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Miniaturization as a smart strategy to achieve greener sample preparation approaches: A view through greenness assessment

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ABSTRACT

Green Analytical Chemistry has been a cornerstone in every stage of method development, including sample preparation. In this sense, extraction techniques have evolved from conventional ones to a wide variety of miniaturized extraction techniques. This new generation of approaches has been developed not only to improve the analytical performance but also the safety for the operator and the environment. To evaluate the impact and sustainability of a method, several green metric tools have been described. In the first part of this review, some of these green metric tools were summarized to highlight their advantages and disadvantages. Then, AGREEprep was selected as the most suitable tool to evaluate several published methods for diverse applications in prominent fields in Analytical Chemistry. The influence of miniaturization of sample preparation strategies was discussed in terms of the use of less and safer solvents, reduced waste generation and energy consumption, and enhanced portability and throughput.

1. Introduction

Analytical Chemistry is an area in continuous development and evolution due to the demands claimed by the society. Since a few decades ago, the concern about environmental damage has increased within the Analytical Chemistry field, resulting in new requirements about greenness of new developed analytical methodologies. To this regard, different principles were set up as a roadmap to follow, making researchers aware of this need. In 2013 Namieśnik and co-workers presented the 12 principles of Green Analytical Chemistry (GAC) [1], based on the adaptation of the 12 principles of Green Chemistry [2] to analytical processes. Later on, in 2021 Pawliszyn and co-workers promoted the concept of White Analytical Chemistry (WAC) [3], which can be considered an extension of GAC making it closer to the idea of sustainable development. WAC has also a more holistic view and it considers the functionality (evaluated according to the "blue principles") of the developed method to reach a compromise between analytical performance (evaluated according to the "red principles") and greenness (evaluated according to the "green principles"). The main pillars of the green principles of the WAC are related to the elimination or reduction in the use of chemical substances, the minimization of energy consumption and waste, the promotion of automation and high throughput, and the increase in the safety for the operator. All these inputs are requested for the development of green methodologies but keeping in mind that a good analytical performance must be ensured. As a result of these principles, direct analysis of samples is preferred. However, sample preparation is a step that can be hardly avoided in most cases due to the complexity of the sample or the need to preconcentrate the analytes. In this sense, a working group of international researchers coordinated by Psillakis recently presented the ten principles of Green Sample Preparation (GSP) [4], in turn based on the principles of GAC, where the necessity of a sample preparation stage is assumed.

Following these trends in sample preparation, several microextraction techniques were presented as alternatives to classical extraction techniques (*i.e.,* liquid-liquid extraction (LLE) and solid-phase extraction (SPE)). Since 1990, when solid-phase microextraction (SPME) [5] was presented as the first microextraction technique, a wide variety of both liquid and solid-based microextraction techniques has been developed. In this sense, within the following three decades, some techniques such as single drop microextraction (SDME) [6–9], stir bar sorptive extraction (SBSE) [10], dispersive solid-phase extraction (DSPE) [11] or dispersive liquid-liquid microextraction (DLLME) [12], among others, were also developed and successfully implemented in research laboratories. Likewise, microextraction techniques have followed over the decades the trend of miniaturization of analytical system seeking to exploit its benefits [13]: reduced consumption of reagents and

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solvents; reduced waste generation; improved sensitivity, rapidity and portability; and reduced power consumption. In addition, these already miniaturized extraction techniques have evolved towards downscaling with special emphasis not only on reducing the amounts of extraction phases but also on reducing sample volume. These developments have led to a whole new generation of techniques beyond microextraction, which have been recently coined as 'ultramicroextraction' techniques [14], evidencing the advances in this field toward a greener and more sustainable sample preparation.

The description of the different microextraction techniques is out of

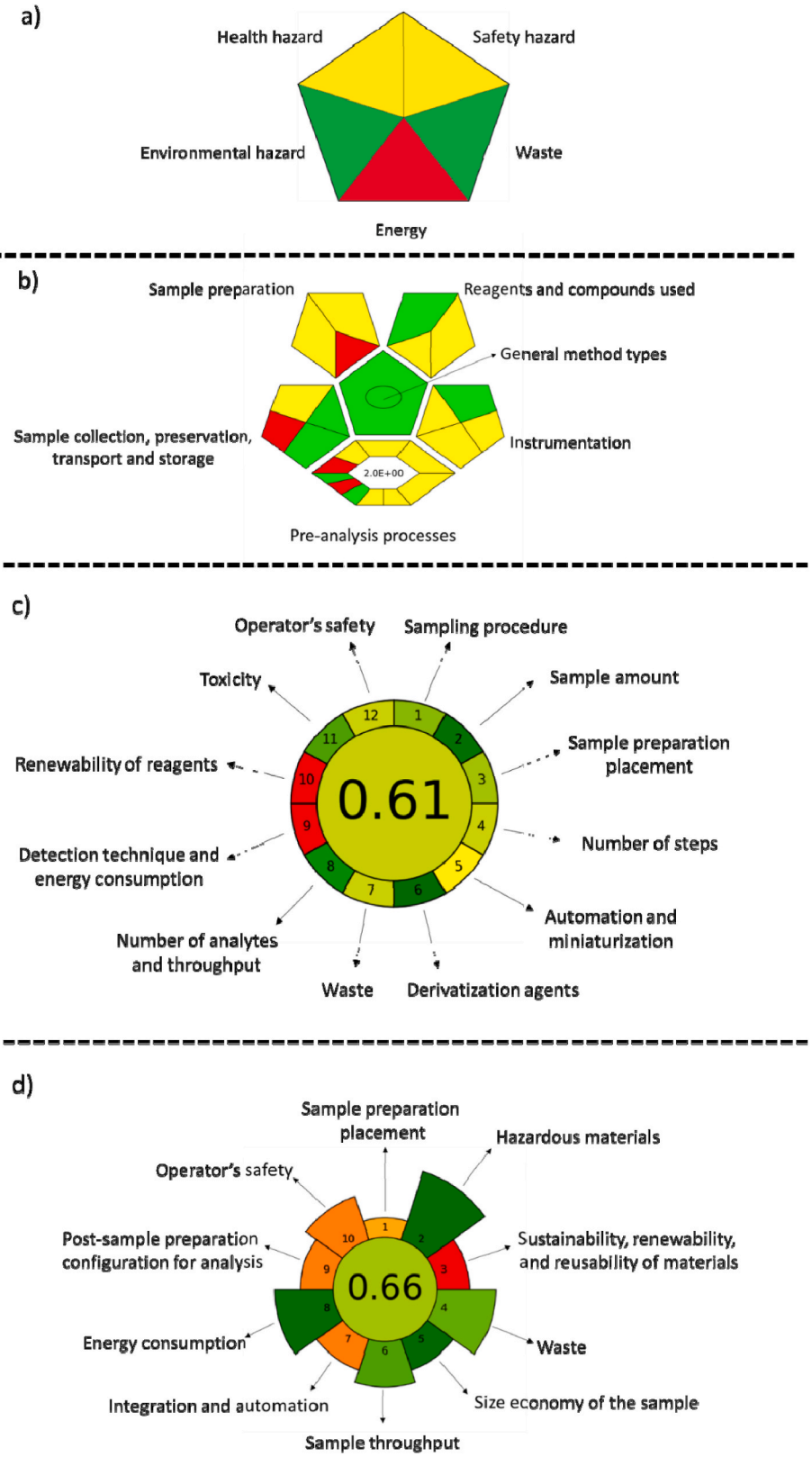


Fig. 1. Information provided by pictograms from (a) modified NEMI, (b) ComplexGAPI, (c) AGREE and (d) AGREEprep.

the scope of this review, so the reader is encouraged to revisit some other reviews and book chapters [15–19] for this purpose. Instead, the aim of this review is to evidence the importance of miniaturization in the development of greener methodologies, not only in an empirical or conceptual way as recently reviewed [14], but also in a tangible way through the use of green metric tools. Specifically, the influence of miniaturization in terms of greenness, comparing conventional extraction techniques, microextraction techniques and further-downscaled strategies is evaluated.

2. Green metrics

Different green metric tools have been described over the last two decades to assess the greenness of analytical methods. They provide a general idea of how in accordance with the principles of Green Chemistry a method is, in such a way they can guide scientists to more sustainable practices. However, each green metric tool adopts its own rules and considerations to evaluate the greenness of a methodology. For instance, some of them are focused on the entire analytical process and others on a specific step. Furthermore, depending on the criteria considered in each green metric, there is a wide variation in the score given by each tool. For this reason, it is obvious that to compare methods from the greenness point of view it should be done by using the same metric tool or, conversely, inconclusive or wrong results will be obtained.

In the following subsections, some of the most relevant available green metric tools are summarized. The advantages and disadvantages of each of them are discussed to select the most suitable green metric tool for further evaluations based on our goal.

Different interesting reviews have been recently published about the most used green metric tools [20–23]. In these reviews, a wide description and discussion of each metric tool has been done applying them to different case studies and analyzing their advantages and limitations. In general terms, it is concluded that each tool presents its disadvantages and is appropriate for certain applications, but none is considered as a universal one. Therefore, the improvement of the existing ones or the development of the definitive green assessment metric tool is still highly encouraged.

2.1. National Environmental Methods Index and its variant

National Environmental Methods Index (NEMI) [24] is the oldest green metric tool. It is based on four categories: Persistent, Bioaccumulative and Toxic (PBT), hazardous, corrosive and waste. These categories are represented as portions of a circle, and a semi-quantitative two color-based score is used for assessing each one, employing green for the most eco-friendly option and white for the worst. Later on, Raynie and Driver [25] proposed the modified NEMI, based on green chemistry properties as an upgrade of the traditional NEMI. It presents five different categories to assess the greenness of a method: energy, environmental, health, safety, and waste. These five sections are represented in a pentagon pictogram. A semi-quantitative three color-based score is used, employing green for the most eco-friendly option, yellow for the medium and red for the worst. To determine the color of each category, predetermined values are set. Fig. 1a shows an example of the modified NEMI and the significance of each category.

It is true that this metric tool is a very simple and visual tool, making easy to know in which of the five areas a method needs to be improved (qualitative information). However, these five areas are very general, and many aspects are considered in each one. It contemplates the hazards of the chemicals used, but not the amount of them, which is an essential variable to consider. Besides, high cut-off values and wide ranges of waste amount and environmental hazard are considered (*i.e.,* less than 50 g to obtain a green pictogram, 51–250 g to obtain a yellow one, and more than 250 g to obtain a red one). These values are more appropriate for the evaluation of classic methods or conventional extraction techniques. However, microextraction techniques are rarely over the 50 g set as the first cut-off value. Thus, mainly green pictograms are obtained when evaluating microextraction techniques by this metric tool and no differences are observed in the score. Some other aspects as sample preparation placement, sample amount, throughput and automation are not considered. Moreover, it is just a semi-quantitative tool, what complicates the comparison of different methods. It should be noted that in this metric tool the entire analytical process is considered, not paying special attention to the sample preparation step, what should be taken into account depending on the scope of the comparison.

2.2. Analytical Eco-Scale

Namieśnik and co-workers proposed the Analytical Eco-Scale in 2012 [26], based on the idea that the ideal green analysis has a punctuation of 100. A certain quantity of penalty points is subtracted from the initial attributed 100 points due to those parameters of a method that are not in agreement with the ideal green analysis, considering chemicals amount, chemicals hazard, energy consumption, occupational hazard, and waste generation. Each of these parameters are considered for the entire analytical process. Hence, the final Analytical Eco-Scale score is calculated as 100 minus the total penalty points. Depending on the obtained score, the analytical procedure is 'excellent' when the score is over 75, 'acceptable' when the score is within 75 and 50, and 'inadequate' when the score is under 50.

This green metric tool is simple and quantitative, and it is useful to compare the greenness between different analytical methods just by comparing a numeric result obtained after the subtraction of the penalty points. In this case, as the amount of reagents and hazards in the entire method are considered (*i.e.*, both sample preparation and measurement steps), there is a major contribution in the total values from the analysis technique (*e.g.*, chromatographic analysis) compared to the extraction technique. This is even more accentuated when microextraction techniques are used due to the low amounts employed in these approaches. So, it is not possible to properly compare between different microextraction methodologies. Furthermore, the cut-off values (*e.g.,* less than 10 mL or g get 1 sub-total penalty point, 10–100 mL or g get 2 sub-total penalty points, and more than 100 mL or g get 3 sub-total penalty points) have a wide range, and more accurate ranges should be considered for a better assessment. Reagents, solvents, and instruments are considered individually, so a high number of penalty points are assigned for methods where several of them are used, even if low amounts of them or low energy consumption are required. Furthermore, as only a final score is given, it does not give qualitative information about the areas that need to be improved. Due to this fact, it is also not possible to compare some specific categories between different methods. Moreover, some important categories in the microextraction field as sample preparation placement, sample amount, throughput and automation are not considered. As in the case of NEMI, Analytical Eco-Scale evaluates the entire analytical process and not only the sample preparation step.

2.3. Green Analytical Procedure Index and its variant

Green Analytical Procedure Index (GAPI) tool was developed by Płotka-Wasylka in 2018 [27]. It is based on five fractionated pentagons referred to (i) sample collection, preservation, transport and storage, (ii) sample preparation, (iii) reagents and compounds used, (iv) instrumentation, and (v) general method types. Depending on the environmental impact of each parameter, a semi-quantitative three color-based score is used, employing green for the most eco-friendly, yellow for the medium and red for the worst option, according to a preset threshold for each parameter. Later on, Płotka-Wasylka and Wojnowski upgraded the GAPI, creating the Complementary GAPI (ComplexGAPI) [28]. In this tool, the five pentagons and the parameters described in each one, and the three color-based score remained intact. The novelty was the addition of a hexagon, which refers to pre-analysis processes such as

synthesis of extraction materials, inside which appears a number termed e-factor that represents the ratio of the mass of waste generated in the synthesis process to the total mass of product generated. Furthermore, to facilitate the use of the tool, permitting the generation of ComplexGAPI pictograms, a freely accessible software is available [28]. In Fig. 1b, an example of the ComplexGAPI and the significance of each category is shown.

This metric tool is very complete due to the high number of aspects that are considered, but, as a result, it is often difficult to assess methods greenness due to the high amount of information needed, which is not usually available on the publications. Moreover, the volume of hazardous chemicals is not considered in an independent way and the cut-off values of the amount of reagents and solvents are too high for the assessment of microextraction techniques (*e.g.*, less than 10 mL or g to obtain a green pictogram, 10–100 mL or g to obtain a yellow one, and more than 100 mL or to obtain a red one), and thus they are more appropriate for classical methods or conventional extraction techniques. The resulting graph is very visual due to the three-colored scale employed. It is easy to semi-quantitatively compare categories of different methods and to qualitatively assess the improvement areas, but it is a tool with a huge amount of information in a same graph, what makes difficult to compare whole analytical procedures. ComplexGAPI assesses the entire analytical method including the sample preparation step, and the pre-analytical processes, such as the synthesis of the extraction materials. Hence, it is the most complete tool described until now even though not completely helpful because of its semi-quantitative nature.

2.4. Analytical GREEnness metric approach and variant

Pena-Pereira, Wojnoski and Tobiszewski developed the Analytical GREEnness Metric Approach and Software (AGREE) in 2020 [29]. To evaluate the greenness of a method, they converted the 12 principles of GAC into scores, each one from 0 to 1, depending on the degree of correlation between the developed method and the principle. Each category presents a different weight according to the importance of this principle and it is used to calculate the final score from 0 to 1 as a weighted average. Furthermore, depending on the obtained punctuation in each category and in the final score, a gradual color scale from green to yellow to red is assigned. A clock-like graph is obtained, where the total numerical score and the corresponding color score of the method are placed in the middle, and around them, the 12 principles (properly labelled from 1 to 12) with the color-based scale are represented in surrounding arcs. The weight of each principle is represented by the size of the corresponding surrounding arc (Fig. 1c).

As previously stated, even though sample preparation is preferred to be eliminated in an ideally green method, it is usually crucial for complex samples. In this sense, while AGREE evaluates the entire analytical method, these same authors along with Psillakis developed the Analytical greenness metric for sample preparation (AGREEprep) [30] a few years later. AGREEprep is based on the 10 principles of GSP evaluating (i) sample preparation placement, (ii) use of hazardous materials, (iii) sustainability, renewability, and reusability of materials, (iv) waste generation, (v) sample size, (vi) sample throughput, (vii) integration and automation of processes, (viii) energy consumption, (ix) subsequent configuration for analysis, and (x) operator's safety. The graph and the scores are represented and calculated as in AGREE, but each principle has the same extension for the arc and the weigh is represented for what stands out from the circle. An example of AGREEprep and the significance of each category is shown in Fig. 1d.

This is a quantitative and very visual tool that allows to compare the entire sample preparation procedure by looking at the final score or a specific category of it between different methods. Furthermore, due to the wide visual scale of colors, it is easy to identify in what criteria the evaluated method is not green enough and to compare categories between different methods. Therefore, AGREEprep provides both quantitative (final score and color scale) and qualitative (which category needs improvement) information, being a very complete tool for greenness evaluation. It is worthy to mention that a numeric value must be inserted for the evaluation of some criteria like hazardous materials, waste, sample size, throughput, and energy consumption, being a continuous variable instead of a preestablished interval. This allows to fairly evaluate the scale of sample preparation approaches thanks to the absence of high cut-off values as describes in previous metrics. Furthermore, a user-friendly software to carry out the evaluation is available [30], making it easily applicable. As main disadvantage, it is a hugely focused tool for the sample preparation step, which does not fully consider other stages of the analytical procedure such as synthesis of extraction sorbents or subsequent measurement analysis. In this case, AGREE tool should be employed.

As it was mentioned above, the aim of this review is to compare the greenness of the sample preparation step of different methods and to evaluate how the miniaturization of extraction techniques, from the conventional ones (*i.e.,* LLE and SPE) to the most advanced microextraction approaches, has influenced their greenness. Thus, as AGREEprep presents a great potential in this sense, and it provides both quantitative and qualitative information, it was selected for further evaluations.

2.5. Other green metrics

In addition to the green metrics described before, during the last years other ones have been developed, which are discussed below.

In 2019, Campíns and co-workers developed the hexagon-CALIFICAMENT [31]. This tool is based on a hexagon divided in six main parameters referred to (i) residues, (ii) carbon footprint, (iii) toxicity/safety, (iv) figures of merit 1 (sample treatment and method characteristics and calibration), (v) figures of merit 2 (quality control and accuracy), and (vi) economic cost. Penalty points are assigned to the parameters that are not in agreement with the ideal green analysis. Finally, depending on the total penalty points obtained in each parameter, values from 0 to 4 (being 0 the best and 4 the worst) are calculated.

The same year, Nowak et al. developed the RGB metric [32]. This one uses the three primary colors to represent the three main attributes of the evaluated method: red for the analytical performance, green for the compliance with the green chemistry principles and blue for the productivity/practical effectiveness. For each parameter three intervals of concordance are assigned (higher than 66.6 % (satisfaction), between 66.6 % and 33.3 % (tolerance) or lower than 33.3 % (lack of acceptance)), and the reference values are: the "lowest satisfactory value" for the score of 66.6 % and the "lowest acceptable value" for the score of 33.3 %. So, comparing these intervals with the developed method values, a final punctuation is obtained. Combining the RGB values obtained, the method is rated with one of the nine final method colors (from the best to the worst: white, magenta, yellow, cyan, red, blue, green, gray, and black). Furthermore, an additional parameter called "Method Brillance" can be calculated to give a quantitative value.

Most recently, in 2023, Kiwfo et al. developed the Need, Quality and Sustainable (NQS) index (%) [33]. This tool assesses the analytical procedure in terms of "Need" (the actual need for analytical procedures for such purposes), the WAC as "Quality", and the accordance with the sustainable development goals as "Sustainability". The percentage of "Quality" is calculated using the RGB 12 algorithm proposed by Nowak et al., "Need" is calculated depending on the characteristics of the method respect to the preset needs represented in the Koel's pyramid, and "Sustainability" is calculated as the percentage of agreement with the 17 sustainable development goals.

All these green metric tools described so far in this section are focused on the evaluation of the entire analytical process. However, very recently, Pino and co-workers developed a tool called Sample Preparation Metric of Sustainability (SPMS) [34], which is focused on the sample preparation step like AGREEprep. It is based on the assessment of

nine parameters divided into four different categories: (i) sample information (sample amount), (ii) extractant information (amount, nature, and reusability), (iii) sample preparation procedure information (number of steps, extraction time, need of additional steps after extraction, and sample throughput), and (iv) energy consumption and waste. Depending on the value of each parameter, a predetermined score for each parameter and a total score of the method are obtained. Furthermore, a clock diagram where each parameter (except reusability, which appears as a star-like mark if it is the case) is represented by a small square colored (green for successful, yellow for acceptable, orange for tolerable, and red for inadequate), is obtained. The squares are grouped in the four main categories, and a central square is in the middle showing the total score of the method (from 0 to 10) and a color code (from red to green).

3. Green evaluation of methods in different fields of application

To demonstrate the benefits of microextraction techniques in terms of greenness compared to conventional extraction techniques, different analytical methods in which the same groups of analytes are determined have been evaluated employing the AGREEprep tool. Specifically, four of the most important fields of application in Analytical Chemistry were selected to include the widest possible variety of analytical methods: biological fluids, environmental waters, personal care products, and food.

Regarding biological matrices, two applications were selected, namely, the determination of corticoids and oxidative stress biomarkers (*i.e.,* 8-hydroxy-2′-deoxyguanosine and 8-hydroxy-2′-deoxiadenosine). Both groups of analytes provide valuable information about different biological processes, so their determination is important for the diagnostic of diverse pathologies. While corticoids are related to Cushing's syndrome, stress and type 2 diabetes mellitus [35–37], among other diseases, oxidative stress biomarkers are related with cancer, heart failure or diabetes [38], among others.

The determination of UV filters and parabens were selected as applications in the environmental field because they are worrying emerging pollutants which are used in a wide range of daily products. Regarding UV filters, they are employed in cosmetic products to protect the skin from solar radiation. Then, they can reach the environment by direct or indirect sources and alter fauna and flora [39]. As lipophilic UV filters are the most used, only those methods determining several of them were selected. Parabens are employed as preservatives and bactericides in a wide range of products. As UV filters, they can reach the environment, and due to their harmful character and potential accumulation in aqueous environmental compartments, their monitorization is recommended [40].

Within personal care products, some compounds are prohibited or their maximum concentration is restricted to ensure consumer safety, and their determination in the final product as well as during the manufacture process is an important task [41]. Among them, allergens were selected as a model application.

In the field of food analysis, acrylamide has become a widely studied substance because of its mutagenic, neurotoxic, and carcinogenic characteristics [42]. So, due to its high toxicity it is important to control its concentration in food to avoid health problems.

It should be pointed out that in some cases, especially when samples are solid, a prior step to extraction may be needed. However, it has not been considered in the AGREEprep to fairly evaluate the extraction procedure, which is the objective of this review.

For each application, mainly two conventional extraction techniques have been compared with three microextraction techniques. The selection of the reported methods was made based on the analytes determined, either in order to cover the largest number, or due to the high interest of the determination of the analyte(s). The widest variety of techniques was intended for each application. In some cases, the same technique is employed for an application, but other characteristics of the method make their AGREEprep scores very different, being their comparison also very interesting.

Even though AGREEprep allows to change the weights for each criterion, the weights given by default were selected in order not to bias the results. Besides, the reader should be aware that some rules had to be stablished to ensure a fair greenness evaluation. Unfortunately, many specific details about laboratory materials, apparatus or general laboratory processes are usually omitted in the reported publications. For instance, as described by authors of AGREEprep [30], all single-use materials (*e.g.*, pipette tips, centrifuge tubes, conical tubes, etc.) must be considered in waste section. However, it is very complicated to know the exact amount of them used because it is not often reported. Therefore, in regard to this review, it was decided to only consider in waste section the laboratory material where the extraction is carried out (*e.g.*, SPE cartridges, centrifuge microtubes, flat-base inserts, etc.). In Table 1, the weights (in grams) considered in this review for each material, adopted from our own laboratory material, are shown in order to homogenize the evaluation of waste generation.

Regarding sample throughput and energy consumption, not in all cases the brand or the model of the employed apparatus were reported. Therefore, it was not possible to know the number of parallel samples that can be treated simultaneously and the energy consumption of the apparatus, which highly depend on the specific apparatus that each laboratory has. As in the case of weights, to homogenize this information, it was decided to consider some instruments as a reference for all the methods where they were employed. In Table 2, the number of sample positions and the energy consumption considered for each apparatus are shown.

In this way, with all these considerations, we have fairly compared all the methods as if we were going to carry them out in our laboratory. This information may be useful to readers in future method comparisons using the AGREEprep.

Table 3 shows the selected methods and their AGREEprep punctuation. In the Supplementary Material file, the breakdown of the values considered for each criterion in each method is shown.

4. Discussion

As can be seen in Table 3, the general trend is that the employment of microextraction techniques leads to greener methodologies compared to conventional extraction techniques. In general terms, scores for classical extraction techniques (*i.e.,* SPE and LLE) ranged between 0.13 and 0.48 (with an average value of 0.31), while microextraction techniques achieve higher scores between 0.37 and 0.66 (with an average value of 0.54). Hence, the benefits of miniaturization of extraction techniques in terms of greenness is highly evidenced. Furthermore, it is confirmed that analytical chemists are aware of environmental problems and great efforts are made to be in line with principles of GAC and GSP.

The discussion of results has been divided into two main subsections: on the one hand, those aspects related to the scale of the extraction, such as the solvents used (Criterion 2), the amount of waste generated (Criterion 4), and the sample volume (Criterion 5); and, on the other hand,

Table 1
Weights considered for general-use material for the evaluation of waste generation.

Material	m (g)
2–200 μL pipette tip	0.3
1 mL SPE cartridge	1.6
3 mL SPE cartridge	2.5
6 mL SPE cartridge	4.0
0.5 mL microcentrifuge tube	0.5
2 mL microcentrifuge tube	0.9
8 mL tube	4.0
15 mL centrifuge tube	6.6
Flat-base glass insert	0.8

Table 2
Number of sample positions and estimated energy consumption considered for the evaluation of throughput and energy consumption.

Apparatus	Sample positions	Energy consumption (W)
Magnetic stirrer	10	20
Vortex	1	45
SPE Manifold	12	160
Vertical rotary shaker	16	15
Centrifuge	6	440
Microcentrifuge	12	440
Thermo-shaker	24	42
Ultrasound bath	12	330
Nitrogen evaporator	12	400
Water bath	20	960
Orbital shaker	12	250
Vacuum concentrator	48	350
Accelerated solvent extractor	1	500
SDME automated robot	1	40
Thermal desorption instrument	1	650

those about related to operational issues and performance, such as sample throughput (Criterion 6), automation and integration of steps (Criterion 7), and energy consumption (Criterion 8).

Finally, a small discussion about the main aspects to improve to develop greener analytical methods is included.

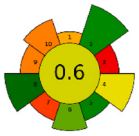
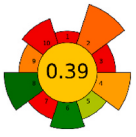
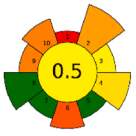
4.1. Sample volume, solvents and waste

As a result of the high efforts in the Analytical Chemistry field to reduce the volumes of solvents, waste and more recently, samples, a greenness comparison between conventional extraction techniques and microextraction techniques is of great interest.

The evolution from conventional techniques to microextraction implies undoubtedly a reduction in the acceptor phase, *i.e.*, the solvent or sorbent used to extract the analytes. Focusing on solvent-based techniques, volumes in the order of milliliters have been used in LLE. For example, 2 mL of *tert*-butyl methyl ether were employed for the determination of corticoids in urine [45], while 5 mL of the same solvent were employed for the determination of allergens in personal care products [65]. However, SDME was employed for the determination of parabens in water using just 3 μL of hexyl acetate [60] and for the determination of acrylamide in food employing 1 μL of octanol [68], among others, having a very positive impact on Criterion 2. In addition, DLLME was used for the determination of corticoids in saliva employing 0.09 g of an ionic liquid and 150 μL of methanol as disperser solvent [46], for the determination of UV filters in water employing 50 μL of chloroform and 250 μL of acetone as disperser solvent [54], and for the determination of acrylamide in food employing 100 μL of dichloromethane and 400 μL of acetonitrile as disperser solvent [69]. In short, the reduction in the volumes of organic solvents is directly related to reduced exposure to toxic substances and waste generation, hence solvent-based microextraction techniques obtain greener scores in Criteria 2 and 4, respectively, than LLE.

Regarding sorbent-based extraction, the sorbent used as extraction phase directly affects to the amount of waste generated. Thus, the grams of waste generated by SPE, due to the employment of extraction cartridges that usually range from 1 to 6 mL, oscillates between 1.6 and 4 g of waste (if its reuse is not considered). As a widespread technique, SPE cartridges were used for the determination of corticoids in urine and plasma [47], oxidative stress biomarkers in urine [51,52], UV filters [56,57] and parabens [62] in water, and acrylamide in food [72] with scores not higher than 0.42 (an average of 0.26). On the other hand, when sorbent-based microextraction techniques are employed, in general terms, less considerable amount of waste is generated. For example, miniaturized stir bar sorptive dispersive microextraction (mSBSDME) was employed for the determination of corticoids in saliva using only 0.5 mg of sorbent [44] and DSPE was employed for the determination of

Table 3
AGREEprep evaluation of the different selected methods.

Analyte(s)	Matrix	Extraction technique[a,b]	Score	Ref.
Corticoids	Urine and serum	***M*-PTME**		[43]
	Saliva	**mSBSDME**		[44]
	Urine	LLE		[45]
	Saliva	**DLLME**		[46]
	Urine and plasma	SPE		[47]
Stress oxidative biomarkers	Saliva and urine	**SPME**		[48]
	Saliva	**DSPE**		[49]
	Urine	**IT-SPME**		[50]

(*continued on next page*)

Table 3 (*continued*)

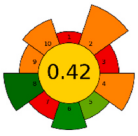
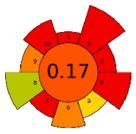
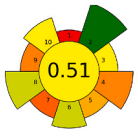
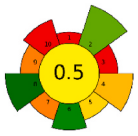
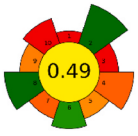
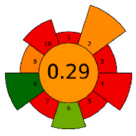
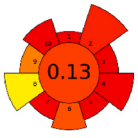
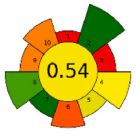
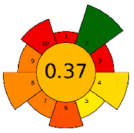
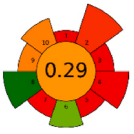
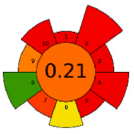
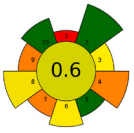
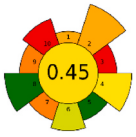
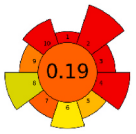
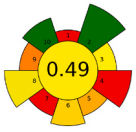
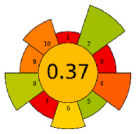
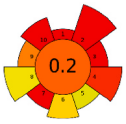
Analyte(s)	Matrix	Extraction technique[a,b]	Score	Ref.
	Urine	SPE		[51]
	Urine	SPE		[52]
Lipophilic UV filters	Environmental water	**SPME**		[53]
		DLLME		[54]
		SBDLME		[55]
		SPE		[56]
		SPE		[57]
Parabens	Environmental water	**SDME**		[58]
		HF-SPME		[59]

Table 3 (*continued*)

Analyte(s)	Matrix	Extraction technique[a,b]	Score	Ref.
		SDME		[60]
		SPE		[61]
		SPE		[62]
Allergens	Hydroalcoholic gels	**HS-SPME**		[63]
	Creams and gels	**SBSDME**		[64]
	Creams, shampoo, deodorants, and perfumes	LLE		[65]
	Creams, shampoo, deodorant, and lipstick	MSPD		[66]
	Baby wipes	PLE		[67]
Acrylamide	Bread, potato chips and biscuits	**SDME**		[68]

(*continued on next page*)

Table 3 (*continued*)

Analyte(s)	Matrix	Extraction technique[a,b]	Score	Ref.
	Coffee	**DLLME**		[69]
	Potato chips	**SPME**		[70]
	Smashed chips	LLE		[71]
	Potato chips and biscuits	SPE		[72]

[a] DLLME: dispersive liquid-liquid microextraction; DSPE: dispersive solid-phase extraction; HF-SPME: hollow-fiber solid-phase microextraction; HS-SPME: headspace solid-phase microextraction; IT-SPME: in-tube solid-phase microextraction; LLE: liquid-liquid extraction; *M*-PTME: magnetic pipette tip microextraction; mSBSDME: miniaturized stir bar sorptive dispersive microextraction; MSPD: matrix solid-phase dispersion; PLE: pressurized liquid extraction; SBDLME: stir bar dispersive liquid microextraction; SBSDME: stir bar sorptive dispersive microextraction; SDME: single drop microextraction; SPE: solid-phase extraction; SPME: solid-phase microextraction.

[b] Microextraction techniques are marked in bold.

oxidative stress biomarkers in saliva using 9 mg of sorbent [49], among others. Furthermore, in some techniques such as SPME the extraction phase is reused several times. Hence, major improvements are achieved in Criterion 4 and, in the case of reusable materials, Criterion 3.

The miniaturization of sorbent-based techniques also implies an affective reduction of the extraction device, which directly results in a decreased use of solvents in the conditioning, washing and/or elution steps. Thus, for SPE procedures, various milliliters of solvents are required, while only few microliters are commonly used in microextraction approaches. However, it is worth mentioning that notable differences have been observed even between some SPE procedures. For example, for the determination of oxidative stress biomarkers, Cervinkova et al. [51] employed 1.8 mL of organic solvent, whereas Shigenaga et al. [52] employed 20 times more volume, which significantly affected the final score (0.42 vs 0.17, respectively). Same occurs for the determination of UV filters, since Díaz-Cruz et al. [57] employed 4 times more volume of organic solvent than Cuderman and Heath [56] (0.13 vs 0.29, respectively). Hence, even if conventional extraction techniques require higher volumes of organic solvents, an effort to reduce them to the maximum should be done. Unlike conventional extraction techniques, some microextraction techniques like SPME or SBSDME can be directly coupled to the thermal desorption of the analytes prior to gas chromatography, decreasing even more solvent consumption or even developing solvent-free methods. This is a relevant input since conventional techniques do not allow this solvent-free desorption. Thus, as mentioned before, the reduction of solvent consumption is closely related to a decrease in waste and exposure to toxic substances (Criteria 2 and 4).

Moreover, a trend to decrease or eliminate the use of non-green solvents has been observed [73], which directly affects Criterion 10. While in some SPE procedures more than 20 mL of hazardous solvents were employed (*e.g.*, methanol), microextraction techniques tend to avoid their use or reduce it under 100 μL. In addition, the replacement of conventional organic solvents (especially the chlorinated ones) by greener solvents is one of the focusses of attention in the field of microextraction [74,75]. Thus, greener scores for Criteria 2 and 10 were obtained when microextraction techniques along with greener solvents are used.

Additionally, an effort to reduce sample volume has also been made in last years, especially in bioanalysis where sample amount is limited and sometimes only few microliters of sample are available. In this regard, magnetic pipette tip microextraction (*M*-PTME) was employed to determine corticoids in only 10 μL of urine or serum [43], mSBSDME was applied for the determination of corticoids in 175 μL of saliva [44], and in-tube SPME (IT-SPME) was used to determine oxidative stress biomarkers in 100 μL of urine [50], among others. In contrast, volumes ranging from 0.5 to 5 mL were used when conventional extraction techniques were employed to analyze biological samples. In other matrices such as environmental water, personal care products or food, this trend is not so significant because the amount of sample is not usually so limited. However, a reduction in sample amount has also been observed for the determination of UV filters or parabens in waters, where volumes ranging from 5 to 30 mL were employed when using microextraction techniques, instead of 100–500 mL when using SPE. Regarding personal care products and food, not a clear trend can be observed from the methods assessed in Table 3. However, the lowest sample amounts employed were reached when using microextraction techniques, obtaining better scores for Criterion 5.

In summary, the employment of microextraction techniques is generally associated with a reduction of sample amount and it directly implies a reduction of waste and usually a reduction in solvents and sorbents, leading to a greener punctuation mainly in Criteria 2, 4 and 5.

4.2. *Throughput, integration of steps and automation*

For routine laboratories, high-throughput methodologies are required due to the high number of samples that need to be treated every day. For that reason, methodologies where several samples can be treated in parallel are required. In this sense, microextraction techniques have a slight advantage over conventional techniques since the miniaturization of the extraction device leads to an effective reduction in physical space, allowing to treat more samples in the same space. However, the difference is not as huge as it is in other aspects because conventional techniques are usually carried out in manifolds (SPE), shakers or ultrasound baths (LLE), where often several samples can be treated in parallel. In some specific cases, as it is the determination of oxidative stress biomarkers, one of the methods based on SPE [51] and the one based on DSPE [49] had the same throughput (*ca.* 48 samples per hour), and it was more than 20 times higher than for IT-SPME [50]. But in general, the trend is like for the determination of acrylamide in food, where the throughput reached by SDME (*ca.* 60 samples per hour) was 10 times higher than for LLE and SPE. Thus, greener punctuations are obtained for microextraction techniques in Criterion 6 and, as high sample throughput is directly correlated with less energy consumption, also good punctuations are obtained for Criterion 8. Additionally, the reduction in physical space of extraction devices would allow its portability to the sampling site (Criterion 1), although depending on the approach, external power supplies would be required.

Regarding number of steps, a clear difference between conventional and microextraction techniques can be noticed again. While SPE usually needs at least 5 steps or more, microextraction techniques generally require from 1 to 4 steps, obtaining greener punctuations in Criterion 7. As an example, for the determination of UV filters in water, 2 or 3 steps were required when employing microextraction techniques (SPME,

DLLME and SBDLME), while 5 or 6 where required when employing SPE. Thus, the reduction in the number of steps is directly correlated with a reduction in total analysis time, increasing sample throughput. Moreover, avoiding time-consuming steps is also important. In this sense, evaporation and reconstitution steps, which are typically performed in SPE and LLE where volumes over 1 mL are usually used, and an elevated time is required to carry out this step, are not recommended.

Regarding automation, due to the complexity of sample preparation and the high cost usually associated with robotic workstations, it is difficult to automate the whole process. Therefore, in most of the methods assessed manual intervention is required. Only the determination of parabens in water employing SDME [58] and the determination of oxidative stress biomarkers in urine employing IT-SPME [50] were totally automated methods obtaining greener punctuations in Criterion 7, but low sample throughput was achieved because of the lack of parallel sample treatment. To achieve automated high-throughput methods, more development on sample preparation apparatus is needed, and it requires more economic investments. Furthermore, the existing commercial automated apparatus are expensive, and a large number of laboratories cannot afford them. In this sense, more research in this area is still needed to develop affordable high-throughput automated apparatus.

4.3. Main improvement areas for green method development

According to the different AGREEprep pictograms shown in Tables 3 and it may be observed that either for conventional extraction or microextraction applications there are three criteria which usually reduce the final green value of the whole method. These are the portability of the sample preparation step (Criterion 1), the reusability of materials (Criterion 3) and the post-sample preparation analysis (Criterion 9). The achievement of these three goals may be the most difficult task in order to obtain the greenest method.

In this sense, for example, the use of chromatographic techniques coupled to mass spectrometry, among other detectors, sometimes is unavoidable due to the high number of analytes and/or their low concentrations in samples. However, an improvement in the greenness of chromatography by optimizing the methods is highly recommended [76]. Some greener separation techniques such as capillary electrophoresis or electrochemical techniques may be employed instead, which positively influence the score, but they are not always a viable alternative. Moreover, the employment of complex instrumentation also undoubtedly hinders the portability of the whole method.

Regarding Criterion 3, not all the required attention has been paid to the reusability of materials for the benefit of the operator, seeking their easy handling. Hence, the single-used and non-biodegradable plastics and extractants are still widely used, which in contrast increases the total waste of the entire method as a consequence.

5. Conclusions

The evolution from conventional extraction techniques to microextraction approaches has provided several advantages in terms of greenness as a result of downscaling, as it has been demonstrated employing the green metric tool AGREEprep. Downscaled methods allow to employ less amounts of extraction phases, both sorbents and solvents, during the sample preparation process that leads to less waste generation. Furthermore, a decrease in the use of hazardous solvents and reagents has been observed, as well as great efforts to replace them with greener alternatives, increasing operator's safety. A reduction in sample amount, especially in bioanalysis, where sample amount is normally limited, has also been made over the decades. Moreover, the reduction in the number of steps in microextraction techniques has led to a slightly increase in sample throughput, decreased energy consumption per sample and greater integration of steps, making methods easier and faster. Regarding automation, low throughput was obtained with fully automated methodology due to the complexity of sample preparation process. Besides, expensive apparatuses complicate the affordability of automation for many laboratories. Therefore, it is crucial that further research should be done in this line. Even though great advances have been already made thanks to the employment of microextraction techniques, future research should be focused on the development of further miniaturized microextraction techniques with greater agreement with principles of Green Sample Preparation to obtain a perfect score of 1.00, or as close as possible, with outstanding analytical performance. In particular, more attention should be paid to those criteria that tend to be more controversial, such as the portability of the sample preparation stage, the reuse of materials, and the post-analysis, which does not always depend directly on the extraction technique itself, but rather on the application. In addition, more awareness is needed in the scientific community for the use of these miniaturized techniques, not only in the research field where they are already practically implemented, but also in routine analysis laboratories.

From this review it is also inferred that the use of metric tools is very useful to assess the greenness of a method and allows researchers to confirm whether their method complies with the GAC and GSP principles. AGREEprep has been used here since it presents a great potential to evaluate the sample preparation stage. It should be noted that for a fair evaluation between methods published by different authors, a consensus on some criteria is necessary. Since much of this information (*e.g.*, laboratory materials or apparatus used) is unfortunately often not available from published methods, or they differ between laboratories, we have included some of these values in this review that may be used in a general way to fairly evaluate different methods.

CRediT authorship contribution statement

Guillem Peris-Pastor: Methodology, Validation, Investigation, Data curation, Writing – original draft. Cristian Azorín: Methodology, Validation, Investigation, Data curation, Writing – original draft. José Grau: Methodology, Conceptualization, Writing – review & editing, Supervision. Juan L. Benedé: Methodology, Conceptualization, Writing – review & editing, Supervision. Alberto Chisvert: Methodology, Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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