Green Analytical Chemistry 10 (2024) 100125

Contents lists available at ScienceDirect

Green Analytical Chemistry

journal homepage: www.elsevier.com/locate/greeac

Green and sustainable evaluation of methods for sample treatment in drug analysis

Héctor Martínez-Pérez-Cejuela [a], Emanuela Gionfriddo [a], Pilar Campíns-Falcó [b], José Manuel Herrero-Martínez [b], Sergio Armenta [b,*]

[a] Department of Chemistry, University at Buffalo, The State University of New York, Buffalo, NY 14260-3000, United States
[b] Department of Analytical Chemistry, University of Valencia, Dr. Moliner 50, 46100, Burjassot, Valencia, Spain

ARTICLE INFO

Keywords:
Green analytical chemistry
Drug analysis
Solid phase extraction
Solid phase microextraction
Liquid phase microextraction
Hexagon
AGREEprep
SPMS

ABSTRACT

The time-consuming nature of many sample treatment protocols continues to be recognized as the main bottleneck of analytical procedures, which, in most cases, use long analysis times, excessive amounts of solvents and reagents, and energy. However, the increased awareness of the need for environmental protection and the development of Green Analytical Chemistry (GAC) has led to significant advancements in the field of sample treatment. Today, advances in the miniaturization of sample treatment techniques, the development of sorbents with tunable properties, and greener alternatives (for example from waste valorization) are gaining interest in the analysis of psychoactive compounds. Indeed, techniques offering rapid sample preparation, minimal use of solvents, reagents, and energy, and a significant reduction of waste generation are chosen by the scientific community. In recent years, non-toxic extraction media, such as ionic liquids, and deep eutectic solvents have emerged as alternatives to petroleum-based solvent extractions. Similarly, sorbent-based techniques have benefited from engineered materials and nanotechnology, incorporating novel sorbents with optimized physicochemical properties to enhance extraction efficiency and selectivity. To meet sustainability standards, GAC provides a wealth of information in the field of green awareness, and the application of green metrics has been extended into the literature in the past decade. These tools serve as guides and encourage scientists to develop the most appropriate methodologies that focus on both analytical performance and sustainability, using different concepts and criteria. This article collects and discusses examples of different sample preparation techniques applied to drug analysis, highlighting their strategies for greening the processes and their evaluation using tools such as HEXAGON, Analytical greenness metric for sample preparation (AGREEprep), and Sample preparation metric of sustainability (SPMS) tools.

1. Introduction

Modern analytical chemistry faces new challenges in analyzing complex samples and ensuring the sustainability of chemical processes. The growing environmental awareness prompts examination of the new analytical procedures that cannot neglect their impact on nature safety and human well-being. Advanced and miniaturized analytical tools enhance the ability to analyze complex samples with minimal reagents, consumables, and energy. Sample treatment is still the most labor-intensive and time-consuming analytical step, accounting for 60–80 % of the total analysis time [1]. This step can affect both, analytical performance (e.g. accuracy and precision) and the green characteristics of the method [2]. In the context of drug analysis in biological specimens, sample treatment is usually a pre-requisite mainly due to: i) incompatibility of most biological samples with direct introduction into analytical instrumentation, due to their complexity, and ii) low concentration of analytes in most biological samples, which can be below detection limits of common analytical instruments. In this sense, solid-phase, liquid-liquid, and solid-liquid extractions are the most used techniques to enable clean-up and preconcentration of drugs from biological samples. These traditional extractions commonly require large quantities of samples and solvents, they are time-consuming and hard to automate, which are inconsistent with the principles of Green Analytical Chemistry (GAC). In this sense, twelve principles of GAC have emerged to account for different aspects of the analytical procedure, suggesting the greenest solutions for each aspect of the analytical workflow [3].

* Corresponding author.
E-mail address: sergio.armenta@uv.es (S. Armenta).

https://doi.org/10.1016/j.greeac.2024.100125
Received 17 May 2024; Received in revised form 25 June 2024; Accepted 25 June 2024
Available online 2 July 2024

Contemporary analytical approaches prioritize miniaturization and automation, resulting in techniques like micro solid-phase extraction (μ-SPE) and solid-phase microextraction (SPME). Moreover, advancements in liquid phase microextraction (LPME) techniques address challenges such as solvent usage and time consumption. Furthermore, the use of alternative solvents has been one of the top priorities of GAC, since it affects many aspects of the method including operator safety, degradability, analytical performances, etc. Green solvents such as ionic liquids (IL) and deep eutectic solvents (DESs), among others, can be employed in extraction approaches to replace conventional solvents typically obtained from fossil sources. While efficient workflows using a low number of steps are highly advisable, key aspects such as cleanup, preconcentration, solvent and instrument compatibilities, and reproducibility need to be always considered, as reported in the White Analytical Chemistry guidelines [4].

To meet sustainability standards, GAC provides a wealth of information in the field of green awareness, and green assessment has been extended into the literature in the past decade with the appearance of different quantifying metrics of method sustainability. These tools not only serve as guides to the scientists, but also encourage them to change the most unsuitable practices from an environmentally friendly point of view. Concretely, some of the factors that are analyzed are chemical usage, energy consumption, sample volume extraction times, waste production, etc. Recently, several reviews have discussed previously proposed green metrics [5–7]. Up to 17 different metrics were created with the Chemical Hazard Evaluation for Management Strategies (CHEMS-1) [8], National Environmental Methods Index (NEMI) [9], Analytical Method Volume Intensity (AMVI) [10], Analytical Eco-scale [11], Green Analytical Procedure Index (GAPI) [12], RGB [13], HEXAGON [14], Analytical Method Greenness Score (AMGS) [15], Blue applicability grade index (BAGI) [16], Analytical Greenness Calculator (AGREE) [17], AGREEprep [18], and sample preparation metric of sustainability (SPMS) [19], being the most used. In addition, it is more than likely that soon new greenness metric tools will be proposed in the scientific literature. The different proposed green metrics take into account various concepts and criteria, advantages and disadvantages; however a comprehensive description of them is out of the scope of this paper. This review focuses on the impacts of various sample preparation methods for drug monitoring, it emphasizes their approaches towards eco-friendly processes and evaluates them using tools such as HEXAGON, AGREEprep, and SPMS.

2. Description of the selected GAC metrics

Among the wide variety of green metrics available, AGREEprep and SPMS have been selected to evaluate the impact provided by the sample treatment step on method greenness. On the other hand, HEXAGON has been selected because it evaluates the whole analytical method globally, considering relevant aspects such as carbon footprint, the associated cost of analysis, the consumption of reagents and solvents, and the time needed for the analysis of the samples. Interestingly, these parameters are not commonly included in other metrics and allow the reader to have a holistic point of view of the method.

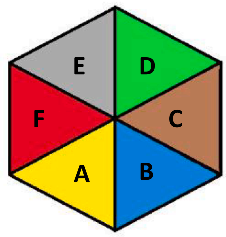
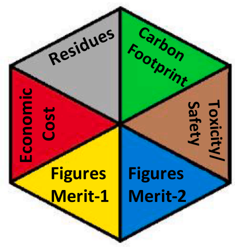
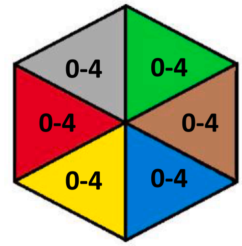
AGREEprep [18] is a metric exclusively evaluating the sample preparation step based on ten criteria with scores from 0 (unfulfilled) to 1 (fulfilled), whose weights can be modulated. This tool employs user-friendly and free-to-download software (mostwiedzy.pl/AGREEprep). The criteria score is as follows: 1) Favors in-field sample preparation so as to minimize wasted time and the use of material and energy (1 point as default weight); 2) Greener chemicals to reduce the cost, environmental impact, and risk to operator (5 point as default weight); 3) Sustainable materials with remarkable stability and degradability (2 point as default weight); 4) Decrease the waste generation since handling, storing and disposing waste consumes resources, time, effort and money (4 point as default weight); 5) Miniaturization which decreases energy needed, chemicals and others (2 point as default weight); 6) Increase the throughput to favor the energy efficiency and operator safety (3 point as default weight); 7) Integrate and automate to keep a method simple and efficient (2 point as default weight); 8) Low energy-demand (4 point as default weight); 9) Greener configuration in the sample analysis avoiding evaporation steps and using low-energy demand equipment if possible (2 point as default weight); 10) Safety to the operator: the lesser hazard symbols it contains the reagent, the better (3 point as default weight). The assessment outcome is depicted as a circular pictogram with a numerical score at its center. This score, ranging from 0 to 1, reflects the overall effectiveness of the sample preparation process in terms of environmental friendliness. The circle is divided into 10 segments, each representing a performance criterion, with the length and color of each segment indicating the weight and performance level of that criterion, respectively.

SPMS is another metric to assess the sample treatment, which highly encourages its use when the evaluation is fitted to this process by itself [19]. In this sense, sampling and analytical techniques to quantify the analytes are not considered in the calculations. The metric is simple to apply and also offers an open-source Excel sheet in the supplementary material. The results are reported with a clock-like diagram, displaying the greenness outcome of the main sample preparation parameters and a total score. Among its additional novel features compared to other metrics, this approach notably takes into account the impact of extraction time for each procedural step, rather than solely focusing on the number of steps or samples processed within a given timeframe. Furthermore, it assigns greater significance to the extractant nature within the metric. The parameters evaluated are 9 including sample amount (1); amount of extractant (2); nature of extractant (3); number of steps (4); extraction time (5); additional steps after extraction (6); simultaneous samples (7); energy consumption (8); total waste (9); reusability (extra asterisk mark). The potential colors, their corresponding numeric ratings, and the qualitative assessments are as follows: green denotes "successful", yellow signifies "acceptable", orange indicates "tolerable", and red represents "inadequate".

The HEXAGON tool jointly considers green chemistry principles in cooperation with environmental impact and economic cost aspects to quantitatively assess the sustainability of analytical methods [14]. Objective criteria are evaluated through the definition of penalty points divided into five different blocks, namely, figures of merit, toxicity and safety, residues, carbon footprint, and economic cost. For each block, the overall qualification is scaled from 0 to 4 and it is depicted on a regular hexagonal pictogram that allows a user-friendly comparison of analytical procedures (see Table 1). Eventually, the arithmetic mean (Sav) of the 0–4 scale is computed in order to compare analytical methods from a single data [20]. HEXAGON is in line with the Green and Sustainable chemistry philosophy, also balancing the figures of merit needed for solving a given problem. The lower the score, the better the adaptation of the analytical procedure to greenness and sustainability aspects, which leads to reliable analytical results. Table 1 summarizes the studied aspects and the contributions of the penalty points established. In addition, the scales of the different triangles are included.

In reference to parameters related to sample treatment and preparation, the best option, without penalty points corresponds to a methodology, that does not use preservation and storage conditions for the sample, requiring a micro amount of it. Furthermore, the use of reagents or solvents, dilution, or concentration steps for achieving the required concentrations for the chosen technique is not necessary. In contrast, a throughput higher than 50 samples per week is positively considered. In general, no more than four situations were considered for each item to facilitate the application of the metric tool for the other considered aspects different from the best option. In addition, if non-sample treatment is required, it can impact all triangles of the hexagon as demonstrated in [20].

Table 1
Description of the HEXAGON tool.

Hexagon Triangle	Name of triangles / Scale	Maximum penalty points (PP) Scaled between 0 and 4
	 	
A (0 to 4) in function of penalty points (PP) **0** [0,5] **1**[6,15] **2**[16,25] **3**[26,35] **4**[36,45]	**Figures of merit 1** ***Sample treatment and preparation***: preservation, storage, amount, reagents/solvents used; amount of reagents/solvents; instrumental vs adequacy to the method, number of weekly samples, pretreatment. ***Methods characteristics***: method categories, operational mode, portability, method/sample, analytes/sample, time of analysis/sample, robustness. ***Calibration***: frequency, required time, number of standards, linear adjustment R^2, LOD and LOQ limits, working range and linearity, precision.	18 10 17
B (0 to 4) in function of PP **0** [0,4] **1**[5,8] **2**[9,12] **3**[13,16] **4**[17,23]	**Figures of merit 2:** ***Quality control***: frequency, time required, number of standards ***Accuracy***: frequency, time required, concentration levels, magnitude/size, selectivity	10 12
C(0 to 4) in function of PP **0** [0,5]; [0,2] **1**[6,12]; [3,5] **2**[13,18];[6,9] **3**[19,25]; [10,14] **4**[26,33]; [15,22]	**Toxicity/ Safety:** ***Toxicity***: health and environmental hazards ***Safety***: physical hazards	27 25
D(0 to 4) in function of PP **0** [0, 0.1] **1**[0.1,0.5] **2**[0.6,1] **3**[1.1, 2] **4**[higher 2]	**Carbon footprint:** **Kg CO_2 equivalents**	quantitative
E (0 to 4) in function of PP **0** [0,5] **1**[6,10] **2**[11,15] **3**[16,20] **4**[21,24]	**Residues:** **Amount** **Waste treatment** **Disposable material**	10 3 5
F (0 to 4) in function of PP **0** [0,5000] **1**[5000,15000] **2**[15000,30000] **3**[30000,50000] **4**[higher 50000]	**Economic cost:** **Equipment, salary, material, electricity (€)**	quantitative

3. Description and GAC evaluation of the selected sample treatment methods applied in drug analysis

3.1. Solid-phase extraction (SPE) and dispersive SPE

Solid-phase extraction (SPE) is the most widely used extraction technique as sample treatment, which is based on the analyte distribution between sorbent (solid) and sample (liquid). The sorbent can be either immobilized, as inSPE, which performs an exhaustive extraction, or use in a non-immobilized form, such as dispersive-SPE (d-SPE), which is an equilibrium-based extraction technique. Both approaches contain the same steps: a) conditioning of the sorbent; b) sample loading; c) washing interferents from the matrix; and d) elution of the retained analytes. From the green point of view, SPE and D-SPE traditionally use large amounts of chemicals and samples due to their macroscopic sizes, several steps during the process (e.g. evaporation, centrifugation, etc.), and manual operation. However, the development of new methodologies has mainly overcome these problems. Despite these two different configurations, greening solid-based extractions are mainly addressed with similar approaches, being size reduction one of the most prominent ones since reduces sample, sorbent usage, and solvents. Furthermore, integrating steps and automating can lead to more efficient methods in terms of analytical performance and environmental friendliness. On the other hand, the development of new sorbents with natural- or bio-based materials such as ILs, DESs, and carbon-based has allowed the improvement of characteristics such as cost-efficiency, biodegradability, and lower toxicity. Also, the use of 3D printing has been reported in the last decade as a revolutionary technology for creating desired devices coated with solid resins to increase the extraction efficiency of commercially available devices. Finally, building highly efficient materials with novel characteristics will directly impact the method's greenness since a lower amount of sorbent would be required, and it could be reused, among others. It is worth noting that, in the latter case, special attention should be paid to the synthesis process since it is always one of the most intricate parts of greening sorbent-based extraction, and hence, more efforts should be made in this aspect.

Table 2 shows some examples of SPE and -SPE in green analytical chemistry, which have been evaluated with AGREEprep, SPMS, and HEXAGON. Selected references included several matrices such as urine, illicit or street drugs, and oral fluids. As a general comment on the use of AGREEprep, it can be observed that when SPE is selected as the extraction method, the overall greenness decreases. The main reason is the intrinsic nature of the off-line extraction method as well as the use of organic solvent in the conditioning and elution steps. The more different organic solvents used, the more risk to the operator [21]. In this sense, when miniaturization is done, for instance, pipette-tip SPE [22], the score can reach higher values (0.57), decreasing the waste generation and sample requirements too. Interestingly, conventional SPE [23] compared to paper-based devices [24] gave green scores of 0.36 and 0.76 being both used for the analysis of synthetic cannabinoids, encouraging the use of natural-based materials as well as solvent-less desorption techniques. On the other hand, it can be clearly seen how the use of large volumes of samples to obtain high preconcentration factors is penalized [25], although they are from highly-available origin (e.g. environmental water). This aspect should be reconsidered in the following updates of the different green metrics. For example, the sample could be considered waste when LPME is used as a sample treatment technique because the sample (urine, water,…) would be saturated with the organic solvent used for analyte extraction. However, in SPE or SPME processes, the sample is not altered except for the extraction of the analytes of interest (along with other compounds that may be completely or partially retained in the solid sorbent). The use of derivatization processes should be also avoided as much as possible [26–28] since additional reagents and steps are being added to the sample pretreatment.

A general criticism of the methodology is the possibility of applying different weights to each analyzed factor. This can be advantageous for experienced GAC users who can discern which factors may hold higher importance in the developed methodology. However, for most users, it may lead to confusion and arbitrariness. Additionally, it complicates the comparison of methodologies when the authors have not applied consistent weighting criteria.

SPMS as green assessment of the sample treatment is a metric with ranges, which increases the overall score of all methods (5.26 to 8.00), being punished by the disk-based extraction for the use of 100 mL of the sample as in AGREEprep [25]. Incredibly, metal-organic framework (MOF)-SPE can obtain 36 % with AGREEprep, whereas SPMS scores 80 % of total greenness. This is mainly explained because the latter metric takes into account different parameters as described in Section 2. It is remarkable the qualitative parameters that this tool displays regarding reusability (asterisk) and multisample treatment (bold line), which allow you to consider important features in sustainability. As previously commented, the use of derivatizing agents is also penalized here in the procedure evaluation [26–28].

Regarding the application of HEXAGON to the SPE and related procedures, it can be seen that values of s_{av} ranging from 0.86 to 1.67 were obtained, providing a good performance. As it has been aforementioned in the previous section, the lower the score, the better the adaptation of the analytical procedure to greenness and sustainability aspects. Differences between the several applications are mainly related to carbon footprint, related mainly with run time, and toxicity as can be seen in their HEXAGONs. The associated estimation of the annual economic cost of the analytical procedures also impacts the obtained results. Analytical procedures using simple instrumentation such as UV–visible spectroscopy or ion mobility spectrometry provided better values for HEXAGON and s_{av}= 1.16 and 0.86, respectively due to their simplicity and suitability to solve the analytical problem.

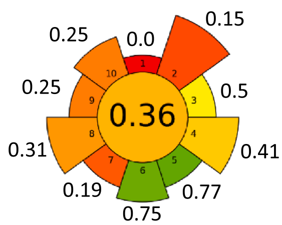
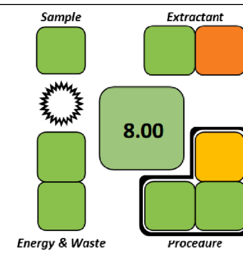
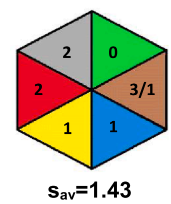
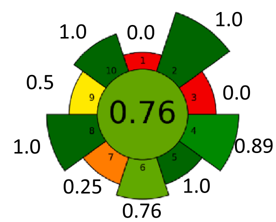
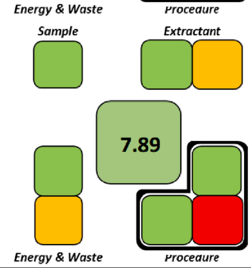
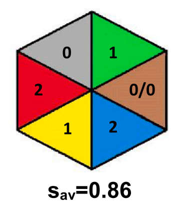
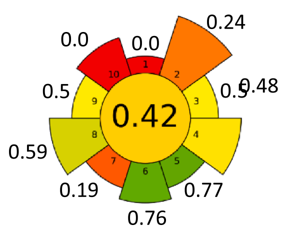
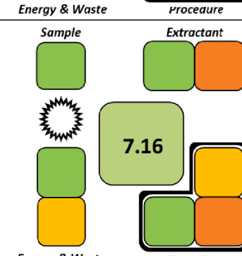
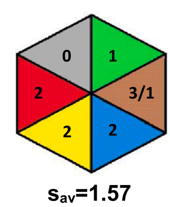
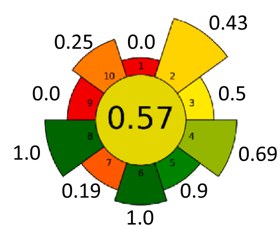
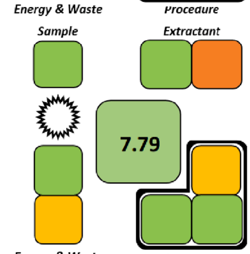
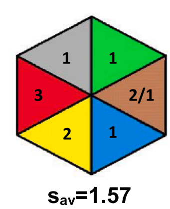
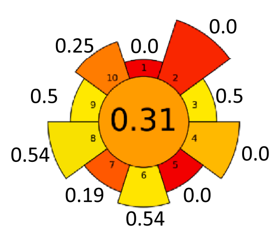
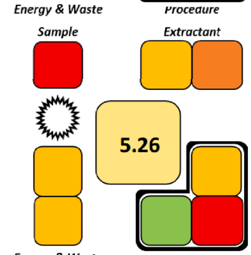
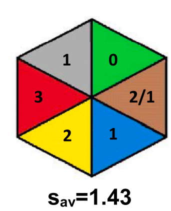
The main disadvantage of HEXAGON is the lack of dedicated software, Excel file, or web page, where users can easily enter data and obtain the characteristic green rating.

3.2. Solid phase microextraction (SPME)

Solid-phase microextraction (SPME) is a sample preparation method based on a non-exhaustive extraction principle. Since its first appearance in 1989 [29], significant efforts have been invested to further develop this technique in terms of cost-efficiency, robustness, and analytical performance. Nowadays, previous problems of fragility and high cost are amended, due to technological advancements (e.g. flexible metal alloys or stainless steel as supports for the extraction phase). Furthermore, in-batch synthesis or lab-made devices allow the preparation of more affordable extractive phases. All these facts have encouraged the scientific community to expand the applicability of SPME to many different fields in analytical chemistry [30–32]. Existing formats of SPME devices can be very diverse, such as fibers, blades, arrows, thin films, etc. However, most of them can efficiently extract the target analyte from solid, liquid, or gaseous phases in complex samples. The miniaturized nature of microextraction devices, as well as their portability, is of special interest in low-available sample applications (clinical bioanalysis), hard-to-reach sampling sites, and in vivo analysis without biopsy [33,34].

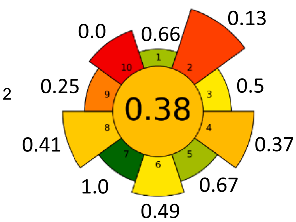
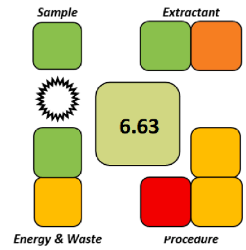
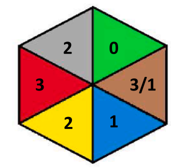
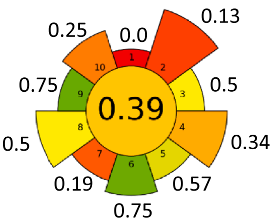
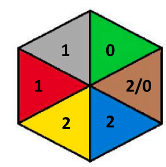
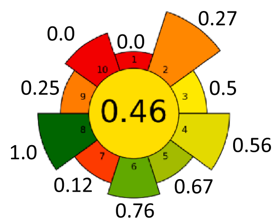
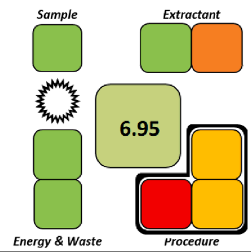
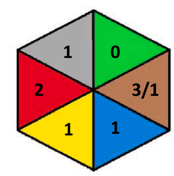
SPME is considered environmentally friendly due to its intrinsic properties, requiring low amounts of sorbents, chemicals, and samples, with consequent production of a limited amount of waste after use. Furthermore, the SPME device sustainability can be significantly enhanced by developing reusable and/or bio-based sorbent phases. Regarding the desorption process, various methods can be found in the literature by using liquid or thermal desorption. When using a liquid desorption process (normally using mixtures of organic solvents and water), a low amount of solvent is required (usually less than 1 mL). However, the use of greener alternative solvents such as DESs or ILs is preferred from a sustainable point of view. Solvent-less thermal

Table 2
Green evaluation of recently reported SPE-based methods for the analysis of drug abuse in biofluids (AGREEprep, analytical greenness metric for sample preparation; SPMS, sample preparation metric of sustainability; Ref., reference).

Analytes	SPE device & Method	Reagents/sorbent and sample[1]	Green assessment			Ref.
			AGREEprep	SPMS	HEXAGON	
8 Synthetic cannabinoids	SPE cartridge and LC-FLD	NH_2-UiO-66 (25 mg), 3500 µL of MeOH:ACN (50:50, v/v), and 500 µL of oral fluids				[23]
7 Synthetic cannabinoids	MOF-based PAD and IMS	NH_2-UiO-66@paper (1 cm^2) 100 µL of oral fluids				[24]
Cocaine	m-SPE and IMS	m-MIPs of organic polymer (5 mg), 100 µL acetic acid, 50 µL of NH_3, 500 µL of $CHCl_3$, 1200 µL MeOH, and 500 µL of oral fluids				[21]
23 psychoactive substances	Pipette tip-SPE and UHPLC-MS/MS	Organic monolith (5 mg), 200 µL isopropanol; 300 µL 1% acetic acid MeOH; 200 µL oral fluids				[22]
Ecgonine methyl ester	Disk-m-SPE and IMS and UHPLC-MS/MS	Organic monolith (one unit); 10 mL carbonate buffer 0.1 M; 5 mL acetic acid 1% MeOH; 200 mL surface water and wastewater				[25]

(continued on next page)

Table 2 (*continued*)

3 amphetamines	Online SPE (20 mm × 2.1 mm i.d. ODS-C18 column) HPLC-FLD	2 mL ethyl acetate, 2 mL of n-hexane, 1 mL urine			 s_{av}=1.67	[28]
2 amphetamines	C18 SPE cartridge - UV	1 mL MeOH, 1.5 mL N_2CO_3 buffer, 0.5 mL NQS reagent and 1 mL ACN Pharmaceutical tablets and 2 mL urine			 s_{av}=1.16	[26]
Ephedrine enantiomers	SPE cartridge (BioMOF) and HPLC-FLD	0.25 mL $NaHCO_3$, 0.125 mL FMOC and 0.125 mL DCC sample in 0.2 mL ethanol and 0.8 mL hexane			 s_{av}=1.17	[27]

Abbreviations: SPE, solid-phase extraction; LC, liquid chromatography; FLD, fluorescence detection; MOF, metal-organic framework; PAD, paper-based analytical device; IMS, Ion-mobility spectrometry; m-MIPs, magnetic molecularly imprinted polymers, UHPLC-MS/MS, ultra-high performance liquid chromatography-tandem mass spectrometry; NQS, 1,2-naphthoquinone-4-sulfonate; FLD, fluorescence detection; FMOC, 9-fluorenylmethyl chloroformate; DCC, dicyclohexylcarbodiimide.
[1]The chemicals used in the synthesis process and in posterior analysis with analytical instrumentation have not been considered for comparison purposes.

desorption, commonly used in SPME-gas chromatography (GC) applications, is suitable for reducing solvent use. Moreover, the direct coupling of SPME with analytical detectors (such as mass spectrometers (MS)) can improve the overall greenness of the method, avoiding the use of chromatographic techniques and reducing analysis time, energy demands, and waste generation. SPME-MS offers rapid and sensitive analysis, making it a promising technique for efficient and eco-friendly analysis. Furthermore, the introduction of robotics and autosamplers into the analytical workflow has led to easy automation and increased throughput while decreasing operator risks.

Table 3 describes representative examples of drug analysis using SPME methods and their green evaluation [35–42]. Three different metrics have been used to evaluate the greenness of these procedures, AGREEprep, SPME, and HEXAGON. It should be noted that due to the difficulty of comparing commercial devices with homemade ones in terms of greenness, the reagents and apparatus involved in the synthetic process of extractive phases have not been evaluated in this overview.

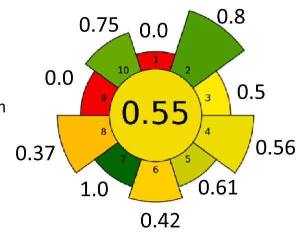
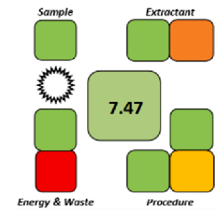
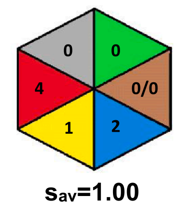
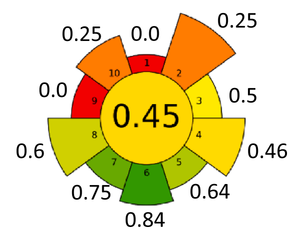
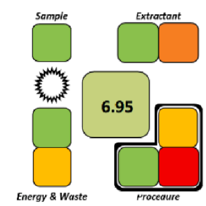
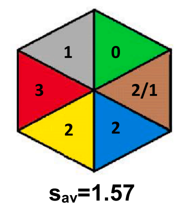
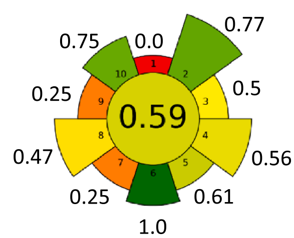
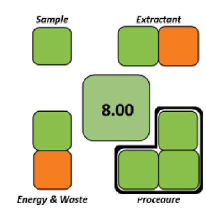
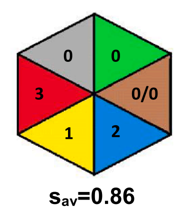
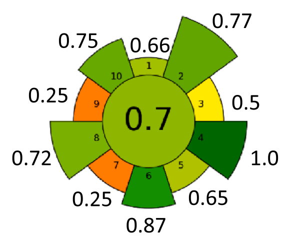
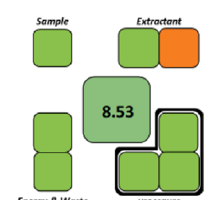
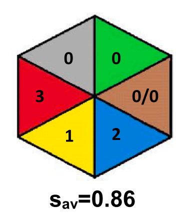
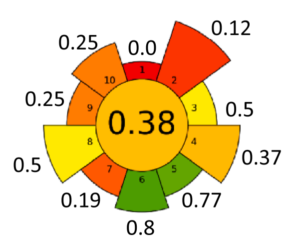
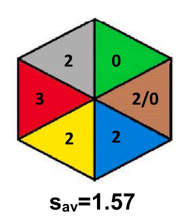
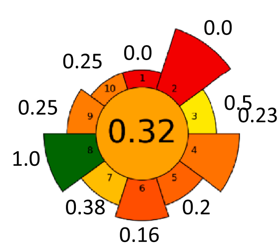
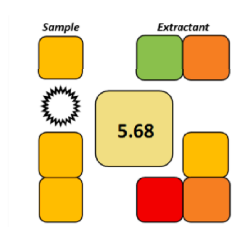
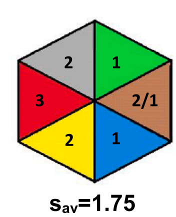
All the SPME methods in Table 3 scored in the range of 0.32–0.7 with the AGREEprep, indicating from poor to suitable greenness. Since only the sample pretreatment is evaluated method, the use of direct desorption in the analytical system allows greener marks, decreasing solvents, waste, and risks [35,37,38]. On the contrary, the use of liquid chromatography with diode array detection (LC-DAD) decreases the final greenness since an elution solvent is used and highly penalized even at low quantities [36,39–42]. As in the SPE Section, the use of derivatizing agents is penalized, and also, when rinsing steps of the fibers are conducted with organic solvents to prepare for the next cycle [36,39,40].

SPMS is a metric exclusively focused on evaluating the sample preparation procedure, which confirms the green aspects of all the SPME methods evaluated here (scores of 5-68-8.53). The high energy consumption of MS, solvents in the mobile phases, and/or off-line procedures are not decreasing the final sustainability score. In SPMS, the higher marks are attributed to miniaturized procedures with a low number of steps and low energy consumption. Hence, the enhancement of greenness using cellulose-based materials [39], automation [35], and high-throughput [36] is remarkable. Most of the procedures evaluated in Table 3 require low temperatures and extraction times, increasing their respective green scores. The reusability of SPME fibers is one of the main sustainable aspects of the methodology and the multi-sample treatment is highly encouraged, represented by an asterisk and bold surrounding, respectively. In this latter point, the use of on-line desorption [35,40–42] hinders the simultaneous processing of multiple samples since only one sample at a time can be desorbed at the analytical equipment.

Regarding the application of HEXAGON to the SPME and related procedures, values of s_{av} ranging from 0.86 to 1.83 were obtained, providing an appropriate performance. The best results have been obtained for those methodologies that use thermal desorption, due to the absence of solvent/reagents consumption and waste generation. Once again, differences related to carbon footprint, and the estimation of the annual economic cost of the analytical procedures were observed. Methods using LC-tandem mass spectrometry (LC-MS/MS) and GC–MS/MS are penalized versus those using simple and less expensive instrumentation such as LC-DAD when all techniques are suitable for solving the analytical problem. Finally, it should be highlighted that the combined use of the miniaturized LC method with on-line in-tube (IT)-SPME for analyzing drugs in oral fluids [41] provided good results regarding greenness and sustainability with a s_{av} = 1.08.

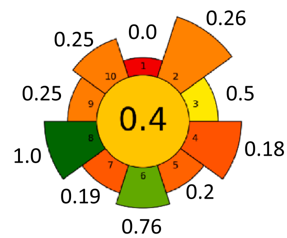
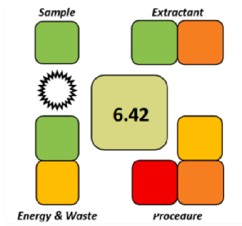
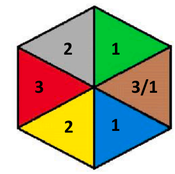
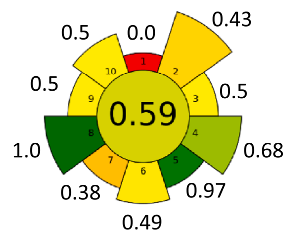
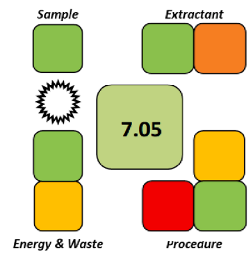
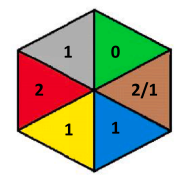
In general, an update of the different green metrics is highly desired, which includes critical aspects regarding the functionality of the technique to solve the proposed analytical problem (fit-for-purpose), and furthermore, addressing the underlying real problem. In the words of

Table 3
Green evaluation of recently reported SPME-based methods for the analysis of drug abuse in biofluids (AGREEprep, analytical greenness metric for sample preparation; SPMS, sample preparation metric of sustainability; HEXAGON; Ref., reference).

Analytes	SPME device & Method	Reagents and sample[1]	Green assessment: AGREEprep	Green assessment: SPMS	Green assessment: HEXAGON	Ref.
1 benzodiazepine, 2 opioids, and others	SPME-PDMS/DVB-fiber-GC/MS	1500 µL serum and plasma				[35]
110 doping substances	SPME-C18-blades-LC/MS (Orbitrap)	1250 µL of MeOH; 500 µL of ACN; 1200 µL of urine				[36]
9 Illicit drugs	SPME-PDMS/DVB-thin film-MS	1.5 mL of beverages, urine, and plasma				[37]
7 drugs of abuse and pesticides	SPME-Mesh strand-PAN/HLB -MS (DART)	1.5 mL for grape juice and milk and 0.75 mL for oral fluid				[38]
5 antidepressants	Sol-gel graphene-cellulose FPSE-LC-DAD	2250 µL ACN and 2250 µL MeOH, 500 µL of urine				[39]
3 amines	Carbowax-templated resin SPME fibers HPLC-FLD	1 mL of 25 mM FMOC, 0.5 mL borate buffer, 10.2 mL ACN 25 mL river/tap water				[40]

(continued on next page)

Table 3 (*continued*)

3 amphetamine enantiomers	Carbowax-templated resin SPME fibers HPLC-FLD	0.5 mL borate buffer, 0.25 mL chiral derivatization agent (OPA-NAC) 1.25 mL of urine and pharmaceutical tablets			 s_{av}=1.83	[41]
3 amphetamines	PDMS@SWCNTs IT-SPME cLC-FLD	250 µL carbonate buffer, 250 µL 0.1 mM FMOC solutions 125 µL			 s_{av}=1.08	[42]

Abbreviations: PDMS, polydimethylsiloxane; DVB, divinylbenzene; GC/MS, gas chromatography coupled to mass spectrometry detection; LC, liquid chromatography; PAN/HLB, polyacrylonitrile hydrophilic-lipophilic balance; SPME, solid-phase microextraction; FPSE, fabric phase sorptive extraction; SBSE, stir bar sorptive extraction; DAD, diode array detection; MCN, magnetic carbon nanoparticle; FMOC, 9-fluorenylmethyl chloroformate; FLD, fluorescence detector; OPA-NAC, o-phthaldialdehyde–N-acetyl-L-cysteine; SWCNTs, single-wall carbon nanotubes; IT-SPME cLC, in-tube SPME capillary LC.
[1]The chemicals used in the synthesis process and in posterior analysis with analytical instrumentation have not been considered for comparison purposes.

Prof. Koel [43], scientists should develop functional methods tailored to specific purposes that are as economical as possible in terms of the amount of unnecessary data, material, reagents, waste, energy, costs, and analysis time. Using the example of the analysis of drugs in biological fluids or environmental/wastewater samples, scientists should consider how many analytes need to be analyzed to determine if a driver is under the influence of drugs or to estimate drug consumption in a city. If the answer suggests that a multianalyte method with the appropriate selectivity and/or sensitivity is needed, single-analyte methods should be heavily penalized, even if the method itself is sustainable and green, its application in such cases is not suitable. In this sense, HEXAGON considers certain aspects of the methodology, including calibration and validation procedures (figures of merit) in the evaluation of its sustainability, being aligned with the paradigm that was later proposed under the name of White Analytical Chemistry [4].

3.3. Liquid phase microextraction (LPME)

Liquid–liquid extraction (LLE), is based on the partition coefficient of analytes between two immiscible solvents. LLE is very simple and requires no complex analytical instrumentation. However, it is considered time-consuming, difficult to automate, labor intensive, and with high consumption of organic solvents [44]. The use of large amounts of solvents causes environmental pollution, health hazards, and increased operational costs due to waste management [45]. To overcome the aforementioned drawbacks of LLE, liquid-phase microextraction (LPME) was developed. LPME is a miniaturized sample treatment procedure based on LLE, in which only several µL of solvent are required to concentrate analytes instead of hundreds of mL required in conventional LLE. Dispersive liquid-liquid microextraction (DLLME) is one of the most usually employed LPME techniques that uses a few µL of extraction solvent in combination with a dispersive solvent [46]. The use of dispersive solvents generates a cloudy solution formed by fine droplets that improve the mass-transfer processes of analytes from the aqueous phase to the organic phase, enhancing extraction efficiency and reducing extraction time. As an alternative to DLLME, extraction of the analytes by micro volumes of extraction solvent in an LPME configuration, without the use of a disperser solvent, has been also proposed [47].

In addition to the reduction of the amount of solvent consumption associated with analyte extraction, the use of alternative green solvents with improved features has been one of the top priorities. Green solvents such as ILs and DESs, among others, can be employed in extraction approaches to replace conventional solvents typically obtained from fossil sources, improving the "greenness" of the analytical procedure [48].

Table 4 describes representative examples of LPMD procedures and their green evaluation using several metrics [49–53]. Three different metrics have been used to evaluate the greenness of these procedures.

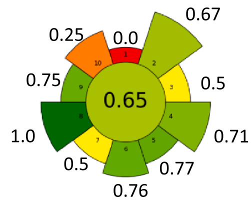
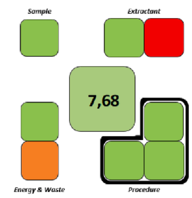
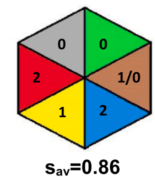
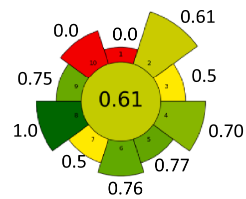
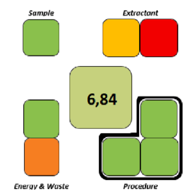
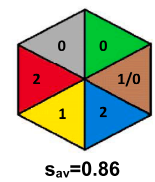
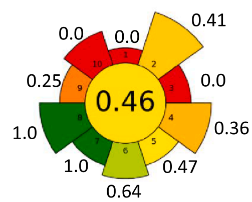
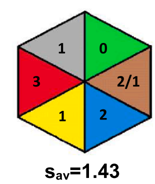
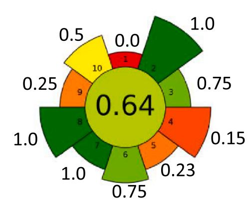
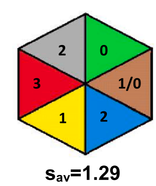
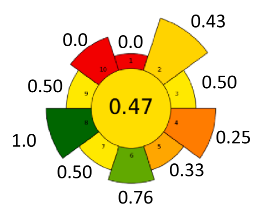
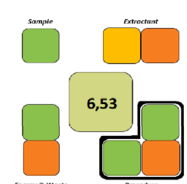
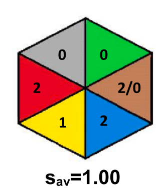
All the methods included in Table 4 scored in the range of 0.46–0.65 with the AGREEprep metric, indicating moderate greenness. Significant scores were attained in key aspects, particularly aspects regarding the amount of reagents (and solvents), integration and automation, and energy consumption, respectively. The advantages of these methodologies rely on their simplicity, short sample treatment times, and their ability to handle simultaneously multiple samples. On the other hand, certain noteworthy aspects exerting a negative influence on the overall score include the necessity for laboratory-based treatment post-collection (aspect 1) and the utilization of large sample volumes (aspect 5).

Comparing the methodologies that used alternative solvents, it can be noted that the use of dangerous solvents, such as dichloromethane or chloroform, leads to penalization in aspects number 2, 3, and 10. It should be also highlighted that the use of large volumes of urine as biological fluid compared to reduced volumes of saliva increases penalization of aspects 4 and 5, despite its relatively high availability and non-invasive sampling

Similar results were obtained using SPMS as a GAC metric (scores of 5.37–8.11). As it has been aforementioned, in SPMS, the importance of sample volume is minimized, and higher relevance is attributed to miniaturization, the use of low amounts of natural solvents, the integration of steps, and low energy consumption. As it can be observed, all the procedures evaluated in Table 4 require low temperatures and extraction times.

The evaluation of the selected LPME methodologies by the HEXAGON metric provided results ranging from 0.86 to 1.43. The best results have been obtained for those methodologies using less toxic reagents,

Table 4
Green evaluation of recently reported DLLME and LPME-based methods for the analysis of drug abuse in biofluids (AGREEprep, analytical greenness metric for sample preparation; SPMS, sample preparation metric of sustainability; HEXAGON; Ref., reference).

Analytes	Analytical method	Reagents and sample	Green assessment			Ref.
			AGREEprep	SPMS	HEXAGON	
MDPV	LPME and IMS	100 µL of $CHCl_3$ + 500 µL saliva sample				[49]
MDMA	LPME and IMS	100 µL of $CHCl_3$ + 50 NaOH 0.1 M + 500 µL saliva sample				[50]
12 opiates, amphetamine and their derivates, cocaine and its metabolites	DLLME and CE-TOF-MS	1 mL of 1 M NaOH; 1.4 mL i-PrOH + 0.6 mL CH_2Cl_2 and 4 mL urine				[51]
20 pharmaceuticals	NADES-DLLME and LC-MS/MS	300 µL of NADES (L-menthol:octanoic acid) + 250 µL i-PrOH and 20 mL urine				[52]
Methamphetamine	IL-DLLME and HPLC	0.5 mL of methanol containing [C_8MIM][PF_6] (50 µL) + 10 mL sample				[53]

Abbreviations: LPME, liquid-phase microextraction; IMS, ion mobility spectrometry; DLLME, dispersive liquid-liquid microextraction; CE-TOF-MS, capillary electrophoresis time-of-flight MS; NADES, natural deep eutectic solvents; LC-MS/MS, liquid chromatography-mass tandem; IL, ionic liquid; HPLC, high-performance liquid chromatography.
[1]The chemicals used in the synthesis process and in posterior analysis with analytical instrumentation have not been considered for comparison purposes.

with a reduced carbon footprint and simple-to-use analytical instrumentation, such as ion mobility spectrometry or conventional LC.

4. Conclusions and future trends

Sample treatment techniques have now evolved towards miniaturized procedures that use engineered sorbents with advanced properties and natural and/or less toxic solvents than traditional alternatives. In the frame of drug analysis in biological specimens, SPE, SPME, and LPME are the most used sample treatment techniques. Undoubtedly, microextraction techniques including SPME and LPME are greener than conventional SPE in many perspectives such as consumption of solvent, energy efficiency, and waste generation, and, thus, the average metrics for both techniques were better than those obtained for SPE. Greening analytical methodologies involve more than just reducing solvent usage or using bio-inspired sorbents; it requires comprehensive efforts to minimize environmental impact and promote sustainability throughout the procedure, implies a reduction of the number of steps, analysis time, and costs associated with analysis, and involves the simultaneous analysis of samples and also multianalyte determinations. These tools serve as a guide and encourage scientists to develop the most appropriate methodologies by considering both analytical features and sustainability. The different proposed green metrics have different concepts and criteria, their own advantages and disadvantages, but in all cases provide significant data for comparative purposes.

The number of papers that have applied each of the different green metrics published up to date is continuously increasing, and it is expected that this trend will continue in the same way. Moreover, the number of metrics is also expected to continue to increase shortly, reflecting the high level of interest in this topic among the scientific community.

For future perspectives, the green metrics should consider additional environmental factors such as water usage, economic factors such as cost-effectiveness or viability, and analytical performance features. By incorporating these metrics into a regulatory framework, industries and laboratories can select/adopt greener and more sustainable analytical procedures. To facilitate this, the use of these metrics should be as easy as possible, achieved through the creation of apps, Excel files, or web pages, where users can easily enter the necessary values and obtain the characteristic green rating. On the other hand, the creation of more and more metrics can play a counterproductive role, fostering bewilderment and a sense of arbitrariness among users. Therefore, it would be useful to harmonize the different criteria into a single and, easy-to-apply metric proposal. In this way, the application of a single tool that incorporates the most significant aspects of method greenness would greatly facilitate the comparison between different treatments and analytical techniques.

CRediT authorship contribution statement

Héctor Martínez-Pérez-Cejuela: Writing – review & editing, Writing – original draft, Conceptualization. **Emanuela Gionfriddo:** Writing – review & editing, Writing – original draft, Conceptualization. **Pilar Campíns-Falcó:** Writing – review & editing, Writing – original draft, Conceptualization. **José Manuel Herrero-Martínez:** Writing – review & editing, Writing – original draft, Conceptualization. **Sergio Armenta:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

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

Acknowledgements

PID2021-125459OB-I00 and PID2021-124554NB-I00 funded by MCIN/AEI/10.13039/501100011033 and by “ERDF A way of making Europe” by the European Union. This research was also funded by the CIAICO/2022/183 grant and PROMETEO Program 2020/078 from the Generalitat Valenciana. The authors also thank the State University of New York at Buffalo and the UB Research Foundation for funding.

References

[1] L. Nováková, Advances in sample preparation for biological fluids, LCGC Int. 29 (2016) 9–15.
[2] G.M. de la, S. A, Green analytical chemistry: theory & practice, in: B. Damià (Ed.), Compr. Anal. Chem. Ser., Elsevier, 2011.
[3] A. Gałuszka, Z. Migaszewski, J. Namieśnik, The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices, TrAC Trends Anal. Chem. 50 (2013) 78–84, https://doi.org/10.1016/j.trac.2013.04.010.
[4] P.M. Nowak, R. Wietecha-Posłuszny, J. Pawliszyn, White analytical chemistry: an approach to reconcile the principles of green analytical chemistry and functionality, TrAC Trends Anal. Chem. 138 (2021) 116223, https://doi.org/10.1016/j.trac.2021.116223.
[5] M.M. Abdelrahman, Green analytical chemistry metrics and life-cycle assessment approach to analytical method development, in: M.H. El-Maghrabey, V. Sivasankar, R.N. El-Shaheny (Eds.), Green Chem. Anal. Sample Prep. Proced. Instrumentation, Data Metrics, Sustain, Springer International Publishing, Cham, 2022, pp. 29–99, https://doi.org/10.1007/978-3-030-96534-1_2.
[6] M. Shi, X. Zheng, N. Zhang, Y. Guo, M. Liu, L. Yin, Overview of sixteen green analytical chemistry metrics for evaluation of the greenness of analytical methods, TrAC Trends Anal. Chem. 166 (2023) 117211, https://doi.org/10.1016/j.trac.2023.117211.
[7] M. Sajid, J. Płotka-Wasylka, Green analytical chemistry metrics: a review, Talanta 238 (2022) 123046, https://doi.org/10.1016/j.talanta.2021.123046.
[8] M.B. Swanson, G.A. Davis, L.E. Kincaid, T.W. Schultz, J.E. Bartmess, S.L. Jones, E. L. George, A screening method for ranking and scoring chemicals by potential human health and environmental impacts, Environ. Toxicol. Chem. 16 (1997) 372–383, https://doi.org/10.1002/etc.5620160237.
[9] L.H. Keith, L.U. Gron, J.L. Young, Green analytical methodologies, Chem. Rev. 107 (2007) 2695–2708, https://doi.org/10.1021/cr068359e.
[10] R. Hartman, R. Helmy, M. Al-Sayah, C.J. Welch, Analytical method volume intensity (AMVI): a green chemistry metric for HPLC methodology in the pharmaceutical industry, Green Chem. 13 (2011) 934–939, https://doi.org/10.1039/C0GC00524J.
[11] A. Gałuszka, Z.M. Migaszewski, P. Konieczka, J. Namieśnik, Analytical Eco-Scale for assessing the greenness of analytical procedures, TrAC Trends Anal. Chem. 37 (2012) 61–72, https://doi.org/10.1016/j.trac.2012.03.013.
[12] J. Płotka-Wasylka, A new tool for the evaluation of the analytical procedure: green analytical procedure index, Talanta 181 (2018) 204–209, https://doi.org/10.1016/j.talanta.2018.01.013.
[13] P.M. Nowak, P. Kościelniak, What color is your method? Adaptation of the RGB additive color model to analytical method evaluation, Anal. Chem. 91 (2019) 10343–10352, https://doi.org/10.1021/acs.analchem.9b01872.
[14] A. Ballester-Caudet, P. Campíns-Falcó, B. Pérez, R. Sancho, M. Lorente, G. Sastre, C. González, A new tool for evaluating and/or selecting analytical methods: summarizing the information in a hexagon, TrAC Trends Anal. Chem. 118 (2019) 538–547, https://doi.org/10.1016/j.trac.2019.06.015.
[15] M.B. Hicks, W. Farrell, C. Aurigemma, L. Lehmann, L. Weisel, K. Nadeau, H. Lee, C. Moraff, M. Wong, Y. Huang, P. Ferguson, Making the move towards modernized greener separations: introduction of the analytical method greenness score (AMGS) calculator, Green Chem. 21 (2019) 1816–1826, https://doi.org/10.1039/C8GC03875A.
[16] N. Manousi, W. Wojnowski, J. Płotka-Wasylka, V. Samanidou, Blue applicability grade index (BAGI) and software: a new tool for the evaluation of method practicality, Green Chem. 25 (2023) 7598–7604, https://doi.org/10.1039/D3GC02347H.
[17] F. Pena-Pereira, W. Wojnowski, M. Tobiszewski, AGREE—analytical GREEnness metric approach and software, Anal. Chem. 92 (2020) 10076–10082, https://doi.org/10.1021/acs.analchem.0c01887.
[18] W. Wojnowski, M. Tobiszewski, F. Pena-Pereira, E. Psillakis, AGREEprep – Analytical greenness metric for sample preparation, TrAC Trends Anal. Chem. 149 (2022) 116553, https://doi.org/10.1016/j.trac.2022.116553.
[19] R. González-Martín, A. Gutiérrez-Serpa, V. Pino, M. Sajid, A tool to assess analytical sample preparation procedures: sample preparation metric of sustainability, J. Chromatogr. A. 1707 (2023) 464291, https://doi.org/10.1016/j.chroma.2023.464291.
[20] A. Ballester-Caudet, R. Navarro-Utiel, I. Campos-Hernández, P. Campíns-Falcó, Evaluation of the sample treatment influence in green and sustainable assessment of liquid chromatography methods by the HEXAGON tool: sulfonate-based dyes determination in meat samples, Green Anal. Chem. 3 (2022) 100024, https://doi.org/10.1016/j.greeac.2022.100024.
[21] A. Sorribes-Soriano, F.A. Esteve-Turrillas, S. Armenta, A. Montoya, J.M. Herrero-Martínez, M. de la Guardia, Magnetic molecularly imprinted polymers for the selective determination of cocaine by ion mobility spectrometry, J. Chromatogr. A 1545 (2018) 22–31, https://doi.org/10.1016/j.chroma.2018.02.055.
[22] A. Sorribes-Soriano, A. Valencia, F.A. Esteve-Turrillas, S. Armenta, J.M. Herrero-Martínez, Development of pipette tip-based poly(methacrylic acid-co-ethylene glycol dimethacrylate) monolith for the extraction of drugs of abuse from oral fluid samples, Talanta 205 (2019) 120158, https://doi.org/10.1016/j.talanta.2019.120158.
[23] H. Martínez-Pérez-Cejuela, M. Conejero, P. Amorós, J. El Haskouri, E.F. Simó-Alfonso, J.M. Herrero-Martínez, S. Armenta, Metal-organic frameworks as promising solid-phase sorbents for the isolation of third-generation synthetic cannabinoids in biological samples, Anal. Chim. Acta 1246 (2023) 340887, https://doi.org/10.1016/j.aca.2023.340887.
[24] H. Martínez-Pérez-Cejuela, P. García-Atienza, E.F. Simó-Alfonso, J.M. Herrero-Martínez, S. Armenta, Micro-paper-based analytical device decorated with metal-organic frameworks for the assay of synthetic cannabinoids in oral fluids coupled to ion mobility spectrometry, Microchim. Acta 190 (2023) 271, https://doi.org/10.1007/s00604-023-05844-6.
[25] A. Sorribes-Soriano, R. Arráez-González, F.A. Esteve-Turrillas, S. Armenta, J. M. Herrero-Martínez, Development of a molecularly imprinted monolithic polymer disk for agitation-extraction of ecgonine methyl ester from environmental water, Talanta 199 (2019) 388–395, https://doi.org/10.1016/j.talanta.2019.02.077.
[26] P. Campíns Falcó, C. Molins Legua, A. Sevillano Cabeza, R. Porras Serrano, Derivatization of amphetamine and methamphetamine With 1,2-naphthoquinone 4-sulfonic acid into solid-phase extraction cartridges. Determination of amphetamine in pharmaceutical and urine samples, Analyst 122 (1997) 673–677, https://doi.org/10.1039/A701134B.
[27] J. Navarro-Sánchez, A.I. Argente-García, Y. Moliner-Martínez, D. Roca-Sanjuán, D. Antypov, P. Campíns-Falcó, M.J. Rosseinsky, C. Martí-Gastaldo, Peptide metal-organic frameworks for enantioselective separation of chiral drugs, J. Am. Chem. Soc. 139 (2017) 4294–4297, https://doi.org/10.1021/jacs.7b00280.
[28] R. Herráez-Hernández, P. Campíns-Falcó, A. Sevillano-Cabeza, On-line derivatization into Pre columns for the determination of drugs by liquid chromatography and column switching: determination of amphetamines in urine, Anal. Chem. 68 (1996) 734–739, https://doi.org/10.1021/ac9505076.
[29] R.P. Belardi, J.B. Pawliszyn, The application of chemically modified fused silica fibers in the extraction of organics from water matrix samples and their rapid

transfer to capillary columns, Water Qual. Res. J. 24 (1989) 179–191, https://doi.org/10.2166/wqrj.1989.010.

[30] V. Jalili, A. Barkhordari, A. Ghiasvand, A comprehensive look at solid-phase microextraction technique: a review of reviews, Microchem. J. 152 (2020) 104319, https://doi.org/10.1016/j.microc.2019.104319.

[31] W. Zhou, M.N. Wieczorek, H. Javanmardi, J. Pawliszyn, Direct solid-phase microextraction-mass spectrometry facilitates rapid analysis and green analytical chemistry, TrAC Trends Anal. Chem. 166 (2023) 117167, https://doi.org/10.1016/j.trac.2023.117167.

[32] J.M. Kokosa, A. Przyjazny, Green microextraction methodologies for sample preparations, Green Anal. Chem. 3 (2022) 100023, https://doi.org/10.1016/j.greeac.2022.100023.

[33] A. Napylov, N. Reyes-Garces, G. Gomez-Rios, M. Olkowicz, S. Lendor, C. Monnin, B. Bojko, C. Hamani, J. Pawliszyn, D. Vuckovic, In vivo solid-phase microextraction for sampling of oxylipins in brain of awake, moving rats, Angew. Chem. Int. Ed. 59 (2020) 2392–2398, https://doi.org/10.1002/anie.201909430.

[34] A. Roszkowska, M. Yu, V. Bessonneau, L. Bragg, M. Servos, J. Pawliszyn, Metabolome profiling of fish muscle tissue exposed to benzo[a]pyrene using in vivo solid-phase microextraction, Environ. Sci. Technol. Lett. 5 (2018) 431–435, https://doi.org/10.1021/acs.estlett.8b00272.

[35] N.H. Godage, E. Gionfriddo, Biocompatible SPME coupled to GC/MS for analysis of xenobiotics in blood plasma, J. Chromatogr. B 1203 (2022) 123308, https://doi.org/10.1016/j.jchromb.2022.123308.

[36] E. Boyaci, K. Gorynski, A. Rodriguez-Lafuente, B. Bojko, J. Pawliszyn, Introduction of solid-phase microextraction as a high-throughput sample preparation tool in laboratory analysis of prohibited substances, Anal. Chim. Acta 809 (2014) 69–81, https://doi.org/10.1016/j.aca.2013.11.056.

[37] M.F. Mirabelli, E. Gionfriddo, J. Pawliszyn, R. Zenobi, Fast screening of illicit drugs in beverages and biological fluids by direct coupling of thin film microextraction to dielectric barrier discharge ionization-mass spectrometry, Analyst 144 (2019) 2788–2796, https://doi.org/10.1039/C8AN02448K.

[38] G.A. Gómez-Ríos, T. Vasiljevic, E. Gionfriddo, M. Yu, J. Pawliszyn, Towards on-site analysis of complex matrices by solid-phase microextraction-transmission mode coupled to a portable mass spectrometer via direct analysis in real time, Analyst 142 (2017) 2928–2935, https://doi.org/10.1039/C7AN00718C.

[39] A. Lioupi, A. Kabir, K.G. Furton, V. Samanidou, Fabric phase sorptive extraction for the isolation of five common antidepressants from human urine prior to HPLC-DAD analysis, J. Chromatogr. B 1118–1119 (2019) 171–179, https://doi.org/10.1016/j.jchromb.2019.04.045.

[40] R. Herráez-Hernández, C. Cháfer-Pericás, J. Verdú-Andrés, P. Campíns-Falcó, An evaluation of solid phase microextraction for aliphatic amines using derivatization with 9-fluorenylmethyl chloroformate and liquid chromatography, J. Chromatogr. A 1104 (2006) 40–46, https://doi.org/10.1016/j.chroma.2005.11.121.

[41] C. Cháfer-Pericás, P. Campíns-Falcó, R. Herráez-Hernández, Application of solid-phase microextraction combined with derivatization to the enantiomeric determination of amphetamines, J. Pharm. Biomed. Anal. 40 (2006) 1209–1217, https://doi.org/10.1016/j.jpba.2005.09.025.

[42] A. Argente-García, Y. Moliner-Martínez, E. López-García, P. Campíns-Falcó, R. Herráez-Hernández, Application of carbon nanotubes modified coatings for the determination of amphetamines by In-tube solid-phase microextraction and capillary liquid chromatography, Separations 3 (2016) 7, https://doi.org/10.3390/chromatography3010007.

[43] M. Koel, Do we need green analytical chemistry? Green Chem. 18 (2016) 923–931, https://doi.org/10.1039/c5gc02156a.

[44] R.E. Clement, C. Hao, Liquid–liquid extraction: basic principles and automation, Compr. Sampl. Sample Prep. 2 (2012) 51–63, https://doi.org/10.1016/B978-0-12-381373-2.00063-6.

[45] A. Sarafraz-Yazdi, A. Amiri, Liquid-phase microextraction, TrAC Trends Anal. Chem. 29 (2010) 1–14, https://doi.org/10.1016/j.trac.2009.10.003.

[46] M. Sajid, Dispersive liquid-liquid microextraction: evolution in design, application areas, and green aspects, TrAC Trends Anal. Chem. 152 (2022) 116636, https://doi.org/10.1016/j.trac.2022.116636.

[47] J.M. Kokosa, Selecting an extraction solvent for a greener liquid phase microextraction (LPME) mode-based analytical method, TrAC Trends Anal. Chem. 118 (2019) 238–247, https://doi.org/10.1016/j.trac.2019.05.012.

[48] S. Armenta, F.A. Esteve-Turrillas, S. Garrigues, M. de la Guardia, Alternative green solvents in sample preparation, Green Anal. Chem. 1 (2022) 100007, https://doi.org/10.1016/j.greeac.2022.100007.

[49] M. de las N. Peiró, S. Armenta, S. Garrigues, M. de la Guardia, Determination of 3,4-methylenedioxypyrovalerone (MDPV) in oral and nasal fluids by ion mobility spectrometry, Anal. Bioanal. Chem. 408 (2016) 3265–3273, https://doi.org/10.1007/s00216-016-9395-1.

[50] S. Armenta, S. Garrigues, M. de la Guardia, J. Brassier, M. Alcalà, M. Blanco, Analysis of ecstasy in oral fluid by ion mobility spectrometry and infrared spectroscopy after liquid–liquid extraction, J. Chromatogr. A. 1384 (2015) 1–8, https://doi.org/10.1016/j.chroma.2015.01.036.

[51] I. Kohler, J. Schappler, T. Sierro, S. Rudaz, Dispersive liquid–liquid microextraction combined with capillary electrophoresis and time-of-flight mass spectrometry for urine analysis, J. Pharm. Biomed. Anal. 73 (2013) 82–89, https://doi.org/10.1016/j.jpba.2012.03.036.

[52] P. García-Atienza, S. Armenta, The use of deep eutectic solvent-liquid phase microextraction as green analytical sample treatment for the analysis of drugs in urine, Green Anal. Chem. (2024). Accepted f.

[53] R. Wang, X. Qi, L. Zhao, S. Liu, S. Gao, X. Ma, Y. Deng, Ionic-liquid-based dispersive liquid–liquid microextraction coupled with high-performance liquid chromatography for the forensic determination of methamphetamine in human urine, J. Sep. Sci. 39 (2016) 2444–2450, https://doi.org/10.1002/jssc.201600170.