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AGREEprep – Analytical Greenness Metric for Sample Preparation

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Abstract

This work proposes for the first time, a metric tool that gives prominence to sample preparation. The developed metric (termed AGREEprep) was based on 10 categories of impact that were recalculated to 0-1 scale sub-scores, and then used to calculate the final assessment score. The criteria of assessment evaluated among others the choice and use of solvents and reagents, waste generation, energy consumption, sample size, and throughput. Assessment was also based on the possibility to differentiate between criteria importance by assigning them weights. The assessment procedure was performed using an open access, intuitive software that produced an easy-to-read pictogram with information on the total performance and structure of threats. A compiled version of the open access software can be obtained from mostwiedzy.pl/AGREEprep. The applicability of AGREEprep was successfully demonstrated using six different methods as case studies.

Keywords
Green analytical chemistry; Green metrics; Sample preparation; Green chemistry; Sustainability assessment

1. Introduction

Green Analytical Chemistry (GAC) aims to minimize the negative environmental impact of chemical analyses by addressing critical issues such as the generation of toxic laboratory waste and the use of solvents and reagents that are hazardous to human health or the environment [1]. The importance of GAC was highlighted soon after the introduction of Green Chemistry (GC) and emerged as a specific branch of GC, in part due to the inability of the GC principles [2] to address the demands of the analytical field. In this direction, only one principle of GC (i.e., real-time analysis for pollution prevention) is directly related to Analytical Chemistry, whereas the rest are either loosely related to chemical analysis or not related at all. For this reason, the 12 principles of GAC were introduced almost a decade ago [3], providing a more suitable framework towards greener analytical chemistry practices. In subsequent years, several self-assigned 'green analytical methods' appeared in the literature that exclusively focused on the improvement of one particular principle of GAC, systematically ignoring other GAC aspects. In an attempt to assess and harmonize the compliance of analytical methods with GAC assumptions, several metrics of varying comprehensiveness have been reported in the literature [4–12]. They are based on the incorporation of different criteria, and the generic response of the assessment can be highly variable in both complexity and appearance. The National Environmental Methods Index (NEMI) pictograms [5], derived from Yes/No responses to four specific criteria, was the first (and rather simplistic) approach proposed in the literature. In 2012, the analytical eco-scale metric tool [6] was reported assigning penalty points to different criteria that were subtracted from an ideal score of 100 in such a way that the closer the numerical value was to 100, the greener the method. In an attempt to provide a more complete and refined output, different advanced metric tools were recently developed, namely, the Green Analytical Procedure Index (GAPI and recently reported ComplexGAPI) [7,12], the RGB model [8,9], the Analytical GREEnness Metric Approach (AGREE) [10] and the hexagon-CALIFICAMET [11]. These tools generally provide easy to read pictograms that map the degree of compliance of evaluated criteria within the framework of GAC. The use of metrics for the assessment of analytical methods can be synergistically complemented with other tools focusing on a specific and problematic step of the analytical method. In this connection, different metrics and tools focused on specific aspects of analytical methods (e.g.

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chromatographic separations) [13–15], which proved helpful in identifying improvable aspects of separation approaches that could go unnoticed or overlooked if only a more comprehensive tool was to be employed. Surprisingly, analogous tools for the assessing the greenness of the sample preparation step have not been reported in the literature.

Sample preparation is a key step in the analytical procedure that is essential for the separation and enrichment of target analytes, the removal or minimization of matrix interferences and/or to ensure compatibility with the measurement technique. At the same time, sample preparation has been identified as one of the most critical steps from the GAC point of view [16], mainly because of the typical substantial requirements in solvents (solvent extraction techniques), sorbents (solid-phase based extractions), reagents (for derivatization reactions or the removal of impurities), acids or bases (for pH correction or mineralization), energetic inputs (heating, stirring, cooling) and other consumable materials or devices (such as cartridges, pipettes or pipette tips, glassware). For this reason, the first principle of GAC suggested avoiding sample preparation and instead, using direct analytical techniques [3]. However, the possibility to incorporate direct analytical methodologies in all applications is rather limited [17–19], rendering the implementation of sample preparation strategies critical to tackle analytical challenges. Admittedly, the first principle of GAC has led to a common misconception that omitting the sample preparation step is a green approach, fully neglecting the necessity of this step and the technological advances in the area [20]. In this context, several mature and modern sample preparation approaches exist that do not adversely affect human health or the environment and can lead to more efficient and metrologically improved methodologies [21].

Hitherto, the greenness of sample preparation methods is assessed using metric tools anchored in the 12 principles of GAC. However, the philosophy of the GAC approach renders these metric tools inadequate for providing sufficient levels of accuracy and specificity and, as such, gauging progress toward greening sample preparation. The wide range of parameters that influence the greenness of sample preparation creates the need to develop a specific metric system for sample preparation. The present work aims for the first time, to bridge the abovementioned gap by offering a powerful yet user-friendly tool that will enable assessing the environmental impact of sample preparation, the most critical step in settling green analytical methods. The proposed metric tool gives prominence to sample preparation, and predicts as well as detects aspects that could be improved for greening the critical step of sample preparation.

2. Criteria and scores calculation

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The assessment criteria are created on the basis of the ten principles of green sample preparation (GSP) given below [20]:

1. Favor *in situ* sample preparation
2. Use safer solvents and reagents
3. Target sustainable, reusable, and renewable materials
4. Minimize waste
5. Minimize sample, chemicals and materials amounts
6. Maximize sample throughput
7. Integrate steps and promote automation
8. Minimize energy consumption
9. Choose the greenest possible post-sample preparation configuration for analysis
10. Ensure safe procedures for the operator

Each of the criteria is recalculated and after quantitative evaluation, the outcome of fulfilling the criterion is reflected as an impact score on a scale from 0 (not fulfilling) to 1 (fulfilling). Criteria 2, 4, 5, 6 and 8 apply logarithmic functions. They are applied to meet the demands of modern sample preparation science. Application of logarithmic functions allows to more easily differentiate between typical microextraction techniques impacts. The assessment criteria, summarized in **Figure 1**, are discussed below.

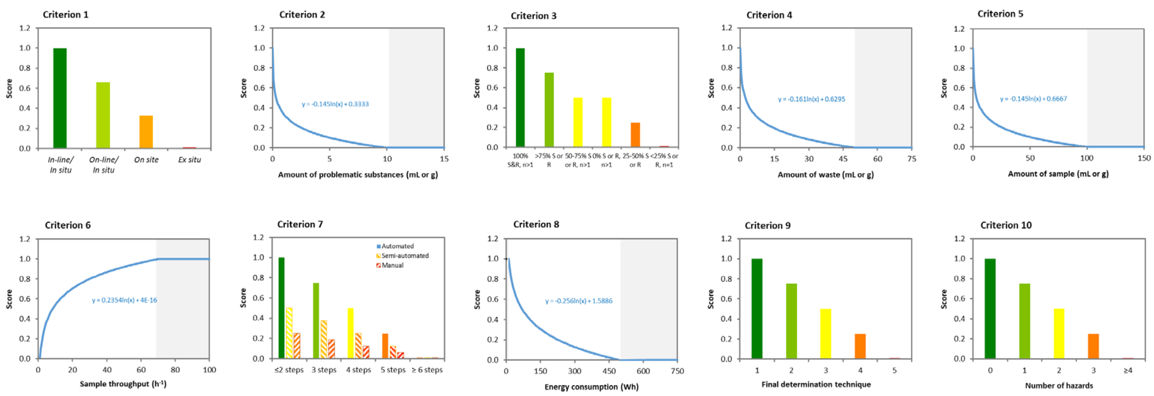

Figure 1. Graphical representation of the functions applied for the assessment of the evaluated criteria.

Criterion 1. Favor in situ sample preparation

The first criterion favors *in situ* sample preparation so as to minimize wasted time and the use of material and energy. Moreover, problems of sample degradation due to improper storage during transport are avoided. *In situ* sample preparation also includes low- or even non-invasive *in vivo* sample preparation that is non-lethal and, as such, eliminates the need to remove living

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organisms from their habitat. To assess this criterion, four categories were considered (depicted in **Figure 2**) and the scores are as follows:

- *In-line/In situ* – score: 1 – sample preparation is carried out in the investigated object. It usually integrates sampling and sample preparation. Good examples can be *in-vivo* SPME application or the use of passive samplers;
- *On-line/In situ* – score: 0.66 – sample preparation is performed *in situ*, sampling and sample preparation are performed in the same place using permanently installed devices with the overall operation being typically fully automated;
- *On site* – score: 0.33 – sample preparation is performed on site, with the sample preparation device being brought to the sampling site;
- *Ex situ* – score: 0 – sample preparation is performed in the laboratory after sample collection and transportation.

Criterion 1 is related to many other criteria that follow utilization of solvents, reagents, energy consumption and generation of wastes and strongly depends on the mode of sample preparation.

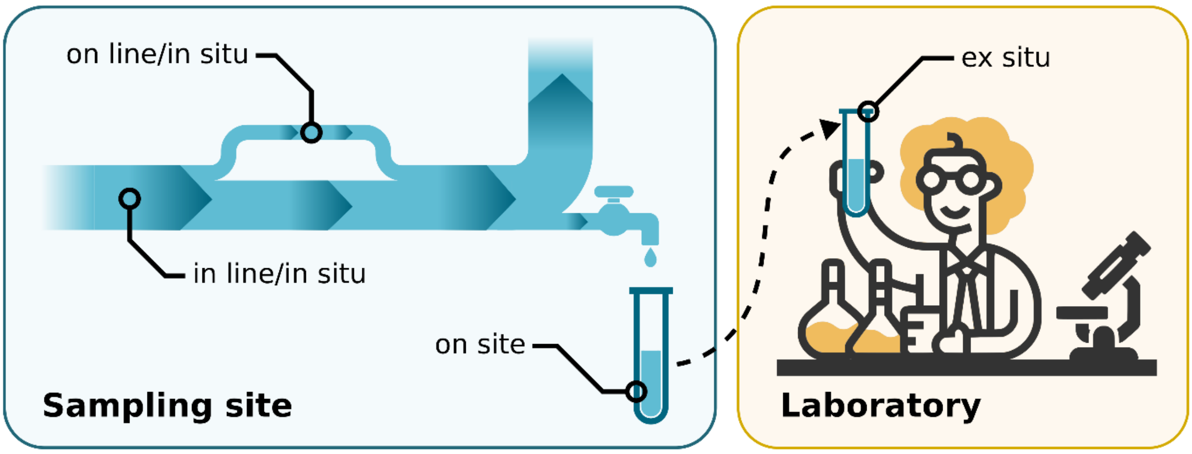

Figure 2. Schematic representation of the four categories used to assess criterion 1

Criterion 2. Use safer solvents and reagents

The cost, environmental impact and safety of sample preparation procedures are often driven by the use of solvents and other auxiliary chemicals. The second principle of GSP suggests using safer solvents and reagents that possess improved inherent properties and little or no toxicity to humans or the environment. This principle also aims at the reduction of hazardous reagents such as the acids and bases used in derivatization and digestion reactions. The adoption of solvent-free and reagent-less sample preparation procedures is the optimum

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condition to be attained in the second principle, and this condition yields a score of 1 to this corresponding criterion. In the worst-case scenario where sample preparation methods make use of more than 50 mL or 50 g of hazardous solvents and reagents, the assigned score for this principle is 0. Otherwise, the score is calculated according to the following equation:

$$Score = -0.145 \times \ln(\text{amount of hazardous substances in g or mL}) + 0.3333$$

It is noted that the mass of the substance should be included if the substance is toxic via one of the exposure pathways or if it is labeled as bioaccumulative or persistent.

Criterion 3. Target sustainable, reusable, and renewable materials

Materials must be stable during their (ideally extended) useful life and then degrade once they are no longer functionally necessary. In this context, the third criterion assesses the use of sustainable or renewable materials in sample preparation methods. Materials of bio-based origin are favored over fossil-based and other depleting chemicals. Moreover, the use of renewable/regenerable materials (including solid waste with the additional benefit of increasing its life-cycle) is also promoted. Following the third principle of GSP, this criterion also promotes materials that can be used several times over those of disposable nature. Reusability refers to the ability of the material to be used again after a regeneration step such as thermal desorption in the case of solid sorbents. If the information on the sustainability of the chemicals used for fabricating the material is not available, it is advised to treat the materials as non-sustainable ones. To calculate the score for this criterion three parameters are taken into account as follows:

- Only sustainable and renewable materials are used several times, Score: 1.0
- > 75% of reagents and materials are sustainable or renewable, Score: 0.75
- 50-75% of reagents and materials are sustainable or renewable and can only be used once, Score: 0.50
- Materials are not sustainable or renewable and are used several times, Score: 0.5
- 25-50% of reagents and materials are sustainable or renewable, Score: 0.25
- < 25% of reagents and materials are sustainable or renewable and can only be used once, Score: 0.0

Criterion 4. Minimize waste

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Creating, handling, storing and disposing of waste consumes resources, time, effort and money. Sample preparation technologies and methods should be designed to prevent waste generation and this is the focus of the fourth principle. Greenness metric systems dedicated to chemical synthesis assessment are based on the ratio of the mass of substrates to the mass of the product(s), such as E-factor [22] or atom economy [23]. In analytical chemistry, and particularly in sample preparation, all material inputs can be treated as wastes. This is because no materials are incorporated into the final product, which is the analytical result. The function to relate the mass or volume of the generated waste is as follows:

$$Score = -0.161 \times \ln(sample\ mass\ or\ volume\ in\ g\ or\ mL) + 0.6295$$

Sample preparation methods that result in the generation of less than 1 g of waste give scores of > 0.5 in this impact category. The fundamental question here is "what should be considered as a waste?". Clearly, any material added to the sample should be treated as waste. Waste includes, among others, solvents applied in extraction processes, derivatization reagents, or acids or bases applied for mineralization or sample pH correction. The mass of waste is also made up of the mass of consumable materials such as single-use glassware, SPE cartridges, sorbents and filters. In addition, the sample itself should be treated as waste if it gets contaminated with toxic substances during the sample preparation step. To exemplify this, a water sample that is in contact with a solid or pseudo-liquid sorptive material cannot be treated as waste. However, a water sample subjected to liquid-liquid extraction becomes a waste since it will become saturated by solvent during the extraction step. In cases where the waste is neutralized or recovered, its mass should be subtracted from the total mass of wastes generated during sample preparation. This criterion assesses the mass of waste only, as the hazards of chemicals utilization are covered by other criteria.

Criterion 5. Minimize sample, chemicals and materials amounts

The size of the sample impacts the energy demand and the amounts of solvents, reagents and other materials to be used in an analytical procedure. Accordingly, smaller sample sizes reduce the time, effort, costs, and resources, next to increase the potential for automation or portability. However, one should keep in mind that sample representativeness must always be ensured and that an excessive reduction of the sample size may deteriorate the analytical characteristics of the overall analytical method. The function for calculating the score for this criterion is the following:

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$$Score = -0.145 \times \ln(sample\ mass\ or\ volume\ in\ g\ or\ mL) + 0.6667$$

It should be noted that procedures where only analytes are collected (as seen with passive samplers), the mass of sample (i.e., collected analytes) is negligible and a score equal to 1 is assigned.

Criterion 6. Maximize sample throughput

Criterion 6 is related to the speed of the overall sample preparation procedure, optimum values for which can be achieved in two ways. The first one is related to the application of fast sample preparation procedures so that many samples can be prepared in a series of steps. The second one is treating several samples in parallel as seen in the 96-well format. To assess sample throughput the number of samples that can be prepared in one hour (in series or in parallel) is recalculated to the score according to the formula:

$$Score = 0.2354 \times \ln(\text{number of prepared samples per hour})$$

Criterion 7. Integrate steps and promote automation

Sample preparation methods commonly consist of multi-step procedures that can result in material loss, increased expenditure of energy and chemicals, as well as time loss. The pursuit of operational simplicity through the integration of steps is a trend in sample preparation with a positive impact on the greenness of the method. Moreover, automation increases sample throughput, lowers the consumption of reagents and solvents, waste generation, minimizes human intervention, and, as such, error involved and potential exposure to harmful substances. The simplification and minimization of the number of involved steps is expressed in sub-scores as follows:

- ≤ 2 steps, Score: 1.0
- 3 steps, Score: 0.75
- 4 steps, Score: 0.5
- 5 steps, Score: 0.25
- ≥ 6 steps, Score: 0.0

The degree of automation is recalculated into a sub-score in the following way:

- fully automated systems, Score: 1.0

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- semi-automated systems, Score: 0.5
- manual systems, Score: 0.25

The final score for principle 7 is the product of both sub-scores.

Criterion 8. Minimize energy consumption

Sample preparation methods and technologies should strive to be as energy-efficient as possible. To measure the impact in this principle the total energetic requirement is estimated and expressed in watt-hour (Wh) per sample. It is noted that if several samples are treated in series or in parallel using the same device, then the energetic requirement of the device is divided by the number of samples run simultaneously.

Depending on whether the criterion is fully, partially or not satisfied, the energy demand is recalculated to the score as follows:

- for < 10 Wh per sample, Score = 1
- for values between 10 and 500 Wh per sample, $Score = -0.256 \times \ln(\text{Wh/sample}) + 1.5886$
- for > 500 Wh per sample, Score = 0

It should be mentioned that the typical values used for calculating energy requirements of the instrumentation used, are the ones listed by manufacturers. Although these refer to maximum values and not the actual power output of analytical instruments (typically 40% of the maximum values), the scores remain valid for comparative reasons [10].

Criterion 9. Choose the greenest possible post-sample preparation configuration for analysis

Sample preparation methods are versatile, in a way that a number of measurement and instrumental techniques can be used for further analysis. The ninth principle of GSP suggests carefully selecting the greenest option that is relatively simple, low energy demanding and leads to consumption of the least amount of chemicals. It is acknowledged however that the final choice depends on the analytical needs in terms of method performance or is simply based on availability. The impact of the final determination step can be significant or negligible, depending on the technique that is applied. The scores assigned to the most widely used final determination techniques are the following:

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- Simple, readily available detection (information technology and communications equipment such as smartphones, desktop scanners, etc.), Score: 1
- Molecular optical spectroscopic techniques (e.g. UV-vis spectrophotometry, fluorimetry, chemiluminescence, etc.), surface analysis techniques, voltammetry, potentiometry, Score: 0.75
- Gas chromatography with non-mass spectrometry (MS) detection, atomic absorption spectroscopy, Score: 0.5
- Liquid chromatography (due to mobile phase consumption, usually being or containing organic solvents), gas chromatography with quadrupole mass spectrometric detection, Score: 0.25
- Advanced mass spectrometry techniques (due to high energetic requirements), inductively coupled plasma - optical emission spectroscopy (ICP-OES), ICP-MS (due to noble gas consumption), Score: 0

For the assessment where no specific final determination is pointed, and more than one can be applied, it is advisable to select the option less problematic final determination technique.

Criterion 10. Ensure safe procedures for the operator

GSP seeks to reduce the environmental impact of sample preparation methods and at the same time protect operators from potential harm. The tenth principle considers the basic hazards of the procedure by counting the threats expressed with pictograms labelling chemicals used in the procedure – toxicity to aquatic life (toxicity to humans is not expressed with safety pictograms), bioaccumulation potential, persistence, flammability, oxidazability, explosiveness and corrosiveness. In addition, physical hazards are included in this criterion, such as compressed gases. The number of identified hazards of chemical or physical nature is used to calculate the score for this criterion:

- no hazards, Score: 1
- 1 hazard, Score: 0.75
- 2 hazards, Score: 0.5
- 3 hazards, Score: 0.25
- 4 or more hazards, Score: 0

The hazards can be easily derived from the MSDS of substances as the number of different pictograms can be taken as input data to this criterion.

3. Weights for criteria

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A closer study of the ten criteria used to assess the greenness of sample preparation, 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 volumes of used solvents, energy requirements or assuring safety for the analyst. Therefore, we suggest the default weights to be applied in any assessment and give the option to assessor to change them provided that justify these changes. In case of changing the weights, we suggest preparing the justification to explain the importance of the criteria that is adjusted to the assessor's requirements.

Table 1. The default weights for the analysis

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

The highest weight was given to criterion 2 since the solvents and reagents used have a great impact on the greenness of the sample preparation method. Criterion 1 was given a lowest weight as it has some impact on sample preparation greenness but still, it is possible to achieve a considerable degree of greenness even if procedures are not carried out *in situ*. Criterion 3, with weight 2, describes the origin and disposal of materials and reagents, including consumables and the assigned weight is not higher due to its semi-quantitative nature. Criteria 4, 8 and 10 dealing with waste, energetic demand and safety of operator are crucial points to consider in greenness assessment and were therefore given high weight (4, 4 and 3 respectively). Criterion 5 is indeed important as it is related to the miniaturization degree of the sample and, as such, to the consumption of reagents, solvents and generation of wastes. The

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latter features are covered by other criteria and to avoid double penalization of the same weakness/shortcoming, a weight equal to 2 was assigned. Sample throughput and final determination (criteria 6 and 9) assess the impact of sample preparation on the entire analytical procedure and are given weights equal to 3 and 2, respectively. Criterion 6 is important as sample preparation with a high throughput potentially allows obtaining a large amount of analytical information or preparation of set of samples within a short time. Criterion 9 concerns post-sample preparation configuration for analysis and a weight equal to 2 is given as too little sample preparation and, as a result, obtaining a seemingly good assessment score might necessitate the use of a sophisticated and non-green final determination technique. Finally, criterion 7 was given a weight equal to 2 as it is possible to achieve a considerable degree of greenness even if procedures have a larger number of steps and/or are not automated.

4. The assessment result

The assessment result is a colorful round pictogram with the number in the center. The inner circle color and the assigned overall score indicate the overall sample preparation greenness performance. The possible values of the overall score lie in the range from 0 to 1. An overall score of 0 means it has the worst performance in all criteria, while an overall score of 1 represents the best performance in all criteria or no sample preparation step. Around the circle, there are 10 parts, each corresponding to one of the performance criteria. The length of each part reflects the weight assigned to the respective assessment criterion while the color of each part visualizes the performance in this criterion. Adopting this structure for the assessment result allows to:

- Compare the general performance of procedures;
- Compare the procedures in respective criteria, find strong and weak points of the procedures or aspects to be improved;
- Get information on the assessor's point of view on the importance of criteria or contribution of criteria to the final result.

5. Assessment examples

AGREEprep was used for the assessment of different procedures intended for the determination of phthalate esters in water samples (**Figure 3**). The first procedure was EPA standard 8061A [24], accompanied by method 3510C [25] for separatory funnel liquid-liquid extraction (LLE). The procedure was performed *ex situ*, and consumed 180 mL (3 times extraction with 60 mL) of dichloromethane and sulfuric acid or sodium hydroxide for pH

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adjustment (only 180 mL of dichloromethane was considered in the 2nd criterion as hazardous materials). It was assumed that no reagents originated from renewable or sustainable sources. Substantial amounts of waste were generated since the excessively large sample volume (1L) became hazardous waste after being in contact with dichloromethane. The sample throughput was estimated to be ~1.5 h^{-1}. The procedure was manual and required ≥ 6 steps; on the other hand, the method did not consume energy. Method 3510C used a GC-MS system for phthalates determination (GC-MS technique was included in the assessment), while method 8061A used GC-ECD increasing the final score to 0.2. Sulfuric acid was accompanied by four pictograms and excluding it from the assessment would result in a 0.22 final score (dichloromethane has two hazard pictograms). The final result for this LLE-based procedure was 0.17, corresponding to a poor performance with the only good score given in the "energy consumption" criterion.

The next assessed method was based on solid-phase extraction (SPE) and the procedure aimed at the *in situ* determination of endocrine-disrupting compounds [26]. Sample preparation proceeded in the *on-line/in situ* mode, consumed 6 mL of acetonitrile for column conditioning (acetonitrile has Category 4 of toxicity "harmful if swallowed, in contact with skin or if inhaled" but it is not categorized as toxic). Neither sustainable nor renewable materials were applied and the amount of waste was calculated to be 6.5 g or mL – 6 mL of acetonitrile and a very tiny cartridge of mass estimated to be less than 0.5 g. The sample size was 20 mL and ~6 samples could be prepared in an hour. It should be noted that the sample throughput of the analytical method was low due to the time needed for HPLC separation. The sample preparation method involved three steps and was fully automated. There was no exact information on the energy demand but this was a microfluidic system with neither heating nor cooling demands, so energy demand per prepared sample was assumed to be low and equal to 30 Wh. The final determination proceeded with HPLC and acetonitrile was labelled with two hazard pictograms. The final score was 0.54 and there were no genuinely low respective criteria scores. It should be noted that although in-situ sample preparation and automation were the net advantages of this procedure, low weights have been considered to assess the corresponding criteria.

The manual dispersive liquid-liquid microextraction (DLLME) procedure [27] considered here was performed *ex situ*, demanded 0.75 mL of acetonitrile (not counted) and 0.04 mL of carbon tetrachloride. The reagents were neither from sustainable nor renewable sources. The volume of water was 5 mL, and counted as waste since it was in contact with carbon tetrachloride and acetonitrile. The extraction procedure was manual and including the centrifugation step time was estimated to be ~10 minutes yielding a 6 h^{-1} sample throughput. It is acknowledged

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however, that analysts may choose to perform more extractions simultaneously and, as such, improve the score in this criterion. Three sample preparation steps were identified that were not automated. A ~50 Wh estimation of power demand per sample was considered and the use of HPLC at the next procedural step. There were 4 different hazards pictograms – 2 from carbon tetrachloride and 2 from acetonitrile. The total score was 0.38, which was rather low, despite this being a microextraction-based procedure. A slightly higher score (0.43) was obtained when 6 simultaneous extractions were considered (sample throughput: 36 h^{-1}).

The fourth assessed procedure was based on the solid-phase microextraction (SPME) technique [28], performed *ex situ*. The method used ~0.63 g of NaCl as a salting-out agent, which was not considered as a hazardous material in criterion 2 but was counted as waste, together with the sample amount. The SPME fiber was reusable while the salt was treated as a sustainable material. The water sample volume was 3.5 mL and the extraction took 1.5 h so the analytical throughput is 0.66 h^{-1}. Sample preparation consisted of a one-step manual procedure and the energy demand was estimated to be ~90 Wh. The final determination technique was GC-MS and no procedural hazards were identified. Although SPME is a green technique, this particular procedure had a few drawbacks that lowered the final score to 0.55 namely, *ex situ* mode, very low sample throughput, no automation and quite problematic final determination.

The fifth assessed procedure was based on the application of molecularly imprinted polymers (MIPs) and solid-phase extractants that were applied directly in water samples [29]. The procedure was *ex situ* mode and involved the use of 5 mL of dichloromethane for desorption. The sorbent itself was a sustainable material but dichloromethane, used in considerably large amounts, yielded for criterion 3 the score "less than 25% of material is from sustainable or renewable sources". The total amount of waste included dichloromethane and filter paper. The sample volume was 200 mL while sample throughput was estimated as ~0.5 h^{-1}. The procedure was manual with 2 steps, used GC-MS as the final determination technique and 2 hazards were identified, both related to the application of dichloromethane. The determination of energy consumption was not straightforward as a magnetic stirrer, vacuum pump and oven were applied, and it was not stated how many samples can be treated simultaneously during sorbent separation and filter drying. It was decided to (arbitrarily) use a 400 Wh estimate value for the energy demand. The final score was 0.17.

In another SPE procedure, polyamidoamine dendrimer-grafted magnetic nanoparticles were incorporated [30]. The method was a 3-step, manual and *ex situ* procedure that involved the use of 0.25 mL of toxic methanol, but all reagents – the sorbent, ethanol and methanol were from renewable sources. The amount of waste was estimated to be 6.5 mL. The water sample

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volume was 40 mL and sample preparation throughput was 2 h^{-1}. Power demand was estimated as ~60 Wh and GC-MS was used, two hazards are identified in criterion 10. The resulting score was 0.4.

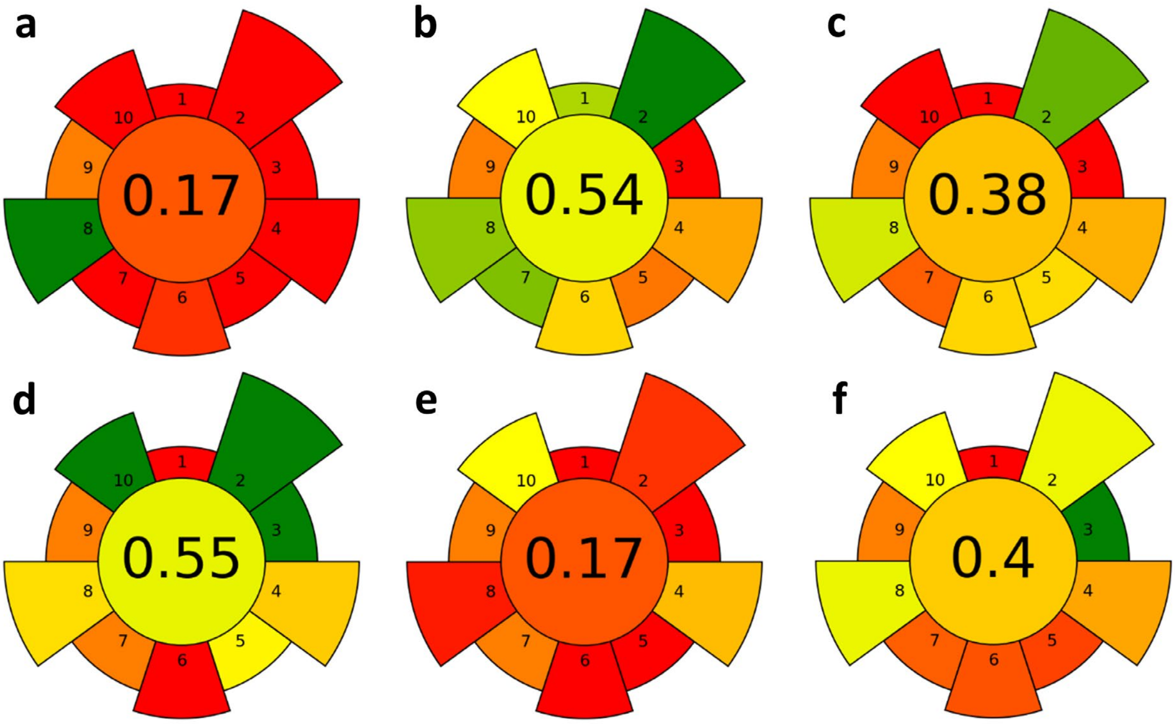

Figure 3. The results of AGREEprep assessment of procedures for phthalate esters determination: a – EPA 8061A based on LLE [24,25]; b – automated SPE [26]; c – DLLME [27]; d – SPME [28]; e – MIP-based SPE [29]; f – magnetic particles-based SPE [30].

The assessment results shown in **Figure 3** depict the general performance of the procedures, while colors distributions allow comparing the nature of threats and hazards. A direct comparison between the results reveals that both the LLE (a) and MIP-based SPE (e) procedures were the least green methods assessed here with final scores of 0.17. Conversely, the procedures based on SPME (d) and on-line SPE (b) were definitely the greenest ones but still, the results (0.55 and 0.54, respectively) were far from being ideal. In the case of the SPME method (d), the manual mode and sample throughput needed improvement and the choice on post-sample preparation configuration of analysis also lowered the final score. Regarding the on-line SPE method (b), the overall score was mainly affected by the lack of sustainable or renewable materials, size economy of the sample and post-sample preparation configuration of analysis.

For comparison purposes, the above methodologies were also assessed by AGREE [10], our published comprehensive tool devised for the assessment of analytical methodologies on which

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AGREEprep is based. In AGREE, the final score (in the 0-1 range) is derived from the application of the 12 principles of GAC. **Figure 4** shows the overall results of the assessment of the six methodologies considered. For simplicity and in agreement with most of the publications where the AGREE tool is applied, equal weights were selected for assessing the 12 principles of GAC. An important point to consider is that the AGREE scores on the six studied methodologies lie in a narrower range (ranged from 0.37 to 0.52) than those obtained with the AGREEprep metric tool (score range 0.17-0.55). This observation points out the importance of having a metric tool dedicated to sample preparation, since wider score ranges allow a better classification of the methods next to interpretation of results. For example, the two least green analytical methods according to AGREE, namely EPA 8061A based on LLE-GC-ECD (a) and MIP-based SPE-GC-MS (e), also received the lowest scores with AGREEprep. However, the difference in terms of greenness between the remaining four methods is less clear, when evaluating the overall AGREE scores (ranged from 0.45 to 0.52). Moreover, while there is a reasonable relative agreement between the ranking scores received by AGREE and AGREEprep, certain aspects obviously differ. One point to consider is that in AGREE, sample preparation has by default a negative connotation; a requirement imposed by the first principle of GAC. In addition, the improvement of certain aspects of sample preparation methods might not be reflected in the corresponding AGREE score even if non-negligible improvements are achieved. This could be the case with the reduction of the energy consumption required to carry out sample preparation in a method that formerly involved extensive energy consumption during the sample preparation and the analytical measurement steps. Thus, AGREEprep can be invaluable in shedding light on improvable aspects of sample preparation methods that could go unnoticed if only a more general tool is used. It is acknowledged however that the combined use of these two metric tools for assessing the greenness of both sample preparation and analytical methods, respectively, can help identifying weaknesses of the overall analytical procedure and point toward greener alternatives.

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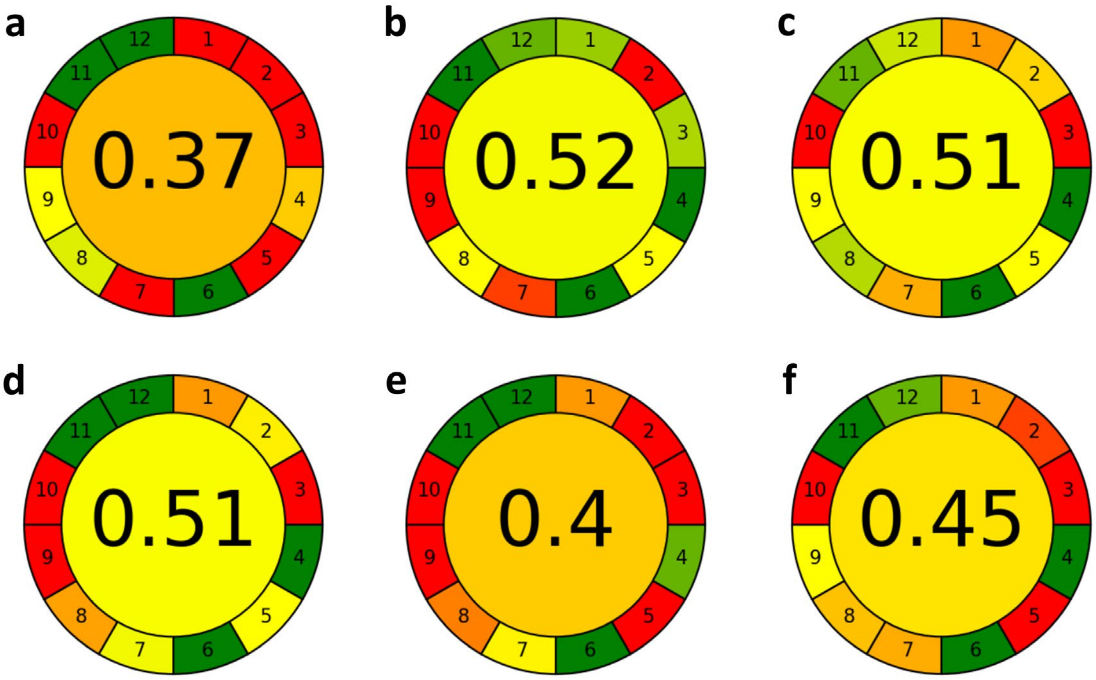

Figure 4. The assessment results with AGREE of procedures for phthalate esters determination: a – EPA 8061A based on LLE-GC-ECD [24,25]; b – automated SPE-LC-DAD-MS [26]; c – DLLME-HPLC-VWD [27]; d – SPME-GC-MS [28]; e – MIP-based SPE-GC-MS [29]; f – magnetic particles-based SPE-HPLC-VWD [30].

6. Conclusions

AGREEprep is the first tool designed for the assessment of analytical sample preparation greenness. It considers 10 criteria that cover different aspects contributing to the overall sample preparation greenness. AGREEprep was applied to 6 sample preparation procedures for the determination of phthalate esters in water samples and was successful in identifying the differences in greenness, structures of threats and points to be improved. compared to our published assessment tool (AGREE) a wider score range was found that provided sufficient levels of accuracy and specificity in assessing the greenness of the studied methods. This result was expected, taken that AGREEprep is a specific tool to assess sample preparation methods. The assessment with AGREEprep is easy to perform, and an intuitive software makes the entire interaction process efficient both for introducing values and reading output. A compiled version of the open access software can be obtained from mostwiedzy.pl/AGREEprep, and the code is available at git.pg.edu.pl/p174235/agreeprep. For details regarding the software see the documentation in Supplementary Materials.

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