

AGREEprep – Analytical Greenness Metric for Sample Preparation

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Abstract

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

32 **Keywords**

33 Green analytical chemistry; Green metrics; Sample preparation; Green chemistry;
34 Sustainability assessment

35

36 **1. Introduction**

37 Green Analytical Chemistry (GAC) aims to minimize the negative environmental impact of
38 chemical analyses by addressing critical issues such as the generation of toxic laboratory waste
39 and the use of solvents and reagents that are hazardous to human health or the environment [1].
40 The importance of GAC was highlighted soon after the introduction of Green Chemistry (GC)
41 and emerged as a specific branch of GC, in part due to the inability of the GC principles [2] to
42 address the demands of the analytical field. In this direction, only one principle of GC (i.e.,
43 real-time analysis for pollution prevention) is directly related to Analytical Chemistry, whereas
44 the rest are either loosely related to chemical analysis or not related at all. For this reason, the
45 12 principles of GAC were introduced almost a decade ago [3], providing a more suitable
46 framework towards greener analytical chemistry practices. In subsequent years, several self-
47 assigned ‘green analytical methods’ appeared in the literature that exclusively focused on the
48 improvement of one particular principle of GAC, systematically ignoring other GAC aspects.
49 In an attempt to assess and harmonize the compliance of analytical methods with GAC
50 assumptions, several metrics of varying comprehensiveness have been reported in the literature
51 [4–12]. They are based on the incorporation of different criteria, and the generic response of
52 the assessment can be highly variable in both complexity and appearance. The National
53 Environmental Methods Index (NEMI) pictograms [5], derived from Yes/No responses to four
54 specific criteria, was the first (and rather simplistic) approach proposed in the literature. In
55 2012, the analytical eco-scale metric tool [6] was reported assigning penalty points to different
56 criteria that were subtracted from an ideal score of 100 in such a way that the closer the
57 numerical value was to 100, the greener the method. In an attempt to provide a more complete
58 and refined output, different advanced metric tools were recently developed, namely, the Green
59 Analytical Procedure Index (GAPI and recently reported ComplexGAPI) [7,12], the RGB
60 model [8,9], the Analytical GREEnness Metric Approach (AGREE) [10] and the hexagon-
61 CALIFICAMET [11]. These tools generally provide easy to read pictograms that map the
62 degree of compliance of evaluated criteria within the framework of GAC. The use of metrics
63 for the assessment of analytical methods can be synergistically complemented with other tools
64 focusing on a specific and problematic step of the analytical method. In this connection,
65 different metrics and tools focused on specific aspects of analytical methods (e.g.

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

70 Sample preparation is a key step in the analytical procedure that is essential for the separation
71 and enrichment of target analytes, the removal or minimization of matrix interferences and/or
72 to ensure compatibility with the measurement technique. At the same time, sample preparation
73 has been identified as one of the most critical steps from the GAC point of view [16], mainly
74 because of the typical substantial requirements in solvents (solvent extraction techniques),
75 sorbents (solid-phase based extractions), reagents (for derivatization reactions or the removal
76 of impurities), acids or bases (for pH correction or mineralization), energetic inputs (heating,
77 stirring, cooling) and other consumable materials or devices (such as cartridges, pipettes or
78 pipette tips, glassware). For this reason, the first principle of GAC suggested avoiding sample
79 preparation and instead, using direct analytical techniques [3]. However, the possibility to
80 incorporate direct analytical methodologies in all applications is rather limited [17–19],
81 rendering the implementation of sample preparation strategies critical to tackle analytical
82 challenges. Admittedly, the first principle of GAC has led to a common misconception that
83 omitting the sample preparation step is a green approach, fully neglecting the necessity of this
84 step and the technological advances in the area [20]. In this context, several mature and modern
85 sample preparation approaches exist that do not adversely affect human health or the
86 environment and can lead to more efficient and metrologically improved methodologies [21].
87 Hitherto, the greenness of sample preparation methods is assessed using metric tools anchored
88 in the 12 principles of GAC. However, the philosophy of the GAC approach renders these
89 metric tools inadequate for providing sufficient levels of accuracy and specificity and, as such,
90 gauging progress toward greening sample preparation. The wide range of parameters that
91 influence the greenness of sample preparation creates the need to develop a specific metric
92 system for sample preparation. The present work aims for the first time, to bridge the
93 abovementioned gap by offering a powerful yet user-friendly tool that will enable assessing
94 the environmental impact of sample preparation, the most critical step in settling green
95 analytical methods. The proposed metric tool gives prominence to sample preparation, and
96 predicts as well as detects aspects that could be improved for greening the critical step of
97 sample preparation.

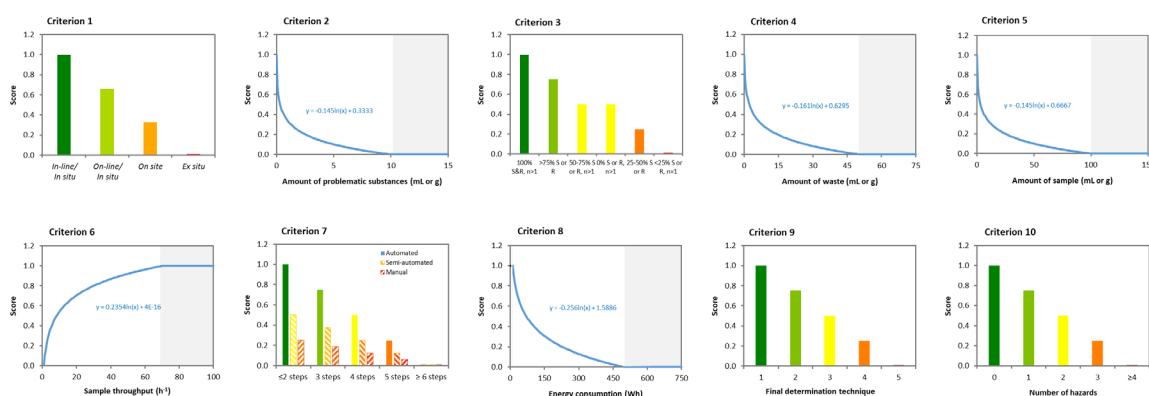
98 99 **2. Criteria and scores calculation**



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

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

112
113 Each of the criteria is recalculated and after quantitative evaluation, the outcome of fulfilling
114 the criterion is reflected as an impact score on a scale from 0 (not fulfilling) to 1 (fulfilling).
115 **Criteria 2, 4, 5, 6 and 8 apply logarithmic functions. They are applied to meet the demands of**
116 **modern sample preparation science. Application of logarithmic functions allows to more easily**
117 **differentiate between typical microextraction techniques impacts.** The assessment criteria,
118 summarized in **Figure 1**, are discussed below.



119
120 **Figure 1.** Graphical representation of the functions applied for the assessment of the evaluated criteria.

122 **Criterion 1. Favor *in situ* sample preparation**

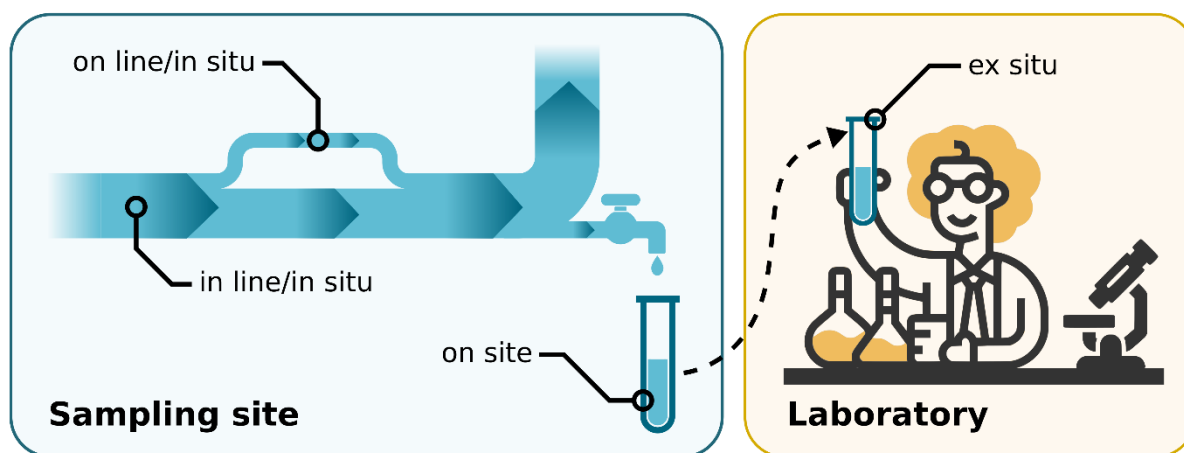
123 The first criterion favors *in situ* sample preparation so as to minimize wasted time and the use
124 of material and energy. Moreover, problems of sample degradation due to improper storage
125 during transport are avoided. *In situ* sample preparation also includes low- or even non-invasive
126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

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

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

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

142



143

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

145

146 **Criterion 2. Use safer solvents and reagents**

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

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

157

$$158 \text{ Score} = -0.145 \times \ln(\text{amount of hazardous substances in g or mL}) + 0.3333$$

159

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

162

163 ***Criterion 3. Target sustainable, reusable, and renewable materials***

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

176 - Only sustainable and renewable materials are used several times, Score: 1.0

177 - > 75% of reagents and materials are sustainable or renewable, Score: 0.75

178 - 50-75% of reagents and materials are sustainable or renewable and can only be used once,
179 Score: 0.50

180 - Materials are not sustainable or renewable and are used several times, Score: 0.5

181 - 25-50% of reagents and materials are sustainable or renewable, Score: 0.25

182 - < 25% of reagents and materials are sustainable or renewable and can only be used once,
183 Score: 0.0

184

185 ***Criterion 4. Minimize waste***



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

194

$$195 \text{ Score} = -0.161 \times \ln(\text{sample mass or volume in g or mL}) + 0.6295$$

196

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

211

212 ***Criterion 5. Minimize sample, chemicals and materials amounts***

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

220

$$221 \quad \text{Score} = -0.145 \times \ln(\text{sample mass or volume in g or mL}) + 0.6667$$

222

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

226

227 **Criterion 6. Maximize sample throughput**

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

234

$$235 \quad \text{Score} = 0.2354 \times \ln(\text{number of prepared samples per hour})$$

236

237

238 **Criterion 7. Integrate steps and promote automation**

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

247 - ≤ 2 steps, Score: 1.0

248 - 3 steps, Score: 0.75

249 - 4 steps, Score: 0.5

250 - 5 steps, Score: 0.25

251 - ≥ 6 steps, Score: 0.0

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

253 - fully automated systems, Score: 1.0

254 - semi-automated systems, Score: 0.5

255 - manual systems, Score: 0.25

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

257

258 ***Criterion 8. Minimize energy consumption***

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

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

266

267 - for < 10 Wh per sample, Score = 1

268 - for values between 10 and 500 Wh per sample, $Score = -0.256 \times \ln(\text{Wh/sample}) +$
269 1.5886

270 - for > 500 Wh per sample, Score = 0

271

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

276

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

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

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

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

302

303 ***Criterion 10. Ensure safe procedures for the operator***

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

- 312 - no hazards, Score: 1
- 313 - 1 hazard, Score: 0.75
- 314 - 2 hazards, Score: 0.5
- 315 - 3 hazards, Score: 0.25
- 316 - 4 or more hazards, Score: 0

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

319

320 **3. Weights for criteria**

321 A closer study of the ten criteria used to assess the greenness of sample preparation, shows that
 322 they are not equal in terms of their importance. For example, selecting *in-situ* sample
 323 preparation or choosing to integrate steps is presumably less significant in terms of greenness
 324 than the volumes of used solvents, energy requirements or assuring safety for the analyst.
 325 Therefore, we suggest the default weights to be applied in any assessment and give the option
 326 to assessor to change them provided that justify these changes. In case of changing the weights,
 327 we suggest preparing the justification to explain the importance of the criteria that is adjusted
 328 to the assessor's requirements.

329

330 **Table 1.** The default weights for the analysis

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

331

332 The highest weight was given to criterion 2 since the solvents and reagents used have a great
 333 impact on the greenness of the sample preparation method. Criterion 1 was given a lowest
 334 weight as it has some impact on sample preparation greenness but still, it is possible to achieve
 335 a considerable degree of greenness even if procedures are not carried out *in situ*. Criterion 3,
 336 with weight 2, describes the origin and disposal of materials and reagents, including
 337 consumables and the assigned weight is not higher due to its semi-quantitative nature. Criteria
 338 4, 8 and 10 dealing with waste, energetic demand and safety of operator are crucial points to
 339 consider in greenness assessment and were therefore given high weight (4, 4 and 3
 340 respectively). Criterion 5 is indeed important as it is related to the miniaturization degree of the
 341 sample and, as such, to the consumption of reagents, solvents and generation of wastes. The



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

353

354 **4. The assessment result**

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

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

369

370 **5. Assessment examples**

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



376 adjustment (only 180 mL of dichloromethane was considered in the 2nd criterion as hazardous
377 materials). It was assumed that no reagents originated from renewable or sustainable sources.
378 Substantial amounts of waste were generated since the excessively large sample volume (1L)
379 became hazardous waste after being in contact with dichloromethane. The sample throughput
380 was estimated to be $\sim 1.5 \text{ h}^{-1}$. The procedure was manual and required ≥ 6 steps; on the other
381 hand, the method did not consume energy. Method 3510C used a GC-MS system for phthalates
382 determination (GC-MS technique was included in the assessment), while method 8061A used
383 GC-ECD increasing the final score to 0.2. Sulfuric acid was accompanied by four pictograms
384 and excluding it from the assessment would result in a 0.22 final score (dichloromethane has
385 two hazard pictograms). The final result for this LLE-based procedure was 0.17, corresponding
386 to a poor performance with the only good score given in the “energy consumption” criterion.
387 The next assessed method was based on solid-phase extraction (SPE) and the procedure aimed
388 at the *in situ* determination of endocrine-disrupting compounds [26]. Sample preparation
389 proceeded in the *on-line/in situ* mode, consumed 6 mL of acetonitrile for column conditioning
390 (acetonitrile has Category 4 of toxicity “harmful if swallowed, in contact with skin or if
391 inhaled” but it is not categorized as toxic). Neither sustainable nor renewable materials were
392 applied and the amount of waste was calculated to be 6.5 g or mL – 6 mL of acetonitrile and a
393 very tiny cartridge of mass estimated to be less than 0.5 g. The sample size was 20 mL and ~ 6
394 samples could be prepared in an hour. It should be noted that the sample throughput of the
395 analytical method was low due to the time needed for HPLC separation. The sample
396 preparation method involved three steps and was fully automated. There was no exact
397 information on the energy demand but this was a microfluidic system with neither heating nor
398 cooling demands, so energy demand per prepared sample was assumed to be low and equal to
399 30 Wh. The final determination proceeded with HPLC and acetonitrile was labelled with two
400 hazard pictograms. The final score was 0.54 and there were no genuinely low respective criteria
401 scores. It should be noted that although *in-situ* sample preparation and automation were the net
402 advantages of this procedure, low weights have been considered to assess the corresponding
403 criteria.

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



410 however, that analysts may choose to perform more extractions simultaneously and, as such,
411 improve the score in this criterion. Three sample preparation steps were identified that were
412 not automated. A ~ 50 Wh estimation of power demand per sample was considered and the use
413 of HPLC at the next procedural step. There were 4 different hazards pictograms – 2 from carbon
414 tetrachloride and 2 from acetonitrile. The total score was 0.38, which was rather low, despite
415 this being a microextraction-based procedure. A slightly higher score (0.43) was obtained when
416 6 simultaneous extractions were considered (sample throughput: 36 h^{-1}).

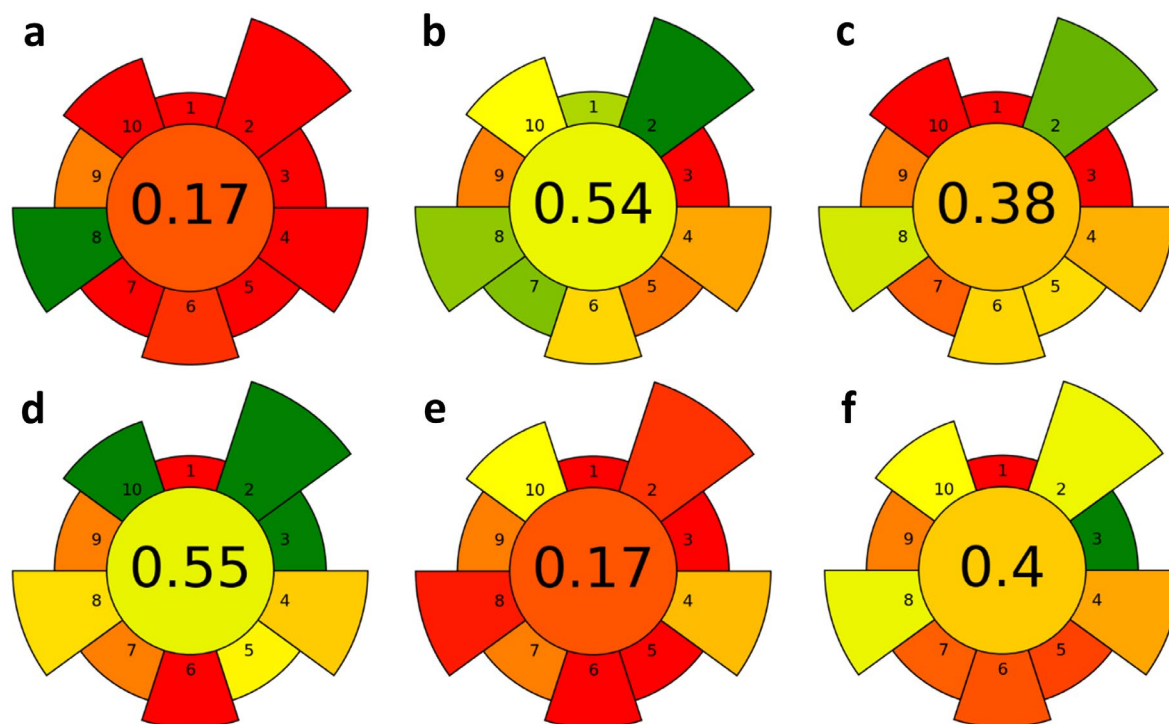
417 The fourth assessed procedure was based on the solid-phase microextraction (SPME) technique
418 [28], performed *ex situ*. The method used ~ 0.63 g of NaCl as a salting-out agent, which was
419 not considered as a hazardous material in criterion 2 but was counted as waste, together with
420 the sample amount. The SPME fiber was reusable while the salt was treated as a sustainable
421 material. The water sample volume was 3.5 mL and the extraction took 1.5 h so the analytical
422 throughput is 0.66 h^{-1} . Sample preparation consisted of a one-step manual procedure and the
423 energy demand was estimated to be ~ 90 Wh. The final determination technique was GC-MS
424 and no procedural hazards were identified. Although SPME is a green technique, this particular
425 procedure had a few drawbacks that lowered the final score to 0.55 namely, *ex situ* mode, very
426 low sample throughput, no automation and quite problematic final determination.

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

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



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

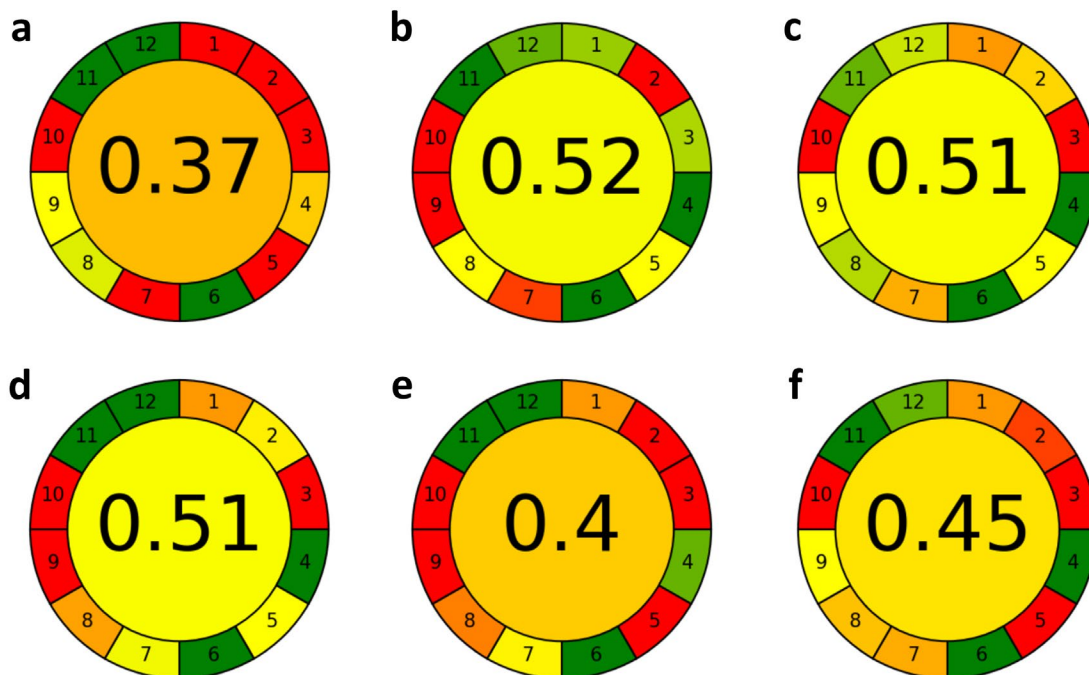


447
448 **Figure 3.** The results of AGREEprep assessment of procedures for phthalate esters determination: a – EPA
449 8061A based on LLE [24,25]; b – automated SPE [26]; c – DLLME [27]; d – SPME [28]; e – MIP-based SPE
450 [29]; f – magnetic particles-based SPE [30].

451
452 The assessment results shown in **Figure 3** depict the general performance of the procedures,
453 while colors distributions allow comparing the nature of threats and hazards. A direct
454 comparison between the results reveals that both the LLE (a) and MIP-based SPE (e)
455 procedures were the least green methods assessed here with final scores of 0.17. Conversely,
456 the procedures based on SPME (d) and on-line SPE (b) were definitely the greenest ones but
457 still, the results (0.55 and 0.54, respectively) were far from being ideal. In the case of the SPME
458 method (d), the manual mode and sample throughput needed improvement and the choice on
459 post-sample preparation configuration of analysis also lowered the final score. Regarding the
460 on-line SPE method (b), the overall score was mainly affected by the lack of sustainable or
461 renewable materials, size economy of the sample and post-sample preparation configuration of
462 analysis.

463
464 For comparison purposes, the above methodologies were also assessed by AGREE [10], our
465 published comprehensive tool devised for the assessment of analytical methodologies on which

466 AGREEprep is based. In AGREE, the final score (in the 0-1 range) is derived from the
467 application of the 12 principles of GAC. **Figure 4** shows the overall results of the assessment
468 of the six methodologies considered. For simplicity and in agreement with most of the
469 publications where the AGREE tool is applied, equal weights were selected for assessing the
470 12 principles of GAC. An important point to consider is that the AGREE scores on the six
471 studied methodologies lie in a narrower range (ranged from 0.37 to 0.52) than those obtained
472 with the AGREEprep metric tool (score range 0.17-0.55). This observation points out the
473 importance of having a metric tool dedicated to sample preparation, since wider score ranges
474 allow a better classification of the methods next to interpretation of results. For example, the
475 two least green analytical methods according to AGREE, namely EPA 8061A based on LLE-
476 GC-ECD (a) and MIP-based SPE-GC-MS (e), also received the lowest scores with
477 AGREEprep. However, the difference in terms of greenness between the remaining four
478 methods is less clear, when evaluating the overall AGREE scores (ranged from 0.45 to 0.52).
479 Moreover, while there is a reasonable relative agreement between the ranking scores received
480 by AGREE and AGREEprep, certain aspects obviously differ. One point to consider is that in
481 AGREE, sample preparation has by default a negative connotation; a requirement imposed by
482 the first principle of GAC. In addition, the improvement of certain aspects of sample
483 preparation methods might not be reflected in the corresponding AGREE score even if non-
484 negligible improvements are achieved. This could be the case with the reduction of the energy
485 consumption required to carry out sample preparation in a method that formerly involved
486 extensive energy consumption during the sample preparation and the analytical measurement
487 steps. Thus, AGREEprep can be invaluable in shedding light on improvable aspects of sample
488 preparation methods that could go unnoticed if only a more general tool is used. It is
489 acknowledged however that the combined use of these two metric tools for assessing the
490 greenness of both sample preparation and analytical methods, respectively, can help identifying
491 weaknesses of the overall analytical procedure and point toward greener alternatives.



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

497 6. Conclusions

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

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