario 2 (promoting safe chemicals/materials) was essentially the same as the one obtained when applying the default weights, owing to the green and “safe” features of the SPME technology. Overall, the results found here show that the analytical goals must be carefully considered and related to the analytical characteristic of the method under evaluation, as they may influence the final score of the AGREEprep assessment. At the same time, if the method under evaluation already satisfies the analytical goals of the analyst, then changes in weights are not necessary.

4. Assessment examples

Analysts can easily assess their own developed procedures, as they are familiar with all the parameters and can state input values for all ten criteria in a straightforward manner. On the contrary, the assessment of procedures found in the literature can be challenging taken that several essential data are not reported (e.g., the volumes of some reagents or the energetic requirements of the electrical appliances/instruments used). It is advised to make estimations based on individual laboratory experience with similar procedures.

To assist with the familiarization of analysts with AGREEprep assessment some more examples are given in the supplementary file (**Fig. S4-S6**). Two of the evaluated methods (**Fig. S4 and S5**) consist of official standard methods that are open access to the public [16,17] and

the evaluation report generated from AGREEprep is included, showing the values inserted in the AGREEprep software. The rest of the examples consist of procedures that were included in a recent open access publication [18]. The pictograms together with the justification for input data are given in Fig. S6 of the Supplementary Information.

5. Conclusions and future directions

AGREEprep is the first metric focusing on sample preparation that can quantify the technical aspects of the procedure and identify points of improvements. This walkthrough tutorial elucidated all aspects of the greenness assessment and will serve as a comprehensive guide to new users. AGREEprep is accompanied by a quick to use software that generates a pictogram visualizing the performance of the method. The different parts of the pictogram allow the identification of weak and strong points of the method as well as the fast comparison of different methodologies.

The use of green metrics is undoubtedly critical and the integration of AGREEprep as a standard laboratory procedure in new and existing sample preparation methods will not only gauge progress toward greening sample preparation but also allow harmonization of procedures in terms of their environmental impact. In particular, the systematic comparison of newly developed methods with existing or even better, official standard methods will provide concrete evidence of the urgent need to replace traditional and hazardous sample preparation practices with modern and powerful alternatives that have the additional benefit of being greener. It is envisaged that the spread of AGREEprep practice will strengthen the link between academia and the private sector, giving the latter the arguments to adopt powerful techniques that at the same time, create a shared value by addressing productivity and societal needs.

Declaration of Competing Interest

None.

Acknowledgements

This article is based upon work from the IUPAC project No. 2021-015-2-500 "Greenness of official standard sample preparation methods".

This article is based upon the work from the Sample Preparation Study Group and Network, supported by the Division of Analytical Chemistry of the European Chemical Society.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sampre.2022.100025.

References

- [1] A. Gałuszka, 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, doi:10.1016/j.trac.2013.04.010.
- [2] Á.I. López-Lorente, F. Pena-Pereira, S. Pedersen-Bjergaard, V.G. Zuñin, S.A. Ozkan, E. Psillakis, The ten principles of green sample preparation, *TrAC Trends Anal. Chem.* 148 (2022) 116530, doi:10.1016/j.trac.2022.116530.
- [3] W. Wojnowski, M. Tobiszewski, F. Pena-Pereira, E. Psillakis, AGREEprep – Analytical greenness metric for sample preparation, *TrAC Trends Anal. Chem.* 149 (2022) 116553, doi:10.1016/j.trac.2022.116553.
- [4] A. Plum, G. Braun, A. Rehorek, Process monitoring of anaerobic azo dye degradation by high-performance liquid chromatography–diode array detection continuously coupled to membrane filtration sampling modules, *J. Chromatogr. A* 987 (2003) 395–402, doi:10.1016/S0021-9673(02)01906-4.
- [5] J. Pan, C. Zhang, Z. Zhang, G. Li, Review of online coupling of sample preparation techniques with liquid chromatography, *Anal. Chim. Acta.* 815 (2014) 1–15, doi:10.1016/J.ACA.2014.01.017.
- [6] F. Pena-Pereira, C. Bendicho, D.M. Pavlović, A. Martín-Esteban, M. Díaz-Álvarez, Y. Pan, J. Cooper, Z. Yang, I. Safarik, K. Pospiskova, M.A. Segundo, E. Psillakis, Miniaturized analytical methods for determination of environmental contaminants of emerging concern – A review, *Anal. Chim. Acta.* 1158 (2021) 238108, doi:10.1016/j.aca.2020.11.040.
- [7] K. Grudpan, S.K. Hartwell, S. Lapanantnoppakun, I. McKelvie, The case for the use of unrefined natural reagents in analytical chemistry—A green chemical perspective, *Anal. Methods* 2 (2010) 1651–1661, doi:10.1039/c0ay00253d.
- [8] M. Tobiszewski, Analytical chemistry with biosolvents, *Anal. Bioanal. Chem.* 411 (2019) 4359–4364, doi:10.1007/s00216-019-01732-2.
- [9] I. Choudhury, Renewable and Sustainable Materials, in: *Ref. Modul. Mater. Sci. Mater. Eng.* (2016) 1–2, doi:10.1016/B978-0-12-803581-8.04095-9.
- [10] B. Raccary, P. Loubet, C. Peres, G. Sonnemann, Life cycle assessment of sample preparation in analytical chemistry: a case study on SBSE and SPE techniques, *Adv. Sample Prep.* 1 (2022) 100009, doi:10.1016/J.SAMPRE.2022.100009.
- [11] EPA Standard Methods IO-2, Integrated sampling of suspended particulate matter (SPM) in ambient air, 1999. <https://www.epa.gov/sites/default/files/2019-11/documents/overvw2.pdf>.
- [12] M.B. Hicks, W. Farrell, C. Aurigemma, L. Lehmann, L. Weisel, K. Nadeau, H. Lee, C. Moraff, M. Wong, Y. Huang, P. Ferguson, Making the move towards modernized greener separations: Introduction of the analytical method greenness score (AMGS) calculator, *Green Chem* 21 (2019) 1816–1826.
- [13] F. Pena-Pereira, W. Wojnowski, M. Tobiszewski, AGREE - Analytical GREENness Metric Approach and Software, *Anal. Chem.* 92 (2020) 10076–10082, doi:10.1021/acs.analchem.0c01887.
- [14] M. Moeder, S. Schrader, U. Winkler, R. Rodil, At-line microextraction by packed sorbent-gas chromatography–mass spectrometry for the determination of UV filter and polycyclic musk compounds in water samples, *J. Chromatogr. A* 1217 (2010) 2925–2932, doi:10.1016/j.chroma.2010.02.057.
- [15] F. Hernández, T. Portolés, E. Pitarch, F.J. López, Target and nontarget screening of organic micropollutants in water by solid-phase microextraction combined with gas chromatography/high-resolution time-of-flight mass spectrometry, *Anal. Chem.* 79 (2007) 9494–9504, doi:10.1021/ac071551b.
- [16] USEPA, Method 3015A – Microwave assisted acid digestion of aqueous samples and extracts, 2007. <https://www.epa.gov/sites/default/files/2015-12/documents/3015a.pdf>.
- [17] USEPA, Method 3060A – Alkaline digestion for hexavalent chromium, 1996. <https://www.epa.gov/sites/default/files/2015-12/documents/3060a.pdf>.
- [18] A.O. Hay, R. Trones, L. Herfindal, S. Skrede, F.A. Hansen, Determination of methotrexate and its metabolites in human plasma by electromembrane extraction in conductive vials followed by LC-MS/MS, *Adv. Sample Prep.* 2 (2022) 100011, doi:10.1016/J.SAMPRE.2022.100011.