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Combined extraction and microextraction techniques: recent trends and future perspectives

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Declarations of interest: none

Abstract

The latest advancements in the analytical sample preparation indicate a trend of combining different extraction techniques with targeting an improvement in separation, cleanup, detection limits, enrichment factors, and dealing with complex matrices. This manuscript identifies mainly two groups of combined sample preparation techniques. The first group integrates conventional or enhanced extraction techniques with microextraction. The second group combines microextraction with each other. The objectives and merits of each combination are critically appraised with respect to nature of the samples, analytical figure of merits, and certain application scenarios. Green aspects of combined extraction methods are described with some examples. At the end, a brief account is provided on accomplishments, limitations, and future directions.

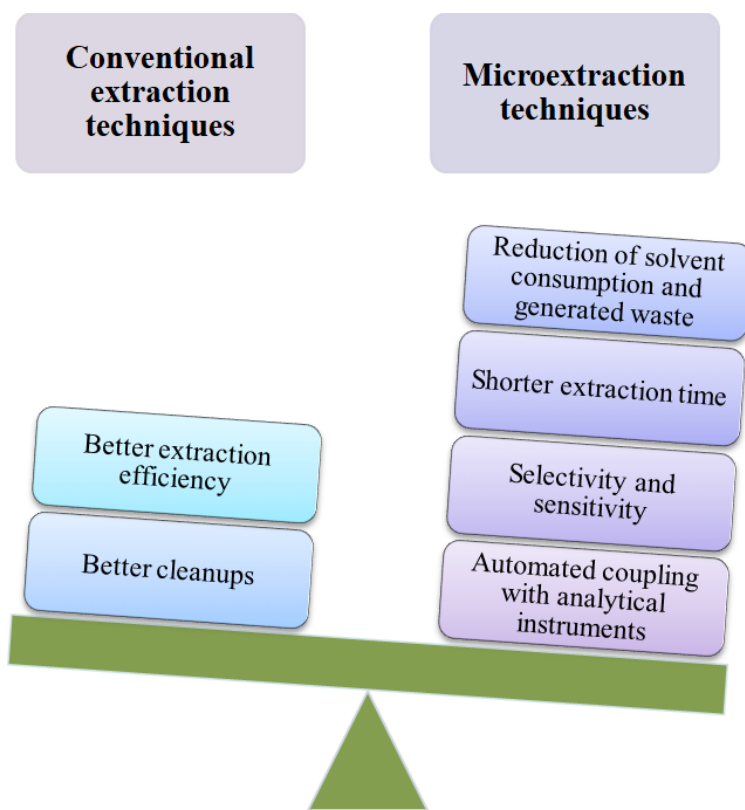
Keywords

Combined extraction techniques; Sample preparation; Microextraction; Preconcentration; Chromatographic analysis; Enrichment factors; Green Analytical Chemistry

1. Introduction

Despite all the major advancements in analytical instrumentation, sample preparation is still of critically importance in the determination of target analytes in various matrices. The requirement of sample preparation arises from several facts including the demand of trace level analysis, the new regulatory obligations, and the complex nature of the sample matrices that are not compatible with analytical instrumentation for direct analysis. In this

37 way, sample preparation is performed to get better separation, clean up, and enrichment of
38 analytes. It is also performed to bring the analytes into a medium that is compatible with
39 analytical instruments [1]. Both conventional extraction and microextraction techniques
40 have been widely adopted as sample preparation methods and they have their own merits
41 and demerits. Generally, conventional extractions provide better extraction efficiency and
42 cleanups as they are exhaustive in nature. In contrast, equilibrium based microextraction
43 techniques are directed toward the reduced use of solvents and extracting phases,
44 miniaturizing the dimensions of extracting devices, and automated coupling with analytical
45 instruments. Such objectives are also in accordance with the principles of green analytical
46 chemistry [2]. At the same time, microextraction are efficient in terms of extraction time,
47 sensitivity, selectivity, enrichment factors and extraction performance (Figure 1).



48

49 Figure 1. Advantages of conventional extraction and microextraction techniques.

50 Recently, a trend has been seen combining conventional and micro- extraction techniques
51 together as well as microextraction techniques with each other. A combination of sample
52 preparation methods is a viable way to introduce a new extraction approach that may
53 synergistically originate advantages from current individual methods, yet with its own
54 innovative merits [3]. Such combinations may overcome the disadvantages of individual
55 techniques and provide benefits specifically related to certain scenario or applications.
56 Recently, combined sample preparation techniques are shown to be excellent approaches
57 for improving the extraction performance through analyte separation, enrichment, and
58 coping with complex matrices and, thus enhancing the quality of the entire analysis [4].



59 This review aims to critically examine and discuss the combined methods and appraise
60 their role in improving overall efficiency of the analytical process from extraction to
61 determination. In addition, it can provide a guidance on the selection of combined methods
62 when dealing with a particular type of extraction challenges or complex matrices.

63 Combined sample preparation techniques can be broadly classified into two categories

- 64 (i) Conventional or enhanced extractions combined with microextraction
- 65 (ii) Binary Miniaturized or microextraction techniques.

66 In this article, only certain trends are highlighted instead of comprehensively covering all
67 the published literature. The articles published in 2015 or later were mainly considered.

68

69 **2. Conventional or enhanced extraction techniques combined with** 70 **microextraction techniques**

71 Liquid phase extraction is associated with a high organic solvents consumption as well as
72 generation of high volume of wastes. Moreover, long time extraction is needed, which
73 involves high energy consumption what impact on an incremental cost. Thus, in order to
74 accelerate the extraction process as well as to improve the analyte separation, the
75 implementation of other extraction technologies, applying different mechanisms such as
76 ultrasound and microwave energy has been promoted. Lowering the final costs through
77 reduction of extraction time and energy consumption are the main objectives of these
78 methods. In addition, enhanced conventional extraction techniques are sustainable, due to
79 the fact that they protect the environment as well as consumers' health. In addition, they
80 are enhancing the economically and innovatively competitiveness of industries. Moreover,
81 application of these techniques in combination with novel microextraction techniques
82 brings additional advantages such as improving the target isolation, and, therefore,
83 enhancing the quality of the whole analysis. The information on microwave- and
84 ultrasound assisted extraction as well as conventional extraction techniques such as Soxhlet
85 and extraction with mechanical agitation are presented in Table 1.

86 Conventional or enhanced extraction techniques combined with microextraction can be
87 categorized into two types based on the nature of the samples i.e. solid and liquid samples

88 **2.1. Combined techniques for the solid samples**

89 In this combination, conventional or enhanced extraction technique is used for the
90 dissolution or releasing of analytes from the solid samples into a liquid medium. The liquid
91 medium containing analytes is further subjected to microextraction to achieve the goals
92 related to sample cleanup and preconcentration of the analytes. The examples of this
93 category include microwave or ultrasound assisted extraction combined with
94 microextraction techniques.

95 **2.1.1. Microwave assisted extraction combined with microextraction**

96 Microwave radiation has ability to penetrate and produce heat inside the biological/solid
97 samples in presence of the polar solvents. Compared to traditional solvent extraction,
98 microwave assisted extraction (MAE) derives benefits from microwave irradiation. The
99 extraction efficiency of MAE is dependent on many factors, including extraction solvent,



100 extraction temperature and time, as well as liquid-to-solid ratio. MAE is relatively greener
101 method compared to liquid-liquid extraction (LLE) because it utilizes very low volume of
102 solvents and generates less waste. Moreover, it is efficient in terms of extraction, time, and
103 energy.

104 MAE is a preferable choice particularly when the analytes are to be extracted from solid
105 samples such as plants, sediments, soil, meat, rice etc. It can be performed simultaneously
106 or prior to microextraction. MAE digests/dissolves the solid samples into a suitable solvent
107 with the aid of microwave energy and resulting extract can be further concentrated with
108 microextraction. This combination provides high enrichment factors and better sensitivity.

109 *2.1.1.1. Microwave assisted extraction followed by dispersive liquid liquid* 110 *microextraction*

111 Dispersive liquid-liquid microextraction (DLLME) is a technique that offers the
112 unbeatably quick extraction rates, however this is accompanied by extensive human
113 manipulation which lead to extra steps that could be a gateway for sample loss, inadvertent
114 contamination, and poor automation. However, when applied with enhanced conventional
115 extraction techniques including microwave assisted extraction, these disadvantages are
116 limited.

117 The first application combining MAE and DLLME was reported in 2011 for extraction of
118 N-nitrosamines in meat samples. MAE was performed using 10 mL of 0.05 M NaOH and
119 this extract was subjected to DLLME. DLLME utilized only 20 μ L of carbon tetrachloride
120 as an extraction solvent. Due to use of NaOH in MAE and extremely small volume of
121 organic solvent in DLLME, this method can be considered relatively environment friendly.
122 MAE provided good extraction efficiency from complex food samples which was not only
123 confirmed by good recoveries but also by the quantification which was possible using
124 aqueous calibration. The enrichment factors were in between 220 and 342. Low LODs
125 were obtained due to the enrichment of analytes provided by DLLME [5].

126 MAE-DLLME-derivatization was used for extraction of haloanisoles and halophenols in
127 cork stoppers and oak barrel sawdust and then final determination by GC-ECD. The
128 method is fascinating from several features such as MAE was performed using methanol
129 and the same extract was employed as disperser solvent in forthcoming DLLME. In
130 DLLME, extraction solvent, derivatizing reagent, and methanolic extract were combined
131 and rapidly injected into an aqueous solution containing potassium carbonate leading to
132 cloudy solution. Moreover, DLLME and derivatization was performed in a single step [6].
133 MAE-DLLME for extraction of polyamine in turkey breast meat [7], pharmaceutical
134 antimicrobials in fish [8], nitrosamines in food samples[9], PAHs in smoked rice [10], and
135 pesticides from pulp and pericarp of Litchi fruit [11].

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140 *2.1.1.2. Dynamic microwave assisted extraction followed by single drop*
141 *microextraction*

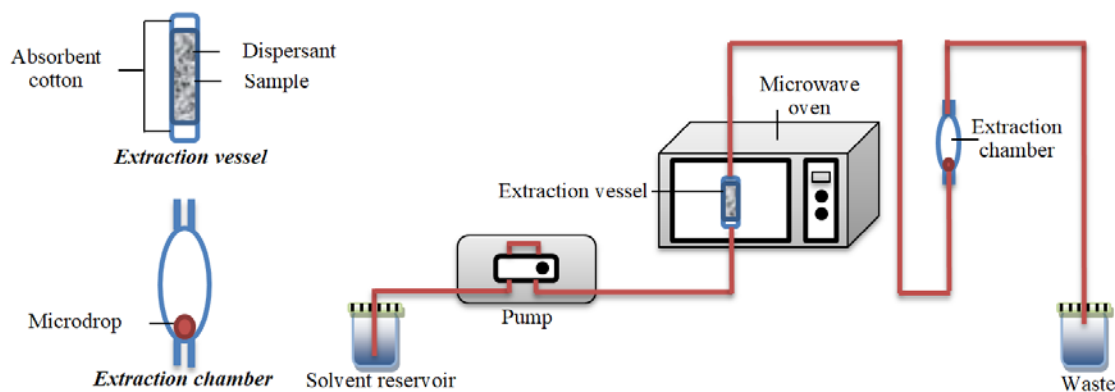
142 Single-drop microextraction (SDME) has become a popular liquid-phase microextraction
143 technique due to the fact that it is inexpensive, nearly solvent-free and easy to
144 operate. From the other site, stirring is mainly performed to accelerate the extraction
145 kinetics by minimizing the interfacial film thickness, which affects the extension of the
146 extraction time as well as lowering extraction efficiency. To overcome these limitations,
147 SDME can be combined with MAE.

148 Traditional MAE is performed at high pressure and temperature that may cause partial
149 decomposition of some target compounds. Moreover, after every extraction cycle, vessels
150 need to be cooled and extract need to be filtered or centrifuged that leads to longer time
151 consumption. However, dynamic MAE (DMAE) can resolve these issues by continuous
152 provision of fresh solvents and transfer of analytes out of the vessel right after completion
153 of extraction process. Furthermore, the extract is amenable to online filtration and DMAE
154 can be coupled with other extraction techniques.

155 The key objective of this combination is the extraction of analytes in complex solid
156 matrices. DMAE was combined online with single drop microextraction (SDME) for
157 extraction of organophosphorus pesticides (OPPs) in tea samples. The microdrop was held
158 in a specially designed chamber that allows the introduction of the microdrop at the bottom
159 of the filled chamber through a micro syringe. A continuous flow of aqueous solution can
160 be passed through the microdrop by means of a microinfusion pump. The droplet formed
161 was quite stable. The generation of the bubbles would push the microdrop to float up
162 slightly, and then the microdrop returns back once the bubbles pass through. This dynamic
163 system provides quick equilibrium achievement. The setup is shown in Figure 2. This
164 combination provides clean up, extraction, separation, and enrichment in a single step
165 process. This method provided LODs of 0.4 to 1.7 $\mu\text{g}/\text{kg}$ [12].

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169 Figure 2. Schematic diagram of DMAE-SDME system [12].

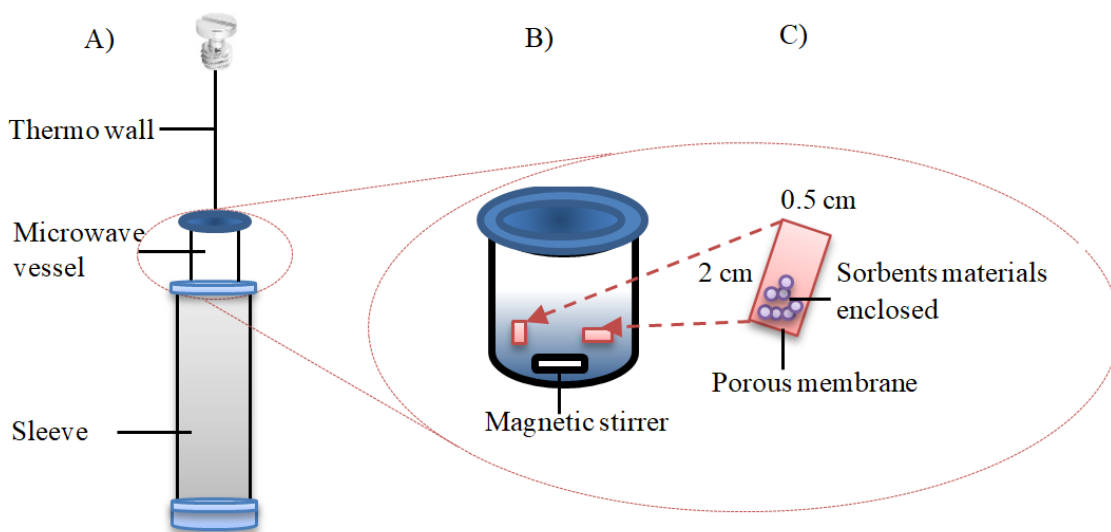
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171 In another work, DMAE was coupled with continuous flow microextraction (CFME) for
172 extraction of OPPs in the vegetables. In the extraction chamber, single drop was suspended
173 at the tip of microsyringe. There was a cooling bath containing ice between CFME and
174 DMAE unit [13].

175 *2.1.1.3. Simultaneous microwave assisted extraction and micro-solid phase* 176 *extraction*

177 In this approach solid sample, extraction solvent, and a membrane bag consisting of sorbent
178 (μ -SPE device) are taken together in a microwave vessel and subjected to MAE. With this
179 strategy, digestion and extraction takes place simultaneously. Solid sample is digested with
180 the help of the microwave irradiation in a suitable solvent and target analytes are released
181 to the same solvent. These analytes simultaneously adsorb on the sorbent inside the porous
182 membrane bag. The protection of the sorbent inside the porous bag is highly suitable for
183 complex matrices as the membrane allows the analytes pass through while interfering
184 complex matrices cannot. After the extraction, μ -SPE device is taken out of the microwave
185 vessel and analytes are back-extracted into a suitable solvent, a part of which is injected to
186 analytical instrument for the quantitation. This approach was used for extraction of
187 parabens in human ovarian cancer tissues and finally their analysis by HPLC-UV [3]. The
188 schematic diagram of this combination is shown in the Figure 3.

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191 Figure 3. Schematic representation of A) MASE – μ -SPE setup, B) μ -SPE system and
192 C) enlarge image of extraction device (not drawn to scale) [3].

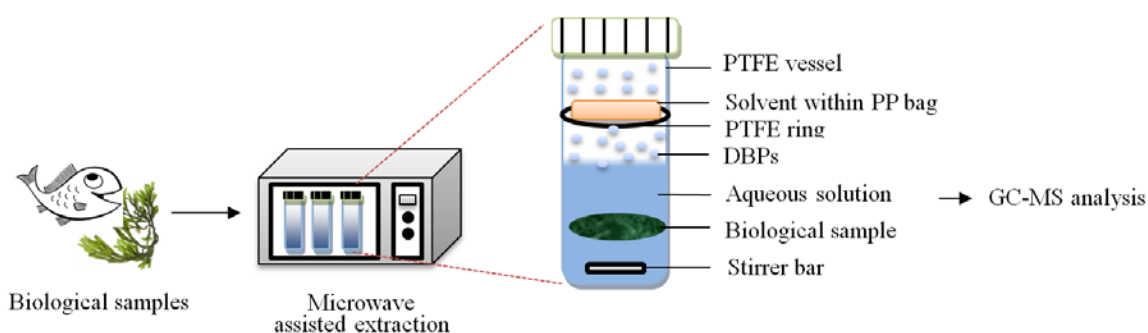
193 *2.1.1.4. Simultaneous microwave assisted extraction and liquid phase* 194 *microextraction*

195 The headspace liquid phase microextraction (HS-LPME) is a very popular technique and
196 thus, have been described in many papers. This is because this method is very useful for
197 the extraction of wide range of compounds including volatile and semi-volatile organic

198 compounds in various types of analyses. However, to reduce the time of extraction,
199 HS-LPME could be coupled with MAE.

200 A single-step microwave assisted headspace liquid-phase microextraction (MA-HS-
201 LPME) method was developed for extraction of trihalomethanes (THMs) and haloketones
202 (HKs) in biological samples. In this method, an optimum amount of biological sample
203 along with optimum volume of acid was taken inside the microwave vessel. Within the
204 vessel, a porous membrane bag filled with extraction solvent was supported on a PTFE
205 ring over a certain height above the sample. This set up was then subjected to microwave
206 irradiation to get simultaneous digestion of biological samples and extraction of target
207 analytes in headspace into the solvent containing porous membrane bag (LPME device).
208 The schematic is shown Figure 4.

209



210

211 Figure 4. Schematic of extraction methods using MA-HS-LPME system [4].

212

213 2.1.2. Ultrasound assisted extraction and microextraction

214 Ultrasound assisted extraction (UAE) has some advantages for extraction of solid samples
215 (natural products, sediments, etc.) due to flexible and adjustable nature of ultrasonic
216 energy. UAE is rapid and significantly increases extraction yield. This is because it has the
217 plenty of power to break up the inner structures of the solid samples (plant cells, tissues,
218 sediments, etc.) and provides high contact surface between sample and extracting phase.
219 UAE extract can be further combined with microextraction to derive benefits of better
220 cleanup, sensitivity and enrichment factor. The most popular microextraction technique
221 that is coupled with UAE is DLLME.

222 The first study combining UAE and DLLME was reported in 2011 for extraction and
223 preconcentration of OPP residues in tomato samples. UAE was performed at small scale
224 (5 mL solvent). Briefly, the sample was homogenized and subjected to UAE in acetone.
225 No clean-up or evaporation were required after extraction. UAE extract was further
226 concentrated by DLLME and injected to gas chromatography–flame photometric detection
227 (GC–FPD) for final determination [14]. UAE was used for elution of PCBs from marine
228 sediments into the extraction solvent under optimum conditions. The extract was then dried
229 under nitrogen stream and reconstituted using 1 mL of the extraction solvent. This extract
230 was then used for DLLME. This method provided LODs in the range of 0.021 to
231 0.057 ng/g, GC-MS being the final determination instrument. The authors did not discuss

232 the enrichment factors achieved, however, one obvious advantage of UAE is to convert the
233 sample into a form which can be combined with microextraction [15].

234 UAE-DLLME was also used for extraction and enrichment of acrylamide from various
235 bread samples. Before DLLME, analyte was derivatized using xanthyrol, GC-MS being
236 the final instrument for analysis [16]. Another example is extraction of Ochratoxin A and
237 citrinin in fruit samples were extracted. The fruit samples were first extracted with 1%
238 acetic acid in acetonitrile by UAE. After centrifugation, the upper phase (acetonitrile) was
239 further employed as disperser solvent in the subsequent DLLME. This is a green aspect
240 that allows the use of extraction solvent of first technique to be disperser solvent of the
241 other technique leading to reduction of overall solvent consumption [17]. The other
242 examples are listed in Table 2.

243 ***2.1.3. Ultrasound-microwave synergistic extraction combined with microextraction***

244 Combining UAE and MAE with microextraction provides synergistically enhanced
245 extraction performance. Ultrasound-microwave synergistic extraction (UMSE) was
246 combined with headspace solid phase microextraction (HS-SPME) for extraction of
247 volatile components in tobacco. UMSE-HS-SPME combines separation, extraction, and
248 enrichment in a single step. UMSE-HS-SPME provided more type of volatile components
249 compared to MAE-HS-SPME and HS-SPME, favoring synergistic effects. These effects
250 were explained with the help of SEM images of ultrasound and microwave irradiated
251 tobacco during extraction [18]

252 The key characteristics of conventional extractions combined with microextractions are
253 provided in Table 2.

254 **2.2. Combined techniques for liquid samples**

255 In this combination, conventional technique is used for the cleanup and isolation of target
256 analytes from relatively large volume of liquid samples. The analytes in the extract of the
257 conventional technique are further concentrated using microextraction approach. The
258 example of this category is hyphenation of solid phase extraction with other
259 microextraction approaches.

260 **2.2.1. Solid phase extraction combined with microextraction techniques**

261 Solid phase extraction (SPE) is combined with microextraction to achieve certain goals
262 related to matrix complexity. SPE provides both concentration and cleanup of the target
263 analytes. SPE is usually selected to deal with dirty or complex matrices. However, it
264 requires large volume of elution solvent and thus decreases enrichment factors (EFs).
265 Microextraction alone can provide reasonably high EFs but still they have some challenges
266 to deal with complex matrices. Large volume SPE extracts can be further enriched by
267 microextraction and this combination will provide both cleanup and high EFs [19].

268 SPE and solidified organic drop microextraction (SODME) was coupled for extraction of
269 total, suspended, dissolved, organic, and inorganic arsenic species (speciation) in tea leaves
270 and tea infusions after combining with electrothermal vaporization ICP-MS. SPE was
271 performed using a micro PTFE column with titanium dioxide as an adsorbent. NaOH
272 solution was used for desorption of retained analytes. For SODME, chelating reagent along

273 with few microliters of organic solvent (extracting phase) was added to extract of SPE and
274 stirred. After the extraction, organic drop was solidified by placing the vial in an ice bath.
275 Organic phase was separated and melted and made up to 100 μL . Only 10 μL extract was
276 injected in ETV-ICP-MS. This method provided very low LODs (ppt levels) as well as
277 enrichment factors of 500 folds for As (III) and As (V). The method also showed good
278 tolerance against very high concentration of common interfering ions mainly due to
279 selective chelating reagent [19].

280 DLLME alone cannot provide proper cleanup when dealing with complex matrix. A kind
281 of sample preparation is needed. The combination of SPE and DLLME can provide better
282 cleanups as well as enhanced EFs. This combination is widely used for extraction in
283 complex matrices. This is a good choice for cleanup and preconcentration of large volume
284 samples as well as their preconcentration. EFs using DLLME mostly in the range of
285 50–1000, which still cannot fulfill the requirement of the ultra-trace residue analysis.
286 However, SPE combined with DLLME can provide very high EFs (up to 50,000), and it
287 can be also used in complex matrices [20].

288 SPE-DLLME combination was used for the extraction of chlorophenols in aqueous
289 samples [21]. SPE-DLLME was also used for extraction of OPPs in water samples before
290 their determination by GC-MS. The elution solvent of SPE was used as disperser solvent
291 in DLLME. This method resulted in very high enrichment factors and excellent LODs in
292 the range of pg/L , which were not attainable using either of the methods alone [22]. SPE-
293 DLLME-SFO was used for extraction of parabens in different matrices and EFs up to 1886
294 were reported [23]. Similarly, some other studies reported even higher EFs, for example
295 up to 2615 for extraction of OPPs in water [24], up to 7873 for amide herbicides in water
296 [25], up to 9405 for extraction of PBDEs in water [20], up to 18,000 for extraction of
297 chlorophenols in water [21], up to 21,000 for extraction of OPPs in water [26].

298 The values for enrichment factors depend on the selection of different parameters related
299 to both SPE and DLLME. The selection of sample volume, suitable sorbent and elution
300 solvent in SPE, and extraction solvent in DLLME are more critical. The analytical
301 instrument can also have substantial effect on the sensitivity.

302 SPE-DLLME was developed for the extraction of eight pyrethroids in cereal samples
303 which were further determined by GC-MS. LOQs with combined method were almost 10
304 times better than SPE alone except for few analytes [27]. Similarly, SPE in combination
305 with ion pair based surfactant assisted DLLME-SFO followed by graphite furnace atomic
306 absorption spectroscopy was used for determination and speciation of mercury. The LOD
307 was 0.009 $\mu\text{g/L}$ [28]. SPE-DLLME was also employed for extraction of different analytes
308 in water [24], honey [29], human urine and plasma [30]. The analytical features of SPE-
309 DLLME are provided in Table 3.

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314 **3. Miniaturized or microextraction techniques combined with each other**

315 Combined or binary microextraction techniques are also used to accomplish certain goals
316 related complex matrices, analyte isolation, and preconcentration. These techniques are
317 mostly used for liquid samples. However, QuEChERS followed by other microextraction
318 technique, is a combination which is also used for the solid samples.

319

320 *3.1. Dual or tandem dispersive liquid-liquid microextraction*

321 DLLME has been widely accepted as an extraction technique both in its original and
322 modified formats due to low consumption of toxic solvents. Dual or tandem DLLME
323 involves coupling of two DLLME procedures. The major aim of this combination is to
324 reduce the interferences that are co-eluted in the first DLLME by back extracting the
325 analytes into the extraction solvent of second DLLME. In case, derivatization is combined
326 with DLLME, second DLLME can remove excess catalysts and derivatizing reagents that
327 otherwise may cause serious interference in separation and detection of target analytes.

328 To introduce further greenness in the procedure and deal with complex matrices, various
329 variations in the original DLLME have been proposed. For example, the use of the toxic
330 organic dispersants can be avoided by using surfactants. However, these surfactants can
331 damage the stationary phase inside the capillary columns. To resolve this, reverse-phase
332 DLLME and standard DLLME can be coupled. Such coupling was used for extraction of
333 phenylpropenes in the oil samples. In the first DLLME oil sample was diluted using n-
334 hexane and analytes are extracted using 160- μ L of 0.2 mM Triton X-100 in acetonitrile
335 following all conventional procedure of DLLME. Then to the extract of first DLLME
336 (110 μ L), water and ethyl acetate was added and analytes were extracted back into ethyl
337 acetate. The solvent of the first extract served as a dispersant in the second DLLME. The
338 purpose of the second DLLME was to reduce the concentration of the surfactant [31].

339 In another work, tandem-DLLME (TDLLME) was consisted of two hyphenated DLLME
340 methods; the first was accompanied by air agitation in the presence of ultrasound
341 irradiation and the last with only several air agitation cycles. The need of this combination
342 arises from the situation when in first DLLME interference are co-eluted with analytes
343 resulting in low sample cleanup. In the second DLLME analytes are extracted into
344 relatively small volume of the extracting phase leading to further cleanup and
345 preconcentration. The selection of extraction parameters such as extraction solvents, pHs
346 are dependent on the nature of the target analytes and target instrumentation. The example
347 of this kind is TDLLME of beta blockers in human plasma and pharmaceutical wastewater
348 samples [32].

349 TDLLME was also used for the extraction of doxepin, citalopram, and fluvoxamine in
350 aqueous samples. This method provided a high sample clean-up, and suitable for complex
351 matrices. In the first DLLME, the analytes in an aqueous sample were extracted (by
352 adjusting pH) into an organic solvent. This step provides a low sample cleanup as some
353 interferences may coextract. In second DLLME, these analytes were simply back-extracted
354 into an aqueous acceptor phase and sample cleanup was significantly enhanced. This step
355 can also solve the problem of the final extract that should be aqueous with some
356 instruments. The overall extraction time was 7 min, and very simple equipment was



357 required for this whole process [33]. TDLLME combining USAEME and AADLLME was
358 used for extraction of tricyclic antidepressant drugs (TCA) wastewater and human plasma
359 samples. Enrichment factors were in between 50 – 101 [34].

360 Dual DLLME can also be combined with derivatization. As an example of this, facile
361 microwave assisted derivatization (MAD) was performed between forward-UADLLME
362 and back-UADLLME. Because of complex matrix and low concentrations of target
363 analytes (PPD and PPT) in rat plasma, the objective of forward-UADLLME was cleanup
364 and enrichment. MAD was used for enhancing the detection sensitivity of target analytes.
365 However, the excess use derivatization reagents and catalysts cause severe interferences in
366 detection. The purpose of the back-UADLLME was removal of these excess reagents and
367 simultaneously enriching derivatized analytes before LC–MS analysis [35]. Key features
368 of TDLLME methods are listed in Table 4.

369 ***3.2. Electromembrane extraction combined with liquid phase microextraction***

370 Hollow fiber liquid-phase microextraction (HF-LPME) in three phase mode is performed
371 by using a supported liquid membrane (SLM) which is an organic solvent impregnated in
372 the pores of a hollow fiber membrane. The acceptor phase is aqueous and it is filled inside
373 the lumen of the hollow fiber. The extraction is based on passive diffusion of neutral
374 species from the sample through the SLM and into the acceptor solution. Although HF-
375 LPME offers tremendous cleanup due to the high selectivity of the SLM and good
376 enrichment factors due to the adjustable ratio between the sample volume and the acceptor
377 volume. However, LPME is not suitable for simultaneous extraction of acidic and basic
378 drugs.

379 Electromembrane extraction (EME) is a miniaturized sample preparation technique, which
380 offers many benefits such as low cost, simple operation, and fast extraction as well as green
381 in nature. EME is also used to selectively extract charged analytes using SLM using electric
382 field and finally into acceptor phase. It provides isolation and cleanup. EME has mostly
383 been used for extraction of basic drugs and acidic drugs individually. Recently, EME has
384 also been used for simultaneous group separation of basic and acidic drugs at a certain
385 sample pH, where the acidic drugs were negatively charged and the basic drugs were
386 positively charged. However, recoveries were very low in such instances.

387 The coupling of EME and LPME has been proposed for single step and simultaneous
388 extraction and clear group separation of acidic and basic drugs with some reasonably high
389 recoveries. The concept took advantage of the fact that low sample pH is optimum pH for
390 the extraction of basic analytes by EME and basic analytes by LPME. Compared to dual
391 EME, this combination provided uniform electric field distribution as well as purity of the
392 separated drugs. Basic drugs were extracted exhaustively by EME while slightly lower
393 recoveries for acidic drugs were obtained because a small fraction of acidic drugs were
394 trapped in SLMs of both EME and LPME. This combination has good potential for
395 extraction in biological samples. Moreover, the low cost device can be used for single
396 extraction to avoid any carry over effects [36].

397

398



399 **3.3. Hollow fiber supported liquid membrane and DLLME**

400 This combination was used for extraction of HF-DLLME for direct extraction of pesticides
401 in grape juice samples. This combination resulted in reduction of some steps involved in
402 conventional DLLME. It is important here to describe some procedural details to
403 understand the underlying objectives of this combination.

404 Previously washed and dried HF membrane was cut into pieces of 2.0 cm length. A
405 stainless-steel wire with diameter equal to the inner diameter of HF membrane was passed
406 through the silicone septum with polypropylene screw cap. HF membrane piece was
407 slipped over the stainless-steel wire in a way that its outer surface and the pores were
408 available for the extraction of the analytes. This porous membrane fixed on the stainless-
409 steel wire was then impregnated with dodecanol by direct immersion. Then it was fixed on
410 the glass vial containing grape juice, buffer solution (to adjust pH), solution containing a
411 mixture of the analytes and a solution containing a mixture of extraction and disperser
412 solvent. The mixture was stirred to transfer the target analytes to SLM. After the extraction,
413 HF membrane was removed from the sample and from the stainless-steel wire and to
414 transfer it to an Eppendorf flask containing desorption solvent. This method does not
415 involve centrifugation like standard DLLME methods and is less laborious [37].

416 The same combination of HF-DLLME with derivatization was used for extraction of
417 aflatoxins in soybean juice followed by HPLC-FD determination. The main benefit of this
418 method is the use of non-chlorinated solvent and insignificant amounts of organic solvents
419 [38].

420 **3.4. Stir-bar sorptive extraction followed by DLLME**

421 Stir bar sorptive extraction (SBSE) is performed by coating the sorbent on a stir-bar which
422 is stirred in the sample solution for an optimum time. The analytes are then desorbed
423 thermally for GC and with solvent for HPLC. SBSE has similar advantages like SPME but
424 EFs are much higher in case of SBSE. SBSE has been combined with DLLME-SFO for
425 extraction of PAHs in water samples. The extracted PAHs were quantified using HPLC-
426 UV. This combination provided very low LODs (0.0067 – 0.010 ppb) and very high EFs
427 (1630 – 2637) [39].

428 **3.5. Dispersive/magnetic solid phase extraction combined with DLLME**

429 Here we describe some examples of single and two-step DSPE-DLLME and their
430 advantages in sample preparation, which mainly rely on purifying target analytes as well
431 as minimizing matrix effect.

432 Single step combination utilizes the benefits of both adsorption and solvent extraction in
433 addition to the in-situ derivatization of the analytes. High enrichment factors can be
434 obtained using this combination. This method was used for the extraction of aliphatic
435 amines on the atmospheric fine particles. The disperser solvent (0.3 mL) was distributed
436 into two parts, extraction solvent and derivatizing reagent was added to first part and 3 mg
437 of the reduced graphene oxide was added to the second part and ultrasonicated for 1 min.
438 First part was rapidly mixed to the sample solution and then the second part was added.
439 Mixture was vortex agitated for 7 min and then centrifuged. The upper aqueous layer was
440 carefully withdrawn by a syringe. The acetone (100 μ L) was added to the remaining

441 mixture to desorb the analytes with aid of sonication. After that it was centrifuged, and
442 supernatant was transferred to a glass micro-insert and it was dried and reconstituted in
443 20 μ L of acetone. High enrichment factors in the range of 307 – 382 were obtained [40].

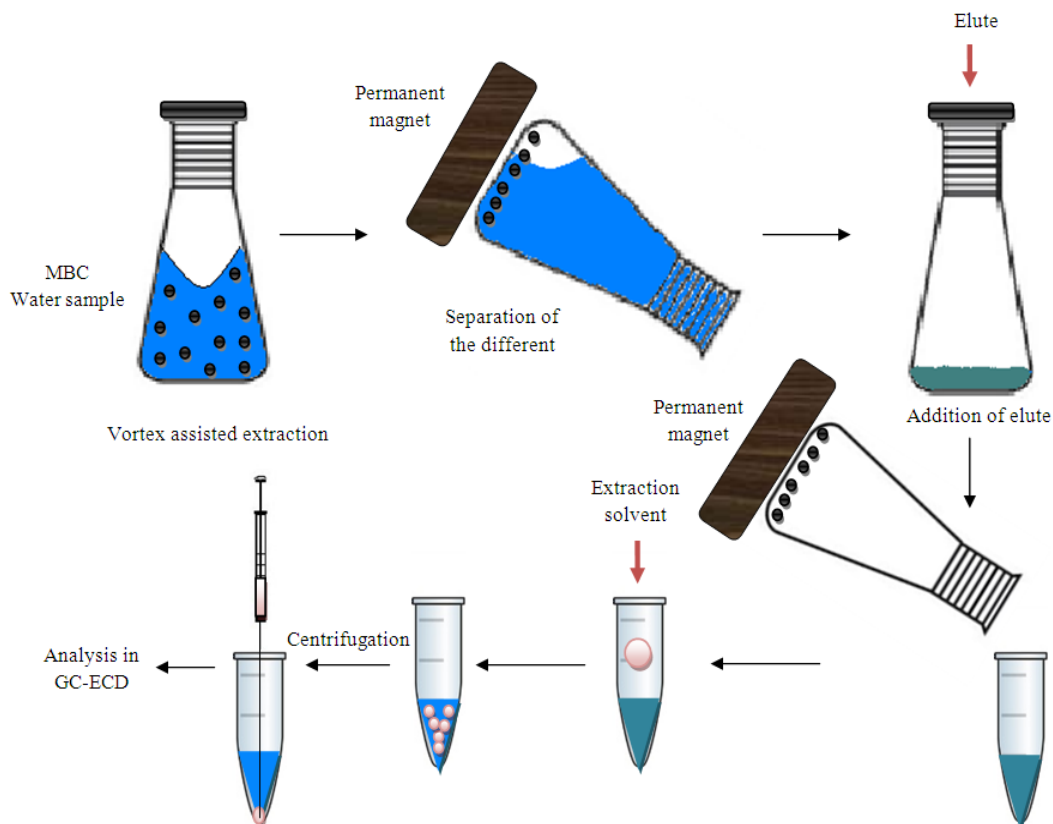
444 In the two-step combination, DSPE is performed first with the objectives of better sample
445 clean up using selective adsorbent. The method was designed for extraction of benzoylurea
446 insecticides in soil and sewage sludge. The analytes were first leached from the certain
447 amount of the sample into acetone with aid of sonication. After filtration, activated carbon
448 was used for DSPE to selective cleanup co-eluting colored species. Again, the filtered
449 acetone was used for VA-DLLME-SFO. Acetone not only worked as leaching solvent but
450 the dispersive solvent for DLLME. 1-undecanol was used as extraction solvent [41].

451 Nano polypyrrole based MSPE was followed by DLLME for extraction of megestrol
452 acetate and levonorgestrel in biological samples prior to their determination by HPLC-UV.
453 In DLLME, sedimented phase was separated using filtration based phase separation.
454 Reasonably high EFs (3680 – 3750) were obtained with corresponding LODs of
455 0.03 ng/mL [42]. Octadecyl modified magnetic silica nanoparticles based MSPE was also
456 combined with DLLME for extraction of phthalates in water. The eluent of MSPE was
457 used as disperser for following DLLME. This combination eliminates the step of
458 evaporative concentration. The average EFs of 20000 were obtained with LODs lying in
459 part per trillion range. This method can be beneficial for ultra-trace analysis in complex
460 matrices [43].

461 Magnetic matrix solid phase dispersion (MMSPD) was also combined with DLLME. The
462 extract of MMSPD was further subjected to DLLME. This combination provided LODs
463 lower than MMSPD or DLLME alone [44]. The schematic is shown in the Figure 5.

464

465



466

467 Figure 5. Schematic procedure of the MMSPD assisted DLLME method [44].

468

469 **3.6. Quick, Easy, Cheap, Effective, Rugged, and Safe Method Followed by DLLME**

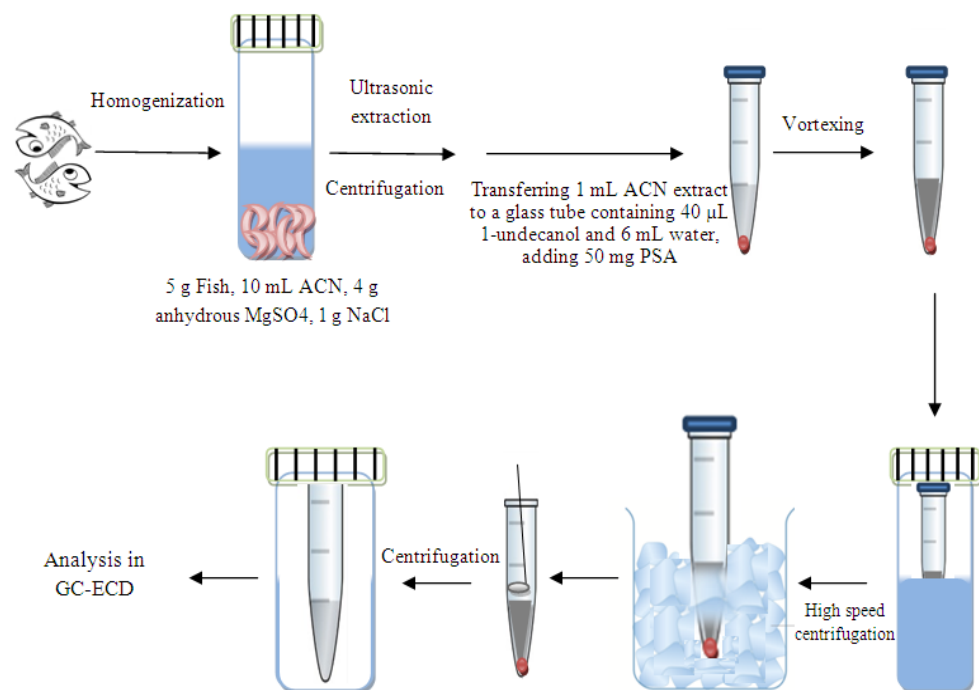
470 Quick, Easy, Cheap, Effective, Rugged, and Safe Method (QuEChERS) is initially
 471 developed for sample cleanup. The complex biological and environmental samples are first
 472 treated with QuEChERS using acetonitrile as a solvent. Despite the fact QuEChERS can
 473 provide an efficient cleanup but the EFs are not very high. The cleaned extracts then can
 474 be employed for microextraction to achieve low LODs through attainment of high EFs.
 475 The other advantage is better chromatographic separations. DLLME is a rapid, easy to
 476 operate, efficient microextraction technique which provides very high EFs.

477 The initial work combining QuEChERS with DLLME was reported in 2011 for extraction
 478 of multi pesticide residues in maize samples prior to their determination by GC-MS. Apart
 479 from the high EFs, DLLME provided better cleanup of some polar matrix components
 480 maximizing the sensitivity of single quadruple MS. The enrichment was about ten times
 481 than QuEChERS alone. The LODs were in between 8 to 55 $\mu\text{g}/\text{kg}$ [45].

482 QuEChERS-IL-DLLME was also used to extract bis-phenol A (BPA) in canned food
 483 samples. The acetonitrile extract (1 mL) obtained from QuEChERS was subjected to
 484 IL-DLLME. IL was used as extraction phase while acetonitrile from first part worked as
 485 disperser solvent. The used IL, 1-hexyl-3-methylimidazolium
 486 bis(trifluoromethylsulfonyl)imide $[\text{C}_6\text{mim}][\text{Tf}_2\text{N}]$ has lower viscosity, surface tension, and

487 water solubility, and higher density than water; it is greener alternative to conventional
488 DLLME solvents (haloalkanes). In this way, this combination provided various
489 advantages. EF of 98 was obtained for BPA [46].

490 For the complex matrices like fish DLLME cannot be used alone, a cleanup is usually
491 required. QuEChERS was combined with DLLME based on solidification of floating
492 organic droplet (SFOD) for determination of organochlorine pesticides (OCPs) in fish.
493 SFOD relies on the use of the extraction solvent with density lower than water and melting
494 point near the room temperature. ACN worked as dispersive solvent while 1-Undecanol
495 was the extraction solvent [47]. The procedural steps of this combination are indicated in
496 the Figure 6.



497

498 Figure 6. The combination of QuEChERS-DLLME (SFOD) [47].

499 There are several other examples where this combination was successfully applied for the
500 extraction of analytes from complex matrices. In most of the cases, acetonitrile of
501 QuEChERS was employed as dispersive solvent for DLLME which is a green aspect of
502 this combination. QuEChERS-DLLME was used for preconcentration of pesticide residues
503 in fatty food [48], OPPs in milk samples [49], and diflubenzuron and chlorbenzuron in
504 fruits [50].

505 The key characteristics of binary microextraction are provided in Table 5.

506 4. Comparison and scope of combined extraction methods

507 Microwave or ultrasound assisted extraction combined with microextraction is usually
508 used for solid samples. Here, microwave or ultrasound assisted extraction releases analytes
509 from the solid samples into the suitable solvent. The analytes in the extract of MAE or
510 UAE are further concentrated using microextraction. The combination serves the purpose

511 of analyte release, cleanup, and further enrichment. With this combination, EFs up to 300
512 have been reported. Although, LODs are highly dependent on the sensitivity of the final
513 determination instrument, LODs down to low ppb levels have been achieved.

514 SPE-DLLME has been widely used for large volume liquid samples. SPE performs both
515 separation and cleanup of the analytes while DLLME can further concentrate the analytes
516 into microliter range of extraction solvent. This combination has provided ultrahigh EFs
517 (up to 50000 times) and LODs in some cases in the ppq range.

518 Binary microextractions are also designed to address certain challenges of sample
519 preparation. For example, in dual or tandem DLLME, the interferences that are co-eluted
520 in the first DLLME are removed by back extracting the analytes in second DLLME. In
521 case, derivatization is combined with DLLME, second DLLME can remove excess
522 catalysts and derivatizing reagents that otherwise may cause serious interference in
523 separation and detection of target analytes. EFs up to 200 have been reported using tandem
524 or dual DLLME. QuEChERS can provide better cleanup for complex samples, but EFs are
525 not very high. Its combination with DLLME can significantly improve EFs.
526 Dispersive/Magnetic SPE-DLLME takes advantage of both adsorption and solvent
527 extraction. EFs as high as 21000 and LODs as low as ppt range were achieved.

528

529 **5. Green Analytical Chemistry and combined extraction methods**

530 The role and impact of Green Analytical Chemistry (GAC) has significantly increased on
531 all analytical procedures. Some of the GAC principles emphasize on the reduction of
532 energy, miniaturization and automation of methods, reduction in the use of toxic reagents
533 and solvents, integration of analytical processes, minimizing sample size or number of
534 samples, and avoiding derivatization [51].

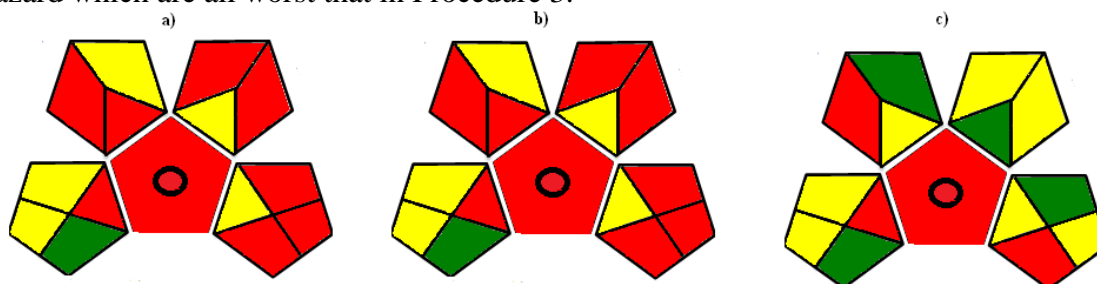
535 Above presented literature depicts some combined extraction methods which present
536 several opportunities to move toward GAC practices. For example, the use of relatively
537 greener energy sources such as microwave and ultrasound for extraction applications is
538 described [5,9]. This will reduce the impact on the environment and the analyst compared
539 to conventional heating sources.

540 In order to present the differences in the green nature of selected procedures [52, 53, 54]
541 based on LLE (Procedure 1 [52]), UAE (Procedure 2 [53]) and UAE-DLLME (Procedure
542 3 [54]) for target compound determination in oil samples, a Green Analytical Procedure
543 Index (GAPI) and Analytical Eco-Scale were applied. GAPI is a “green” assessment tool
544 of analytical methodologies which rates analytical methods against amount and type of
545 waste, environmental hazard and chemical health, and energy requirements [55]. This tool
546 presents information on the entire analytical protocol, from sampling, through sample
547 preparation to final determination. The second tool named Analytical Eco-Scale, is a tool
548 based on penalty points (PPs) which are subtracted from a base of 100. Penalty points are
549 assigned for each reagent/ chemical compound relating to the amount, chemicals
550 utilization, occupational hazards, high energy consumption, and generation of waste [56].
551 In the case of analytical procedures comparison, this one is assigned as greener and more
552 economical, which is characterized by the highest score.



553 The evaluation of examined procedures using GAPI and Analytical Eco-Scale tool is
554 presented in Figure 7 and Table 6, respectively.

555 Taking into consideration examined, it is visible at first glance that Procedure 3: UAE-
556 DLLME can be considered greener than the other two methodologies. This is mainly
557 because a microextraction instead of extraction at macro scale is performed, thus less
558 reagents/solvents are applied affecting the reduction of generated waste. The main critical
559 point of Procedure 1 and 2 are extraction procedure performed at macro scale, the character
560 and aliquot of solvents and reagents used, aliquot of generated waste and occupational
561 hazard which are all worst than in Procedure 3.



562

563 Figure 7. Assessment of the green profile of evaluated procedures (Procedure 1 [52],
564 Procedure 2 [53] and Procedure 3 [54]) using GAPI tool.

565 In solvent based extraction, it is not possible to eliminate the extraction solvents completely
566 but their quantities can be significantly decreased. Solvent based microextraction are best
567 examples of this. However, when integration of two analytical extraction techniques is only
568 a viable way to cope with complex matrices or certain application scenario in sample
569 preparation, there should be some ways to reduce the use of reagents and solvents. This
570 has been demonstrated in many combined methods that extraction solvent of first technique
571 can be used as disperser solvent of the upcoming DLLME [41,47]. Another development
572 with regards to GAC in combined methods is the use of greener solvents such as ionic
573 liquids, surfactants [31].

574 In order to deal with certain type of solid samples (tissues, plant, meat etc.), a kind of
575 pretreatment or digestion is required. This increases overall steps related to pretreatment
576 and then extraction. The one solution is to perform pretreatment/digestion and extraction
577 in a single step. Combined extraction methods based on simultaneous digestion and
578 extraction have been discussed above [3,4]. In some cases, these combined methods,
579 reduce the number of steps as well as the requirement of special equipment [37].

580 The 6th principle of the GAC says avoid derivatization. However, this is not possible to
581 eliminate such derivatizations due to certain limitations related to nature of the analytes
582 and available instrumentation. Different ways to make derivatization process greener
583 include use of less-toxic reagents and solvents, and in situ derivatization using
584 microextraction [57]. This has been practiced in combined extractions [6,35].

585

586

587

588 6. Conclusion and future recommendations

589 The idea of combining different extraction techniques together mostly arises from the
590 special extraction and analysis requirements or underlying limitations of individual
591 approaches. In most of the cases, the combined methods provide a better way of dealing
592 with complex matrices, enhanced cleanups, ultra-high enrichment factors, and trace level
593 detection. In some cases, they also reduce the overall number of steps associated with an
594 individual extraction procedure, or eliminate some procedural steps or reduce the
595 requirement of the electric or special equipment.

596 Based on the literature presented above, it can be suggested that microwave/ultrasound
597 assisted extractions combined with microextraction can be a preferable choice for solid
598 samples. This combination can provide extraction as well as high enrichment factors.
599 Simultaneous MAE and μ -SPE or LPME can provide single step digestion and extraction
600 [3]. SPE-DLLME is a good choice for high volume liquid samples; SPE can provide
601 extraction as well as better clean up, while DLLME can further concentrate the target
602 analytes leading to improved sensitivity of detection. In some cases, EFs of more than
603 50,000 have been attained. Tandem DLLME can provide efficient sample clean up while
604 dealing with complex matrices. Dispersive/magnetic SPE combined with DLLME takes
605 benefit of both adsorption and solvent extraction. QuEChERS can provide an efficient
606 cleanup but the EFs are not very high, however, its combination with DLLME can serve
607 the purpose.

608 Some difficulties may also arise while combining these methods. When each method is
609 performed separately in the combination, it increases overall number of steps as well as
610 extraction time compared to any individual method. Combined methods may have
611 limitations in certain aspects such as requirement of certain volume of the sample and
612 extraction time, to get an efficient performance. For example, in SPE-DLLME, SPE part
613 usually requires a large volume sample. On the other hand, this combination provides not
614 only better cleanups also very high enrichment factors and detection limits. In such cases,
615 the analyst should decide what preferred analytical figure of merits in his analysis are. It
616 has also been noticed that most of the combined methods involve one extraction followed
617 by other, this can be time-consuming and laborious compared to individual techniques.

618 The online coupling of these methods is challenging and it should be considered for future
619 research in this area. Another aspect that needs additional research efforts is the automation
620 of such combinations with analytical instruments as it can greatly reduce the human effort
621 and chances of error. In addition to that these methods should not be developed for the sake
622 of the new combination but with clear objectives and as a solution to existing problems.
623 Different variables involved in combined methods such as time of extraction, number of
624 steps, use of solvents and reagents, and requirement of energy sources should be considered
625 in accordance with recent trends of GAC.

626

627

628

629



630 **Acknowledgements**

631 Muhammad Sajid would like to thank and acknowledge Center for Environment and Water
632 (CEW), Research Institute, King Fahd University of Petroleum and Minerals, Saudi
633 Arabia.

634 **Declarations of interest:** none

635

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Table 1. Main characteristics, advantages and limitations of enhanced and conventional extraction technologies

Issue	Conventional methods		Enhanced extraction techniques	
	<i>Soxhlet</i>	<i>Extraction with mechanical agitation</i>	<i>Microwave-assisted</i>	<i>Ultrasound-assisted</i>
Force of driving	Heat	Solvent contact	Microwave power	Acoustic cavitation
Sample size	1-30 g	1-30 g	1-10 g	1-30 g
Extraction time	6-24 h	Several hours	3-30 min	10-60 min
Solvent amount	150-500 mL	50-500 mL	10-40 mL	50-200 mL
Power amount	High	High	High	Moderate
Advantages	Not use of sophisticated equipment	Not use of sophisticated equipment	Fast. Easy to handle. Moderate use of solvent.	Safe (atmospheric pressure and ambient temperature). Easy to handle. Moderate use of solvent. Reproducible.
Limitations	Exposure risk to organic vapors. Thermo-labile compounds degradation.	Spills risk. Exposure to organic vapors. Thermo-labile compounds degradation. Filtration step is required.	Explosion risk (solvent must absorb microwave power). Filtration step is required. Expensive.	Filtration step is required.

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Table 2. Key characteristics of conventional extractions combined with microextractions

Combination	Analytes	Matrix	Instrument	EFs	LODs (ppb)	Ref.
MAE-UADLLME	Pyrethroids residues	Litchi fruit	HPLC-UV	56.4 – 68.3	1.15–2.46	[11]
MAE-DLLME	Polyamines	Meat	HPLC-UV	190 – 305	0.24 – 0.42	[7]
MAE-SPP-DLLME	Antimicrobial pharmaceuticals	Fish	LC-MS/MS		(4.54 – 101.3) ×10 ⁻⁶	[8]
MAE-DLLME	PAHs	Smoked rice	HPLC-UV	258 - 307	0.05 – 0.12	[10]
MAE-DLLME	Nitrosamines	Food	GC-MS		0.1 – 0.5	[9]
DMAE-SDME	OPPs	Tea	GC-MS		0.4 – 1.7	[12]
DMAE-CFME	OPPs	Vegetables	GC-MS		0.59 – 1.57	[13]
MASE-μ-SPE	Parabens	Human ovarian cancer tissues	HPLC-UV	27 – 314	0.005 – 0.024	[3]
MAE-DLLME	Aromatic amines	Hamburger patties	HPLC-UV	112 – 174	0.06 – 0.21	[58]
MA-HS-LPME	Trihalomethanes and haloketones	Fish tissue and alga	GC-MS		0.051 – 0.110	[4]
UAE-DLLME	PCBs	Marine sediments	GC-MS	-	0.021 – 0.057	[15]
UAE-DLLME	Acrylamide	Bread	GC-MS	230	0.54	[16]
UAE-DLLME	Acrylamide	Potato chips	GC-MS	192	0.6	[59]
UAE-DLLME	Ochratoxin A and citrinin	Fruit	HPLC-FLD		0.06 – 0.16	[17]
USL-SPE-DSLLME	OPPs	Soil samples	GC-MS	6890–8830	0.012 – 0.2	[60]

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941 **Table 3.** List of methods combining SPE and microextraction

Combination	Analytes	Matrix	Instrument	EFs	LODs (ppb)	Ref.
SPE-SODME	Arsenic species	Tea leaves and tea infusions	ETV-ICP-MS	500	0.000046 – 0.000072	[19]
SPE-DLLME	PBDEs	Water	GC-MS	6838 – 9405	0.04 – 0.16	[20]
SPE-DLLME	Chlorophenols	Water	GC-ECD	4390 – 17870	0.0005 – 0.1	[21]
SPE-DLLME	OPPs	Water	GC-MS		0.000038 – 0.000230	[22]
SPE-DLLME-SFO	Parabens	Water, shampoo, mouth rinse solution.	HPLC-UV	245 – 1886	0.3 – 1.7	[23]
SPE-DLLME	OPPs	Water	HPLC-UV	2219 – 2615	0.021 – 0.15	[24]
SPE-DLLME	Amide herbicides	Water	GC-MS	6593 - 7873	0.002 – 0.006	[25]
SPE-DLLME	OPPs	Water	GC-FPD	15160 – 21000	0.0002 – 0.0015	[26]
SPE-DLLME	Pyrethroids	Cereals	GC-MS	18.1 – 25.7	0.2 – 4.0	[27]
SPE-SA-DLLME-SFO	Hg ²⁺	Fish, sand, cigarette, pine leaf, well water, river water	GFAAS	1540	0.009	[28]
SPE-DLLME	Pyrethroids	Honey	GC-MS		0.02 – 0.04	[29]
SPE-DLLME	Benzodiazepines	Human urine and plasma	HPLC-UV		0.07 – 0.7	[30]

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Table 4. Key features of tandem-DLLME methods

Combination	Analytes	Matrix	Instrument	EFs	LODs (ppb)	Ref.
TDLLME	Beta blockers	Human plasma and pharmaceutical wastewater	HPLC-UV	75 – 100	0.8 – 1.0	[32]
TDLLME	Pharmaceutical drugs	Aqueous matrices	HPLC-UV	63 – 94	3 – 10	[33]
TDLLME	TCAs	Wastewater and plasma	HPLC-UV	50 – 101	0.7 – 1.0	[34]
DUADLLME-MAD	PPD and PPT	Rat plasma	UHPLC-MS/MS	164 – 182	0.010 – 0.015	[35]

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Table 5. List and analytical features of the methods based on binary microextraction

Combination	Analytes	Matrix	Instrument	EFs	LODs (ppb)	Ref.
SBSE-DLLME-SFO	PAHs	Water	HPLC-UV	1630 – 2637	0.0067 – 0.010	[39]
DSPE-DLLME	Aliphatic amines	Atmospheric fine particles	GC-MS	307 – 382	0.03 – 0.09	[40]
DSPE-VA-DLLME	Benzoylurea insecticides (BUs)	Soil and sludge	HPLC-UV	104 – 118	0.08 – 0.56	[41]
MMSPD-DLLME	PCBs	Water	GC-ECD		0.00005 – 0.0001	[44]
MSPE-DLLME	Megestrol acetate and levonorgestrel	Biological samples	HPLC-UV	3680 – 3750	0.03	[42]
MSPE-DLLME	Phthalates	Water	GC-FID	17749 – 21278	0.002 – 0.003	[43]
QuEChERS-IL-DLLME	BPA	Canned food	HPLC-UV	98	0.1	[46]
QuEChERS-DLLME (SFOD)	OCPs	Fish	GC-ECD		0.65 – 1.58	[47]
QuEChERS-DLLME	Pesticide residues	Oil seeds	GC-MS	6 – 17	0.01 – 12.17	[48]
Modified QuEChERS-DLLME-SFO	OPPs	Milk	GC-FPD	159 - 213	0.1 – 0.3	[49]
Acetonitrile-based extraction with DLLME	Diflubenzuron and chlorbenzuron	Fruits	HPLC-UV		5.0	[50]

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979 Table 6. Calculated PPs for evaluated analytical procedures for PAHs determination in oil samples (Procedures 1-3)

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PROCEDURE 1: LLE-SPE [52]		PROCEDURE 2: UAE-SPE [53]		PROCEDURE 3: UAE-DLLME [54]	
Reagents	PPs	Reagents	PPs	Reagents	PPs
n-hexane: 16 mL	16	Acetonitrile: 27 mL	16	Water: 3 mL	0
N,N-dimethyl formamide: 8 mL	8 4	Internal standard	4	Acetone: 1 mL	4
Internal standard	0	Dichlorometane: 70 mL	6	Toluene: 100 µL	3
Saline solution: 50 mL	0	n-hexane: 20 mL	16		
Dichloromethane: 20 ML	6				
Acetonitrile: 1 mL	8				
	Σ 42		Σ 42		Σ 7
Instruments	PPs	Instruments	PPs	Instruments	PPs
Transport	1	Transport	1	Transport	1
GC-MS	2	LC-FD	2	GC-MS	2
Occupational hazard	2	Occupational hazard	2	Occupational hazard	1
Centrifugation	1	Waste	5	Waste	3
Sonification	1	Centrifugation	1		
Waste	5				
	Σ 12		Σ 11		Σ 7
Total PPs: 54		Total PPs: 53		Total PPs: 14	
Score: 46		Score: 47		Score: 86	