

Research Article

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Greenness of dispersive microextraction using molecularly imprinted polymers

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Abstract: Molecularly imprinted polymers (MIPs) as materials with determined levels of selectivity and specificity for designated analytes have recently gained much attention in various application fields. However, with the growing adoption of green analytical chemistry (GAC) principles, it is essential to investigate the greenness of MIP synthesis and its subsequent application in sample preparation, as well as to evaluate the “green” nature of the developed analytical methodologies, such as dispersive solid-phase microextraction (DSPME). Accordingly, the main objective of this research was to evaluate the greenness of MIP-based glycidyl methacrylate synthesis and MIP use as a DSPME sorbent prior to high-performance liquid chromatography with mass spectrometry (HPLC-MS). The green perspective of MIP-DSPME prior to HPLC-MS was investigated using various analytical metric tools such as the Analytical Eco-Scale, the Green Analytical Procedure Index, and Analytical GREENness (AGREE). Since these analytical tools are not fully implementable for the assessment of the greenness of the MIP synthesis, some alternative approaches were used to optimize the synthesis parameters to make the MIP DSPME sorbent as close as possible to the GAC principles. The calculated AGREE score (0.62) and 91 points in the Analytical Eco-Scale for the proposed DSPME technique using MIP indicated a high level of greenness.

Keywords: DSPME, analytical eco-scale, GAPI, AGREE, green sorbent, magnetic MIP

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1 Introduction

The concept of greenness is defined by the use of methods and techniques that reduce or eliminate large amounts of reagents and solvents that can be dangerous to people and the environment [1–3]. The 12 principles of green analytical chemistry (GAC) act as the main guideline for examining greenness in analytical methods (Figure 1) [4,5].

The latest trends in the development of analytical methods focus on developing solventless extraction methods with a minimal amount of solvents and reducing steps to diminish the energy used [6]. Several metrics have been developed to evaluate the greenness of analytical procedures [7–9]. Analytical Eco-Scale, the Green Analytical Procedure Index (GAPI), and the Analytical GREENness (AGREE) are the most widely used analytical metrics due to their applicability to the majority of analytical procedures [3,10,11].

The Analytical Eco-Scale categorizes the method by deducting points out of 100 for each component, which makes it less environmentally friendly. Depending on the final score, the method may be excellent, acceptable, or inadequate green [12]. AGREE is a straightforward and comprehensive evaluation approach based on 12 GAC principles transformed into a 0–1 scale. The final result of the greenness is clearly shown in the middle of the AGREE pictogram, while the color shows the visual output of how green the method is [10]. GAPI considers the reagents, the procedures, and instrumentation, thus studying the entire analytical procedure. Presented as several pentagrams colored in different colors depending on the greenness, it enables an easy visual comparison of several methods. The red–yellow–green color scale represents the high, medium, and low environmental effects [7], enabling the weakest points in analytical procedures to be visually observed [13]. Neither GAPI nor AGREE takes the synthesis step into account before sample preparation; the total greenness of the analytical approach remains unclear [14].

In most cases, it is impossible to perform direct instrumental analytical measurements in real environmental and material samples due to the very low concentration



Figure 1: Twelve principles of GAC.

levels at which analytes are usually found and the presence of interfering substances. For this reason, preconcentration of analytes and removal of interferences are needed prior to instrumental analysis [15]. Extraction procedures are recommended because of their ability to increase the detection limit of the analytical technique as well as to eliminate matrix interferences [16]. However, conventional extraction methods are limiting in terms of the economy of the process and the excessive use of chemicals. In order to overcome these challenges, the current trend of green chemical analysis led to the development of extraction on a micro-scale. Thus, microextraction techniques have been intensively investigated over the last few years [17–19], particularly their green aspects [20–22].

Dispersive solid-phase microextraction (DSPME) mainly consists of two steps: the adsorption (extraction) and the desorption process [23]. As one of the surface-dependent techniques implies direct contact between the solid sorbent and the studied solution in order to increase the contact surface between them and significantly reduce extraction and desorption time, as well as extract consumption [24]. All these advantages are consistent with green chemistry.

Meanwhile, molecularly imprinted polymers (MIPs) have attracted growing attention as DSPME sorbent in the separation and extraction of analytes from complex matrices [25–27], making the extraction technique easier,

simpler, quicker, and more efficacious [28]. Since during synthesis, the target molecule is added to the mixture and then removed, leaving recognition sites, MIPs contain specific and selective binding sites for target analytes [29]. MIP shows the possibility of recognizing not only the target molecule but also its fragments or molecules similar in shape and functional groups as the target one [24]. Magnetic MIP has been found to have an application as a sorbent in DSPME. Due to its magnetic properties, it can be removed with an external magnet.

Due to its capacity to detect multiple compounds simultaneously and high selectivity and sensitivity, high-performance liquid chromatography with mass spectrometry (HPLC-MS) is widely used in complex matrices [30] [31–33]. Despite the major progress in instrumental techniques, removing interferences is a critical step in sample preparation. Therefore, clean-up and efficient sample preparation while maintaining an eco-friendly environment, low cost, and short analysis time are the key goals to improve analytical methods.

In this research, different strategies for the preparation of magnetic MIP, as well as its incorporation into DSPME, are discussed from a green perspective. Additionally, the ecological impact of the proposed MIP-based DSPME-HPLC-MS was investigated, and the greenness level of the method was evaluated using the Analytical Eco-Scale, GAPI, and AGREE.

2 Materials and methods

2.1 Chemicals, reagents, and instruments

The following chemicals were used to synthesize the magnetic MIP. Glycidyl methacrylate, 2,2'-azobisisobutyronitrile (AIBN), cyclopentanol, 1-tetradecanol, and pentaethylenehexamine (PEHA) were obtained from Merck KGaA (Darmstadt, Germany). Poly(*N*-vinyl pyrrolidone) (PVP) was purchased from BASF SE (Ludwigshafen, Germany). Iron(II, III) oxide (nanopowder, <50 nm particle size [TEM], 98% trace metal basis), tetraethyl orthosilicate (TEOS), 3-methacryloxypropyltrimethoxysilane (MPS), ammonium hydroxide (25 wt%), ethylene glycol dimethacrylate (EGDMA), and aniline (ReagentPlus, 99%) were obtained from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). Acetonitrile as an eluent in the DSPME method was purchased from Carlo Erba Reagents GmbH (Emmendingen, Germany). Chemicals were of analytical grade and used as received. The polymer carrier was synthesized using the *in situ* suspension polymerization method, and the magnetic MIP was obtained using the core-shell surface imprinting method. The resulting polymer is labeled as MIP-PEHA [24]. As for the instrumentation, an ultrasonic and oil bath was used for synthesis in order to avoid excessive energy consumption and maintain the temperature as long as possible. Dispersive microextraction was vortex-assisted (Vortex Stirrer, VORX-005-001, V05, Labbox Labware S.L., Spain).

A methodological evaluation of the proposed DSPME method was made using ComplexGAPI v.0.2 beta (open-source and downloadable from <https://mostwiedzy.pl/complexgapi>) [13] and AGREE software v.0.5 (freely available and downloadable from <https://mostwiedzy.pl/AGREE>) [10].

High-performance liquid chromatography-mass spectrometry (HPLC-MS, TSQ Quantum Access Max, Thermo Fisher Scientific, Waltham, MA, USA) fitted with C-18 Thermo Scientific Accucore aQ column (100 mm length, 2.1 mm I.D., particle size 2.6 μm) was used for the determination of aromatic amines. Data were processed using Thermo Xcalibur software (version 3.0, Thermo Fisher Scientific, Waltham, MA, USA). The mobile phase was methanol and water (80:20 v/v), the flow rate was 0.3 mL·min⁻¹, the injection volume was 5 μL , and the total run time was 5 min.

2.2 MIP synthesis

The polymer carrier was synthesized using the *in situ* suspension polymerization method, and the magnetic MIP was obtained using the core-shell surface imprinting method [24]. Many

conditions are varied in order to obtain the most effective and applicable polymer. First, the polymerization method chosen for the synthesis of MIP was *in situ* suspension polymerization to minimize waste generation. The choice of functional monomer, cross-linkers, and polymerization conditions is generally based on an already optimized synthesis [34,35]. In order to obtain a magnetic nanocomposite with the highest synthesis yield, as well as a suitable form for multiple applications, the stirring rate and the amount of stabilizing agent poly(*N*-vinyl pyrrolidone) (PVP) were varied. In general, there are two phases involved in the preparation of magnetic nanomaterials: (i) preparation of the magnetic core and (ii) additional modification in order to crosslink the magnetite nanoparticles into a polymer structure. A further process (surface imprinting) is also used for additional polymer functionalization and improvement of its potential to be extracted [36]. The ratio of the ligand to template used for imprinting is a very important step leading to the success of the synthesis. Preliminary results (template/ligand ratio 1:1, 1:2, 1:4) showed that the best ratio is 1:4, which favors greenness due to using a smaller amount of chemicals. Rinsing the template to make an imprint was done by a Soxhlet, using methanol and acetic acid, but a greener approach was also used by ultrasound-assisted extraction. With a view to obtaining an MIP that will respond in the best way to the 12 principles of greenness, small, spherical particles were obtained, and the centrifuge step and Soxhlet extraction were replaced. Figure 2 shows that the optimized procedure is greener than the old one.

2.3 DSPME optimization

Many factors influence the efficiency of microextraction, and the classic approach to conducting experiments is long and involves a large consumption of chemicals, energy, etc. The design of experiment (DoE) needs to be carried out, which enables the simultaneous assessment of a number of factors that can affect the system using a minimum number of experiments while simultaneously obtaining sufficient information about their importance and mutual interactions. The design, selected factors, their levels, and obtained responses are presented in our previous work [24]. Effective factors were optimized in two steps using the Plackett–Burman design as the screening step and the Box–Benken design as the optimization step. In the first step, the influence of variables was tested to obtain an efficient DSPME method. Each variable was examined at two levels, a high (+1) and a low (–1) level: pH (2–10), mass of sorbent (10–50 mg), type and volume of desorption solvent

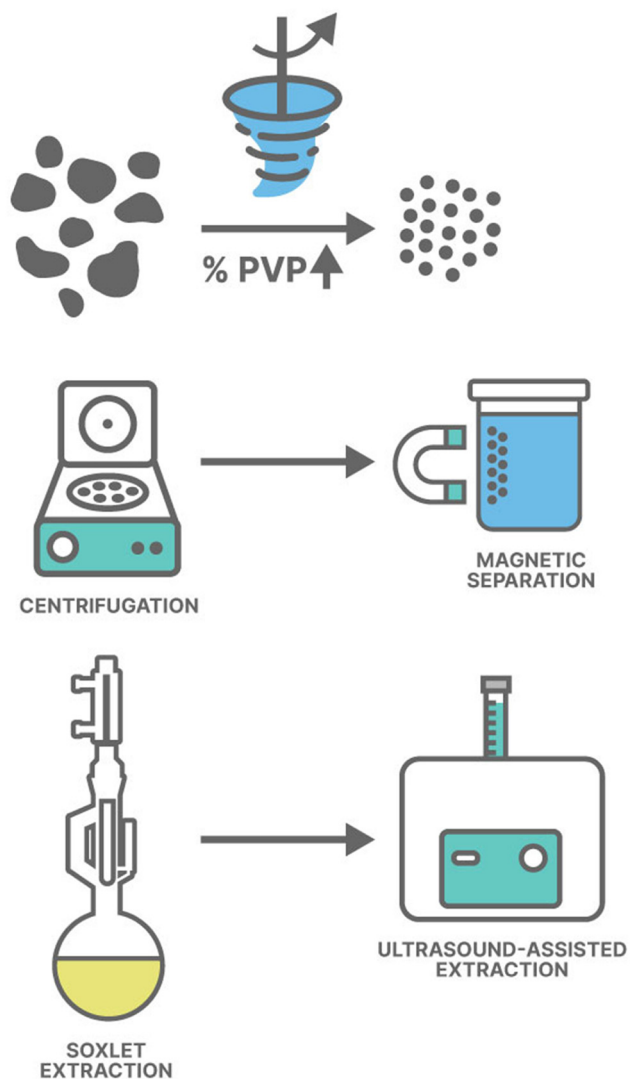


Figure 2: Synthesis optimization steps.

(methanol or acetonitrile; 200–700 μL), ion strength (0–1 wt%), type of extraction and desorption (vortex-ultrasonic), extraction and desorption time (1–5 min), and extraction and desorption

temperature (10–40°C). The following steps involved optimizing the most significant factors from the previous step (pH, volume of desorption solvent, and extraction temperature).

The optimized MIP-DSPME method included 10 mL of aqueous extract, 50 mg of magnetic polymer sorbent, and 450 μL of eluent (acetonitrile). The pH of the sample was 6, while both sorption and desorption were performed at room temperature (25°C) for 1 min each, assisted by a vortex. The acetonitrile phase was filtered and measured directly on the HPLC-MS. It is not enough that the method is effective; it should also respect the 12 principles of greenness. To evaluate the greenness of the developed MIP-DSPME-HPLC technique, Analytical Eco-Scale, GAPI, and AGREE were applied (Figure 3).

3 Results

3.1 Improvement of MIP synthesis toward greenness

In order to turn the synthesis towards a green approach, it is shown which prerequisites need to be met in order to guarantee a green preparation technique, as well as which variables should be tuned to enhance the ecological properties of the synthetic process. As a result, our focus is to investigate ideas of greenness, working out all steps of the synthesis so that they satisfy the principles of green chemistry. Since there is no explicit agreement on the conditions that must be addressed to ensure a green preparation method or which parameters must be optimized to improve the environmental features of the synthetic procedure, magnetic nanomaterials are, in fact, referred to as “green materials” in many studies even though their synthesis conditions do not meet the Green Chemistry requirements. It is

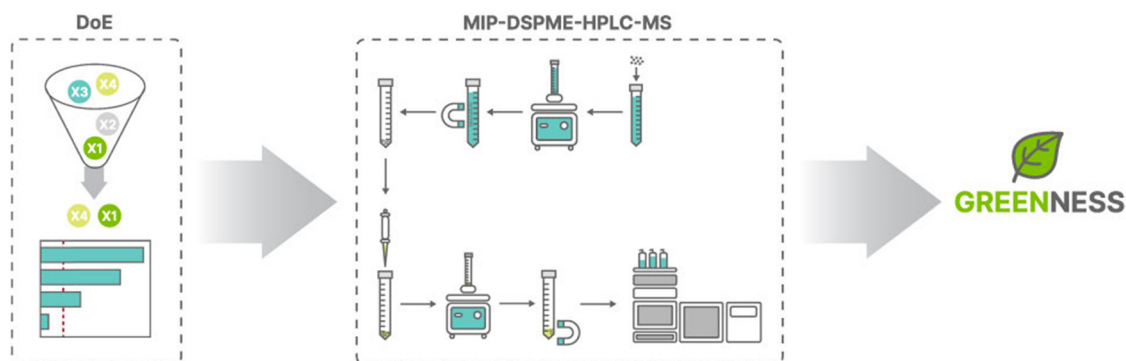


Figure 3: Proposed MIP-DSPME-HPLC-MS toward greenness.

important to note that either AGREE or GAPI cannot be used to evaluate the greenness of synthesis. However, they are suitable tools for evaluating the greenness of analytical methods, such as microextraction, which will be discussed below. Our efforts were focused on obtaining the best possible MIP that would satisfy the principles of green chemistry (Table 1).

The synthesis was performed *in situ*, which is a good indicator of greenness. A small amount of sample was used, and the measurements were performed off-line. In step with greenness, the number of steps in the preparation procedures was reduced after optimization and the centrifugation step was replaced by a magnetic polymer. Semi-automated and miniaturized methods, as well as procedures without derivatization, represented green performance. The transition to a greener approach is reflected in the reduction of waste during synthesis, the use of a smaller amount of reagents, and the performance of synthesis by *in situ* suspension polymerization. The tedious Soxhlet extraction and using a large amount of eluent to wash the template were replaced by ultrasound and a small amount of acetonitrile. This replacement was improved by minimizing the energy used for synthesis. The synthesis parameters were varied, and thus, larger quantities of finer particles were obtained.

3.2 MIP-based DSPME

To evaluate the green approaches of the developed DSPME technique, three metric systems based on the GAC principles were applied, namely Analytical Eco-Scale, GAPI, and AGREE [10,12,13].

The Analytical Eco-Scale is based on deducting points out of 100 for each process component, making it less environmentally friendly. The scale contains certain penalty points (PPs) for reagents used, safety, instrumentation, and working conditions. If the proposal method receives a score of over 75, it represents “excellent green analysis,” between 50 and 75, “acceptable green analysis,” and below 50, “inadequate green analysis.” Table 2 shows the Eco-scale result of the proposed MIP-DSPME-HPLC-MS method. Based on points, this technique is counted as an “excellent analytical technique.” This type of extraction on solid-phase sorbents significantly reduces the reagents used, resulting in such a high score.

The GAPI metric uses a pictogram to classify the greenness of each step of an analytical methodology, applying a color scale. The GAPI consists of five pentagrams corresponding to different analytical phases affecting the environment. Each pentagram consists of a different number of fields. In GAPI, sample preparation, procedures, reagents, and

Table 1: A possible step toward a greener synthesis

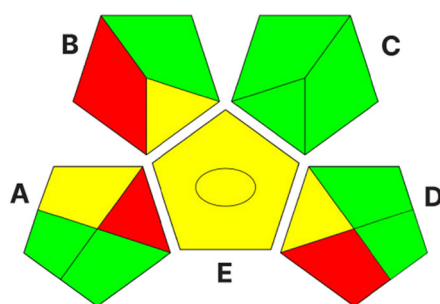
Green analytical chemistry principles	Score of the proposed synthesis	Current/Possible solution
Direct analytical techniques should be applied to avoid sample treatment.		Not applicable
Minimal sample size and minimal number of samples are goals		MMIPs has reliable trace level detection ability from a complex sample and reduced sample size and number
<i>In situ</i> measurements should be performed		Off-line position of analytical devices
Integration of analytical processes and operations saves energy and reduces the use of reagents		Magnetic MIP, no centrifugation step, instead of Soxhlet removing the template with the help of ultrasound with a small amount of AcN
Automated and miniaturized methods should be selected		Direct <i>in situ</i> synthesis, energy saving
Derivatization should be avoided		No derivatization
Generation of a large volume of analytical waste should be avoided and proper management of analytical waste should be provided		Reutilization, ultrasonic extraction of template
Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time		Multi-analyte detection with single or dual templated based MMIPs
The use of energy should be minimized		Magnetic separation, Ultrasound-assisted template extraction
Reagents obtained from renewable sources should be preferred		Use of greener reagents such as deep eutectic solvents, ionic liquids
Toxic reagents should be eliminated or replaced		Aqueous medium polymerization / Solvents such as deep eutectic solvents and ionic liquids, less toxic alternatives dummy templates
The safety of the operator should be increased		Proper guidelines, supervision and skillful staff / Use of greener chemicals to avoid threats to the environment

Table 2: Analytical Eco-Scale result of the proposed MIP-DSPME-HPLC-MS method

Reagent	PPs
Acetonitrile	4
Instrumentation	PPs
HPLC-MS	2
Vortex	0
Occupational hazard	Analytical process (0)
Hazard	Emission of hazard to the atmosphere (0)
Waste	3
Total PPs	9
Eco-Scale score	91
Comment	Excellent green method

instrumentation are evaluated. According to their effect on the environment, each field is color-coded. Red indicates a hazardous impact, while green and yellow indicate a low and medium impact. The first pentagram refers to sampling (collection, preservation, transport, and storage). The scales of extraction, the greenness of the reagents used, as well as the need for additional treatments are associated with the second pentagram. The amount, health, and safety hazards of chemicals used belong to the third pentagram. The fourth pentagram comprises four fields related to instrumentation (energy consumption, workplace hazards, waste amount, and waste management). The type of method associated with the fifth pentagram has only one field with or without a circle, depending on whether the method is suitable for both qualitative and quantitative analysis or only quantitative analysis is possible.

Figure 4 shows a GAPI pictogram of the proposed method with an explanation of each field. All three colors are present in the first pentagram associated with sample preparation. The red color represents off-line sample collection; storage and transport are not required, corresponding to the green color, while normal conditions are sufficient for storage (yellow). In the second pentagram, yellow fits the micro type of extraction, while red indicates

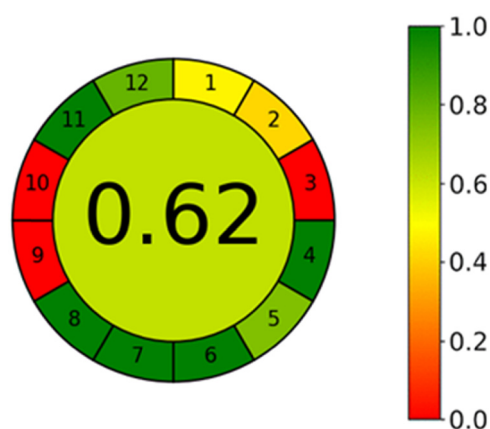
**Figure 4:** GAPI of the MIP-DSPME-HPLC-MS.

the use of no green solvent, and green is for no additional treatments. No special health and safety hazards and a small amount of reagents led to all green pentagrams.

Regarding instrumentation and waste, a small amount of waste is marked in green, but the waste treatment is shown in red. The energy consumption of this technique is yellow, while hermetic sealing corresponds to green. One yellow field with a circle in it represents simple procedures for sample preparation and the possibility of both qualitative and quantitative analysis.

AGREE was created based on the 12 GAC principles. On the AGREE scale, the greenness of certain principles is evaluated from 0 to 1, where 0 represents the least greenness and 1 is the greatest. The values on the scale match the corresponding color (the tone shifts from green to red from 1 to 0), and the outcome is a graph in the form of a clock, with the overall score and color represented in the center.

Figure 5 shows the AGREE pictogram of the MIP-DSPME-HPLC-MS technique previously described in the Experimental section. Each segment of the pictogram is colored according to greenness. The score corresponding to GAC principle 1, which refers to direct analytical techniques to avoid sample treatment, is marked in yellow due

**Figure 5:** AGREE of the MIP-DSPME-HPLC-MS.

- A:** Sample collection, transport, storage
- B:** Sample preparation
- C:** Reagents and chemicals
- D:** Instruments
- E:** Method type

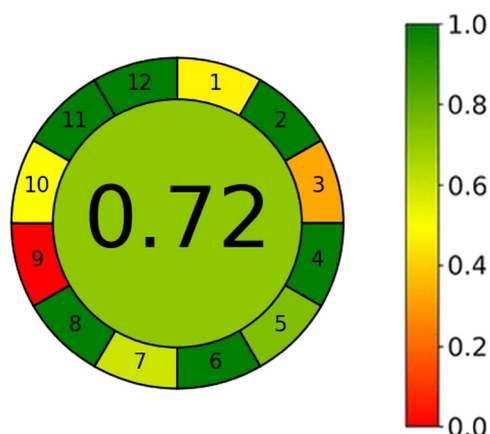


Figure 6: AGREE of the HPLC-MS.

to the impossibility of direct analysis. The pictogram clearly shows that only three fields are marked in red, namely field numbers 3, 9, and 10, which correspond to the GAC principles related to the possibility of online analysis, the technique that uses the highest energy, and the use of renewable reagents. The greenness of the method is reflected in the use of a smaller sample, so the second field on the pictogram is colored yellow. All other principles are colored in different shades of green, making the developed DSPME technique excellent for greenness. The sample preparation procedure involves a few steps (Field 4), and the proposed method is miniaturized and semi-automated (Field 5). Derivatization of the sample, representing green performance, is not required (Field 6). The method itself uses a very small amount of reagents, so the amount of waste is also minimal (Field 7). Dispersive microextraction on an MIP is suitable for measuring a large number of analytes due to the type of polymer, which has the ability to recognize it, and the duration of the microextraction itself is very short, which enables the examination of a large number of samples (Field 8). The method does not involve using toxic reagents (Field 11), and the threats can be toxicity to aquatic life (Field 12). Figure 5 shows the overall score in the middle of the pictogram with a value of 0.62,

and the light green color indicates that the proposed DSPME technique using MIP as a sorbent is green.

3.3 Evaluation of HPLC-MS greenness

The greenness evaluation of the HPLC-MS method using AGREE software is presented in Figure 6. This method involves an off-line sample preparation step, while the volume of the injecting sample is 5 μL , and the analytical device is positioned at-line. The number of distinctive steps is less than 3. The procedure is semi-automated. No derivatization agents are needed. The amount of waste is in the range of 1–10 mL. Multi-analytes are determined. The red color originates from the instrument itself, which consumes a lot of energy. Some of the reagents are bio-based, there are no toxic reagents, and operator safety is secured.

Three key GAPI aspects were examined for HPLC-MS greenness assessment: sample preparation, use of reagents and solvents, and instrumentation assessment (Figure 7). Direct collection of the sample is impossible (red color), and at-line measurement is represented in yellow. Preservation and transport are the greenest options, while normal conditions (yellow) are required for storage. Sampling does not require preparation techniques such as dilution or sonication, so it is classified as a green method. Regarding the type of extraction, microextraction (yellow) was used prior to HPLC measurement. No solvent is used for sample preparation before analysis on the instrument, which represents in-line sampling (green color). The last step in sample preparation refers to additional treatments, which are unnecessary in this case (green).

Regarding reagents and solvents, the amount of reagent used for one complete analysis belongs to the green method <10 mL. The following steps related to health and safety are determined. According to the classification, HPLC-MS belongs to the non-green method, as far as the use of energy and non-green chemicals is concerned. This analytical method is

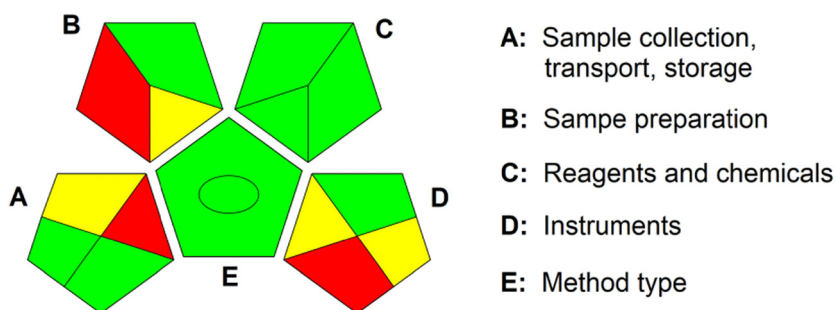


Figure 7: GAPI of the HPLC-MS.

performed in closed environments, so occupational hazards are less likely (green color). The last part refers to waste and waste treatment. Waste generated by the HPLC-MS method is ranked in green, with no recycling treatment (red color).

Table 3 shows a detailed calculation of the Analytical Eco-Scale for HPLC-MS. The decisive factor for the result and deduction of points refers to the reagents used and their number.

Using these three assessment tools, the full picture of the greenness profile of the proposed HPLC-MS method is given.

Different extraction methods are used to determine organic analytes before instrumental measurement. A comparison of the proposed MIP-DSPME-HPLC-MS method with other published methods in terms of greenness is shown in Table 4. The results of the AGREE score and the Analytical Eco-Scale were calculated using the AGREE software and by subtracting points from the ideal green method (100 base points), respectively.

4 Discussion

4.1 Principles of greenness evaluation

According to Principle 1 (P1), the sample preparation step should be avoided by performing direct analytical techniques. This method allows for immediate *in situ* measurement and eliminates the requirement for sample preparation, which usually reduces the use of hazardous reagents, solvents, and energy. The time and the number of analytical stages needed for the analysis overall are greatly decreased by direct measurement. Nevertheless, direct analysis is not always possible due to the nature of the analyte, the intricacy of the sample matrix, and the features of the analytical technique, which require greater sensitivity or selectivity.

In line with Principle 2 (P2), minimal sample size leads to less waste generation, which is consistent with green chemistry. A smaller number of samples can be obtained by using non-invasive methods of field screening, as well as by using statistical calculations for the selection of sampling sites. Reliable results from miniaturized methods can be obtained even with a significant reduction in sample size. However, too small the number of samples or the sample size can lead to unreliable results, which could lose the representativeness of the sample.

According to Principle 3 (P3), measurements should be made *in situ*, and instruments should be as close as possible to the sampling location. Miniaturized analytical systems and field portable instruments represent the best solution, leading to the greening of analysis. In this way, expensive and time-consuming transport is eliminated, which contributes to air pollution and eliminates wasting energy for sample storage. Sample preparation is reduced to a minimum, consumption of reagents is minimized, and the time between two analyses is shorter. There are four possible modes to the distance between the instrument and the sampling location: in-line, online, at-line, and off-line sampling.

Principle 4 (P4) refers to the integration of analytical processes. Reducing the number of analytical steps leads to energy savings and reduction of reagent use and time. The number of individual steps in the analytical procedure can be reduced by integration and performing some operations simultaneously. The greenest approach involves three or fewer steps, while more steps move away from the green approach.

Based on Principle 5 (P5), an automated method aligns with greenness because it reduces the risk of accidents and exposure to solvent vapors, thereby increasing operator safety, while miniaturized methods consume less energy and reagents.

Derivatization refers to Principle 6 (P6). Using reagents in the derivatization process results in the creation of toxic waste. In cases where derivatization is necessary (to improve the response of the analyte), it is desirable to use more environmentally acceptable reagents.

Waste and waste management belong to Principle 7 (P7). Possible ways to reduce waste are reflected in reducing the number of steps in the analytical procedure as well as the amount of solvents and reagents used. Proper management of analytical waste involves recycling, degradation, or passivation to reduce toxicity.

Principle 8 (P8) refers to the possibility of analyzing multiple analytes and estimating multiple parameters simultaneously compared to methods that use one analyte at a time. Multianalyte or multiparameter methods significantly

Table 3: Analytical Eco-Scale result of the HPLC-MS method

Reagent	PPs
Methanol	4
Instrumentation	PPs
Energy consumption	2
Occupational hazard	Analytical process (0)
Hazard	Emission of hazard to the atmosphere (0)
Waste	3
Total PPs	9
Eco-Scale score	91
Comment	Excellent green method

Table 4: Comparison of the proposed method with other published methods

Type of extraction	Vortex-assisted DLLME-DES-UHPLC-DAD ^a	HF-SLM-HPLC-FLD ^b	Menthhol-based DES-DLLME-UPLC-MS/MS ^c	QUECHERS SF- μ SPE/HPLC-UV ^d	MIP-based DSPME-HPLC-MS
Analytes	Sulfonamides	Fluoroquinolone	Sulfonamides	Anti-diabetic drugs	Aromatic amines
Solvent/sorbent	Thymol/acetic acid DES	Sodium phosphate buffer	Menthhol/octanoic acid DES	Cur-HNADES/PVA ^e	MIP-PEHA
Amount	150 μ L	100 mM	150 μ L	2 mg	50 mg
Extraction time (min)	2	45	11	3	1
Matrix	Water samples	Chicken liver	Milk-based infant formulas	Wastewater, serum, plasma real samples	Textile wastewater
Desorption solvent	150 μ L AcN	/	500 μ L ethanol	500 μ L MeOH/AcN	450 μ L AcN
Analytical Eco-Scale	73 [*]	86 [*]	73 [*]	/	91 ^{**}
AGREE	0.64 [*]	0.46 [*]	0.6 [*]	0.61 [*]	0.62 ^{**}
Type of instrument	UHPLC-DAD	HPLC-FLD	UPLC-MS/MS	HPLC-UV	HPLC-MS
Mobile phase	0.1% HCOOH (aq)/90% AcN in EtOH	AcN/0.1% HCOOH (aq)	0.1% HCOOH (aq)/EtOH	Phosphate buffer/MeOH/AcN mixture	MeOH/water (80:20 v/v)
Flow rate (mL·min ⁻¹)	1	0.35	0.3	0.8	0.3
Injection volume (μ L)	/	5	2	20	5
Reference	[39]	[40]	[41]	[42]	This work

*Taken from the cited reference. **Assessed by the author. ^aDLLME-DES-UHPLC-DAD –dispersive liquid–liquid microextraction – deep eutectic solvent-ultra-high-performance liquid chromatography coupled with diode array detection. ^bHF-SLM-HPLC-FLD – hollow fiber liquid-phase microextraction combined with HPLC with a fluorescence detector. ^cUPLC-MS/MS – Ultra-performance liquid chromatography-tandem mass spectrometry. ^dQueChERS SF- μ SPE/HPLC-UV – quick easy cheap effective rugged safe method based on a syringe filter and on micro-solid phase extraction followed by HPLC with ultraviolet detection. ^eCur-HNADES/PVA – curcumin immobilized thymol:menthol hydrophobic natural DES into the polyvinyl alcohol electrospun nanofibers.

reduce time and the amount of reagents and solvents compared to methods that use one analyte at a time.

According to Principle 9 (P9) the main goal is to minimize the energy used. The goal is to reduce every source of energy (energy required for sample preparation, analytical measurement, transport, storage, and preservation) to a minimum.

Considering Principle 10 (P10), preference should be given to bio-based reagents and reagents obtained from renewable sources. Using chemicals from renewable sources is very promising, so bio-based chemicals are the greenest approach.

The elimination or replacement of toxic reagents corresponds to the Principle (P11). This principle implies removing or replacing toxic reagents with greener alternatives whenever possible.

Operators' safety is taken into account in Principle 12 (P12). If possible, operational hazards (sound, inhalation) should be removed or reduced to a minimum. Additionally, the dangerous use of solvents, especially in large quantities, should be strictly avoided. The operator's safety may be endangered by toxic, flammable, and explosive reagents. Automation and miniaturization of the procedure have a positive impact on operator safety.

GAC principles are taken into account by AGREE software and GAPI in the overall greenness assessment. One field on the AGREE circle corresponds to one principle, while with GAPI, the principles are divided into three parts (sample preparation, reagents and solvents, and instrumentation).

4.2 Greenness profile of MIPs

In order to get a complete picture of the MIP's greenness, each of the 12 principles should be explained in detail. An *in situ* sampling procedure is performed. Advantages of this procedure include the reduction of reagents required for the synthesis and minimizing the waste generation due to the entire sorbent produced in one reactor. For example, in bulk polymerization, grinding and sieving steps are necessary, while in the suspension polymerization procedure, these steps are not involved, thus decreasing time and energy consumption, as well as the risk for operators. GAC principle 2 favors using a small amount of sample, which in the case of synthesis refers to smaller particles. Initially, large particles with irregular shapes were obtained. In order to obtain smaller spherical particles, the number of revolutions and the amount of stabilizer were varied. Thus, spherical, porous particles with a small diameter, which

have a wide range of applications due to their large contact surface, were obtained, accelerating extraction.

It is necessary to avoid or find an alternative for operations requiring a large energy and chemical consumption, where possible. This synthesis optimization reduces the number of steps in several ways. First, the centrifugation step is avoided by using a magnetic polymer, whose magnetic properties provide rapid extraction, simplifying its separation from the sample and skipping several steps, such as centrifugation and filtration. Since using derivatizing agents in sample preparation involves an additional step that has a negative effect on the sample, the derivatization step is avoided. Reducing waste generation and minimizing the consumption of hazardous reagents is of great importance for green chemistry. MIPs use a single template during imprinting, which can later recognize a large range of similar molecules, thereby reducing the amount of chemicals used for synthesis and unnecessarily synthesizing new MIPs. The large amount of analytical waste generated by washing the template by Soxhlet extraction takes a lot of time, energy, and solvents. Ultrasound-assisted extraction allows less time consumption, as well as the extraction, with the help of a smaller number of reagents in small quantities.

4.3 Greenness profile of the proposed MIP-DSPME-HPLC-MS

Dispersive microextraction is one of the types of microextractions with many advantages compared to others. One of the main advantages is that everything takes place in one reactor, thus reducing waste and drastically shortening the method, which corresponds to P4 and P7. What makes the developed microextraction even greener is the short time of sorption (1 min) and desorption (1 min) (P5, P8). Everything is performed at room temperature, so no additional energy consumption is required (P8). Centrifugation is avoided using a magnetic polymer, which is easily separated by an external magnetic field. Pre-designed computer programs for microextraction optimization (DoE) greatly reduced unnecessary time and waste (P4, P8). Additional treatments, often carried out prior to DSPME, require more energy and reagents, which will harm the process overall. The impossibility of online analysis (P1) and non-green and renewable solvents (P9) makes this method less green. There is an explanation for each of these weak points of the method. As the lack of automation is the primary limiting factor when evaluating green approaches in analytical chemistry, future trends

should shift to the development of automated in-line analysis that will improve the overall greenness of the procedures. The presented DSPME method on MIP uses a minimal amount of AcN (450 μL), which does not represent a serious problem for waste disposal. After the DSPME method, the measurement is performed on HPLC-MS, which, according to AGREE, counts as the technique that uses the highest energy, but this technique is far more sensitive and more innovative than the others offered.

4.4 HPLC-MS through greenness

Assessing the greenness of the analytical method is necessary for its credibility and sustainability.

Direct analysis is often not possible in chromatographic methods due to the impossibility of portability (P1). In the future, it is necessary to consider how this could be solved and enable *in situ* measurement. According to P2, a smaller sample amount means a more environmentally friendly method. If there is a delay between sampling of introducing and obtaining results, it corresponds to in-line or at-line sampling, which is considered a slightly less green sampling technique, which is characteristic of most reported methods of liquid chromatography (P3) [37]. The sample volume recorded on the instrument was 5 μL , making this step eco-convenient. Because derivatization is not required, P6 corresponds to greenness. In support of P7, the amount of solvents used and waste generated have been reduced, owing to the low flow rate of 0.3 $\text{mL}\cdot\text{min}^{-1}$, the short injection volume of 5 μL , and the 5 min total run time. The possibility of analyzing a large number of analytes simultaneously significantly contributes to reducing analysis time and energy consumption. Although HPLC with mass spectrometry as a detector is classified as the least green method due to high energy consumption (P9), the ability of this analytical technique to extract chemical fingerprints from trace levels of analytes is not always achievable with other greener methods. A combination of water and an organic solvent is most often used as a mobile phase in HPLC. Acetonitrile and methanol are suitable HPLC solvents with good chromatographic properties, such as miscibility with water, high purity, low viscosity of their aqueous solutions, and chemical reactivity with most samples. However, in terms of ecological impact and health safety, acetonitrile and methanol may not be the best choices (P10 and P11). Therefore, methanol could be replaced with a less toxic solvent [38]. Operators' safety is adequately regulated. Hermetic sealing eliminates the possibility of dangerous solvent evaporation (P12).

4.5 Greenness of other reported approaches and future perspectives

A comparison of the proposed MIP-DSPME-HPLC-MS method with other published extraction methods for organic analytes in terms of greenness was made. To analyze different sulfonamides in a water sample, Mostafa et al. used thymol-based DES-DLLME [39]. The value on the AGREE scale of this method was 0.64, while subtracting PPs in the Analytical Eco-Scale resulted in 73. Moema et al. used HF-SLM-HPLC-FLD to determine the fluoroquinolone residues in chicken livers [40]. According to the Analytical Eco-Scale, this method was classified as an excellent green method, but the AGREE and GAPI values were not in accordance with that, as shown by the AGREE score of 0.46. The fact that the Analytical Eco-Scale gave a mathematical assessment without further detailed study of the analytical method explains why it gave better results than AGREE and GAPI.

In contrast, AGREE and GAPI provide information on the entire procedure and consider all the individual steps of the proposed method. In order to determine sulfonamide residues in infant milk formulas, DES based on menthol and octanoic acid was used as an extraction solvent in DLLME-UPLC-MS/MS [41]. The calculated AGREE score (0.6) indicated a high level of method greenness, while 73 points in the Analytical Eco Scale indicated an adequately green method. Table 3 shows the results for QuEChERS SF- μSPE /HPLC-UV [42]. Cur-HNADES/PVA was used to extract anti-diabetic drugs from real samples (wastewater, serum, and plasma). The AGREE graph shows a score of 0.61 for the procedure.

As for energy consumption, the UPLC method consumes less energy for one analysis compared to HPLC. Due to heavy energy consumption by a mass spectrometry detector, HPLC-MS made most methods vulnerable to the green aspect. According to Greenness assessment tools, HPLC-MS consumes the most energy. The miniaturized UPLC technique reduces energy consumption and waste generated by analysis. However, energy consumption, according to greenness, is higher when coupled with an MS detector (in this case, tandem mass spectrometer). Although the coupled methods are classified as the least green method (due to high-energy consumption), the capability of such analytical techniques should be emphasized in terms of significantly better sensitivity, which is not possible to achieve with some other, according to greenness, greener methods. Also, when evaluating methods, the composition of the mobile phase should be considered, as well as the quantity, injection volume, and total run time, given that all of this affects the final energy consumption and the amount of solvents used. Less total run

time, injection volume, and flow rate reduce the amount of solvent used, thus waste and energy consumption. For a greener method, it is preferable to use greener organic solvents, if possible, and if such a solvent is an appropriate replacement in terms of the efficiency of the method. Methanol and acetonitrile, as organic solvents, are the most often used in HPLC analysis. Regarding the greenness, ethanol is a more suitable solvent than the two mentioned (ethanol > methanol > acetonitrile).

New trends are directed toward using greener solvents, such as ionic liquids, deep eutectic solvents, and magnetic nanomaterials, to reduce process steps. The main drawback of the mentioned research is off-line measurement. Future aspects should be shifted to the possible in-line sampling strategies, thus avoiding and solving the problem of the preservation, transportation, and storage of the sample. This would also reduce the amount of waste produced and enable effective and easier analysis. Some of the proposed solutions for improvements in chromatography are miniaturization, thanks to micro-chip HPLC, portable HPLC, and substitution of stationary phase in terms of size and nature [37].

5 Conclusions

In addition to green analytical techniques, there is a need for the use of greener sorbents. Following the principles of green economy, synthesis steps could be improved, including using magnetic sorbents, selecting appropriate polymerization strategy, reducing or replacing organic solvents with alternative greener solvents, and decreasing energy consumption. Several analytical metrics, such as Analytical Eco-Scale, GAPI, and AGREE, were used to evaluate the greenness of the proposed MIP-DSPME-HPLC-MS. The limitations of different extraction types, such as using a large amount of solvent and long analysis time, are solved by dispersive approaches combined with a highly selective sorbent such as a magnetic MIP. The results showed that the reported method meets the requirements of green chemistry and is suitable for use. The overall score in the middle of the AGREE pictogram for MIP-DSPME-HPLC-MS and HPLC-MS, with values of 0.62 and 0.71, respectively, and a light green color, indicates that the assessed procedures are green. According to the Analytical Eco-Scale, MIP-DSPME-HPLC-MS and HPLC-MS are categorized as excellent green methods.

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