



# Greening sample preparation: An overview of cutting-edge contributions

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Sample preparation is a paramount step of the analytical process that has undergone significant improvements within the framework of green chemistry. The present review reports on cutting-edge advances toward greener analytical methods involving sample preparation. Remarkable contributions published in the last two years on the implementation of greener solvents and adsorbents in sample preparation approaches, integration of unitary steps, miniaturization, and automation are discussed. Concluding remarks and additional considerations for further improvements are also addressed.

## Addresses

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## Introduction

The twelve principles of green chemistry (GC) paved the pathway toward greener chemical processes [1,2]. However, a single GC principle, aimed at the development of analytical methods for real-time analysis of pollution prevention, is directly related to analytical chemistry (11th principle), and the fact that certain paramount aspects of analytical methodologies were vaguely related with GC principles led to more specific principles of green analytical chemistry (GAC), as shown in Table 1 [3,4]. Most of GAC principles are directly or indirectly related to sample preparation that, in light of the 1st GAC principle [3], should be avoided whenever possible. Nevertheless, sample preparation approaches have remarkably improved their greenness profile in recent years.

This work reports on relevant advances achieved in the last two years in the field. Selected contributions toward greener methodologies involving sample pretreatment

approaches are discussed, with focus on the implementation of advanced materials and neoteric solvents, as well as on the integration of steps, miniaturization, and automation. In addition, further considerations toward greener analytical methods involving sample preparation are presented.

## Recent advances in sample preparation

In this section, the implementation of materials in sample preparation, integration of unitary steps, automation, and miniaturization in the period under review are discussed, and selected works are presented as illustrative examples. It should be noted that selected contributions have been deliberately associated with a single representative category for the sake of avoiding redundancy.

## Implementation of novel materials in sample preparation

The implementation of greener materials in analytical methods can be directly or indirectly associated with different GAC principles [3], including those that promote the replacement or removal of toxic reagents (11th principle) and the implementation of renewable reagents (10th principle), which can also be aligned with the 12th principle if safety conditions are enhanced. The use of renewable feedstocks and biobased solvents as replacement to commonly used petroleum-derived solvents is also promoted toward greener extraction processes [5]. Some recent examples can be highlighted in this sense, including, among others, Cyrene, a biobased solvent that has shown excellent extractability for bioactive compounds [6], or diethyl carbonate, used for enrichment of chlorophenols [7]. Alternatively, solvents such as deep eutectic solvents (DESs) and switchable polarity solvents (SPSs) are being used in sample preparation. Both hydrophilic and hydrophobic DESs are used in liquid-phase microextraction (LPME). Recent applications of hydrophilic natural DESs include, for instance, their application in microwave-assisted extraction for elemental analysis of plants [8]. Hydrophobic DESs have been introduced more recently [9,10]. Thus, mixtures of coumarin and thymol have demonstrated much potential for extraction of ionizable compounds with a wide polarity window [11]. Besides, DESs decomposition has been exploited for extraction of hydrophobic compounds [12]. SPSs reversibly modify their properties abruptly in response to an external stimulus, being appealing for separation purposes. Some SPSs, including tertiary amines (e.g. N,N-

Table 1

## Principles of green analytical chemistry [3].

Principle number	Principles of green analytical chemistry
1	Direct analytical techniques should be applied to avoid sample treatment.
2	Minimal sample size and minimal number of samples are goals.
3	<i>In situ</i> measurements should be performed.
4	Integration of analytical processes and operations saves energy and reduces the use of reagents.
5	Automated and miniaturized methods should be selected.
6	Derivatization should be avoided.
7	Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided.
8	Multianalyte or multiparameter methods are preferred versus methods using one analyte at a time.
9	The use of energy should be minimized.
10	Reagents obtained from renewable sources should be preferred.
11	Toxic reagents should be eliminated or replaced.
12	The safety of the operator should be increased.

dimethylcyclohexylamine, N,N-diethylethanamine, or N,N-benzyl dimethylamine), have been proposed for extracting target compounds from environmental samples [13] or biological fluids [14,15]. However, addition of concentrated NaOH (10–20 M) was required to trigger phase separation. Recent reviews devoted to the application of greener solvents in analytical chemistry can be found [16,17].

A wide number of contributions have focused on the development of advanced materials that can overcome certain drawbacks of commercially available adsorbents, including thermal and mechanical stability, robustness, and versatility. Alternative biosorbents and biocomposites, such as agarose gel impregnated with fructose corn syrup [18] or halloysite–chitosan [19], have been proposed. Recent related reviews can be consulted for more details [20,21]. Recycled natural materials and materials obtained by reagentless procedures have also demonstrated their applicability as adsorbents. Thus, recycled cork wine stoppers have been proposed as floating adsorbents for paraben extraction from waters, showing a lifetime of 5 extractions per desorption cycles [22]. Furthermore, the reagentless preparation of solid-phase microextraction (SPME) fibers by formation of carbonaceous nanoparticles using a candle flame allowed for determination of long-term retention of mercury (<5% loss after 9 days) [23].

### Integration of unitary steps

The 4th principle of GAC focuses on integrating processes and operations [3]. Benefits include lower use of

reagents and energy, simplification, reduced manipulation, and enhanced sample throughput. Thus, in-drop [24] and thin film configuration [24,25] allow integrating sample cleanup, extraction, enrichment, and sensing of target analytes in a straightforward manner. Particularly, cellulose substrates, widely used in paper-based analytical devices [26], have demonstrated their convenience as platforms for simultaneous enrichment and sensing of analytes with noninstrumental detection. Nanomaterials have been used for optical sensing [24,25], although natural indicators (e.g. butterfly pea flower extract) [27] have also found applicability in these systems.

Analytical approaches that enable real-time measurements to be performed are particularly advantageous [28], in accordance with the recommendation to carry out *in situ* analysis whenever possible (3rd principle) [3]. Notwithstanding this, the integration of sample preparation and transportation to the laboratory, that is, in-transit sample preparation, can be particularly beneficial in clinical analysis. Accordingly, in-transit electroextraction of pharmaceuticals from blood involving polymer inclusion membranes enabled the analysis at arrival in a central laboratory with no additional sample processing [29].

Other remarkable approaches include an ‘all-in-one’ paper-based sampling chip for in-device protein analysis that integrates immunocapture, protein reduction, alkylation, and tryptic digestion [30] before liquid chromatography–mass spectrometry (MS) analysis, a downsized tip-based method that enabled automated isolation of proteins and on-tip protease digestion for urinary proteomics by liquid chromatography–MS/MS [31], or a spintip-based approach for glycoproteome analysis of *in vivo* tissues [32].

### Miniaturization and automation

The 5th principle of GAC refers to the preference for automated and miniaturized methods [3]. Many efforts have been devoted to the development of analytical methods with remarkable levels of miniaturization and automation. Downsized sample preparation approaches have significantly contributed to a wide reduction of solvent consumption, thus receiving a very favorable acceptance. Thus, SPME minitips have been proposed to process sample volumes of as small as 1  $\mu\text{L}$ , in accordance with the 2nd principle, being particularly beneficial for extraction of different compounds present in biofluids, tissues, and cells [33]. Readers are referred to a recent review on the topic for further details [34].

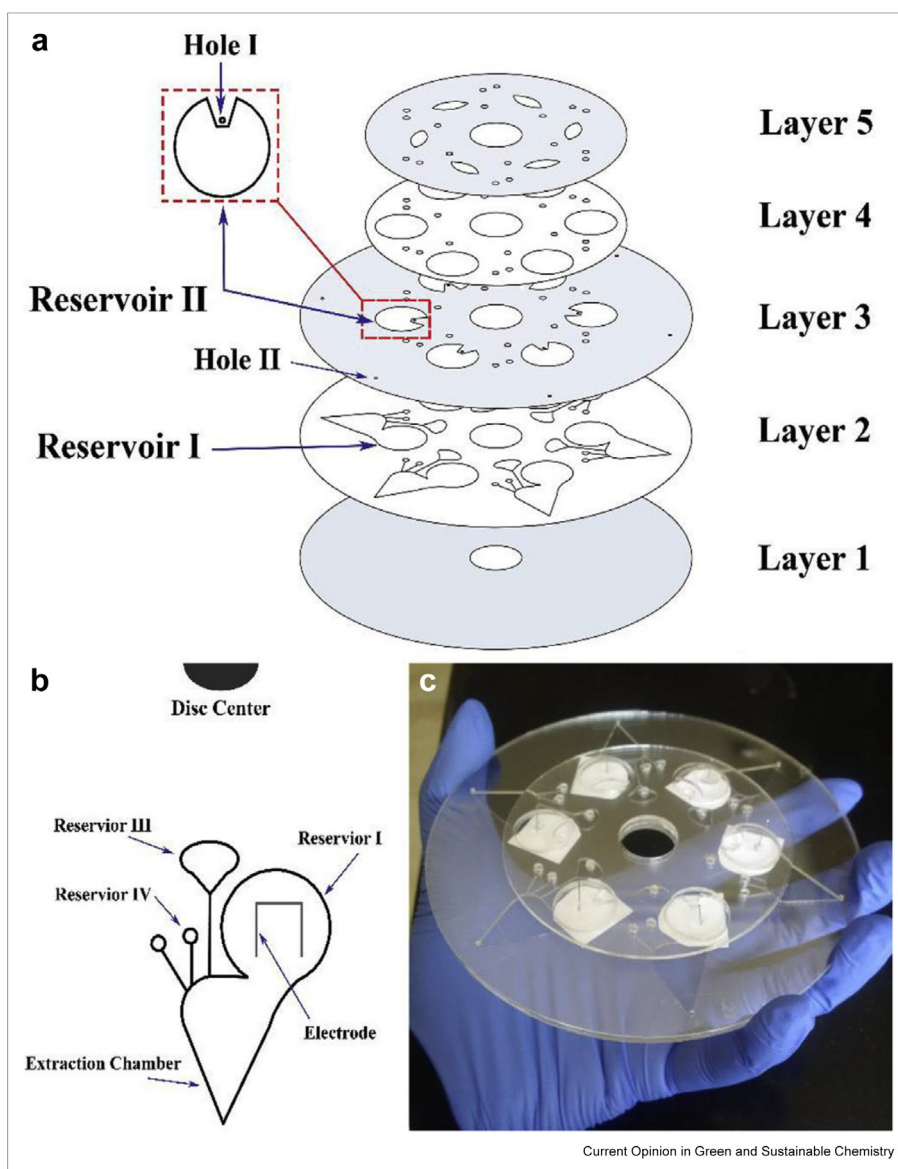
Microfluidic systems enable performing several unitary steps in a miniaturized fashion, in agreement with the 4th and 5th GAC principles. These systems have ability to reproducibly dispense highly reduced volumes.

Remarkable contributions involving LPME approaches in microfluidic systems have been reported. For instance, a microfluidic chip-based LPME has been designed for determining fluoroquinolones in urine, reaching extraction efficiencies of 35–62% within 7 min using highly reduced sample and extractant volumes (10 and 4  $\mu\text{L}$ , respectively) [35]. Furthermore, a lab-on-a-disc device has been designed to perform sequential drop-to-drop electromembrane extraction and dispersive liquid–liquid microextraction of tricyclic antidepressants in biofluids (Figure 1) [36]. The microfluidic compact disc platform enabled miniaturization and integration of two sample preparation approaches by modifying the disc spin speed, enabling six parallel

extractions. Centrifugal microfluidic discs have been used to perform an efficient cleanup from microvolume samples of considerable complexity (e.g. human serum) before analysis of small-molecule metabolites [37]. Furthermore, the first proteome sample preparation on a digital microfluidic chip was recently reported, integrating the major steps, that is, cell lysis, protein extraction, reduction/alkylation, and proteolytic digestion, on-chip [38].

Outstanding improvements have also been achieved in automation of analytical systems. Apart from improved analytical characteristics, automated systems avoid personal errors, provide improved sample throughput,

Figure 1



(a) A schematic view of the designed limit of detection (LOD) device consisting of five layers. (b) A schematic of one of the extraction units in layer 1. (c) Fabricated LOD device. Reprinted with permission from Elsevier [36].

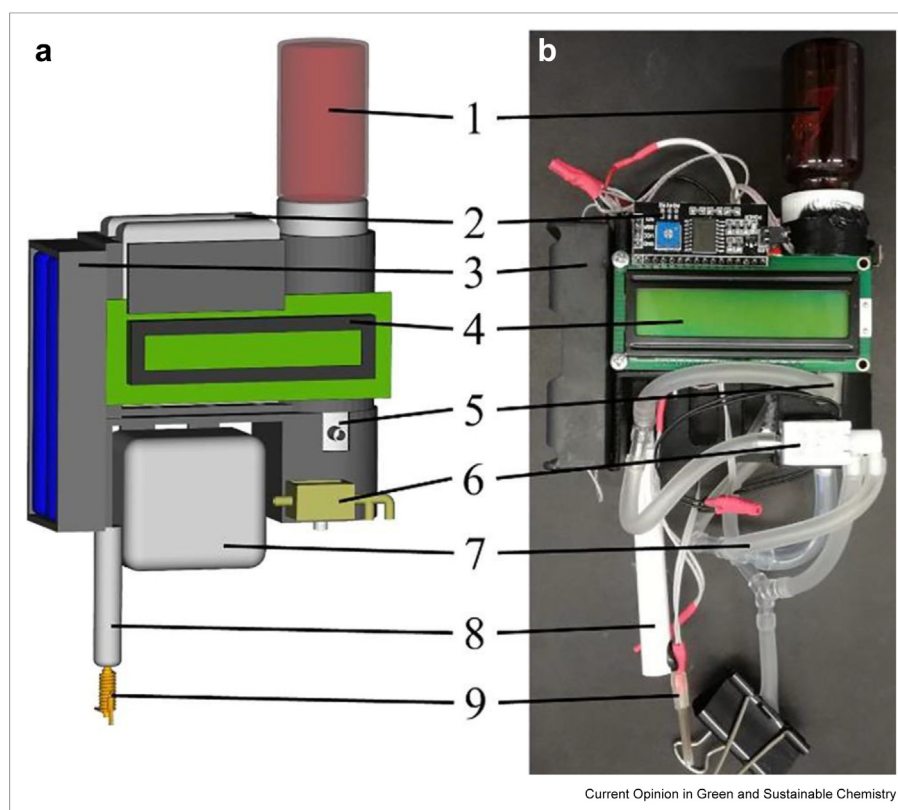
and minimize personal exposure. In the last years, several strategies that enable the automation of sample preparation, analytical separation, and/or detection have been reported with different levels of complexity. Thus, a robotic purification system has been proposed for solid phase extraction automation involving preloaded pipet tip columns that processed 12 samples in 17 min [39]. Different robot-assisted LPME approaches have been proposed [40–42].

Developments toward high-throughput parallel sample preparation have been reported exploiting 96-well plate systems, which provide excellent sample throughput and precision, especially when autosamplers are involved. They have been used for simultaneous extraction of target compounds with a sample processing time of less than 1 min in immersed single-drop microextraction [43]. Besides, a miniaturized array gas membrane system, containing a heating platform to assist volatile transfer, has been reported for determination of volatile compounds by surface-enhanced Raman spectroscopy [44]. This approach allowed analyzing 96 samples within 45 min. Besides, a

microfluidic open interface device enabled direct coupling of biocompatible SPME with MS–MS for determining tranexamic acid in plasma, with a sample throughput of  $120 \text{ h}^{-1}$  [45].

Fully automated systems that comply with the 5th principle, including sampling, sample preparation, and analysis, have been reported [46]. Notable contributions also deal with the design of portable instrumentation that integrates virtually all steps. Accordingly, handheld, portable, and automated instrumentation has demonstrated collection of air samples, enrichment of airborne analytes by in-tube monolithic adsorbents, and desorption and detection of volatile amines (Figure 2) [47]. In addition, the automated coupling of three-phase electromigration and capillary electrophoresis (CE) allowed integration of online sample cleanup, preconcentration, separation, and detection with a minimal modification in the electrode configuration (Figure 3) [48]. Furthermore, a system that integrates solvent extraction and CE–UV, enabling desalting, protein precipitation, solvent extraction, in-line CE stacking, separation, and

Figure 2



Design of a portable automated sample collection–preparation system. (a) Schematic diagram and (b) photograph. (1) vacuum and pressure depo with a pressure sensor, (2) electronic modules, (3) battery holder, (4) liquid-crystal display (LCD) screen, (5) vacuum and pressure pump, (6) solenoid 3-way air valve, (7) vacuum and pressure distribution and piping modules, (8) heating element holder, (9) heating element. Reprinted with permission from Elsevier [47].

detection of tyrosine kinase inhibitors in plasma samples with no intervention, has also been reported [49].

### Further considerations toward greener sample preparation and concluding remarks

Analytical chemistry trends toward miniaturization of analytical systems, integration of steps, automation, and implementation of advanced materials have been consolidated, and recent contributions involving sample preparation demonstrate strong commitment of the field toward greener methodologies. Notwithstanding this, certain considerations can be brought forward.

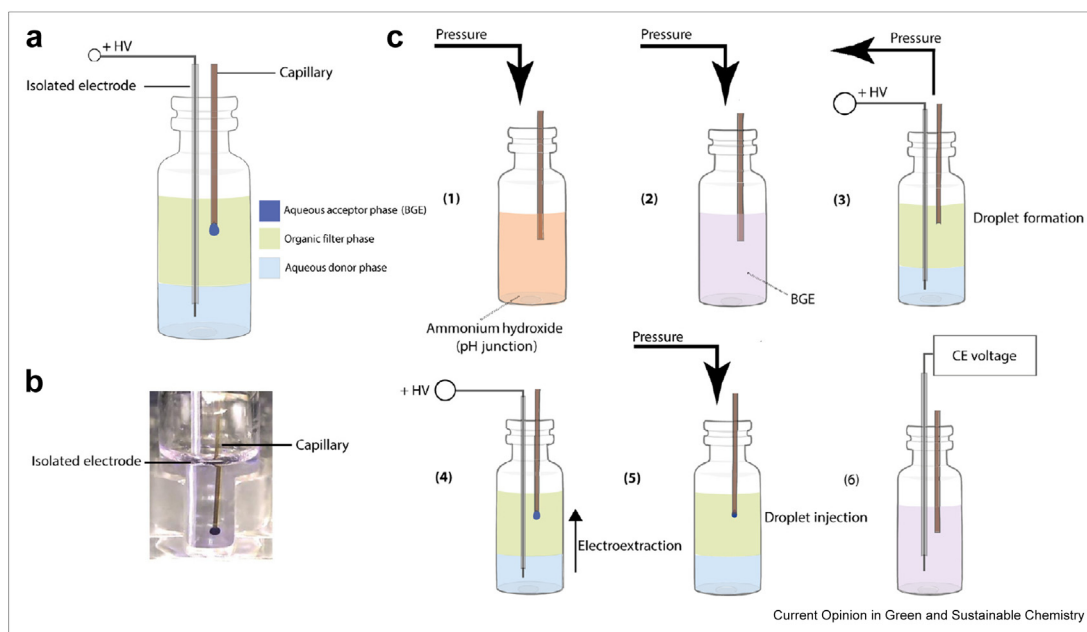
Removal or replacement of harmful chemicals by alternatives showing none or reduced environmental, health, and safety (EHS) issues is consistently taken into account. Nevertheless, there is still room for improvement. Particularly, the replacement of petrol-derived solvents by biobased alternatives is an attractive option. It should be emphasized, however, that the bioderived nature of these solvents does not assure the absence of EHS issues in all cases. In general, the continuing progress toward greener solvents [50] should be exploited in sample preparation, although (at least) comparable analytical performance should be ensured. On the other hand, the development of advanced

materials for sample preparation, still a hot topic, usually makes use of chemicals characterized by undesirable EHS characteristics. Emphasis on greening synthetic procedures would significantly contribute to overall greener sample preparation approaches.

Miniaturization of analytical systems has undergone considerable advances in the last decades [34]. Solventless and reagentless methods are a reality nowadays, thanks to downsized sample preparation approaches. Further miniaturization of sampling/sample preparation devices is expected to deal with challenging point-of-need analyses. Automation is another recurring objective. Although batch methods are still prevailing, microfluidic and mesofluidic systems involving sample preparation are gaining steady attention, and higher degrees of automation are being achieved. Besides, the integration of unitary steps (4th principle) is implicit in these systems.

Analysis time is not explicitly outlined in any of the 12 principles of GAC despite the paramount role of this parameter in analytical chemistry. This is especially true when dealing with a large number of samples to be run, for example, in clinical analysis. Further contributions aimed at acceleration of extraction processes deserve additional attention [51]. In addition, the development

Figure 3



(a) Schematic representation of the three-phase microelectroextraction setup and (b) actual setup incorporating the modified electrode configuration used during experiments (bottom of the vial not visible). (c) Schematic representation of the key steps in the extraction procedure in the CE–UV system: (1) injection of ammonium hydroxide, (2) injection of BGE, (3) application of negative pressure, (4) application of voltage, (5) retraction of droplets using pressure, (6) vial switch to BGE and start of CE separation. Reprinted with permission from Elsevier [48].

of straightforward multiplexed systems, wherein sample preparation can play a key role, would enable the simultaneous determination of target analytes, in accordance with the 8th GAC principle. Besides, the implementation of vanguard—rearguard approaches is highly recommended for efficient decision-making, especially when a large number of samples are processed. Vanguard—rearguard strategies aim at first screening (yes/no binary responses) and subsequently analyzing positive and/or inconclusive samples by a confirmatory method [52]. The development of screening methods can dramatically reduce the number of samples to be processed, thus leading to reduced analysis times and, importantly, lower consumption of reagents, solvents, and/or energy, and lower generation of wastes. However, vanguard—rearguard approaches still represent underexplored strategies. The development of screening methods, wherein sample preparation steps can be integrated for improved sensitivity and/or selectivity, should be considered further.

A number of tools have been reported in the literature to evaluate analytical methods from the point of view of GC. Three tools have been reported in the last two years, namely, RGB model [53], AGREE [54], and HEXAGON [55], that complement previously reported metrics [56]. These tools provide information on green aspects of reported methodologies that complement the required validation aspects and can be used to identify strengths and limitations of these methods for further improvements.

Last but not least, the pace of replacement of reference methods by reliable greener alternative methodologies is still much slower than recommendable. In fact, greening current reference methods would have a strongly positive effect, bearing in mind their extensive use. Thus, a greater focus on the advances achieved in the field within the framework of GC is highly advised when selecting reference methodologies.

### 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.

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  - \*\* of outstanding interest
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